Molecular Phylogenetics and Evolution 215 (2026) 108487



Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev



Phylogenomics of Amazonian squirrel monkeys (*Saimiri*: Primates, Cebidae)

Michelle P. Mercês [a,b], Hazel Byrne [b], Natalie Finnegan [b], Maria L. Harada [c], Fernanda P. Paim [d], Felipe E. Silva [d,e,f], Anita Stone [g], Marcela G.M. Lima [c], Anthony B. Rylands [h], José de Sousa e Silva Júnior [a], Jessica W. Lynch [b,*]

[a] *Museu Paraense Emílio Goeldi, Belém, PA, Brazil*
[b] *University of California, Los Angeles, CA, the United States of America*
[c] *Universidade Federal do Pará, Belém, PA, Brazil*
[d] *Instituto de Desenvolvimento Sustentável Mamirauá, Tefé, AM, Brazil*
[e] *New York University, NYC, NY, the United States of America*
[f] *Université libre de Bruxelles (ULB), Brussels, Belgium*
[g] *California Lutheran University, Thousand Oaks, CA, the United States of America*
[h] *Re:wild, Austin, TX, the United States of America*

ARTICLE INFO

Keywords:
Amazonia
ddRadseq
Gene flow
Phylogenetics
Platyrrhine primates
Shared ancestry
Time tree

ABSTRACT

Phylogenetic relationships among squirrel monkeys (genus *Saimiri*) are still poorly resolved. Morphology-based taxonomies range from two to 12 taxa, while molecular phylogenies have recovered up to 17 different lineages. The last species account lists 11 taxa: *Saimiri boliviensis boliviensis*, *S. boliviensis peruviensis*, *S. vanzolinii*, *S. oerstedii oerstedii*, *S. oerstedii citrinellus*, *S. sciureus*, *S. collinsi*, *S. cassiquiarensis*, *S. albigena*, *S. macrodon* and *S. ustus*. Here we gathered a phylogenomic dataset for *Saimiri* using *double digest* restriction-site associated DNA sequencing (ddRadseq) to construct a phylogeny for squirrel monkeys in Amazonia. All the phylogenomic analyses strongly supported the division of the genus in two main clades, corresponding to the Gothic- and Roman-arch groups based on morphology, and provided strong support for five major lineages: *S. boliviensis* + *S. vanzolinii*; *S. ustus*; *S. cassiquiariensis*; *S. macrodon*; and *S. sciureus* + *S. collinsi*. Structure analyses showed evidence for population clusters based on geography within these lineages, but also shared ancestry across clusters, and Dsuite analyses showed evidence for gene flow across the Gothic species. Our time-calibrated tree confirmed that the diversification of Amazonian *Saimiri* occurred during the Pleistocene.

1. INTRODUCTION

The relationship between squirrel monkeys, *Saimiri* Voigt, 1831, and their sister group, the capuchin monkeys (genera *Cebus* and *Sapajus*), has been well-established morphologically (Rosenberger, 1981, 2011) and via mitochondrial and nuclear genomic data (Chiou et al., 2011; Springer et al., 2012; Kuderna et al., 2023). Squirrel monkeys and capuchin monkeys diverged about 13.8 million years ago (12.1–16.1 Ma 95 % HPD) according to mtGenome data (Chiou et al., 2011), or 18.33 Ma (16.8–19.9 Ma 95 % HPD) based on nuclear genomic data (Kuderna et al., 2023). However, intrageneric relationships are still poorly resolved in *Saimiri*, with substantial discordance between morphological and molecular (mitochondrial) studies (e.g., Hershkovitz, 1984; Thorington, 1985; Lavergne et al., 2010; Lynch Alfaro et al., 2015; Ruiz García et al., 2015) and important Amazonian taxa missing from the most recent genomic analyses (Kuderna et al., 2023).

Squirrel monkeys occur throughout the Amazon Basin, with a distribution spanning from east of the Andes in Colombia to the transition zone between Amazonia and Cerrado in the state of Maranhão, Brazil. The woodlands of Bolivia form the southern limit to their distribution, while their northern limit is the Río Orinoco in Venezuela, extending eastwards to the Atlantic Ocean in the Guianas. Two Central American taxa, disjunct from the Amazonian forms, inhabit lowland rain forest in Costa Rica and Panama (Hershkovitz, 1984; Thorington, 1985). Hershkovitz (1984) and Thorington (1985) each revised the genus, and the most recent taxonomic review is by Groves (2001). The three reviews diverge significantly in the taxa considered valid, and the number of species and subspecies. MacLean (1964) divided *Saimiri* into two species groups, Roman and Gothic, based on the shape of the arch of white above the eyes. Although initially incredulous (P. Hershkovitz, pers.

* Corresponding author.
E-mail address: jwlynx@g.ucla.edu (J.W. Lynch).

https://doi.org/10.1016/j.ympev.2025.108487
Received 22 June 2025; Received in revised form 5 October 2025; Accepted 25 October 2025
Available online 30 October 2025
1055-7903/© 2025 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

comm. 1966, in Cooper, 1968), in his 1984 revision, Hershkovitz (1984) validated MacLean's observation as taxonomically and geographically valid. Members of the Gothic Group are characterized by whitish hairs forming a Gothic arch above the eyes and also have a bushy tail pencil, while members of the Roman Group have a narrow, rounded, Roman arch above the eyes and a thin tail pencil.

Hershkovitz (1984) based his review on external characters, chromosome number, geographic distribution and behavior, and considered nine taxa to be valid: two in the Roman Group – *S. boliviensis boliviensis*, *S. b. peruviensis* – and seven in the Gothic Group – *S. sciureus sciureus*, *S. s. albigena*, *S. s. cassiquiarensis*, *S. s. macrodon*, *S. oerstedii oerstedii*, *S. o. citrinellus*, and *S. ustus*. Hershkovitz (1987) subsequently recognized three more subspecies of *S. boliviensis* (*S. b. pluvialis*, *S. b. jaburuensis*, and *S. b. vanzolinii*).

Thorington (1985) considered just two species of squirrel monkeys to be valid: one monotypic, *S. madeirae* [later synonymized with *S. ustus*], and another, *S. sciureus*, with four subspecies (*S. sciureus sciureus*, *S. s. boliviensis*, *S. s. cassiquiarensis*, and *S. s. oerstedii*).

Groves (2001) recognized five species as valid: *S. oerstedii* with two subspecies (*S. o. oerstedii* and *S. o. citrinellus*), *S. sciureus* with four subspecies (*S. s. sciureus*, *S. s. albigena*, *S. s. cassiquiarensis* and *S. s. macrodon*), *S. ustus*, *S. boliviensis* with two subspecies (*S. b. boliviensis* and *S. b. peruviensis*), and the more recently discovered *S. vanzolinii* Ayres, 1985.

The compilation of Rylands et al. (2013) modified Groves' (2001) proposal by separating *S. cassiquiarensis* and *S. macrodon* as distinct species from *S. sciureus*, and classifying *albigena* as a subspecies of *S. cassiquiarensis* rather than *S. sciureus*, making *S. sciureus* monotypic. They also considered *S. collinsi* to be a species, following Lavergne et al. (2010). Mercès et al. (2015), using mitochondrial genetic data and morphological data, provided evidence that *S. collinsi* is a species distinct from *S. sciureus*.

The mitogenomic appraisal by Chiou et al. (2011) recovered two main clades concordant with Hershkovitz' (1984) morphological hypothesis: one composed of the Roman group (*Saimiri b. boliviensis* and *S. b. peruviensis*) and the other made up of the Gothic group (*S. macrodon* (*S. s. sciureus* (*S. o. oerstedii* + *S. o. citrinellus*))). More complete *Saimiri* phylogenies with mitochondrial genomes have, however, consistently shown a complex topology with paraphyly for both *S. macrodon* and *S. ustus*, as well as species relationships that are not concordant with morphological hypotheses for species similarity (Janiak et al., 2022; Finnegan et al., 2025). Phylogenetic relationships recovered in other recent molecular studies based on mitochondrial loci are also substantially discordant from the morphological evidence. In the molecular phylogeny of Lavergne et al. (2010), which was based on one mitochondrial locus (cytochrome *b*), the Roman group clade (*S. b. boliviensis* and *S. b. peruviensis*) was recovered as sister to some members of the Gothic *S. sciureus* clade (terminal taxa: *macrodon, albigena, cassiquiarensis*). *Saimiri s. sciureus* was recovered as paraphyletic and Lavergne et al. (2010) proposed the revalidation of *collinsi* as a subspecies of *ustus*. The molecular phylogeny of Lynch Alfaro et al. (2015) included all taxa considered as valid by Rylands et al. (2013); *S. macrodon* and *S. ustus* were each recovered as paraphyletic and *S. vanzolinii* was not recovered as sister to the *boliviensis* group despite Roman-arch morphology. A molecular phylogeny based on two other mitochondrial loci (COI and COII) also recovered *S. macrodon* and *S. ustus* as each polyphyletic (Ruiz García et al., 2015). Ruiz García et al. (2015) proposed that *Saimiri* should comprise just three species: *S. sciureus*, *S. vanzolinii* and *S. oerstedii*. The most complete mitogenome phylogeny (Finnegan et al., 2025) provides the overall topology: (((*S. boliviensis peruviensis*) (*S. boliviensis boliviensis*))((((*S. cassiquiarensis*)(*S. macrodon**)) (*S. macrodon**))(*S. ustus**))((((*S. oerstedii citronellus*)(*S. oerstedii oerstedii*))(*S. sciureus*))(*S. ustus**)))). Here the Gothic and Roman types are separated, but *S. vanzolinii* was not sampled, and *macrodon* and *ustus* are both polyphyletic. Since emigration from the natal group among squirrel monkey populations is sex-biased in a species-specific way (Mitchell et al., 1991; Boinski et al., 2002; Blair and Melnick, 2012), i.e. males or females are more likely to disperse depending on the species, the population relationships based in mtDNA (maternal inheritance) will not necessarily correspond to population relationships based in nuclear DNA. The mtDNA paraphyly within species may also be the result of incomplete lineage sorting and/or cross-species admixture within the species evolutionary history.

In one of the first studies that included nuclear genes, Springer et al. (2012) recovered the following relationship among *Saimiri* species: (*S. boliviensis* (*S. ustus* (*S. sciureus* (*S. oerstedii*))). However, one of the limitations of this study is that it used captive specimens with just one sample per species. Most recently Kuderna et al. (2023)'s work using UltraConserved Elements (UCEs) sampled from whole nuclear genomes produced a topology of (((*S. oerstedii*)(*S. ustus*))((*S. sciureus*) ((*S. cassiquiarensis*)(*S. macrodon*))), with different samples of *S. sciureus* paraphyletic; a second topology based on the Kuderna et al. (2023) dataset placed *S. sciureus* as the sister to all other Gothic taxa sampled. The differences of topology across these studies could be a result of incomplete lineage sorting, introgression, or sex-biased migration. In sum, the relationships amongst squirrel monkeys have still not been resolved with the limited nuclear genomic studies to date.

Divergence dating estimates among extant *Saimiri* taxa are incongruent across previously published studies. Lavergne et al. (2010) estimated a Pliocene divergence at ~4.3 Ma (2.4–6.6 Ma 95 % HPD), while other studies estimated the initial divergence at ~1 to 1.5 Ma [1.5 Ma (1.1–2.9 Ma 95 % HPD) using BEAST, or 1.10 Ma (0.72–1.88 Ma 95 % HPD) using multidivtime (Chiou et al., 2011); 1.36 Ma (1.1–1.91 Ma 95 % HPD) (Lynch Alfaro et al., 2015)]. Springer et al. (2012) estimated the Pleistocene divergence within *Saimiri* at ~1.75 Ma (1.06–2.88 Ma 95 % HPD), while Perelman et al. (2011) recovered an earlier divergence for the genus at ~2.24 Ma (1.05–3.73). The UCEs study by Kuderna et al. (2023) placed the crown *Saimiri* diversification at 1.75 Ma (1.35–2.15 Ma 95 % HPD), but that dataset was missing taxa from the Roman-arch group and so certainly underestimated the crown diversification time.

The decrease in the cost of generating genomic data has made genomic methods available to the study of phylogeny and population genetics of many taxa. One type of genomic sampling is *double digest* restriction-site associated DNA sequencing (ddRADseq) that generates reduced representation libraries of complete genomes. The ddRADseq methodology uses two restriction enzymes to digest genomic DNA into several DNA fragments. These fragments are size selected to reduce the number to be sequenced (Peterson et al., 2012), resulting in libraries that represent a subset of homologous loci from across the genome. Valencia et al. (2018) developed a protocol to use ddRADseq across the entire radiation of Platyrrhini and verified that it is suitable for phylogenetic studies in diverse taxonomic levels and evolutionary time scales. It has recently been used by Martins and colleagues (Martins et al., 2023) to study robust capuchin monkey phylogenomics.

Here we use ddRADseq to generate a genome-wide molecular dataset for morphologically identified *Saimiri* of known geographic origin (see Fig. 1) to elucidate the phylogenetic relationships among *Saimiri* taxa in Amazonia and their evolutionary history in the region. We assess these results for congruence with evidence from mitochondrial markers and morphology, as well as previous nuclear genomic evidence. Phylogenomic approaches have already provided new insights into platyrrhine primate diversity and evolution (Callicebinae – Byrne et al., 2016, Byrne et al., 2018; *Cebuella* – Boubli et al., 2018; *Sapajus* – Lima et al., 2018, Martins et al., 2023; *Mico* – Silva et al., 2018, Costa-Araújo et al., 2019; Cebidae, Pitheciidae and Atelidae – Valencia et al., 2018; and *Plecturocebus* – Boubli et al., 2019, *Cacajao* – Silva et al., 2022). Most recently Kuderna et al. (2023) provide an overarching primate phylogeny based on genomic sampling of UCEs. Our geographic sampling of squirrel monkey taxa and populations here complements and extends the Amazonian squirrel monkey sampling by Kuderna et al. (2023). We have denser and geographically broader sampling in the Amazon, including three Amazonian squirrel monkey species (*Saimiri collinsi*, *S. vanzolinii*, and *S. boliviensis*) not sampled in Kuderna et al. (2023). We also extend

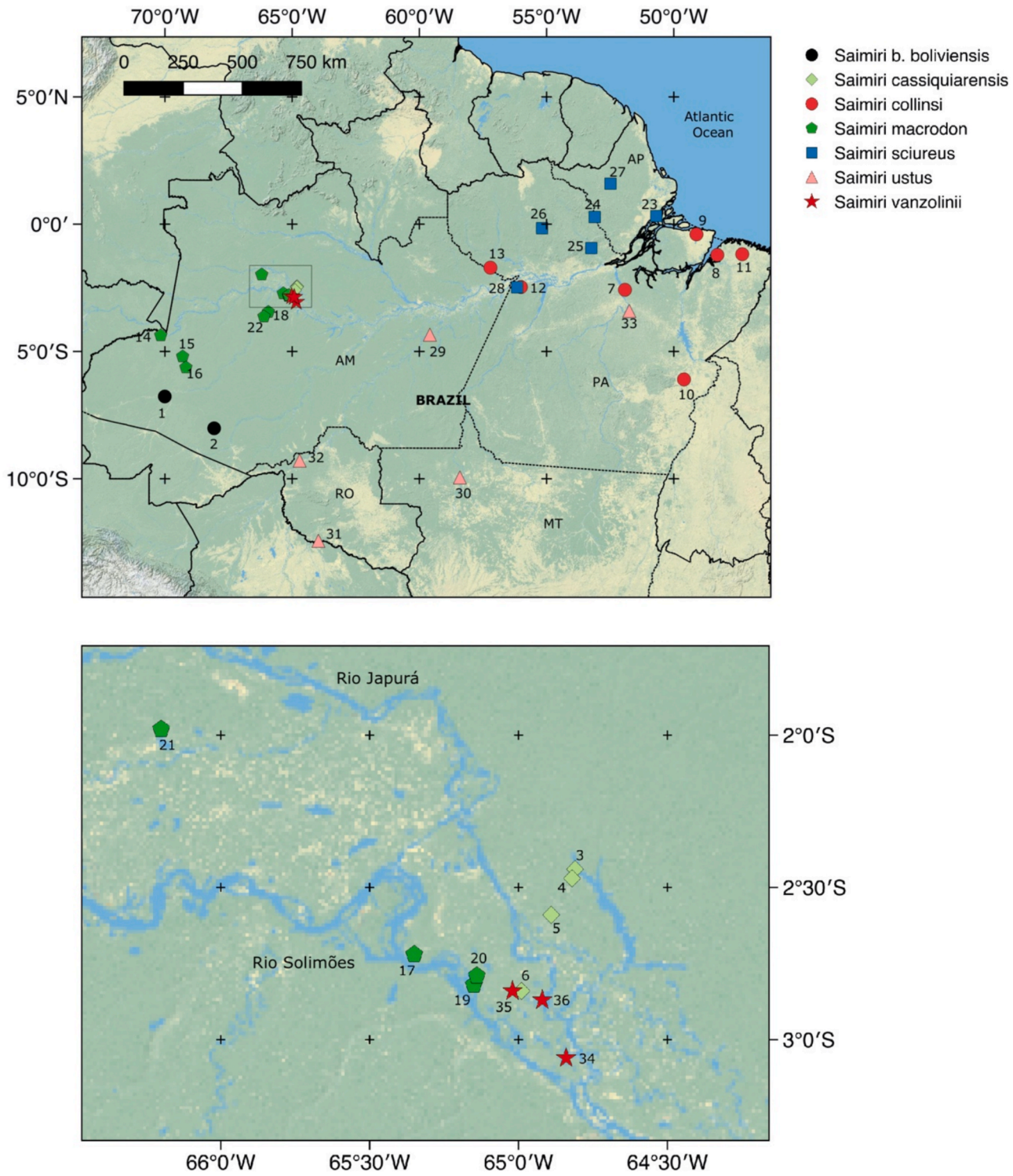

Fig. 1. Sampled localities for the species of *Saimiri*. Numbers correspond to the sample numbers in Table 1. Species names are based on Rylands et al. (2013, 2024) and Mercês et al. (2015). The second inset map corresponding to the box noted in the first map shows the localities sampled in the Solimões-Japurá interfluvium. State acronyms: AM: Amazonas; AP: Amapá; MT: Mato Grosso; PA: Pará; RO: Rondônia.

the geographic range of sampling for *S. sciureus*: Kuderna et al. (2023) included only two samples for *S. sciureus*, both at the edge of their geographic distribution, north of the Faro/Juruti region (see our results) that resulted in paraphyly for this taxon in their tree; in contrast, our *S. sciureus* sampling included 10 samples from six different locations in eastern Pará state and Amapá state, allowing us to compare populations from Faro/Juruti to both *S. sciureus* and *S. collinsi*. Our sample also included individuals from both *S. macrodon* 1 and *S. macrodon* 2 populations, the two considered as potentially distinct squirrel monkey taxa based on morphological studies (Paim et al., 2013) and phylogenetics based on mitochondrial DNA (Lynch Alfaro et al., 2015). While the time tree in Kuderna et al. (2023) used UltraConserved Elements (UCEs) for genomic sampling due to a focus on constructing a phylogeny for all primates and thus the need for a relatively deep time depth for

divergences (from ~65 Ma), our phylogeny and time tree constructed with ddRADseq allows for better resolution of the shallow time depth of divergences in the *Saimiri* clade (from ~2 Ma). The time-tree presented by Kuderna et al. (2023) focused on resolving "primate families, tribes and genera as monophyletic" (p. 908) but did not report support for topology at the species level, either in the main tree, or in the supplemental trees, except to say the main tree "has strong support at most nodes" (p. 908). In our study, we provide a fully resolved topology for the *Saimiri* phylogeny at the species level. We also provide evidence for shared ancestry through Structure analyses using single-nucleotide polymorphisms (SNPs) from the ddRAD dataset, and for gene flow across Gothic squirrel monkey taxa using DSuite ABBA-BABA analyses.

2. MATERIAL AND METHODS

2.1. Taxon sampling and DNA extraction

A total of 47 *Saimiri* tissue samples were collected from museum voucher specimens and ten *Saimiri* blood samples from live wild individuals in the field (Fig. 1, Table 1). Six live individuals were captured by F. P. Paim with Tomahawk traps at trapping stations in Mamirauá, Brazil. The trapping methodology is described in detail in Paim and Rabelo (2015), with information on ethical considerations and authorization numbers from Brazilian authorities. Four live individuals were trapped during annual capture by A. Stone and P. Castro at Ananim, Brazil; 3 mL of blood were collected from the femoral vein of each individual, and samples were preserved at −80 °C as described in Stone et al. (2015). All procedures at Ananim were approved by the Brazilian organization ICMBio (permit number 32242-1).

Tissue samples were obtained from the following institutions: Museu Paraense Emílio Goeldi (MPEG), Belém, Pará, Brazil; Universidade Federal do Pará (UFPA), Belém, Pará, Brazil; Instituto de Desenvolvimento Sustentável Mamirauá (IDSM), Tefé, Amazonas, Brazil; Universidade Federal do Mato Grosso (UFMT), Cuiabá, Mato Grosso, Brazil; Instituto de Pesquisas Científicas e Tecnológicas do Estado do Amapá (IEPA), Macapá, Amapá, Brazil. Tissue samples and Mamirauá blood samples were exported under CITES permit 18BR029277/DF; and exportation of Ananim blood samples to the United States was under CITES export permit 17BR023549/DF.

DNA was extracted from blood and muscle tissues using the Qiagen DNeasy Blood & Tissue Kit according to the manufacturer's protocol. After extraction, we quantified DNA with a Qubit 2.0 Fluorometer.

2.2. ddRADseq library preparation and sequencing

After normalization, a total of 100 ng of genomic DNA for each sample was sent for ddRAD library preparation with the SphI-MluCl enzyme pair, size selected to focus on fragments of 300 ± 30 bp [at the Genome Sequencing Facility at the University of Texas, San Antonio (GSAF)]. With this size selection window, we estimated that we could generate sufficient coverage (6 ×) for a genotyping set of ~100,000 ddRAD loci by targeting a total of 2–4 million reads per sample. Subsequently the samples were sequenced on one lane of HiSeqX (400 million reads, for a target of 4 million 2 × 150 bp PE reads per sample), an Illumina platform, by Novogene (USA). Our protocol and pipelines were modified from the protocol of Valencia et al. (2018).

2.3. Quality control

Raw reads were quality checked with FASTQC (Andrews, 2010). We used the bbmap tool bbduk.sh to trim any adapter sequence present at the 3′ end of the reads using a kmer length of 22 with a maximum of 3 mismatches, discarding any reads smaller than 30 bp, with the "tbo" and "tpe" options. We used the bbmap tools bbduk.sh and reformat.sh to remove phiX contamination and to reverify pairing, respectively. We trimmed the 3' end of reads to remove bases with quality score less than

Table 1
List of samples, coordinates and voucher codes. Species names are based on morphological assessment of samples. Map IDs are shown in Fig. 1. *The specimens noted with asterisks are from Faro and Juruti, the farthest west collection sites for *S. collinsi* or *S. sciureus* for our study, west of the rios Trombetas and Madeira, on both sides of the Rio Amazonas. These localities were flagged in Mercês et al. (2015) as areas with species contact and possibly gene flow/hybridization. Abbreviations for institutions: IDSM (Mamirauá Institute for Sustainable Development), MPEG (Emilio Goeldi Museum, Pará), UFPA (Federal University of Pará), UCLA/CalLu (University of California Los Angeles/Cal Lutheran University), IEPA (Institute of Scientific Research and Technology), UFMT (Federal University of Mato Grosso), SAZoo (Santa Ana Zoo).

Map ID	Species	Latitude	Longitude	Voucher number	Institution
1	*Saimiri b. boliviensis*	−6.77	−70.01	FES056	IDSM
2	*Saimiri b. boliviensis*	−8.02	−68.07	RS050	MPEG
3	*Saimiri cassiquiarensis*	−2.44	−64.81	LB719	UFPA
4	*Saimiri cassiquiarensis*	−2.47	−64.82	LB735	UFPA
5	*Saimiri cassiquiarensis*	−2.59	−64.89	LB738	UFPA
6a	*Saimiri cassiquiarensis*	−2.84	−64.99	SC4.2	IDSM
6b	*Saimiri cassiquiarensis*	−2.84	−64.99	SC4.4	IDSM
7a	*Saimiri collinsi*	−2.58	−51.92	LB463	UFPA
7b	*Saimiri collinsi*	−2.58	−51.92	LB459	UFPA
7c	*Saimiri collinsi*	−2.58	−51.92	LB389	UFPA
8	*Saimiri collinsi*	−1.21	−48.29	MIC01	MPEG
9a	*Saimiri collinsi*	−0.4	−49.11	MAR_5	MPEG
9b	*Saimiri collinsi*	−0.4	−49.11	MAR_4	MPEG
10	*Saimiri collinsi*	−6.1	−49.6	LB603	UFPA
11a	*Saimiri collinsi*	−1.18	−47.31	AF4	UCLA/CalLu
11b	*Saimiri collinsi*	−1.18	−47.31	AM3	UCLA/CalLu
11c	*Saimiri collinsi*	−1.18	−47.31	AM6	UCLA/CalLu
11d	*Saimiri collinsi*	−1.18	−47.31	AF13	UCLA/CalLu
12a	*Saimiri collinsi**	−2.47	−56.01	LB801	UFPA
12b	*Saimiri collinsi**	−2.47	−56.01	LB796	UFPA
12c	*Saimiri collinsi**	−2.47	−56.01	LB798	UFPA
13a	*Saimiri collinsi**	−1.71	−57.21	CN30	MPEG
13b	*Saimiri collinsi**	−1.71	−57.21	CN15	MPEG
14a	*Saimiri macrodon*	−4.37	−70.17	LB125	UFPA
14b	*Saimiri macrodon*	−4.37	−70.17	LB128	UFPA
14c	*Saimiri macrodon*	−4.37	−70.17	LB127	UFPA
15a	*Saimiri macrodon*	−5.2	−69.31	RDSC03	MPEG
15b	*Saimiri macrodon*	−5.2	−69.31	RDSC27	MPEG
16a	*Saimiri macrodon*	−5.63	−69.18	RDSC30	MPEG
16b	*Saimiri macrodon*	−5.63	−69.18	RDSC47	MPEG
16c	*Saimiri macrodon*	−5.63	−69.18	LB1304	UFPA
17a	*Saimiri macrodon*	−2.72	−65.35	LB791	UFPA
17b	*Saimiri macrodon*	−2.72	−65.35	LB762	UFPA
18	*Saimiri macrodon*	−3.45	−65.94	BJ002	IDSM
19	*Saimiri macrodon*	−2.82	−65.15	SM9.2	IDSM
20	*Saimiri macrodon*	−2.79	−65.14	SV15.1	IDSM
21	*Saimiri macrodon*	−1.98	−66.2	AP030	IDSM

(continued on next page)

Table 1 (*continued*)

Map ID	Species	Latitude	Longitude	Voucher number	Institution
22	*Saimiri macrodon*	−3.63	−66.11	BJ024	IDSM
23a	*Saimiri sciureus*	0.32	−50.69	LB561	UFPA
23b	*Saimiri sciureus*	0.32	−50.69	LB560	UFPA
24a	*Saimiri sciureus*	0.28	−53.11	AP124	IEPA
24b	*Saimiri sciureus*	0.28	−53.11	AP104	IEPA
25a	*Saimiri sciureus*	−0.94	−53.24	CN276	MPEG
25b	*Saimiri sciureus*	−0.94	−53.24	CN243	MPEG
26a	*Saimiri sciureus*	−0.16	−55.18	CN297	MPEG
26b	*Saimiri sciureus*	−0.16	−55.18	LB1849	UFPA
27	*Saimiri sciureus*	1.59	−52.49	AP221	IEPA
28	*Saimiri sciureus**	−2.48	−56.16	LB806	UFPA
29	*Saimiri ustus*	−4.34	−59.59	LB556	UFPA
30a	*Saimiri ustus*	−9.95	−58.41	MSN147	UFMT
30b	*Saimiri ustus*	−9.95	−58.41	MSN145	UFMT
31	*Saimiri ustus*	−12.44	−63.97	RGB02	MPEG
32	*Saimiri ustus*	−9.29	−64.71	JIR9791	MPEG
33	*Saimiri ustus*	−3.41	−51.75	LB468	UFPA
34	*Saimiri vanzolinii*	−3.06	−64.84	LB727	UFPA
35	*Saimiri vanzolinii*	−2.84	−65.02	SV6.2	IDSM
36	*Saimiri vanzolinii*	−2.87	−64.92	SV1.4	IDSM
–	*Sapajus robustus*			robustus	UCLA/ SAZoo

20 with the bbmap tool bbduk.sh.

We demultiplexed the sequence reads per sample in iPyRAD, allowing for no barcode mismatches. We used the program Cutadapt (Martin, 2010) to remove restriction enzyme recognition sites from the 5′ end of reads with an error rate of 0.25, remove all reads with more than 5 % Ns, and remove all reads now smaller than 30 bp.

We performed additional quality filtering using iPyRAD v.0.9.56 (Eaton and Overcast, 2016). Bases with a Phred quality score of less than 20 were turned into undetermined sites (Ns), reads were processed using iPyRAD's strict filter_adapter setting (=2) with raw edges trimmed for quality, and reads with more than ten undetermined sites (Ns) or reads shorter than 35 bp were discarded. We used the reference pipeline implemented in iPyRAD to generate ddRADseq data matrices and SNPs calling by mapping our quality-filtered ddRAD-seq reads onto the *Saimiri boliviensis* reference genome from GenBank (accession number GCF_000235385.1). Consensus sequences were retained if the minimum depth of coverage was at least 6×, and if they contained no more than 5 % undetermined sites (Ns), 5 % heterozygous sites, and two alleles after error correction. Final datasets with different combinations of taxa for downstream analyses were then constructed (Table 2) with the following parameters: a minimum of ~ 50–75 % of samples at a locus, a maximum of 10 indels at a locus, maximum shared Hs as a proportion at 0.5, and max SNPs as a proportion at 0.2.

2.4. Phylogenomic analyses and divergence dating analyses

We ran a Bayesian phylogenomic analysis of unlinked SNPs using the MrBayes tool on ACCESS v.3.2.2, with 56 samples (dataset 'unlinked_SNPs'; Table 2), using the GTR + F + gamma rate heterogeneity model with four chains and 2,000,000 generations, sampled every 100 generations with a 25 % burn in. The GTR + F + gamma rate heterogeneity model was the closest analogue model available in MrBayes to GTR + F + R3, determined by ModelFinder (Kalyaanamoorthy et al. 2017) to be the best substitution model for this dataset.

We ran a Maximum likelihood (ML) analysis on RAXML-NG (Kozlov et al., 2019) using our 'all_SNPs' dataset including linked SNPs, employing the GTR + G (gamma) substitution model and performing 250 searches for the best-scoring ML tree, with node support assessed

Table 2
Summary of the final ddRADseq datasets, including number of samples, SNPs and dataset usage. All datasets can be found on Dryad.

Dataset	No. samples	SNPs	Minimum sample coverage Initial Filter	SNP minimum sample coverage	Usage
unlinked_SNPs	56	30,430	42	42	MrBayes
all_SNPs	56	518,558	42	2	RAXML IQTREE Dsuite
all_clades	56	208,638	42	2	Parallel Structure
Roman	5	20,512	42	2	Parallel Structure
macrodon	15	113,220	42	10	Parallel Structure
ustus	6	32,138	4	2	Parallel Structure
Eastern	25	70,099	18	2	Parallel Structure
TimeTree	13 + Outgroup	297,748	2	11	BEAST2

using 500 bootstrap replicates. We also ran an ML analysis with the 'all_SNPs' dataset on IQTree v.3.0.1 (Wong et al., 2025) using the GTR + F + R3 model, determined by ModelFinder (Kalyaanamoorthy et al. 2017) to be the best substitution model for this dataset, with 1000 ultrafast bootstrap replicates. We additionally filtered the 'all_SNPs' dataset to only include SNPs with a 90 % sample coverage minimum (n = 81,936 SNPs) and performed 250 ML searches and 1100 bootstrap replicates using the same parameters as the initial ML analysis to examine whether data coverage levels impacted topology for these datasets.

Phylogeny and diversification times were jointly estimated under an optimized relaxed clock model (Douglas et al., 2021) in the program BEAST2 v.2.7.7 (Bouckaert et al., 2019) for the "TimeTree" dataset (Table 2). A Yule speciation process and the GTR + G substitution model was used, and the ucld.Mean prior was set to an exponential distribution (mean = 0.05). To obtain the posterior distribution of the estimated divergence times, one calibration point on the root node (capuchin monkey/squirrel monkey divergence) was implemented with a lognormal distribution to set hard minimum and soft maximum bounds. A minimum age of 12.5 Ma was used based on the fossil *Neosaimiri* Stirton, 1951, based in Hartwig and Meldrum (2002) and a soft maximum bound was set at 26 Ma based on the fossil *Branisella boliviana* Hoffstetter, 1969, from the Deseadan fauna of La Salla (MacFadden, 1990; see Byrne et al., 2016). The log standard deviation (0.8) and log mean (1.287) were set such that 95 % of the prior distribution falls before the maximum age to create the soft maximum bound.

To construct the time tree, we conducted two replicate runs of 100 million MCMC generations, sampling every 10,000 generations. We visualized the sampling distributions using Tracer v.1.7.2 (Rambaut et al., 2018) to evaluate convergence and effective sample size after a burn-in of 10 %. Independent runs were combined with the first 10 % of samples of the posterior distribution discarded as burn-in using LogCombiner v.2.7.7 and the maximum credibility tree with mean heights was generated in TreeAnnotator v.2.7.7 and trees were visualized in treeio v.1.30.0 (Wang et al., 2020) and FigTree v.1.4.4.

2.5. Bayesian clustering analyses

To better understand the structure of squirrel monkey genetic diversity from our genomic data in the context of previous taxonomic classifications and phylogenetic results, we conducted population structure analyses of the "all_clades" dataset using the ParallelStructure tool on ACCESS v.2.3.4 on the CIPRES Science Gateway v.3.3 server

(Miller et al., 2010). This dataset was generated by taking the "all_SNPs" dataset and filtering out sites where variability was due to ambiguous bases (all IUPAC ambiguity codes). ParallelStructure (Besnier and Glover, 2013) is an R-based implementation of the program Structure (Pritchard et al., 2000) which allows for the assessment of the number of lineages present in a sample set and the degree of shared ancestry found across different geographic clades. We used the admixture model and no putative origins specified for individuals. For each dataset, 10 runs at each value of K were performed with a MCMC length of 300,000 steps, which included a burn-in of 50,000 steps. We ran the Structure analyses for five groups (Table 2): The first included all clades retrieved in molecular analyses (dataset = "all_clades" for ParallelStructure; K = 1–7), the second included samples from the *Saimiri vanzolinii* + *S. boliviensis* clade (dataset = "Roman"; K = 1–6), the third included all samples found from the *S. macrodon* clade (dataset = "macrodon"; K = 1–6), the fourth included samples from the *S. ustus* clade (dataset = "ustus"; K = 1–6), and the fifth included samples from the *S. collinsi* + *S. sciureus* clade (dataset = "Eastern"; K = 1–6).

For the "all_clades" set and the "macrodon" set, we also ran additional analyses to account for a lack of convergence in certain K runs, potentially due to too much low-coverage data. For the "macrodon" analysis, we filtered to sites where at least 67 % of individuals were represented (n = 20,775 SNPs) and ran K 1–6. For the "all_clades" analysis, we ran an analysis from a subset of the data (every fifth site from Main_STRUCTURE; n = 41,729 SNPs) for K 1–14. We also filtered the "ustus" dataset to sites where at least 67 % of individuals were represented (n = 24,145 SNPs) despite convergence in the full dataset to check that consistent K populations were chosen between runs with different data subsets.

We identified the most likely number of clusters for each dataset using LnP(D). If several K values had similar LnP(D) scores, the assignment of the additional clusters was evaluated to check if they were informative or assigned equally to the putative populations. We also implemented the *ad hoc* statistic delta K (Evanno et al., 2005) in the program Structure Harvester (Earl and vonHoldt, 2012) to detect the most appropriate number of clusters. The program CLUMPP (Jakobsson and Rosenberg, 2007) was used to combine and average individual cluster outputs generated by independent runs, and the combined data were visualized using the R package ggplot2 v.3.5.1 (Wickham, 2016).

2.6. Gene flow analyses

To assess for gene flow across the Gothic arch *Saimiri* species and putative subspecies, we used Dsuite (Malinsky et al., 2021) to calculate Patterson's *D* and f_4-ratio statistics using the 'all_SNPs' dataset, considering the Roman arch *Saimiri* taxa as the outgroup. We used the Dtrios command with the −t option to generate results in concordance with species relationships, with one analysis using a tree at the currently recognized species level (Outgroup [*S. boliviensis* + *S. vanzolinii*], (*S. ustus*, (*S. cassiquiarensis*, (*S. macrodon*, (*S. sciureus*, *S. collinsi*)))), and a second analysis using a tree at the subspecies or population level consistent with taxa and topology from Fig. 2. We calculated *f*-branch statistics using the Fbranch command and adjusted the p-values using the R p.adjust function with the "fdr" correction method (Benjamini & Hochberg, 1995). We plotted the results using dtools.py included with Dsuite, setting f-branch = 0 where the adjusted p-value was less than 0.01. *F*-branch values were not calculated between *S. collinsi* and *S. sciureus* in the species-level analysis, as in the species level tree we lumped *S. collinsi* with *S. collinsi* Faro + Juruti so *S. collinsi* and *S. sciureus* were considered sister taxa.

2.7. Research data deposit, citation and linking

Our ddRAD datasets and datasets used for generating phylogenies and structure analyses are available upon publication at the Dryad repository here: https://doi.org/10.5061/dryad.djh9w0wc0.

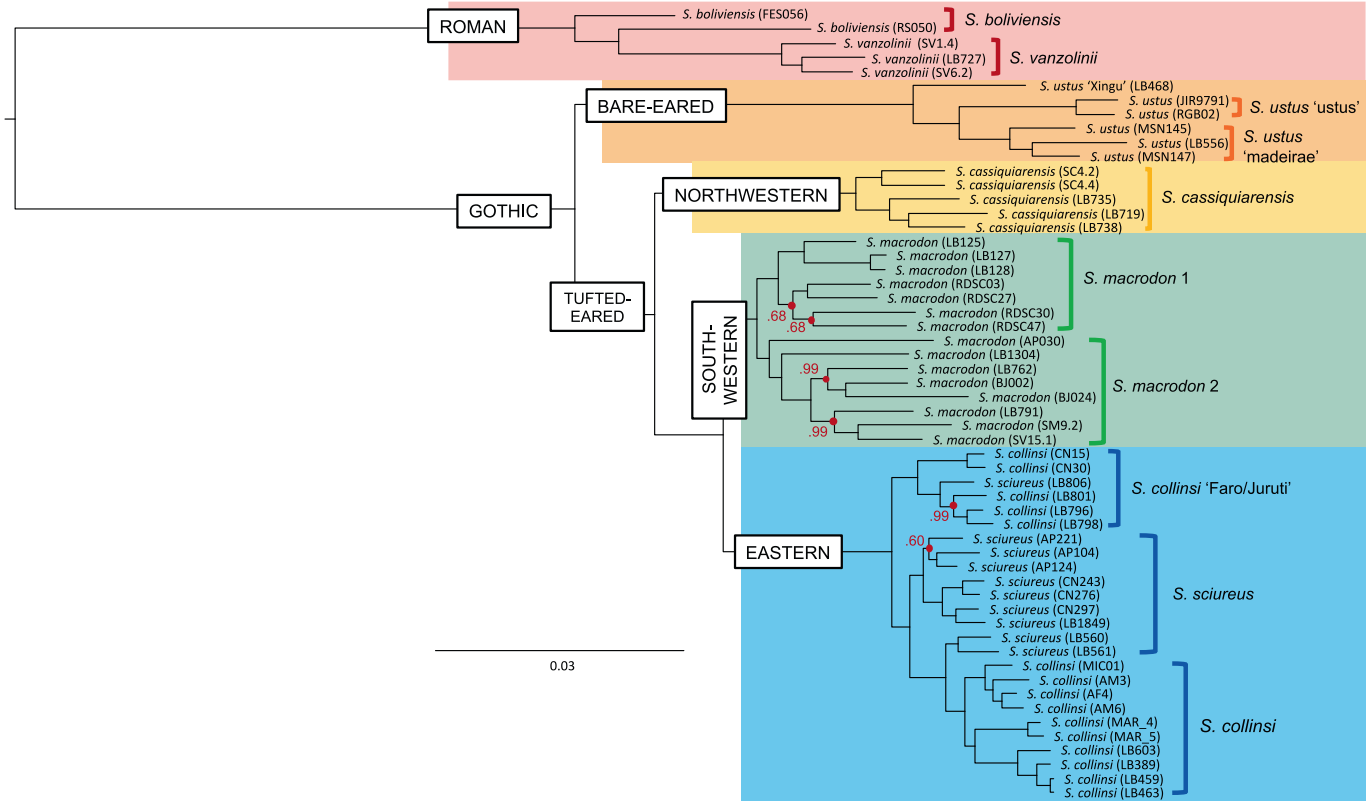

Fig. 2. Amazonian *Saimiri* Bayesian phylogeny using 75 % Coverage threshold of 'unlinked_SNPs' dataset, GTR + F + gamma rate heterogeneity substitution model with four chains and 2,000,000 generations, 56 taxa. Numbers at each node represent posterior probability when less than 1.

3. RESULTS

3.1. ddRADseq data assembly

The ddRADseq samples for the main dataset had an average of 7 million raw reads after initial demultiplexing and preliminary filtering in Cutadapt, which were subsequently quality filtered into an average of 6.96 million reads per sample using iPyRAD. Supplemental Table S1 provides data assembly sequencing information per *Saimiri* sample used in the study. We mapped an average of 258,936 stacks per sample to the reference genome, for a filtered average of 105,844 loci. The average depth was 36.6 across the high depth clusters. The "Main_RAXML" dataset contained 518,558 ddRADseq SNPs, while the other datasets contained between 20,512 and 297,748 SNPs [time tree includes *Sapajus robustus* as outgroup]. Mean heterozygosity varied among *Saimiri* individuals (mean 0.0016, range 0.0003–0.0099) and species (mean 0.0018, range 0.001–0.0033). On average, *S. cassiquiarensis* individuals showed the most heterozygosity (0.0033), with *S. boliviensis* (0.0024) and *S. macrodon* (0.002) intermediate, and *S. sciureus* (0.001), *S. ustus* (0.001), *S. vanzolinii* (0.001) and *S. collinsi* (0.001) the least heterozygous.

3.2. Phylogenomic inferences

We generated four phylogenetic trees using the concatenated ddRADseq data matrices (Fig. 2, Supplemental Figs. S1–S3). All phylogenetic trees showed largely congruent topology except that the all_SNPs-IQtree and all_SNPS-RAXML tree placed sample AP030 as sister to all other *S. macrodon* samples, whereas the Bayesian and U-SNP ML tree placed AP030 within the *S. macrodon* 2 clade. All phylogenies divided *Saimiri* into two clades corresponding to the morphology-based Roman- and Gothic-arch groups, related to the shape of the white arch above the eyes. In the Gothic-arch group, divergence occurred first between bare-eared and tufted-eared taxa. Tufted-ear taxa were divided into three geographical clades. All five major geographical/morphological clades (*S. boliviensis* + *S. vanzolinii*; *S. ustus*; *S. cassiquiariensis*; *S. macrodon*; and *S. sciureus* + *S. collinsi*) were highly supported, with bootstrap values of 100 in the RAXML trees, and a posterior probability of 1 for the Bayesian tree. In the phylogeny and subsequent STRUCTURE analyses, we added additional information to species names for some subclades within major groups when they corresponded to noted geographical morphological variations that have been reported in the literature (*S. ustus* 'ustus', *S. ustus* 'madeirae': Thorington, 1985), and *S. ustus* 'Xingu' (described here), *S. macrodon* 1 and 2 (Paim et al., 2013), *macrodon* 2 AP030 (described here), and *S. collinsi* and *S. sciureus* from 'Faro and Juruti' (Mercês et al., 2015, Lynch Alfaro et al., 2015).

The Roman clade was composed of *S. boliviensis* and *S. vanzolinii*. *Saimiri boliviensis* was recovered as paraphyletic for all analyses, but with poor support for the topology. The Gothic group clade was composed of four lineages, here called the *S. ustus* or bare-eared clade, the *S. cassiquiarensis* or northwestern Amazon clade, the *S. macrodon* or western Amazon clade, and the *S. sciureus* + *S. collinsi* or eastern Amazon clade. In the Gothic group, the *S. ustus* clade representing the bare-eared squirrel monkeys diverged first. In tufted-eared squirrel monkeys, *S. cassiquiarensis* diverged first, and *S. macrodon* was recovered as sister to *S. sciureus* + *S. collinsi*. *Saimiri collinsi* was recovered as paraphyletic, with a western *S. collinsi* clade from Faro and Juruti (and including one specimen identified as *S. sciureus*) the sister group to an all *S. sciureus* + Marajó and eastern Pará *S. collinsi* clade.

3.3. Divergence time analysis

With the "Time_Tree" dataset, using BEAST2 we generated one time tree that jointly estimated phylogeny and diversification times (Fig. 3,

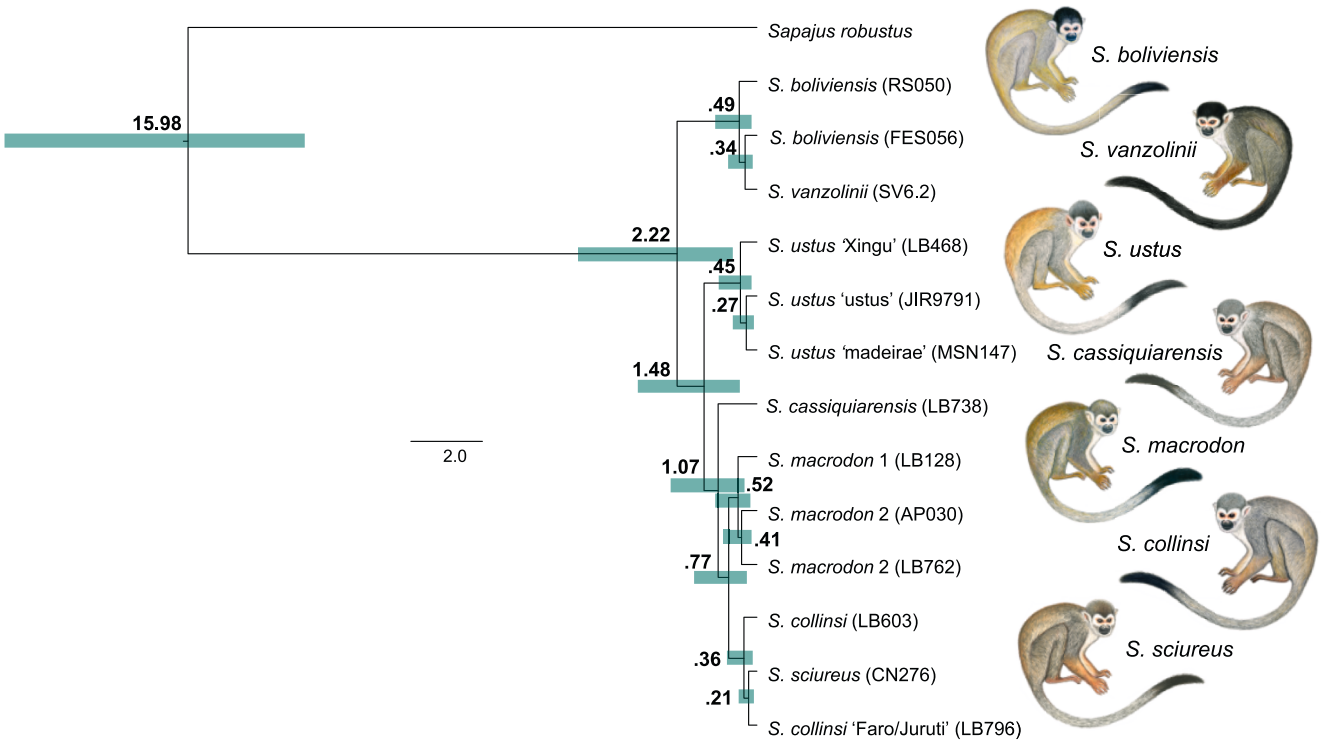

Fig. 3. A time-calibrated phylogeny inferred with ddRADseq "time" dataset. All nodes received full support (posterior probability of 1). Blue bars represent 95% highest posterior density (HPD) intervals. 95% HPD ranges for each node can be found in Supplemental Table S2. Bolded numbers represent average common ancestor heights in millions of years. Representatives are included for each of the geographical variants that are known to have some genetic and/or morphological support for being distinct species or subspecies. Illustrations by Stephen D. Nash. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Supplemental Table S2). *Saimiri* and capuchin monkeys (*Cebus* and *Sapajus*) are estimated to have diverged in the Miocene (Fig. 3; 15.98 Ma: 95 % HPD = 12.9–21.23 Ma). The estimated divergence time for the split between the Gothic and Roman *Saimiri* lineages is at 2.22 Ma (95 % HPD = 0.66–5 Ma). The topology of the time tree (Fig. 3) was fully resolved and is concordant with the five main *Saimiri* clades in the larger phylogeny (Fig. 2), but the two trees are not always concordant for the diversification order within clades.

3.4. Bayesian clustering analyses

Bayesian clustering analyses were performed using five datasets of ddRADseq: "all_clades" with all *Saimiri* taxa, "Roman" with the *S. boliviensis* and *S. vanzolinii* samples, "ustus" with all the *S. ustus* samples, "macrodon" with all *S. macrodon* samples, and "Eastern" with all the *S. collinsi* and *S. sciureus* samples.

For the "all_clades" Structure dataset, the mean likelihood score increased until K = 5, where it decreased significantly in both dataset analyses. It then increased again at K = 6, and beyond that, kept decreasing. Similarly, when examining the delta K, K = 6 showed a local peak. When K = 6 was assumed, each major clade recovered from the phylogeny (*S. boliviensis* + *S. vanzolinii*, *S. ustus*, *S. cassiquiarensis*, *S. macrodon*, and *S. sciureus* + *S. collinsi*) represented its own cluster (Fig. 4A). Some samples of *S. macrodon* 1 shared a low percentage of ancestry with the *S. collinsi* + *S. sciureus* cluster, and some samples of *S. macrodon* 2 shared a low percentage of ancestry with the *S. cassiquiarensis* cluster. All *S. cassiquiarensis* samples shared some ancestry with the *S. macrodon* 2 cluster.

We tested the number of clusters for the *S. boliviensis* + *S. vanzolinii* clade with the dataset "Roman." The highest mean likelihood occurred at K = 1, indicating no evidence for population structure. However, K = 2 showed a similar mean likelihood value. For K = 2, one cluster corresponded to *S. boliviensis* and the other to *S. vanzolinii*, with no samples showing shared ancestry (Fig. 4B).

For the "ustus" dataset, representing the *S. ustus* or bare-eared clade, the most likely mean likelihood occurred at K = 1 in both the full dataset and 67 % coverage analyses, indicating no evidence for population structure. Delta K did not show a clear peak, which may indicate there are no clear cluster alternatives to K = 1.

For the "macrodon" dataset, we tested for western Amazon squirrel monkey (*Saimiri macrodon)* clusters. Highest mean likelihood occurred when K = 2, and an estimated delta K also chose K = 2 when data was filtered. Unfiltered data failed to converge past K = 1. When K = 2 was assumed, one cluster corresponded to *S. macrodon* 2, while the other was found in all specimens from the *S. macrodon* 1 clade, though the *S. macrodon* 1 clade samples all showed mixed ancestry (Fig. 4C).

For the eastern Amazon clade (*Saimiri collinsi* + *S. sciureus* clade), we used the "Eastern" dataset. Mean likelihood was highest at K = 3, and

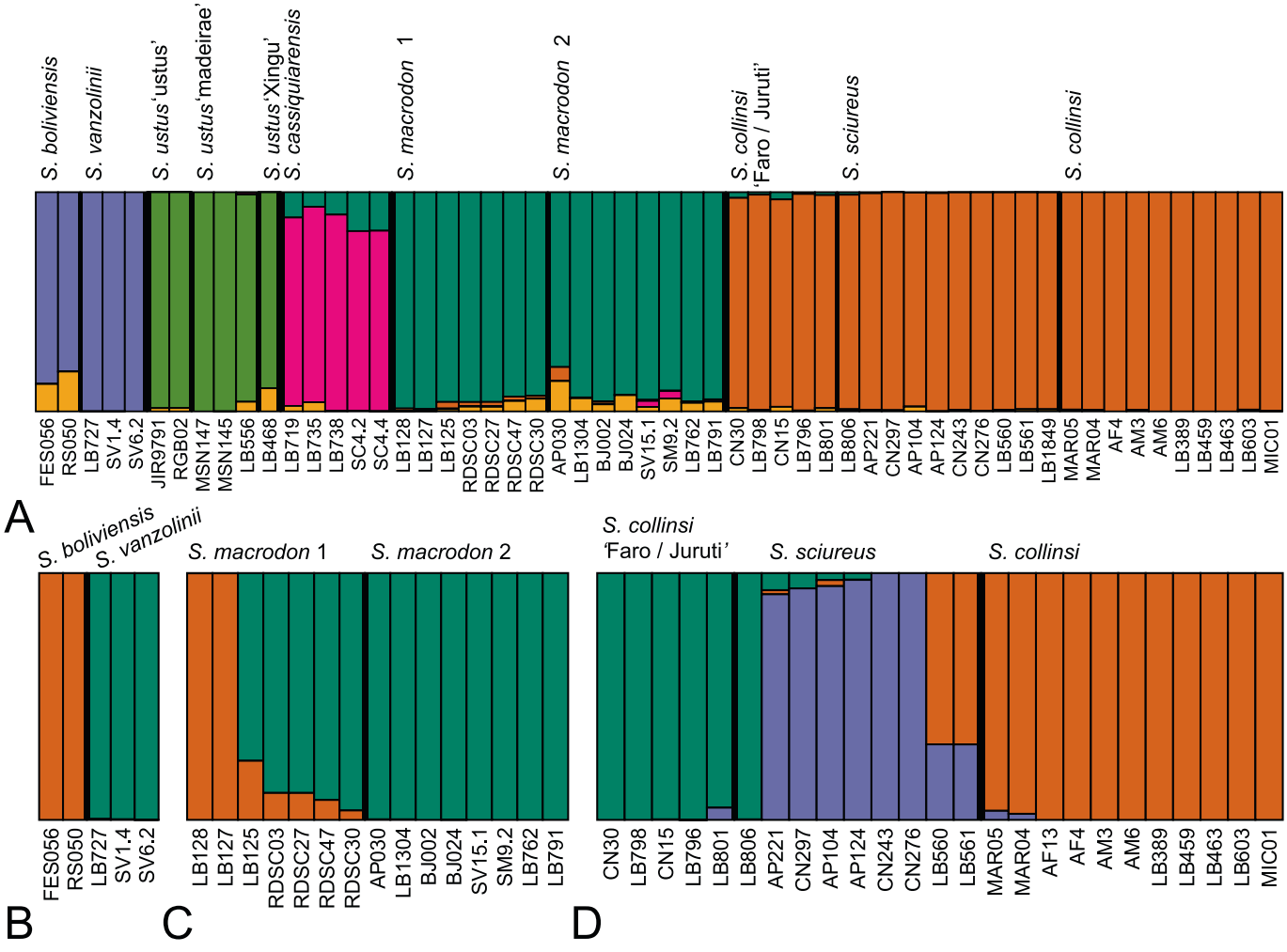

Fig. 4. Genetic structure analyses from full datasets, where species labels are assigned based on morphological assessment. (A) Genetic structure analyses of the "all_clades" dataset at K = 6. (B) Genetic structure analyses of the "Roman" dataset at K = 2. (C) Genetic structure analyses of the "macrodon" dataset at K = 2. (D) Genetic structure analyses of the "Eastern" dataset at K = 3. Note that morphologically LB806 is considered *S. sciureus* but was assigned to the *S. collinsi* 'Faro/Juruti' cluster genetically.

delta K selected K = 2. When K = 3 is assumed, one cluster is assigned to Western *S. collinsi/sciureus* samples from Faro and Juruti, one to *S. sciureus* samples in Amapá state, and the last to southeastern *S. collinsi* samples from Marajó Island and southern Pará (Fig. 4D). One sample from western *S. collinsi* 'Faro and Juruti' and four samples from Southeastern *S. collinsi* shared ancestry with the *S. sciureus* cluster; some *S. sciureus* samples shared ancestry with both the western *S. collinsi* 'Faro and Juruti' and southeastern *S. collinsi* clusters. When K = 2 is assumed, one cluster is assigned to samples from Faro and Juruti, and one to *S. collinsi*. *S. sciureus* samples showed mixed ancestry (Supplemental Fig. S4).

3.5. Dsuite gene flow analyses within the Gothic group

We found significant signals of gene flow within the Gothic group in our Dsuite analyses. At the species level, we found four signals of gene flow, two of which were above an *f*-branch value of 0.05 (Fig. 5A, Supplemental Tables S3 and S4). In our analysis at the subspecies or population level, we found 31 signals of gene flow, four of which exceeded the *f*-branch value of 0.05 (Fig. 5B, Supplemental Tables S5 and S6). Both analyses show evidence of gene flow between *S. ustus* and the lineage leading to the Eastern species (*S. sciureus* and *S. collinsi*), and the population level analysis confirms that these gene flow events occurred between *S. ustus* 'Xingu' and *S. ustus* 'madeirae' and the ancestral lineage to the Eastern clade. The second strong signature for gene flow in the species-level analysis was found between *S. macrodon* and *S. sciureus*, which was significant in the population-level analysis for both *S. macrodon* 1 and *S. macrodon* 2, but did not exceed an *f*-branch value of 0.05. The highest *f*-branch values in the population-level analysis occurred between *S. collinsi* 'Faro and Juruti' and *S. sciureus* (*f*-branch = 0.2293), and between *S. ustus* 'Xingu' and *S. ustus* 'ustus' (*f*-branch = 0.1712).

4. DISCUSSION

The topology of our phylogenetic trees showed strong congruence with morphological hypotheses and with earlier nuclear phylogenies that included fewer specimens and fewer taxa (Springer et al., 2012; Kuderna et al., 2023). However, we recovered different species relationships without the species paraphyly observed in previous phylogenetic analyses that were restricted to a small number of mitochondrial loci (Lynch Alfaro et al., 2015; Ruiz García et al., 2015) or even whole mitochondrial genomes (Janiak et al., 2022; Finnegan et al., 2025). *Saimiri boliviensis* was recovered together with *S. vanzolinii* in the Roman clade, matching the species grouping proposed by Hershkovitz (1984) that was based on morphology and behavior. The first species occurs from Bolivia to northwestern Brazil in the states of Acre and Amazonas, and the second is endemic to the Reserva de Desenvolvimento Sustentável Mamirauá, located in the Solimões-Japurá interfluvium, in the state of Amazonas, Brazil. The topology recovered in this study is discordant with previous mitochondrial phylogenetic analyses in which *S. vanzolinii* was retrieved as sister to *S. ustus* (Lynch Alfaro et al., 2015), or as sister to *S. collinsi* (Ruiz García et al., 2015). Lynch Alfaro et al. (2015) considered the Roman morphology of *S. vanzolinii* to be a convergence, as the species did not group with the other Roman species (*S. b. boliviensis* and *S. b. peruviensis*). The incongruence between nuclear and mitochondrial signals could instead provide evidence of incomplete lineage sorting and/or past or present introgression and/or sex-biased dispersal, as seen in other recent rapid primate diversifications, such as the macaque radiation (Tosi et al., 2003), the baboon radiation (Zinner et al., 2009; Rogers et al., 2019) and uakari monkeys (Hermosilla-Albala et al., 2024; Silva et al., 2024). The two *S. boliviensis* samples did not form a clade, and more sampling will be needed to better understand Roman arch squirrel monkey relationships. The taxon *S. boliviensis* currently includes two subspecies, *S. b. boliviensis* and *S. b. peruviensis*, the latter of which was not sampled in this study.

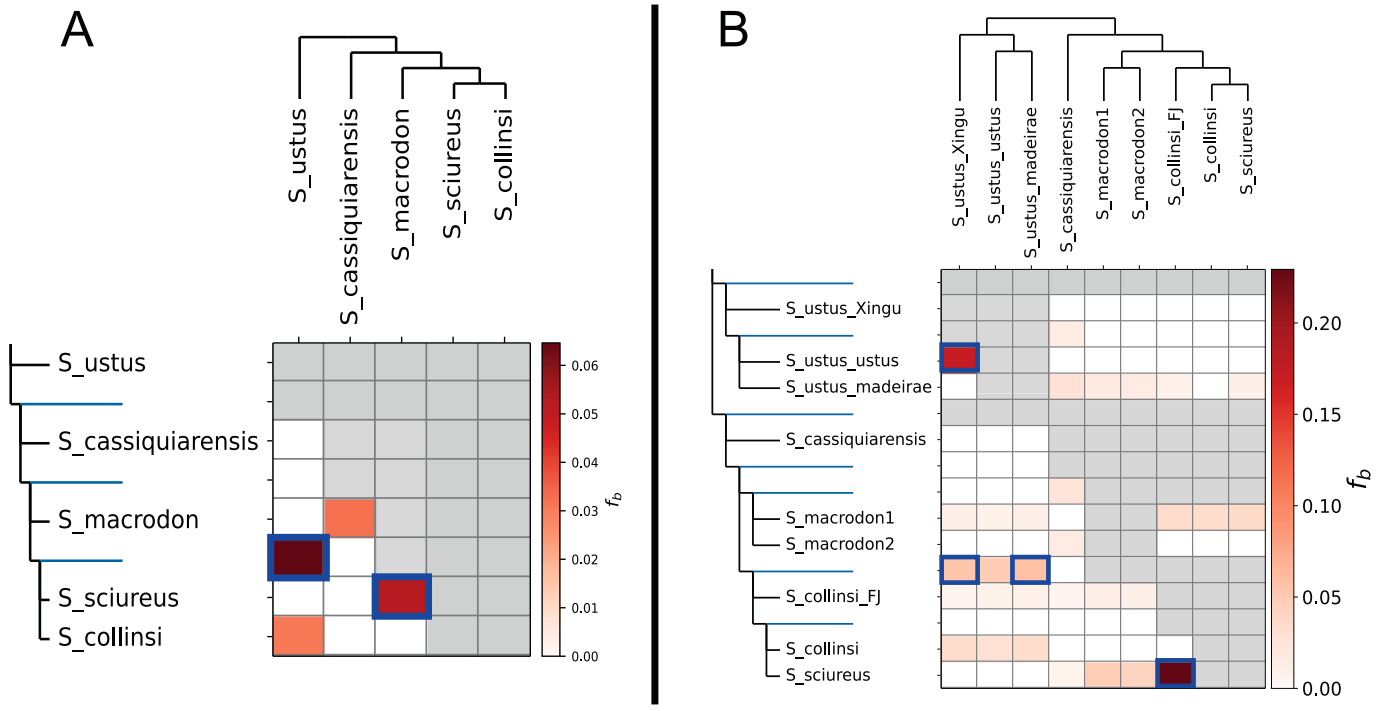

Fig. 5. Matrices of *f*-branch values for the Gothic group of *Saimiri* from the Dsuite analysis of ddRADseq data, as visualized with dtools.py. (A) Species-level analysis based on current recognized morphological species. (B) Population-level analysis using Gothic clades recovered in *Saimiri* phylogeny from Fig. 2. S_collinsi_FJ refers to *S. collinsi* 'Faro and Juruti.' *F*-branch values indicate the proportion of excess allele sharing between population/species (columns) and branches (rows) compared to that expected under the multispecies coalescent model. Following Malinsky et al. (2021) we expect that *F*-branch values above 0.05 (with blue border for square) point to most likely cases of gene flow or introgression. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The other major group of Amazonian squirrel monkeys, the Gothic clade is widespread throughout almost all of the Amazon Basin, and all species present a more similar morphology, with the exception of the *S. ustus* clade, that can be easily differentiated by the absence of ear-tufts. The bare-eared squirrel monkey (*Saimiri ustus*) was retrieved as a paraphyletic species by Lynch Alfaro et al. (2015)—one *S. ustus* lineage was retrieved as sister to Central American and Guianan squirrel monkeys, while the other *S. ustus* lineage was sister to *S. collinsi* + *S. vanzolinii*. In the present study, however, we recovered two *S. ustus* clades that were reciprocally monophyletic. Our phylogeny included one individual from the left bank of the Rio Xingu at the far eastern border of the bare-eared squirrel monkeys' distribution, here called *S. ustus* 'Xingu,' that was sister to the rest of the *ustus* clade. As we only had one sample of this population, further investigation with more samples will be necessary to understand the distribution and genetic structure of monkeys in this region. The monophyly of *S. ustus* corroborates the morphology shared among the bare-eared taxa and their geographical distribution from the right bank of the Rio Purus to the left bank of the Rio Xingu, including the state of Rondônia and the northwest of the state of Mato Grosso in Brazil (Fig. 6). While morphological analyses have suggested *S. ustus* may comprise two or even three different geographic species or subspecies (Thorington, 1985), and mtDNA analyses suggest divergent clades of *S. ustus* (Lynch Alfaro et al., 2015; Ruiz García et al., 2015; Janiak et al., 2022; Finnegan et al., 2025), the ddRAD results here confirmed monophyly but we did not find additional population structure in our analyses.

Thorington (1985) treated the squirrel monkeys from the Guiana Shield, east of the Rio Negro, and the Brazilian Shield, east of the Rio Xingu, and those from Colombia, Ecuador and northern Peru as a single subspecies *S. sciureus sciureus*. He argued that the geographical variation in coat color and skull morphology in these squirrel monkeys lacked any concordance that would provide a compelling argument for more than this one subspecies. *Saimiri s. cassiquiarensis*, *S. s. boliviensis* and *S. s. oerstedii* (Panama and Costa Rica) were the other subspecies he identified. Thorington also recognized *Saimiri madeirae* Thomas, 1908, with "nearly naked ears" (p.22) as a distinct species in the interfluvium of the rios Xingu and Madeira, but Groves (2001) determined that it is a synonym of *Saimiri ustus* (I. Geoffroy Saint-Hilaire, 1844). Groves (2001, 2005) placed *albigena* (eastern Llanos of Colombia), *cassiquiarensis* and *macrodon* as subspecies, and *S. collinsi* as a synonym, of *S. sciureus*.

The monophyly of the tufted-eared squirrel monkeys recovered here agrees with their morphology, although in previous molecular studies using mtDNA markers this group was paraphyletic (Lavergne et al., 2010; Lynch Alfaro et al., 2015; Ruiz García et al., 2015, Janiak et al., 2022; Finnegan et al., 2025). Elsewhere *S. cassiquiarensis* was recovered as sister to *S. macrodon* (see Kuderna et al., 2023), but our results here position *S. cassiquiarensis* as the first to branch off in the tufted-eared group, sister to the *S. macrodon* + *S. sciureus* + *S. collinsi* lineage. Our Bayesian cluster analyses revealed evidence for shared ancestry between some *S. cassiquiarensis* and *S. macrodon* individuals, which may help clarify previously affirmed phylogenetic relationships. Lynch Alfaro et al. (2015) recovered *S. cassiquiarensis* as sister to *S. macrodon* B, a clade that was distributed in the Inambari center of endemism. We did not sample *S. albigena* from the Colombian Llanos or *S. oerstedii* from Central America in this study.

Paim et al. (2013) observed that three species of *Saimiri* occur in the Reserva de Desenvolvimento Sustentável Mamirauá (RDSM), one of which they identified as *S. sciureus macrodon* (*sensu* Hershkovitz, 1984). Although the external morphology of *S. macrodon* in the RDSM differs from that of specimens from Ecuador, Colombia and adjacent areas in Brazil, they referred to it as *Saimiri sciureus macrodon* 2 (Paim et al., 2013). *Saimiri macrodon* 2 in our phylogeny corresponds to the specimens from Mamirauá and in the Solimões-Japurá interfluvium, with some specimens on both banks of lower Rio Juruá. Costello et al. (1993) considered this phenotype to be a result of hybridization between *S. madeirae* (= *S. ustus*) and other *Saimiri* species. However, our Dsuite analyses do not reveal evidence for gene flow between *S. ustus* and *S. macrodon* 2. Our STRUCTURE analyses suggest that *S. macrodon* 1 has ancestry from two different lineages: the same lineage as *S. macrodon* 2, and another lineage that may extend farther west into the Peruvian and Bolivian Amazon. Our samples from *Saimiri macrodon* 1 are limited to the west of the Brazilian Amazon. Previous morphological analyses (Paim et al., 2013) and mitochondrial DNA analyses (Lynch Alfaro et al., 2015) suggested there were two clades within *S. macrodon*. While within *Saimiri macrodon* we too found evidence for ancestry from two different population clusters, all of our *S. macrodon* 1 individuals had ancestry from *both* population clusters.

The eastern Amazonian *Saimiri sciureus* clade includes *S. sciureus* and

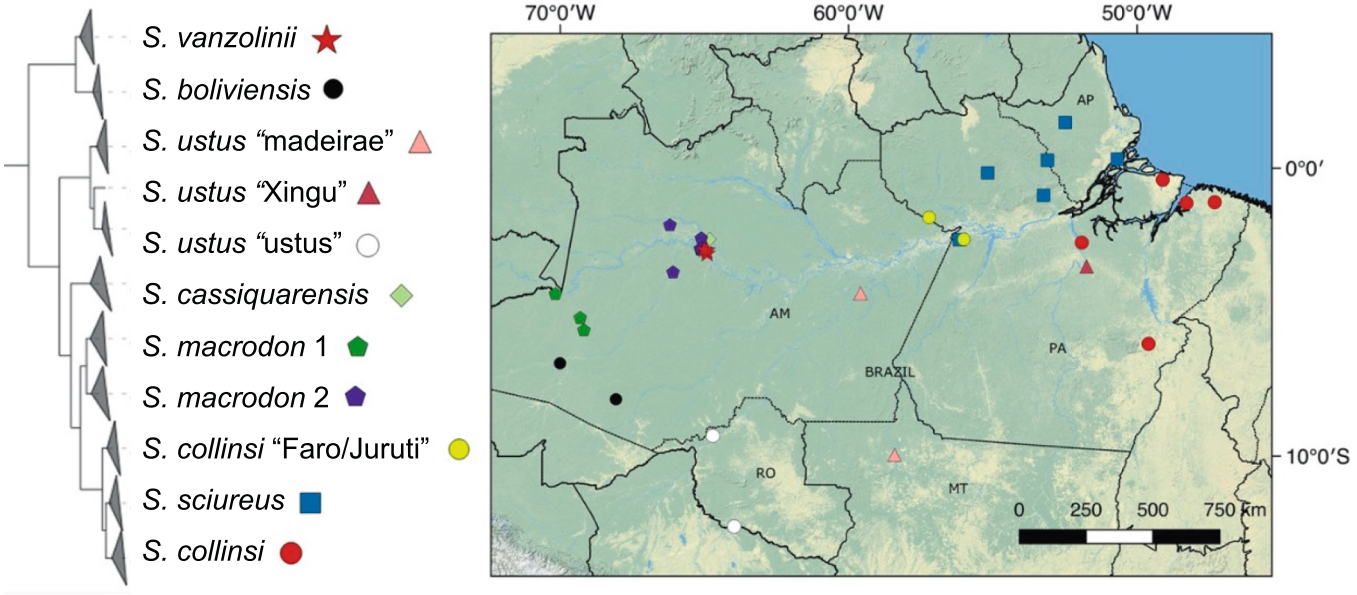

Fig. 6. Collecting localities of specimens included in molecular analyses, and corresponding clade structure found in the ddRadseq phylogeny, with taxon names associated with them (tree to the left). Acronyms: AM: Amazonas; AP: Amapá; MT: Mato Grosso; PA: Pará; RO: Rondônia.

S. collinsi as sister taxa, as well as a third clade that we provisionally refer to as *S. collinsi* 'Faro and Juruti'. In four phylogenetic studies with mitochondrial loci (Lavergne et al., 2010; Lynch Alfaro et al., 2015; Mercês et al., 2015; Ruiz García et al., 2015), *S. sciureus* and *S. collinsi* never appeared as sister taxa, rather *Saimiri sciureus* was always retrieved as sister to *S. oerstedii* (Boinski and Cropp, 1999; Cropp and Boinski, 2000; Lavergne et al., 2010; Chiou et al., 2011; Springer et al., 2012; Lynch Alfaro et al., 2015; Mercês et al., 2015; Ruiz García et al., 2015) and *S. collinsi* was placed sister to *S. ustus* (Mercês et al., 2015) or *S. ustus* + *S. vanzolinii* (Lynch Alfaro et al., 2015). As we did not have access to samples of *S. oerstedii*, we are unable to consider the whole history of the diversification of *Saimiri*; however, the phylogeny from Kuderna et al. (2023) using UCE genomic sampling placed *S. oerstedii* as sister to *S. ustus*, with the three northern tufted-eared Amazonian taxa (*sciureus, macrodon*, *cassiquiarensis*) forming a clade together, or alternately, *S. sciureus* forming the sister taxon to all other Gothic squirrel monkeys. Regarding the lineage '*S. collinsi* Faro and Juruti', the specimens in this clade are all found quite near to the Rio Amazonas and to the west of the rios Trombetas and Madeira, in contrast to the rest of the *S. collinsi* and *S. sciureus* samples. Considering morphology and mitochondrial loci, Mercês et al. (2015) found that the specimens from Faro and Juruti have a complex evolutionary situation. Unexpectedly based on species distributions, specimens with the morphology and haplotypes of *S. collinsi* were found on the northern side of the Rio Amazonas and those of *S. sciureus* on the southern side. *Saimiri collinsi* 'Faro and Juruti' was retrieved in their phylogeny as sister to *S. collinsi*, while in the present study *S. collinsi* 'Faro and Juruti' was recovered as sister to *S. collinsi* + *S. sciureus*, although in our time tree, *S. collinsi* was sister to *S. sciureus* + *S. collinsi* 'Faro and Juruti'. Mercês et al. (2015) argued that the Faro and Juruti region presented unique fluvial dynamics that might have facilitated the crossing or passive transport of primates. *Saimiri collinsi* 'Faro and Juruti' includes the westernmost samples for this group (see Fig. 6), and the phylogenetic and structure results suggest this population may have been isolated first, as squirrel monkeys moved eastward across the rios Trombetas and Madeira to expand into the eastern Amazon, first north of the Rio Amazonas and later to Marajó Island and south into southern Pará, and the states of Tocantins and Maranhão (Mercês et al., 2017). In our Structure analyses, at K = 3. *S. collinsi*, *S. sciureus* and *S. collinsi* 'Faro and Juruti' each formed their own lineages, whereas at K = 2, *S. collinsi* and *S. collinsi* 'Faro and Juruti' were two different lineages, and *S. sciureus* samples showed ancestry from both lineages. In our Dsuite analyses, we found strong evidence for gene flow between *S. sciureus* and *S. collinsi* 'Faro and Juruti'.

Our BEAST analysis estimated the age for diversification of modern squirrel monkeys at ~ 2.22 Ma. This is comparable to previous studies, that estimated divergence age in the crown of *Saimiri* at 1.75 Ma (95 % HPD = 1.06–2.88) using 69 nuclear and ten mitochondrial loci (Springer et al., 2012), at 1.5 Ma (95 % HPD = 1.12–190) using a BEAST analysis with mitogenomic data (Chiou et al., 2011) and at 1.36 (95 % HPD = 1.1–1.91) using mitochondrial loci (Lynch Alfaro et al., 2015). The diversification of *Saimiri* evidently occurred during the early Pleistocene and is, as such, more recent than in most other Neotropical primate genera, including titi monkeys (Byrne et al., 2016) and gracile capuchin monkeys (Boubli et al., 2012). The sister species of *Cebuella* (Boubli et al., 2018) present a similar divergence time (2.24, 95 % HPD = 1.70–2.96), around the Pliocene/Pleistocene boundary. The diversification of *Cacajao* (uakaris) in the Amazon occurred largely within the last million years, based on a ddRAD phylogeny (1.13 Ma, 95 % HPD = 0.67–1.72) (Silva et al., 2022), and the diversification of *Sapajus* (robust capuchins) in the Amazon begins even later, at about 500 ka based on a ddRAD phylogeny (Martins et al., 2023).

Pleistocene climate changes seem to have played an important role in the intraspecific diversity of the squirrel monkeys. Diversification of the Amazonian trumpeters (*Psophia*) is evident before the major glacial cycles (Ribas et al., 2012), as is the diversification in both genera of Amazonian titi monkeys (Byrne et al., 2018), but Amazonian *Saimiri* diversification in both the Gothic group and the Roman group occurred during the last 1 Ma, coincident with the major glacial maxima. The Pleistocene climate fluctuations promoted changes in forest structure and composition in Amazonia (Wang et al., 2017), although the extent of change and fragmentation of the rainforest to seasonal or dry forest is still debated (Baker et al., 2020; Sato et al., 2021; Maksic et al., 2022; Kern et al., 2023). These changes might have resulted in the diversification of squirrel monkeys due to their ecological requirements and due to isolation in forest refugia. Paim et al. (2018) have shown that differences in habitats (structure, flooding cycles and floristic composition) are related to differences in habitat use by *Saimiri*. These aspects might well have been major drivers for *Saimiri* diversification; the intermittent connections between fragmented forests during interglacial periods may explain the multiple signatures of gene flow found across *Saimiri* taxa in our study.

5. CONCLUSION

The genome-wide data generated with ddRADseq data matrices provides more topological certainty compared to previously published multilocus-based or UCE-based nuclear phylogenies. Our data set is the largest molecular study for squirrel monkeys (*Saimiri*). Our phylogenomic data provide strong support for two major clades within *Saimiri*, the Gothic and Roman species groups. This is concordant with mitogenomic data (Chiou et al., 2011) and nuclear genes (Springer et al., 2012) that had fewer taxa and smaller samples. In our BEAST analysis, the median estimated age for the *Saimiri* crown was similar to studies with mitogenomic and nuclear evidence, in the Pleistocene epoch (Chiou et al., 2011; Springer et al., 2012).

Altogether, our phylogeny based on genome-wide ddRADseq data was not congruent with mitochondrial loci phylogenies (Lavergne et al., 2010; Lynch Alfaro et al., 2015; Ruiz García et al., 2015; Finnegan et al., 2025), and combining information across different types of genetic and morphological studies points strongly to multiple cases of introgression during rapid speciation events across this widespread taxon's short evolutionary history. Our ddRAD phylogeny distinguished five major lineages: the Roman group (*S. b. boliviensis* + *S. vanzolinii*) and within the Gothic group the bare-eared group *S. ustus* and the tufted-eared group, distinguished in three geographic clades: Northwestern *S. cassiquiarensis*; southwestern *S. macrodon;* and eastern *S. sciureus* + *S. collinsi*. Evidence for further species divisions within these clades was mixed, and several taxa need more research. Additional sampling of *S. boliviensis* and *S. macrodon* outside of Brazil and more sampling of *S. ustus* within Brazil are of particular importance. A comparison to *S. albigena* in the Colombian Llanos and to *S. o. oerstedii* and *S. o. citronellus* in Central America will also be necessary to fully understand the extant *Saimiri* diversification.

CRediT authorship contribution statement

Michelle P. Mercês: Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Hazel Byrne:** Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis. **Natalie Finnegan:** Writing – review & editing, Methodology, Formal analysis. **Maria L. Harada:** Supervision. **Fernanda P. Paim:** Writing – review & editing, Funding acquisition, Data curation. **Felipe E. Silva:** Writing – review & editing, Funding acquisition, Data curation. **Anita Stone:** Writing – review & editing, Funding acquisition, Data curation. **Marcela G.M. Lima:** Writing – review & editing, Data curation. **Anthony B. Rylands:** Writing – review & editing, Writing – original draft. **José de Sousa e Silva Júnior:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Jessica W. Lynch:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We are grateful to the curators and museums that released tissue and blood samples for this study: João Valsecchi do Amaral (IDSM), Cláudia Regina Silva (IEPA), Suely Aparecida Marques-Aguiar (MPEG), Rogério Vieira Rossi (UFMT), Maria Lúcia Harada (UFPA), and André Ravetta. Support to MPM's PhD research was provided in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001, and in part by CAPES/PDSE - 47/2017 (process number 88881.187682/2018-01). Support for AS and JWL's research was provided respectively by NSF-BCS 2140666 and 2140675. FES received support from the Fonds National de la Recherche Scientifique (F.R.S.-FNRS, Belgium; 40017464), Brazilian National Council for Scientific and Technological Development (CNPq) (Processes 303286/2014-8, 303579/2014-5, 2000502/2015-8, 302140/2020-4, 300365/2021-7, 301407/2021-5, 301925/2021-6), the Margot Marsh Biodiversity Foundation (SMA-CCO-G0023, SMA-CCOG0037), the Primate Conservation Inc. (1713 and 1689) and the Gordon and Betty Moore Foundation (Grant 5344, to the Mamiraua Institute). The ddRADseq data were generated with a grant from Re:wild's Primate Action Fund and the Margot Marsh Biodiversity Foundation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2025.108487.

Data availability

Our ddRAD datasets and datasets used for generating phylogenies and structure analyses are available upon publication at the Dryad repository here: DOI: 10.5061/dryad.djh9w0wc0

References

Andrews, S., 2010. FastQC: A quality control tool for high throughput sequence data. Available: http://www.bioinformatics.babraham.ac.uk/projects/fastqc.

Ayres, J.M., 1985. On a new species of squirrel monkey, genus *Saimiri*, from Brazilian Amazonia (Primates, Cebidae), Pap. Avuls. Zool. S. Paulo 36, 147–164. https://doi.org/10.11606/0031-1049.1983.36.p147-164.

Baker, P.A., Fritz, S.C., Battisti, D.S., Dick, C.W., Vargas, O.M., Asner, G.P., Martin, R.E., Wheatley, A., Prates, I., 2020. Beyond refugia: New insights on quaternary climate variation and the evolution of biotic diversity in tropical South America, in: Rull, V., Carnaval, A. (Eds.), Neotropical Diversification: Patterns and Processes. Springer, Cham. DOI: 10.1007/978-3-030-31167-4_3.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Royal Statis. Soci. Series B (Methodological) 57 (1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Besnier, F., Glover, K.A., 2013. ParallelStructure: an R package to distribute parallel runs of the population genetics program STRUCTURE on multi-core computers. PLoS One, e70651. https://doi.org/10.1371/journal.pone.0070651, 29 8(7).

Blair, M.E., Melnick, D.J., 2012. Genetic evidence for dispersal by both sexes in the central American squirrel monkey, *Saimiri oerstedii citrinellus*. Am. J. Primatol. 74, 37–47. https://doi.org/10.1002/ajp.21007.

Boinski, S., Cropp, S.J., 1999. Disparate data sets resolve squirrel monkey (*Saimiri*) taxonomy: implications for behavioral, ecology and biomedical usage. Int. J. Primatol. 20, 237–256. https://doi.org/10.1023/A:1020522519946.

Boinski, S., Sughrue, K., Lara, S., Quatrone, R., Henry, M., Cropp, S., 2002. An expanded test of the ecological model of primate social evolution: competitive regimes and female bonding in three species of squirrel monkeys (*Saimiri oerstedii*, *S. boliviensis*, and *S. sciureus*). Behaviour 139, 227–261. https://doi.org/10.1163/156853902760102663.

Boubli, J.P., Rylands, A.B., Farias, I.P., Alfaro, M.E., Lynch Alfaro, J.L., 2012. *Cebus* phylogenetic relationships: a preliminary reassessment of the diversity of the untufted capuchin monkeys. Am. J. Primatol. 74 (4), 381–393. https://doi.org/10.1002/ajp.21998.

Boubli, J.P., da Silva, M.N., Rylands, A.B., Nash, S.D., Bertuol, F., Nunes, M., Mittermeier, R.A., Byrne, H., Silva, F.E., Röhe, F., Sampaio, I., 2018. How many pygmy marmoset (*Cebuella* Gray, 1870) species are there? a taxonomic re-appraisal based on new molecular evidence. Mol. Phylogenet. Evol. 120, 170–182. https://doi.org/10.1016/j.ympev.2017.11.010.

Boubli, J.P., Byrne, H., da Silva, M.N., Silva-Júnior, J.S., Araújo, R.C., Bertuol, F., Gonçalves, J., Melo, F.R., Rylands, A.B., Mittermeier, R.A., Silva, F.E., 2019. On a new species of titi monkey (Primates: *Plecturocebus* Byrne et al., 2016), from Alta Floresta, southern Amazon. Brazil. Mol. Phylogenet. Evol. 132, 117–137. https://doi.org/10.1016/j.ympev.2018.11.012.

Bouckaert, R., Vaughan, T.G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., et al., 2019. BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. PLoS Comput. Biol. 15 (4), e1006650. https://doi.org/10.1371/journal.pcbi.1006650.

Byrne, H., Rylands, A.B., Carneiro, J.C., Alfaro, J.W.L., Bertuol, F., da Silva, M.N., Messias, M., Groves, C.P., Mittermeier, R.A., Farias, I., Hrbek, T., 2016. Phylogenetic relationships of the New World titi monkeys (*Callicebus*): first appraisal of taxonomy based on molecular evidence. Front. Zool. 13 (1), 10. https://doi.org/10.1186/s12983-016-0142-4.

Byrne, H., Lynch Alfaro, J.W., Sampaio, I., et al., 2018. Titi monkey biogeography: Parallel Pleistocene spread by *Plecturocebus* and *Cheracebus* into a post-Pebas Western Amazon. Zool. Scr. 47, 499–517. https://doi.org/10.1111/zsc.12300.

Chiou, K.L., Pozzi, L., Lynch Alfaro, J.W., Di Fiore, A., 2011. Pleistocene diversification in living squirrel monkeys (*Saimiri* spp.) inferred from complete mitochondrial sequence. Mol. Phylogenet. Evol. 59, 736–745. https://doi.org/10.1016/j.ympev.2011.03.025.

Cooper, R.W., 1968. Squirrel monkey taxonomy and supply. In: Rosenblum, L.A., Cooper, R.W. (Eds.), The Squirrel Monkey. Academic Press, New York, pp. 1–29.

Costa-Araújo, R., de Melo, F.R., Canale, G.R., Hernández-Rangel, S.M., Messias, M.R., Rossi, R.V., Silva, F.E., da Silva, M.N.F., Nash, S.D., Boubli, J.P., Farias, I.P., Hrbek, T., 2019. The Munduruku marmoset: a new monkey species from southern Amazonia. PeerJ 7, e7019.

Costello, R.K., Dickinson, C., Rosenberger, A.L., Boinski, S., Szalay, F.S., 1993. Squirrel monkey (genus *Saimiri*) taxonomy. a multidisciplinary study of the biology of species. In: Kimbel, W., Martin, L. (Eds.), Species, Species Concepts and Primate Evolution. Plenum Press, New York, pp. 177–210, 10.1007/978-1-4899-3745-2_8.

Cropp, S., Boinski, S., 2000. The central American Squirrel Monkey (*Saimiri oerstedii*): introduced hybrid or endemic species? Mol. Phylogenet. Evol. 16 (3), 350–365. https://doi.org/10.1006/mpev.2000.0814.

Douglas, J., Zhang, R., Bouckaert, R., 2021. Adaptive dating and fast proposals: Revisiting the phylogenetic relaxed clock model. PLoS Comput. Biol. 17 (2), e1008322. https://doi.org/10.1371/journal.pcbi.1008322.

Earl, D.A., VonHoldt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4 (2), 359–361. https://doi.org/10.1007/s12686-011-9548-7.

Eaton, D.A.R., Overcast, I., 2016. iPYRAD: Interactive assembly and analysis of RADseq data sets. Bioinformatics 36 (8), 2592–2594. https://doi.org/10.1093/bioinformatics/btz966.

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14, 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x.

Finnegan, N., Lima, M.G.M., Lynch, J.W., 2025. Mitochondrial DNA for phylogeny building: Assessing individual and grouped mtGenes as proxies for the mtGenome in platyrrhines. Am. J. Primatol. 87, e70017. https://doi.org/10.1002/ajp.70017.

Groves, C.P., 2001. Primate Taxonomy. Smithsonian Institution Press, Washington, DC.

Groves, C.P., 2005. Order Primates. In: Wilson, D.E., Reeder, D.M. (Eds.), Mammal Species of the World: A Taxonomic and Geographic Reference. Johns Hopkins University Press, Baltimore, MD, pp. 111–184, 10.1644/06-MAMM-R-422.1.

Hartwig, W.C., Meldrum, D.J., 2002. Miocene platyrrhines of the northern Neotropics. In: Hartwig, W.C. (Ed.), The Primate Fossil Record. Cambridge University Press, Cambridge, pp. 175–188.

Hermosilla-Albala, N., Silva, F.E., Cuadros-Espinoza, S., et al., 2024. Whole genomes of amazonian uakari monkeys reveal complex connectivity and fast differentiation driven by high environmental dynamism. Commun. Biol. 7, 1283. https://doi.org/10.1038/s42003-024-06901-3.

Hershkovitz, P., 1984. Taxonomy of squirrel monkeys, genus *Saimiri* (Cebidae, Platyrrhini): a preliminary report with description of a hitherto unnamed form. Am. J. Primatol. 7 (2), 155–210. https://doi.org/10.1002/ajp.1350060402.

Hershkovitz, P., 1987. Uacaries, New World monkeys of the genus *Cacajao* (Cebidae, Platyrrhini): a preliminary taxonomic review with the description of a new subspecies. Am. J. Primatol. 12 (1), 1–53. https://doi.org/10.1002/ajp.1350120102.

Jakobsson, M., Rosenberg, N.A., 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23 (14), 1801–1806. https://doi.org/10.1093/bioinformatics/btm233.

Janiak, M.C., Silva, F.E., Beck, R.M.D., de Vries, D., Kuderna, L.F.K., Torosin, N.S., Melin, A.D., Marquès-Bonet, T., Goodhead, I.B., Messias, M., da Silva, M.N.F., Sampaio, I., Farias, I.P., Rossi, R., de Melo, F.R., Valsecchi, J., Hrbek, T., Boubli, J.P., 2022. Two hundred and five newly assembled mitogenomes provide mixed evidence for rivers as drivers of speciation for amazonian primates. Mol. Ecol. 31, 3888–3902. https://doi.org/10.1111/mec.16554.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., Jermiin, L.S., 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. Nat. Methods 14, 587–589. https://doi.org/10.1038/nmeth.428.

Kern, A.K., Akabane, T.K., Ferreira, J.Q., Chiessi, C.M., Willard, D.A., Ferreira, F., Sanders, A.O., Silva, C.G., Rigsby, C., Cruz, F.W., Dwyer, G.S., 2023. A 1.8 million-year history of Amazon vegetation. Quat. Sci. Rev. 299, 107867.

Kozlov, A.M., Darriba, D., Flouri, T., Morel, B., Stamatakis, A., 2019. RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. Bioinformatics 35 (21), 4453–4455. https://doi.org/10.1093/bioinformatics/btz305.

Kuderna, L.F.K., Gao, H., Janiak, M.C., Kuhlwilm, M., Orkin, J.D., Bataillon, T., Manu, S., Valenzuela, A., Bergman, J., Rousselle, M., Silva, F.E., Agueda, L., Blanc, J., Gut, M., de Vries, D., Goodhead, I., Harris, R.A., Raveendran, M., Jensen, A., Marques Bonet, T., 2023. A global catalog of whole-genome diversity from 233 primate species. Science 380 (6648), 906–913. https://doi.org/10.1126/science.abn7829.

Lavergne, A., Ruiz-García, M., Catzeflis, F., Lacote, S., Contamin, H., Mercereau-Puijalon, O., Lacoste, V., Thoisy, B., 2010. Phylogeny and phylogeography of squirrel monkey (genus *Saimiri*) based on cytochrome b genetic analysis. Am. J. Primatol. 72, 242–253. https://doi.org/10.1002/ajp.20773.

Lima, M.G., Silva-Júnior, J.S., Černý, D., Buckner, J.C., Aleixo, A., Chang, J., Zheng, J., Alfaro, M.E., Martins, A., Di Fiore, A., Boubli, J.P., Lynch, J.W., 2018. A phylogenomic perspective on the robust capuchin monkey (*Sapajus*) radiation: first evidence for extensive population admixture across South America. Mol. Phylogenet. Evol. 124, 137–150. https://doi.org/10.1016/j.ympev.2018.02.023.

Lynch Alfaro, J.W., Boubli, J.P., Paim, F.P., Ribas, C.C., da Silva, M.N.F., Messias, M.R., Röhe, F., Mercês, M.P., Júnior, J.S.S., Silva, C.R., Pinho, G.M., 2015. Biogeography of squirrel monkeys (genus *Saimiri*): South-central Amazon origin and rapid pan-amazonian diversification of a lowland primate. Mol. Phylogenet. Evol. 82, 436–454. https://doi.org/10.1016/j.ympev.2014.09.004.

MacFadden, B.J., 1990. Chronology of Cenozoic primate localities in South America. J. Hum. Evol. 19, 7–21. https://doi.org/10.1016/0047-2484(90)90010-9.

MacLean, P.D., 1964. Mirror display in squirrel monkey *Saimiri sciureus*. Science 146, 950–952. https://doi.org/10.1126/science.146.3646.950.

Maksic, J., Venancio, I.M., Shimizu, M.H., Chiessi, C.M., Piacsek, P., Sampaio, G., Cruz, F.W., Alexandre, F.F., 2022. Brazilian biomes distribution: past and future. Palaeogeogr. Palaeoclimatol. Palaeoecol. 585, 110717. https://doi.org/10.1016/j.palaeo.2021.110717.

Malinsky, M., Matschiner, M., Svardal, H., 2021. Dsuite - Fast *D*-statistics and related admixture evidence from VCF files. Mol. Ecol. Resour. 21, 584–595. https://doi.org/10.1111/1755-0998.13265.

Martin, M., 2010. Cutadapt removes adapter sequences from high-throughput sequencing reads. Embnet J. 5–7, 0.14806/ej.17.1.200.

Martins, A.B., Valença-Montenegro, M.M., Lima, M.G.M., Lynch, J.W., Svoboda, W.K., Silva-Júnior, J.d.S.e., Röhe, F., Boubli, J.P., Fiore, A.D., 2023. A new assessment of robust capuchin monkey (*Sapajus*) evolutionary history using genome-wide SNP marker data and a Bayesian approach to species delimitation. Genes 14 (5), 970. https://doi.org/10.3390/genes14050970.

Mercês, M.P., Alfaro, J.W.L., Ferreira, W.A., Harada, M.L., Silva-Júnior, J.S., 2015. Morphology and mitochondrial phylogenetics reveal that the Amazon River separates two eastern squirrel monkey species: *Saimiri sciureus* and *S. collinsi*. Mol. Phylogenet. Evol. 82, 426–435. https://doi.org/10.1016/j.ympev.2014.09.020.

Mercês, M.P., de Paula, W.S., Silva-Júnior, J.S., 2017. New records of *Saimiri collinsi* Osgood, 1916 (Cebidae, Primates), with comments on habitat use and conservation. Mammalia 82 (5), 516–520. https://doi.org/10.1515/mammalia-2017-0050.

Miller, M., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Gateway Computing Environments Workshop (GCE). New Orleans, LA. doi.10.1109/GCE.2010.5676129.

Mitchell, C.L., Boinski, S., Van Schaik, C., 1991. Competitive regimes and female bonding in two species of squirrel monkeys (*Saimiri oerstedii* and *S. sciureus*). Behav. Ecol. Sociobiol. 28, 55–60. https://doi.org/10.1007/BF00172139.

Paim, F.P., Rabelo, R.M., 2015. Methods for capturing wild squirrel monkeys in a floodplain forest: a comparison of two techniques and two species. Neotrop. Primates 22, 7–11. https://doi.org/10.62015/np.2015.v22.146.

Paim, F.P., Silva Júnior, J.S., Valsecchi, J., Harada, M.L., Queiroz, H.L., 2013. Diversity, geographic distribution and conservation of squirrel monkeys, *Saimiri* (Primates, Cebidae), in the floodplain forest of Central Amazon. Int. J. Primatol. 34, 1055–1076. https://doi.org/10.1007/s10764-013-9714-8.

Paim, F.P., Valenta, K., Chapman, C.A., Paglia, A.P., de Queiroz, H.L., 2018. Tree community structure reflects niche segregation of three parapatric squirrel monkey species (*Saimiri* spp.). Primates 59 (4), 395–404. https://doi.org/10.1007/s10764-013-9714-8.

Perelman, P., Johnson, W.E., Roos, C., Seuánez, H.N., Horvath, J.E., Moreira, M.A.M., Kessing, B., Pontius, J., Roelke, M., Rumpler, Y., Schneider, M.P.C., Silva, A., O'Brien, S.J., Pecon-Slattery, J., 2011. A molecular phylogeny of living primates. PLoS Genet. 7 (3), e1001342. https://doi.org/10.1371/journal.pgen.1001342.

Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., Hoekstra, H.E., 2012. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One 7 (5). https://doi.org/10.1371/journal.pone.0037135.

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959. https://doi.org/10.1093/genetics/155.2.945.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A., 2018. Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. Syst. Biol. https://doi.org/10.1093/sysbio/syy032.

Ribas, C.C., Aleixo, A., Nogueira, A.C., Miyaki, C.Y., Cracraft, J., 2012. A palaeobiogeographic model for biotic diversification within Amazonia over the past three million years. Proc. Roy. Soc. Lond. Biol. 279 (1729), 681–689. https://doi.org/10.1098/rspb.2011.1120.

Rogers, J., Raveendran, M., Harris, R.A., Mailund, T., Leppälä, K., Athanasiadis, G., Schierup, M.H., Cheng, J., Munch, K., Walker, J.A., Konkel, M.K., 2019. The comparative genomics and complex population history of *Papio* baboons. Sci. Adv. 5 (1). https://www.science.org/doi/10.1126/sciadv.aau6947.

Rosenberger, A.L., 1981. Systematics: the higher taxa. In: Coimbra-Filho, A.F., Mittermeier, R.A. (Eds.), Ecology and Behavior of Neotropical Primates. Academia Brasileira de Ciências, Rio de Janeiro, pp. 9–27.

Rosenberger, A.L., 2011. Evolutionary morphology, platyrrhine evolution, and systematics. Anat. Rec. 294, 1955–1974. https://doi.org/10.1002/ar.21511.

Ruiz García, M., Luengas-Villamil, K., Leguizamon, N., de Thoisy, B., Gálvez, H., 2015. Molecular phylogenetics and phylogeography of all the *Saimiri* taxa (Cebidae, Primates) inferred from mt COI and COII gene sequences. Primates 56 (2), 145–161. https://doi.org/10.1007/s10329-014-0452-0.

Rylands, A.B., Mittermeier, R.A., Bezerra, B.M., Paim, F.P., Queiroz, H.L., 2013. Species accounts of Cebidae. In: Mittermeier, R.A., Rylands, A.B., Wilson, D.E. (Eds.), Handbook of the Mammals of the World, Volume 3. Primates. Lynx Edicions, pp. 390–413.

Rylands, A.B., Mittermeier, R.A., Lynch, J.W., Jerusalinsky, L., Strier, K.B., Cortés-Ortiz, L., Link, A., de la Torre, S., de Melo, F.R., Canale, G.R., Boubli, J.P., Cornejo, F. M., Sechrest, W., 2024. Illustrated Checklist of the Neotropical Primates. Lynx Edicions, Barcelona https://www.lynxeds.com/product/neotropical-primates/.

Sato, H., Kelley, D.I., Mayor, S.J., Colvo, M.M., Cowling, S.A., Prentice, I.C., 2021. Dry corridors opened by fire and low CO2 in amazonian rainforest during the last Glacial Maximum. Nat. Geosci. 14, 578e585. https://doi.org/10.1038/s41561-021-00777-2.

Silva, F.E., Costa-Araújo, R., Boubli, J.P., Santana, M.I., Franco, C.L., Bertuol, F., Nunes, H., Silva-Júnior, J.S., Farias, I., Hrbek, T., 2018. In search of a meaningful classification for amazonian marmosets: should dwarf marmosets be considered *Mico* congenerics? Zool. Scripta 47 (2), 133–143. https://doi.org/10.1111/zsc.12278.

Silva, F.E., Valsecchi do Amaral, J., Roos, C., Bowler, M., Röhe, F., Sampaio, R., Cora Janiak, M., Bertuol, F., Ismar Santana, M., Silva Jr., J., Rylands, A.B., Gubili, C., Hrbek, T., McDevitt, A.D., Boubli, J.P., 2022. Molecular phylogeny and systematics of bald uakaris, genus *Cacajao* lesson, 1840 (Primates: Pitheciidae), with the description of a new species. Mol. Phylogenet. Evol. 173. https://doi.org/10.1016/j.ympev.2022.107509.

Silva, F.E., Luna, L.W., Batista, R., Röhe, F., Gubili, C., Farias, I.P., Boubli, J.P., 2024. Impact of Quaternary amazonian river dynamics on the diversification of uakari monkeys (genus *Cacajao*). J. Biogeogr. 51, 1505–1517. https://doi.org/10.1111/jbi.14844.

Springer, M.S., Meredith, R.W., Gatesy, J., Emerling, C.A., Park, J., Rabosky, D.L., Stadler, T., Steiner, C., Ryder, O.A., Janečka, J.E., Fisher, C.A., 2012. Macroevolutionary dynamics and historical biogeography of primate diversification inferred from a species supermatrix. PLoS One 7 (11), e49521. https://doi.org/10.1371/journal.pone.0049521.

Stone, A., Castro, P.H.G., Monteiro, F.O.B., Ruivo, L.P., Silva Júnior, J.de S., 2015. A novel method for capturing and monitoring a small Neotropical primate, the squirrel monkey (*Saimiri collinsi*). Am. J. Primatol. 77, 239–245. https://doi.org/10.1002/ajp.22328.

Thorington Jr., R.W., 1985. The taxonomy and distribution of squirrel monkey (*Saimiri*). In: Rosenblum, L.A., Coe, C.L. (Eds.), Handbook of Squirrel Monkey Research. Plenum Press, New York, pp. 1–33, 10.1007/978-1-4757-0812-7_1.

Tosi, A.J., Morales, J.C., Melnick, D.J., 2003. Paternal, maternal, and biparental molecular markers provide unique windows onto the evolutionary history of macaque monkeys. Evolution 57 (6), 1419–1435. https://doi.org/10.1111/j.0014-3820.2003.tb00349.x.

Valencia, L.M., Martins, A., Ortiz, E.M., Di Fiore, A., 2018. A RAD-sequencing approach to genome-wide marker discovery, genotyping, and phylogenetic inference in a diverse radiation of primates. PLoS One 13 (8), e0201254. https://doi.org/10.1371/journal.pone.0201254.

Wang, L.G., Lam, T.T.Y., Xu, S., Dai, Z., Zhou, L., Feng, T., Guo, P., Dunn, C.W., Jones, B. R., Bradley, T., Zhu, H., Guan, Y., Jiang, Y., Yu, G., 2020. treeio: an R package for phylogenetic tree input and output with richly annotated and associated data. Mol. Biol. Evol. 37 (2), 599–603. https://doi.org/10.1093/molbev/msz240.

Wang, X., Edwards, R.L., Auler, A.S., Cheng, H., Kong, X., Wang, Y., Cruz, F.W., Dorale, J. A., Chiang, H.W., 2017. Hydroclimate changes across the Amazon lowlands over the past 45,000 years. Nature 541 (7636), 204–207. https://doi.org/10.1038/nature20787.

Wong, T.K., Ly-Trong, N., Ren, H., Baños, H., Roger, A.J., Susko, E., Bielow, C., De Maio, N., Goldman, N., Hahn, M.W., Huttley, G., 2025. IQ-TREE 3: Phylogenomic Inference Software using Complex Evolutionary Models. DOI: 10.32942/X2P62N.

Wickham, H., 2016. Data Analysis, in: ggplot2. Use R! Springer, Cham. DOI: 10.1007/978-3-319-24277-4_9.

Zinner, D., Groeneveld, L.F., Keller, C., Roos, C., 2009. Mitochondrial phylogeography of baboons (*Papio* spp.)—indication for introgressive hybridization? BMC Evol. Biol. 9 (1), 83. https://doi.org/10.1186/1471-2148-9-83.