Titi monkey biogeography: parallel Pleistocene spread by Plecturocebus and Cheracebus into a post-Pebas Western Amazon


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<tr>
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<td>Byrne, H, Lynch Alfaro, JW, Sampaio, I, Farias, I, Schneider, H, Hrbek, T and Boubli, JP</td>
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**INTRODUCTION**

The study of the biogeographic history and phylogeography of forest-dwelling Neotropical vertebrates has focused strongly on diversification patterns among avian taxa, while many Neotropical mammal groups have been comparatively little studied in this context. Biogeographic scenarios derived from avian diversification patterns have been employed to make inferences about the historical biogeography of Amazonia, the evolution of the Amazonian drainage system and biotic connections between the Amazon and Atlantic forest of eastern Brazil (e.g., Aleixo & Rossetti, 2007; Batalla-Filho, Fjelda, Fabre, & Miyaki, 2013; Fernandes, 2013; Ribas, Aleixo, Nogueira, Miyaki, & Cracraft, 2012). Major Andean
uplift in the Neogene and subsequent landscape change, including the evolution of the Pebas lacustrine system and the establishment of the Amazonian drainage system, have often been invoked as the primary drivers of avian speciation in the Neotropics through the division and isolation of formerly continuous populations (e.g., Fernandes, Wink, Sardelli, & Aleixo, 2014; Ribas et al., 2012). In contrast, other studies have highlighted the role of dispersal suggesting that an organism’s ability to persist and disperse in the landscape is the main driver of avian speciation in lowland Neotropical rainforests (Burney & Brumfield, 2009; Smith et al., 2014). Although avian taxa are evidently important models, it is necessary to investigate diversification patterns and speciation mechanisms across a range of widespread Neotropical vertebrate taxa to generate a broader synthesis of the historical biogeography of the Neotropics.

Neotropical primates are a diverse, widespread mammalian clade with five families, 21 genera and around 171 species (Byrne et al., 2016; Estrada et al., 2017; Mittermeier, Rylands, & Wilson, 2013; Rylands et al., 2016). There have been some recent investigations into the biogeographic history of Neotropical primates in a robust phylogenetic framework (e.g., Buckner, Lynch Alfaro, Rylands, & Alfaro, 2015; Lima et al., 2017; Lynch Alfaro, Boubli, et al., 2015). Diversification patterns among capuchin monkeys have been used to test “out of the Amazon” versus “reinvasion of the Amazon” hypotheses to explain sympatry between robust (Sapajus) and gracile (Cebus) capuchins in the Amazon (Lima et al., 2017), while squirrel monkey diversification in the Pleistocene has been associated with the transition from a lacustrine to a riverine system in Amazonia and the occupation of a lowland wetland niche (Lynch Alfaro, Boubli, et al., 2015). Among all Neotropical primate lineages, however, titi monkeys (Callicebinae; Pitheciidae) are unrivalled in their distribution across space and time, forming one of the most widespread and species-rich groups, making them an interesting primate model for the study of Amazonian and South American historical biogeography.

Titi monkeys were considered monogeneric (Callicebus Thomas, 1903) for much of their taxonomic history; however, the most recent classification (Byrne et al., 2016) recognises 33 species across three genera: Cheracebus Byrne et al. (2016) for the widow titis; Callicebus Thomas (1903) for the eastern Brazil titis; and Plecturocebus Byrne et al. (2016), comprised of the P. donacophilus and P. moloch species groups. Their evolutionary history has only recently been elucidated using molecular evidence (Byrne et al., 2016; Carneiro et al., 2016; Hoyos et al., 2016), providing new insights into the relationships among callicebine lineages and an appropriate phylogenetic framework to investigate the biogeographic history of the clade. The subfamily Callicebinae diverged from their sister clade (Pitheciinae) in the early Neogene, c. 18–20 Ma (Byrne et al., 2016; Jameson Kiesling, Yi, Xu, Sperone, & Wildman, 2015; Perelman et al., 2011); thus, titi monkeys comprise one of the oldest lineages of extant New World primates. Based on recent molecular dating analyses, the ancestors of titti genera appeared in the late Miocene, while most extant species diverged in the Pleistocene (Byrne et al., 2016; Hoyos et al., 2016). A deeper understanding of the diversification of extant titti monkey taxa has the potential to provide insight into the biogeographic history of South America since the late Miocene, as well as the biotic connections between the Amazon and Atlantic forest. Furthermore, species-level divergences in the Amazonian clades can shed light on the processes and mechanisms of speciation in Amazonia.

Callicebinae have an extensive range spread across nearly all ecogeographic zones inhabitable by nonhuman primates in the Neotropics (Jameson Kiesling et al., 2015; except Mesoamerica), with each genus or group showing a distinct distributional pattern (Figure 1). Callicebus are entirely extra-Amazonian and are isolated from all other tittis by over 500 km of drier habitats (the cerrado shrubby savannas). They are endemic to eastern Brazil from south of the Rio São Francisco as far as the state of São Paulo, predominantly in the Atlantic forest biome but also in neighbouring arboreal Caatinga regions. The widow titti (genus Cheracebus) occur in the Amazon and Orinoco basins from the eastern foothills of the Andes to the Rio Branco and the Rio Purus (north and south of the Rio Amazonas, respectively). The moloch group of Plecturocebus occurs throughout the southern and western Amazon basin, as far east as the Rio Tocantins, as well as some isolated regions in Colombia (P. ornatus and P. caquetensis). Sympathy among tittis occurs between species of Cheracebus and Plecturocebus in the Amazon, west...
of the Rio Purus. The *donacophilus* group of *Plecturocebus* occupies forest patches and gallery forests in wooded savannas, the Pantanal and Chaco scrublands of Bolivia, Brazil, Peru and Paraguay (Ferrari et al., 2013; Hershkovitz, 1990; Printes, Jerusalinsky, Sousa, Rodrigues, & Hirsch, 2013). The extant distribution of titi monkeys makes the study of their biogeographic history particularly interesting, especially when interpreted in the light of phylogenetic relationships (e.g., the sister clade relationship between *Callicebus* and *Plecturocebus*) and estimated lineage divergence times.

A recent review of Neotropical primate biogeography (Lynch Alfaro, Cortés-Ortiz, Di Fiore, and Bouli, 2015) highlighted the family Pitheciidae as the group most urgently in need of further biogeographic research. As with all New World primate lineages, little information regarding the biogeography of Callicebinae can be derived from the fossil record. The oldest fossil with a definite resemblance to modern titi monkeys (*Miocallicebus villaviega* Takai et al., 2001) comes from La Venta fauna of the middle Miocene (c. 12–11 Ma), in the modern-day Tatacoa Desert of Colombia (Kay, Meldrum, & Takai, 2013). It is the only fossil to document the callicebine lineage; however, the material is limited (consisting of one maxillary fragment), with little else known about *Miocallicebus*. The few previous attempts to understand the biogeographic history of Callicebinae have been confounded by taxonomic and phylogenetic uncertainty (e.g., Hershkovitz, 1963, 1988; Kinzey, 1982; Kinzey & Gentry, 1979).

The earliest detailed hypotheses for callicebine biogeography were proposed by Hershkovitz (1963, 1988) and focused on the upper Amazon Basin as the centre of origin for each major lineage. From this area, he suggested that ancestral stock for each clade dispersed downstream to lowland areas through newly available habitats following receding floodplains during the Pleistocene, with fluvial dynamics important in driving and maintaining species-level diversification. His biogeographic scenarios are elaborate and contain details about the putative historical distribution of each lineage (Hershkovitz, 1988). However, they are based on species relationships inferred from Hershkovitz’s metachromism hypothesis (the evolution of pelage colouration via specific unidirectional pathways), which has not been corroborated by molecular phylogenetic analyses (e.g., see Jacobs, Larson, & Cheverud, 1995). An evaluation of facial colouration in Neotropical primates found no support for the metachromism hypothesis and much higher support for a model assuming no constraints on colour change (Santana, Lynch Alfaro, & Alfaro, 2012). Owing to Hershkovitz’s strict adherence to this hypothesis, it is difficult to extract further details from his biogeographic reconstructions that are interpretable in the light of our current understanding of titi monkey evolutionary history. The first biogeographic scenario derived from an explicit phylogenetic hypothesis is that of Hoyos et al. (2016) who suggested that the central Amazon region was the ancestral area of origin for *P. cupreus* and closely related taxa, with subsequent dispersal westwards following the southern bank of the Rio Amazonas towards the foothills of the Andes, and then northwards along the eastern Andes into Colombia. Since Hoyos et al. (2016) focuses solely on select species of the genus *Plecturocebus*, broader biogeographic patterns remain unclear.

In this study, through a statistical biogeographical approach, we reconstructed the biogeographic history of Callicebinae to better understand their pattern of diversification and the processes responsible for shaping present-day distributions. Our primary aim was to infer the geographic origin of Callicebinae and of the major titi clades, and the history and mode of their colonisation of South America since the late Miocene. Statistical methods based on maximum likelihood were applied to a time-calibrated molecular phylogeny, allowing the comparison of models to determine the relative importance of vicariance and dispersal in forming the current distributions of callicebine taxa. This approach has been used to better understand the biogeographic history of other Neotropical primates (e.g., Buckner et al., 2015; Lima et al., 2017; Lynch Alfaro, Bouli, et al., 2015).

We investigated whether the ancestral populations to extant titi monkeys were widespread across South America or had a more regionally restricted ancestral range, with the aim to shed light on the patterns and processes of genus and species-level diversification, as well as the present-day sympatry between *Cheracebus* and *Plecturocebus* in the Amazon. If callicebine genus and/or species-level divergences represent the fragmentation of a widespread ancestral range owing to a series of ecological or geological vicariant events, we expect our biogeographic model and reconstruction to emphasise vicariance and the ancestral range should span across multiple regions. In the case of a narrow ancestral range, dispersal should be emphasised and the ancestral areas should be restricted to one or two regions. The results of our biogeographic analyses are interpreted in the light of the evolution of the South American and Amazonian landscape, the biotic connections between the Amazon and Atlantic forest since the late Miocene and the drivers of titi monkey diversification.

### 2 | METHODS

#### 2.1 | Molecular data set

A molecular data set was assembled comprising 11 nuclear and two mitochondrial loci (Supporting Information File S1, Table S1). These loci were chosen to maximise taxonomic coverage while minimising missing data across the data set, as only mitochondrial sequences were available for some species. Twenty individuals from 19 species of Callicebinae were represented in the data set, as well as one taxon in the
process of description (P. cf. moloch; J. P. Boubli, unpublished; see also Byrne et al., 2016). A total of 400 sequences were included: 197 sequences belonging to callicebine taxa generated by Byrne et al. (2016), Hoyos et al. (2016) or Perelman et al. (2011) and 203 sequences retrieved from GenBank belonging to 16 species of Platyrhini (N = 5) and Catarhini (N = 11) selected as outgroups in order to include nodes with reliable fossil calibrations.

Six new sequences were obtained to include titi species for which no or little molecular sequence data were available. Three tissue samples were collected from museum voucher specimens from the National Institute of Amazonian Research (INPA; Cheracebus lucifer, CTGAM703) and the Rio de Janeiro Primate Center (CPRJ; Plecturocebus vieirai, 4654; Callicebus personatus, 2466). DNA was extracted from blood and muscle tissues using the Qiagen DNeasy Blood & Tissue Kit according to manufacturer's protocol. New sequences were generated for COI (N = 3), CYTB (N = 2) and RAG1 (N = 1) (see primer information in Supporting Information Table S2 in Appendix S1). The PCRs were carried out in a total volume of 50 μl, containing approximately 30 ng of genomic DNA, 4 μl of dNTPs (200 μM each), 5 μl 10X PCR buffer (100 mM Tris–HCL, 500 mM KCl, 15 mM Mg2+), 1 μl of each forward and reverse primer (0.2 μM) and 0.25 μl of TaKaRa Taq DNA polymerase (1 Unit). The amplification cycles were carried out under the following conditions, initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturing at 94°C for 1 min, primer annealing for 1 min (see annealing temperature in Supporting Information Table S2 in Appendix S1) and extension at 72°C for 1 min; a final extension was carried out at 72°C for 5 min. PCR products were analysed on 1.5% agarose gels and then purified using ExoSap (Werle, Schneider, Renner, Volker, & Fiehn, 1994). Fluorescent dye-terminator (ddNTP) sequencing was performed following the manufacturer's recommended protocol for BigDye sequencing (Applied Biosystems). Subsequent to the cycle sequencing reaction, the products were precipitated with 100% Ethanol/125 mM EDTA solution, re-suspended in Hi-Di formamide and resolved on an ABI 3130xl automatic sequencer (Applied Biosystems).

Consensus sequences were generated from forward and reverse reads using Geneious R7.1 (Biomatters). GenBank accession numbers for all sequences are provided in Supporting Information Table S1 in Appendix S1. Each locus was aligned independently using the MUSCLE algorithm in Geneious R7.1 (Biomatters) and subsequently combined in a matrix with a total alignment length of 9,175 bp. PartitionFinder (Lanfear, Calcott, Ho, & Guindon, 2012) was used to determine the optimal partitioning scheme and the best-fit substitution models for each partition under the Bayesian information criterion (BIC). The analysis was run using the greedy search algorithm and linked branch lengths (see Supporting Information Table S3 in Appendix S1 for selected partitioning scheme).

For some callicebine species in our molecular data set, sequences were obtained from two different individuals, namely Callicebus nigrifrons, Callicebus personatus and Plecturocebus donacophilus (denoted composite individuals in Supporting Information Table S1 in Appendix S1; typically, mitochondrial data originated from one individual and nuclear data from another). Both P. donacophilus individuals are captive specimens from the United States, and thus, likely from the same captive population of P. donacophilus in that country. For C. nigrifrons, both individuals are wild-caught from the same region of Minas Gerais (SE of Belo Horizonte) and loci sequenced for both individuals are highly similar. Both C. personatus specimens are wild-caught from the State of Espírito Santo, but finer location data are only available for one individual, with no comparative sequence data between them (Byrne et al., 2016; Perelman et al., 2011). Species relationships among the three species of Callicebus included in this study are well-resolved in both nuclear and mitochondrial phylogenies (when available, e.g., see additional files 3 and 4 in Byrne et al., 2016), and thus, any uncertainty surrounding C. personatus is not expected to strongly impact phylogenetic reconstruction.

2.2 | Phylogenetic analysis

Phylogeny and divergence times were jointly estimated under an uncorrelated lognormal relaxed clock in the program BEAST v. 1.8.4 (Drummond, Suchard, Xie, & Rambaut, 2012). The partitioning scheme and best-fit models chosen by PartitionFinder were implemented, and a Yule speciation process was used. We conducted two replicate runs of 20 million generations, sampling every 2,000 generations. The sampling distributions were visualised using Tracer v. 1.6 to evaluate convergence, performance and burn-in. We combined the runs using LogCombiner v. 1.8.4 and generated the maximum credibility tree using a burn-in of 10% in TreeAnnotator v. 1.8.4. To obtain the posterior distribution of the estimated divergence times, we used six calibration points with lognormal priors to set hard minimum and soft maximum bounds. Calibration times were chosen based on well-established, dated fossil taxa (see Supporting Information Table S4 in Appendix S1); (a) Callicebinae/Pitheciinae (95%: 15.7–26.0 Ma); (b) Cebinae/Saimirinae (95%: 12.5–26.0 Ma); (c) Hominoid/Cercopithecoid (95%: 21.0–30.0 Ma); (d) Hominoid/Pongo (95%: 12.5–18.0 Ma); (e) Homo/Pan (95%: 5.0–10.0 Ma); and (f) Theropithecus/Papio (95%: 3.5–6.5 Ma).
2.3 | Biogeographic analyses

We divided the distribution of titi monkeys into eight geographic regions (Pantepui, Napo, Inambari, Rondônia, Pará, Wet & Dry Savannas, North Atlantic Forest, South Atlantic Forest) and coded each taxon for presence/absence in each of these regions based upon The IUCN Red List of Threatened Species 2008 and/or more recent studies referenced here. Single letter codes correspond to those in Figures 2 and 4, Supporting Information Figure S2. The geographic regions were broadly defined by major biomes or vertebrate centres of endemism in the Amazon, primarily following Cracraft (1985) and Silva, Novaes, and Oren (2002) (Figure 2; see also description of area delineations in Supporting Information Table S5 in Appendix S1). The Pará region represents both the Tapajós and Xingu areas of endemism. The Pantepui region covers the highlands of the Guiana Shield (from west of the Brazil-Guyana border) in the Duida and parts of the Gran Sabana subcentres of the Pantepui area of endemism and the lowlands of Imeri (Cracraft, 1985; Silva et al., 2002). Geographic regions were assigned for the north and the south Atlantic forest, delineated by the Rio Doce (Carnaval et al., 2014); forests in the southern Atlantic forest are cooler than lowland forests in the northern Atlantic forest, and studies have indicated that the distinction between these
regions is important for many primates (Kinzey, 1982). We also assigned a region for the wet and dry savanna ecosystems; the Wet & Dry Savannas region is comprised largely of the seasonally flooded Pantanal and Llanos de Moxos (Beni) savannas, and the dry wooded Chaco and Chiquitano savannas, and the southernmost tip of the Amazon basin. It broadly follows the Central Grasslands area of Jameson Kiesling et al. (2015) but is refined to the known limits of extant titi monkey distributions.

Our biogeographic analyses were based on time-calibrated trees from the BEAST analysis with the outgroup taxa removed. Two approaches were employed to perform ancestral-area estimations across the phylogeny of Calliacebinae. We first used the R package “BioGeoBEARS” (Matzke, 2013a) to reconstruct the biogeographic history of Calliacebinae under alternative models implemented in a likelihood framework. Anagenetic processes that can be reconstructed (all models) are dispersal (A → AB) and extinction (AB → B). Cladogenetic processes include narrow sympathy (A → A, A), widespread sympathy (ABC → ABC, ABC), subset sympathy (ABC → A, ABC), narrow vicariance (ABC → A, BC; at least one daughter lineage occupies one area after vicariance) and widespread vicariance (ABCD → AB, CD; both daughter lineages occupy more than one area after vicariance). Each model allows for a different subset of biogeographic processes implemented as free parameters estimated from the data (see Matzke, 2013b, fig. 1); widespread and subset sympathy are not allowed in DIVALIKE models; widespread sympathy and widespread vicariance are not allowed in DEC models; and subset sympathy, widespread vicariance and narrow vicariance are not allowed in BAYAREALIKE models. Jump dispersal (A → A, B) is an additional cladogenetic process that can be inferred in the DIVALIKE, DEC and BAYAREALIKE models in BioGeoBEARS with the estimation of the J parameter (+J models; DIVALIKE+J, DEC+J and BAYAREALIKE+J). Jump dispersal (also referred to as founder-event speciation) corresponds to a scenario where dispersal to a new region and divergence were coincident, that is, when a new population is founded from a rare “long-distance” colonisation event and instantly genetically isolated from the ancestral population (Matzke, 2013b, 2014). To account for the influence of distance on dispersal, we also implemented distance-based dispersal models in BioGeoBEARS (+X models; DIVALIKE+X, DIVALIKE+J+X, DEC+X, DEC+J+X, BAYAREALIKE+X, BAYAREALIKE+J+X). For +X models, base dispersal rates are multiplied by distance to the power of X, and X is implemented as a free parameter calculated from the data. Distance has no effect on dispersal when X is estimated as 0 (equivalent to non-X models), whereas when negative values are estimated for X, dispersal probability decreases as distance increases (Van Dam & Matzke, 2016). Distances were calculated as the physical distance between the centre of the geographic areas (see the distance-dependent dispersal matrix in Supporting Information Table S6 in Appendix S1). A summary of the 12 models implemented in BioGeoBEARS is presented in Table 2. Further information on each of the biogeographic models as implemented in BioGeoBEARS can be found in Matzke (2013b, 2014) and Van Dam and Matzke (2016).

We set the maximum number of ancestral areas at a given node to four for all biogeographic analyses given that extant species of Callicebinae have relatively restricted geographic distributions and to avoid intractability of the analyses. We compared the twelve different BioGeoBEARS models for statistical fit using the corrected Akaike information

### TABLE 2

Comparison of the biogeographic processes allowed and the use of distance-dependent dispersal for each of the models implemented in BioGeoBEARS

<table>
<thead>
<tr>
<th>Model</th>
<th>Sympathy</th>
<th>Vicariance</th>
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<th>Distance-dependent dispersal (X)</th>
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<td></td>
<td>Narrow</td>
<td>Widespread</td>
<td>Subset</td>
<td>Narrow</td>
</tr>
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<td>NO</td>
<td>NO</td>
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<tr>
<td>DIVALIKE+J+X</td>
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<td>NO</td>
<td>YES</td>
<td>YES</td>
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<tr>
<td>DEC</td>
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<td>NO</td>
<td>YES</td>
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<tr>
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</tr>
<tr>
<td>DEC+X</td>
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<tr>
<td>DEC+J+X</td>
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criterion (AICc) and interpreted the biogeographic history of Callicebinae based on the ancestral areas estimated at each node under the best-fit BioGeoBEARS model(s). We performed Biogeographic Stochastic Mapping (BSM) simulations in BioGeoBEARS to estimate the overall probability of the different biogeographic processes under the specified phylogeny, parameters and models (Dupin et al., 2016; Matzke, 2016). BSM simulations were conducted across 1,000 stochastic maps using the best-fit BioGeoBEARS models (based on AICc scores) and checked for convergence.

The second approach to ancestral-area estimations was using the Bayesian framework implemented in the software RASP 3.2 (Yu, Harris, Blair, & He, 2015), which reconstructs ancestral states over a posterior distribution of trees. The biogeographic history of titi monkeys was reconstructed under the Bayesian DIVA (S-DIVA), Bayesian DEC (S-DEC) and Bayesian Binary MCMC (BBM) models, all of which allows anagenetic dispersal and extinction. The same cladogenetic processes are allowed in the S-DIVA, S-DEC and BBM models in RASP as in the DIVALIKE, DEC and BAYAREALIKE models implemented in BioGeoBEARS, respectively. All analyses were run on a random subset of 1,000 trees from the BEAST analysis. The BBM chains were run for 5 million generations (sampling every 1,000 generations, a temperature of 0.1 and 10% burn-in), and state frequencies were estimated under the F81 + G model.

3 | RESULTS

3.1 | Phylogeny

Our phylogenetic results showed monophyly for each of the three callicebine genera (Figure 3), the sister relationship between the P. donacophilus and the P. moloch species group, and are broadly concordant with previous molecular genetic analyses (Byrne et al., 2016; Carneiro et al., 2016; Hoyos et al., 2016). Phylogenetic relationships within the subfamily Callicebinae were largely resolved with most nodes showing strong support. The most notable result is the division of the P. moloch group into four major lineages that diversified rapidly in the early Pleistocene. At 2.24 Ma, the progenitor of a clade containing P. hoffmannsi, P. miltoni and P. cinerascens diverged from the ancestor of the Eastern (here containing P. bernhardi, P. cf. moloch, P. vieirai and P. moloch) and Western (here containing P. brunnus, P. caligatus, P. ornatus, P. caquetensis, P. discolor and P. cupreus) clades. Subsequently, P. hoffmannsi diverged from the ancestor of P. cinerascens + P. miltoni at c. 2.02 Ma, and the Eastern and Western clades diverged at c. 1.95 Ma. The descriptive names for the Eastern and Western clades are based on the geographic distribution of each clade in the Amazon, although the Western clade also occurs in the central Amazon, and members of both clades are found in Rondônia (P. bernhardi and P. brunnus). The phylogenetic relationships between P. miltoni, P. cinerascens and all other P. moloch group taxa were previously unresolved (e.g., see Byrne et al., 2016). The rapid diversification of the P. moloch group into four major lineages in the early Pleistocene, as found in this study, is further supported by a large independent molecular data set generated using reduced representation genomic sequencing techniques (H. Byrne, unpublished). This is the first known molecular phylogeny to include Cheracebus lucifer, which is recovered as sister to C. purinus, while C. lugens is the earliest diverging widow titi species represented. A summary of posterior probability and age estimates with 95% HPD intervals are reported in Supporting Information Table S7 in Appendix S1, and the full timetree including outgroup taxa is presented in Supporting Information Figure S1.

3.2 | Biogeographic reconstruction

In total, we performed 15 different biogeographic analyses using BioGeoBEARS (12) and RASP (3). Of the 12 BioGeoBEARS models evaluated, the DIVALIKE+J+X model (ΔAICc = 0) produced the best statistical fit to the data (Table 3). Both jump dispersal (+J) and distance-dependent dispersal (+X) are components of this model. Figure 4 depicts the ancestral areas with the greatest probability at each node under the DIVALIKE+J+X model. The second best model, DEC+J+X (ΔAICc = 3.47), estimated identical most likely states at most nodes with similar support values as the best-fitting model with exception to the diversification of Cheracebus (discussed below; see Supporting Information Figure S2D). Together, these two best-fit models comprise 0.95 of the relative likelihood according to corrected Akaike weights (AICc; Table 3), and the strong concordance between the diversification patterns recovered under these models is viewed as strong support for the inferred biogeographic scenario. We interpret the biogeographic history of Callicebinae based on the ancestral areas estimated under these two models (DIVALIKE+J+X and DEC+J+X) throughout the remainder of this article.

Our results support a widespread ancestral population to all extant titi monkeys occurring through the Napo, Wet & Dry Savannas and South Atlantic Forest regions in the late Miocene. Genus-level diversification events were characterised by vicariance of ancestral range resulting in the isolation of Cheracebus in the Napo at c. 10.24 Ma, followed by the isolation of Plecturocebus in the Wet & Dry Savannas region and Callicebus in the South Atlantic Forest at c. 8.08 Ma. The Plecturocebus species groups diverged from each other in the Pliocene, c. 3.78 Ma, through a jump dispersal event when the progenitor of the P. moloch group dispersed from the Wet & Dry Savannas into Rondônia.

Cladogenetic events among the Amazonian titis of the P. moloch group were characterised by jump dispersal or
occurred within the same geographic region (referred to as narrow sympatry). The initial divergence within the *P. moloch* group occurred within Rondônia between the ancestor of the Eastern + Western clade and the ancestor of the clade containing *P. hoffmannsi, P. cinerascens* + *P. miltoni*. The divergence between the Eastern and Western clades was explained by jump dispersal of the progenitor of the Western clade from Rondônia into Inambari at c. 1.95 Ma. Among the Eastern clade taxa, *P. bernhardi* diverged when the ancestor of *P. cf. moloch* + *P. vieirai* + *P. moloch* dispersed into the Pará region at c. 1.31 Ma, and subsequent divergence of these latter taxa occurred within Pará. The species of the Western clade of the *P. moloch* group showed a more complex pattern of spatial diversification centred on Inambari and characterised by three cladogenetic dispersal events to Rondônia (*P. brunneus*) and Napo (*P. ornatus, P. caquetensis*).

The DIIVALIKE+J+X model estimated that the ancestral population to extant *Cheracebus* taxa was widespread through Pantepui, Napo and Inambari, with the earliest divergence characterised by vicariance resulting in the isolation of the ancestor to *C. purinus + C. lucifer* in Inambari and *C. lugens* in Pantepui and Napo. Subsequently, *C. purinus* and *C. lucifer* diverged via jump dispersal when *C. lucifer* dispersed back to the Napo region. The DEC+J+X model (see Supporting Information Figure S2D), however, recovered a different pattern where the ancestral *Cheracebus* population occurred through Pantepui and Napo, with the earliest divergence characterised by “subset sympatry” resulting in
the ancestor to *C. purinus* + *C. lucifer* in Napo, and subsequently, *C. purinus* and *C. lucifer* diverged via a jump dispersal event when *C. purinus* colonised Inambari.

The above reconstruction of the diversification of Callicebinae is based on the ancestral states with the greatest probability at each node under the best-fit models (DIVALIKE+J+X and DEC+J+X). The most probable ancestral areas recovered at most nodes under these models showed good support (see graphic depictions of state probabilities in Supporting Information Figure S2B,D, and a summary of all probable states at each node in Supporting Information Table S7 in Appendix S1). The greatest uncertainty is found at the root nodes, which is the characteristic of biogeographic methods due to a lack of direct information about historical distributions and extinct taxa (Landis, 2016). All analyses, however, within the DIVA and DEC model sets inferred the most likely ancestral area of origin for Callicebinae across Napo, Wet & Dry Savannas, and South Atlantic Forest, and some included Rondônia, thus providing stronger support for an ancestrally widespread population.
to extant titis across these regions. At nodes corresponding to the generic divergences in the best-fit models, all ancestral states estimated with a probability >0.05 are widespread across multiple areas (see state probabilities for node 1 and 2 in Supporting Information Table S7 in Appendix S1). This is viewed as additional support for the divergence of callicebine genera through vicariance. Overall, the greatest ambiguity in our reconstruction of callicebine biogeographic history is related to the timeframe during which titi monkeys colonised Rondônia. The most probable states estimated in the best-fit models suggest that the ancestor to the $P. molo$ group colonised Rondônia through a jump dispersal event. In contrast, the alternative most probable states in the best-fit models and the most probable states in nearly all DIVA and DEC models (DIVALIKE; DIVALIKE+J; DIVALIKE+X; DEC; DEC+X) suggest that Rondônia was colonised earlier in the Neogene by either the ancestral population to $Plecturocebus$ or to Callicebinae (see Supporting Information Figure S2A,C,G,H,I; Supporting Information Table S7 in Appendix S1). In these latter scenarios, the progenitor to $Plecturocebus$ was widespread across the Wet & Dry Savannas and Rondônia, and a vicariant event led to the divergence between the $P. molo$ and $P. donacophilus$ species groups.

For all model sets (DEC, DIVALIKE, BAYAREALIKE), the addition of both jump dispersal (+J models) and distance-based dispersal (+X models) conferred large improvements to likelihood. Negative values were estimated for X in all +X models indicating that there is a strong relationship between distance and dispersal rates such that dispersal probability decreases with increasing distance. Importantly, however, despite the interaction between distance and dispersal, the results of the best-fit model, DIVALIKE+J+X, are highly comparable to DIVALIKE+J, where distance has no impact on dispersal probability (see Supporting Information Figure S2H). For the DIVALIKE+J model, slightly different ancestral areas were recovered at a small number of nodes, the most significant of which was the inclusion of Rondônia in root ancestral states, as noted above. The biogeographic processes recovered under both models, however, are identical: early vicariant events marking divergence of the genera, while almost all further cladogenesis is explained by jump dispersal or occurred within one area (narrow sympathy).

The impact of the jump dispersal (J) parameter on likelihood scores across all +J models is significant (Table 3). It is evident that founder-event speciation (jump dispersal) is an important process in explaining current species distributions from both the statistical fit of the +J models (e.g., the strongly significant $p$-values for the likelihood ratio tests, Table 3) and the proportion of jump dispersal events in the reconstructions. BSM simulations that estimate the overall probability of different biogeographic processes under the specified phylogeny, parameters and model, lend further support to jump dispersal (J) as a strong contributor to explaining the data. Event counts derived from 1,000 simulated histories under both the DIVALIKE+J+X and DEC+J+X models (where the J parameter is ~0.4) indicate that about ~38% of the cladogenetic events were jump dispersal, 34–37% occurred within a single geographic area (narrow sympathy), and only around 17–25% were vicariance (Supporting Information Table S8 in Appendix S1). These counts show the highest occurrence of jump dispersal events between Inambari and Napo (see Supporting Information Table S9 in Appendix S1).

The results from each of the RASP models are broadly comparable to the corresponding BioGeoBEARS analyses (S-DIVA to DIVALIKE, S-DEC to DEC and BBM to BAYAREALIKE+J), with the same most likely states

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Note. *Likelihood ratio test; chi-squared test between LnL.
recovered at most nodes (see Supporting Information Figure S2M,N,O).

4 | DISCUSSION

4.1 | Study limitations

The study of the biogeography of titis is limited by the absence of a species-level phylogeny with all callicebine taxa represented. Explicit phylogenetic hypotheses are a necessary component of understanding the patterns of diversification and the processes driving extant and historical distributions. The *P. donacophilus* group is particularly poorly represented in the current study owing to the lack of available sequence data for most taxa, and little information about their spatial diversification can be inferred from our reconstruction apart from the putative origin of this clade in the Wet & Dry Savannas area, which comprises the majority of their current geographic distribution (except *P. oenanthe*). Given the unusual and disjunct distributions between the species of the *P. donacophilus* group, in particular, *P. oenanthe* in the Andean foothills of Peru, a phylogenetic framework including these taxa will be required for a comprehensive reconstruction of the biogeographic history of Callicebinae at species-level and to assess the biogeographic hypotheses presented in this study.

Overall, callicebine species are little studied, which is both a driving factor and a limiting factor in the present study. The species-level taxonomy of Callicebinae is still contentious and much uncertainty surrounds the geographic distribution of many taxa as broadly recognised (e.g., Ferrari et al., 2013). Species’ distributions, as presented on the IUCN, represent approximate hypotheses largely based on the work of van Roosmalen, van Roosmalen, and Mittermeier (2002) and Hershkovitz (1988, 1990) that have not been investigated in the field for many titi species across their hypothesised range. In addition, monophyly across the hypothesised range for most species has yet to be explicitly tested. Four new species have been formally described since 2010 (as well as one resurrected taxon; Defer, Bueno, & García, 2010; Gualda-Barros, Nascimento, & Amaral, 2012; Dalponte, Silva, & Silva-Júnior, 2014; Vermeer & Tello-Alvarado, 2015), and given the many uncertainties/unknowns, further changes to the species-level taxonomy of each of the callicebine genera are likely. New species descriptions and/or modifications to hypothesised species’ distributions may impact how accurately callicebine taxa are represented in our biogeographic analyses. In the light of the above limitations, we advocate caution over strict interpretation of our results; however, the overall diversification patterns recovered here are considered to be significant; that is, divergence of the three callicebine genera was driven by vicariance of widespread ancestral populations from the northwestern Amazon to the Atlantic forest and the divergence of Amazonian taxa primarily through jump dispersal from a narrow area of origin.

4.2 | Diversification in the Neogene: Connection between the Amazon and Atlantic forest

Different timescales have been proposed for the formation of the current Amazonian drainage system. The “Old Amazon” geologic model suggests that by 7 Ma a transcontinental drainage system and major rivers in the Amazon had established, the Pebas lake and floodplain system of the western Amazon had disappeared, and from then on this region bore the key geographic features of the current landscape (Hoorn et al., 2010). In contrast, the “Young Amazon” model infers a more recent Pliocene to Pleistocene origin for the transition from a lacustrine system to floodplain to lowland forest in the western Amazon (Campbell, Frailey, & Romero-Pittman, 2006; Latrubesse et al., 2010; Rossetti, Toledo, & Göes, 2005). This model implies that lowland forest was only established in the western Amazon following this Plio–Pleistocene transition, and thus, the diversification of some Amazonian biota may have been associated with the availability of suitable habitat to colonise and disperse across the western Amazon. The patterns of diversification reconstructed here across callicebine taxa are considered more consistent with a “Young Amazon” model, and thus, we summarise our interpretation of the biogeographical history of Callicebinae under this timescale. Among other New World primates, the diversification of *Saimiri* (Lynch Alfaro, Boubli, et al., 2015), as well as basal divergences in *Cebuella* and *Aotus* across the Rio Solimões (Boubli et al., 2018), have also been associated with the “Young Amazon” geologic model. Below, our biogeographic results are considered in relation to Neotropical biogeography and the evolution of the South American landscape since the late Miocene.

Prior to genus divergences, the ancestral population to all modern titis was widespread between the Napo, Wet & Dry Savannas and South Atlantic Forest regions in the late Miocene (Figure 5a). Despite occurring in surrounding regions, titis were absent from the western Amazon (Inambari), which is consistent with the proposal that the western Amazon was inhospitable to upland forest lineages owing to the extensive Pebas system (Aleixo & Rossetti, 2007). These wetlands would have also extended into parts of Napo; thus, this interpretation assumes that titi monkeys were restricted to the region around the lake in the current Andean foothills of the Napo region. The existence of Callicebinae towards the western extreme of the Napo region in the late Miocene is supported by the fossil *Miocallicebus* Takai, 2001, dated to c. 11–12 Ma (Kay et al., 2013). *Miocallicebus* belongs to the La Venta fauna of the modern-day Tatacoa Desert, Magdalena.
Valley, Colombia, which would have been contiguous with the Napo region in the late Miocene prior to the formation of the eastern Andes. Similar patterns among other Neotropical primates have been associated with a broad ancestral distribution in an arc along the west bank of the Pebas wetlands (Buckner et al., 2015), suggesting that Napo was at least intermittently connected to the southern regions through the Andean foothills west of the Inambari region. This is consistent with the proposal that land connections existed between the Andean foothills and Brazilian Shield around the southern rim of the Pebas system during this period (Wesselingh & Salo, 2006).

The earliest divergence within the subfamily Callicebinae at c. 10.24 Ma was characterised by a vicariant event that disrupted connectivity between the Napo and southern regions (Figure 5b), consistent with vicariance across the relatively geologically stable terra firme centres of the Andean foothills (of Napo) and the Brazilian Shield (parts of the Wet & Dry Savannas region) (Aleixo & Rossetti, 2007). This vicariance may have been associated with the period of strong and widespread Andean uplift that begun around the late Miocene (e.g., Garzione et al., 2008; Gregory-Wodzicki, 2000). Once the link around the southern rim of the Pebas system disappeared, the region of the Napo occupied by the *Cheracebus* ancestor remained largely isolated until suitable lowland forest habitat developed in the Western Amazon in the Plio–Pleistocene (under the “Young Amazon” model). The *Callicebus + Plecturocebus* ancestor remained widespread across the Wet & Dry Savannas and South Atlantic Forest until another vicariant event at c. 8.08 Ma led to their disjunction, isolating the ancestor of *Callicebus* in the South Atlantic Forest and *Plecturocebus* in the Wet & Dry Savannas region (Figure 5c). Overall, this scenario is consistent with diversification patterns seen for avian taxa where most upland suboscine lineages (suborder Tyranni) found in the southern Atlantic forest originated in the Miocene and have closely related taxa distributed in the northwestern

**FIGURE 5**  (a–d) Graphical depiction of approximate historical distributions and diversification patterns for Callicebinae in the Neogene
Amazon, with the highest concentration found around the tropical Andes of Ecuador, Colombia and Peru (see Batalha-Filho et al., 2013: figs 1a and 2).

All New World primate genera found in the Atlantic forest have closely related sister taxa in the Amazon (Lynch Alfaro, Cortés-Ortiz, et al., 2015). Similar patterns among avian taxa in the Amazon and Atlantic forest have been associated with a Miocene corridor between the northwestern Amazon and the southern Atlantic forest along the southern Cerrado in Mato Grosso and the transition towards the Chaco of Bolivia and Paraguay, while younger Plio–Pleistocene divergences correspond to connections between the northeastern Atlantic forest and the eastern extreme of the Amazon through the Caatinga (Batalha-Filho et al., 2013; see also, Ledo & Colli, 2017). Our results suggest that titis spread to the Atlantic forest via a southern pathway in the Miocene (prior to \( c. \) 10.2 Ma), providing support for the proposal that the northwestern Amazon and the southern Atlantic forest were connected along the western edge of the Brazilian Shield and the transition towards the Chaco of Bolivia and Paraguay (Batalha-Filho et al., 2013). Our reconstruction suggests that this corridor for Callicebinae disappeared in two stages: first the Wet & Dry Savannas region lost connectivity with northwestern Amazon (Napo) c. 10–10.5 Ma, and around 2 million years later, the Atlantic forest became isolated following the closure of the link along the modern-day Chaco/southern Cerrado. Lineages of plants found in the Cerrado began to diversify around 9 Ma (Simon et al., 2009), and it is possible that ecological changes early in the transition to the Cerrado biome disrupted this connection to the Atlantic forest and played a role in the divergence of Callicebus and Plecturocebus.

Following these major vicariant events, the ancestor to the genus Plecturocebus was restricted to the Wet & Dry Savannas region until the progenitor of the \( P. \) moloch group jump dispersed into Rondônia at \( c. \) 3.8 Ma (Figure 5d). An alternative scenario suggests that the ancestral range of Plecturocebus extended into Rondônia prior to a vicariant event leading to the isolation of the \( P. \) moloch group in Rondônia and the \( P. \) donacophilus group in the Wet & Dry Savannas region. Both scenarios, however, suggest that Plecturocebus first colonised southern Rondônia, invading from the Wet & Dry Savannas region, and that divergence between the \( P. \) donacophilus and \( P. \) moloch groups may have been associated with the Madeira watershed, through the ability to disperse across or around this major geological feature/fluvial dynamics.

The ancestral population to the eastern Brazil taxa of the genus Callicebus was widespread across the Atlantic forest regions (north and south) by \( c. \) 2.9 Ma. The initial divergence occurred by vicariance isolating \( C. \) nigrifrons in the south and \( C. \) personatus + \( C. \) coimbrai in the north (Figure 5d). This scenario suggests that \( C. \) nigrifrons is among the earliest diverging extant callicebine species and this distinction is particularly important given this species’ near threatened IUCN classification; although \( C. \) nigrifrons has the largest range among Callicebus species (see Supporting Information Figure S2 in Appendix S2), the region it inhabits is highly developed with extensive forest loss and fragmentation. The divergence between \( C. \) personatus and \( C. \) coimbrai occurred within the North Atlantic Forest, with \( C. \) personatus subsequently invading some parts of the south. The eastern Brazil species not represented in this study, namely \( C. \) barbarabrownae and \( C. \) melanochir, are from the northern section of the distribution of Callicebus and likely form a clade with \( C. \) personatus and \( C. \) coimbrai.

## 4.3 Diversification in the Pleistocene: Invasion of the western Amazon

Species-level diversification of the Amazonian clades, Cheracebus and the \( P. \) moloch group, occurred in an explosive manner from the early Pleistocene to \( c. \) 0.5 Ma (Figure 6). The \( P. \) moloch group showed a complex pattern of diversification with a nonmonophyletic assemblage of taxa endemic to Rondônia, the inferred area of origin for this clade. The initial major division among \( P. \) moloch group taxa occurred within Rondônia at \( c. \) 2.24 Ma (Figure 6a). Current distributions correlate with the Rio Roosevelt-Aripuanã such that the Rondônian members of the Western and Eastern clades (\( P. \) brunneus and \( P. \) bernhardi) are found on the left bank and the clade containing \( P. \) hoffmannsi, \( P. \) cinerascens and \( P. \) miltoni is restricted to the right bank, although the range of \( P. \) cinerascens also extends upstream of the headwaters of the Rio Roosevelt (e.g., see Ferrari et al., 2000). Based on similar patterns in some avian taxa, Fernandes (2013) proposed several mini interfluvial areas of endemicity for Rondônia that correspond to the subdivisions found among \( P. \) moloch group taxa.

One proposed explanation for the complex diversification patterns seen in this region is changing river dynamics in the Plio–Pleistocene, whereby current tributaries of the Rio Madeira such as the Rio Roosevelt-Aripuanã may have captured a significant part of the Madeira or even Tapajós drainages (Fernandes, Wink, & Aleixo, 2012). Furthermore, evidence for an alluvial mega-fan involving the Rio Roosevelt-Aripuanã, and a smaller alluvial fan involving the Rio Jiparanã, indicates the existence of a much wider and more complex drainage system in the Pleistocene (Latrubesse, 2002). This complex history of river system evolution may have played an important role in influencing historical distributions in Rondônia. We reason that the initial division within the \( P. \) moloch group was associated with the Rio Roosevelt-Aripuanã, whether through jump dispersal over this barrier or vicariance owing to river dynamics, restricting the ancestor to \( P. \) hoffmannsi, \( P. \) cinerascens + \( P. \) miltoni on the right bank to the northern part of Rondônia. We further suggest that the occurrence of \( P. \) cinerascens to the south
of the headwaters of the Rio Roosevelt-Aripuanã represents recent range expansion that could be related to fluvial dynamics associated with this river since the late Pleistocene. Rondônia is the only geographic area with members from more than one of the four major *P. moloch* group lineages (notably, containing taxa from all four) and is of evident importance to understanding the biogeographic history of this group. This pattern of shared lineages in the Rondônia region appears to be common as similar patterns are observed in birds (e.g., Fernandes, 2013; Thom & Aleixo, 2015), lizards (de Oliveira, de Carvalho, & Hrbek, 2016) and squirrel monkeys (Lynch Alfaro, Boubli, et al., 2015). Studies with a specific focus on the biogeography of the *P. moloch* group will provide insight into diversification dynamics within Rondônia at a finer scale.

After the initial major divergence, the ancestral population to the Western + Eastern *P. moloch* group clade was isolated briefly in the southern section of Rondônia on the left bank of the Rio Roosevelt-Aripuanã. At around 1.95 Ma, these clades diverged when the progenitor of Western taxa dispersed to Inambari via jump dispersal across the upper Rio Madeira (Figure 6b). The Western clade subsequently spread westward across Inambari, reaching the Rio Solimões and dispersing into Napo by c. 1.2 Ma. Under the Young Amazon model, this diversification pattern suggests that the wetlands had receded and suitable lowland forest habitat had begun to be established in the western Amazon by the early Pleistocene allowing the rapid colonisation of Inambari and Napo. Species divergences within the Western clade were largely characterised by “island hopping” between Inambari and Napo across the Rio Solimões-Amazonas, although jump dispersal back across the upper Rio Madeira to southern Rondônia led to the divergence of *P. brunneus* (Figure 6d).

Further details about the exact dispersal patterns should be interpreted with caution given the important taxa missing from this clade in this study, such as *P. toppini, P. aureipalatii* and potentially *P. urubambensis* (see Vermeer & Tello-Alvarado, 2015), as well as the availability of only mitochondrial data for some species for phylogenetic inference (see Supporting Information Table S1 in Appendix S1).

The progenitor of the Eastern clade remained in Rondônia to the left bank of the Rio Roosevelt-Aripuanã where *P. bernhardi* diverged when the ancestor of the remaining Eastern clade taxa dispersed eastwards over or around the headwaters of the Rio Tapajós-Juruena into the Pará region at c. 1.31 Ma (Figure 6c). *Plecturocebus cf. moloch* is restricted to the Alta Floresta region and dispersing into Napo by c. 1.2 Ma. Under the Young Amazon model, this diversification pattern suggests that the wetlands had receded and suitable lowland forest habitat had begun to be established in the western Amazon by the early Pleistocene allowing the rapid colonisation of Inambari and Napo. Species divergences within the Western clade were largely characterised by “island hopping” between Inambari and Napo across the Rio Solimões-Amazonas, although jump dispersal back across the upper Rio Madeira to southern Rondônia led to the divergence of *P. brunneus* (Figure 6d).

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between the Rios Juruena and São Manuel (J. P. Boubli, unpublished) and likely diverged from the ancestor of *P. vieirai + P. moloch* as they dispersed eastwards from Rondônia (Figure 6d).

The two best fitting BioGeoBEARS models inferred different scenarios for the diversification of the widow titis (genus *Cheracebus*). The DEC+J+X reconstruction is discussed here as the most probable ancestral states at these nodes have a higher probability, and it is more consistent with extant species distributions. Under this model, the initial divergence between *C. lugens* and *C. lucifer + C. purinus* at c. 1.84 Ma occurred within the Napo region and current distributions suggest that it was associated with the Rio Caquetá-Japurá. As such, we propose that ancestral *Cheracebus* began to expand out of the Andean foothills of the northwestern Amazon following newly established forest habitat in the Plio–Pleistocene. This ancestral population was divided when it reached and dispersed southwards over the Rio Caquetá, or by vicariance owing to river dynamics, leaving the ancestor to *C. lucifer + C. purinus* to the south and *C. lugens* to the north and in the Pantepui region (Figure 6b). The ancestral population to *C. lucifer + C. purinus* spread eastwards along the south of the Rio Caquetá-Japurá, diverging when the ancestor of *C. purinus* (and likely, *C. regulus*) dispersed across the Rio Solimões into Inambari at c. 1.23 Ma (Figure 6c).

Titi monkeys in the Amazon (*Cheracebus* and the *P. moloch* group) are found in terra firme regions and occasionally in seasonally inundated black-water forests (Igapó), but they are generally absent from white-water flooded forest habitats known as Varzéa (Haugaasenn & Peres, 2005; Peres, 1997). It is likely that the extensive lake and floodplains of the Western Amazon were largely inhospitable to titi monkeys and colonisation of this region would have depended upon the availability of suitable lowland forest habitats. Under the “Young Amazon” model, this is suggested by the absence of ancestral range from Inambari throughout the late Miocene and Pliocene, and the dispersal of both *Plecturocebus* and *Cheracebus* taxa to Inambari from other regions in the Pleistocene. This biogeographic reconstruction also sheds light on the present-day sympatry between *Cheracebus* and *Plecturocebus* species across the Napo and Inambari regions. The ancestors to these clades were likely isolated at opposing extremes of the Amazon in the terra firme centres of the Andean foothills and Brazilian Shield by the Pebas system in the late Miocene, evolving in isolation in these distinct regions for 8–9 million years. Range expansion from the east (*Plecturocebus*) and north-west (*Cheracebus*) in the Pleistocene following the establishment of lowland forest in the western Amazon has led to sympathy (see Figure 1) but occupation of distinct niches (discussed below).

The largely cosmopolitan distribution of the *P. moloch* group across the Amazon is notable: from east of the Rio Tocantins in eastern Amazon, to the Rio Beni in northern Bolivia, and north-west as far as the Andean foothills of the Meta department in Colombia, entirely absent only from the Pantepui region. Differences in ecology/habitat preference and dispersal ability may have allowed the *P. moloch* group to spread much more widely across the Amazon than *Cheracebus* within a similar period. Rather than soil type, the structure of forest vegetation is thought to best explain differences in habitat preference between *Cheracebus* and *Plecturocebus* (Defler, 1994). *Cheracebus* species occur in forests that are tall and well-stratified, mostly in undisturbed highland terra firme regions, but they have also been recorded in well-developed Igapó habitats. Taxa of the *P. moloch* clade are frequently found in poorly stratified low forests including disturbed, secondary and liana-rich habitats in terra firme and seasonally inundated regions, where they are often located at the edge of large gaps in the canopy (Defler, 1994; Haugaasenn & Peres, 2005; Peres, 1997). As poorly stratified gallery forests are often in low-lying areas along waterways, rare jump dispersal events through passive transfer across riverbanks may occur more frequently among taxa of the *P. moloch* group, facilitating passive dispersal across major Amazonian rivers and the rapid colonisation of the greater part of the Amazon within the last 2 million years. This scenario is consistent with the diversification pattern based on founder-event speciation (jump dispersal) reconstructed in this study for the *P. moloch* group clade, even across major rivers such as the Solimões and Madeira. Additionally, owing to their ability to thrive at edge and in disturbed habitats, it is possible that *P. moloch* group taxa have been able to disperse more broadly through the use of temporary, secondary or developing lowland forests; forests at the edge of their range; and/or forests along the river network of the western and central Amazon, while the dispersal of *Cheracebus* species may depend upon the establishment of well-developed forests and connectivity between them.

### 4.4 The drivers of titi monkey diversity in Amazonia

Titi monkeys are one of most species-rich groups of New World primates with 33 recognised species (Byrne et al., 2016). The Amazonian *P. moloch* group includes 16 species (Byrne et al., 2016), plus one more in process of description (*P. cf. moloch*; J. P. Boubli, unpublished), while the genus *Cheracebus* includes six species. The *P. moloch* group began diversifying at c. 2.2 Ma, and as discussed above, subsequently spread relatively rapidly and broadly across the Amazon. A deeper understanding of species-level diversification in these clades can add to our understanding of the mechanisms of speciation in Amazonia in the Pleistocene.

A number of biogeographic models have been proposed for the Amazon basin, the most frequently invoked of these is the riverine barrier hypothesis (Wallace, 1852), which identifies river dynamics as primarily responsible for the
isolation and diversification of Amazonian biota. Larger Amazonian rivers frequently delineate the distributions of *Cheracebus* and *P. moloch* group taxa, with sister taxa often found on opposite banks, and river dynamics have been proposed as an important speciation force among Callicebinae (e.g., Ayres & Clutton-Brock, 1992; Boubli et al., 2015; Hershkovitz, 1988). Similar patterns in Amazonian avian taxa have been taken to suggest that the establishment of current Amazonian drainage system led to the division of formerly continuous populations and promoted their divergence (e.g., Ribas et al., 2012). Our biogeographic reconstruction suggests that cladogenetic events among *Cheracebus* and the *P. moloch* group taxa in the Pleistocene are rarely explained by vicariance, and the major Amazonian tributaries delineated in this study were largely established prior to species-level diversification in these clades. Rather than vicariance owing to landscape change such as river formation, our results support a sequential, long-distance dispersal model of speciation by “island hopping” across pre-existing river barriers, or dispersal around the headwaters. These jump dispersal events were sufficiently rare to allow divergence in isolation after dispersal, pointing to major Amazonian rivers as relatively strong barriers to gene flow in these titi clades. Comparable dispersal models of speciation have also been proposed for Amazonian birds, for example, Smith et al. (2014) summarised the principal driver of avian speciation in lowland Neotropical rainforests as “dispersal and differentiation on a matrix previously shaped by large-scale landscape events” and also highlighted the importance of an organism’s ability to disperse and persist in the landscape. Ayres and Clutton-Brock (1992) proposed that rivers constrain the dispersal of Amazonian primates, and related speciation and the geographic range size of Neotropical primate lineages to their ability to cross ecological barriers.

Dispersal across white-water rivers of the western Amazon (such as the Ucayali, Juruá and Purús) may have been mediated by shifts in river course, transposing land to the opposite side of the channel and facilitating the passive transfer of callicebine populations. The dynamic nature of white-water rivers suggests that they may form more permeable ecological barriers to the dispersal of some Amazonian forest-dwelling lineages (Gascon et al., 2000). The general absence of titi monkeys from white-water flooded forest habitats, however, likely limits the frequency of dispersal events owing to shifts in the course of white-water rivers. Thus, major rivers divide the Amazonian landscape and appear to form significant (but not completely impenetrable) barriers to the spread of titis, limiting gene flow and promoting genetic isolation after a rare dispersal event. Overall, however, we suggest that titi monkey diversity in the Amazon is best explained by lineage-specific dispersal ability (and habitat preference), which is limited by riverine and other ecological barriers to varying degrees across callicebine taxa. As noted earlier, habitat preferences and the seemingly greater dispersal ability of the *P. moloch* group taxa likely influenced their more cosmopolitan distribution across Amazonia, as well as higher number of species, in comparison with the widow titis of the genus *Cheracebus*. Other Amazonian rivers not delineated by the geographic regions in this study will also have played a role in callicebine diversification, including the Rios Caquetá, Purús, Juruá and Roosevelt-Aripuanã.

In support of this mode of speciation, other sources of molecular and phenotypic evidence indicate that dispersal across major rivers, or around the headwaters, is an ongoing process, albeit infrequent. For example, a specimen classified as *P. cupreus* based on morphology and collection locality has a cytochrome *b* gene with the closest genetic affinity to *P. moloch*, which may suggest a relatively recent dispersal event into the central/western Amazon and subsequent introgression with *P. cupreus* (Hoyos et al., 2016; notably, however, *P. moloch* was the only species from *P. moloch* group included in this study that was not a member of the Western clade). Vermeer and Tello-Alvarado (2015) recorded *P. discolor* at localities on the right bank of the Rio Ucayali (based on phenotype), when it was previously thought to be distributed only to the left bank, and these authors suggested that it was a result of the shifting course of this river. It should be noted, however, that both of the above scenarios could also be a result of unsorted ancestral polymorphism.

## CONCLUSIONS

In this study, we reconstructed a well-resolved phylogeny for Callicebinae that supports the diversification of the *P. moloch* group into four major lineages early in the Pleistocene. Our phylogenetic results clarify aspects of callicebine evolutionary history and species relationships, such as the relationship between *P. cinerascens* + *P. miltoni* and the other taxa of the *P. moloch* group. Our biogeographic results indicate that the diversification of titi monkey genera initiated in the late Miocene via the fragmentation of a widespread ancestor distributed across the northwestern Amazon, the present-day wet and dry savannas of Bolivia and Brazil, and the southern Atlantic forest of eastern Brazil, isolating the progenitors of *Cheracebus*, *Plecturocebus* and *Callicebus* in these regions, respectively (Figure 5). Species-level diversification of Amazonian taxa (*P. moloch* group and *Cheracebus*) occurred as they spread across the Amazon in the Pleistocene, largely characterised by a sequential, long-distance “island-hopping” dispersal model of speciation from a narrow area of origin (likely the northwestern Amazon for *Cheracebus* and Rondônia for the *P. moloch* group).

This study comprises the first large-scale reconstruction of the biogeography of titis and investigations into their evolutionary history in the context of Amazonian and South American...
biogeography, and adds to our understanding of the patterns and processes of diversification among primates, and more generally, forest-dwelling vertebrate lineages in the Neotropics. It provides a starting point for future research focusing specifically on each major titi monkey clade, with increased taxonomic sampling (particularly for the P. donacophilus group) and subdivisions within each geographic region, to allow a more in-depth investigation of their biogeographic history and the processes promoting their diversification at a finer scale.

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