



# Spatiotemporal Diversification of Tree Squirrels: Is the South American Invasion and Speciation Really That Recent and Fast?

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Tree squirrels (Sciurinae, Sciurini) represent a diverse radiation that successfully colonized Europe, Asia and the Americas during the Miocene-Pliocene, but information on their evolutionary history remains unclear. In the Neotropics, they have been shown to exhibit the highest rate of diversification amongst all arboreal squirrels, with strikingly high species accumulation rates in the past 3 Mya. In this study, we investigated the tempo and mode of diversification of tree squirrels using a mitogenome dataset that includes 43 Sciurini species. Our results corroborate the date of origin of the tribe Sciurini around 14 Mya (13.4–15.5) but suggest that their ancestral area was most likely in North America. This is in contrast to previous findings that suggested that the ancestors of this tribe occupied Eurasia. We estimated that cladogenetic events leading to the Eurasian lineages occurred twice at 10.5 and 9.7 Mya. Current North American genera originated in a temporal window from 6.2–2.3 Mya, and the origin of the Neotropical radiation was estimated to have occurred around 6 Mya in northwestern South America, in the Pacific dominion. Remarkably, our results indicate that tree squirrels entered South America at an earlier date than previously estimated. This could have happened either through a land corridor connecting the Caribbean islands or through the Panamanian land bridge. Most cladogenetic events in Eurasia and North America appear to have occurred either late in the Miocene or in the Pleistocene, while the majority of Neotropical cladogenetic events occurred along the Pliocene—right after the South American invasion. We found a fairly constant speciation rate for tree squirrels (averaging 0.29), which contrasts with the peak of lineage accumulation observed in the Pliocene. The absence of fluctuations

in the diversification rate may be the result of several extinction events that were responsible for equalizing the number of lineages maintained over time. Finally, we conclude that the South American invasion was not as recent as previously inferred, but the diversification there was indeed very fast.

**Keywords:** ancestral range, founder-event speciation, Isthmus of Panama, mitogenome, Neotropics, Sciurini

## INTRODUCTION

Tree squirrels (Sciurinae, Sciurini) represent a diverse radiation that successfully colonized forested landscapes of Europe, Asia and the Americas during the Miocene and Pliocene (Mercer and Roth, 2003; Pečnerová et al., 2015). The timing of the origin of the tribe is imprecise and has been estimated to have occurred from 19 to 13 million years ago (Mya; Mercer and Roth, 2003; Fabre et al., 2012; Pečnerová et al., 2015; Zelditch et al., 2015). In addition, the uncertainties regarding the location of their ancestral geographic range have also contributed to the blurred inferences of the evolutionary history of tree squirrels. Pečnerová and Martínková (2012) suggested that Sciurini originated in the northern hemisphere, not distinguishing between Eurasia and North America. A similar result was found by Rocha et al. (2016), who proposed a Holarctic distribution for the ancestor of tree squirrels. Subsequently, Pečnerová et al. (2015) estimated a more restricted ancestral range of Sciurini, confined to the Palearctic region.

Discrepancies on inferences about the spatiotemporal diversification of Sciurini are likely a result of the limited datasets, in terms of geographic and taxonomic coverage, employed by previous analyses. None of the aforementioned studies included more than a third of the South American tree squirrel species and also missed some Central and North American taxa (sensu Thorington et al., 2012; Vivo and Carmignotto, 2015); therefore, the lack of so many taxa and presumed lineages might mislead phylogenetic and, consequently, biogeographic inferences. A recent comprehensive phylogenomic study conducted by Abreu-Jr et al. (2020), that included samples from 40 of the 43 recognized species of Sciurini (sensu Thorington et al., 2012; Vivo and Carmignotto, 2015; Hope et al., 2016), revealed an underestimated diversity on the genus and species group levels within the tribe and clarified several important points with regards to the phylogenetic relationships and systematics of tree squirrels. This study did not find support for the recognition of two species previously considered as valid—*Sciurus richmondi* sensu Thorington et al. (2012) and *Microsciurus venustus* sensu Vivo and Carmignotto (2015)— and found evidence for the recognition of six additional lineages that might represent species to be named or revalidated. This new taxonomic proposal suggested a diversity of 46 species within Sciurini which were tentatively organized in 14 genera coined by previous authors (see Abreu-Jr et al., 2020). The mitogenome dataset produced in that study is highly suitable to investigate the tempo and mode of evolution of tree squirrels, especially across the Neotropical region, where the taxonomic and geographic sampling were strengthened.

In the Neotropics, tree squirrels have been shown to exhibit the highest rate of diversification amongst all arboreal squirrels (a non-phylogenetic ecological category that includes members of four subfamilies; Roth and Mercer, 2008), with strikingly high species accumulation rates in the past three million years, presumably after the final closure of the Panamanian land bridge and the establishment of the Great American Biotic Interchange (GABI). The GABI is one of the most significant events of transcontinental fauna dispersal that occurred during the temporal window from the late Pliocene to the late Pleistocene, wherein several mammal lineages migrated from North America to South America and the other way around (Simpson, 1983; Stehli and Webb, 1985; Woodburne, 2010). Among rodents, tree squirrels and sigmodontine rodents arguably shared a very successful history of diversification and colonization of South America accompanying the GABI (Patterson and Pascual, 1972; Simpson, 1983).

However, alternative scenarios proposed either a diversification at the generic level in tropical portions of North and Central America, followed by an invasion of South America, after the Panamanian land bridge arose; or an early entrance in South America, sometime in the Miocene, prior to the establishment of the Panamanian land bridge and the GABI (see detailed explanation in D'Elia, 2000). For sigmodontine rodents, current hypotheses suggest an early arrival in South America (Smith and Patton, 1999; Stepan et al., 2004; Parada et al., 2013), in the Miocene, supporting the scenario proposed by Hershkovitz (1966, 1969) and Reig (1984). For squirrels, current hypotheses sustain the entrance of this group in South America during the GABI, after the Panamanian land bridge arose (Mercer and Roth, 2003), but results so far are still inconclusive, as most of the South American squirrel diversity remained unstudied.

These rodents are an ideal group of mammals that can help to address a wide range of biogeographic questions, such as the timing of the dispersal events across the Americas and colonization within South America, and the potential relevance of specific areas as sources of diversity within the Neotropics. The South American continent has a history of major geological (such as the Andes uplift and the Amazon basin formation) and climatic (such as the Pleistocene climate fluctuations) changes that occurred in the past 10 million years. These events gave rise to a great variety of environments and new habitats that leveraged the rapid diversification of several lineages of the tree of life (Hoorn et al., 2010).

In this study, we test current hypotheses on the tempo and mode of diversification of tree squirrels (Pečnerová and Martínková, 2012; Pečnerová et al., 2015) employing a comprehensive dataset (mitogenome data) and a diverse

taxonomic coverage (including 43 Sciurini putative species; Abreu-Jr et al., 2020). We provided a time-scaled phylogeny to infer the timing of the origin and diversification of the main lineages, and performed biogeographic analyses to estimate ancestral ranges and evaluate the drivers of diversification at a global scale. In addition to the broad-scale analysis that includes all Sciurini species—and since the Neotropics is home of the most diverse assemblage of species—we also performed a finer-scale analysis focused exclusively on the diversification processes of Neotropical taxa, to unravel their colonization history across Central and South Americas. Finally, we tested the hypothesis that tree squirrels represent a rapid-diversified radiation (Roth and Mercer, 2008) by estimating speciation rates and investigating diversification rate fluctuations through time.

## MATERIALS AND METHODS

### Dataset Taxonomic Composition

Our dataset is composed of 14 genera and 43 species of Sciurini delimited in a recent contribution (Abreu-Jr et al., 2020). Taxonomic identifications at the genus and species levels follow Abreu-Jr et al. (2020). At the species level, those authors kept the museum identifications for several specimens, especially those housed at the American Museum of Natural History (AMNH) and at the Smithsonian National Museum of Natural History (USNM), which had been made by some of the main authorities on tree squirrel taxonomy (e.g., R. W. Thorington and M. de Vivo). For material not previously identified, they performed identifications by examining the morphology of the vouchers, consulting original descriptions and other relevant literature. In cases of unviability to examine vouchers, original museum identifications were accepted if (i) those identifications correspond to the known geographic distribution of the taxon in question, and (ii) phylogenetic analyses of their DNA sequences were consistent with the museum identification. The currently valid species of tree squirrels not included in this study are: *Tamiasciurus fremonti* sensu Hope et al. (2016), *Microsciurus santanderensis* and *M. simonsi* sensu Vivo and Carmignotto (2015). We used as outgroup two representatives of the tribe Pteromyini (the sister-tribe of Sciurini): *Glaucomys volans* (USNM 569823) and *Hylopetes phayrei* (USNM 584420). A complete list of specimens analyzed in this study accompanied by geographic information and GenBank accession numbers for complete mitogenomes is provided as (Supplementary Table S1).

### DNA Matrix, Alignment and Saturation Test

We selected the 13 mitochondrial protein-coding genes (CDS) from one sample of each of the 43 putative species of Sciurini to perform the analyses. The CDS matrix was aligned using MUSCLE (Edgar, 2004), with up to eight iterations. We investigated the presence of saturation in our dataset by plotting transitions and transversions against nucleotide divergence in DAMBE7 (Xia, 2018).

### Divergence Times Estimate

Divergence times were estimated using a Bayesian framework in BEAST 2.6.1 (Bouckaert et al., 2019). We applied a single nucleotide substitution model (GTR) with a gamma category of 4 to our dataset and implemented a lognormal relaxed clock with a Yule tree prior. The analysis was conducted by running 600 million generations of Markov chain Monte Carlo (MCMC), sampling every 60,000 generations. The results of MCMC runs were visualized in Tracer 1.7 (Rambaut et al., 2018) to confirm a minimum of 200 effective sample size for all parameters. A time-calibrated tree was generated with TreeAnnotator v2.6.0 (Bouckaert et al., 2019), considering 10% of burnin and selecting the maximum clade credibility as the target tree. We do not specify posterior limit for the nodes and we selected mean heights for the common ancestor heights. BEAST analysis was performed in the CIPRES Science Gateway (Miller et al., 2010).

We employed four calibrations points representing three distinct types of priors: (i) fossil record – the first known fossil record of *Tamiasciurus hudsonicus*, dated from the Irvingtonian (from 1.9 to 0.25 Mya; Steele, 1998), was implemented as uniform prior for the root of *Tamiasciurus*; (ii) fossil record – *Sciurus alleni* was first reported from the Late Pleistocene (from 0.126 to 0.0117 Mya; Jakway, 1958) and it was constrained as uniform prior for the split between *S. alleni* and *S. oculatus*; (iii) geological events plus fossil record – the crown of Nearctic *Sciurus* was constrained with uniform prior between 13.6 and 7.4 Mya, considering that *Sciurus* was present in North America since the late Miocene (Clarendonian; *Sciurus olsoni*; Emry et al., 2005) and this radiation supposedly arrived from Eurasia (Pečnerová et al., 2015), thus the colonization occurred before the initial opening of the Bering Strait about 7.4 Mya (Marincovich and Gladenkov, 1999); (iv) secondary estimation – we constrained the root of Sciurini with log-normal prior with offset of 13.4 Mya ( $M = 0.5$  and  $S = 1.0$ ), according to results of Upham et al. (2019), which is currently the most comprehensive phylogeny of mammals.

### Ancestral Range and Diversification Events Estimate

We estimated the ancestral range of the phylogenetic nodes and investigated the historical events (e.g., vicariance, dispersal) that might be evoked to explain the diversification pattern of the tribe Sciurini. These analyses were performed employing a maximum likelihood framework using the R package BioGeoBEARS (Matzke, 2013). The time-scaled topology provided by BEAST with only one individual per species was used as the input tree. Six models of evolution—which distinctively incorporate vicariance, dispersal and extinction throughout the cladogenesis—were tested, as follows: (1) Dispersal-Extinction-Cladogenesis (DEC; Ree, 2005), (2) DEC + founder-event speciation (“jump”; DEC + J), (3) Dispersal-Vicariance Analyses (DIVALIKE; Ronquist, 1997), (4) DIVALIKE + J, (5) Bayesian inference of historical biogeography for discrete areas (BAYAREALIKE; Landis et al., 2013), and (6) BAYAREALIKE + J. The best-fitting model was selected based on

the Akaike Information Criterion weights (AICw; Wagenmakers and Farrell, 2004).

These biogeographic analyses were performed in two geographic scales: global and Neotropical. The first included all Sciurini species and we investigated their colonization history across large continental areas; species were coded as occupying: (1) Eurasia, (2) Borneo, (3) North America, (4) Central America, and (5) South America. The second focused exclusively on the diversification of Neotropical taxa. For this finer-scale analysis, we followed the Neotropical biogeographical dominions proposed by Morrone (2014) to categorize the current geographical range of species, which were coded as occurring at: (1) the Mexican transition zone, (2) the Mesoamerican dominion, (3) the Pacific dominion, (4) the Boreal Brazilian dominion, (5) the South Brazilian dominion, (6) the South-eastern Amazonian dominion, and (7) the Parana dominion.

## Lineage Through Time and Speciation Rates

We investigated the diversification pattern of Sciurini by constructing a lineage through time (LTT) plot using the R package Ape (Paradis and Schliep, 2019). Speciation rates and the presence and temporal location of putative diversification rate shifts were explored by employing the Bayesian Analysis of Macroevolutionary Mixtures (BAMM; Rabosky, 2014; Rabosky et al., 2017). We implemented a speciation-extinction model, run for 10 million generations and sampled every 2,000 generations in BAMM v2.5.0. We incorporated a sampling frequency of 0.93, considering that we have sampled about 93% of the extant species of Sciurini (43 out of the 46 putative species; Abreu-Jr et al., 2020). Results of BAMM analysis were processed and visualized using the R package BAMMtools (Rabosky et al., 2014).

## RESULTS

### Phylogeny of the Tribe Sciurini

Our final mitogenome alignment was 11,278 bp long and included 45 species (43 ingroup and two outgroup). The mean sequence length per sample was 10,360 bp (ranging from 1,393 to 11,278 bp) and the overall matrix missing data (including gaps and undetermined characters) was 7.4%. We found no evidence for nucleotide substitution saturation in our sequences that could influence the phylogenetic signal (Supplementary Figure 1). The maximum clade credibility tree obtained from our BEAST analysis recovered similar relationships to those previously reported by Abreu-Jr et al. (2020), with all nodes strongly supported (PP  $\geq$  0.98), excepted by the node of the genus *Echinosciurus*, which did not receive a significant support (PP < 0.95).

### Model Selection in Ancestral Range Estimates

The analyses performed with BioGeoBEARS at the global scale recovered DEC + J as the most suitable model to explain the

diversification of Sciurini (LnL =  $-47.61$ , AICwt = 0.82; Table 1), considering distinct evolutionary events, such as dispersal, extinction and vicariance. The ancestral range estimation focused on the Neotropical taxa recovered that the model BAYAREALIKE + J better fit our data (LnL =  $-68.79$ , AICwt = 0.85; Table 2), favoring dispersal-extinction scenarios rather than vicariance events.

## Spatiotemporal Diversification of Sciurini at a Global Scale

The origin of the tribe Sciurini was estimated at 14.1 Mya (13.4–15.5; Figure 1; see also Supplementary Figure 2) and its ancestral geographic range was inferred with highest probability to North America (58.6%; Figure 1). An area including both North America and Eurasia was estimated with the second highest probability of occurrence for the ancestor of Sciurini (30.6%; Supplementary Table S2). The first lineage to diverge within the tribe was the genus *Tamiasciurus*. All of the known species of this genus are from North America and its ancestor was also estimated to have occurred in North America. The subsequent diversification event, leading to the Borneo endemic genus *Rheithrosciurus*, occurred at 10.5 Mya (9.5–11.7) and the ancestral range of this node was also in North America. Likewise, the ancestral ranges of the nodes leading to the Eurasian genus *Sciurus* (at 9.7 Mya), the North American genus *Hesperosciurus* (at 7.6 Mya), and the clade including both North American genera *Neosciurus* and *Parasciurus* (at 6.2 Mya), were most likely to be in North America. In summary, diversification events generating the major lineages from North America and Eurasia took place in the mid-late Miocene, whereas most speciation events involving extant species in those lineages occurred in the Pleistocene (Figure 1).

The large clade including exclusively Neotropical taxa (marked with a black star in Figure 1) originated with highest probability in South America at 5.9 Mya (5.2–6.5). Within this clade, the Central American radiation (including genera *Microsciurus*, *Syntheosciurus*, and *Echinosciurus*) originated at 4.6 Mya (3.8–5.3) and the South American lineage (including genera *Leptosciurus*, *Simosciurus*, *Guerlinguetus*, “*Microsciurus*,” and *Hadroskiurus*) at 5.0 Mya (4.3–5.6). Therefore, these Central and South American radiations, as well as most Neotropical genera, originated in the early-mid Pliocene and experienced a rapid diversification, with speciation events occurring mostly in the Pliocene and in the Plio-Pleistocene transition, and only a few during the Pleistocene (Figure 1).

The ancestral range estimation of the tribe Sciurini on a global scale (Figure 1) allows the inference of seven founder-event speciation and three anagenetic dispersals, while it does not indicate the occurrence of vicariance events during the evolutionary history of tree squirrels (see Supplementary Figures 3, 4). The founder events (i.e., a jump of a lineage to a new area of occurrence outside of the ancestral range of the node; Matzke, 2013) were inferred for the ancestors of (1) the genus *Rheithrosciurus* from North America to Borneo, (2) the Eurasian genus *Sciurus* from North America



**TABLE 1** | Results of the six biogeographic models implemented by BioGeoBEARS on the ancestral range estimation of Sciurini in a global scale.

Model	LnL	Parameters	d	e	j	AIC	AICwt
DEC	-52.54	2	0.018	0.007	0	109.1	0.016
<b>DEC + J</b>	<b>-47.61</b>	<b>3</b>	<b>0.007</b>	<b>1.00E-12</b>	<b>0.023</b>	<b>101.2</b>	<b>0.820</b>
DIVALIKE	-54.74	2	0.022	1.00E-12	0	113.5	0.002
DIVALIKE + J	-49.39	3	0.009	1.00E-12	0.025	104.8	0.140
BAYAREALIKE	-73.16	2	0.020	0.078	0	150.3	1.80E-11
BAYAREALIKE + J	-51.33	3	0.006	1.00E-07	0.031	108.7	0.020

Fits of alternative models were compared using AIC values and AIC weights (AICwt). The model that provided the best fit to our data is in bold.

**TABLE 2** | Results of the six biogeographic models implemented by BioGeoBEARS on the ancestral range estimation of Sciurini in a Neotropical scale.

Model	LnL	Parameters	d	e	j	AIC	AICwt
DEC	-73.30	2	0.025	2.00E-08	0	150.6	0.025
DEC + J	-72.82	3	0.023	1.00E-12	0.008	151.6	0.015
DIVALIKE	-79.16	2	0.031	4.10E-09	0	162.3	7.20E-05
DIVALIKE + J	-77.19	3	0.026	1.00E-12	0.012	160.4	0.0002
BAYAREALIKE	-71.81	2	0.013	0.160	0	147.6	0.110
<b>BAYAREALIKE + J</b>	<b>-68.79</b>	<b>3</b>	<b>0.012</b>	<b>0.038</b>	<b>0.014</b>	<b>143.6</b>	<b>0.850</b>

Fits of alternative models were compared using AIC values and AIC weights (AICwt). The model that provided the best fit to our data is in bold.

to the Palearctic region, (3) the large Neotropical radiation from North America to South America, (4) the Central American clade from South America to Central America, (5) the clade *Parasciurus alleni* + *P. oculatus* from North America to Central America, (6) *Parasciurus nayaritensis* from North America to Central America, and (7) *Microsciurus* “species 1” from Central America to South America. Anagenetic dispersals included (1) the colonization of Central America by *Hesperosciurus aberti*—although this species occurs in Central America mainly in the Sierra Madre Occidental and there is a discussion whether this region is part of the Nearctic region (Holt et al., 2013) or it belongs to the Neotropical region (Morrone, 2014)—, (2) colonization of South America by *Syntheosciurus granatensis*, and (3) colonization of Central America by the ancestor of *Leptosciurus boquetensis* and *L. isthmus*.

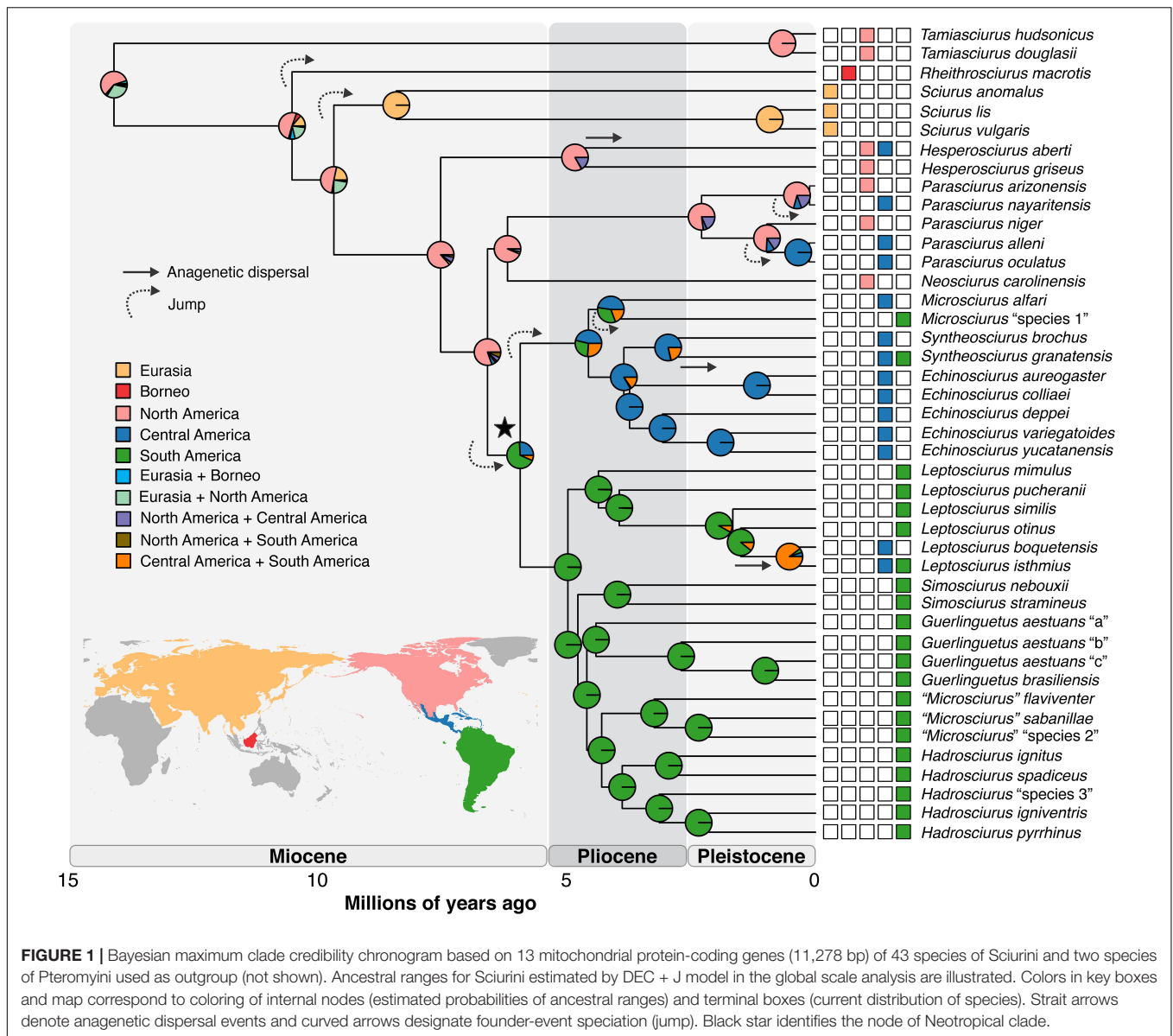
## Spatiotemporal Diversification of Sciurini in the Neotropics

Our biogeographic analyses focused on Neotropical Sciurini suggested with highest probability (92.2%) that this large clade originated in the northwestern portion of South America in the Pacific dominion (Figure 2; Supplementary Table S3). The Pacific dominion was also recovered with highest probability as the ancestral range of the Central American (93.6%) and South American (99.0%) major radiations. Within the Central American radiation, two genera originated in this same region: *Microsciurus* at 4.1 Mya (3.3–5) and *Syntheosciurus* at 3 Mya (2.1–3.7). The genus *Echinosciurus* originated at 3.7 Mya (3–4.4) in an area including the Mexican transition zone and the Mesoamerican dominion.

With regards to the South American radiation, the ancestors of the first two genera occupied the northwestern portion of

the continent, west of the Andes, in the Pacific dominion: *Leptosciurus* that originated at 4.4 Mya (3.6–5.1) and *Simosciurus* that originated at 4 Mya (3.1–4.7). The following genera had their origins on the east side of the Andean cordillera, along the Amazon basin. The genus *Guerlinguetus* originated at 4.4 Mya (3.9–5.1) in the Boreal Brazilian dominion. The ancestor of the genera “*Microsciurus*” and *Hadrosociurus* occupied a large area including the Boreal Brazilian and the South Brazilian dominions. The origin of “*Microsciurus*” dated to 3.2 Mya (2.5–3.7) and the origin of *Hadrosociurus* was estimated to 3.9 Mya (3.6–4.9). In summary, the majority of the cladogenetic events observed for Neotropical tree squirrels took place in the northern portion of South America and southern Central America during the Pliocene. Two major dispersal events apparently led to the colonization of Mesoamerica by the genus *Echinosciurus* and the colonization of the east of the Andes by the most recent common ancestor of the genera *Guerlinguetus*, “*Microsciurus*” and *Hadrosociurus* (Figure 2).

The scenario recovered by this analysis allows the inference of four founder-event speciation and five anagenetic dispersal events during the diversification of the tribe Sciurini across the Neotropics, and, once again, no vicariance event was inferred (see Supplementary Figures 5, 6). The founder-event speciation corresponds to the jump of the ancestors of (1) the genus *Echinosciurus* from the Pacific dominion to the Mexican transition zone and Mesoamerican dominion, (2) the genera *Guerlinguetus*, “*Microsciurus*” and *Hadrosociurus* from the Pacific dominion to the Boreal Brazilian dominion, (3) (*Guerlinguetus aestuans* “b”, (*G. aestuans* “c”, *G. brasiliensis*) from the Boreal Brazilian dominion to the South Brazilian dominion, and (4) (*Guerlinguetus aestuans* “c”, *G. brasiliensis*) from the South Brazilian dominion to Boreal Brazilian, South-eastern Amazon, and Parana dominions. Anagenetic dispersal



occurred with the colonization of (1) the Pacific dominion by *Echinosciurus variegatoides*, (2) the South Brazilian dominion by the ancestor of “*Microsciurus*” and *Hadroskiurus*, (3) the Boreal Brazilian and Parana dominions by the ancestor of *Guerlinguetus aestuans* “c” and *G. brasiliensis*, (4) the Pacific dominion by “*Microsciurus*” *sabanillae*, and (5) the Pacific dominion by *Hadroskiurus igniventris*.

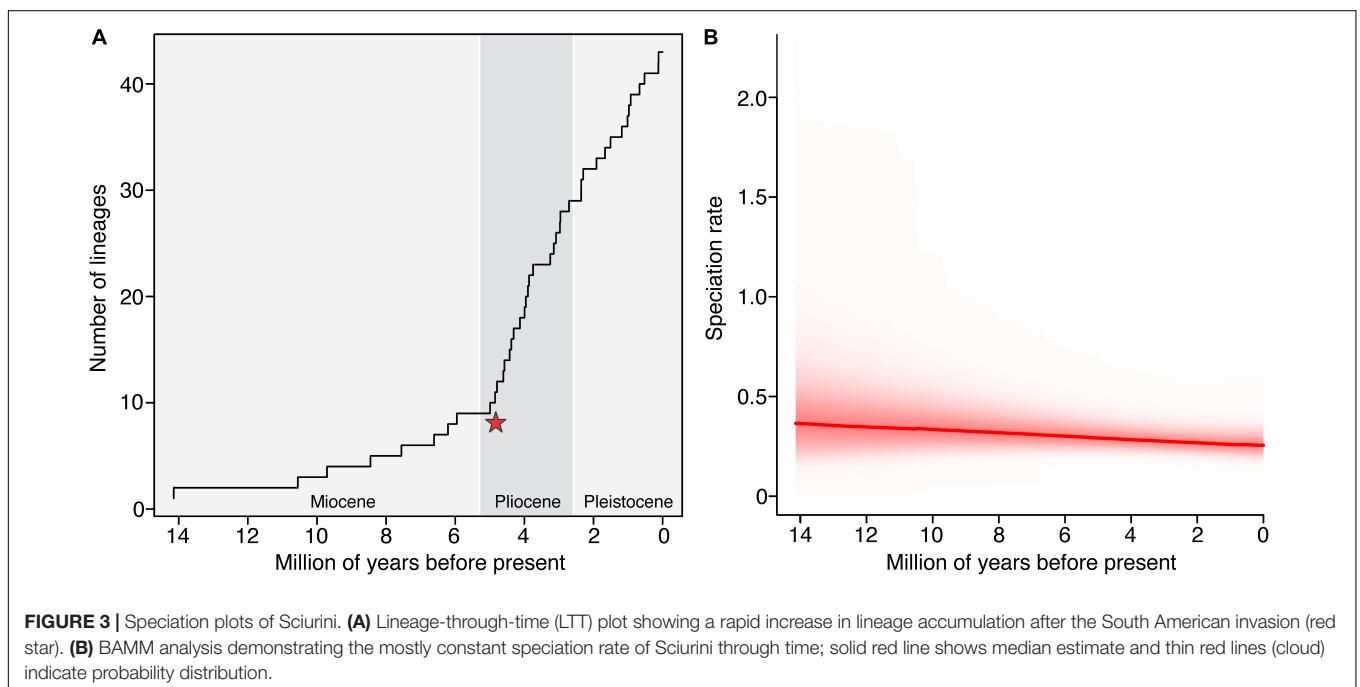
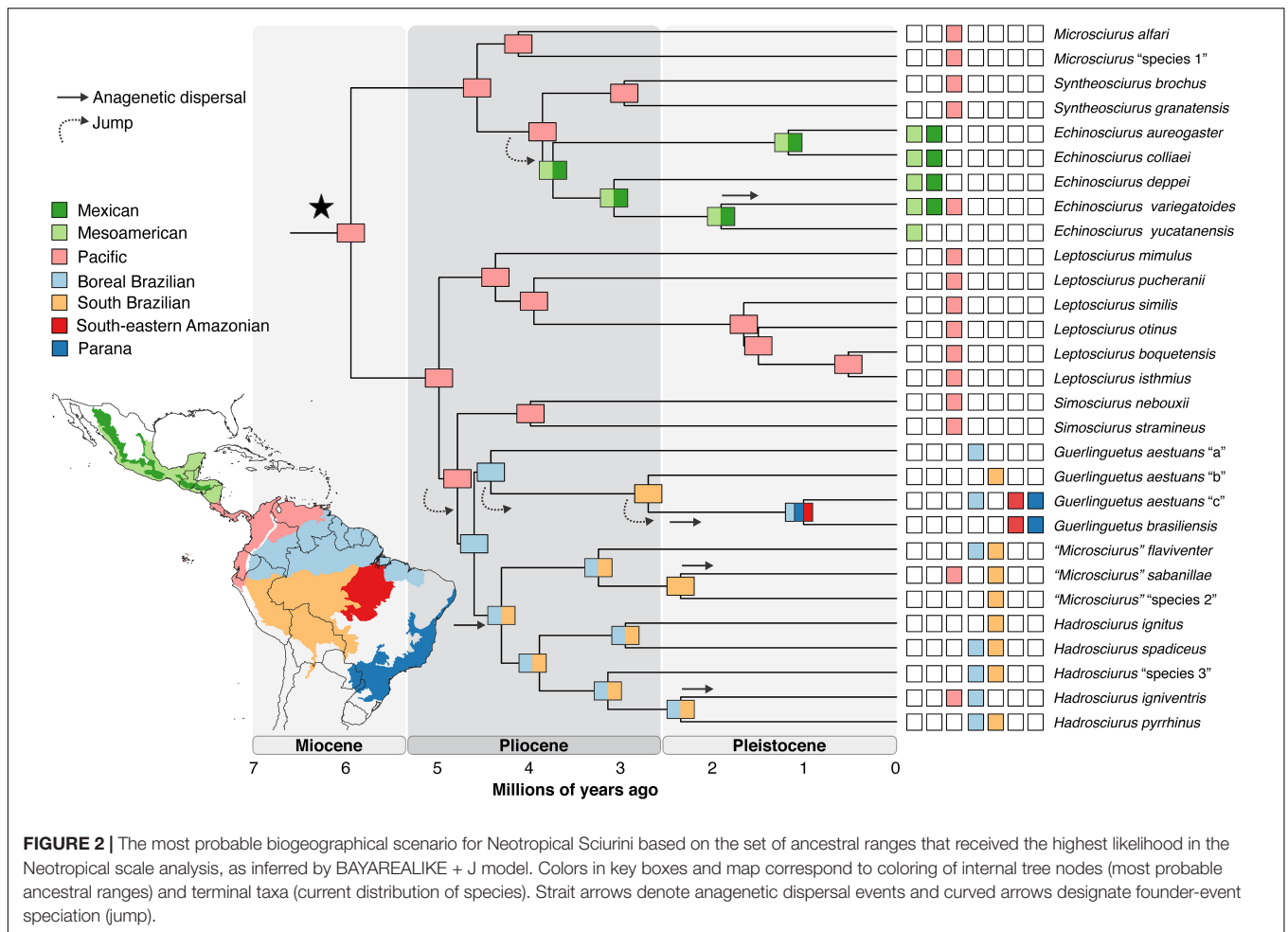
### Diversification Rates of the Tribe Sciurini

The LTT plot indicated an initial low and constant net diversification rate from about 14 to 10.5 Mya, followed by a moderate increase from 10.5 to 5 Mya, and an acceleration on the diversification rate from 5 Mya to the present (Figure 3A). This period of rapid inflation on the number of lineages, along the Pliocene and Pleistocene, coincides with the South American invasion and colonization by tree squirrels (Figure 3A; red star).

The Bayesian approach, however, showed a pattern of constant speciation rate through time, with no inflection point and with a slight decrease on the magnitude of speciation over time (Figure 3B). The BAMM analysis also recovered the null model “no shifts” on speciation rate as the most probable (0.66) to represent the diversification of Sciurini. The mean speciation rate of Sciurini was 0.29.

### DISCUSSION

The phylogenetic hypothesis presented here is concordant with the inferences depicted in Abreu-Jr et al. (2020), as we employed the same taxa and data source. We included in our analyses 43 of the 46 currently recognized species of tree squirrels (see Abreu-Jr et al., 2020). Previous biogeographic analyses have included up



to 28 Sciurini species, and less than a third of South American tree squirrel species (Pečnerová and Martínková, 2012; Pečnerová et al., 2015). Therefore, this is the most comprehensive study investigating the biogeographic history of the tribe Sciurini, both in number of species and geographic coverage.

The models with best fitting scores on each biogeographic analysis are distinct in their criteria, as one detects vicariant events (DEC) and the other (BAYAREALIKE) does not (Matzke, 2013). Both approaches employed to investigate the range of evolution of tree squirrels (analysis of the whole tribe and analysis focused on Neotropical taxa) did not recover vicariant events during the evolutionary history of Sciurini. These results suggest that vicariance was not a main driver of the diversification processes of tree squirrels at either global or local (Neotropical) scales (but extinctions might mask instances of vicariance; see discussion below). Dispersal events, on the other hand, were commonly recovered and founder-events were estimated to have happened more times than anagenetic dispersals in both approaches.

Our time estimation for the origin of Sciurini was similar to the result of Mercer and Roth (2003) and Upham et al. (2019), supporting a more recent date around 14 Mya compared to the estimation of Zelditch et al. (2015) and Pečnerová et al. (2015) around 18 and 19 Mya, respectively. This is also relatively more recent compared to the origin of the other tribes of the family Sciuridae that have been hypothesized to have happened before 18 Mya (Roth and Mercer, 2008). Our estimates suggested that the most recent common ancestor of Sciurini probably inhabited exclusively North America (near 60% of probability). This is in contrast to previous findings that suggested that the ancestors of this tribe originated in Eurasia or in a large area that included Eurasia and North America (Pečnerová and Martínková, 2012; Pečnerová et al., 2015; Rocha et al., 2016). Despite the higher probability for an exclusive North American origin, our results do not rule out the possibility of an origin that included both the Nearctic and Palearctic regions (supported as the ancestral range of Sciurini with about 30% of probability).

Pečnerová and Martínková (2012) suggested two alternative working hypotheses for the initial diversification and dispersal of tree squirrels. The first one postulates that the ancestor of the genus *Sciurus* dispersed from North America to Eurasia after diversification from *Tamiasciurus*. This ancestor diverged again in Eurasia and returned to North America via the Bering land bridge. The second hypothesis claims that ancestors of both *Tamiasciurus* and *Sciurus* originally occupied Eurasia and colonized the Americas in two distinct occasions, also crossing over Beringia. However, the results obtained by Pečnerová and Martínková (2012) do not allow them to choose conclusively between these two hypotheses and our results indicate a higher likelihood for a third distinct scenario. We found that the ancestors of *Tamiasciurus*, *Rheithrosciurus*, and the ancestor of the clade from which *Sciurus* diverged, occupied the Nearctic region with highest probability. Our results suggest that the ancestors of *Rheithrosciurus* and *Sciurus* dispersed from North America to Eurasia in two independent events, through subsequent jump dispersal events some thousand years apart (Figure 4). The cladogenetic

events leading to *Rheithrosciurus* dated at 10.5 Mya and to the Eurasian *Sciurus* at 9.7 Mya, which supports the colonization of the Palearctic region by these two lineages before the opening of the Bering Strait around 7.4 Mya (Marincovich and Gladenkov, 1999).

*Rheithrosciurus macrotis*, endemic to Borneo (Thorington et al., 2012), possibly colonized the island overland from southeast Asia and has been isolated there since its arrival. Borneo is connected to mainland areas through the Sunda Shelf, and the distribution of shallow marine carbonates and the depths of sea water on the Sunda Shelf indicate the presence of past dispersal routes from the continent (perhaps via Java) to Borneo (Hall, 2001). Unlike *Rheithrosciurus*, the genus *Sciurus* successfully spread throughout Asia and Europe, and its species are currently widespread in the Palearctic region (Thorington et al., 2012).

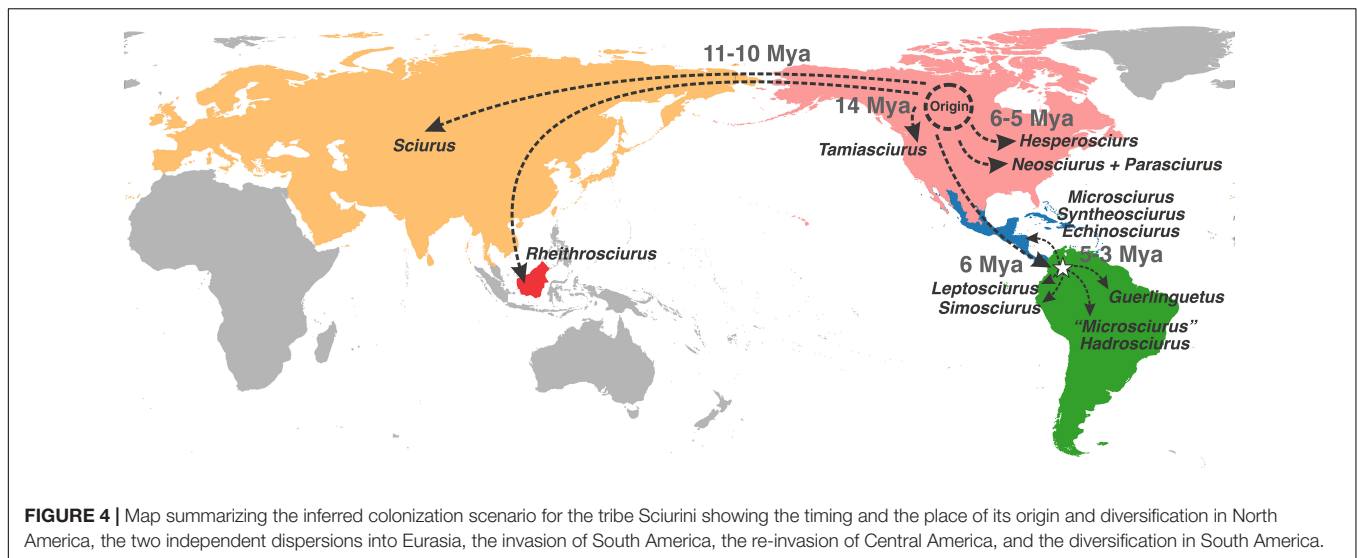
Besides *Tamiasciurus*, the remaining Nearctic genera, *Hesperosciurus*, *Parasciurus*, and *Neosciurus*, also had their ancestors occupying North America. The estimated origin of *Hesperosciurus* dated to the late Pliocene (around 4.8 Mya), *Parasciurus* to the late Pleistocene (around 2.3 Mya), and *Neosciurus* diverged in the late Miocene (around 6.2 Mya). Thus, despite exhibiting the same ancestral range, the North American groups originated in distinct periods, during a relatively large temporal window (about 3.9 Mya) compared to the South American genera (see below).

The ancestral range of the large Neotropical clade was in South America, which indicates that the colonization of Central America by Neotropical taxa was via movement north back from South America (Figures 1, 4). Remarkably, our estimates indicate that this clade originated around 6 Mya, an earlier date than ever suggested. Previous studies had estimated that tree squirrels colonized South America around 3 Mya (Mercer and Roth, 2003; Roth and Mercer, 2008; Pečnerová et al., 2015) and they argued that the South American invasion was conditioned by the closure of the Isthmus of Panama.

The date of the Panamanian land bridge formation—allegedly the moment that triggered the GABI—has gained a lot of attention and discussion in the scientific literature in the past decade. Current biological data suggest an early emergence of the Isthmus, around 6–8 Mya, and indicate that the biotic turnover associated with GABI was much longer and more complex than traditionally recognized, beginning perhaps as early as the Oligocene–Miocene transition (Bacon et al., 2015; Montes et al., 2015; Jaramillo et al., 2017; Molnar, 2017). However, comprehensive geological, paleontological, and also biological reviews pointed to scant evidence and analytical bias in some of the aforementioned studies, and sustained the conventional hypothesis of the formation of the Panamanian Isthmus *sensu stricto* around 3–2.8 Mya (Lessios, 2008, 2015; Leigh et al., 2014; Marko et al., 2015; O’Dea et al., 2016).

In addition to this ongoing discussion, Agnolin et al. (2019) proposed an alternative hypothesis to explain biotic interchanges between North and South America based on Pre-GABI land connections. The hypothesis from Agnolin et al. (2019) relies on paleontological and geological evidences for a large corridor of shallow waters and emergent spans of land connecting North and South America through the Caribbean islands during the





Oligocene-Miocene, known as GAARlandia (Iturralde-Vinent and MacPhee, 1999). The presence of GAARlandia could help to explain the occurrence of early migrants (from the Miocene) in North and South America (see Pinto-Sánchez et al., 2012), considering the lack of strong geological evidence to sustain the early closure of the Panamanian Isthmus. Hershkovitz (1972); Savage (1974) and Reig (1984) also defended a much earlier entrance of land mammals in South America, in the early Miocene, prior to the GABI and the emergence of the land bridge, based on the patterns of diversity and endemism of sigmodontine rodents.

Our results for the first entrance of tree squirrels in South America could either fit this alternative route of colonization through GAARlandia or the traditional route recognized via the Panamanian Isthmus. Even if the complete establishment of the Isthmus occurred in the Pliocene, it might have been permeable to biota migration since the Oligocene (Molnar, 2008; Eizirik, 2012; Carrillo et al., 2014; Bloch et al., 2016). Marshall (1979) already defended that events of marine regression on Upper Miocene would have favored dispersion between South and Central/North America, via the Isthmus, suggesting its permeability. The timeframe of this invasion is also similar to what has been shown for sigmodontine rodents (Smith and Patton, 1999; Steppan et al., 2004; Parada et al., 2013). To the best of our knowledge, no previous hypothesis ever suggested that these two distinct lineages of rodents may have shared a parallel and similar history of invasion and dispersal throughout South America and re-invasion of Central America. During a similar lapse of time, sigmodontine rodents appear to have been more successful in adapting to a greater variety of ecosystems (including grasslands, shrublands, wetlands) and spreading as far as the temperate zone at the southern border of the continent, reaching a diversity of hundreds of species (Burgin et al., 2018). Squirrels, on the other hand, remained restricted to the mid-northern portion of the continent, inhabiting only dense and continuous forests (Vivo and Carmignotto, 2015), exhibiting much less species diversity.

The predominantly Central American radiation (including the genera *Microsciurus*, *Syntheosciurus* and *Echinosciurus*) is more likely to have originated in Central America around 4.6 Mya (probably southern Central America as suggested by the analyses focused on Neotropical taxa, see below), from a jump dispersal from South America. Also, most ancestors of the extant species within this radiation occurred in Central America. Two species, one from the genus *Microsciurus* (*M.* “species 1”) and one from the genus *Syntheosciurus* (*S. granatensis*), colonized back to South America (Figure 1).

The major South American radiation is slightly older than the Central American one, with its date of origin estimated to around 5 Mya. Within this lineage all the ancestors occupied South America, except the most recent common ancestor of *Leptosciurus boquetensis* and *L. isthmius*, estimated to have inhabited Central America (Figure 1). Previous studies (Pečnerová and Martínková, 2012; Pečnerová et al., 2015) suggested a directional diversification process following the latitudinal gradient from North America to South America giving rise to all Neotropical taxa. Our results, supported by ancestral range estimation of Sciurini in both global and Neotropical scales, do not corroborate this hypothesis, and indicate a much more complex pattern, with distinct colonization events from South to Central America and vice-versa and perhaps through distinct dispersal routes.

Several Central American vertebrate lineages that originated along the Pliocene-Pleistocene boundary have been shown to exhibit their ancestral ranges in northern South America (Barrantes, 2009; Saldarriaga-Córdoba et al., 2017; Maestri et al., 2019). Savage (2002) proposed a two-step dispersal pulse hypothesis to explain amphibians and reptiles of South America inhabiting Middle America. The first colonization episode, according to him, occurred about 3.5 Mya when the sea level lowered and many taxa were able to invade Mesoamerica as far as Mexico. The second episode took place in the Pleistocene and the majority of taxa could not disperse further than southern Nicaragua or Costa Rica. The first episode described by Savage

(2002) coincides with the diversification and dispersion of the genus *Echinosciurus* to the Mesoamerican dominion and Mexican transition zone (see **Figure 2**), and the second episode coincides with the dispersal into Central America by *Leptosciurus boquetensis* and *L. isthmius* (see **Figure 1**). Both episodes of Central American colonization can be plausibly explained via the traditional dispersal route across the Isthmus of Panama.

The biogeographic analysis focused on Neotropical taxa recovered the origin of this clade in the Pacific dominion, which ranges from southern Central America to northwestern South America. This was estimated to have occurred in the late Miocene (around 6 Mya), when the Panamanian land bridge was still emerging and during the final uplift stages of the Andean Cordillera (Hoorn et al., 2010; Stange et al., 2018). This region was also the ancestral range of four genera: *Microsciurus* and *Syntheosciurus* (composed of Central and South American species), *Leptosciurus* (composed of species occupying mainly the northwestern portion of South America), and *Simosciurus* (composed of two species occurring in the coast of Ecuador and Peru). Within the mainly Central American radiation, a founder-event speciation is inferred, leading to the colonization of the Mexican transition zone and Mesoamerican dominion by the ancestor of the genus *Echinosciurus* around 3.7 Mya. Moreover, among these species, *E. variegatoides* colonized back to the Pacific dominion through anagenetic dispersal. Some groups of sigmodontine rodents also had their origin estimated to be in the late Miocene or Pliocene in northern South America, suggesting that this region has played an important role on the origin and diversification of forest rodents (Maestri et al., 2019). In the mid-Pliocene, when several of the cladogenetic events of the Central American radiation have been estimated, important geological events such as the development of the Sierra Madre oriental and occidental and the volcanism of the Trans-Mexican Volcanic Belt were taking place (Sedlock et al., 1993). This intense geologic activity culminating with the formation and expansion of highland forests might have played a crucial role on the diversification of several biotic components (see Morrone, 2010), perhaps including the tree squirrels.

The ancestors of the remaining South American genera (*Guerlinguetus*, “*Microsciurus*”, *Hadrosociurus*) occupied areas east of the Andes with highest probability. This was the point (approximately 4.8 Mya) in the evolutionary history of Sciurini that tree squirrels crossed over the Andes and through a founder-event colonized large forested areas in the eastern portion of South America. *Guerlinguetus* showed the highest probability of ancestral occupancy in the Boreal Brazilian dominion, which includes the northern bank of the Amazon basin and the Guiana Shield. The ancestors of the genera “*Microsciurus*” and *Hadrosociurus* occupied an area including the Boreal Brazilian and South Brazilian dominions. Within *Guerlinguetus*, the ancestor of three species (*G. aestunas* “b”, *G. aestunas* “c” and *G. brasiliensis*) colonized the South-eastern Amazonian dominion through a founder-event speciation. Through another founder event speciation, the ancestor of *G. aestunas* “c” + *G. brasiliensis* colonized the South-eastern Amazonian dominion. Subsequently, it expanded its range,

reaching the Boreal Brazilian dominion and, for the first and only time in the tree squirrel diversification, the Atlantic Forest on the coast of Brazil (see **Supplementary Figure 6** for details). South-eastern Amazon and Atlantic Forest were the last two areas occupied by tree squirrels in South America.

The timing of Atlantic Forest colonization by tree squirrels (around 1 Mya) is comparable to the timing of the entrance of some species of sigmodontine rodents (such as species in the genera *Euryoryzomys*, *Hylaeamys* and *Oligoryzomys*; Steppan and Schenk, 2017; Maestri et al., 2019). At least two main distinct spatiotemporal routes have been proposed between the Amazon and the Atlantic Forest (Costa, 2003; Batalha-Filho et al., 2013; Ledo and Colli, 2017): older (middle to late Miocene) connections between southeastern Atlantic Forest and western Amazonia (the SE-NW bridge), and younger (Pliocene and Pleistocene) connections across northeastern Brazil (the NE bridge). As our results indicate a young invasion, squirrels might have used NE routes, possibly favored by interglacial periods of the Pleistocene, as the NE bridge hypothesis assumes.

Within the genera “*Microsciurus*” and *Hadrosociurus*, most species remained restricted to the Amazon basin and adjacent areas (along Boreal Brazilian and South Brazilian dominions). Only one species in each genus (“*Microsciurus*” *sabanillae* and *Hadrosociurus igniventris*) dispersed to the eastern slopes of the Andean cordillera and invaded the border of the Pacific dominion (**Figure 2**).

All South American genera originated from 4.4 to 3.2 Mya. This very short temporal window (about 1.2 My) overlaps with the South American Land Mammal Ages (SALMAs) of the Montehermosan (from about 6.8 to 4 Mya) and the Chapadmalalan (from about 4 to 3.4 Mya) (Flynn and Swisher, 1995). At this time, the Andean uplift was complete and the Amazon basin was in its final stages of formation with a large supply of Andean-derived sediments (Hoorn et al., 2010). These massive geological changes produced a great variety of new ecosystems in South America that favored the diversification of many groups of organisms.

South American genera usually do not overlap their distribution, except in a small area on the northern bank of the Amazon river, where one species of *Guerlinguetus* (*G. aestuans* “c”) is sympatric with two species of *Hadrosociurus* (*H. igniventris* and *H. spadiceus*), and in the western Amazon basin, where species from “*Microsciurus*” and *Hadrosociurus* are mostly sympatric. It is important to mention that these last two genera are the most morphologically dissimilar among all South American forms. Species of “*Microsciurus*” are small squirrels with head and body length ranging from 125 to 180 mm, and species from *Hadrosociurus* are large, with head and body length ranging from 220 to 325 mm (Vivo and Carmignotto, 2015). It is also noteworthy that along lowland areas in the western Amazon basin, species of “*Microsciurus*” are more commonly found in non-flooded upland forests, while species of *Hadrosociurus* inhabit mainly seasonally inundated floodplain forests (Emmons and Feer, 1997). Besides tree squirrels, South America is the home of another remarkable radiation of Sciuridae, the Neotropical pygmy squirrel, *Sciurillus pusillus*. This singular radiation diverged early in the history of the family

(about 35 Mya) and is included in its own subfamily, Sciurillinae (Mercer and Roth, 2003; Steppan et al., 2004; Roth and Mercer, 2008). The diversification and colonization of South America by the Neotropical pygmy squirrels are enigmatic pieces on the evolutionary history of this group.

Regarding speciation events generating the 43 extant species of Sciurini analyzed here, three species originated in the Miocene, 17 in the Pliocene, and 23 in the Pleistocene. The events in the Miocene were: the diversification of the Borneo endemic species *Rheithrosciurus macrotis*, the Eurasian *Sciurus anomalus*, and the North American *Neosciurus carolinensis*. During the Pliocene, the great majority of speciation events took place in South America, where ten species (from all genera) originated; in Central America five species originated (also from all genera) and in North America only one speciation event occurred (within the genus *Hesperosciurus*). In the Pleistocene, eight speciation events occurred in South America (within the genera *Leptosciurus*, *Guerlinguetus*, “*Microsciurus*”, and *Hadrosociurus*), six in North America (within the genera *Tamiasciurus* and *Parasciurus*), and six in Central America (within the genera *Echinosciurus* and *Lepstosciurus*). In summary, most speciation events in North America occurred late in the Pleistocene, and in South America the majority occurred during the Pliocene or in the Pliocene-Pleistocene transition. Moreover, most cladogenetic events in South and Central America took place in the Pliocene. In North America and Eurasia, cladogenetic events occurred mainly early in the Miocene or late in the Pleistocene (Figure 1).

Pleistocene climatic fluctuations were probably a determining factor in the diversification process within North American lineages, for instance across the genera *Tamiasciurus* (see Arbogast et al., 2001; Chavez et al., 2011) and *Parasciurus*. In the Neotropics, Pleistocene fluctuations apparently played a secondary role, as dozens of speciation events occurred before the intensification of climatic cycles. It seems that the Central and South American lineages rapidly adapted to the new (and perhaps vacant) environments and experienced explosive diversification. This is quite different from the history of other rodent radiations in South America, rats and spiny rats, where most of the speciation events took place in the Pleistocene (Fabre et al., 2012; Steppan and Schenk, 2017).

Our analyses revealed a mostly constant (slightly decreasing) speciation rate through time for the tribe Sciurini. However, we observed a peak of lineage accumulation since the beginning of the Pliocene, which coincides with the Neotropical invasion, followed by a rapid diversification in this area. The constancy of the speciation rate (with no shifts during the Pliocene) might indicate that several extinctions may have occurred during the evolutionary history of tree squirrels. In this way, the number of species that went extinct possibly equalized the evident increase in species accumulation since the Pliocene and, therefore, the number of species maintained over time is similar to the number of extinct species. This could also strengthen the prevalence of founder-event speciation and overlook the presence of vicariant events in the ancestral range estimates of Sciurini, as extinctions might be expected to eliminate instances of vicariance and inflate instances of “jump” dispersal. Moreover, our analysis yielded a mean diversification rate around 0.29 for tree squirrels, which is slightly higher than the estimate of Zelditch et al. (2015) and

similar to the diversification rate of muroid rodents in the same time frame (Steppan and Schenk, 2017). Finally, we conclude that the South American invasion was not as recent as previously inferred, but the speciation was indeed very fast, mostly restricted to a short period of time in the Pliocene, much earlier than for other groups of rodents.

## DATA AVAILABILITY STATEMENT

The dataset generated for this study can be found in the Figshare Repository (<https://doi.org/10.6084/m9.figshare.12288185.v1>). GenBank accession numbers for all complete mitogenomes are provided in the **Supplementary Table S1**.

## AUTHOR CONTRIBUTIONS

EA-J, and SP conceived the project. EA-J analyzed the data. EA-J, SP, AP, and JM interpreted and discussed the results. EA-J wrote the manuscript with contributions from SP, MT, DW, AP, JM. SP, AP, DW, and JM secured funding for this project. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2020.00230/full#supplementary-material>

**FIGURE S1** | Nucleotide substitution saturation plot. The number of transitions (s) and transversions (v) in the mitochondrial genome dataset is plotted against the F84 distances.

**FIGURE S2** | Bayesian maximum clade credibility chronogram based on 13 mitochondrial protein-coding genes (11,278 bp) of 43 species of Sciurini and two species of Pteromyini used as outgroup. The mean heights along with the 95% highest posterior density intervals of dates are shown at each node.

**FIGURE S3** | Chronogram employed on the range evolution estimation of the tribe Sciurini on a global scale, showing the numbers of the nodes.

**FIGURE S4** | The most probable biogeographical scenario for Sciurini based on the set of ancestral ranges that received the highest likelihood in the global scale analysis, as inferred by DEC + J model. Colors in key boxes and map correspond to coloring of boxes within the tree (most probable ancestral ranges) and at terminals (current distribution of species).

**FIGURE S5** | Chronogram employed on the range evolution estimation of Sciurini on a Neotropical scale, showing the numbers of the nodes.

**FIGURE S6** | The most probable biogeographical scenario for Sciurini based on the set of ancestral ranges that received the highest likelihood in the Neotropical scale analysis, as inferred by BAYAREALIKE + J model. Colors in key boxes and map correspond to coloring of boxes within the tree (most probable ancestral ranges) and at terminals (current distribution of species).

**TABLE S1** | List of specimens analyzed in this study, with geographic information and GenBank accession numbers for complete mitochondrial genomes. Taxonomic identifications follow Abreu-Jr et al. (2020). Voucher acronyms refer to the following scientific collections: American Museum of Natural History, United States (AMNH); Field Museum of Natural History, United States (FMNH); University of Kansas Natural History Museum, United States (KU); Museu Nacional da Universidade Federal do Rio de Janeiro, Brazil (MN); Museu Paraense Emílio Goeldi, Brazil (MPEG); Museum of Southwestern Biology, University of New Mexico, United States (MSB); Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Peru (MUSM); Museum of Vertebrate Zoology, University of California, United States (MVZ); Smithsonian National Museum of Natural History, United States (USNM).

**TABLE S2** | Alternative states of ancestral range estimated by DEC + J model in the global scale analyses. Node numbers are the same depicted in the phylogeny of **Supplementary Figure S3**.

**TABLE S3** | Alternative states of ancestral range estimated by BAYAREALIKE + J model in the Neotropical scale analyses. Node numbers are the same depicted in the phylogeny of **Supplementary Figure 5**. Areas acronyms are: Mexican transition zone (MEX), Mesoamerican dominion (MES), Pacific dominion (PAC), Boreal Brazilian dominion (BBR), South Brazilian dominion (SBR), South-eastern Amazonian dominion (SEA), and Parana dominion (PAR).

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