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The genus *Habromys*: an untold biogeographic history

Deborah V. Espinosa-Martínez[1,3], César A. Ríos-Muñoz[2*], and Joaquín Arroyo-Cabrales[3]

[1]Posgrado en Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Ciudad de México, México. E-mail: dvem@ciencias.unam.mx (DVE-M)
[2]Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad Universitaria, CP 04510, Ciudad de México, México.
[3]Laboratorio de Arqueozoología, Subdirección de Laboratorios y Apoyo Académico, Instituto Nacional de Antropología e Historia, Moneda 16 Centro, CP 06060, Ciudad de México, México. E-mail: joaquin_arroyo@inah.gob.mx (JA-C)
[*]Corresponding author: cesar.rios@unam.mx

The genus *Habromys* includes seven species of rodents restricted to Mesoamerican cloud forests across the mountain systems of central and eastern Mexico and northern Central America. Previous studies have suggested that diversification within the genus largely shaped by vicariant scenarios in which range expansion was followed by isolation driven by habitat fragmentation. However, these hypotheses have not been formally evaluated within a time-calibrated framework using explicit model-based approaches. Here, we reconstructed the biogeographic history of *Habromys* using a time-calibrated phylogeny based on mitochondrial genes, previously used in published studies, and evaluated alternative parametric biogeographic models implemented in BioGeoBEARS. The DEC+j was the best-supported model explaining geographic range evolution, indicating that founder-event speciation and within-area cladogenesis (narrow and subset sympatry) were the most frequent processes shaping diversification, whereas vicariance and anagenetic dispersal/extinction were inferred as comparatively uncommon. Divergence-time estimates suggest that crown diversification in *Habromys* began during the Pliocene, whereas most interspecific divergences occurred during the middle Pleistocene. These patterns are consistent with a biogeographic history driven by climatic oscillations that periodically increased connectivity among cloud forest habitats and later promoted isolation in montane regions. Overall, our results support a scenario in which repeated colonization and isolation events associated with Pliocene–Pleistocene climatic fluctuations played a greater role than vicariant processes linked to mountain building.

Keywords: Ancestral reconstruction area, cloud forest, Cricetidae, distribution, Mesoamerica, parametric biogeography.

El género *Habromys* incluye siete especies de roedores restringidas a los bosques mesófilos de montaña de Mesoamérica, distribuidos en los sistemas montañosos del centro y este de México y el norte de Centroamérica. Estudios previos han sugerido que la diversificación dentro del género estuvo determinada principalmente por escenarios vicariantes, en los cuales una expansión del área de distribución fue seguida por aislamiento impulsado por la fragmentación del hábitat. Sin embargo, estas hipótesis no han sido evaluadas formalmente dentro de un marco temporal calibrado ni mediante enfoques explícitos basados en modelos. En este estudio reconstruimos la historia biogeográfica de *Habromys* utilizando una filogenia calibrada temporalmente basada en genes mitocondriales previamente empleados en estudios publicados, y evaluamos modelos biogeográficos paramétricos alternativos implementados en BioGeoBEARS. El modelo DEC+j fue el mejor soportado para explicar la evolución del rango geográfico, indicando que la especiación por efecto fundador y la cladogénesis dentro de área (simpatría estrecha y simpatría por subconjunto) fueron los procesos más frecuentes que moldearon su diversificación, mientras que la vicarianza y la dispersión/extinción anagenética fueron inferidas como relativamente poco comunes. Las estimaciones de tiempo de divergencia sugieren que la diversificación en *Habromys* inició durante el Plioceno, mientras que la mayoría de las divergencias interespecíficas ocurrieron durante el Pleistoceno medio. Estos patrones son consistentes con una historia biogeográfica impulsada por oscilaciones climáticas que incrementaron periódicamente la conectividad entre los bosques mesófilos y posteriormente promovieron el aislamiento en regiones montañosas. En conjunto, nuestros resultados apoyan un escenario en el que eventos repetidos de colonización y aislamiento asociados a las fluctuaciones climáticas del Plioceno–Pleistoceno desempeñaron un papel más importante que procesos vicariantes vinculados al levantamiento de los sistemas montañosos.

Palabras clave: Biogeografía paramétrica, bosques mesófilos de montaña, Cricetidae, distribución, Mesoamérica, reconstrucción de áreas ancestrales.

The genus *Habromys* Hooper and Musser 1964 (Cricetidae: Neotominae) includes seven species of rodents (*H. chinanteco*, *H. delicatulus*, *H. ixtlani*, *H. lepturus*, *H. lophurus*, *H. schmidlyi*, and *H. simulatus*), all endemic and ecologically restricted to Mesoamerican cloud forest from central and eastern Mexico to northern Central America (León-Paniagua *et al.* 2007; Ceballos 2014; Ramírez-Pulido *et al.* 2014; Contreras-Medina *et al.* 2024). The species are considered unique due to their arboreal habits and high ecological specialization (Colunga Salas 2014; 2016; Marines-Macías *et al.* 2018). In some species, populations may occur at low densities (Colunga Salas 2016; Castañeda-Rico *et al.* 2023) and small home ranges (Marines-Macías *et al.* 2018), which has contributed to their recognition as threatened at the national level (*H. simulatus*: subject to special protection, SEMARNAT 2010) and the international level (*H. delicatulus*: endangered, *H. ixtlani*: critically endangered, and *H. lophurus*: near threatened, [Vázquez 2017; Álvarez-Castañeda *et al.* 2018; Emmons *et al.* 2019]).

The geographic distribution of *Habromys* species is restricted or highly fragmented. *H. delicatulus* and *H. schmidlyi* occur in the Trans-Mexican Volcanic Belt (Romo-Vázquez *et al.* 2005); *H. simulatus* has a disjunct distribution in the Sierra Madre Oriental and eastern Trans-Mexican

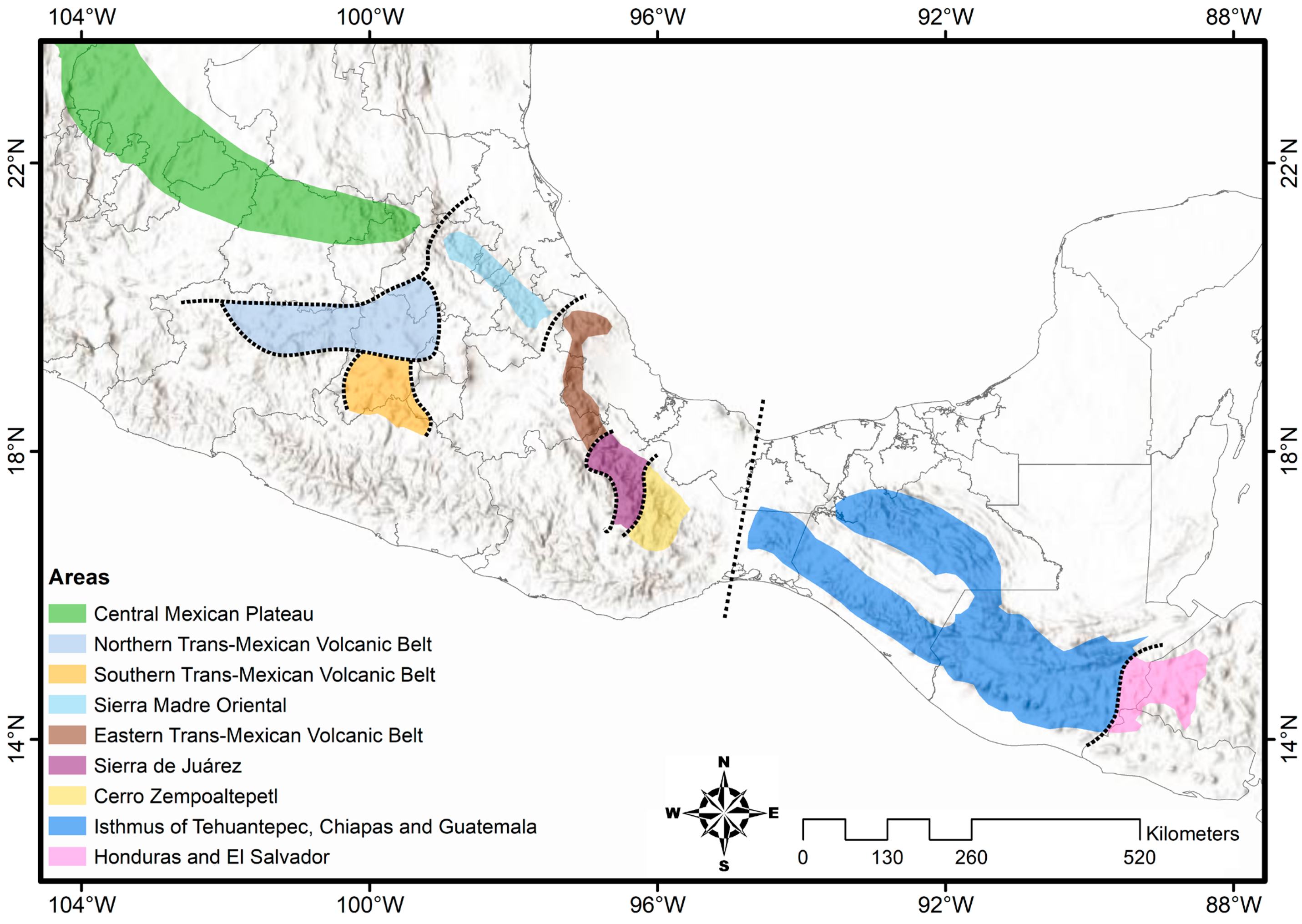

Figure 1. Distribution areas of the species included in the analysis: *Peromyscus boylii* (Central Mexican Plateau), *Habromys delicatulus* (Northern Trans-Mexican Volcanic Belt), *H. schmidlyi* (Southern Trans-Mexican Volcanic Belt), *H. simulatus* (Sierra Madre Oriental and eastern Trans-Mexican Volcanic Belt), *H. ixtlani* and *H. chinanteco* (Sierra de Juárez), *H. lepturus* (Cerro Zempoaltepetl), *H. lophurus* (Isthmus of Tehuantepec, Chiapas, Guatemala, Honduras, and El Salvador). *Peromyscus slevini* (not illustrated) is found only on Santa Catalina Island in the Gulf of California.

Volcanic Belt (León-Paniagua *et al.* 2007; Castañeda-Rico *et al.* 2011); while *H. chinanteco* and *H. ixtlani* are found in the Sierra de Juárez and *H. lepturus* in Cerro Zempoaltepetl, all part of the Sierra Madre de Oaxaca (León-Paniagua *et al.* 2007; Contreras-Medina *et al.* 2024); and *H. lophurus* is distributed in the mountains east of the Isthmus of Tehuantepec in southeastern Mexico (Chiapas), through southern and central Guatemala (Sierra de las Minas), to El Salvador (Hall 1981; León-Paniagua *et al.* 2007; Contreras-Medina *et al.* 2024) (Figure 1).

In addition to formal descriptions of the species (Merriam 1898; Osgood 1904; Goodwin 1964; Robertson and Musser 1976; Carleton *et al.* 2002; Romo-Vázquez *et al.* 2005), published works have focused on identifying new distributional records (Briones-Salas *et al.* 2012), ecological aspects and genetic diversity for some species (Castañeda-Rico *et al.* 2011; 2023; Colunga Salas 2014; 2016; Marines-Macías *et al.* 2018), analyzing the effect of Pleistocene climate change (León-Paniagua and Guevara 2019), and understanding phylogenetic relationships within the genus (León-Paniagua *et al.* 2007; Rogers *et al.* 2007; Castañeda-Rico *et al.* 2011; 2023). Nevertheless, studies have emphasized phylogenetic relationships and distributional patterns rather than explicit hypothesis testing of biogeographic processes.

The first phylogenetic hypotheses for *Habromys* were presented by Rogers *et al.* (2007), based on the complete mitochondrial cytochrome-b gene (1143 bp), and by León-Paniagua *et al.* (2007), based on mitochondrial *ND3*, *tRNA-Arg*, *ND4L*, and *ND4* sequences (1131 bp). Both studies supported the monophyly of *Habromys* using broad taxonomic sampling within Neotominae, and recovered geographically structured lineages largely associated with montane regions of Mexico and northern Central America, suggesting that isolation among cloud forest massifs has promoted diversification. Rogers *et al.* (2007), which included six of the seven recognized species, emphasized the role of the Isthmus of Tehuantepec as a major geographic barrier potentially driving divergence between lineages west and east of the isthmus, and suggested that this vicariant event may predate Pleistocene climatic fluctuations. They also proposed the Cajonos River as a potential vicariant barrier separating *H. ixtlani* and *H. lepturus*. León-Paniagua *et al.* (2007) included all described species of *Habromys* and additionally documented geographically structured lineages within *H. simulatus* and *H. lophurus*, suggesting a history of range expansion followed by diversification associated with fragmentation of montane forests during climatic oscillations, and suggesting that the Isthmus of Tehuantepec is not a major force in the differentiation of the genus. Subsequent work supported the differentiation of two evolutionary units within *H. simulatus* using microsatellite data (Castañeda-Rico *et al.* 2011), whereas the phylogeographic structure suggested for *H. lophurus* remains untested. However, although these studies provided a critical phylogenetic framework, their biogeographic interpretations were not evaluated under explicit model-based approaches, and the relative roles of biogeographic processes in shaping current distributions remain unclear.

Parametric biogeography provides a statistical framework to test alternative evolutionary scenarios by explicitly modeling biogeographic processes (anagenetic: dispersal, extinction, range switching; and cladogenetic: within-area cladogenesis, vicariance, founder-event) through time, involved in the reconstruction of biogeographic history. In addition to considering a temporal framework that allows identifying the congruence of biogeographic events, and relating them to independent geographic information (Sanmartin 2012). Based on a time-calibrated phylogenetic hypothesis and knowledge of species distributions, the geographic evolution of lineages is modeled using a stochastic process that determines the rate of change between areas, allowing the reconstruction of ancestral areas and inference of processes that shaped the distribution of a given taxon (Sanmartin 2012; Matzke 2013b). Despite the availability of a phylogenetic hypothesis and well-documented geographic distributions, *Habromys* has not been examined using parametric biogeographic methods that explicitly compare alternative evolutionary scenarios.

Given the strong association of *Habromys* with cloud forests, the high fragmentation of these habitats, and the presumably limited dispersal ability of these rodents, we hypothesize that vicariance played an important role in the diversification of the genus among montane regions during climatic fluctuations of the late Neogene and Pleistocene (León-Paniagua *et al.* 2007; Rogers *et al.* 2007; León-Paniagua and Guevara 2019). We evaluated this scenario against alternative processes such as founder-event speciation and within-area cladogenesis. Specifically, we predict that (1) ancestral areas will be reconstructed in eastern Mexican and Central American highlands, (2) diversification events will coincide with major geographic barriers such as the Isthmus of Tehuantepec and the separation of the Trans-Mexican Volcanic Belt, Sierra Madre Oriental, Sierra de Juarez and Cerro Zempoaltepetl, and (3) dispersal events will be limited and mostly restricted to adjacent mountain systems. To test these predictions, we conducted a model-based historical biogeographic analysis using a time-calibrated phylogeny and explicit parametric models. Here we reanalyzed the mitochondrial dataset of León-Paniagua *et al.* (2007), complemented with an additional sequence for *H. simulatus* from Castañeda-Rico *et al.* (2014), to evaluate alternative parametric models of historical biogeography.

Materials and methods

Phylogenetic analysis. We performed a phylogenetic analysis including 31 individuals representing the seven species described for the genus *Habromys* and 14 outgroup species (one individual per species), following the taxon sampling and outgroup selection by León-Paniagua *et al.* (2007). Partial mitochondrial sequences of ND3 and ND4 were obtained from GenBank (Supplementary Material 1), following León-Paniagua *et al.* (2007), with one additional *H. simulatus* sequence from Castañeda-Rico *et al.* (2014). The two gene fragments were concatenated for subsequent analyses. Sequences were aligned in MEGA11 (Tamura *et al.* 2021) with the Multiple Sequence Comparison algorithm based on Log-Expectation (MUSCLE) (Edgar 2004) under default settings.

We inferred a time-calibrated phylogeny using BEAST 2.7.7 (Bouckaert *et al.* 2019). We selected the substitution model by running Modeltest 3.6 on our alignment, which recovered HKY model (Hasegawa *et al.* 1985) with invariant sites, four Gamma categories as the best-fit model, consistent with León-Paniagua *et al.* (2007). To ensure comparability with previous analyses, we implemented this same model in BEAST. We implemented a relaxed log-normal molecular clock, and the Calibrated Yule speciation model (Heled and Drummond 2012). The speciation rate (birthRateY) was modeled using a Gamma distribution with parameters $\alpha = 3$ and $\beta = 0.09$. Node ages were calibrated using four secondary fossil-based constraints taken from León-Paniagua *et al.* (2007). For each calibrated node, we implemented an MRCA (most recent common ancestor) priors with a lognormal distribution and enforced monophyly (Table 1), these priors constrain node ages while enforcing monophyly. For the relaxed molecular clock, Gamma distributions were used with parameters $\alpha = 3$ and $\beta = 0.00667$ for the mean (ucldMean), and $\alpha = 4$ and $\beta = 0.05$ for the standard deviation (ucldStdev).

Twenty million generations were run, sampling every 1000 generations. We assessed the stationarity of the Markov

Table 1. Parameters used in the calibration of each internal node. The ages used in the divergence time estimates for the nodes of the *Habromys* genus phylogeny were taken from León-Paniagua *et al.* (2007).

Nodes-Groups	Calibration
Neotominae	14.8 Ma ($M = 7.1$, $S = 0.01$, *mean in real space* = true, *offset* = 7.7)
Onychomys-Peromyscus-Habromys	10.3 Ma ($M = 4.9$, $S = 0.15$, *mean in real space* = true, *offset* = 5.36)
Peromyscus-Habromys	4.69 Ma ($M = 2.25$, $S = 0.05$, *mean in real space* = true, *offset* = 2.44)
Habromys simulatus	0.78 Ma ($M = 0.4$, $S = 0.1$, *mean in real space* = true, *offset* = 0.38)

Table 2. Summary of statistics for selecting the best model obtained in BioGeoBEARS. LnL = likelihood, d = dispersion parameter, e = extinction parameter, j = founder-event parameter, AIC = Akaike Information Criterion, and AIC_wt = weighted Akaike Information Criterion.

Model	LnL	Number of parameters per model	d	e	j	AIC	AIC_wt
DEC	-40.4	2	0.07	0.13	0	84.79	0.000023
*DEC+j	-28.92	3	1E-12	1E-12	0.31	63.85	0.8
DIVALIKE	-37.91	2	0.059	0.026	0	79.82	0.0003
DIVALIKE+j	-30.62	3	0.0064	1E-12	0.14	67.24	0.15
BAYAREALIKE	-46.09	2	0.15	0.54	0	96.19	0.000000076
BAYAREALIKE+j	-31.59	3	0.0007	0.031	0.17	69.18	0.055

Chain Monte Carlo (MCMC) in Tracer v.1.7.2 (Rambaut *et al.* 2018), ensuring that the effective sample sizes (ESS) were > 200 for all priors, indicating adequate sampling of the posterior distribution. The Maximum Clade Credibility Tree (MCCT) and the most probable common ancestor were obtained using TreeAnnotator v.2.7.7, discarding 25% of burn-in. The maximum credibility tree was visualized in FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Ancestral area estimation. The ancestral areas for the genus *Habromys* were estimated using the BioGeoBEARS package (Matzke 2013a) in R v.4.3.3 (R Core Team 2024). To reduce the number of geographic states, we initially retained one representative per species of the genus was initially used. In the case of *H. schmidlyi*, two individuals were considered because they do not form a monophyletic group and one of the individuals is a sibling of *H. delicatulus*. This non-monophyly has been previously reported and may reflect incomplete lineage sorting or taxonomic complexity. On the other hand, for *H. simulatus* and *H. lophurus*, León-Paniagua *et al.* (2007) and Castañeda-Rico *et al.* (2011) report that there are two well-differentiated populations in two different areas in each species, so we decided to retain one individual from each of the populations in order to reconstruct biogeographic processes involved (Figure 1). Considering that BioGeoBEARS analyses have a computational limit associated with the number of states (number of combinations of relationships between the areas used, Matzke 2013a, b), we pruned most outgroups, leaving only *Peromyscus boylii* (distributed in the south and southwest of the United States of America and northern Mexico, up to the Central Mexican Plateau [Kalcounis-Rueppell and Spoon 2009]) and *P. slevini* (distributed on Santa Catalina Island in the Gulf of California [Álvarez-Castañeda and Cortés-Calva 2002]), as both are recovered as a sister lineage to *Habromys* (León-Paniagua *et al.* 2007).

To estimate the biogeographic history of the genus, we considered three sets of biogeographic models implemented in BioGeoBEARS: (1) Dispersal-Extinction-Cladogenesis (DEC, Ree *et al.* 2005; Ree and Smith 2008), (2) the likelihood version of the Dispersal-Vicariance Analysis (DIVALIKE, Matzke 2013a, b), which differs from the original version of DIVA (Ronquist 1997; Ronquist and Sanmartín 2011) in that it is not based on parsimony methods, but rather on a likelihood interpretation, and (3) the likelihood version of the BAYAREA model (BAYAREALIKE, Matzke 2013a, b) which, unlike the original version of BAYAREA (Landis *et al.* 2013), considers cladogenesis as part of the evolutionary process. Likewise, models that consider speciation because of the founder effect (parameter +j, Matzke 2013b, 2014) were also tested: DEC+j, DIVALIKE+j, and BAYAREALIKE+j. The best model was selected based on the likelihood values (LnL) and the Akaike Information Criterion (AIC) values obtained from model fitting (Table 2).

We performed biogeographic stochastic mapping (BSM) under the best-fitting DEC+j model to estimate the frequency of biogeographic events across the evolutionary history of *Habromys*. We generated 50 stochastic map replicates and recorded, for each replicate, the number of inferred events corresponding to anagenetic dispersal, within-area cladogenesis (narrow sympatry), subset sympatry, vicariance, and founder-event speciation. The resulting frequency distributions were used to assess the relative contribution of each process.

Results

Phylogenetic analysis. Our phylogenetic hypothesis recovered the same overall topology as León-Paniagua *et al.* (2007), consistent with the use of the same mitochondrial markers (ND3 and ND4) and substitution model HKY+I+G. Some differences were observed in the relationships among individuals of *H. lophurus*, *H. lepturus*, *H. delicatulus*, *H. schmidlyi*, and *H. simulatus*, reflected in moderate posterior probabilities (0.5–0.8) for several internal nodes (Figure 2).

Divergence-time estimates indicate that the neotomine clade containing *Habromys* diversified during the Miocene, whereas crown diversification within *Habromys* began in the Pliocene, with most interspecific divergences occurring during the Pleistocene.

Ancestral area estimation. DEC+j was the best-supported model explaining the geographic range evolution of *Habromys*, with an AIC weight of 0.80, compared to much lower values for the other six models tested (Table 2). Biogeographic stochastic mapping under this model indicated that founder-event speciation (+J) and within-area cladogenesis (narrow and subset sympatry) were inferred as the most frequent processes shaping range evolution and diversification in *Habromys* in the mountain systems of Mexico and the highlands of northern Central

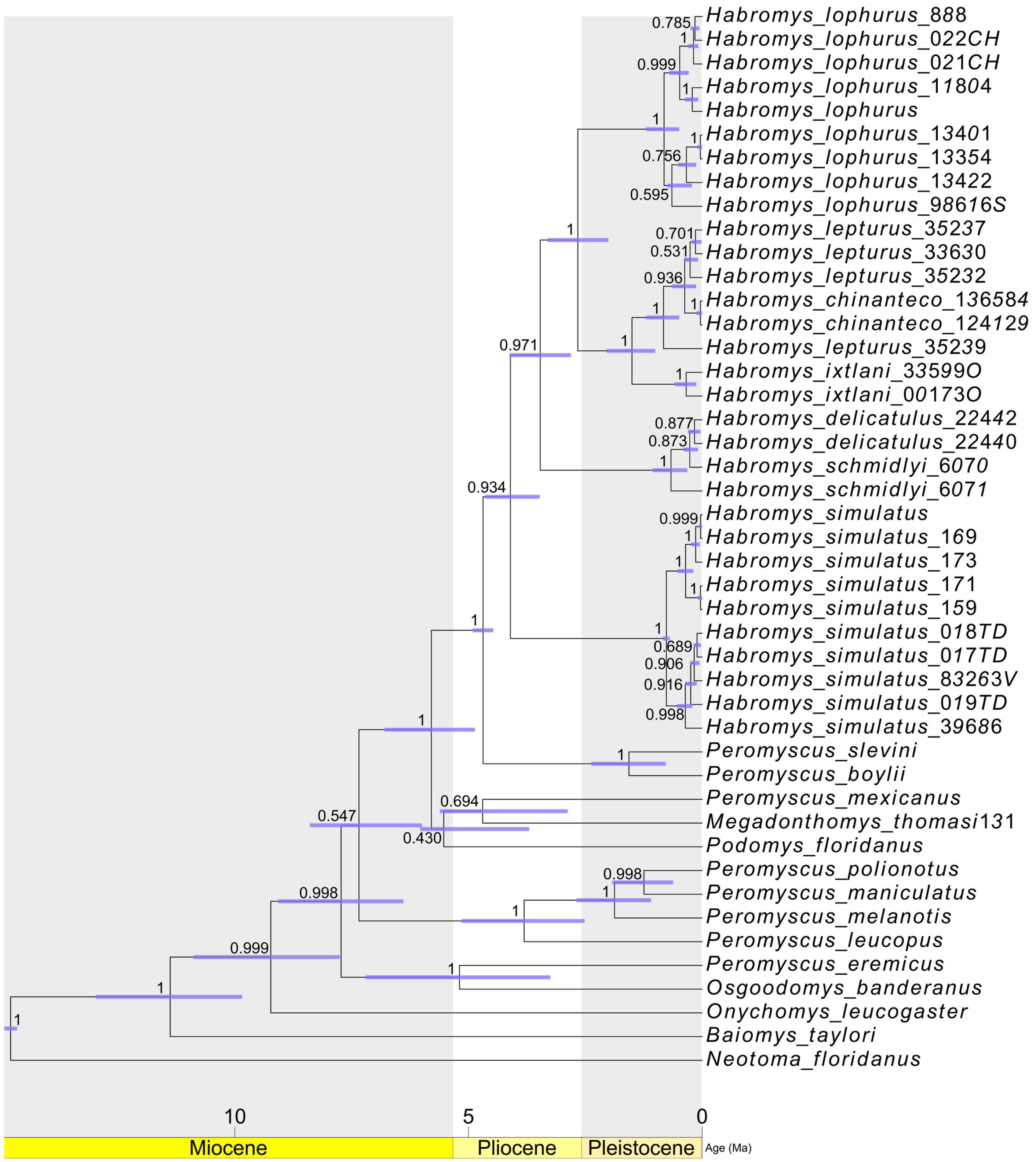

Figure 2. Phylogenetic reconstruction dated for the genus *Habromys* and its external groups based on information from León-Paniagua *et al.* (2007)

America, whereas anagenetic dispersal/extinction and vicariance were comparatively uncommon (Figs. 3 and 4; Supplemented Material 2).

Crown *Habromys* was inferred to originate in the Sierra Madre Oriental (SMO) at 4.69 Ma via founder-event speciation from the outgroup range (Baja California + Central Mexican Plateau) (Figure 3; Supplemented Material 2). The split leading to *H. simulatus* was dated at ~4.1 Ma, and this lineage was inferred to have colonized the eastern Trans-Mexican Volcanic Belt during the Middle Pleistocene (0.77 Ma) through a founder-event (Figure 3, Supplemented Material 2). A subsequent founder-event from the SMO to the southern Trans-Mexican Volcanic Belt (S-TMVB) was inferred at ~4.1 Ma. Within S-TMVB, *H. schmidlyi* was

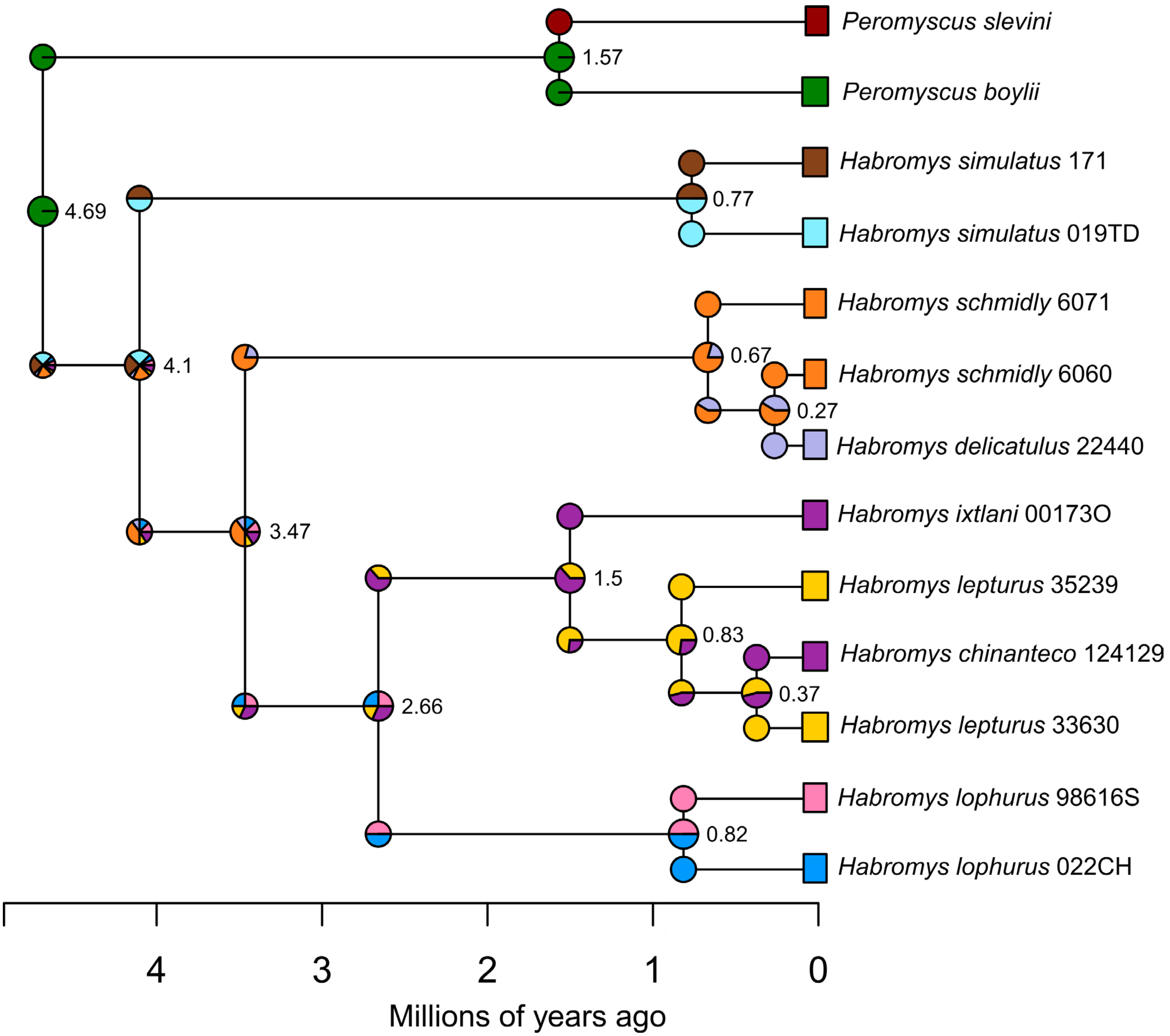

Figure 3. Hypothesis of ancestral area estimation for the genus *Habromys*. The main process involved was the founder-event present in all nodes, with the exception of sympatry events in the species *H. delicatulus* and *H. lepturus*. The colors of the branches correspond to those of the areas in Figure 1.

inferred to originate via within-area cladogenesis (narrow sympatry) at 0.67 Ma, followed by a founder-event into the northern Trans-Mexican Volcanic Belt, where *H. delicatulus* originated at 0.27 Ma (Figure 3; Supplemented Material 2).

A founder-event from the S-TMVB to the Sierra de Juárez (SJ) in the Sierra Madre de Oaxaca was inferred at 3.47 Ma (Figure 3). Within the SJ, diversification among *H. ixtlani*, *H. lepturus*, and *H. chinanteco* was dated at 2.66 Ma at the end of the Pliocene. A founder-event was inferred at 1.5 Ma from the SJ to Cerro Zempoaltepetl, by the lineage that includes *H. lepturus*, whereas *H. ixtlani* remains in SJ. This event was followed by within-area cladogenesis (narrow sympatry) at 0.83 Ma, associated with the divergence of *H. lepturus*. A subsequent founder-event from Cerro Zempoaltepetl back to the SJ was inferred at 0.37 Ma, associated with the divergence of *H. chinanteco* (Figure 3; Supplemented Material 2). Finally, a founder-event from the SJ into the Isthmus of Tehuantepec and the highlands of Chiapas and Guatemala was inferred at 2.66 Ma, followed by colonization of Honduras and El Salvador at 0.82 Ma. Diversification within *H. lophurus* was inferred to occur in these Central American highland areas (Figure 3; Supplemented Material 2).

Discussion

Previous studies on the diversification of the genus *Habromys* suggest that its presence in the mountain systems of central and eastern Mexico and northern Central America is the result of vicariant scenarios, in which range expansion was followed by isolation associated with habitat fragmentation (León-Paniagua *et al.* 2007; Rogers et al. 2007). However, these hypotheses were not formally evaluated in a time-calibrated framework that allows explicit comparison among alternative biogeographic processes. Our results indicate that the biogeographic history of *Habromys* is best explained by repeated founder-event speciation, whereas within-area cladogenesis (narrow sympatry) was inferred only for the nodes involving the lineages leading to *H. schmidlyi* and *H. lepturus* (Figure 3; Table 2; Supplemented Material 2).

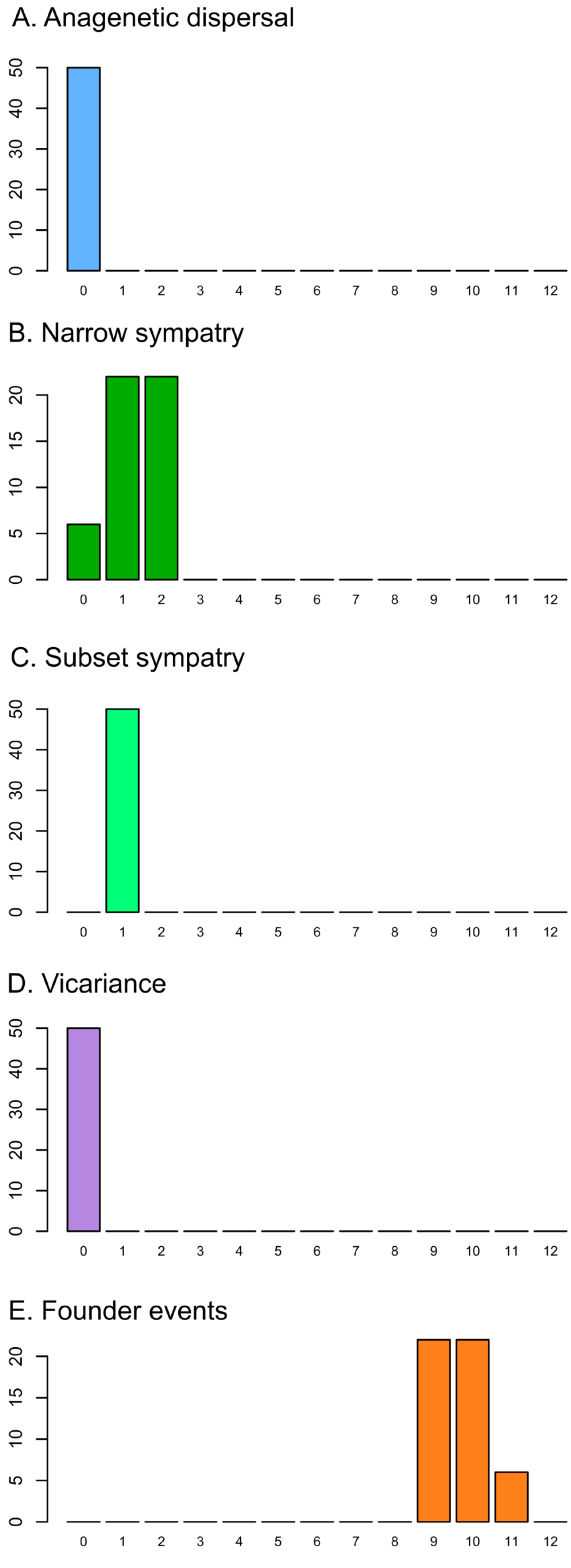

Figure 4. Distribution of biogeographic processes counts across 50 biogeographical stochastic mapping (BSM) replicates under the DEC+J model. Panels show the frequency of inferred events for five processes: (A) Anagenetic dispersal, (B) Narrow sympatry, (C) Subset sympatry, (D) Vicariance, and (E) Founder events. The results indicate a strong predominance of founder-event speciation relative to other biogeographic processes.

Founder-event speciation, unlike speciation by vicariance, occurs when a new area is colonized by a small number of individuals through long-distance dispersal or rare dispersal events. Such events may be facilitated when a geographical barrier disappears (due to various factors such as climate changes, continental collisions, the formation of mountain ranges, or changes in sea level). When the barrier reappears or a new one forms, populations on both sides of the barrier begins to separate, reducing gene flow and allowing them to differentiate into different species (geodispersion). Therefore, barriers are not static elements, but rather dynamic throughout history (Lieberman and Eldredge 1996; Matzke 2013c; Wiley and Liberman 2011). In *Habromys*, the best-fitting model (DEC+j, Table 2) supports a biogeographic scenario dominated by multiple founder-events speciation events from the early Pliocene to the middle Pleistocene (Figure 3, Supplemented Material 2), consistent with the climatic oscillations that periodically expanded and contracted cloud forest habitats, and not with the formation of mountain systems. This differs from the scenario proposed by León-Paniagua *et al.* (2007), who interpreted the diversification of *Habromys* and the closely related genera (*Podomys*, *Osgoodomys*, and *Megadontomys*) as the result of an initial range expansion followed by vicariant differentiation driven by climatic fragmentation of montane forests.

The Pliocene has been considered a transitional climatic period characterized by warm climates that changed rhythmically every 40 000 years to large-scale glacial-interglacial periods, which preceded the Pleistocene conditions that occurred with greater frequency and magnitude (Dowsett *et al.* 2010; Lawrence *et al.* 2010; Salzmann *et al.* 2011). In this context, the founding-events occurred during the Pliocene leading to the colonization of the southern Trans-Mexican Volcanic Belt (4.69 Ma), Sierra Madre Oriental (4.1 Ma), Sierra de Juárez (3.47 Ma), and the Isthmus of Tehuantepec, Chiapas, and Guatemala (2.66 Ma), coincide with warm periods of this era (Haywood *et al.* 2009; Salzmann *et al.* 2011; Jennings *et al.* 2025). These have been associated with the dynamics of cloud forests and may have increased their connectivity, which correspond to the habitat of *Habromys* species, whose processes associated solely with the Pliocene have been overshadowed by Pleistocene climate changes (Sosa *et al.* 2016).

Although colonization of the areas began during Pliocene, the diversification of the species that make up *Habromys* within their current ranges took place during the Middle Pleistocene (1.5–0.27 My) (Figs. 2 and 3). This coincides with the Middle Pleistocene Transition (MPT, 1.2–0.5 My), when the frequency of glacial periods changed from approximately every 41 000 years to approximately every 100 000 years, and remained so until the Late Pleistocene (Head and Gibbard 2005; Head *et al.* 2008; Sun *et al.* 2019; Herbert 2023). In the case of *Habromys*, our divergence-time estimates suggest that their presence distribution areas coincide with glacial periods that took place during the MPT (Cohen and Gibbard 2019; 2022), which coincides with León-Paniagua and Guevara (2019). The colonization of the eastern Trans-Mexican Volcanic Belt from the Sierra Madre Oriental by *H. simulatus* (Figure 3) is temporally consistent with a glacial period that took place 0.77 Ma. Likewise, the colonization southern and northern Trans-Mexican Volcanic Belt, 0.67 and 0.27 Ma respectively (Figure 3), shows that the presence and diversification of *H. schmidlyi* and *H. delicatulus* are also the result of glacial periods that occurred during the MPT (Cohen and Gibbard 2019; 2022).

Unlike the findings of Rogers *et al.* (2007), who argue that the colonization the Sierra de Juárez and Cerro Zempoaltepetl resulted from a single vicariant event occurred in the valley of the Cajonos River, our results suggest a biogeographic scenario were an initial colonization of the Sierra de Juárez by *H. ixtlani* (1.5 Ma) took place during a glacial period prior to the onset of the MPT (Head and Gibbard 2005; Head *et al.* 2008; Cohen and Gibbard 2019; Sun *et al.* 2019; 2022; Herbert 2023); subsequently, the colonization of Cerro Zempoaltepetl (0.83 Ma) and the recolonization of the Sierra de Juárez (0.37 Ma) by *H. chinanteco* and *H. lepturus* also coincide with glacial periods that occurred during the MTP (Cohen and Gibbard 2019; 2022). As for the presence of *H. lophurus* in the Isthmus of Tehuantepec, Chiapas and Guatemala, and Honduras and El Salvador (0.82 Ma) may be associated with the same glacial episode that led to the presence of *H. lepturus* on Cerro Zempoaltepetl. This differs from other studies that have proposed that the presence of *H. lophurus* in the mountain systems of northern Central America is the result of vicariance events due to the presence of the Isthmus of Tehuantepec, which acts has acted as a geographical barrier to the dispersal of *Habromys* and other small mammals (Carleton *et al.* 2002; Rogers *et al.* 2007; León-Paniagua and Guevara 2019). However, it is necessary to consider that the cold and dry conditions that occurred during glacial periods may have promoted the contraction of cloud forests, restricting them to possible refuges, as has been suggested for other periods in Mesoamerica (Toledo 1982; Graham 1999).

The evolution of *Habromys* and its distribution areas is the result of glacial and interglacial events during the Pliocene and Pleistocene epochs. The oldest events allowed the colonization of new areas, while the most recent ones generated isolation and, therefore, the differentiation of each species within its distribution area. This is consistent with the proposal by León-Paniagua and Guevara (2019), who mention that glacial events allowed connections through the formation of corridors between mountainous areas, enabling dispersal events between them, while during warm periods these processes were prevented because valleys and lowlands acted as barriers for species of the genus *Habromys*. However, it is worth mentioning that the climatic changes associated with the Last Glacial Maximum, although they may have had effects on distribution (León-Paniagua and Guevara 2019), did not coincide with major inferred divergence events in our phylogeny.

Our results suggest that the biogeographic history of *Habromys* has been strongly shaped by long-term climatic fluctuations, in which colonization and subsequent isolation processes appear to have played a greater role than vicariant scenarios linked to the formation of Mesoamerican mountain systems. Nevertheless, our inferences are based on a phylogeny reconstructed from mitochondrial genes, which represent a single maternally inherited locus. Mitochondrial datasets may not fully reflect the species tree due to processes such as incomplete lineage sorting, introgression, or sex-biased dispersal, potentially affecting both divergence-time estimates and ancestral-area reconstructions. Therefore, although our results support a founder-event dominated biogeographic scenario, future studies incorporating multilocus nuclear data or phylogenomic datasets will be critical to test the robustness of this hypothesis and to evaluate whether alternative processes such as vicariance or anagenetic dispersal gain support under different phylogenetic reconstructions.

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Declaration of Artificial Intelligence use

We declare that the authors have not used any Artificial Intelligence in the elaboration of this paper.

Author contributions

Conceptualization: Deborah V. Espinosa-Martínez and César A. Ríos-Muñoz; Methodology: Deborah V. Espinosa-Martínez and César A. Ríos-Muñoz; Supervision: Joaquín Arroyo-Cabrales; Drafting: Deborah V. Espinosa-Martínez, César A. Ríos-Muñoz, and Joaquín Arroyo-Cabrales.

Supplementary data

SD1. Genbank accession numbers of specimens used in the construction of the dated phylogenetic reconstruction. **SD2.** Cladogenetic biogeographic events are inferred from biogeographical stochastic mapping (BSM) under the DEC+J model. The table shows the divergence time (Ma), process type, ancestral area, descendant areas (1 and 2), and the dispersal areas in founder events. A = X, B =, C =, D =, E =, F =, G =, H =, I = y J =. Geographic areas correspond to those shown in Fig. 1: A = Isthmus of Tehuantepec + Chiapas + Guatemala; B = Honduras + El Salvador; C = Sierra Madre Oriental; D = Eastern Trans-Mexican Volcanic Belt; E = Northern Trans-Mexican Volcanic Belt; F = Southern Trans-Mexican Volcanic Belt; G = Cerro Zempoaltepetl; H = Sierra de Juárez; I = Baja California; J = Central Mexican Plateau.

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