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Biogeography of squirrel monkeys (genus *Saimiri*): South-central Amazon origin and rapid pan-Amazonian diversification of a lowland primate

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ABSTRACT

The squirrel monkey, *Saimiri*, is a pan-Amazonian Pleistocene radiation. We use statistical phylogeographic methods to create a mitochondrial DNA-based timetree for 118 squirrel monkey samples across 68 localities spanning all Amazonian centers of endemism, with the aim of better understanding (1) the effects of rivers as barriers to dispersal and distribution; (2) the area of origin for modern *Saimiri*; (3) whether ancestral *Saimiri* was a lowland lake-affiliated or an upland forest taxa; and (4) the effects of Pleistocene climate fluctuation on speciation. We also use our topology to help resolve current controversies in *Saimiri* taxonomy and species relationships. The Rondônia and Inambari centers in the southern Amazon were recovered as the most likely areas of origin for *Saimiri*. The Amazon River proved a strong barrier to dispersal, and squirrel monkey expansion and diversification was rapid, with all speciation events estimated to occur between 1.4 and 0.6 Ma, predating the last three glacial maxima and eliminating climate extremes as the main driver of squirrel monkey speciation. *Saimiri* expansion was concentrated first in central and western Amazonia, which according to the "Young Amazon" hypothesis was just becoming available as floodplain habitat with the draining of the Amazon Lake. Squirrel monkeys also expanded and diversified east, both north and south of the Amazon, coincident with the formation of new rivers. This evolutionary history is most consistent with a Young Amazon Flooded Forest Taxa model, suggesting *Saimiri* has always maintained a lowland wetlands niche and was able to greatly expand its range with the transition from a lacustrine to a riverine system in Amazonia. *Saimiri vanzolinii* was recovered as the sister group to one clade of *Saimiri ustus*, discordant with the traditional Gothic vs. Roman morphological division of squirrel monkeys. We also found paraphyly within each of the currently recognized species: *S. sciureus*, *S. ustus*, and *S. macrodon*. We discuss evidence for taxonomic revision within the genus *Saimiri*, and the need for future work using nuclear markers.

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1. Introduction

1.1. Models for recent Amazonian biotic speciation

Two predominant models explaining patterns of Amazonian biotic diversity include the riverine barrier hypothesis (Wallace, 1852) and the Pleistocene forest refuge hypothesis (Haffer, 1969). Historically these two models have been separated on a temporal scale, with major river formation in the Amazon, including the Amazon River itself, hypothesized have happened in the Upper Miocene (Hoorn et al., 2010), several million years before the Pleistocene climate fluctuation that generated forest refugia. In contrast, more recent analyses of Amazonian stratigraphy and paleoenvironment (Campbell et al., 2006; Latrubesse et al., 2010; Nogueira et al., 2013) suggest a much younger, Plio-Pleistocene history for the transition from a lacustrine system in western Amazonia to the current drainage system (Ribas et al., 2012). This "Young Amazon" geologic model also implies that different regions of Amazonia may have undergone distinct rates of landscape change, with the most dynamic area being the western portion (west of the Madeira River), today covered by the Solimões sedimentary basin (Aleixo and de Fátima Rossetti, 2007). Under this model the western Amazon has only recently transitioned from a lacustrine system to floodplain to lowland forest and retains many patches of seasonally inundated forest (Hoorn 1994; Hoorn et al., 1995; Campbell et al., 2001; Rossetti et al., 2005).

Ribas et al. (2012) used biogeographic evidence for diversification of the trumpeters (*Psophia*; Psophiidae), a widely distributed Amazonian bird genus, as a way to evaluate the riverine barrier vs. the refuge hypotheses during the Plio-Pleistocene. Their analyses (Ribas et al., 2012) support a strong role for river dynamics, and no such role for glacial climate cycles and refugia, at least for the causation of the origin of current trumpeter species. The intense diversification in the last 2.5 million years found in several other Amazonian avian taxa has been related to the formation of major rivers in the Amazon in the Plio-Pleistocene (d'Horta et al., 2013; Ribas et al., 2009; Fernandes et al., 2012, 2014; Sousa-Neves et al., 2013, see also Smith et al., 2014).

The squirrel monkeys, genus *Saimiri*, are another recent pan-Amazonian radiation that can serve to evaluate Amazonian diversification dynamics in the last two million years. Biogeography models applied specifically to primate diversification include Wallace (1852) and Ayres and Clutton-Brock (1992) for primate river barriers and Kinzey (1982) for primate Pleistocene refuges. However, our present study provides the first explicit statistical phylogeographic test for evolutionary diversification of a primate genus across all Amazonian centers of vertebrate endemism.

1.2. Squirrel monkeys as an example of recent pan-Amazonian diversification

The squirrel monkeys, genus *Saimiri*, occur in each of the areas of vertebrate endemism in the Amazon (*sensu* Cracraft, 1985; Ribas et al., 2012; Silva et al., 2005), as well as in a restricted coastal area within Central America (Hershkovitz, 1984). Squirrel monkeys are one of only four genera of neotropical primates that are universally distributed throughout the Amazon (Ferrari, 2004). The taxonomy for squirrel monkeys has been greatly disputed based on morphological studies, with results ranging from 1 to 16 terminal taxa (for reviews see Hershkovitz, 1984, 1987; Thorington, 1985; Costello et al., 1993; Boinski and Cropp, 1999; Cropp and Boinski, 2000; Groves, 2001, 2005; Rylands et al., 2013). Species relationships

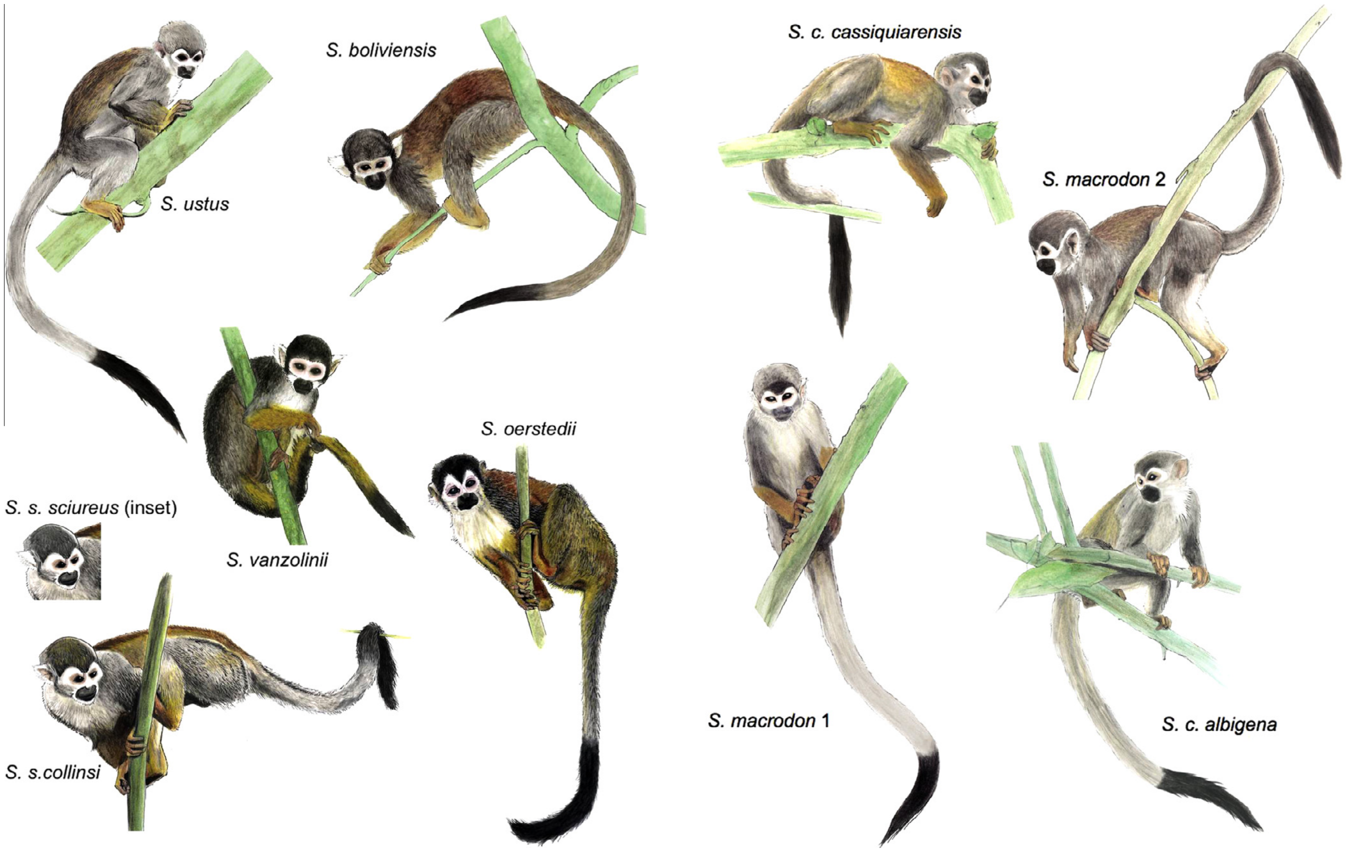

Fig. 1. Saimiri morphological diversity, following species classification by Rylands et al. (2013). Note that morphological differences shown for *Saimiri macrodon* 1 and *Saimiri macrodon* 2 are based on Paim et al. (2013) and it is not yet determined how closely either of these correspond geographically to *Saimiri macrodon* A, B and C recovered from the genetic work in this paper. All drawings by Amisha Gadani.

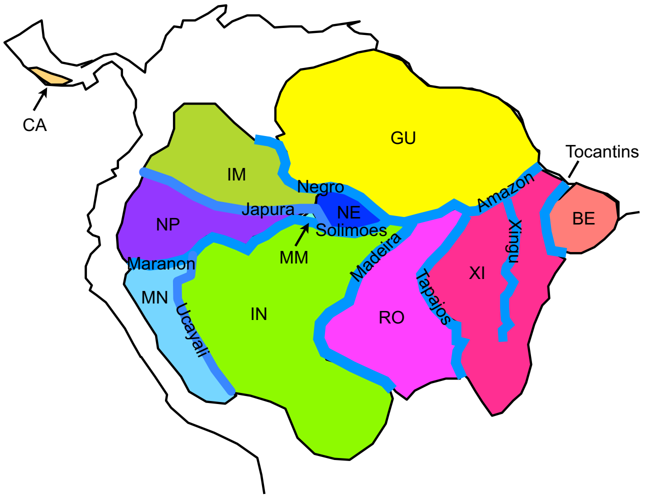

Fig. 2. Centers for vertebrate endemism. CA = Central America; GU = Guiana; IM = Imeri; NP = Napo; NE = Negro; XI = Pará (Xingu and Tapajós); RO = Rondônia; MM = Mamirauá; IN = Inambari; MN = Marañón; BE = Belém. Major Amazonian rivers are also indicated on map.

within *Saimiri* are also disputed, in part because of the extremely subtle nature of the morphological differences across taxa (Fig. 1).

We obtained genetic sequence data from all currently recognized squirrel monkey taxa following Rylands et al. (2013): *S. oerstedii oerstedii*, *S. o. citronellus*, *S. cassiquiarensis cassiquiarensis*, *S. c. albigena*, *S. macrodon*, *S. ustus*, *S. sciureus sciureus*, *S. s. collinsi*, *S. boliviensis boliviensis*, *S. b. peruviensis*, and *S. vanzolinii.* We include genetic sequences from all the areas of vertebrate endemism in the Amazon as well as from Central America (see Fig. 2), to analyze squirrel monkey relationships and divergence times and to test recent hypotheses for Pleistocene biotic diversification in Amazonia. The "Young Amazon" model suggests that the current Amazonian drainage system was established between 2 and 3 Ma, and that major Amazonian tributaries were formed within the last 2 million years (Ribas et al., 2012). If this is true, the modern Amazon River was in place prior to the initial diversification of modern *Saimiri* (1.5 Ma, 1.1–1.9 Ma 95% HPD, based on mitochondrial genome data; Chiou et al., 2011; 1.75 Ma, 1.1–2.9 Ma 95% HPD, based on 69 nuclear and 10 mtDNA markers, Springer et al., 2012), or formed during the initial diversification of *Saimiri* (2.24 Ma, 1.1–3.7 Ma 95% HPD, nuclear and mitochondrial genes: Perelman et al., 2011) [note that Lavergne et al. (2010) recover a significantly earlier date for the initial divergence of *Saimiri* (~4.3 Ma, 2.4–6.6 Ma 95% HPD) using cytochrome *b* sequences]. However, in the "Young Amazon" model, the major tributaries of the Amazon were formed *during or after* the onset of *Saimiri* diversification, and could have had an important role in this process. In contrast the "Old Amazon" (Hoorn et al., 2010) model of Amazonian river formation posits that the formation of the Amazon River *and all of its tributaries* were established long before the diversification of the squirrel monkeys, even using Lavergne et al.'s (2010) older dates for squirrel monkey diversification.

Significant controversy exists over the taxonomy, species relationships, and number of terminal taxa within *Saimiri* based on morphological and molecular data. Fig. 3a–d summarizes the main hypotheses for species and subspecies relationships based on morphological and molecular data, using a geographic framework. Hershkovitz (1984, 1987) placed *S. boliviensis* and *S. vanzolinii* as sister taxa, and as sister to all other squirrel monkeys, with other species including *S. oerstedii*, *S. sciureus*, and *S. ustus* (Fig. 3a). Thorington (1985) hypothesized that *S. ustus* was the only differentiated taxon, and that all other squirrel monkeys should be lumped as *S. sciureus* (Fig. 3b). Costello et al. (1993) suggested all Amazonian squirrel monkeys were one species, *S. sciureus*, as differentiated from *S. oerstedii* from Central America (Fig. 3c). Lavergne et al. (2010) proposed another model, based on molecular evidence, with a Western Amazonian origin to squirrel monkeys, and subsequent dispersal north and east (not pictured; see Around the Amazon in Section 1.3). A second aim of this study is to recover the main squirrel monkey clades based on the most complete sampling to date across their range, and to use this information as evidence for resolving key issues in squirrel monkey taxonomy (see Section 3.1 for more details).

One relevant aspect of squirrel monkey behavioral ecology is that, unlike in most other primate genera, sex-biased dispersal varies by species or population (reviewed in Boinski et al., 2005a,b). Boinski et al. (2005a,b) report that in Costa Rica, *Saimiri oerstedii* males remain in their natal group and females emigrate; in Peru, *S. boliviensis* males disperse and females stay in their natal group; and in Suriname, *S. sciureus* males and females tend to leave their natal group, although some females stay in their natal group. However, these behavioral observations were incongruent with molecular estimates of sex-biased dispersal, at least for *Saimiri oerstedii*, for which Blair and Melnick (2012) found, using mitochondrial DNA (mtDNA) and microsatellite markers, that both males and females leave their natal groups, and males disperse longer distances than females. Neither molecular nor observational data for dispersal patterns are available for several other *Saimiri* species. Mitochondrial DNA (mtDNA), the genetic marker analyzed in the present study, is inherited through the maternal line only, and so reflects the movement of females over time, such that when females are philopatric, mtDNA haplotypes tend to show a high degree of geographic structure within species (i.e. cynomolgus macaques: Shiina et al., 2010), whereas when females regularly disperse from their natal groups, within-species mtDNA patterns tend to have less geographic signal (i.e. hamadryas baboons: Hapke et al., 2001, but see Whinney et al., 2004). A third aim of the study is to examine within species mtDNA variation to see the extent to which mtDNA is structured geographically in squirrel monkey species. Given that squirrel monkeys are such a recent radiation and that there are short branch lengths between several successive diversification events, incomplete lineage sorting may also affect the mtDNA patterns in *Saimiri*. This is particularly likely if there is female philopatry, which can extend coalescent times for species (Tosi et al., 2003).

1.3. Hypotheses for diversification dynamics of Saimiri

In this study, through statistical phylogeographic analysis of widely sampled squirrel monkey mitochondrial DNA sequences, we infer (1) the spatial correlation between large Amazonian rivers and current lineage distributions, as a proxy for the role of drainage evolution on the origin of these taxa; (2) the distribution of the common ancestor to all modern squirrel monkeys—by center of endemism; (3) whether *Saimiri* was initially a lowland lake-affiliated or an upland forest taxa; and (4) the effect of climate fluctuation on squirrel monkey distribution (by assessing whether squirrel monkey speciation occurred before, during or after the Glacial Maxima period). We also compare models to determine the relative importance of vicariance, dispersal and extinction in forming the current geographical distribution for squirrel monkey taxa. Below we provide alternative hypotheses for both the biogeographic history of the *Saimiri* radiation (Hypotheses 1a–1d) and for the timing of speciation events in that radiation (Hypotheses 2a–2b).

(1a) Unconstrained Dispersal (Fig. 3c): If squirrel monkeys are not strongly affected by either river barriers or retreating forests due to Pleistocene climate change, squirrel monkeys will show

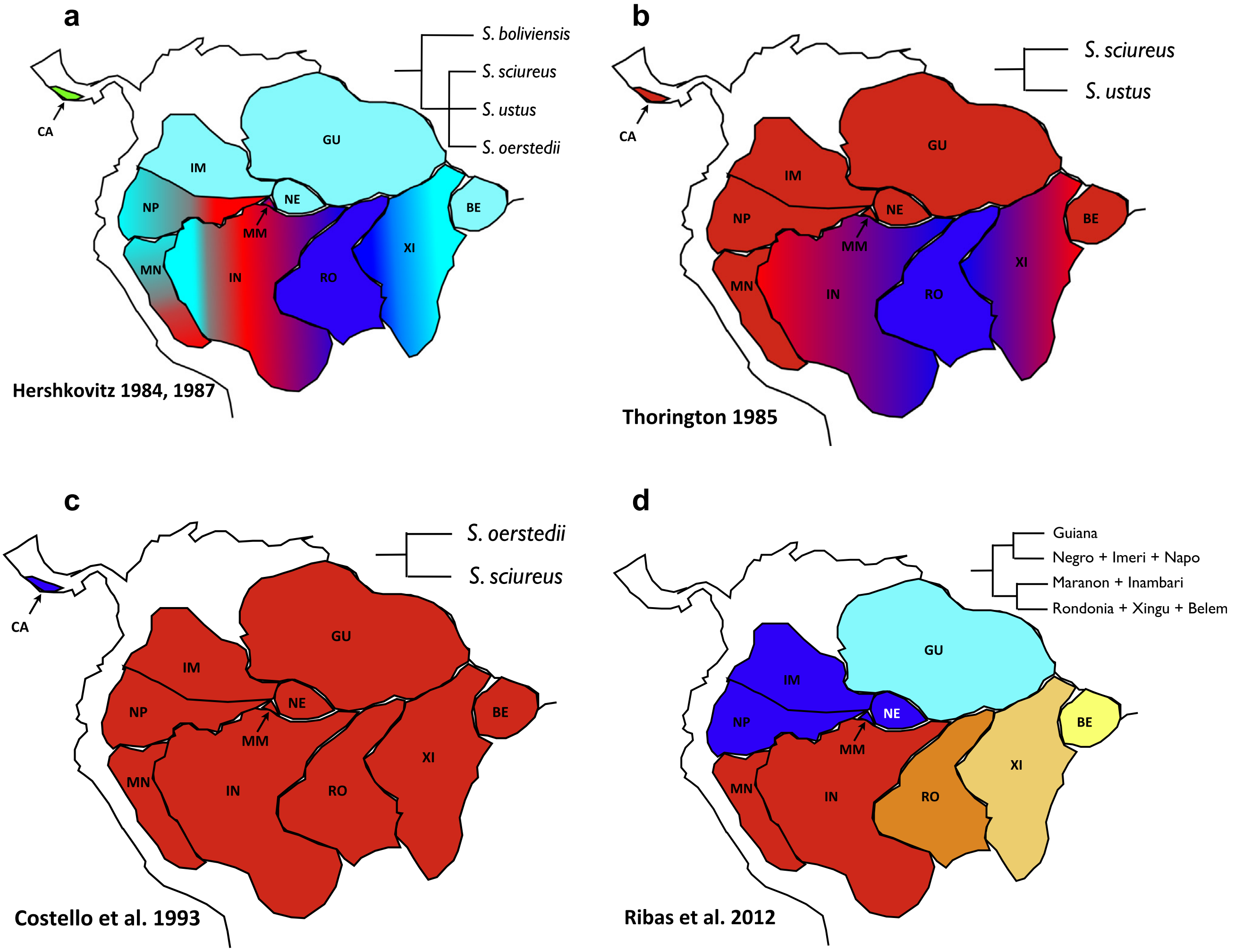

Fig. 3. Alternate predictions for species relationships in *Saimiri* in a geographical framework. Closer colors indicate relatively closer relationships. (a) Hershkovitz (1984) placed *S. boliviensis* (IN, MN, NP) as sister to all other squirrel monkeys, and separated *S. oerstedii* (CA), *S. sciureus* (NP, IM, MM, NE, GU, XI, BE, including *collinsi*, *albigena*, *cassiquiarensis*, and *macrodon*), and *S. ustus* (IN, RO, XI) into species. Hershkovitz (1987) places *S. vanzolinii* (MM) as sister to *S. boliviensis*. (b) Thorington (1985) considered there to be only two squirrel monkey species: *S. ustus* (IN, RO, XI) and *S. sciureus* (CA, NP, IM, MM, NE, GU, MN, IN, XI, BE). (c) Costello et al. (1993) also suggested two squirrel monkey species, *S. oerstedii* (CA) and *S. sciureus* (NP, IM, MM, NE, GU, MN, IN, RO, XI, BE, or all Amazonian forms). (d) Ribas et al. (2012) found a Pleistocene signature of Amazonian biotic relationships for avian fauna, that they suggested was a result of vicariance due to river formation of the major Amazonian tributaries; if squirrel monkeys show this same pattern, we would expect *S. s. sciureus* to be sister to *S. c. cassiquiarensis* + *S. c. albigena* + *S. vanzolinii*; *S. macrodon* and *S. boliviensis* to be sister taxa; and *S. ustus* to be sister to *S. s. collinsi*, with the taxa north and south of the Amazon River the most different from one another. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

isolation by geographic distance, but divergences and distributional patterns will not correspond with river barriers in Amazonia. Under these conditions, we predict that squirrel monkey species are not geographically defined by areas of vertebrate endemism. Instead, we expect a more cosmopolitan signature, with the mixing of haplotypes across river barriers, for one large panmictic or gradient species throughout both the north and south Amazon, as suggested by Costello et al. (1993) based on morphological data (see Fig. 3c). Ayres and Clutton-Brock (1992) characterized *Saimiri* as a habitat generalist, able to thrive in terra firma and flooded forests (both *varzea* and *igapó*), and suggested that this allowed squirrel monkeys to be efficient at crossing rivers despite their small size. Ferrari (2004) also offered *Saimiri* as an exception to the rule that small-bodied primates are restricted by river barriers, citing the fact that *Saimiri* is identical on both banks of the lower Amazon River.

(1b) “Old Amazon” River Barriers: If all major Amazonian rivers are already in place before the origin of crown *Saimiri* (Hoorn et al., 2010), and rivers are in fact barriers to *Saimiri* dispersal, then with subsequent rare dispersal events across river barriers, we expect to observe decreasing diversity as *Saimiri* moved away from its origin, with founder effects occurring at each crossing event, leading to nested low-diversity subclades farther away from the original population. Expected species relationships depend on the location of the original population and the direction of dispersal. The *Saimiri* radiation is too young to be explained by vicariance through river formation under the Old Amazon model, because all the major rivers would have been in place for millions of years before the start of the *Saimiri* radiation; thus pure dispersal, rather than vicariance, would be expected to explain modern squirrel monkey distribution. Dispersal in this scenario would also fit with ‘jumps’ in the Dispersal-Vicariance with founder parameter model (DIVAj) in the BioGeoBears analysis (see Methods section).

Based on their mtDNA analysis, Lavergne et al. (2010, p. 250 for map) propose a Western Amazonian origin, followed by diversification between ancestral stock of *S. macrodon* and *S. boliviensis*. Further movement happens eastward, from both south of the Amazon (*S. macrodon* generates *S. ustus* and then *S. ustus* generates *S. s. collinsi*) and north of the Amazon (*S. macrodon* generates *S. c. albigena* and *S. c. cassiquiarensis*; *S. cassiquiarensis* generates *S. s. sciureus*). Finally, *S. sciureus* expands west to generate *S. oerstedii*. In this model, there is no crossing of the Amazon River, only one crossing of the Ucayali River by *S. macrodon* to expand north. This "Around the Amazon" reconstruction is a particular case of the "Old Amazon" founder's effect model; Lavergne et al. (2010) point out that this model is supported by higher levels of genetic diversity in *S. boliviensis* and *S. macrodon* compared to the other clades.

(1c) "Young Amazon" River Formation—Terra Firme taxa (Fig. 3d): Following Ribas' et al.'s (2012) model, Pleistocene river formation will have a strong effect on the structure of *Saimiri* phylogenetic relationships, with speciation events based on the formation of the Amazon, Madeira, Tapajós, Negro, Xingu and Tocantins rivers. If we consider *Saimiri* to be an 'upland' widespread forest taxon, as are most arboreal mammals, then geographical species relationships will mirror those for trumpeters in Ribas et al. (2012), with the major divisions through vicariance occurring first across the Amazon/Solimões River (formed 2–3 Ma), then across the Madeira River (formed 1–2 Ma), the Tapajós River (1.3–0.8 Ma), the Rio Negro (~1 Ma), and finally Xingu and Tocantins rivers (0.8–0.3 Ma)—see Fig. 3d [all river formation age estimates from Ribas et al., 2012]. The central western Amazon may be the most dynamic area for new colonization, due to a relatively recent reduction of the Amazon Lake and subsequent floodplains in that area (Aleixo and de Fátima Rossetti, 2007). In this model, during the beginning of *Saimiri* diversification the central western Amazon is not available for terra firme taxa (Aleixo and de Fátima Rossetti, 2007: see page S445, Fig. 1b for map); 'upland' forest species would be subject to vicariance across three terra firme Centers: the Guianan Shield (corresponding to the Guiana Center in Fig. 2), the Brazilian Shield (corresponding to the Rondônia, Pará, and Belém Centers), and the Eastern foothills of the Andes (corresponding to the Marañón Center). Dispersal from these terra firme centers would be a directional radiation, with dynamic colonization of the recently available habitat in the post-lacustrine Western Amazon. This is not just a classical widespread then cut by vicariance scenario, it involves dispersion (expansion of distributions) to the west when the lacustrine system drains and terra firme develops in the west. However, the exact timing during the Pleistocene of the opening of the western Amazon to arboreal primates is unknown.

(1d) "Young Amazon" River Formation – Flooded forest taxa: In contrast to upland species described above, in the Pleistocene a lowland water-affiliated mammal species could radiate out in all directions from the Western Amazon (corresponding to the Inambari Center) (Aleixo and de Fátima Rossetti, 2007: see page S445, Fig. 1a for map), and this expansion could show dispersal both inward in western Amazonia to colonize new floodplain forest and seasonally inundated forest and beyond western Amazonia along newly established riverways. One characteristic that unites all living squirrel monkeys is their affinity for water, with a habitat preference for inundated forests, river edges, and lake margins (Rylands and Mittermeier, 2013). Modern squirrel monkeys, such as *S. ustus*, do also use terra firme forest, but it tends to be in the dry season when they are searching more widely for fruit (Peres, 1999). In fact, for some species, like *S. macrodon*, populations may be scarce or absent from terra firme forest if it is far from rivers or lakes (Rylands and Mittermeier, 2013). Our own field research in Brazil reveals that squirrel monkeys in general are much rarer in terra firme forest, and can be completely absent in these types of forest, especially when there are no large bodies of water nearby (F. Röhe, pers. comm.). In Central America as well, the extremely restricted distribution of *Saimiri oerstedii* is accredited to its specialization in a lowland coastal niche (Ford, 2005). The evidence, then, suggests the common ancestor to squirrel monkeys was a lowland water-affiliated taxon; neither an 'upland' species restricted to the Guiana and Brazilian centers and Andes foothills, nor a species able to widely colonize a lacustrine habitat itself, but instead a lowland lake-edge tropical forest niche taxon. We hypothesize that squirrel monkeys may have diversified first in a ring around the Amazon Lake, making incursions into the lake when floodplain forest arose, and following rivers out of the lake to colonize suitable riverine habitats.

(2a) Pre-Glacial Maximum Speciation: Under Ribas et al.'s (2012) Model, most biotic diversification (origin of species) in the Amazon predates the glacial maxima that begin around 0.9 Ma. This precludes climate-induced forest reduction and expansion as a main driver for speciation. However, the refugia could still be important in shaping current intraspecific diversity. We will compare both the divergence dates between species and the timing of within-species diversification to the timing of glacial maxima.

(2b) Glacially-driven Speciation: If most *Saimiri* speciation or diversification events occur within the last 0.9–0.02 Ma, peaking at the Last Glacial Maximum at 0.026–0.019 Ma when the forest was likely at its most reduced, this would lend support for Pleistocene refuges as main drivers of squirrel monkey speciation.

2. Materials and methods

2.1. Taxon sampling and distribution

We obtained DNA from fresh museum tissues in Brazil ($N = 32$) and older specimens from museums in the United States ($N = 37$), as well as sequences from 49 individuals from GenBank, for a total of 118 *Saimiri* samples (Supplementary Materials, Table 1). Taxa included all recognized species and subspecies of squirrel monkey (following Rylands et al., 2013, see Introduction, Section 1.2). Fig. 4 shows sample locations, color-coded by species identification (following Rylands et al., 2013), and Table 1 identifies the samples for each locality ($N = 68$ localities, 100 samples identified to mapped location, maximum of 4 individuals per locality). If latitude and longitude were not available for specimens, we estimated their

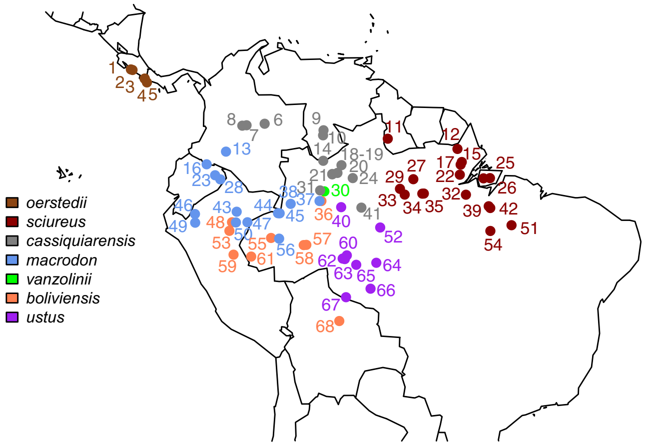

Fig. 4. Map of South and Central America with *Saimiri* sample locations color-coded by species following Rylands et al. 2013. Map numbers correspond to sample numbers in Table 1 and Supplementary Table 1, as well as in Fig. 5. Samples identified only to country or region are not included on the map, but are listed in Table 1 and Supplementary Table 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Samples used in the study, including those from specific localities (see Fig. 4) and those with known provenance only to country or region. "Center" signifies the center of vertebrate endemism coded for each sample; the centers of endemism are described in detail in Table 2 and Fig. 2.

Map code	Sample IDs from TimeTree (Fig. 5)	Center	Lat	Long
1	*S. o. citronellus* 1	CA	9.53	−84.50
2	*S. o. citronellus* 2A, 2B	CA	9.48	−84.34
3	*S. o. citronellus* 3	CA	8.65	−83.15
4	*S. o. oerstedii* 4	CA	8.63	−83.16
5	*S. o. oerstedii* 5A, 5B	CA	8.28	−82.94
6	*S. c. albigena* 6A, 6B	IM	4.28	−71.49
7	*S. c. albigena* 7	IM	4.13	−73.25
8	*S. c. albigena* 8	IM	4.09	−73.66
9	*S. c. cassiquiarensis* 9A, 9B	GU	3.65	−65.77
10	*S. c. cassiquiarensis* 10A, 10B	GU	3.16	−65.81
11	*S. s. sciureus* 11	GU	2.83	−59.52
12	*S. s. sciureus* 12	GU	1.84	−52.74
13	*S. macrodon* 13	IM	1.58	−75.25
14	*S. c. cassiquiarensis* 14	GU	0.70	−65.79
15	*S. s. sciureus* 15	GU	0.58	−52.32
16	*S. macrodon* 16	NP	0.36	−77.13
17	*S. s. sciureus* 17A, 17B	GU	0.32	−52.43
18	*S. c. cassiquiarensis* 18	GU	0.27	−64.02
19	*S. c. cassiquiarensis* 19	NE	−0.48	−64.41
20	*S. c. cassiquiarensis* 20	NE	−0.58	−64.91
21	*S. c. cassiquiarensis* 21	NE	−0.61	−64.92
22	*S. s. sciureus* 22A, 22B	GU	−0.65	−52.51
23	*S. macrodon* 23	NP	−0.70	−76.31
24	*S. c. cassiquiarensis* 24	NE	−0.98	−62.92
25	*S. s. collinsi* 25A, 25B	XI	−0.98	−49.58
26	*S. s. collinsi* 26	XI	−1.00	−50.21
27	*S. s. sciureus* 27A, 27B	GU	−1.08	−57.03
28	*S. macrodon* 28	NP	−1.10	−75.80
29	*S. s. sciureus* 29	GU	−2.02	−58.33
30	*S. vanzolinii* 30A, 30B, 30C	MM	−2.26	−65.68
31	*S. c. cassiquiarensis* 31A, 31B, 31C	MM	−2.26	−65.72
32	*S. s. collinsi* 32A, 32B, 32C	RO	−2.47	−56.01
33	*S. s. sciureus* 33	RO	−2.48	−56.16
34	*S. s. sciureus* 34	GU	−2.57	−57.87
35	*S. s. collinsi* 35	XI	−2.58	−51.92
36	*S. b. boliviensis* 36	IN	−3.19	−65.97
37	*S. macrodon* 37A, 37B	IN	−3.19	−65.97
38	*S. macrodon* 38	NP	−3.47	−68.96
39	*S. s. collinsi* 39	XI	−3.70	−49.70
40	*S. ustus* 40	IN	−3.79	−64.05
41	*S. c. cassiquiarensis* 41	NE	−3.83	−62.08
42	*S. s. collinsi* 42A, 42B	BE	−3.87	−49.55
43	*S. macrodon* 43A, 43B	NP	−4.23	−74.21
44	*S. macrodon* 44A, 44B, 44C, 44D	IN	−4.37	−70.19
45	*S. macrodon* 45	IN	−4.38	−70.03
46	*S. macrodon* 46	MN	−4.45	−78.27
47	*S. macrodon* 47A, 47B, 47C, 47D	IN	−5.25	−73.16
48	*S. b. peruviensis* 48	MN	−5.25	−74.67
49	*S. macrodon* 49	MN	−5.26	−78.43
50	*S. macrodon* 50	MN	−5.27	−74.28
51	*S. s. collinsi* 51A, 52A	BE	−5.53	−47.48
52	*S. ustus* 52	RO	−5.76	−60.26
53	*S. b. peruviensis* 53	MN	−6.08	−74.92
54	*S. s. collinsi* 54A, 54B	XI	−6.09	−49.54
55	*S. b. boliviensis* 55	IN	−6.77	−70.87
56	*S. macrodon* 56	IN	−6.83	−70.08
57	*S. b. boliviensis* 57	IN	−7.44	−67.44
58	*S. b. boliviensis* 58	IN	−7.46	−67.69
59	*S. b. peruviensis* 59	MN	−8.38	−74.52
60	*S. ustus* 60A, 60B, 60C, 60D	RO	−8.45	−63.50
61	*S. b. boliviensis* 61	IN	−8.57	−72.80
62	*S. ustus* 62	RO	−8.76	−63.90
63	*S. ustus* 63	RO	−8.81	−63.70
64	*S. ustus* 64	RO	−9.17	−60.63
65	*S. ustus* 65A, 65B, 65C	RO	−9.36	−62.59
66	*S. ustus* 66A. 66B	RO	−11.67	−61.19
67	*S. ustus* 67	RO	−12.50	−63.58
68	*S. b. boliviensis* 68	IN	−14.80	−64.23

(*continued on next page*)

Table 1 (*continued*)

Code	Sample IDs from TimeTree (Fig. 5)	Center	Locality information
Samples identified only to country or region, not pictured on map			
A	*S. b. peruviensis* A	NP	IQUITOS, PERU
B	*S. b. boliviensis* B	IN	BOLIVIA
C	*S. b. boliviensis* C	IN	SANTA CRUZ, BOLIVIA
D	*S. b. boliviensis* D	IN	SANTA CRUZ, BOLIVIA
E	*S. macrodon* E	NP	IQUITOS, PERU
F	*S. b. boliviensis* F	IN	BOLIVIA
G	*S. macrodon* G	NP	IQUITOS, PERU
H	*S. oerstedii* H	CA	CENTRAL AMERICA
I	*S. s. sciureus* I	GU	GUYANA
J	*S. s. sciureus* J	GU	GUYANA
K	*S. s. sciureus* K	GU	GUYANA
L	*S. s. sciureus* L	GU	FRENCH GUIANA
M	*S. s. sciureus* M	GU	GEORGETOWN, GUYANA
N	*S. s. sciureus* N	GU	FRENCH GUIANA
O	*S. s. sciureus* O	GU	FRENCH GUIANA
P	*S. s. sciureus* P	GU	KUITARO RIVER, GUYANA
Q	*S. c. cassiquiarensis* Q	NE	AMAZONAS, BRAZIL
R	*S. c. cassiquiarensis* R	GU	RORAIMA, BRAZIL

location based on the locality data on voucher tags or museum databases. Some museum or GenBank samples were identified only to country (French Guiana (*N* = 3), Guyana (*N* = 3), Bolivia (*N* = 2)), or region (Central America (*N* = 1), Roraima, Brazil (*N* = 1), Rio Negro, Brazil (*N* = 1), Kuitaro River, Guyana (*N* = 1)) or were marked as Georgetown, Guyana (*N* = 1); Santa Cruz, Bolivia (*N* = 2); or Iquitos, Peru (*N* = 3), all known distribution centers for captive squirrel monkeys. These specimens are not included on the map (Fig. 4) but are listed in Table 1. Species identity and centers of endemism ascribed to these specimens of less exact provenance should be interpreted with caution, as noted in Supplementary Table 1, which provides more information about each sample.

For museum specimens sequenced for this study, morphological analysis and confirmation of species identification followed Hershkovitz (1984) and Paim et al. (2013). Key distinguishing morphological traits include the arch shape to the cap; the hairiness of the ear; and the tail pencil shape. Key color pattern variations include the crown color; the color of the back; the hand, forearm and wrist color; color of the shoulders; and the color of the outer thighs in contrast to the body. Squirrel monkey skeletal material alone was not identifiable with certainty to the species level, so when pelts were not available for analysis, we based the sample identity primarily on collection location and secondarily on the current and past taxonomic names registered for the specimen. In the wild, there are few areas of known sympatry between squirrel monkeys, but it does occur in Peru between *S. boliviensis peruviensis and Saimiri macrodon* east of the Río Ucayali from about 04°S southward to the Rio Abujao (Aquino and Encarnación, 1994); and parapatry with some cross-species interaction does occur in Mamirauá, Brazil among *S. vanzolinii* and both *S. macrodon* and *S. c. cassiquiarensis* (Paim et al., 2013); and possibly between *S. ustus* and *S. sciureus collinsi* south of the Amazon River at the Tapajós River (Thorington, 1985). Mercês et al. (2015) also point to some spots of syntopy or replacement along the Amazon River between *S. sciureus sciureus and S. s. collinsi.*

2.2. Laboratory procedures

This study analyzed two mitochondrial regions: the mitochondrial control region (D-Loop) and cytochrome *b* (cyt *b*), a dataset based on short sequences from a single locus, the mitochondrial genome. Mitochondrial DNA is available at a much higher copy number than nuclear DNA and is resilient to the processes of degradation over time, facilitating amplification from decades-old museum specimens. This advantage was key for our biogeographic analysis because it allowed us to make use of a wide geographic sampling from known locations across many countries.

In addition, this research builds on a previous study in which Chiou et al. (2011) sequenced a full mitochondrial genome for several squirrel monkey samples using circular overlapping primer pairs. The cyt *b* and D-Loop regions from the following five samples of known provenance from Chiou et al. (2011) are included in our analyses: HQ644338 (*Saimiri macrodon*), HQ644339 (*Saimiri boliviensis boliviensis*), HQ644335 and HQ644336 (*Saimiri oerstedii citronellus*), and HQ64337 (*Saimiri oerstedii oerstedii*).

The cyt *b* and D-Loop sequences obtained in this study were generated from three laboratories: University of California – Los Angeles (UCLA), Universidade Federal do Amazonas (UFAM), and Universidade Federal do Pará (UFPA). There was a strong concordance in terms of expected species relationships, both among the sequences generated across different labs and with the ones harvested from GenBank, including other Chiou et al. (2011) squirrel monkey sequences with whole mitochondrial genome data that were not used in the phylogeny in this paper because of unknown provenance. While we cannot rule out entirely the possibility that some sequences generated in this study could be numts (nuclear insertions of mitochondrial DNA), we outline other preventative lab techniques below.

At UCLA, JWLA, GK, MTTN, and MEA performed extraction, sequencing and analysis for museum samples, including dry 'crusties' salvaged from skeletal material, and wet tissue preserved in alcohol. Museum samples were extracted in Chelex following Barber (2004); downloaded from http://people.bu.edu/pbarber/Web%20Protocols/Protocol2.pdf. To prevent contamination of the degraded museum samples, samples were extracted and set up for PCR in a UV irradiated hood. In addition, all reagents and equipment for museum samples were kept separate, sterilized and UV irradiated. All samples were run with two negative controls: one from the extraction process to ensure the hood was not contaminated and one from PCR setup. For some samples, we performed multiple extractions, PCRs, and sequencing to confirm sequence validity. Preventative lab techniques to control for the possibility of inadvertent amplification of numts in degraded samples included the use of several overlapping internal primers to amplify ~200 bp at a time. We aligned these smaller fragments together to create complete cyt *b* and D-Loop sequences (see Supplementary Materials, Table 2 for primer sequences). PCR amplifications were performed in 20 μl reactions (18 μl reagent mixture + 2 μl template). Standard reactions include 2 μl template, 2 μl 10xAmpliTaqGold (Applied Biosystems) buffer, 10.64 μl ddH20, 1.2 μl (25 mM) MgCl2, 0.8 μl (10 mM) each of forward and reverse primers, 1.8 μl (10 mM) dNTPs, 0.8 μl BSA (10 mg/ml), 0.16 μl (5 units/μl) AmpliTaqGold DNA polymerase (Applied Biosystems). The standard thermal cycling program consisted of 10 min activation at 94 °C; denaturing at 94 °C for 35 s; annealing at 55 °C for 35 s; and extension at 70 °C for 30 s. A final extension cycle was carried out at 72 °C for 10 min. Template volume, number of cycles, annealing temperature, and $MgCl_2$ concentration varied by primer pair and template concentration. All PCR products were further purified for sequencing by EXO/SAP. Sequencing was conducted using an Applied Biosystems 3730xL DNA Analyzer at the Yale Sequencing Lab at Yale University or Cornell University Institute of Biotechnology, with Big Dye Terminator chemistry and AmpliTaq-FS DNA Polymerase.

Fresh tissue samples collected from museum voucher specimens in Brazil were analyzed by IPF and GMP at UFAM. These museum voucher specimens were obtained in the context of an Amazonian-wide faunal inventory project (CNPq/SISBIOTA) carried out in accordance with the appropriate collection permits (IBAMA license No. 005/2005 – CGFAU/LIC). Tissue samples were deposited at Instituto Nacional de Pesquisas da Amazônia (INPA) Mammal Collection, Recursos Genéticos/INPA and CTGA/UFAM tissue collection. At UFAM, total genomic DNA was extracted from muscle tissues preserved in alcohol using the DNeasy Blood & Tissue Kit (QIAGEN). PCR amplification for the two mitochondrial regions were performed with the set of primers listed in Supplementary Table 2. The final volume of each PCR reaction was 15 μl and contained 6.8 μl of ddH2O, 1.2 μl of 25 mM MgCl2, 1.2 μl of 10 mM dNTPs, 1.5 μl of 109 buffer (750 mM Tris–HCl—pH 8.8 at 25 C, 200 mM (NH4)2SO4, 0.1% (v/v) Tween 20), 1.5 μl of each primer (2 μM), 0.3 μl of Taq DNA Polymerase (1 U/μl) and 1 μl of DNA. PCR consisted of an initial denaturation step at 68 °C for 60 s followed by 35 cycles of denaturation at 93 °C for 5 s, primer annealing at 50 °C for 35 s, and primer extension at 68 °C for 90 s, followed by a final extension at 68 °C for 10 min. PCR products were purified using EXO-SAP (Exonuclease—Shrimp Alkaline Phosphatase) following the manufacturer's suggested protocol (Werle et al., 1994). Sequencing was performed on ABI 3130xl automatic DNA analysis system (Applied Biosystems) using the manufacturer's recommended settings. Sample processing at UFPA by MPM and MLH followed the same protocol, with modifications described in Mercês et al. (2015). All new sequences have been deposited to GenBank, with accession numbers KM234491-KM234608 and KM245631-KM245638 (see Supplemental Table 1).

2.3. Phylogenetic analysis

Our phylogenetic analysis builds on the earlier mitochondrial genome analysis of nine squirrel monkey sequences by Chiou et al. (2011). In the present study, rather than maximizing sequence length, we maximize sampling across known localities. We adopted a Bayesian statistical framework for our phylogenetic and biogeographic analyses. Bayesian phylogenetic frameworks offer the advantage of accommodating uncertainty in topology, branch lengths, and other model parameters when making evolutionary inferences. A BEAST xml file was generated from BEAUti 1.7.5 with the following parameters: 118 taxa for 1074 bp of cyt *b* and a subset of 71 taxa for 811 bp D-Loop. Clock models were linked for both genes, but substitution models were unlinked. For both cyt *b* and D-Loop, the nucleotide substitution model used in our analyses was HKY with gamma site heterogeneity model. We linked the tree prior for all trees, and used the coalescent model for Bayesian skyline using 10 groups and a piecewise-constant model, with a random starting tree. We chose the Bayesian skyline model because our sample includes both populations and species, and there is disagreement over how many species are represented within the total sample; the skyline model estimates both coalescent error and phylogenetic error. We used a chain length of 100 million and logged parameters every 50,000 steps. We used Tracer 1.7.5 to assess performance and burnin. In Tree Annotator 1.7.5 we used a Burnin of 200 trees and created a maximum clade credibility tree with mean node heights. We visualized the annotated tree in FigTree 1.4.0.

2.4. Molecular dating

For the clock model in our BEAST analysis, we used an uncorrelated lognormal relaxed clock and a starting estimate of a rate of 1.0 for the sequence data. We based our calibration of the root height of our tree at 1.1–1.9 Ma (mean 1.5 Ma) from dates recovered in Chiou et al. (2011) for *Saimiri* crown age based on whole mitochondrial genome data and six primate fossil calibrations. We recovered the same topology in our BEAST tree as that found in Chiou et al. (2011), with *S. boliviensis* as the sister group to *S. macrodon* + *S. s. sciureus* + *S. oerstedii*, and *S. macrodon* as the sister group to *S. s. sciureus* + *S. oerstedii*. The other taxa in our analysis were not sampled in Chiou et al. (2011).

2.5. Biogeographic analyses

We performed two different types of statistical biogeographic analyses: BEAST phylogeography (Lemey et al., 2009), which uses a Bayesian framework, and BioGeoBears (Matzke, 2013a,b), which uses both a Bayesian and a maximum likelihood framework, depending on the model. Both analyses were performed on the timetree recovered in the BEAST analysis, described above.

We performed the BEAST phylogeography analysis, which incorporates a pure dispersal model, to understand how squirrel monkeys have spread across the landscape over time. We used a discrete traits model and partitioned the samples into geographic regions, considered the 'locations' for this analysis (see below). We used a symmetric substitution model for the discrete traits in the location partition, and we inferred the social network with phylogeographic Bayesian Stochastic Search Variable Selection Procedure (BSSVS). The basic idea with BSSVS is that there are various possible dispersal routes that could explain *Saimiri* distribution. The BSSVS places half of the prior probability on the minimum number of connections to explain present day localities and places the other half on all other possible ways that one could visit areas and still end up with the observed occupation in the tips. Then MCMC samples those possible connections. Connections that do a good job of explaining present day distributions will attract the most visits by the chain (Lemey et al., 2009).

Trees were linked among the two genes and the 'location' trait. We used a strict clock with estimation and starting rate of 0.01 for the location data. For the discrete trait of 'locations', we performed ancestral state reconstruction for states at all ancestors. We used a gamma distribution for the prior on the locations.clock.rate, with an initial value of 0.01, a shape of 0.01, a scale of 1000, and an offset of 0. Details of the BEAST phylogeography model are found in Lemey et al. (2009).

We also performed six different BioGeoBears analyses (Matzke, 2013a,b), to reconstruct the biogeographic history of squirrel monkeys across the Amazon and Central America. The six models included the Dispersal-Extinction Cladogenesis Model (DEC), the Dispersal-Extinction Cladogenesis Model with the founder parameter, called J or Jump (DECj), the Dispersal-Vicariance Analysis (DIVA), the Dispersal-Vicariance Analysis with the founder parameter (DIVAj), Bayesian inference of historical biogeography for discrete areas (BayArea), and the Bayesian inference method with the founder parameter (BayAreaj). Each model allows for a different subset of biogeographic possibilities, such as dispersal, vicariance and extinction (see Matzke, 2013a,b and also Fig. 5 in Buckner et al., 2015). These biogeographic processes are implemented in a maximum likelihood (DEC, DECj, DIVA, DIVAj) or Bayesian (BayArea, BayAreaj) framework as free parameters estimated from the data. For all six BioGeoBears analyses, the possible number of locations at a given node was limited to four to avoid intractability of the model. Reconstructions used a distance dependent model, so that dispersal probability was dependent on the distance between the centers of the 'locations' (see Supplemental Table 3). Finally, we compared the six different models for statistical fit using the Akaike Information Criterion implemented in the R package BioGeoBEARS (Matzke, 2013a,b). It was not possible to directly compare the statistical fit of the BEAST phylogeography analyses with the BioGeoBEARS analyses, so we address the two separately in the results section.

Both the BEAST phylogeography and the BioGeoBears analyses used discrete geographic locations coded from the tips, to perform ancestral state reconstruction to determine the most likely location for each ancestral node. The locations that we used for the biogeographic analyses mirrored known centers of vertebrate endemism, as described in Cracraft (1985) and Ribas et al. (2012), with the addition of the Imeri (*sensu* Silva et al., 2005), Mamirauá, and Central American Centers as key areas of endemism known for *Saimiri*. Fig. 2 depicts these eleven 'locations', and Table 2 describes each 'location' in detail.

3. Results and discussion

3.1. Phylogenetic inferences: Species and species limits

The topology of our time tree (Fig. 5) was largely concordant with previous mitochondrial genetic analyses (Chiou et al., 2011; Lavergne et al., 2010), but we recovered more clades and found paraphyly for some currently recognized species (per Rylands et al., 2013). In our analysis, *Saimiri boliviensis* was recovered as the sister to all other squirrel monkeys, concordant with Chiou et al. (2011), Perelman et al. (2011), and Springer et al. (2012). However, unlike previous morphological (Hershkovitz, 1984) or molecular studies (Lavergne et al., 2010; Chiou et al., 2011; Cropp and Boinski, 2000), we did not find molecular evidence for subspecies differentiation within *S. boliviensis*.

Two samples considered *S. macrodon* (*S. macrodon E* and *G* in Fig. 5) were recovered within the *S. boliviensis* clade; these samples are both from crania from Iquitos, Peru, labeled *S. sciureus* and more recently re-identified as *S. macrodon*. They may be mislabeled, or they may indicate hybridization between *S. boliviensis* and *S. macrodon*, either in captivity or in the wild, both of which have been reported in previous studies (Silva et al., 1992; Hershkovitz, 1984; Thorington, 1985; Costello et al., 1993).

We recovered *S. boliviensis* and *S. vanzolinii* as distinct monophyletic groups, but not sister to each other, as previously considered (Hershkovitz, 1987). Instead, *S. vanzolinii* is sister to eastern Rondônia *S. ustus* (*S. ustus* C), an unexpected finding. *S. vanzolinii* and *S. ustus* have allopatric distributions, so this is unlikely to be a result of modern hybridization. *S. oerstedii* was monophyletic, and its two subspecies, *S. oerstedii oerstedii* and *S. o. citronellus*, reciprocally monophyletic, as found by Blair et al. (2013) in a more extensive study of this group.

The four other currently recognized species (per Rylands et al., 2013), *S. ustus*, *S. cassiquiarensis*, *S. macrodon* and *S. sciureus* may need revision based on our results. *S. sciureus sciureus* from north of the Amazon River, as delineated geographically in Mercês et al. (2015), does not form the sister group to the *S. sciureus collinsi* clade south of the Amazon. *S. macrodon*, *S. c. albigena*, and *S. c. cassiquiarensis* all form a clade together, but do not separate into two distinct clades of *S. macrodon* vs. *S. c. cassiquiarensis* + *S. c. albigena* (as would be expected from the taxonomic arrangement in Rylands et al., 2013). Instead, they form four clades, which correspond to *S. macrodon* A, *S. c. albigena* + *S. macrodon* in Imeri, *S. macrodon* B, and *S. c. cassiquiarensis*. The single *S. macrodon* sample in Imeri from our study is from a locality in the range formerly distinguished for *S. caquetensis* Allen 1916, later considered a synonym of *S. s. macrodon* by Hershkovitz (1984), and within the currently recognized range of *S. macrodon*, not *S. c. albigena*, based on Carretero-Pinzon et al. (2009; see p.60 for map of *Saimiri* distribution in Colombia). Finally, *S. ustus* was paraphyletic in our analysis, with *S. ustus* A forming a clade with *S. s. sciureus* (Guiana) and *S. oerstedii* (Central America), and *S. ustus* B as sister to *S. ustus* C + *S. vanzolinii*. *S. ustus* has a distinct morphology from other squirrel monkeys, with the most striking difference its bare ears in place of the tufted ears found in other species. *S. ustus* considered as a whole (A + B + C) has the most haplotype diversity of any squirrel monkey taxon in this study. There is strong geographic structuring within *S. ustus*, and the apparent paraphyly might be a result of female philopatry and incomplete lineage sorting in this group. Alternatively, this result may support

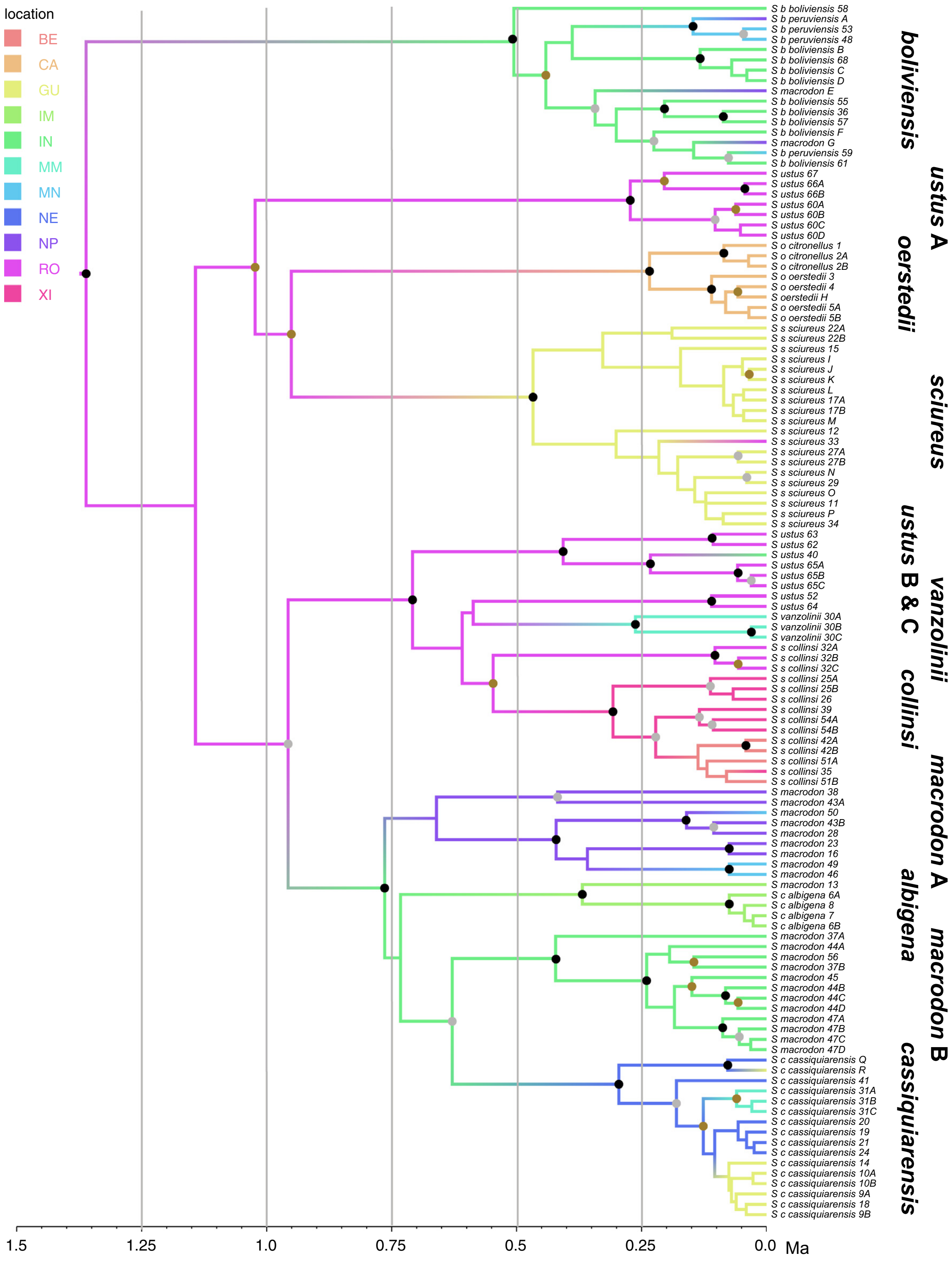

Fig. 5. BEAST phylogeography tree for *Saimiri* evolution by centers of endemism. Samples are coded by species and subspecies names following Rylands et al. 2013, and by map location as in Table 1. The colors in the tree correspond to the centers of endemism (see key and Fig. 2), which are used as the 'locations' in the BEAST phylogeography analysis. Black dots signify nodes with posterior probabilities of 1; grey dots nodes with pp of 0.90–0.99, and brown dots nodes with pp of 0.70–0.89. All other nodes have a pp of less than 0.70.

the presence of cryptic species. This points to the urgent need for studies of autosomal DNA in *S. ustus* and other squirrel monkeys.

In summary, our timetree analysis produced four major clades of squirrel monkeys. We consider each clade separately here:

1. *S. boliviensis* – Inambari, Napo, and Marañón: Our analysis shows clear evidence that *S. boliviensis* is a distinct species, but provided no support for geographic subspecies within this clade, despite the morphological variation in some Peruvian populations.

Table 2
Centers for vertebrate endemism used as ‘localities’ in the BEAST phylogeography and BioGeoBears analyses.

CA	Central America includes samples from the western coast of Costa Rica and Panamá; this is a disjunct distribution from all other *Saimiri* populations
GU	Guiana includes samples north of the Amazon River, east of the Rio Negro, and east of the Orinoco River
BE	Belém includes samples east of the Tocantins River north of where it meets the Araguaia, north of the city Imperatriz, west to Belém, and east to São Luís
XI	We combined the Xingu and Tapajós areas for the Pará Center, which includes the Marajó Archipelago and the area south of the Amazon River between the Araguaia River/Tocantins River and the Tapajós River and Teles Pires River
RO	Rondônia includes samples south of the Amazon/Solimões, east of the Madeira and Guaporé rivers, west of the Tapajós and Teles Pires rivers
IN	Inambari includes samples south of the Solimões/Amazon, west of the Madeira and east of the Ucayali River. It extends farther south into Bolivia than in Silva et al. (2005)
MN	Marañón includes samples from the south and east of the Marañón, and west of the Ucayali River
NP	Napo includes samples from south of the River Japurá/Caquetá and north of the Solimões/Amazon River, and north of the Marañón River (Silva et al., 2005)
IM	Imeri includes samples north of the Japurá/Caquetá and Solimões, south of the Rio Negro starting west of the mouth of the Rio Japurá, and west of the Orinoco. This distribution follows that in Silva et al. (2005), except that it is restricted as west of the Negro region (Borges, 2004), as below
NE	Negro includes samples west of the Rio Negro, north of the Solimões River, going west until the Japurá reaches the Solimões (Ribas et al., 2012, following Borges, 2004)
MM	Mamirauá includes samples on the floodplain forest (or várzea) habitat in the Mamirauá Reserve between the Solimões and the Japurá Rivers in the Tefé Region. This region was separated as unique because of the endemic taxon *Saimiri vanzolinii*, found only in this single habitat (Ayres, 1985; Paim and Queiroz, 2009; Paim et al., 2013)

2. *S. ustus* A, *S. sciureus*, *S. oerstedii*: Rondônia, Guiana, Central America: Despite these three taxa forming a clade together in our topology, there was weak support for the sister relationship between *S. ustus* A and the other two species, and we feel our data is preliminary at best for distinguishing *S. ustus* A as a different species to other *S. ustus* populations; this may be an instance of incomplete lineage sorting. More data will be necessary to resolve the taxonomy for *S. ustus*. *S. oerstedii* and *S. sciureus* have disjunct distributions and are separated by long branch lengths, and we concur with placing *S. oerstedii* into its own species, with *S. o. oerstedii* and *S. o. citronellus* as subspecies, based on genetic, distributional and morphological data. The taxon *S. sciureus* should be restricted to the Guiana center as a separate species.
3. *S. ustus* B & C, *S. vanzolinii*, *S. collinsi*: Rondônia, Mamirauá, Pará, Belém: More genetic and morphological data are needed to understand the relationships amongst *S. ustus*, *S. vanzolinii* and *S. collinsi*. *S. vanzolinii* is morphologically most similar to *S. boliviensis*, and has been considered a subspecies of *S. boliviensis* by some authors. More intense genetic sampling of *S. vanzolinii* is needed to rule out past introgression as the reason its haplotype forms a clade with some *S. ustus* populations. *S. collinsi* appears to be a good taxon, reciprocally monophyletic to its sister clade (*S. ustus*/*S. vanzolinii*). Genetic and morphological sampling in the Tapajós–Xingu interfluvium will be important to understand what is considered the transitional area between *S. ustus* and *S. collinsi* (Thorington, 1985).

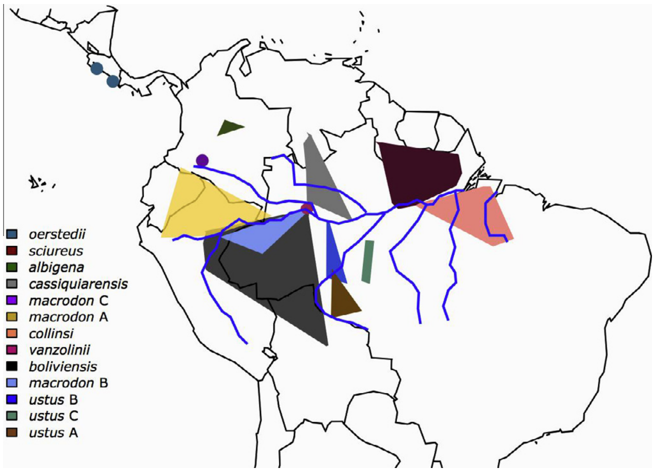

Fig. 6. Minimum clade range for each of the 14 main clades recovered in the study.

4. *S. cassiquiarensis* complex: Inambari, Napo, Marañón, Imeri, Negro, Mamirauá and Guiana: *S. cassiquiarensis* forms a well-supported clade, with reciprocal monophyly from other squirrel monkey clades. The taxon *macrodon* is paraphyletic as currently construed, and more morphological research, building on Paim et al. (2013), is needed to determine how best to categorize this group as a whole. Our interim recommendation is to consider five subspecies of *S. cassiquiarensis*, which correspond to clades *cassiquiarensis*, *albigena*, *macrodon* A (Marañón and Napo Centers), *macrodon* B (Inambari Center) and *macrodon* C (Imeri Center) in our phylogenetic tree; although the proper nomenclature for these taxa requires further taxonomic revision. Interestingly, Hill (1960) also divided this geographic group into five subspecies of *S. sciureus*, based on morphological data: *S. s. cassiquiarensis*, *S. s. codajazensis*, *S. s. caquetensis*, *S. s. macrodon*, and *S. s. petrina*.

For clarity and simplicity, for the remainder of the article, we switch to use a provisional taxonomy concordant with the fourteen clades recovered in our phylogeny, as follows: *S. sciureus*, *S. oerstedii* (*S. o. oerstedii* and *S. o. citronellus*), *S. collinsi*, *S. ustus* (A, B, and C), *S. boliviensis*, *S. cassiquiarensis* (*S. c. cassiquiarensis*, *S. c. albigena*, *S. c. macrodon* A, *S. c. macrodon* B, and *S. c. macrodon* C), and *S. vanzolinii*. Note that the group *S. ustus* and the group *S. c. macrodon* are each paraphyletic in our topology, but we have retained these species or subspecies names and instead delimited the different clades by letters, pending further morphological and molecular analysis. Fig. 6 shows the minimum clade range for each of the 14 clades recovered in this study, as visualized through drawing minimum convex polygons from localities for the genetic samples corresponding to each clade.

3.2. Chronological inferences: Temporal pattern of diversification

Table 3 provides the clade ages and upper and lower 95% HPD for major splits and radiations within the *Saimiri* genus. Table 4 compares mean ages from this study to mean ages for key splits in other studies. Our study provides a much more detailed picture for *Saimiri* diversification than has been available in any past work. However, one limitation to our study is the short length of the mitochondrial sequences, which in combination with a series of rapid speciation events and short branch lengths in the topology lead to low support for several deep nodes in the tree (Fig. 5).

When we compare the mean values for the timing of splits between *Saimiri* species (*S. boliviensis*, *S. cassiquiarensis*, *S. oerstedii*, *S. sciureus*, *S. collinsi*, *S. ustus*, *S. vanzolinii*) to the timing of the Pleistocene Glacial Maxima, we find that all diversification events occurred *prior* to 0.5 mya; in other words, prior to the last three

Table 3
Mean ages and upper and lower 95% HPD for main splits, clades and radiations within the genus *Saimiri*.

Split or clade	Mean age (Ma)	Lower 95% HPD	Upper 95% HPD
S. boliviensis vs. *all other Saimiri*	1.36	1.1	1.91
S. ustus A + *S. oerstedii* + *S. sciureus* vs. *S. collinsi* + *S. cassiquiarensis* + *S. ustus B* + *S. ustus C* + *S. vanzolinii*	1.14	0.7	1.72
S. ustus A vs. *S. oerstedii* + *S. sciureus*	1.02	0.5	1.55
S. oerstedii vs. *S. sciureus*	0.95	0.47	1.48
S. ustus B + *S. ustus C* + *S. vanzolinii* + *S. collinsi* vs. *S. cassiquiarensis*	0.96	0.54	1.5
S. c. macrodon A vs. *S. c. albigena* + *S. c. macrodon B* + *S. c. cassiquiarensis*	0.76	0.41	1.2
S. ustus B vs. *S. ustus C* + *S. vanzolinii* + *S. collinsi*	0.71	0.37	1.15
S. c. albigena vs. *S. c. macrodon B* + *S. c. cassiquiarensis*	0.73	0.33	1.01
S. c. macrodon B vs. *S. c. cassiquiarensis*	0.63	0.28	0.88
S. ustus C + *S. vanzolinii* vs. *S. collinsi*	0.61	0.33	0.96
S. ustus C vs. *S. vanzolinii*	0.59	0.22	0.95
S. boliviensis radiation	0.51	0.24	0.8
S. collinsi radiation	0.55	0.25	0.9
S. sciureus radiation	0.47	0.22	0.74
S. c. macrodon A radiation	0.66	0.33	1.12
S. c. macrodon B radiation	0.42	0.19	0.69
S. c. macrodon vs. *S. c. albigena in Imeri*	0.37	0.13	0.63
S. ustus B radiation	0.41	0.18	0.68
S. c. albigena radiation	0.07	0.02	0.15
S. c. cassiquiarensis radiation	0.3	0.19	0.69
S. ustus A radiation	0.27	0.09	0.48
S. vanzolinii radiation	0.26	0.08	0.52
S. oerstedii oerstedii vs. *S. oerstedii citronellus*	0.23	0.09	0.48
S. ustus C radiation	0.11	0.03	0.25

Table 4
Comparison of mean ages for major events in *Saimiri* evolutionary history in this and other studies.

Split or clade	Mean age (Ma), this study	Mean age (Ma), Chiou et al. (2011) (BEAST)	Mean age (Ma), Chiou et al. (2011) (Multidivtime)	Mean age (Ma), Lavergne et al. (2010)	Mean age (Ma), Perelman et al. (2011)	Mean age (Ma), Springer et al. (2012)
Diversification of modern Saimiri	1.36	1.5	1.1	4.3[a]	2.24	1.73
S. oerstedii + *S. (s.) sciureus* vs. *S.(c.) macrodon*	1.14	1.06	0.87	4.3[a]	1.26[c]	1.05[c]
S. oerstedii vs. *S. (s.) sciureus*	0.95	0.91	0.77	2.3	0.73	0.64
S. ustus + *S. collinsi* vs. *S. cassiquiarensis complex*	0.96			2.6		
S. cassiquiarensis 'complex'	0.76			2		
S. ustus + *S. collinsi* radiation	0.61			1.7[b]		
S. boliviensis radiation	0.51	0.3	0.25	1.1		
S. (s.) scuireus radiation	0.47			0.9		
S. oerstedii oerstedii vs. *S. oerstedii citronellus*	0.23	0.16	0.11			

[a] Lavergne et al. (2010) recovered a topology of *S. boliviensis* sister to *S. (s.) sciureus* and *S. oerstedii*, and *S. macrodon* 'complex' sister to *S. collinsi* and *S. ustus.*

[b] Lavergne et al. (2010) did not include samples of *S. vanzolinii*, and for *S. collinsi* had samples from Marajó Island only.

[c] Perelman et al. (2011) and Springer et al. (2012) did not include *S. (c.) macrodon* in their studies. The date of 1.26 Ma and 1.05 Ma are for their splits between *S. oerstedii* + *S.(s.) sciureus* vs. *S. ustus.*

glacial maxima (Table 3). However, if we consider the minimum age estimate within the 95% HPD, most splits could fall within the Glacial Maxima period, although all diversification events still would have occurred prior to the Last Glacial Maximum, which was the most extreme. When considering the timing of within-species radiations, based on the sampling available in this study (which is likely to underestimate the true age of species diversification), we find that all mean values for species radiations occur between 0.5 Ma and 70 kya, during the Glacial Maxima period, and in fact the span of each of the 95% HPD ranges except *S. cassiquiarensis* and *S. ustus* (sensu lato) are wholly encompassed within the Glacial Maxima period (between 0.9 Ma and 0.015 Ma). This suggests that intra-specific diversification in *Saimiri* occurred simultaneously with major Pleistocene climate fluctuations. However, none of the lower range limits for the 95% HPD for these radiations fall more recently than the Last Glacial Maximum (See Table 3). Two molecular studies of *Saimiri* nuclear markers also confirm the timing of *Saimiri* speciation as occurring before and during the Pleistocene glacial maxima (Perelman et al., 2011; Springer et al., 2012, see Table 4).

Climate-induced forest reduction and expansion probably were not the original drivers for *Saimiri* speciation. However, the refugia could have been important in shaping current intraspecific diversity as well as causing opportunities for contact between species, as we found the most dynamic movement across endemism centers to occur in the very late Pleistocene or Holocene. The most extreme glacial maxima may have affected *Saimiri* intraspecific diversity as there could have been moments of hybridization and introgression between adjacent species, because it was probably easier to cross rivers when ice caps took up more water, making rivers smaller and slower (Maslin and Burns, 2000). Regarding *S. oerstedii*, Ford (2005) suggests that Pleistocene extremes in high water levels may have caused a vicariant distribution in Costa Rica and Panama and consequently the formation of the two current subspecies. In fact, the isolation of *S. oerstedii* in a disjunct distribution in Central America may have been a result of the increasing aridity in northern South America, in the Venezuelan Llanos and the Northern Andes regions.

3.3. Biogeographic inferences

3.3.1. Species relationships within and across areas of endemism, from BEAST phylogeography analyses

From the BEAST phylogeography analysis, Bayes factor tests for significant non-zero rates showed that there have been several historically important dispersal routes for squirrel monkeys (see

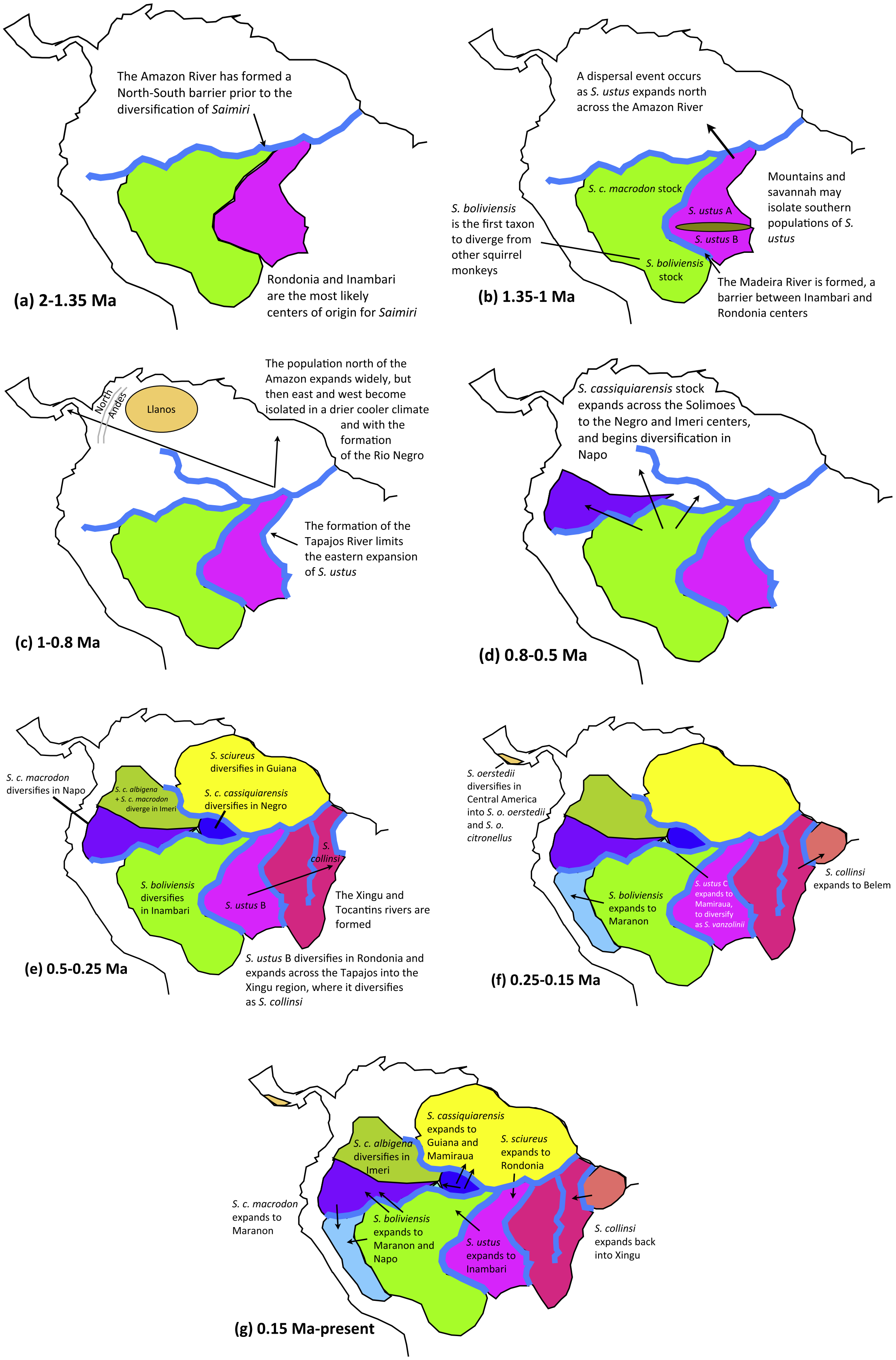

Fig. 7. a–g: Time slices for *Saimiri* evolution, based on BEAST phylogeography analysis, following SPREAD results (see Supplemental File 1). The text in Section 3.3.1. describes the time slices in detail.

Supplementary Materials, Fig. 1 for summary figure and Supplementary Materials, Table 4 for non-zero rates table). All southern Amazon centers are connected in a chain from Belém to Xingu (Pará) to Rondônia to Inambari. Inambari also forms a triangle of connectivity with Marañón and Napo. The only strong connection across the Amazon River to the north is between Rondônia and Guiana. Guiana also shows a dispersal route connection to the Negro region, which in turn is connected to Mamirauá.

The panels in Fig. 7a–g represent time slices in *Saimiri* evolutionary history, and are annotated with major geologic and climatic events for each time slice based on our interpretation of the "Young Amazon" geological model. Below is a corresponding summary of the spread of squirrel monkeys through time, based on our BEAST Phylogeography SPREAD analysis, as visualized in Google Earth (.kml file available as Supplementary Material, File 1).

3.3.1.1. From 2 Ma to 1.35 Ma: Initial diversification in southern Amazon. The root states analysis of the BSVSS placed the highest probability for the origin of *Saimiri* as the Rondônia Center (44%), with the Inambari (23%) as the next most likely center of origin (Supplementary Materials, Fig. 2). The initial diversification and divergence between *S. boliviensis* and the ancestor to all other squirrel monkeys occurred in the Rondônia and Inambari regions in the southern Central Amazon (Fig. 7a). The Amazon River was probably already in place in its present form, or in its final stages of formation, by the onset of *Saimiri* species diversification, and it formed a significant barrier to dispersal north. Warm climates and high water may have facilitated expansion of *Saimiri* along riverways. Our interpretation is that ancestral *Saimiri* was a lakeside lowland taxon, and its distribution changed at this time due to the draining of the lake and the formation of the river system.

3.3.1.2. From 1.35 to 1.00 Ma: Diversification of major groups. The River Madeira formed between 2 and 1 Ma (here and below, dates for river formation follow Ribas et al., 2012). Between 1.35 and 1 Ma, there were two lineages in the Inambari center: *S. boliviensis* and the ancestor of all other taxa. This ancestral lineage probably occupied both Inambari and Rondônia centers and was split by the Madeira River, originating the ancestor of the clade (*S. ustus* A, *S. oerstedii*, *S. sciureus*) in Rondônia. Another split associated with the formation of the Madeira would isolate the ancestor of another eastern clade (*S. ustus* B/C, *S.* vanzolinii, *S. collinsi*) from the western clade (*S. c. macrodon* A, B, C, *S. c. albigena*, *S. c. cassiquiarensis*) (Fig. 7b). A single population expansion event from Rondônia across the Amazon River to the north, from *S. ustus* A stock, could have been possible if there was an exposed Purus arch, with the headwaters of the main channel of the Amazon River being at this central Amazonian location (Nogueira et al., 2013). The following breach of the arch and formation of the transcontinental Amazon river channel completely isolated the northern stock in the Guiana center, and this northern ancestral population (*S. oerstedii*, *S. sciureus*), subsequently reached Central America originating *S. oerstedii*.

3.3.1.3. From 1 Ma to 0.8 Ma: Dropping temperatures and isolation of northern populations. At about 900 kya the first Pleistocene Glacial Maximum hits with the coldest temperatures since the initial diversification of *Saimiri*. At this time, in the Northern Amazon, *S. oerstedii* may have become vastly isolated from *S. sciureus* due to forest retraction and increased aridity with dunes and savannah, particularly in the Llanos region of northern South America (Fig. 7c). In the southern Amazon, the formation of the Tapajós River limits the eastern expansion of *S. ustus*.

3.3.1.4. From 0.8 to 0.5 Ma: Expansion of the S. cassiquiarensis complex into Imeri, Napo and Negro. *S. cassiquiarensis* complex rapidly expands from Inambari into three other Centers: Imeri, Napo, and Negro (Fig. 7d). This expansion could be related to landscape changes due to the transition from lacustrine to riverine landscape, and suggests that *S. c. macrodon* populations became isolated across the Solimões, the Ucayali, the Putamayo, and the Napo rivers during this period. *S. cassiquiarensis* complex is limited in its expansion to the north of Amazon River by the newly formed Rio Negro (∼1 Ma)—this appears to maintain a barrier between the *S. cassiquiarensis* complex and *S. sciureus* in the Guiana center. *S. ustus* diversifies within Rondônia. During cladogenesis of *S. c. macrodon* A, *S. c. macrodon* B, *S. c. cassiquiarensis*, and *S. c. albigena*, cold pulses continue every ∼100 k with repeated Glacial Maxima.

3.3.1.5. From 0.5 to 0.25 Ma: Diversification within Centers of Endemism. During this time slice, *S. boliviensis* diversifies in the Inambari region, *S. sciureus* diversifies in the Guianas, and western *Saimiri* populations diversify within their 'Centers': *S. c. albigena + S. c. macrodon* in Imeri, *S. c. macrodon* A in Napo, *S. c. macrodon* B in Inambari, and *S. c. cassiquiarensis* in Negro center (Fig. 7e).

Also during this time, the Tapajós, Tocantins and Xingu rivers form in the southeastern Amazon. *S. ustus* B diversifies in Rondônia, expands into Pará Region, and populations isolated east of the Tapajós and Xingu, diversify into *S. collinsi*.

3.3.1.6. From 0.25 to 0.15 Ma: Expansion to Mamirauá, Marañón and Belém. This time slice includes the Last Glacial Maximum, the coldest temperatures during the Pleistocene. By this time, all the major rivers are in their current form in the Amazon (Fig. 7f). *S. boliviensis* diversifies and expands into Marañón, *S. oerstedii* diverges into two populations in Central America, *S. sciureus* continues to diversify in isolation in the Guianas; *S. ustus* C diversifies in Rondônia and *S. vanzolinii* diversifies in the Mamirauá center. *S. cassiquiarensis* subspecies continue diversification *in situ* in four regions (Inambari, Napo, Imeri, Negro). *S. collinsi* expands east across the Tocantins River and invades the Belém area.

3.3.1.7. From 0.15 Ma-present: Re-invasions of occupied areas and hybridization. This period is a warming period, returning to temperatures similar to those that occurred at the timing of the initial radiation of *Saimiri*. *S. boliviensis* expands from Inambari to Napo center where *S. c. macrodon* A is already *in situ*. Both *S. boliviensis* and *S. c. macrodon* expand into the Marañón center (Fig. 7g). *S. sciureus* crosses the Amazon River to expand south into Rondônia, into potential contact with both *S. ustus* and *S. collinsi*. *S. ustus* B crosses the Madeira River west and expands into Inambari, into potential contact with *S. boliviensis* and/or *S. c. macrodon*. *S. vanzolinii* diversifies in Mamirauá; *S. c. cassiquiarensis* invades Mamirauá from the Negro center, with potential contact between *S. vanzolinii* and *S. c. cassiquiarensis*. *S. c. cassiquiarensis* also invades the Guiana Region, crossing the Rio Negro, leading to both *S. c. cassiquiarensis* and *S. sciureus* in the Guiana region (however, current field data suggest that these two species may remain isolated from one another on the two sides of the Rio Branco, see Boubli et al., 2015). *S. collinsi* re-invades Pará region from Belém, re-crossing the Tocantins River to the west. The subspecies *S. o. oerstedii* and *S. o. citronellus* diversify *in situ*, isolated in disjunct distributions in Central America.

3.3.2. Model testing of squirrel monkey biogeography based on BioGeoBears analyses

Of the six biogeographic models we evaluated using BioGeoBears, the DECj (ΔAIC = 0; Fig. 8) and DIVAj (ΔAIC = 0.2; Supplementary Fig. 3) models produced the best statistical fit to the data. (AIC scores for all six models are presented in Supplementary Table 5). The DECj and DIVAj analyses converged strongly with each other and the BEAST phylogeography analysis, in that most

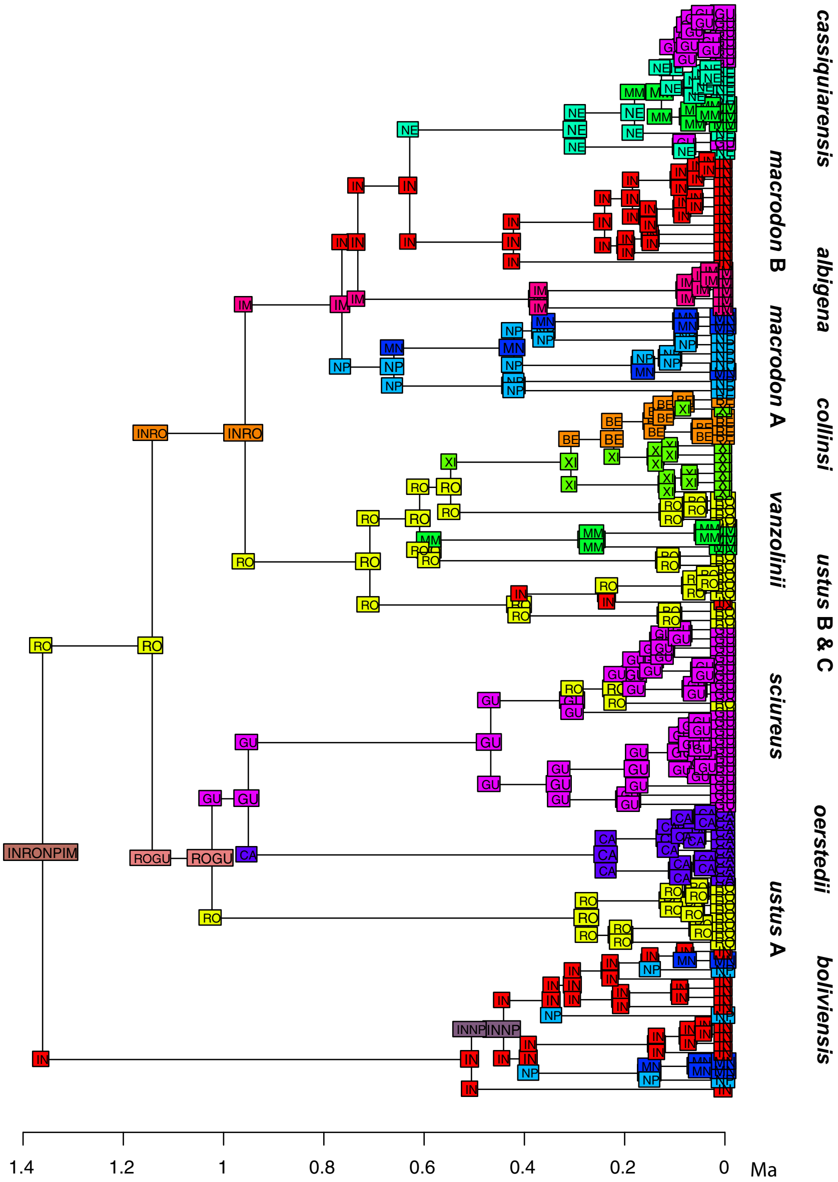

Fig. 8. BioGeoBears unconstrained DECj tree for *Saimiri* evolution by center of endemism. *d* = 0; *e* = 0; *j* = 0.011. Centers of endemism represented by two-letter codes as in Fig. 2 and Table 2.

of squirrel monkey evolutionary history was explained through dispersal, not vicariance, and in that all three analyses recovered Rondônia and Inambari as most likely centers of origin for the modern squirrel monkey radiation. Below we highlight the key differences found in the DECj and DIVAj analyses:

DECj recovered a wider ancestral population to all squirrel monkeys that included not only Rondônia and Inambari (as found by DIVAj) but also Napo and Imeri, suggesting that the ancestral *Saimiri* spanned the area that is now north and south of the Amazon River. DECj also recovered the ancestor to the *S. cassiquiarensis*, *S. collinsi*, *S. ustus* B & C, and *S. vanzolinii* clade as inhabiting both the Inambari and Rondônia regions, whereas DIVAj had the ancestral locations for this clade as Rondônia and Napo. In both the DIVAj and DECj analyses, the ancestor to *S. oerstedii*, *S. sciureus* and *S. ustus* A inhabited both the Rondônia and Guiana regions, spanning what is now the Amazon River. The DECj and DIVAj results disagree in terms of whether the ancestor to *S. oerstedii* and *S. sciureus* was found in Central America (DIVAj) or in the Guiana region (DECj). Both DIVAj and DECj point to a Western/Central Amazon ancestor to all modern squirrel monkeys, and early vicariance events followed by multiple sequential dispersal events leading to the current range of *Saimiri*.

3.3.3. Biogeography of squirrel monkeys

We found clear geographic structure in the topology for squirrel monkeys within the Amazon Basin, with most clades spatially organized within single endemism centers, and sister taxa usually found in adjacent endemism centers. This and the finding that Central American *Saimiri oerstedii* forms a clade nested within Amazonian taxa provide strong evidence against the Costello et al. (1993) division of squirrel monkeys into *S. sciureus* and *S. oerstedii*, or the Unconstrained Dispersal hypothesis (1a, Section 1.2).

Speciation events across endemism centers coincided with Ribas et al.'s (2012) hypothesized timing for the formation of the Amazon, Madeira, Tapajós, Negro, Xingu and Tocantins rivers, and are concordant with the Young Amazon hypothesis for the timing of major river formation in the Amazon. However, the DECj, DIVAj, and BEAST Phylogeography results all point to dispersal, not vicariance, as the driver of most cases of speciation within *Saimiri*. The DECj analysis recovered a wider ancestral population to all squirrel monkeys that included exactly the regions encircling the area where the Amazon Lake is thought to have occurred (Rondônia, Inambari, Napo and Imeri), suggesting that the ancestral *Saimiri* was a lowland water-affiliated taxon (Hypothesis 1d, Section 1.2) that circled the circumference of the lake and split into Northern and Southern populations by vicariance with the formation of the Amazon River. If there was still an exposed Purus arch, it may have acted as a vicariant agent by disorganizing the main channel of the Amazon River at this central Amazonian location about 300 km west of Manaus (Ribas et al., 2012; Nogueira et al., 2013). Subsequent invasions inward into newly habitable floodplains as well as outward along newly formed riverways is most congruent with the rapid burst of mid-Pleistocene speciation and the reticulate morphology found across *Saimiri* species. It is important to remember that the mtDNA tree does not necessarily have the same topology as the "true" species tree, and our knowledge of the exact timing between divergence events and species diversification is limited by the available sampling. However, based on the available data, *Saimiri* response to landscape evolution was strongly influenced by its lowland water-affiliated niche. There seems to be a relationship between drainage of the lake system and diversification in the central-western portion of Amazonia. As expected under the "Young Amazon" model, central western Amazon was a dynamic area for colonization (Aleixo and de Fátima Rossetti, 2007).

The Lavergne et al. (2010) "Around the Amazon" hypothesis predicted a Western Amazonian origin, with subsequent movement eastward, from both south of the Amazon and north of the Amazon. We found congruence with this model in that *S. ustus* expanded eastward from Rondônia to diversify as *S. collinsi* in Pará and Belém centers, and that *S. c. macrodon* diversified north and east into *S. c. albigena* and *S. c. cassiquiarensis* populations. However, we find no evidence for further expansion east to become *S. sciureus*; the *S. sciureus*/*S. oerstedii* stem radiation appears to be an independent event from *S. ustus* stock that became isolated to the north of the Amazon River, perhaps during the end of its formation 1.5–2 Ma. In contrast to expectations in the Old Amazon model and the Around the Amazon model, we found little evidence for reduced genetic diversity in clades farther from the center of origin for modern *Saimiri*. This argues against repeated founder's effects or bottlenecks from isolated 'sweepstakes' dispersal across rivers.

In general small primates are strongly affected by river barriers (Ayres and Clutton-Brock, 1992; Ferrari 2004). Squirrel monkeys are small primates (500–1500 g), but may be able to cross river barriers (Thorington, 1985; Costello et al., 1993; Ayres and Clutton-Brock, 1992; Ferrari, 2004; Paim et al., 2013). Ferrari (2004) used squirrel monkeys as a counter example to the Amazon River as a barrier to dispersal, because the squirrel monkey species along both sides of the mid to lower Amazon was considered the same (*S. sciureus* sensu lato). Our genetic data, and the multidisciplinary study of Mercês et al. (2015), indicate that populations on the two sides of the lower Amazon River (*S. s. sciureus* north of the Amazon and *S. s. collinsi* south of the Amazon) are not sister taxa. However, Mercês et al. (2015) did also encounter some *S. s. sciureus* individuals on the southern bank of the Amazon, and *S. s. collinsi* on the north bank in limited local areas; it is unknown if this is a result of recent human-mediated movement, or if some aspect of local river dynamics has passively moved squirrel monkeys from bank to bank (Mercês et al., 2015). Squirrel monkeys in central Amazonia are floodplain forest specialists and commonly found in Amazonian fluvial islands, so passive crossing of rivers may occur more frequently for them.

In the case of Mamirauá, even small rivers and channels can delimit squirrel monkey movement and decrease interaction between squirrel monkey taxa. Small channels are crossable by squirrel monkeys, but in 9 years of study (2005–2013), the few times *S. cassiquiarensis* individuals crossed the channel into the *S. vanzolinii* distribution and habitat, they tended to cross back to their own side, avoiding direct competition with the other species and apparently preferring to keep within their own preferred habitat type (Paim et al., 2013). This suggests that it may not be the rivers themselves that are barriers *per se*, but that geographic distribution may be determined by ecological factors such as vegetation type and resource niche, and by avoidance of direct competition with other squirrel monkey taxa (Paim et al., 2013). It is clear that the Amazon River has been an important barrier, with only one event of successful expansion across it in over one million years.

Squirrel monkey diversity was highest in the Rondônia and Inambari centers, both in the number of *Saimiri* clades and their distribution throughout the phylogeny. The Madeira River is the largest southern tributary of the Amazon River and splits southern Amazonia with the Inambari center to the west and the Rondônia center to the east. Ferrari (2004) points to Rondônia as a generator of diversity, not only because of Madeira River, but also for other geographic and ecological barriers. The mountain range Serra dos Pacaas Novas, and the rivers Mamoré, Guaporé, Ji-Paraná, and Madeira effectively isolate southern Rondônia from northern Rondônia (Ferrari, 2004). The Ji-Paraná also isolates western Rondônia from far eastern Rondônia and Mato Grosso (Ferrari, 2004). In southern Rondônia, there is Cerrado habitat interspersed with Amazonian rainforest. These barriers may have been instrumental in the genetic diversification of *Saimiri*, which presents several different, non-monophyletic lineages within this area of endemism, though morphologically they have been grouped together as a single taxa, *S. ustus* (see Section 3.4 for more on *S. ustus* morphology). The paraphyly of lineages from the Rondônia area of endemism has been found for birds as well. In a review of avian lineages, Aleixo and de Fátima Rossetti (2007) found distinct or highly divergent taxa replacing each other across a latitudinal gradient on the western part of the Brazilian Shield, along the Madeira River basin. In some cases, these lineages were not even monophyletic, but instead some subgroups had closer phylogenetic affinities with disparate geographic lineages (Aleixo and de Fátima Rossetti, 2007). In *Psophia*, the species from Rondônia (*P. viridis*) shows larger intraspecific divergences than the other species in the genus (Ribas et al., 2012). Two other bird species (*Myrmeciza hemimelaena* and *Glyphorhunchus spirurus*) also show several different non-monophyletic lineages within the Madeira-Tapajós interfluvium (Fernandes et al., 2012, 2013). In fact, recent studies of Rondônia's geological history suggest that this western fringe of the Brazilian Shield is bordered by more recent sedimentary deposits that remain to be characterized or dated (Rossetti et al., 2005).

Within the Inambari Center, two rivers, the Juruá and the Purus, both play important roles as barriers to squirrel monkeys. In Brazil, *S. boliviensis* is largely restricted to within the Juruá and Purus interfluvium, while *S. c. macrodon* is found west of the Juruá and *S. ustus* is found east of the Purus (Thorington, 1985). Our results suggest the Inambari center was key in the early diversification of squirrel monkeys, and that the first southern Amazon splits were among *S. boliviensis*, *S. cassiquiarensis*, and *S. ustus* in Inambari and Rondônia centers. Perhaps these rivers formed relatively early in squirrel monkey evolutionary history, at 2–1 Ma, serving as effective barriers to gene flow across these three morphotypes.

According to our results, *S. vanzolinii* diverged from *S. ustus* about 590 kya (220–950 kya), and began modern diversification at $\sim$260 kya. We find a significant incongruence between these dates and the timing of the formation of the floodplain forest (várzea) habitat where *S. vanzolinii* is now endemic in Mamirauá Reserve, a habitat that is only 5–15 thousand years old (Ayres, 1993). In fact, according to Ayres (1993) all the floodplain forests of the Mamirauá reserve are Pleistocenic or Holocenic in origin, ranging from 100 kya to less than 4 kya. These whitewater floodplain forests go through short and long-term modifications based on erosion and sedimentation (Klammer, 1984; Henderson, 1999). The process of destruction and reconstruction of floodplain habitat can be rapid, constantly transforming the riverways, so rivers in this ecology tend to have many meanders and ox-bow lakes (Sioli, 1984; Henderson, 1999). Klammer (1984) suggests that floodplain forest formation history has probably played an important role in the distribution of Amazonian primate species. It is likely that *S. vanzolinii* has become restricted to a very small habitat because of the evolution of the floodplain forest ecology over time, and it is also likely that all suitable remaining habitat for *S. vanzolinii* will be rapidly lost because of global climate change in conjunction with the inherent dynamic characteristics of floodplain forest.

From the geographic, geologic and genetic evidence, we suggest that *S. vanzolinii* did not initially radiate in the area that is now the Mamirauá Reserve. More likely, the species was formerly more widespread in the Inambari-Solimões area and gradually restricted in this floodplain forest either by competition with other squirrel monkey species or because of changing habitat ecology over time. Presently, three different species or subspecies of squirrel monkey are all sympatric or syntopic within the Mamirauá Reserve: *S. vanzolinii*, *S. c. cassiquiarensis*, and *S. c. macrodon* "2" (Paim et al., 2013). We do not have sequences from *S. c. macrodon* from Mamirauá, but our topology suggests that *S. c. cassiquiarensis* invaded the Mamirauá region much later than *S. vanzolinii*, and perhaps through time was able to exclude *S. vanzolinii* from most habitat types through competition.

3.4. Phylogeny and morphology of squirrel monkeys

Our study using mitochondrial markers found that *S. boliviensis* and *S. vanzolinii* are both distinct and monophyletic groups, but not sister to each other as previously considered. This suggests that Hershkovitz's (1984) principal division between squirrel monkey types, the Gothic vs. Roman cap, is paraphyletic, and that cap shape in terms of the superciliary arch must have evolved in convergence at least once in squirrel monkey evolutionary history. In fact, several of the traits important for distinguishing among squirrel monkey species appear to be reticulate or include cases of convergent evolution. The black cap has evolved independently in *S. boliviensis*, *S. oerstedii*, and *S. vanzolinii*. The distance that the bright yellow or orange coloration extends from the hands (to the wrist only or up to mid-forearm or elbow) is variable across *S. c. macrodon* clades (Paim et al., 2013) and *S. ustus* populations (Thorington, 1985), as well as across species (Paim et al., 2013; Mercês et al., 2015). Another example of convergent evolution with a potential ecological explanation is the degree of blackness on the body itself, which has been hypothesized to increase in squirrel monkeys with amount of rainfall—*S. vanzolinii*, restricted to floodplain forest, has the blackest coat. In short, few traits can be used to distinguish among squirrel monkey species, because morphologically all squirrel monkeys are quite similar to one another, as might be expected given their very recent diversification; and many of these key traits are so labile they may be unreliable for reconstructing evolutionary history because of high incidence of homoplasy. This morphological reticulation along with the very recent evolution of modern *Saimiri* provide some evidence in favor of collapsing all squirrel monkeys into one species, despite the geographic structure to morphological and genetic traits that suggests differentiation across various clades. Wildman and Goodman (2004) support an age based phylogenetic classification (see Jameson Kiesling et al., 2015), and their method would lump all *Saimiri* species together because the entire group diverged less than 2 Ma. In contrast, the geographic structure in molecular markers and the morphological suites of traits that distinguish particular geographic areas would point to various species using the Phylogenetic Species Concept as championed for primates by Groves (2001, 2005).

At the intraspecific level, sampling across multiple locations in our study showed that *S. boliviensis boliviensis* and *S. boliviensis peruviensis* were not two reciprocally monophyletic clades (Fig. 5). This was in contrast to the subspecies division based on morphology by Hershkovitz (1984) and later confirmed in molecular studies by Lavergne et al., 2010; Chiou et al., 2011; Cropp and Boinski, 2000 with one to two sample locations per subspecies. In our wider sampling effort, we found haplotypes were more cosmopolitan and without defined geographic structure. Bolivian, Peruvian and Brazilian haplotypes are mixed throughout the clade of *S. boliviensis*. Similarly, the Guiana center taxon *S. sciureus* had no apparent geographic structure. Samples from Amapá, Brazil; French Guiana; and Guyana were each represented throughout the clade, suggesting gene flow throughout these regions. These cosmopolitan signatures of mtDNA haplotypes for *S. boliviensis* and *S. sciureus* indicate there may be female transfer in these species (see Boinski et al., 2005a,b for discussion of diverse dispersal patterns in *Saimiri*).

In summary, the relationship between morphological similarity and genetic proximity is not straight-forward in *Saimiri*. This may be attributed to several factors: *Saimiri* is a recent and explosive radiation, possibly stemming from a ring of populations around the Amazon Lake. In our time tree we recovered short branch lengths prior to several consecutive divergence events, and there probably has not been enough time for 'coalescence' of the mtDNA genes for clear separation and monophyly for each species. Another complicating factor is that *Saimiri* is known or believed to hybridize across several taxa both in captivity and the wild (Silva et al., 1992; Hershkovitz, 1984; Thorington, 1985; Costello et al., 1993). Squirrel monkey groups have up to 70 or more individuals, and males have extremely high skew in reproductive success (Boinski et al., 2005a,b; Stone, 2013). Squirrel monkey characteristics, such as the ability to disperse rapidly and colonize new areas, the ability to traverse river barriers at a low rate, the extreme male competition for mates and female mate choice, and the ability to hybridize make it likely that select traits of interest to females (i.e. cap shape, hairiness of ears, brightness of forearms) could easily sweep through populations if introduced by novel males. For all these reasons, a clear next step in understanding squirrel monkey morphology and genetics is to sample populations using nuclear genomic markers, to determine if there is evidence for recent hybridization, long-term introgression, or strong positive and sweeping selection for certain traits.

Incomplete lineage sorting may affect the squirrel monkey topology in various instances. The ancestral lineage to *S. sciureus* and *S. oerstedii* split from a *S. ustus* ancestral population in the Rondônia center and dispersed across the Amazon River to the northern Amazon. As a result, the lineages in the Rondônia center became non-monophyletic, with one clade more genetically similar to the *S. sciureus/oerstedii*, and the other clade more genetically similar to the other southern taxa (i.e. *S. cassiquiarensis* complex, *S. collinsi*, *S. vanzolinii*). The lineages identified as *S. ustus* B and C are also non-monophyletic, as one of them is sister to *S. vanzolinii*, which became isolated in Mamirauá, and this clade is sister to *S. collinsi*, from the Brazilian Shield centers of endemism (Rondônia, Pará and Belém). These may mark two cases of incomplete lineage sorting, if we believe that all *S. ustus* is really one single taxon that is panmictic across the southeastern Amazon today. If not, the problem is taxonomic (treating more than one species with the same name) and the biological problem is that there has been little phenotypic differentiation despite the genetic isolation, with little time for diversification and differentiation of haplotypes across species. The same questions arise with *S. c. macrodon* and *S. c. albigena* in the Imeri region.

The paraphyly within each of these currently recognized species (by Rylands et al., 2013): *S. cassiquiarensis*, *S. sciureus* and *S. ustus* could reflect the understudied nature of their areas of geographic distribution, which may contain higher taxon diversity than previously recognized. Paim et al. (2013) provide morphological evidence for the distinction between *S. macrodon* 1 and *S. macrodon* 2, which may correspond respectively to our *S. c. macrodon* B and *S. c. macrodon* A clades, based on specimen locations, but this needs more extensive morphological and molecular sampling to see if the patterns are congruent across entire geographic ranges. Mercês et al. (2015) found genetic, morphological and morphometric distinctions between *S. sciureus* from the Northern Amazon vs. *S. collinsi* south of the Amazon River, congruent with our findings here. In our study, we found three paraphyletic mtDNA clades for *S. ustus*. Decades earlier, Thorington (1985) pointed to morphological diversity within *S. ustus*, stating that populations at the type locality and farther north have yellow hands and wrists, but southern populations have bright orange forearms. Thorington (1985) stated that between the Tapajós and Xingu rivers, there were squirrel monkeys with naked ears, yellow forearms, and a yellow gray crown, a phenotype not documented elsewhere. While this area was not sampled for genetics in this study, our research group confirmed this morphotype in Museu Paraense Emilio Goeldi (MPEG) specimens collected between the Tapajós and Xingu rivers, as well as through field observations at Jacareacanga, Pará State, Brazil, on both sides of the Tapajós River (M. Mercês, pers. comm.). As Thorington (1985) pointed out, if the Tapajós area squirrel monkeys turn out to be a different species from those near the Madeira, the Tapajós monkeys would be *S. ustus* Geoffroy (1845), and the western populations *S. madeirae* Thomas (1908).

Our results suggest that most of the contact between *Saimiri* species has occurred recently, within the last 100 k years, and possibly within a time frame of human-mediated transport. It is clear that squirrel monkeys have the facility to invade and prosper in distant new habitats and new regions when introduced by humans, as has occurred in Florida in the United States; in Recife in the Brazilian northeast; and in southeastern Brazil in the Atlantic Forest in Rio de Janeiro. What is unclear, then, is how much humans have facilitated squirrel monkeys' recent movement across major rivers, such as the Amazon. Essentially all cases where we see the 'wrong' species on the other side of the river, it is found adjacent to the river itself, not distributed farther out into a region populated by other squirrel monkey species (Hershkovitz, 1984; Thorington, 1985; Mercês et al., 2015). Squirrel monkeys are able to swim very well (F. Röhe, pers. comm.), and it appears from our results that the *S. cassiquiarensis* complex has been the most successful in the last million years at repeatedly expanding across major river systems—this complex has spread into seven of the ten centers of vertebrate endemism in the Amazon, which suggests it has crossed the Marañón River, the Solimões, and the Negro.

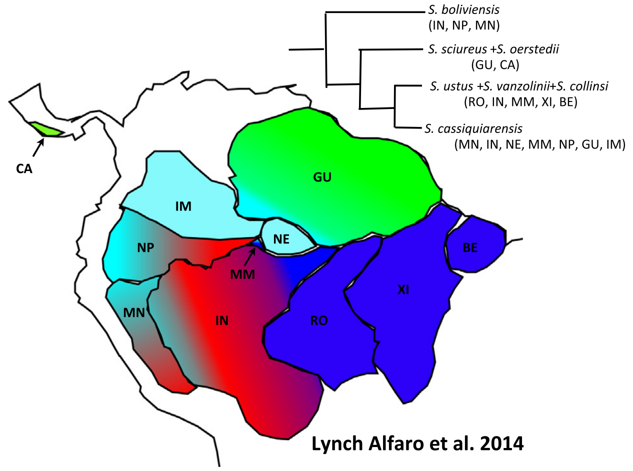

Fig. 9. Hypothesis for major clades in *Saimiri* in a geographical framework, based on results from this study. Closer colors indicate relatively closer relationships. Color gradients indicate more than one species in a particular center of endemism. See Fig. 3 for comparison of earlier predictions about species relationships within squirrel monkeys. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Thorington (1985) believed the current contact between *Saimiri* species or subspecies was due to recent expansion of range subsequent to isolation in Pleistocene refugial forests. He believed that squirrel monkeys in general had the ability to transfer side to side of rivers passively because of changing river meanders, and that this was key to their range expansion, but that when the other side of the river was already populated by squirrel monkeys, the invading group would interbreed and become subsumed in the population there, leaving only evidence of gene flow (Thorington, 1985). Our data partially confirm this model, but suggest that rather than isolation by contracting forest refugia, the Pleistocene was the time of the initial radiation and expansion into unoccupied habitat, and only recently have continued expansions, and perhaps human-mediated transport, put squirrel monkeys back into contact with one another.

4. Conclusions

Fig. 9 gives a geographic summary of the major clades found in this study, in comparison to the hypotheses by Hershkovitz (1984, 1987), Thorington (1985), Costello et al. (1993), and Ribas et al. (2012), depicted geographically in Fig. 3. In our study, we found evidence for the Young Amazon biotic diversification model, as mid-Pleistocene timing of the formation of major Amazonian tributaries such as the Madeira, Negro, Tapajós, Xingu and Tocantins (Ribas et al., 2012) coincided with speciation events for several squirrel monkey taxa in those regions. The Amazon River, in particular, was a nearly impassible barrier for squirrel monkeys over the last 1 Ma, and most of the important dispersal routes for squirrel monkeys were across Southern and Western Amazonia. All squirrel monkey speciation events were recovered as occurring before the Last Glacial Maximum, and the biogeographic reconstructions pointed to squirrel monkey colonization of all centers of endemism prior to the Last Glacial Maximum, suggesting that extreme climate shifts were not instrumental in shaping the modern taxa and geographic distribution for this clade. However, within species diversification occurred almost exclusively during the period of the Glacial Maxima, from 0.9 to 0.02 Ma. The origin of all modern squirrel monkeys was placed in the Rondônia or Inambari centers of endemism in the southern Amazon, or possibly even more widespread including Napo and Imeri centers. Squirrel monkeys rapidly radiated first within the southern Amazon and then throughout the Amazon, perhaps coincident with the draining of the Amazon Lake and the formation of the Amazon River and its major tributaries. This suggests that *Saimiri* was initially a Western lowland lake-affiliated taxon that spread throughout the Amazon with the increasing availability of floodplain forest and lowland riverine habitat.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2014.09.004. These data include Google maps of the most important areas described in this article.

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