



PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE

Facultad de Ciencias Biológicas

Programa de Doctorado en Ciencias Biológicas

Mención en Ecología

TESIS DOCTORAL

TEMPO Y MODO DE LA RADIACIÓN
DE ROEDORES NEOTROPICALES SIGMODONTINOS.

Por

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Tesis entregada a la Pontificia Universidad Católica de Chile en cumplimiento parcial de los requisitos para optar al Grado de Doctor en Ciencias con mención en Ecología

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RESUMEN

La subfamilia de roedores Sigmodontinae (Rodentia, Cricetidae) con cerca de 400 especies reconocidas, es uno de los grupos de mamíferos neotropicales más diverso y ampliamente distribuido. El contexto ecológico parece esencial para entender la evolución de este grupo. Estos roedores han proliferado abundantemente ocupando una gran variedad de hábitats y estilos de vida. Para entender el escenario histórico en el cual floreció este grupo, mediante el análisis de secuencias de ADN se estimaron los tiempos de divergencia de los principales linajes y del ancestro común más reciente de Sigmodontinae empleando relojes moleculares. Se estimó un origen de Sigmodontinae en el Mioceno medio (cerca de 12 en el pasado), mientras que la mayoría de las tribus habrían diversificado durante el Mioceno tardío. Estos resultados junto a la reconstrucción de áreas ancestrales han facilitado entender la evolución de Sigmodontinae. Este grupo se habría originado en la región del Caribe y luego de su entrada en Sudamérica diversificó profusamente. La “oportunidad ecológica” (i.e. abundancia de recursos accesibles) es usualmente considerada como desencadenante del acúmulo de riqueza de especies excepcional en algunos taxa. Se evaluó su influencia en la diversificación de los sigmodontinos examinando la asociación entre el hábitat ocupado y las tasas de especiación inferidas en distintos linajes. Se detectaron varios cambios de tasa de diversificación en varios linajes (ej. dentro de Akodontini). Los resultados sugieren una correlación hábitat ocupado y las tasas de especiación. En un marco más general, se considera

que eventos o condiciones locales pueden haber facilitado el acceso a recursos para estos roedores y cómo esto puede haber influido en la proliferación -o falta de ésta- en algunos grupos dentro de la Subfamilia. Se realizó una breve revisión de aquellos mecanismos explicativos de patrones de riqueza de especies que serían útiles para entender la diversidad sigmodontina. Explicaciones como la influencia del “determinismo local” o la “zona ecológica de origen” parecen marcos promisorios para entender la radiación de este grupo. A modo de conclusión, la aparición de nuevos hábitats, sumado a cambios geológicos y en vegetación durante el Mioceno/Plioceno habrían promovido la diversificación de Sigmodontinae.

ABSTRACT

With about 400 living species and 82 genera, rodents of the subfamily Sigmodontinae comprise one of the most diverse and more broadly distributed Neotropical mammalian clades. Ecological context seems essential to understand the evolution of this group. These rodents have proliferated through a wide variety of habitats and lifestyles. To further understand the historical scenario in which this taxon flourished, the divergence times for the most recent common ancestor of Sigmodontinae and its major lineages was estimated based on the analysis of DNA sequences. The origin of Sigmodontinae was estimated in the middle Miocene about 12 million years before present while most of the tribes radiated through late Miocene. These results together with the ancestral area reconstruction aided to understand the evolution of the subfamily. This group might have originated in the Caribbean region and diversified after invasion of South America. Ecological opportunity (i.e. abundance of available resources) is usually considered as a plausible trigger in the exceptional accumulation of species in certain taxa. The influence of ecological opportunity in the sigmodontine diversification was assessed examining the association between habitat and diversification rates. Several shifts in diversification rates were detected (e.g. within Akodontini). The results suggest a correlation between habitat and speciation rates. Furthermore, in a broader framework, the events that might have influenced the access to resources for these rodents -which in turn might have influenced the proliferation of certain

clades- were examined. Finally a brief revision of the plausible mechanisms behind the pattern of species richness was considered. “Local determinism” or the “ecological zone of origin” seem like promissory fields to explore to understand the historical context of this group. Summarizing new habitats together with geological changes during the Miocene/Pliocene may have promoted the diversification of Sigmodontinae.

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1. INTRODUCCIÓN GENERAL

1.1 Marco Teórico

La extraordinaria riqueza de especies en el Neotrópico ha sido apreciada desde que esta captó la atención de exploradores y naturalistas en el siglo XIX. Dentro de la abundante riqueza de mamíferos se destaca la diversidad de roedores, siendo uno de los grupos más diversos y ampliamente distribuidos la subfamilia Sigmodontinae (Rodentia, Cricetidae) con alrededor de 400 especies vivientes y 85 géneros [1]. El contexto ecológico parece esencial para entender la evolución de este grupo ya que estos roedores han proliferado abundantemente ocupando una gran variedad de hábitats y estilos de vida (ej. [2]). A continuación se reseñan tres aspectos relevantes para entender cuál es la importancia del contexto histórico y ecológico donde se diversificó el grupo.

Tempo de diversificación

La riqueza de especies y el rango ecológico de este grupo despertó el interés entre los naturalistas y biólogos del siglo pasado. Hershkovitz [3] al apreciar la rápida evolución de este grupo por ejemplo, postuló que los sigmodontinos sufrieron “una extensa radiación adaptativa”. Las controversias alrededor de la radiación sigmodontina se han centrado en el origen geográfico del linaje invasor, el lugar donde ocurrió la diversificación basal, y el

momento de entrada en Sudamérica (ver comentarios en [4–6]). Al respecto, existen dos hipótesis principales: La primera postula un "arribo tardío" e interpreta el registro fósil como evidencia de la llegada de los ancestros sigmodontinos a Sudamérica luego de la formación del Istmo de Panamá, lo que habría implicado una rápida acumulación de especies luego de esta fecha [7,8]. Por otro lado, Hershkovitz [2,9] y Reig [10,11] promovieron la hipótesis de un "arribo temprano" donde el ancestro sigmodontino habría llegado a Sudamérica a través del mar, presumiblemente durante el Mioceno. Para estos autores la diferenciación a nivel de tribus, géneros y especies habría ocurrido *in situ* luego del arribo a Sudamérica.

Las estimaciones de tiempo -o “dating”- más recientes, han utilizado genes nucleares y un reloj molecular “smoothed” y calibraciones fósiles por fuera de Sigmodontinae [12]. El objetivo de esta tesis fue la superfamilia Muroidea incluyendo solo 10 taxa sigmodontinos por lo que brindo estimaciones solo para el origen de la subfamilia y Oryzomyalia (definido como el clado que contiene el ancestro común más reciente de las tribus Akodontini, Oryzomyini, Phyllotini, Thomasomyini, y Reithrodontini y todos sus descendientes, excluyendo a Sigmodontini; 12). El origen de Sigmodontinae fue estimado hace 10.6-14.5: años (Ma) y para Oryzomyalia 6.0-8.8 Ma [12]. Es por esto que obtener estimados de tiempo confiables para el origen de Sigmodontinae y de todas las tribus, permitirá conocer si esta es una radiación “explosiva” con gran acumulación de linajes en corto tiempo, o si los linajes se acumularon durante un largo período.

El origen geográfico de Sigmodontinae

La formación de cadenas montañosas, “puentes terrestres” o “puentes continentales”, ríos o cuencas, campos de hielo y otros atributos del paisaje, así como cambios climáticos habrían

promovido la riqueza de especies en ciertas áreas de Sudamérica [13,14]. Durante el Neogeno, varios eventos pudieron haber influido en los patrones de riqueza de especies. El levantamiento Andino puede haber generado oportunidades para la proliferación de especies de distintas formas: a) incrementando la heterogeneidad climática y de hábitat, b) creando un “corredor biótico” para los taxa montanos, c) favoreciendo vicarianza o d) actuando como “species pump” cuando los linajes surgidos dispersan en otros biomas [7]. Los cambios en el clima y tectónica de placas habrían generado cambios en el antepais Andino y las tierras bajas del Este, abriendo “corredores bióticos” adicionales y generando nuevos hábitats. También es relevante tener en cuenta que la historia de la biota de Sudamérica durante el Neogeno estuvo ligada al aislamiento y a sucesivas invasiones a través de los mares y conexiones terrestres. Por una parte, Norteamérica no se conectó con Centroamérica hasta hace 12 Ma [15], y Sudamérica no se conectó a otras masas de tierra hasta el cierre del Istmo de Panamá durante el Plioceno (~ 3.5 Ma, [16]). Teniendo en cuenta el patrón de distribución de especies y de endemismos, los Andes han sido vistos como el área de origen y diversificación de Sigmodontinae [17]. Reig propuso el concepto de área de diferenciación original, “ADO”, como aquel “espacio geográfico dentro del cual un determinado taxón experimentó su principal diferenciación (o cladogénesis) de los taxa subordinados que lo componen” [17]. Según esta hipótesis, las tribus Akodontini, Oryzomyini, Phyllotini and Sigmodontini se habrían originado en los Andes [17]. Para entender la evolución de Sigmodontinae, es relevante entender el contexto geográfico donde radiaron estos grandes grupos. Asimismo, una reconstrucción ancestral de área facilitará entender cómo se sucedieron las transiciones entre regiones o biomas.

¿Influyen elementos como el hábitat ocupado o los molares en la riqueza de los linajes?

Como ya se mencionó, el contexto ecológico es esencial para entender la radiación sigmodontina. Las radiaciones adaptativas son definidas como “la divergencia de los miembros de un mismo linaje en una variedad de distintas formas adaptativas” [18]. La mayoría de las teorías de radiación adaptativa (ej. [7,18]) postulan el inicio de las mismas en presencia de “oportunidad ecológica” (ver también [19,20]). “Oportunidad ecológica” es definida como “la riqueza de recursos accesibles poco usados por otros taxa” [11]. Como lo resume Losos [19] existen 4 formas bajo las cuales los recursos se vuelven accesibles: a) aparición de nuevos recursos (la evolución de un clado provee recursos a otros taxa), b) extinción de las especies que previamente empleaban cierto recurso, c) colonización de un área nueva con ausencia de competidores y d) evolución de un rasgo que permita utilizar el recurso de una forma novedosa. En el caso de los sigmodontinos, estos pueden haberse encontrado ante oportunidad ecológica debido a varios factores. Primero, la colonización de nuevas áreas que habrían permanecido aisladas por largo tiempo ya sea al ingresar a Sudamérica o aprovechando la creación de nuevos hábitats durante el Mioceno. Segundo, los nuevos hábitats emergidos en los Andes pueden haber promovido la proliferación de linajes. Sumado a esto, el levantamiento andino y el enfriamiento global del Mioceno tardío habrían desencadenado el avance de las sabanas y hábitats de vegetación más abierta, lo que implicó un nuevo tipo de recursos disponibles o nuevos hábitats. Asimismo, las transiciones en la morfología dental, particularmente los molares, pueden haber permitido a algunos linajes explotar una mayor variedad de recursos alimenticios. Se ha propuesto que la aparición de molares “simplificados” de 4 lófos (o tetralofodonte, usualmente de corona alta o hipsodonte) habrían permitido la colonización de ambientes abiertos o “pastorales”, mientras que el tipo

pleisomórfico de 5 lofos (o pentalofofonte, usualmente de corona baja o braquiodonte), estarían asociados a taxa de ambientes “silvanos” o de bosque [2]. La diversidad de formas asociadas a distintas áreas y hábitats ocupados por roedores sigmodontinos, otorga un escenario ideal para considerar el rol de distintos “drivers” de diversidad y determinar cómo éstos influyeron en la diversificación o florecimiento de algunas tribus. Simultáneamente, se puede considerar si el patrón de radiación se asemeja a aquellos esperados en una radiación adaptativa [20]. Mediante la reconstrucción de la evolución del hábitat (rango altitudinal, tipo de vegetación ocupada), área ancestral y de morfología molar se podrá evaluar si estos factores se correlacionan con la/s tasa de diversificación.

1.2 Objetivos

En base a los antecedentes ya señalados, los objetivos de esta tesis se pueden resumir de la siguiente manera:

- a)** Reconstruir la filogenia de Sigmodontinae, en base a secuencias de genes mitocondriales y nucleares para examinar la radiación de los roedores sigmodontinos.
- b)** Estimar los tiempos de divergencia para los principales eventos cladogenéticos de Sigmodontinae, incluyendo el tiempo de origen del ancestro común más reciente de la subfamilia y de las tribus que la componen.
- c)** Distinguir aquellos clados que presenten diversidad extraordinaria examinando alejamientos en la tasa de diversificación y el desbalance en la riqueza de spp. dentro de los sigmodontinos.

d) Entender si la transición en morfología dentaria y/o los cambios en hábitat (altitud o tipo de vegetación) han causado un incremento o disminución en la tasa de diversificación, y examinar el modo de evolución de estos rasgos mediante la reconstrucción de caracteres ancestrales.

1.3 Estructura de la tesis

Establecer el tiempo de entrada del ancestro de los sigmodontinos fue esencial para entender el patrón de diversificación de este grupo. A través de inferencia Bayesiana, relojes moleculares relajados y calibraciones fósiles, se estableció una cronología para la radiación sigmodontina, un origen en el Mioceno medio del grupo [*ca.* 12 Ma], y la diversificación de la mayoría de las tribus durante el Mioceno tardío [capítulo 1, artículo publicado en *Molecular Phylogenetics and Evolution*]. Habiendo establecido un marco para entender el *tempo* de evolución de Sigmodontinae, el siguiente gran objetivo (o la siguiente aproximación) se concentró en considerar si el acceso a recursos, hábitat o áreas ocupadas o tipo de molar pueden haber influenciado las tasas de diversificación dentro de Sigmodontinae. Los resultados sugieren una correlación entre rango altitudinal y tipo de vegetación con las tasas de especiación y se destaca el rol de las tierras bajas tropicales con alta especiación [capítulo 2, pronto a someterse a PLOS ONE]. Finalmente, se revisan qué mecanismos explicativos de patrones de riqueza de especies pueden considerarse al examinar la diversidad sigmodontina. Se consideran los eventos que pueden haber facilitado el acceso a recursos en los roedores sigmodontinos, cómo estos pueden haber influido en la proliferación -o falta de ésta- en algunos grupos dentro de la Subfamilia, y se contextualizan los resultados obtenidos previamente [capítulo 3, manuscrito en preparación].

1.4 Modelo de estudio

La subfamilia Sigmodontinae (~ 400 spp.) es un grupo de roedores cricétidos que a su vez forman parte de la superfamilia Muroidea (~1300 spp.). Están distribuidos a lo largo y ancho del Nuevo Mundo, pero su riqueza de especies y endemismos es notable en Sudamérica [21]. Se encuentran prácticamente en todos los hábitats (desierto, bosque tropical, humedal, sabana, estepa, bosque templado, pasturas de altitud, salares y monte) desde el sur de los Estados Unidos hasta la zona más austral de Sudamérica, en Tierra del Fuego [21] [22]. Se encuentran desde el nivel del mar hasta aproximadamente 5,200 metros de altura. Tradicionalmente los géneros sigmodontinos han sido clasificados en grupos, algunos de los cuales han sido definidos formalmente como tribus (ej. [23]). La riqueza de especies no está distribuida de forma homogénea entre estos grupos, ya que tribus como Akodontini y Oryzomini acumulan la mayor parte de la riqueza [1]. Varias hipótesis acerca del *tempo* y modo de evolución de Sigmodontinae han sido propuestas. Para este trabajo se consideraron secuencias mitocondriales [citocromo b] y nucleares [IRBP] de representantes sigmodontinos, obteniendo el muestreo taxonómico más amplio logrado hasta la fecha, así como el acceso a las clasificaciones taxonómicas y literatura más recientes que están disponibles. Se utilizó la bibliografía disponible para recabar datos de hábitat, morfología molar y distribución para las especies consideradas [1] [22]. En esta tesis se presenta una cronología para el origen y diversificación de Sigmodontinae, se revela su biogeográfica histórica, y se analiza el *tempo* y modo de diversificación considerando cómo la evolución de preferencias de hábitat y la morfología dental (molar) han influido en la diversificación de la Subfamilia. Mediante el

análisis de los patrones de evolución de este grupo, se espera contribuir al conocimiento general de cómo se acumuló la biodiversidad en la región.

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CAPÍTULO 1

Datando de una radiación neotropical explosiva: estimados moleculares para Sigmodontinae (Rodentia) y su contribución a la biogeografía histórica.

Dating an impressive Neotropical radiation: molecular time estimates for the Sigmodontinae (Rodentia) provide insights into its historical biogeography

**2. DATING AN IMPRESSIVE NEOTROPICAL RADIATION: MOLECULAR
TIME ESTIMATES FOR THE SIGMODONTINAE (RODENTIA) PROVIDE
INSIGHTS INTO ITS HISTORICAL BIOGEOGRAPHY.**

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Dating an impressive Neotropical radiation: molecular time estimates for the Sigmodontinae (Rodentia) provide insights into its historical biogeography

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Abstract

With about 400 living species and 82 genera, rodents of the subfamily Sigmodontinae comprise one of the most diverse and more broadly distributed Neotropical mammalian clades. There has been much debate on the origin of the lineage or the lineages of sigmodontines that entered South America, the timing of entrance and different aspects of further diversification within South America. The ages of divergence of the main lineages and the crown age of the subfamily were estimated by using sequences of the interphotoreceptor retinoid binding protein and cytochrome *b* genes for a dense sigmodontine and muroid sampling. Bayesian inference using three fossil calibration points and a relaxed molecular clock estimated a middle Miocene origin for Sigmodontinae (~ 12 Ma), with most tribes diversifying throughout the late Miocene (6.9-9.4 Ma). These estimates together with the geographic distribution of the main sigmodontine lineages were used to discuss the timing of entrance into South America and further diversification of the main sigmodontine lineages. Results of analyses of ancestral area reconstructions suggest a distribution for the most recent common ancestor of Sigmodontinae in Central-South America and a South American distribution for the most recent common ancestor of Oryzomyia.

Keywords: Cricetidae, Great American Biotic Interchange, South America, diversification, Muroidea, relaxed molecular clock.

1. Introduction

South America, with its numerous episodes of faunal interchanges (Goin et al., 2012), constitutes an ideal scenario to investigate these patterns of diversification. There has been a considerable debate regarding the time of exchange of land mammals between North and South America (Woodburne, 2010). Particularly, the geographic origin of the invader lineage, the timing of entrance into South America and further diversification of the main lineages within the subfamily Sigmodontinae (family Cricetidae) remain unclear. The species richness and ecological range of this group has sparked considerable interest among evolutionary biologists; for example, according to Hershkovitz (1969), sigmodontines underwent a “pervasive adaptive radiation.” These rodents, with about 400 living species, occupy a variety of habitats and their lifestyles range from semiaquatic to fossorial, arboreal, cursorial, and scansorial. The pattern of distribution is remarkable considering that 68 of the 82 living genera are endemic to South America, whereas only one genus is endemic to Central America (D’Elía, 2003a). Controversies concerning the sigmodontine radiation have focused on the geographic placement of the basal diversification, and the timing that of ancestral forms entered into South America (reviewed in D’Elía, 2000; Pardiñas et al., 2002). As Smith and Patton summarized (1999; see also Steppan et al., 2004), there are two groups of competing hypotheses attempting to explain the biogeographic history of the Sigmodontinae. A “Late-arrival” hypothesis take the fossil record (see below) as evidence that the ancestors of South American sigmodontines reached South America by overland dispersal after the formation of the Panamanian land bridge. Considering this scenario, Simpson (1950) proposed a very rapid radiation of a basal stock once the ancestral form reached South America. As a variation of

this hypothesis, Patterson and Pascual (1972) and Baskin (1978) suggested that some or most of the sigmodontine genera had already diversified in tropical North and Central America before arriving into South America. On the other hand, Hershkovitz (1966, 1972) and Reig (1980, 1984) advanced an "early-arrival" hypothesis in which the ancestor of the sigmodontines reached South America by overwater dispersal, presumably sometime in the Miocene. These authors proposed that differentiation at the tribal, generic, and species level took place *in situ* after the arrival into South America. Marshall (1979) also supported a South American diversification but after a more recent arrival of 5-7 million years ago (Ma), contemporary to the invasion registered for the procyonid carnivore *Cyonasua* ca. 7.3 Ma (Procyonidae; Cione et al., 2007). More recently, Stepan et al. (2004) considered plausible a single origin for the sigmodontine ancestor in the Miocene (see below).

Attempts to understand the historical biogeography and the diversification of sigmodontines have been hampered by the sparse nature of the fossil record. For decades, the oldest known record of a South American sigmodontine was the extinct *Auliscomys formosus* from the Montehermosan, Buenos Aires, Argentina (4.5-5.3 Ma, Pardiñas and Tonni, 1998; Reig, 1978). Recently, Verzi and Montalvo (2008) identified an undetermined cricetid from the Huayquerian (5.7-5.8 Ma), La Pampa, Argentina, but its chronology was questioned by Prevosti and Pardiñas (2009) who would not rule out an early Pliocene age for these fossils. Finally, Nasif et al. (2009) reported a molar fragment resembling those of living *Calomys* from the Late Miocene (7.14 Ma) of northwestern Argentina.

The most recent molecular-based time-estimates of the main cladogenetic events of Sigmodontinae was based on four nuclear genes and used a smoothed-rate molecular clock and fossil calibration points outside Sigmodontinae (Stepan et al., 2004). This study,

focusing on the whole superfamily Muroidea and included only 10 sigmodontine genera; as such, it provided estimates only for the origin of the subfamily and of Oryzomyalia (defined as the clade containing the most recent common ancestor of the tribes Akodontini, Oryzomyini, Phyllotini, Thomasomyini, and Reithrodontini and all of its descendents, excluding Sigmodontini; cf. Steppan et al., 2004:547). The origin of Sigmodontinae was estimated between 10.6 to 14.5 Ma and the origin of Oryzomyalia was estimated between 6.0 and 8.8 Ma (Steppan et al., 2004). Obtaining additional estimates for the timing of the sigmodontine radiation, including estimates for all tribes, would inform us whether this was a case of recent explosive radiation or if the lineages diversified at a constant rate through a long history in South America. Moreover, considering the disparity in species richness among tribes, from two to ca. 130 living species (Weksler and Percequillo, 2011), it would be relevant to determine if the more species-rich tribes (e.g., Akodontini and Oryzomyini) emerged earlier than the others (e.g., Abrotrichini) or if different rates of diversification characterize different tribes.

Thus, the main goal of this study was to infer the time frame of the origin and diversification of the major lineages of the Sigmodontinae using a reconstructed time-calibrated phylogeny based on nuclear and mitochondrial DNA sequence data. We show that a dense taxonomic sampling, which includes sequences of genera belonging to all recognized sigmodontine tribes, as well as several genera of uncertain position (D'Elía, 2003b; D'Elía et al., 2007; Musser and Carleton, 2005), together with the availability of methods for estimating divergence times with an uncorrelated relaxed clock model contribute to the knowledge of the evolution of the South American fauna. Likewise, through ancestral area reconstructions we inferred the geographic distribution of the ancestor of the subfamily.

2. Material and methods

2.1 Character and taxonomic sampling

Analyses were based on DNA sequences of the first exon of the nuclear gene interphotoreceptor retinoid binding protein (IRBP) and mitochondrial cytochrome *b* (cyt *b*). A total of 96 sequences of IRBP and cyt *b*, all gathered from GenBank (Supplementary Material: Table 1), were analyzed. Sigmodontine diversity was thoroughly covered, including representatives of all recognized tribes as well as most of the genera considered as Sigmodontinae *incertae sedis* (see the last sigmodontine classification in D'Elía et al., 2007); as such, 76 of the 82 currently recognized genera were included. We included representatives for which IRBP sequences were available and we also searched for cyt *b* sequences of the same genus. Composite sequences (i.e., IRBP from one species and cyt *b* from another) were constructed for a few genera (see Table 1 in Supplementary Material); available studies have show that these genera are monophyletic (e.g., D'Elía et al, 2003, 2005; D'Elía and Pardiñas, 2004; Smith and Patton, 1999; Weksler, 2003, 2006). Nine genera, *Auliscomys*, *Bibimys*, *Hylaeamys*, *Nesoryzomys*, *Oecomys*, *Oligoryzomys*, *Oryzomys*, *Rhagomys*, and *Thomasomys*, were represented by sequences gathered from different congeneric species. For *Aepeomys*, *Microakodontomys*, *Neusticomys*, and *Rheomys*, there were no cyt *b* sequences; these taxa were represented by ambiguous state characters (i.e., n) for the cyt *b* portion of the alignment. Sigmodontine monophyly and its placement within the family Cricetidae are well

corroborated, however, its sister group is not clear. Similarly, the relationships among main muroid lineages remained mostly unresolved (e.g., Engel et al., 1998; Jansa and Weksler, 2004; Stepan et al., 2004). Therefore, our sampling included, when sequences of both analyzed genes were available, data from two representatives of the other cricetid subfamilies and one representative of all non-cricetid subfamilies of Muroidea (sensu Stepan et al., 2004: 547); these sequences comprised the outgroup.

2.2 Phylogenetic analyses and divergence time estimation.

Sequence alignment was carried out using MUSCLE (Edgar, 2004) with the default settings. The IRBP matrix had 1181 positions of which 650 were variable; one insertion of three base pairs was inferred in the sequence of *Scolomys* and another of six base pairs long in the sequences of *Steatomys*. Muroid cytochrome *b* sequences had variable lengths. Most commonly, sequences were 1140 base pairs long ending in a TAA or TAG stop codon. Other sequences were 1143 bp, ending in a TAA or TAC stop codon. Meanwhile, other sequences did not end with a TAA or TAG stop codon, but had an extra T, which presumably gets polyadenylated to form a stop codon as reported for *Mus* (Bibb et al., 1981). From this alignment it was clear that the position of the indel/s responsible for the difference in gene length was present at the very end of the sequences, but determining its exact position was impossible (i.e., it may corresponded to the codon number 379, 380 or 381). To avoid this problem, we followed D'Elia et al. (2003) and based our analysis on the first 1134 bases of the sequences; this cyt *b* alignment had 655 variable sites.

The Bayesian analysis was conducted in BEAST v1.7.1 (Drummond et al., 2012) using a partitioned dataset that simultaneously estimated substitution model parameters, and dates for cladogenetic events for both genes (henceforth referred to as “combined dataset”). Topology was estimated considering both partitions simultaneously. Additionally, an analysis with a matrix limited to the IRBP dataset was conducted considering the same priors. Substitution rates were estimated from the data; a speciation Yule Process using an initial random tree was set as a prior. The substitution model employed was GTR + Γ + I (cyt b) and TVM + Γ + I (IRBP, modifying the file generated by the program BEAST following instructions at http://beast.bio.ed.ac.uk/Substitution_model_code), with empirical base frequencies, and 4 gamma rate categories was selected using ModelGenerator (Keane et al., 2006). Given that preliminary analysis indicated (considering the *ucl.d.mean* parameter) that sequences did not evolve in a clock-like mode, runs were performed under an uncorrelated lognormal relaxed clock model. Three independent runs of 9.0×10^7 and 6.0×10^7 , generations sampled every 8000 generations were performed with the combined and IRBP dataset respectively. Convergence to stable values was checked with Tracer v.1.5 (Rambaut and Drummond, 2007), obtaining an effective sample size (ESS) greater than 200 (harmonic mean ~1000 for each run) for all parameters and examining the runs through AWTY (Wilgenbusch et al., 2004). Tree and log files (-3.3% and -1.6% burnin for the combined and IRBP datasets respectively) were combined using LogCombiner (Drummond et al., 2012). Trees then were compiled into a maximum clade credibility (MCC) tree using TreeAnnotator (Drummond et al., 2012) to display mean node ages and highest posterior density (HPD) intervals at 95% (upper and lower) for each node.

To estimate divergence times, three calibrations based on the fossil record were incorporated into the analysis. Calibrations were implemented in the form of lognormal prior distributions with lognormal means of 0.01 and lognormal standard deviations of 0.6. We provided a minimum bound for each distribution such that the 5% quantile corresponds to the minimum age of the fossil while the 95% interval allows both for the uncertainty of the fossil age and for the incompleteness of the fossil record. The following calibrations were used (offset, median, 5% and 95% quantiles in Ma respectively): a) the crown clade *Abrothrix* (2.9, 3.91, 3.276 and 5.61) based on the fossil species *Abrothrix kermacki* Reig (see Reig, 1978, 1987), b) the crown clade of *Sigmodon* (4.13, 5.14, 4.506 and 6.84) based on the fossil record of *Sigmodon* spp. (see Peláez-Campomanes and Martin, 2005); and c) the crown clade of Phyllotini (4.95, 5.96, 5.326 and 7.66) based on the fossil species *Auliscomys formosus* Reig (see Pardiñas and Tonni, 1998; Reig, 1978).

Additionally, maximum likelihood (ML) analyses on both datasets were conducted using RAxML GUI v.1.1 (Silvestro and Michalak, 2010), a graphical front-end for RAxML-VI-HP (Randomized Accelerated Maximum Likelihood; Stamatakis, 2006). ML searches were conducted by means of 100 inferences with 100 distinct randomized trees using the general time-reversible (GTR) model with gamma distributed rate heterogeneity. Branch support were recovered as in Shimodaira and Hasegawa (1999) and plotted on the single best-scoring tree of each of the analyses.

2.3 Biogeographic analysis

Sigmodontine ancestral distributions were reconstructed using Bayesian Binary MCMC (BMM) and Dispersal-Vicariance Analysis (S-DIVA) analyses implemented in the program RASP (Reconstruct Ancestral State in Phylogenies) 2.1 alpha (Yu et al., 2010; Yu et al., 2011). In these methods, ancestral ranges are averaged over all trees. To account for uncertainties in phylogeny, we used all of the post-burnin trees obtained with BEAST for the combined dataset. The number of maximum areas was kept as either three or two. The possible ancestral ranges at each node on the MCC Bayesian tree were recovered. Ten MCMC chains were run simultaneously for 5×10^5 generations. The state was sampled every 1000 generations and the temperature for heating the chains was 0.1. A model with all biogeographic events equally likely, similar to a fixed Jukes-Cantor model, was used along with a wide root distribution. Distributions were coded in a character matrix by successive numbers (character states = Continent A-G, see Supplementary Material, Fig. 4 for details). Terminal taxa were coded to represent either the distribution of the genus (for Sigmodontinae) or the subfamily (for the muroid taxa included here) following data given in Musser and Carleton (2005). Additionally, a time-events curve was recovered after the S-DIVA analysis in order to illustrate the events occurring during the sigmodontine diversification. The “standard curve” which has only one event per node was selected.

3. Results

3.1 Phylogenetic relationships

Chronograms were compiled and annotated from 32628 and 22125 (combined and IRBP datasets respectively) post-burnin trees as two MCC trees. Topologies recovered from the analysis of both matrices are congruent; as such only that of the combined dataset is shown (Figs. 1 and 2; the tree obtained with the analysis of the IRBP dataset is shown in Supplementary Material). In addition, these topologies are congruent to those gathered in previous studies (e.g., D'Elia et al., 2006a,b; Martínez et al., 2012; Salazar-Bravo et al., submitted; Weksler, 2003), and as such only the main features will be mentioned. We focused on the ages inferred for the different clades, which are presented as the mean node heights within HPD interval at 95% retrieved by the MCMC at each node (Table 1).

In the Bayesian tree, Tylomyinae was recovered as sister to Sigmodontinae (posterior probability, PP=0.91). Sigmodontinae was highly supported (PP=1; Fig. 1). Sigmodontine tribes, with the sole exception of Ichthyomyini, were recovered monophyletic with strong support (PP=1; Fig. 2). The most basal dichotomy within Sigmodontinae leads to a clade (PP=0.90) composed of *Sigmodon* (PP =1) and *Rheomys* on one side of the node, and to another clade (PP =0.88) composed by the remainder sigmodontines in the other. As such, Ichthyomyini is not recovered monophyletic, given that *Rheomys* is sister to Sigmodontini while *Neusticomys* is sister to Oryzomyia. Within the latter (PP=0.99), all tribes composed of more than one genus are recovered as monophyletic with strong support (i.e., Abrotrichini, Akodontini, Oryzomyini, and Phyllotini all with PP=1; Thomasomyini with PP=0.92). In addition, a clade (PP=0.8; hereafter clade A) composed of the genera *Andinomys*, *Chinchillula*, *Euneomys*, *Irenomys*, *Juliomys*, *Neotomys* and *Punomys* was recovered. The *incertae sedis* genus *Delomys* was sister (PP=0.99) to Phyllotini. Wiedomyini and Abrotrichini formed a well-supported clade (PP=0.97), which was sister (PP=0.98) to the

Delomys-Phyllotini clade. Tree topology of the IRBP solo matrix is similar to the combined dataset. The only notable discrepancy was that the clade *Wiedomys*-Abrotrichini was recovered, with weak support, sister to clade A (see Fig. 1 in Supplementary Material).

Topologies recovered from ML analyses were congruent with those obtained in the Bayesian analyses (see Supplementary Material). The final GAMMA-based score of best tree found in the combined analysis was -52628.7989. The main discrepancy with the Bayesian tree was found with regards to the base of the clade Oryzomyalia (i.e. a paraphyletic Thomasomyini with respect to Reithrodontini is sister to the remaining members of this clade).

3.2 Molecular clock dating

The stem age of Sigmodontinae was estimated to be 15.4 Ma, (HPD 11.91-19.41, mid Miocene, Langhian Age; see Table 1 for additional estimates with the single locus analysis). The estimated crown age of Sigmodontinae was 11.82 Ma (HPD 9.28-14.70, mid Miocene, Servallian Age). Initial divergence within the subfamily began during the Tortonian Age (ca. 10.85 Ma). Several divergence events during the Late Miocene and Pliocene resulted in lineages leading to the emergence of most of the sigmodontine tribes (Abrotrichini, Akodontini, Oryzomyini, Phyllotini, Reithrodontini, Thomasomyini, and Wiedomyini), clade A, and the genus *Delomys* (Table 1). Throughout the Late Miocene the crown groups of the tribes Akodontini, Oryzomyini, Phyllotini, and Thomasomyini and clade A began to radiate (grey line in Fig. 1); meanwhile the crown groups of the tribes Abrotrichini and Sigmodontini began their diversification in the Pliocene.

3.3 Ancestral area reconstruction

Results based on the combined data set of the ancestral area reconstruction using S-DIVA (keeping “maximum area” option as 3) supported a Central-South American range for the most recent common ancestor of Sigmodontinae (marginal probability for Central-South America 99.99%). The range at the most recent common ancestor of Oryzomyia was inferred as South America with 100 % of marginal probability. Inferred ancestral areas for all nodes within the sigmodontine radiation and dispersion events inferred to account for current distributions given the phylogenetic relationships are shown in Supplementary Material Figure 4.. The BMM approach, considering a “maximum area” of two yielded similar results than those of S-DIVA; the combined area Central + South America (72.37%) was inferred as the most probable ancestral region for the same node while South America was the second most likely (14.97%). Meanwhile, for the common ancestor of Oryzomyia, South America appears as the most probable distributional area (89.13%). Considering a “maximum area” of three, a combined area of the three Americas as the most probable ancestral region (66.78%) and South America was the second most likely (24.10%). A time-event curve representing the events inferred along the sigmodontine radiation is given in Supplementary Material, Figure 5

4. Discussion

The study of sigmodontine historical biogeography ought to be seen as one of the most remarkable chapters of Neotropical mammalogy and of the Great American Biotic

Interchange; as such, it has generated much –sometimes acrid-- debate (reviewed in D’Elía, 2000; see also HersHKovitz, 1966; Reig, 1981). Even when most sigmodontines are endemic to South America, there is consensus that the immediate ancestor of the group was not autochthonous to the subcontinent, given that no potential ancestor has been discovered in the South American fossil record. The fossil record indicates that the sigmodontine invasion could have occurred as late as the Late Miocene or Early Pliocene (Nasif et al., 2009; Prevosti and Pardiñas, 2009; Verzi and Montalvo, 2008). Similarly, other questions that need to be answered include the geographic placement of the diversification of the main sigmodontine lineages (i.e., if a single sigmodontine stock entered South America and then radiated in the subcontinent or if multiples lineages invaded South America) and the timing of the invasion/s (Steppan et al., 2004). The present study sheds new light on these issues by considering the geographic distribution of the main sigmodontine lineages and the timing of their diversification.

Previous attempts at dating the sigmodontine radiation with molecular clocks mostly focused on the split of the subfamily from other cricetid lineages and on the diversification of some of their tribes. In spite of the different approaches used, these studies prompted similar results. The split of the lineage leading to Sigmodontinae was estimated ~ 7.6-12.4 Ma considering the mitochondrial ND3, ND4L and ND4 genes (Engel et al., 1998), whereas the sigmodontine crown age was estimated ~ 10-14 Ma based on *cyt b* sequences (Smith and Patton, 1999), and 10.6-10.45 Ma based on the growth hormone receptor, the breast cancer 1, the recombination activating 1, and the proto-oncogene *c-myc* genes (Steppan et al., 2004). These authors used either local clocks, ‘quartet method’ (Engel et al., 1998, Smith and Patton, 1999), or rate-smoothing (Steppan et al., 2004). Our analyses employing an uncorrelated

relaxed-clock framework recovered older estimates for the IRBP dataset considering the stem age and crown age of Sigmodontinae (20.25 and 14.46 Ma respectively) and Oryzomyalia (10.75 Ma, see also Table 1). Estimates for the origin of Sigmodontinae based on the combined dataset (stem age of 15.41, HPD 11.91-19.41 and crown age of 11.82, HPD 9.28-14.7) are younger than those based on IRBP only analysis (stem age of 20.25, HPD 14.96-26.09 and crown age of 14.46, HPD 10.92-18.66). The analysis of the combined dataset suggested the origin of Oryzomyalia as ca. 9.81 (HPD 7.68-12.08) Ma, an average date that is older than the 6-8.8 Ma estimated by Steppan et al. (2004). In addition, our results suggested that the differentiation of the oryzomyalid crown group took place around 7-9 Ma during the late Miocene as an apparent pulse when five tribal-level groups emerged.

Even after considering the uncertainties mentioned above, molecular clock estimates clearly falsify those historical biogeographic scenarios suggesting that sigmodontines radiated in South America after a single stock entered the continent when the Panamanian land bridge was completed (e.g., Simpson, 1950). All sigmodontine lineages of tribal level are older than the closing of the Panamanian Isthmus about three Ma (Bartoli et al., 2005, see grey line in Fig. 1). In addition, despite the reinsertion of putative doubts in recent revisions (e.g., Webb, 2006), the presence of sigmodontines in southern South America previous to the completion of Panamanian Isthmus is firmly established from the fossil record (e.g., Pardiñas, 2000, Pardiñas and Tonni, 1998). Moreover, by the beginning of the Pliocene —about five Ma ago— at least four tribes were present in central Buenos Aires province: Reithrodontini, Phyllotini, Akodontini, and Abrotrichini (Reig, 1978, Pardiñas et al., 2002).

The biogeographic analysis favored a distribution in Central and South America for the most recent common ancestor of Sigmodontinae (Fig. 4, Supplementary Material).

therefore the sigmodontine ancestral distribution as the numbers of sigmodontine lines that invaded South America remains to be solved. We concur with Steppan et al. (2004: 548-549) in that the most plausible scenario involves waif dispersal of one or few lineages into South America through a proto-Caribbean sea. If more than one lineage invaded South America, representatives of three major lineages (i.e., Oryzomyalia, Sigmodontini and Ichthyomyini), each largely distributed in the Americas, might have arrived to the continent well before the completion of the Panamanian Isthmus; if the non-monophyly of Ichthyomyini is corroborated an additional invader lineage may be invoked depending on the phylogenetic position of ichthyomyine genera not yet analyzed (i.e., *Anotomys*, *Chibchanomys*, *Ichthyomys*). The timing of these putative multiple invasions remains unclear and did not necessarily occur at the same time. However, according to our estimates for the origin and radiation of the subfamily the invasions would have been older than those suggested in Marshall's (1979) proposal of waif dispersal across the Bolivar Trough around five or seven Ma. Regarding the isolation of South America, it must be noted that according to paleogeographic reconstructions the Central American Seaway must have existed in the early to middle Miocene (MacFadden, 2006). Given that between 6 and 19 Ma North and Central America would have been a continuum, the Central American Seaway acted as the ultimate barrier to the exchange of land mammals between North-Central and South America (Kirby et al., 2008).

The radiation of Oryzomyalia into its main lineages (i.e., the tribes Abrotrichini, Akodontini, Oryzomyini, Phyllotini, Reithrodontini, Thomasomyini, Wiedomyini and other main lineages such as that leading to clade A), as well as their further diversification, occurred in South America (see also Smith and Patton, 1999). After all, most of the oryzomyalid tribes

are endemic to South America; only *Oryzomyini* is found in the three Americas, while *Thomasomyini* has only one genus that marginally reached Panama (Musser and Carleton, 2005). If the main oryzomyalid radiation had taken place outside South America, most sigmodontine diversity, in particular in phylogenetic terms, would be present in Central and/or North America (see also Reig, 1984, 1986), a scenario that does not occur. Our ancestral area analyses are congruent with this argument (Fig. 4, Supplementary Material). The entrance into a new adaptive zone, a South American continent almost empty of small gnawing mammals, represented a source of ecological opportunities (*sensu* Losos, 2010; Losos and Mahler, 2010), which might have triggered an adaptive radiation leading to the proliferation of the main lineages or tribes of Sigmodontinae. In addition, the diversification of early sigmodontine lineages may have been prompted by a variety of vicariant events. Paleographic reconstructions of the Late Miocene South America indicate a mosaic of emerged land crisscrossed by marine transgressions (particularly the Pebasian and Paranaense at ca. ~10 Ma) marine ingressions might have acted as barriers (Räsänen et al., 1995; Webb, 1995). As seas retreated during marine regressions there was ample opportunity for the expansion through the newly emerged land (Webb, 1995; Wise et al., 2008). As such, after the entrance into a new territory, several events, including the Andean orogeny, the reconnection of South America with North-Central America, the floodbasin system in the Amazonian Miocene, the formation of Orinoco and Amazon drainages and the onset of glacial-interglacial cycles, together with the multiple ecological opportunities they generated, prompted the proliferation of sigmodontines.

After an original pulse of diversification in South America, different lineages (e.g., *Neacomys*, *Oecomys*, and *Rhipidomys*) secondarily invaded Central and North America.

Future phylogenetic studies, including an exhaustive sigmodontine taxonomic sampling, should further clarify if taxa like *Melanomys*, *Nephelomys*, *Sigmodontomys* and *Transandinomys*, which have larger Central American distributions, and the genera *Handleyomys* (as currently delimited that includes the "*alfaroi* species group"), *Oligoryzomys*, *Oryzomys*, and *Sigmodon* that inhabit areas of the three Americas and the genus *Rheomys* that does not inhabit South America, are all direct descendants of the first sigmodontines or if they also represent secondary invasions to North America from South America. The position of most of these taxa, well nested into the sigmodontine radiation, argues in favor of the latter scenario. On the other hand, the position of *Sigmodon* in the tree together with its extensive North American fossil record, allegedly go back to Early Blancan ages (Peláez-Campomanes and Martin, 2005), would suggest that for this genus the former scenario is the one most likely. However, available evidence on the phylogenetic relationships of *Sigmodon* species (Peppers et al., 2002; Henson and Bradley, 2009), suggests that the most recent common ancestor of the genus would have been a South American lineage. Additionally, the phylogenetic closeness between *Sigmodon* and the North American fossil form *Prosigmodon*, an alleged ancestor of the *Sigmodon* line (Peláez-Campomanes and Martin, 2005), has not been properly assessed (see discussion in Prevosti and Pardiñas, in press).

Crown ages of all but one tribe for which more than one genus was analyzed, appear to have originated in the Miocene; crown age of Abrotrichini falls in the Pliocene. The oldest lineage of tribal level seems to be the clade A. However, there is no apparent relation between tribal crown age and species richness. Plausible causes behind the apparent observed differences between the rhythm and tempo of tribal diversification are for the moment unclear. Further studies, after testing the significance of observed differences, may explore if

these are associated to different degrees of morphological and/or functional diversity and if a particular evolutionary novelty (Mayr, 1963) or a particular geographic setting may have favored the diversification of any of these groups. In addition, possible differences in extinction rates among tribal level lineages should be explored; the fossil record shows that Reithrodontini, which includes only one living genus, is comparatively the tribe with most extinct genera (Ortiz et al., 2000; Pardiñas et al., 2002). More in general, when additional unlinked loci are considered more robust time estimates should be achieved. Similarly, future work should incorporate different combinations of priors as calibration points. Finally, considering molecular data for the remaining six genera of South American sigmodontines would help to provide a more complete picture of this impressive rodent radiation.

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FIGURES



Figure 1. Chronogram illustrating the diversification of Muroidea obtained from the Bayesian analysis of a matrix combining IRBP and cyt b gene sequences. Ages are represented as mean node height for a maximum clade credibility tree compiled from post-burnin trees topologies from 3 independent runs (9.0×10^7 generations per run) implemented in BEAST v1.7.1. Bars at the nodes represent the 95% highest posterior density credibility interval of the node's age. Branch support is indicated as Bayesian Posterior Probability (PP).

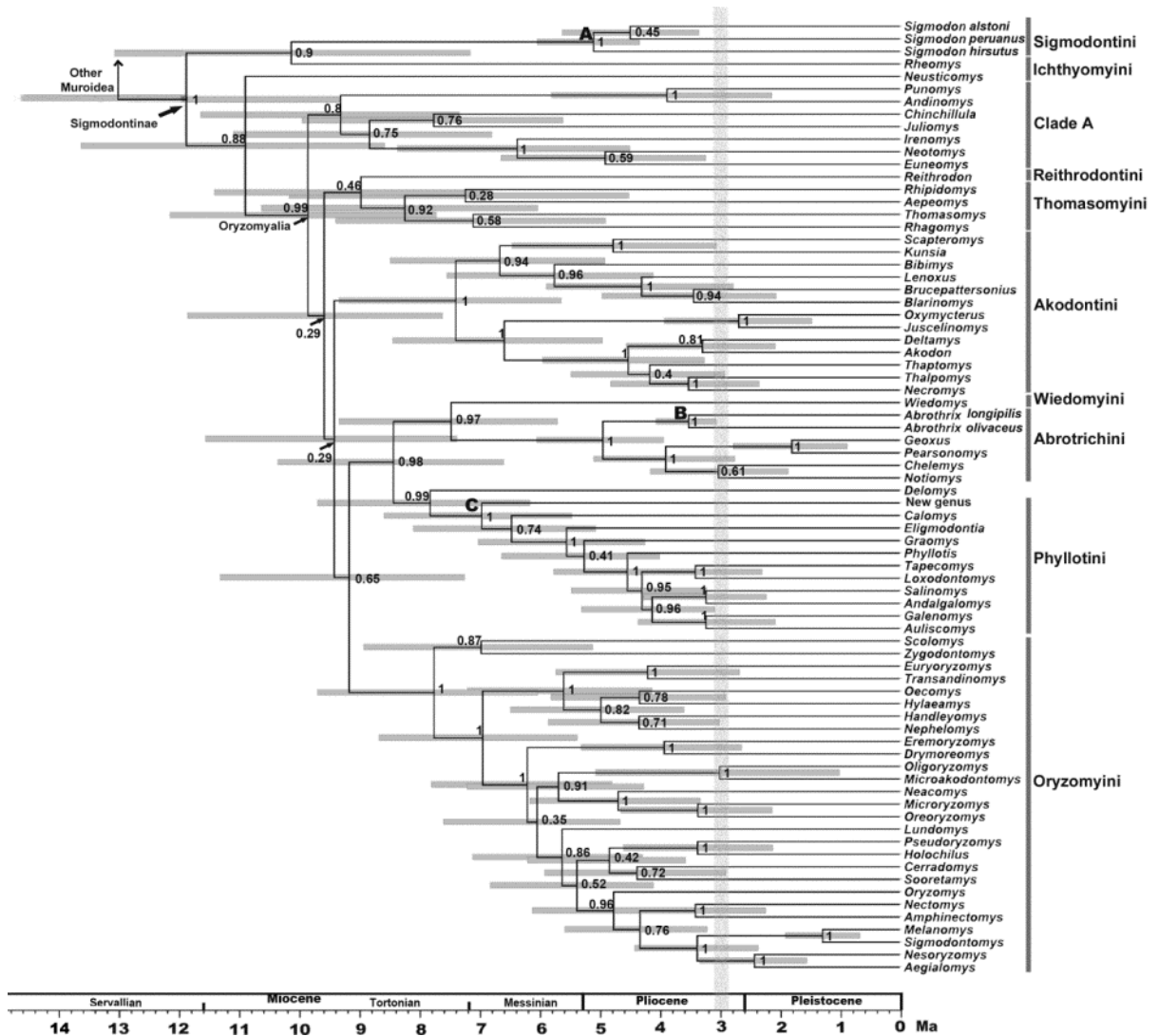


Figure 2. Chronogram illustrating the diversification of Sigmodontinae obtained from the Bayesian analysis of a matrix combining IRBP and cyt b gene sequences. Ages are represented as mean node height for a maximum clade credibility tree compiled from post-burnin trees topologies from 3 independent runs (9.0×10^7 generations per run) implemented in BEAST v1.7.1. Three calibration points were used: A, Sigmodon; B, Abrothrix; and C, Phyllotini. Ages of the nodes of interest are provided in Table 1. Bars at the nodes represent the 95% highest posterior density credibility interval of the node's age. Branch support is indicated as Bayesian Posterior Probability (PP). Estimated time for the closure of the Panamanian land bridge (ca. 3 Ma) is shown as a gray line.

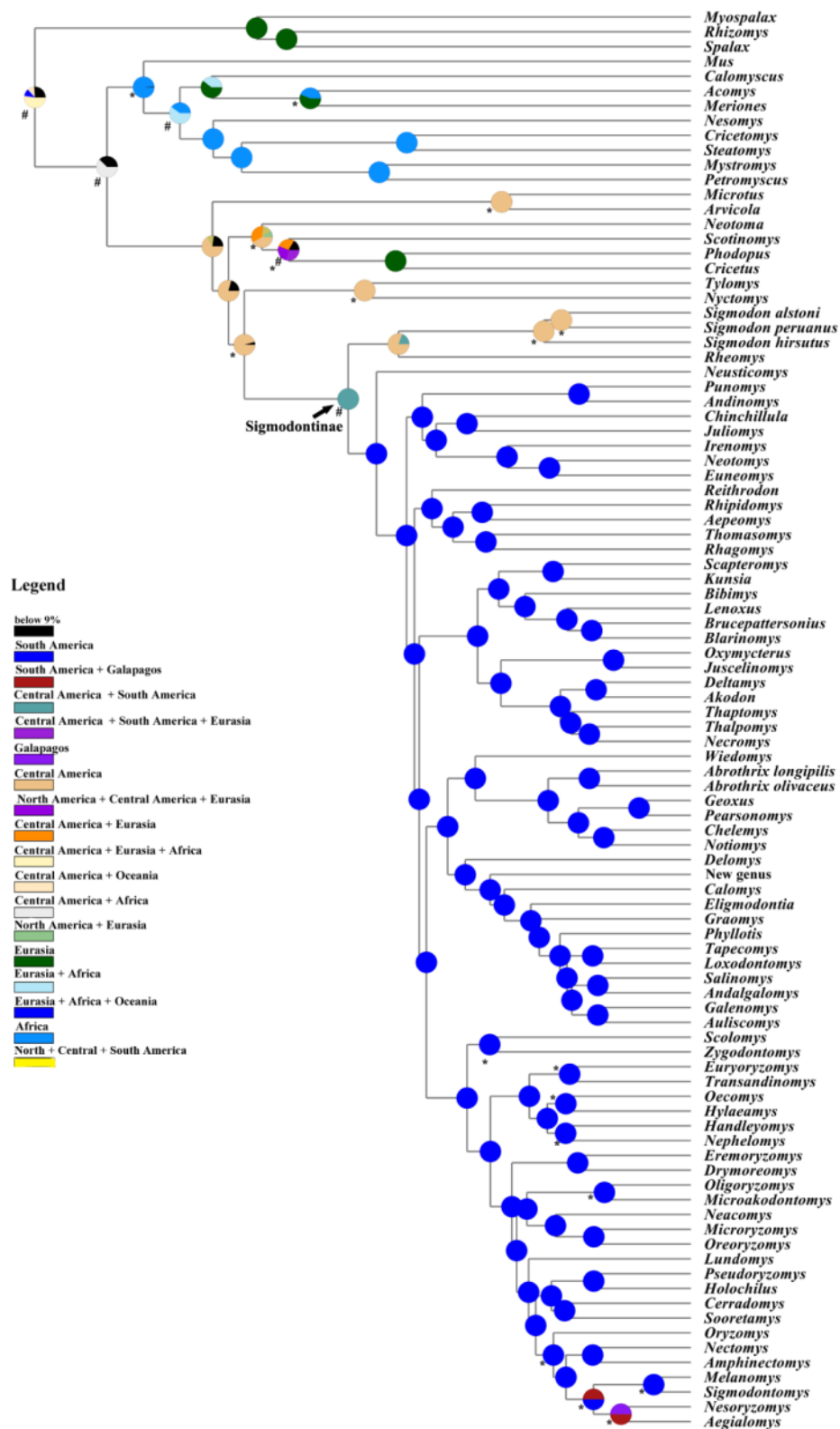


Figure 3. Historical biogeographic analysis of Sigmodontinae. Ancestral distributions were reconstructed with Dispersal-Vicariance Analysis (S-DIVA) in RASP (Reconstruct Ancestral State in Phylogenies) using the maximum clade credibility tree recovered with BEAST for the combined dataset (keeping three “maximum area”). Pie charts at each node show probabilities of alternative ancestral ranges. A “*” close to a node denotes an inferred dispersion event while a “#” marks a vicariance event. Squares at the right of the tips represent the actual area as coded in this analysis

TABLES

Lineage	IRBP/cyt <i>b</i>			IRBP		
	Mean	Std. error	95% HPD	Mean	Std. error	95% HPD
<i>Abrothrix</i>	3.5	1.80E-03	3.06–4.06	3.65	2.60E-03	3.07–4.36
<i>Sigmodon</i>	5.08	3.80E-03	4.33–6.02	5.12	3.90E-03	4.32–6.14
<i>Abrotrichini</i>	4.92	5.40E-03	3.93–6.02	4.91	5.70E-03	3.66–6.33
<i>Akodontini</i>	7.36	1.30E-02	5.62–9.29	6.98	1.40E-02	4.91–9.34
<i>Oryzomyia</i>	9.81	1.60E-02	7.68–12.08	10.71	1.80E-02	8.10–13.67
<i>Oryzomyini</i>	7.72	1.30E-02	6.01–9.64	8.51	1.70E-02	6.18–11.00
<i>Phyllotini</i>	6.93	1.10E-02	5.45–8.54	6.31	6.50E-03	5.19–7.75
Clade A	9.38	1.60E-02	7.33–11.73	9.37	2.00E-02	6.45–12.48
Sigmodontinae (crown)	11.82	2.10E-02	9.28–14.70	14.46	2.50E-02	10.92–18.66
Sigmodontinae (stem)	15.41	3.40E-02	11.91–19.41	20.25	4.10E-02	14.96–26.09
Thomasomyini	8.24	1.60E-02	6.11–10.66	6.96	1.60E-02	4.36–9.69

Table 1. Crown age estimates for the main clades of Sigmodontinae (and one stem age) gathered from the Bayesian analyses, performed in BEAST v1.7.1, of a matrix combining IRBP and cyt *b* gene sequences and another matrix consisting only of IRBP sequences. Ages (Ma) are mean node heights from HPD intervals at 95% (upper and lower) and standard error of the mean (std. error).

SUPPLEMENTARY MATERIAL

Table 1. Taxa included in the phylogenetics analysis of Sigmodontinae and Genbank accession numbers of the IRBP and cyt b DNA sequences used for each taxa.

Species	IRBP	Species	Cyt b
<i>Abrothrix longipilis</i>	AY163577	<i>Abrothrix longipilis</i>	ALU03530
<i>Abrothrix olivaceus</i>	AY277421	<i>Abrothrix olivaceus</i>	AY275111
<i>Aegialomys xanthaeolus</i>	GQ178247	<i>Aegialomys xanthaeolus</i>	EU579479
<i>Aepeomys lugens</i>	DQ003722		---
<i>Akodon azarae</i>	AY163578	<i>Akodon azarae</i>	DQ444328
<i>Amphinectomys savamis</i>	AY163579	<i>Amphinectomys savamis</i>	EU579480
<i>Andalgalomys pearsoni</i>	EU649038	<i>Andalgalomys pearsoni</i>	JQ434418
<i>Andinomys edax</i>	JQ434399	<i>Andinomys edax</i>	JQ434419
<i>Auliscomys pictus</i>	JQ434401	<i>Auliscomys boliviensis</i>	JQ434420
<i>Bibimys chacoensis</i>	AY277435	<i>Bibimys labiosus</i>	DQ444329
<i>Blarinomys breviceps</i>	AY277437	<i>Blarinomys breviceps</i>	AY275112
<i>Brucepattersonius igniventris</i>	AY277438	<i>Brucepattersonius soricinus</i>	AY277486
<i>Calomys lepidus</i>	AY163580	<i>Calomys lepidus</i>	EU579473
<i>Cerradomys scotti</i>	EU649040	<i>Cerradomys scotti</i>	EU579482
<i>Chelemys macronyx</i>	AY277441	<i>Chelemys macronyx</i>	CMU03533
<i>Chinchillula sahamae</i>	JQ434409	<i>Chinchillula sahamae</i>	JQ434422
<i>Delomys sublineatus</i>	AY163582	<i>Delomys sublineatus</i>	AF108687
<i>Deltamys kemp</i>	AY277444	<i>Deltamys kemp</i>	AY195862
<i>Drymoreomys albimaculatus</i>	EU649042	<i>Drymoreomys albimaculatus</i>	EU579487
<i>Eligmodontia typus</i>	AY277445	<i>Eligmodontia typus</i>	AF108692
<i>Eremoryzomys polius</i>	AY163624	<i>Eremoryzomys polius</i>	EU579483
<i>Euneomys chinchilloides</i>	AY277446	<i>Euneomys chinchilloides</i>	AY275115
<i>Euryoryzomys nitidus</i>	EU649041	<i>Euryoryzomys nitidus</i>	EU579485
<i>Galenomys garleppi</i>	JQ434410	<i>Galenomys garleppi</i>	JQ434423
<i>Geoxus valdivianus</i>	AY277448	<i>Geoxus valdivianus</i>	AY275116
<i>Graomys griseoflavus</i>	EU649037	<i>Graomys griseoflavus</i>	EU579472
<i>Handleyomys alfaroi</i>	EU649043	<i>Handleyomys intectus</i>	EU579490
<i>Holochilus brasiliensis</i>	AY163585	<i>Holochilus brasiliensis</i>	EU579496
<i>Hylaeamys laticeps</i>	EU649050	<i>Hylaeamys megacephalus</i>	EU579499
<i>Irenomys tarsalis</i>	AY163587	<i>Irenomys tarsalis</i>	ITU03534
<i>Juliomys pictipes</i>	AY163588	<i>Juliomys pictipes</i>	AF108688
<i>Juscelinomys huanchacae</i>	AY277453	<i>Juscelinomys huanchacae</i>	AF133667
<i>Kunsia tomentosus</i>	AY277455	<i>Kunsia tomentosus</i>	AY275120
<i>Lenoxus apicalis</i>	AY277456	<i>Lenoxus apicalis</i>	LAU03541
<i>Loxodontomys micropus</i>	JQ434412	<i>Loxodontomys micropus</i>	AY275122
<i>Lundomys molitor</i>	AY163589	<i>Lundomys molitor</i>	EU579501
<i>Melanomys caliginosus</i>	EU649052	<i>Melanomys caliginosus</i>	EU340020
<i>Microakodontomys transitorius</i>	EU649054		---
<i>Microryzomys minutus</i>	AY163592	<i>Microryzomys minutus</i>	AF108698
<i>Neacomys musseri</i>	AY163596	<i>Neacomys spinosus</i>	EU579504
<i>Necomys lasiurus</i>	AY277459	<i>Necomys lasiurus</i>	AY273912
<i>Nectomys squamipes</i>	AY163598	<i>Nectomys squamipes</i>	EU340012
<i>Neotomys ebriosus</i>	JQ434413	<i>Neotomys ebriosus</i>	JQ434424

<i>Nephelomys albigularis</i>	EU649057	<i>Nephelomys albigularis</i>	EU579505
<i>Nesoryzomys swarthi</i>	AY163601	<i>Nesoryzomys fernandinae</i>	EU579506
<i>Neusticomys monticolus</i>	EU649036	---	---
New Genus	JQ434417	New Genus	JQ434425
<i>Notiomys edwardsii</i>	AY163602	<i>Notiomys edwardsii</i>	NEU03537
<i>Oecomys bicolor</i>	AY163604	<i>Oecomys mamorae</i>	EU579509
<i>Oligoryzomys nigripes</i>	AY163612	<i>Oligoryzomys fulvescens</i>	DQ227457
<i>Oreoryzomys balneator</i>	AY163617	<i>Oreoryzomys balneator</i>	EU579510
<i>Oryzomys couesi</i>	EU273430	<i>Oryzomys palustris</i>	EU074639
<i>Oxymycterus nasutus</i>	AY277468	<i>Oxymycterus nasutus</i>	EF661854
<i>Pearsonomys annectens</i>	AY851749	<i>Pearsonomys annectens</i>	AF108672
<i>Phyllotis xanthopygus</i>	AY163632	<i>Phyllotis xanthopygus</i>	AY275128
<i>Pseudoryzomys simplex</i>	AY163633	<i>Pseudoryzomys simplex</i>	EU579517
<i>Punomys kofordi</i>	JQ434414	<i>Punomys kofordi</i>	JQ434426
<i>Reithrodon auritus</i>	AY163634	<i>Reithrodon auritus</i>	EU579474
<i>Rhagomys longilingua</i>	DQ003723	<i>Rhagomys rufescens</i>	AY206770
<i>Rheomys raptor</i>	AY163635	---	---
<i>Rhipidomys macconnelli</i>	AY277474	<i>Rhipidomys macconnelli</i>	AY275130
<i>Salinomys delicatus</i>	JQ434415	<i>Salinomys delicatus</i>	EU377608
<i>Scapteromys tumidus</i>	AY163637	<i>Scapteromys aquaticus</i>	AY275132
<i>Scolomys ucayalensis</i>	AY163638	<i>Scolomys ucayalensis</i>	EU579518
<i>Sigmodon alstoni</i>	EU635698	<i>Sigmodon alstoni</i>	EU652907
<i>Sigmodon hirsutus</i>	EU635704	<i>Sigmodon hirsutus</i>	AF425197
<i>Sigmodon peruanus</i>	EU635718	<i>Sigmodon peruanus</i>	AF293395
<i>Sigmodontomys alfari</i>	EU649071	<i>Sigmodontomys alfari</i>	EU340016
<i>Sooretamys angouya</i>	EU649072	<i>Sooretamys angouya</i>	EU579511
<i>Tapecomys primus</i>	JQ434416	<i>Tapecomys primus</i>	AF159287
<i>Thalpomys cerradensis</i>	AY277481	<i>Thalpomys cerradensis</i>	AY273916
<i>Thaptomys nigrita</i>	AY277482	<i>Thaptomys nigrita</i>	AF108666
<i>Thomasomys baeops</i>	AY163642	<i>Thomasomys aureus</i>	TAU03540
<i>Transandinomys talamancae</i>	EU649074	<i>Transandinomys talamancae</i>	EU579514
<i>Wiedomys pyrrhorhinos</i>	AY163644	<i>Wiedomys pyrrhorhinos</i>	EU579477
<i>Zygodontomys brevicauda</i>	AY163645	<i>Zygodontomys brevicauda</i>	EU579521
Outgroup			
<i>Acomys spinosissimus</i>	AY326074	<i>Acomys spinosissimus</i>	AM409396
<i>Arvicola terrestris</i>	AY277407	<i>Arvicola terrestris</i>	AY275106
<i>Calomyscus baluchi</i>	AY163581	<i>Calomyscus baluchi</i>	AY288509
<i>Cricetomys emini</i>	AY326081	<i>Cricetomys emini</i>	AF160613
<i>Cricetus cricetus</i>	AY277410	<i>Cricetus cricetus</i>	AY275109
<i>Meriones unguiculatus</i>	FN984755	<i>Meriones unguiculatus</i>	AF119264
<i>Microtus sikimensis</i>	AY163593	<i>Microtus californicus</i>	EF506071
<i>Mus musculus</i>	AF126968	<i>Mus musculus</i>	AJ512208
<i>Myospalax aspalax</i>	AY326097	<i>Myospalax psilurus</i>	AF326270
<i>Mystromys albicaudatus</i>	AY163594	<i>Mystromys albicaudatus</i>	AF160606
<i>Neotoma lepida</i>	AY163599	<i>Neotoma albigula</i>	AF108704
<i>Nesomys rufus</i>	AY326099	<i>Nesomys rufus</i>	AF160592
<i>Nyctomys sumichrasti</i>	AY163603	<i>Nyctomys sumichrasti</i>	AY195801
<i>Petromyscus collinus</i>	DQ191517	<i>Petromyscus collinus</i>	AF160601
<i>Phodopus sungorus</i>	AY163631	<i>Phodopus sungorus</i>	AF119266
<i>Rhizomys pruinosus</i>	AY326107	<i>Rhizomys sinensis</i>	AF326274
<i>Scotinomys xerampelinus</i>	AY277416	<i>Scotinomys xerampelinus</i>	AF108706
<i>Spalax ehrenbergi</i>	SZU48589	<i>Spalax ehrenbergi</i>	AJ389537
<i>Steatomys parvus</i>	AY326110	<i>Steatomys parvus</i>	AF160599
<i>Tylomys nudicaudus</i>	AY163643	<i>Tylomys nudicaudus</i>	DQ179812

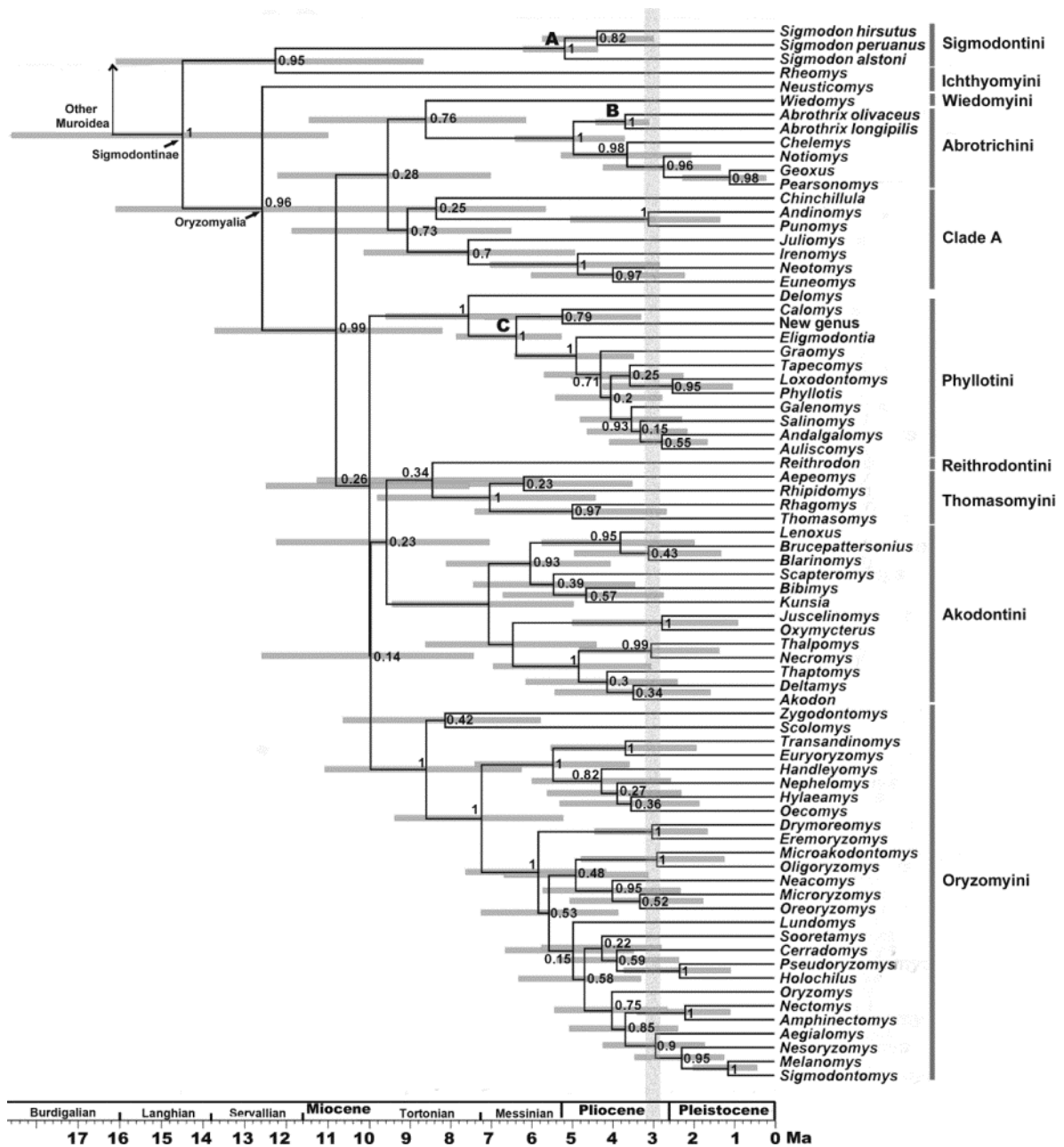


Figure S1. Chronogram illustrating the diversification of Sigmodontinae obtained from the Bayesian analysis of the IRBP matrix. Ages are represented as mean node height of the 95% HPD from a maximum clade credibility tree compiled from post-burnin trees topologies from 3 independent runs (9.0×10^7 generations per run) implemented in BEAST v1.7.1. Three calibration points were used: A, *Sigmodon*; B, *Abrothrix*; and C, Phyllotini. Estimated time for the closure of the Panamanian land bridge (ca. 3 Ma) is shown as a grey line. Branch support is indicated as Bayesian Posterior Probability (PP). Bars at the nodes represent the 95% highest posterior density (HPD) credibility interval of the node's age.

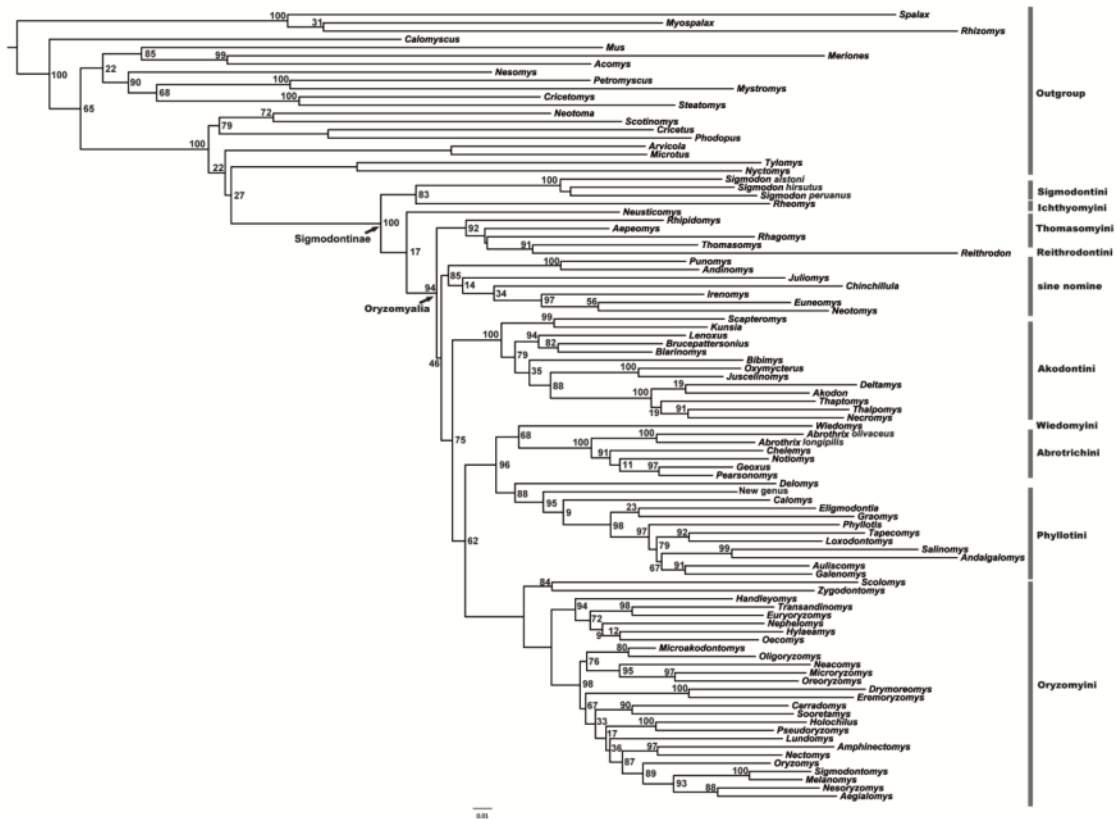


Figure 2. Most likely tree (ln=-52628.7989) tree recovered from the analysis of the combined dataset of sigmontine DNA sequences after 100 inferences using the general time-reversible (GTR) with gamma model of rate heterogeneity. The SH-like branch support values are indicated close to the nodes.

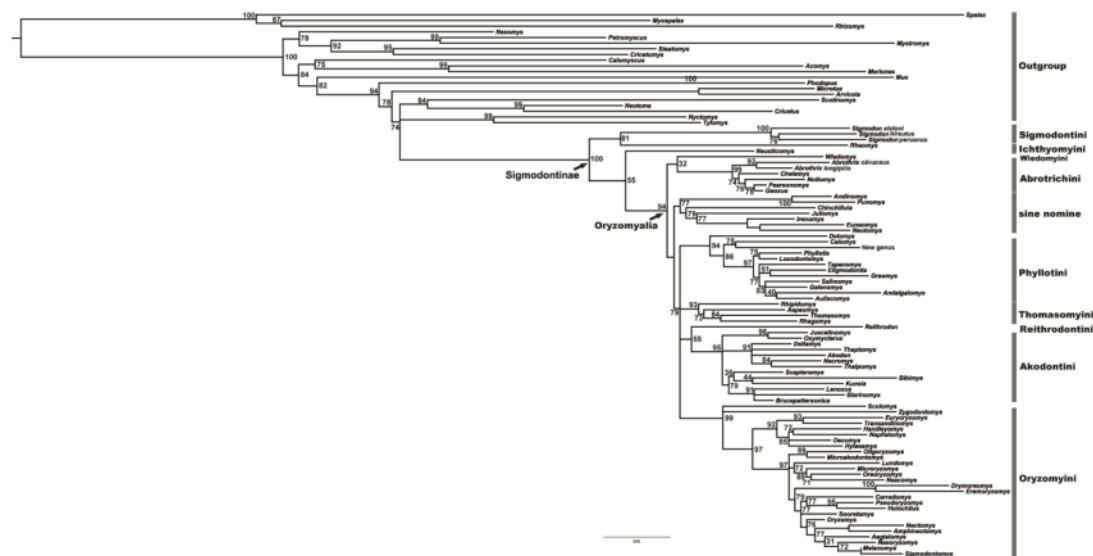


Figure 3. Most likely tree ($\ln=-13434.2136$) tree recovered from the analysis of the IRBP dataset of sigmontine DNA sequences after 100 inferences using the general time-reversible (GTR) with gamma model of rate heterogeneity. The SH-like branch support values are indicated close to the nodes.

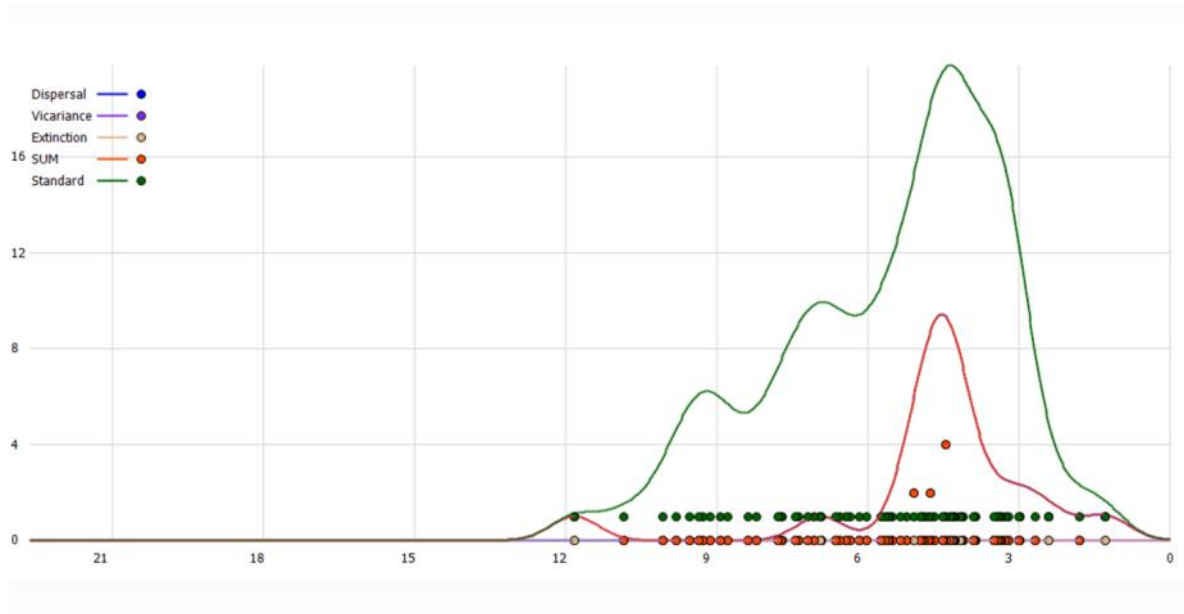


Figure 4. Historical biogeography analysis of Sigmodontinae. Time-event curve considering the S-DIVA reconstruction in RASP. The Y axis is the value of event; the X axis represents time. The SUM curve comprised the sum of all events; including Dispersal, Vicariance and Extinction. The Standard curve is a curve where each node of the tree has only one event. A node density of 2 was selected. Only the events within Sigmodontinae are considered.

CAPITULO 2

Promotores de diversidad en Sigmodontinae (Rodentia, Cricetidae): el rol de la morfología molar y los hábitats emergidos durante el Mioceno/Plioceno

Potential drivers for the diversity of Sigmodontinae (Rodentia, Cricetidae): the role molar morphology and the novel habitats emerged during the Miocene/Pliocene.

**3. POTENTIAL DRIVERS FOR THE DIVERSITY OF SIGMODONTINAE
(RODENTIA, CRICETIDAE): THE ROLE MOLAR MORPHOLOGY AND THE
NOVEL HABITATS EMERGED DURING THE MIOCENE/PLIOCENE.**

Autores: Andrés Parada, Guillermo D'Elía y Eduardo Palma.

En preparación, se enviará a PLOS ONE.

Potential drivers for the diversity of Sigmodontinae (Rodentia, Cricetidae): the role of molar morphology and the novel habitats emerged during the Miocene/Pliocene.

Short title

drivers of sigmodontine diversity

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Abstract

Rodents of the subfamily Sigmodontinae (Rodentia, Cricetidae) comprise one of the most diverse Neotropical mammalian clades (~ 400 living species and 82 genera). These rodents occupy a wide range of habitats and lifestyles and ecological context seems relevant to understand the evolution of this group. Much debate has focused on how transitions in life history have favored the proliferation of certain clades. The aim of this study was to examine whether transitions between lowland and montane habitats, open-habitat and forest or changes in molar architecture promoted bursts in diversifications within Sigmodontinae. For this, we recovered a phylogeny for Sigmodontinae, including a dense taxon sampling of 268 representatives. The obtained phylogeny is consistent with previous studies and indicated that the subfamily and the major lineages appeared during late Miocene. Our ancestral area reconstruction placed the origin of the subfamily in the Caribbean region. Dispersal to other areas was pervasive during the radiation. Tropical lowlands accumulated as much lineage diversity as the remainder of the distribution of these rodents and this area might have served as cradle of species, supporting higher speciation rates. The ancestral areas of several groups were found outside the Andean region contrary to the predictions of previous authors, although some lineages flourished extensively once they reached this area. Our results suggest a slowdown in diversification rates around 1 million years ago. We also detected several lineage-specific rate shifts.

Our likelihood analyses suggest that vegetation type and altitudinal range are correlated with diversification rates. Furthermore, there were more transitions from lowland to higher altitudes and from forest to open vegetation than the opposite. We found no support of differential speciation rates associated with molar architecture. We concluded that dispersal into novel habitats and geologic and vegetation changes during the Miocene/Pliocene promoted diversification within Sigmodontinae.

1. Introduction

The uneven distribution of species richness is one of the most staggering features of biological diversity. There is much debate on why and how this accumulation of such exceptional richness occurred. Nowadays comparative methods facilitate inferring the mode and tempo of speciation at macroevolutionary scales by examining variation in diversification rates among lineages (e.g. [1] [2]) and this has triggered a renaissance of interest in exploring radiations. The rich biota of the Neotropical region [3] [4] [5], with its numerous episodes of faunal interchanges [6], constitutes a scenario of interest to examine broad scale patterns of diversity. During the Neogene, several events might have provided ample opportunity for the biota of South America to diversify. The Andean uplift may have played a role in several ways: a) by increasing climatic and habitat heterogeneity, b) by creating a “biotic corridor” for montane taxa, (c) by favoring vicariance, or (d) acting as a “species pump”, as new lineages dispersed into other areas [7]. The coupling of tectonic and climatic processes resulted in changes of the Andean foreland and eastern lowlands. This in turn caused the emergence of more “biotic corridors” and new habitats. It must be taken into account the particular isolation of South America during the Neogene, since the continent was isolated from all other landmasses until the closure of the Panama Isthmus during Pliocene (~ 3.5 Mya) [8].

The subfamily Sigmodontinae, with about 400 living species and 85 genera, is one of the most species rich and broadly distributed group of Neotropical mammals.

The species richness and ecological range of the Sigmodontinae has for long time fascinated evolutionary biologists. Sigmodontine genera have been assembled into groups, most of which are formally recognized at the tribal level [9] [10] [11], which greatly differ in diversity (D'Elía y Pardiñas, [12]). Tribes with pervasive species richness has been attributed to have experienced adaptive radiation [13]. The ecological context seems essential to the interpretation of evolutionary radiations [14]. The changes in the landscape of South America through the Neogene might have provided ample ecological opportunity [15] [16], i.e., “a wealth of evolutionarily accessible resources little used by competing taxa” [17]. In the case of sigmodontines they might have found themselves in the presence of such favorable circumstances for a number of reasons. First, colonization of new areas which remained isolated for a long period may have provided access to resources not available for other taxa. Second, the heterogeneous Andean highlands may have promoted species diversity [9], which for some groups may have been complemented with further diversification in the recently colonized lowlands. Additionally, changes in the vegetation following the Andean uplift and global cooling at the late Miocene triggered the advance of savannas and more open vegetation habitats, fragmenting forest specialist populations while providing larger habitats for open areas dwellers. Moreover, an issue that deserved be further explored in the context of the whole sigmodontine radiation are transitions in molar architecture that might have enabled some lineages to exploit or access to a wider variety of food resources. It has been proposed that the appearance of a molar with four lophs, known as tetralophodont (usually high crowned or hypsodont), allowed the colonization of open or “pastoral” habitats while the plesiomorphic type of five lophs, or pentalophodont plan (usually low crowned or braquidont), remained associated to taxa from forested habitats [18].

Besides the aforementioned highly influential work of Reig and Hershkovitz [9][19] [18] [20], other researchers had evaluated, together with the timing of the sigmodontine radiation [21], the evolution of molar morphology and habitat preference. For instances, 4 transitions between open and forested habitats were inferred for the tribe Oryzomyini ([22] see also [23]) . Concerning the sigmodontine

mode of evolution, a quick burst of diversification at the base of the large clade of Oryzomyia was inferred [24] [25]. In studies previous to the incorporation of an explicit phylogenetic approach, distinct Andean regions were proposed as “areas of original differentiation” for the most of the sigmodontine tribes [9]; however, recent phylogenetic studies has questioned the generalization of this model [26] [27] [23] [28]. Therefore, it would be of much interest to reconstruct the “ancestral area” for each of the major lineages and test if the fact of inhabiting a region has affected the tempo of sigmodontine diversification and/or if transitions between regions affected diversification rates. Similarly, it of interest to assess if molar types are associated to differential diversification rates. Here we construct the most complete phylogeny of Sigmodontinae and allies based on sequences of a combined matrix (one mitochondrial and one nuclear gene) for 289 species. Considering this expanded dataset, we use multiple fossil-based constraints to infer divergence times.

We aim to: a) examine whether any South American area, in particular the Andes or Amazonia, sustain different diversification rates than other areas; (b) reconstruct the evolution of main habitat type preferences (altitudinal range, occurrence in open or forested habitats), distributional areas, and molar morphology; c) test whether among-lineage variation in diversification rates explain the observed disparity in extant species richness among tribes of Sigmodontinae, and d) test whether transitions in habitat type preferences or lifestyles (altitude, vegetation) and/or molar architecture are associated to changes in diversification rates.

This framework, by clarifying the phylogenetic position and the timing of shifts rates, would greatly enhance our knowledge of sigmodontine evolution by formally assessing for the first time the role of these traits in the context of the whole radiation of the subfamily.

2. Materials and Methods

2.1 Taxon sampling

Analyses were based on DNA sequences of the first exon of the nuclear gene interphotoreceptor retinoid binding protein (IRBP) and the mitochondrial cytochrome b (cyt b); all sequences were gathered from GenBank (Table S1). The sequences were recovered from a total of 289 species of Muroidea. Sigmodontine diversity was thoroughly covered, including representatives of all recognized tribes as well as several species considered *incertae sedis* (see [11]). For those taxa which either no cyt b or IRBP sequences was available, the matrix was completed with ambiguous state characters (i.e., n). Relationships among main muroid lineages remain mostly unresolved (e.g., [29] [30]). Thus, our coverage included data from two representatives of the other cricetid subfamilies and one representative of all non-cricetid subfamilies of Muroidea (sensu [31]); these sequences compose the outgroup.

2.2 Molecular dating and Phylogenetic analysis

Sequences were aligned with MAFFT v.6.925b [32] with auto settings. The IRBP matrix had 1181 positions of which 751 were variable; one insertion of three base pairs was inferred in the sequence of *Scolomys ucayalensis* and another of six base pairs long in the sequences of *Steatomys parvus*. To avoid ambiguous alignment, caused by distinct indels, at the end of the muroid cytochrome b sequences, the analysis was limited to the first 1134 bases of the gene [33]; this cyt b alignment had 874 variable sites.

A Bayesian analysis was conducted in BEAST v1.7.4 [34] that simultaneously estimated substitution model parameters and dates for cladogenetic events. A Birth-Death process with incomplete sampling [35] using an initial random tree was set as a prior. The employed substitution models, selected using

ModelGenerator [36], were TVM +GAMMA+ I for the cyt b gene and TVM + GAMMA for the IRBP gene (implemented by modifying the file generated by the program BEAUti following instructions at http://beast.bio.ed.ac.uk/Substitution_model_code), with empirical base frequencies, and four gamma rate categories. Given that preliminary analysis indicated (considering the *ucl.d.mean* parameter) that sequences did not evolve in a clock like mode, runs were performed under an uncorrelated lognormal relaxed clock model. Three independent runs of 145×10^6 generations, sampled every 40000 generations were performed. Convergence to stable values was checked with Tracer v.1.5 [37], obtaining an effective sample size (ESS) greater than 200 for all parameters. Tree and log files (4.1% burnin) were combined using LogCombiner [34]. Trees then were compiled into a maximum clade credibility (MCC) tree using TreeAnnotator [34] to display mean node ages and highest posterior density (HPD) intervals at 95% (upper and lower) for each node. Fossil calibrations were employed in the form of lognormal prior distributions, providing a minimum bound for each distribution such that the 5% quantile corresponds to the minimum age of the fossil while the 95% interval allows both for the uncertainty of the fossil age and for the incompleteness of the fossil record. We used the following calibrations (offset, median, 5% and 95% quantiles in Mya respectively): (a) the crown clade *Abrothrix* (2.9, 3.91, 3.276 and 5.61) based on the fossil species *Abrothrix kermacki* ([38] [39]), (b) the crown clade of *Sigmodon* (4.13, 5.14, 4.506 and 6.84) based on the fossil record of *Sigmodon* spp. ([40]); and (c) the crown clade of Phyllotini (4.95, 5.96, 5.326 and 7.66) based on the fossil species *Auliscomys formosus* Reig ([38] [41]).

2.3 Area Definitions and Biogeographic Inference

The geographic range for each species was obtained from the catalog of Musser and Carleton [42], updated with ranges portrayed in IUCN (2013) and primary literature. Biogeographic regions corresponded to those regions, subregions or provinces defined by Morrone [43]: (A) Amazonian

subregion excluding Yungas; (B) Caribbean subregion; (C) Chaco province; (D) Andean region excluding Patagonian subregion; (E) Nearctic region; (F) Parana subregion; (G) Patagonian subregion; (H) North Andean- Paramo province; (I) Cerrado/Caatinga provinces; (J) Yungas province; (K) Puna and other provinces considered under the “South American transition zone” but excluding Páramo; (L) Africa; (M) Pampa province; and (N) Eurasia (see Figure 1).

Sigmodontine ancestral distributions were reconstructed using Bayesian Binary MCMC (BMM) analysis implemented in the program RASP (Reconstruct Ancestral State in Phylogenies) 2.1b [44] [45]. We used all the post-burnin trees obtained from the Bayesian analysis, along with a wide distribution at the root, 0.08 of temperature, a maximum of 4 states (areas) allowed for each node, and other settings left at the default values.

2.4 Effects between Geographic Range Evolution and Diversification

We tested if the transition into new areas provided opportunity for radiation for some lineages of Sigmodontinae; specifically we tested if radiation in the Andes or Amazonia leads to increased speciation rates.

In order to consider simple models we established two classification schemes. For the first model (hereafter “Andean model”) species distribution was set as a) present or b) absent in an area composed of Andean, Paramo, Patagonian and Puna. For the second model, (hereafter “tropical lowland model”) we treated the distribution as falling either present or b) absent in an area composed by an area composed of the mostly tropical lowlands of Amazonian, Parana and Cerrado-Caatinga. Since the Yungas province is a transitional zone, we also rerun these models excluding Yungas from the “tropical lowland” distribution, and including it in as part of the “Andean” distribution.

The ClaSSE [46] function in the diversitree package for R ([47] see below) allows to infer, from a phylogeny, geographic range shifts and the effect of range on speciation and extinction rates. This

likelihood-based approach allows the estimation of region-dependent rates of speciation, extinction and range evolution. To reduce the complexity of the analysis, five ClaSSE models – a full parameter model and others with less parameters – were estimated under a ML framework and compared using a likelihood ratio test. Finally, with a method based on Mahler et al. [48] we estimated the lineage density at each node considering the geographic distribution using the function `estDiversity` in `phytools` [49] .

2.5 Analyses of Diversification

To examine the tempo and mode of diversification of Sigmodontinae we used R version 3.0.0 [50] in combination with the packages APE [51], `apTreeshape` [52], `diversitree` [47], GEIGER [53], LASER [54], MEDUSA 0.93-4-20 [2] and `phytools` [49].

Since differential diversification rates would lead to unbalanced phylogenetic trees, we obtained, using `apTreeshape`, the indices of tree shape of Sackin and Colles. We also considered in `apTreeshape` the shape statistic, which is the likelihood ratio under both the Yule and PDA (proportional to distinguishable arrangements) models. Additionally, we tested departures from constant rates for the branching times with the function `diversi.gof` in `ape`; this function computes two tests of branching times distribution using the Cramér–von Mises and Anderson–Darling goodness-of-fit tests [55].

To evaluate the accumulation of lineages-through-time (LTT), based on the MCC tree, we used the gamma statistic [56] to determine whether per-lineage speciation and extinction rates have remained constant. To account for topological uncertainty

we randomly selected a subset of 1000 trees from the posterior distribution of the analysis done with BEAST and rerun the LTT analysis with them.

We estimated the overall diversification rate under simple birth-death model [BD] and pure birth [PB].

Considering time-dependent models we adjusted a Yule model extended to have a varying speciation

rate lambda in 2 and 3 intervals [Y2R and Y23 respectively, [57]]. In order to examine if extant species richness is the result of past slowdowns in speciation rate, increased extinction rates, or both we tested these three models in a likelihood framework (SPVAR, EXVAR, and BOTHVAR). We also tested models that predict slowdowns in the tempo of diversification (density-dependent exponential and density-dependent logistic (DDX and DDL, [58])). We used Akaike's Information Criterion (AIC) scores to compare the fit of these models.

To improve our model fit, we considered species richness associated along a backbone tree (see below) and found the maximum likelihood estimate of the net diversification rate under one and two rate models in GEIGER.

As a way to individualize those nodes where net diversification has changed we used two approaches available in the packages GEIGER and MEDUSA. First we considered the relative cladogenesis (rc) test, that evaluates all alternative scenarios to the null hypothesis of equal diversification in all lineages. Second, we used MEDUSA a method that fits both pure birth and birth-death models to estimate rate shifts on a dated phylogeny accommodating incomplete sampling by using taxonomic richness information. To do so, the MCC tree was pared down to a backbone tree (with all but seven of the tips representing distinct genera) where the known sigmodontine diversity (366 species) was assigned to the tips (see Table S2). After fitting a single model to the entire dataset, a series of shifts are added to the tree in a stepwise procedure until the addition of an extra shift does not improve the AIC score given the appropriate threshold (4.119) considering tree size. MEDUSA analyses were performed over the backbone tree first; then, we pruned the 1000 subsampled trees as described above and run MEDUSA with those trees, to account for the uncertainties of the phylogenetic reconstruction.

2.6 Ancestral State Reconstruction and correlates with diversification

We examined three traits hypothesized to be correlated with increased rates of diversification in Sigmodontinae: (i) altitudinal range (ii) habitat or vegetation type and (iii) molar plan (pentalophodont-tetralophodont). Altitudinal range and habitat type data (presence in open vegetation, forest areas or both types) were obtained from the catalog of Musser and Carleton [42] updated with information displayed at the IUCN site [59] and primary literature. Molar plan was compiled primarily after Steppan [59] and Weksler [22]. We categorized altitudinal range and habitat in all sampled taxa as characters with three states. First we coded species as a) occurring in open vegetation habitats such grassland and shrubland; b) forest; or c) occurring in both habitats, fringe habitats or a mixture of both type of habitats (hereafter “mixed”). Second, species were coded according to their altitudinal range: a) lowlands from 0 to 800 m; b) middle range (up to 3000 m); c) highland (above 3000 m). Molar type was coded as a binary state: a) tetralophodont; or b) pentalophodont. Ancestral state reconstruction was performed in phytools, with the functions `make.simmap` and `describe.simmap`, which fits a continuous-time reversible Markov model for trait evolution and then simulates stochastic character histories. For this procedure, taxa lacking information for these traits were excluded from the analysis. If transition into novel habitat types constituted ecological opportunity prompting sigmodontine cladogenesis, diversification rates would be higher for lineages in these habitats. We used the MuSSE (Multiple State Speciation Extinction) model implemented in diversitree [47] to test whether diversification rates in lineages differed across altitudinal range or vegetation type. We compared the fit of a model where the speciation rate was independent of habitat and of a model where distinct speciation rates along the altitudinal range. We calculated parameters for the unconstrained model and then sequentially constrained each of the model parameters, alone and in combination, to determine if constrained models provided a better fit to the data than the unconstrained model. We constrained the full model into 3 alternative models and one model allowing parameters to vary according to two “epochs” established at one point in the past. We followed a similar procedure to test whether transition in habitat types provided ecological opportunity by comparing a model where diversification

rates were independent of habitat and models that allowed rates to vary. We performed a similar analysis under the BISSE (Binary State Speciation and Extinction) model [47], also implemented in diversitree, testing whether transition in molar plan correlates with differential rates of diversification. We used AIC scores to determine which model provided the best fit to our data. Bayesian posterior distributions of MuSSE (and BiSSE) model parameters were also estimated using Markov Chain Monte Carlo analyses in diversitree. Priors for each parameter used an exponential distribution, and estimated ML model parameters were used as a starting point.

3. Results

3.1 Phylogenetic Analyses

Chronograms were compiled and annotated from 10425 post-burnin trees as a MCC tree (Highest Log Clade Credibility: -67.2176). The topology is congruent to those gathered in previous studies (e.g., [22] [21] [23] [60]). In the Bayesian tree, the pair *Microtus-Arvicola* was recovered as sister to Sigmodontinae (posterior probability, PP = 0.3). Sigmodontinae was highly supported (PP = 1; Fig. 2). The most basal split within Sigmodontinae leads to one clade (PP = 0.99 composed of Sigmodon (PP = 1) and *Rheomys*, and a second clade (PP = 0.96) composed by the remainder sigmodontines in the other. Ichthyomyini is not recovered monophyletic, given that *Rheomys* is sister to Sigmodontini while *Neusticomys* is sister to Oryzomyalia. Within Oryzomyalia (PP = 0.89), all tribes composed of more than one genus are recovered as monophyletic with strong support (i.e., Abrotrichini, Akodontini, Oryzomyini, Phyllotini and Thomasomyini, all with PP = 1). In addition a clade composed by *Andinomys*, *Chinchillula*, *Euneomys*, *Irenomys*, *Neotomys* and *Punomys* (PP=0.61, hereafter clade A) was recovered.

3.2 Divergence time analysis

Our relaxed clock inferred a stem age of 13.95 Mya (11.17-16.79 highest posterior density, HPD) for the origin of Sigmodontine. The crown of Sigmodontinae is inferred as 10.91 Mya (8.88-13.06). During the Late Miocene and Pliocene several split lead to the emergence of most of the sigmodontine tribes (Abrotrichini, Akodontini, Oryzomyini, Phyllotini, Reithrodontini, Thomasomyini, and Wiedomyini) and clade A (see Table 1). Throughout the Late Miocene the stem lineages of the tribes Akodontini, Oryzomyini, Phyllotini, and Thomasomyini and clade A began to diversify. The stem lineages of Abrotrichini and Sigmodontini began to radiate more recently, in the Pliocene.

3.3 Effects between Geographic Range Evolution and Diversification

The BBM method suggests a complex biogeographical history in which dispersal and to some extent vicariance played an important role shaping the current sigmodontine distribution pattern. Through time, there was an increase of dispersal and vicariance events with a peak ~ 1 Mya (Fig. S1). The Caribbean subregion was inferred as the most probable ancestral area for the subfamily Sigmodontinae and also for the clade Sigmodontini-*Rheomys* (Fig. 3, see Table 1 for details). The MRCA of the lineage *Oryzomyia-Neusticomys* most likely inhabited the Paramo province or the Caribbean subregion. The MRCA for *Oryzomyia* occurred in the Puna and in a brief period of time the main lineages colonized the Amazonian and Parana subregions. Members of the tribe Sigmodontini appear to have dispersed into the Nearctic region during the Pliocene. Likewise, it must be noted that the Ichthyomyini representatives included in our analysis (*Neusticomys* and *Rheomys*), which do not appear sister to each other, have a disjunct distribution. After an initial expansion of thomasomyine genera into the Amazonian region, a basal split lead to a lineage that colonized the Paramo and Puna. The MRCA of the Akodontini tribe occurred in the Parana and several dispersion and vicariant events during the Pliocene lead to a widespread distribution across Puna, Chaco Cerrado-Caatinga, Yungas

and Pampa. The MRCA of the tribe Abrotrichini inhabited the Patagonian region, and its descendants reached the Andean region in the last 3 Mya. The Cerrado-Caatinga appears as the ancestral area of the Phyllotini tribe which diversified after the dispersion into the Puna. Within the tribe Phyllotini, a lineage composed of *Calomys* representatives radiated through the Cerrado Caatinga after a second invasion of this region. The ancestral area of the MRCA of the tribe Oryzomyini was the Amazonian region; ca. 5 Mya several dispersal events lead to the expansion of this group through the Caribbean, Parana, and Cerrado-Caatinga regions. Finally, the occurrence of *Oryzomys* in the Nearctic is the result of a recent invasion to this region.

We considered a framework under the ClaSSE models that tested for differential speciation rates among geographic ranges. In both scenarios analyzed models with distinct speciation rates between different regions were preferred over simpler models with a single speciation rate (Table 2). The first model recovered higher speciation rates within the composite area of Amazonas+Cerrado-Caatinga+Parana than within the remainder distribution area of the group. In the second analysis, the estimated speciation rates in the “Andean” area were smaller than that corresponding to the remaining distributional area of the group. When the Yungas were included as part of the “Andean” area and excluded from the “Amazonas” one, the same trends were recovered, although a slight increase in speciation rates in the “Andean” + Yungas region is inferred (results not shown). Results of the lineage diversity estimated for distinct regions are summarized in Fig. 4 and Fig. S2-S3.

3.4 Analyses of diversification

There was a significant imbalance in the tree shape (Colless' index 2.21/0.41; Sackin's index 1.95/0.75, for yule/PDA models, all $p < 0.05$). The tree did not fit the Yule and PDA models according with the likelihood ratio test (shape statistic=3.68 and -4.88 respectively, $p < 0.05$). When branching times along the MCC were examined, the null hypothesis of constant diversification rate was rejected under both the Cramer-von Mises and the Anderson-Darling test ($W_2=23.233$ and $A_2= 5.118$, both

with $p < 0.01$). The LTT plot for Sigmodontinae revealed a decline in diversification toward the present (LTT for the subsampled trees in Fig. S4). The gamma statistic indicated internode distances are clustered early in the phylogeny ($\gamma = -4.691$, $p < 0.01$).

When we tested for significant changes in diversification rate within the Sigmodontinae subfamily, a three-rates variant [Y3R] of the pure-birth model with a rate shift at 1.174 and 1.099 Mya provided the best fit (see Table 3 for details). When taxonomic richness data was paired along a backbone tree, the constant-rate model (all lineages with equal diversification rate) was rejected in favor of a variable rate model with a shift at the MRCA of *Oligoryzomys-Melanomys* (see Table S3). The rc test computed in geiger detected diversification shifts at five nodes: the MRCA of Oryzomyalia, of the *Akodon* division, of a large clade composed by all oryzomyalids but Clade A, Akodontini and Thomasomyini, of a group of species of *Calomys*, and of all Oryzomyini but *Zygodontomys* and *Scolomys* (Fig. S5). The MEDUSA method applied over our backbone tree favored a pure birth model and detected two shifts in net diversification rates (crown node *Oxymycterus-Deltamys*, and crown node clade A, Fig. 5) relative to background levels in Sigmodontinae (Log-likelihood = -283.4466, final corrected AIC = 577.2958, Table S4). The procedure was repeated through 1000 subsampled trees and recovered six shifts in net diversification present in at least 5% of the trees (Fig. 5).

3.5 Ancestral state reconstruction and correlates with diversification

The stochastic mapping of lifestyle or habitat (altitudinal range and vegetation type) and the transitions between molar types (pentadont or tetradont states) are shown in Fig. S6-S8. The fit and parameters of the MuSSE models evaluating the mode of evolution and diversification rates for the transition across vegetation type and altitudinal range are summarized in Table 4. When compared a full model with three speciation rates versus one allowing rates to vary in two epochs, the time-dependent model (or “2-epoch”) with a shift inferred at 2.92 Mya was favored by the AIC score. The

time-dependent model inferred low speciation rates in the mixed habitats, followed by an increase in speciation towards the present. A slowdown in speciation rates is observed in the open vegetation and forest habitats in the later epoch too.

Allowing for separate rates of speciation according to different altitudinal range occupied in the full MuSSE model provided the best fit. In light of the time-dependent model there is a salient slowdown in speciation rate at the highlands and an increase in speciation rate in the middle range towards the present. There was no support for different speciation rates associated to a particular molar type with BiSSE models although the time-dependent model performed better in AIC score (Table S5). While examining evolution across distinct habitats the full models recovered minimal transition rates from highlands to lowlands or from forest to open vegetation. Between vegetation types, the greater transition rates were found from mixed type into the other two habitats; in the case of altitudinal range the greater transition rates were observed from middle range into lowlands. Transitions in molar types were more frequent from the tetralophodont to the pentalophodont condition; the latter morph is inferred as the derived state (see Fig. S8).

4. Discussion

4.1 Historical biogeography and timing of the sigmodontine radiation.

Our analyses, employing a dense taxon sampling, produced a major new timeline for the radiation of Sigmodontinae, examined the historical biogeography for the group and provided a robust framework for examining the influence of habitat on the macroevolution of a large group of neotropical rodents. Given that the phylogenies obtained are in agreement with those recovered in previous studies [citas] here we will focus on the general pattern of diversification. Our estimates of the crown age of Sigmodontinae and its main clades or tribes are consistent with the ones found previously (see [21] and references therein). Our data corroborates a scenario of an early arrival into South America that

predates the closure of the Panamanian land bridge closure. This arrival might have occurred through a proto-Caribbean region via waif dispersal coinciding with a global sea level drop [61].

Our interpretation of the ancestral area reconstruction for Sigmodontinae placed an origin of the subfamily at the Caribbean region during the Late Miocene, an epoch when this somehow large and diverse area was highly fragmented. Following the invasion of South America, sigmodontine lineages dispersed from the Paramo or Puna towards the East colonizing the Amazonian and Parana regions. These dispersal and vicariant events occurred during one of the most intense phases of the Andean uplift and associated important climatic and vegetational changes. In Northern South America, during the Tortonian (late Miocene) open biomes proliferated, and the Messinian (late Miocene) sustained a mixture of tropical evergreen broadleaf forest and tropical savanna [62]. Also, the demise of the megawetlands in Western Amazonia and its replacement by forested habitats started ca. 7 Mya [7]. Similarly, in southern South America by the Tortonian tropical evergreen broadleaf forest occupied mountain slopes whilst tropical savanna dominated the lowlands; the Messinian accentuated the presence of temperate deciduous broadleaf savanna [62].

The Amazonian region has been envisioned both as a cradle for new species and as a museum that acts as preserving species diversity originated elsewhere (see [63]). Our data show that the “tropical lowlands” (an area composed of Amazonian, Cerrado/Caatinga and Parana) accumulated as much lineage diversity as the remainder of the sigmodontine distributional range; in accordance, there was support for a model accounting for higher speciation rates within this area. This area served as the cradle that promoted the radiation of the tribes Akodontini, Oryzomyini and Thomasomyini, an idea somehow advanced by Smith and Patton [10], but that contradicts classical views of sigmodontine diversification that invoke the Andes as the main sigmodontine diversification area [9] [19]. In addition, Southeastern Brazil harbors many genera regarded as Sigmodontinae incertae sedis, (termed as “unique lineages” by Smith and Patton [10]), which are taxa of unclear phylogenetic position within the sigmodontine radiation. For the moment, it is unclear if these genera (e.g. *Delomys*, *Phaenomys*,

Juliomys) are the unique extant representatives of a past more diverse group widely distributed in South America or if they truly represent non-diversified main lineages of sigmodontines.

The relative contribution to diversity by either vicariance, favored by the Andean uplift - with one peak in late middle Miocene ~ 4.5 Mya- or the dispersal and expansion through the lowlands ~ 3.5 Mya might be hard to untangle. The Andean uplift dramatically changed the South American landscape, in particular in the lowland towards the East such as the Amazonian with the reconfiguration of drainage systems. The Andean region, or more broadly speaking the South America western highlands, has been proposed as a promoter of diversity through several mechanisms [7]. Testing this scenario has proven to be a complex task. Reig [9] proposed that the tribes Akodontini, Oryzomyini, Phyllotini and Sigmodontini had its origin and diversification linked to the Andean highlands. Reig envisioned the so called “areas of original differentiation” (AOD) as the areas where a given taxon undergoes most of the cladogenetic events that originated its component taxa of subordinate rank. We found no evidence of an ancestor of the crown group of these tribes in the Andean, Paramo, Patagonian or Puna areas. Only Clade A and Abrotrichini, both of which were not considered by Reig, appeared to have radiated in the Puna and Patagonia respectively. However, according to our BBM analysis after an initial split at the base of the Phyllotini, while one clade flourished through the Cerrado-Caatinga regions another one certainly accumulated its richness in the Puna. We found a similar pattern within the tribe Thomasomyini where the specific diverse lineage of *Thomasomys* proliferated across Puna and Paramo regions. Multiple factors might explain the apparent contradiction in our results and the interpretation advanced by Reig. First, intensive field work in the last two decades have revealed far more richness outside the Andes than it was previously known, at the time that have expanded the range of species previously known as restricted to the Andes. More important, most if not all tribes were distinctly delimited at the times of Reig’s proposition than are currently understood today. As such, prompted by current phylogenetic analyses, after removal of the mostly Andean abrotrichines, the distribution of Akodontini appear less restricted to the Andean

region than envisioned three decades ago. Some similar occurs with Phyllotini and Thomasomyini (see also discussion in [23]). The main akodontine radiation flourished in the Parana region while oryzomyines originated in Amazonian region and the dispersed other areas like Caribbean and Cerrado/Caatinga regions. Moreover, Sigmodontini seem to have originated outside the Andes in the Caribbean region. In general, our analysis suggest that Andes as the a main area for sigmodontine cladogenesis has been overstated given that this region accrued fair less lineage diversity through time here than in the remainder of the distribution of the group. This is not to neglect the role of the Andean uplift prompting sigmodontine diversity; it might have acted as a “species pump” [64] pouring taxa that in turn radiated in the newly emerged habitats. The Andes might have also preserved part of the diversity arising in the initial phase of the radiation.

4.2 Radiation of sigmodontine rodents and correlates of diversification

Analyses (γ -statistic, LTT plots, and likelihood models fitted with single and variable rates) suggest a decrease of rates of diversification close to the present. This slowdown appears to have occurred ca. 1 Mya, during the Pleistocene. Interestingly, Derryberry et al. [65] found a slowdown in the diversification of Neotropical woodcrepers around the same age. Whether this is just a temporal coincidence or points to a scenario accounting for an important fraction of the Neotropical fauna awaits the analysis of additional taxa.

Allowing clades to vary in speciation and extinction rates for evaluating exceptional shifts in diversification rate, we found evidence for two lineage-specific rate shifts: one slowdown in the diversification rate at the MRCA of the Clade A and a rate increase in the stem leading to Akodontini. This scenario holds with the analysis of 1000 subsampled trees that also found additional rate increases at other nodes of Akodontini and Oryzomyini. Additionally rc test detected five shifts, of which one was within *Calomys* and another one close to the base of Oryzomyini.

Andean uplift and changes in vegetation associations are linked and interwoven, and it is particularly difficult to disentangle and quantify their respective contribution to the establishment of Neotropical diversity and in particular that of Sigmodontinae. Our results show that vegetation type and altitudinal range are correlated with diversification rates. Clades that occurred in a mix of forest and open vegetation presented the highest diversification rates. Under a model of two epochs, initially the higher rates were found in lineages associated with forest, then, after 2.92 Mya, there is a rise in the rate for the lineages in mixed vegetation types. Likewise, lowland lineages supported higher diversification rates than highland lineages. It must be noted the categorical definition of “highlands” used here is at best a proxy for other factors, such as temperature, spatial heterogeneity or humidity, which might affect the ecology of organisms. According to the ancestral state reconstruction there were more transitions from lowland to higher altitudes and from forest to open areas than the opposite, which is partially tied to the pervasive proliferation of lowland lineages through late Miocene and the colonization of mixed forest, savanna and dry woodlands.

A close look to the evolution of the genus *Thomasomys* and the crown group *Akodon juniensis*-*A. spegazzinii* reveals a similar pattern: both emerged at the Puna and proliferated through montane and forested habitats. We indeed have an indication based on the analyses with MEDUSA that the lineage leading to *Akodon* (node 99 Fig. 5) showed a shift in diversification rate and that these taxa may be exceptionally species-rich given their age.

Regarding the results obtained under the BiSSE models for the sigmodontine molar type, we should exercise caution invoking acquisition or loss of a fifth loph as a key innovations since we found no correlation of this trait and speciation rates. We cannot exclude the possibility that this trait is associated or covaries with other traits related with life history, and not evaluated here, that provided opportunity to diversify. As it was expected, pentalophodont molar appeared associated with the lineages present inhabiting forest habitat in the ancestral state reconstruction.

As previous studies [e.g.[10]], our topologies identified several deep sigmodontine lineages constituted by a single genus (e.g., those leading to *Delomys*, *Juliomys*) that contrast with those other that proliferated extensively (e.g. Akodontini or Oryzomyini). Historical contingency or local determinism might explain why some clades apparently “failed” to radiate. If early colonizers took over existing resources late arrivals might not radiate. If certain group, due to lack of speciation, fails to preserve transient variation the radiation might fail even when resources are available [66]. Noteworthy, analyzing another impressive radiation Drummond et al. [67] suggested a scenario where the genus *Lupinus* might have experienced repeated ecological release following colonization of new regions rather than undergoing changes in the evolutionary rates per se. We argue that this might be also the case for Sigmodontinae. Considering the relatively recent colonization of some areas, some sigmodontine lineages could still be in the early stages of increasing diversification. [sugiero sacar esta ultima frase, abrotri no exploto como vos decis, eso seguro.]

Summarizing, we suggest a multi-tiered scenario that invokes historical contingency such as the Andean uplift, changes in drainage patterns and dispersal into novel habitats during the Miocene/Pliocene as a plausible explanation for species richness in Sigmodontinae. More effort is needed to understand the patterns and processes linked to the evolution of sigmdontines. It would be of much interest to assess the ecological constraints tied to this group in light of carefully examination of proxies of niche space. Besides gathering more data for sigmodontines not included in this work, more molecular characters and traits associated with lifestyle should be examined to gain an improved appraisal of the history of this group. Currently, we lack evidence of replicated adaptive radiation from habitat specialist (e.g. caviomorph rodents, but see [68]) as found in other systems [69] but such patterns could be detected as additional data is gathered. We consider this combined approach of examining the historical biogeography together with the assessment of the correlates of diversification as a promising way for understanding the evolutionary trends in the Neotropics.

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FIGURES



Figure 1. Map showing areas considered for the analysis of ancestral biogeographic of sigmodontine rodents. Biogeographical regions are: A, Amazonia; B, Caribbean; C, Chaco; D, Andean; E, Neartic; F, Parana; G, Patagonian; H, Paramo; I, Caatinga-Cerrado; J, Yungas; K, Puna; L, Africa; M, Pampa; N, Eurasia. L and N not shown. Colors were chosen to match the ones displayed in the BMM results.

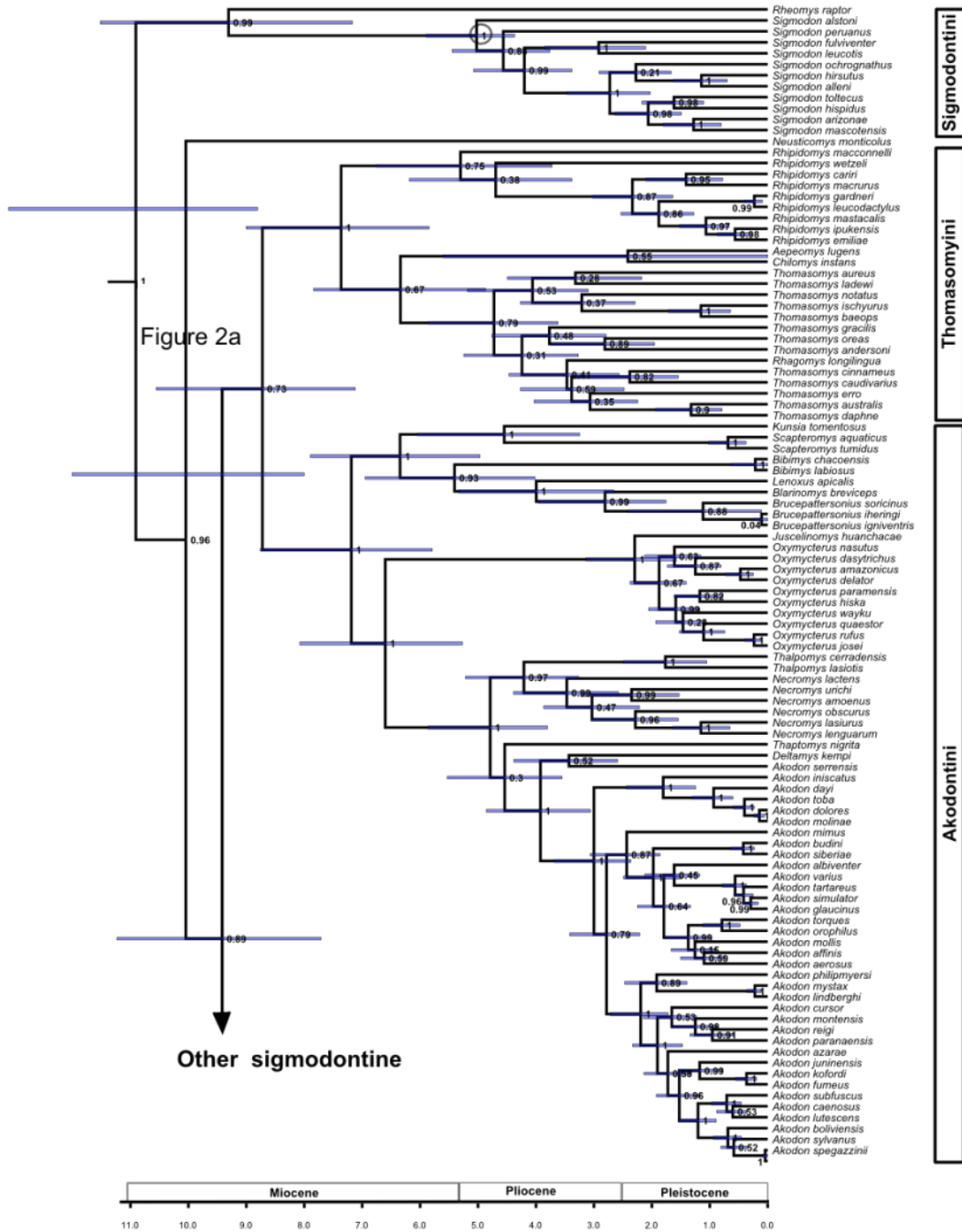


Figure 2. Maximum clade credibility tree for the subfamily Sigmodontinae obtained with BEAST using 289 species. Calibration points considered in the analysis are highlighted with circles. Numbers on nodes indicate posterior probability for the corresponding nodes. Bars represent the 95% highest posterior density (HPD) interval for the divergence times. Tribes indicated on the right.



Figure 3. Ancestral area reconstruction. (A) Graphical results of ancestral distributions at each node of the phylogeny of subfamily Sigmodontinae obtained by BMM. (B) Colour key to possible ancestral ranges at different nodes; black represents other ancestral ranges below 9 % of probability.

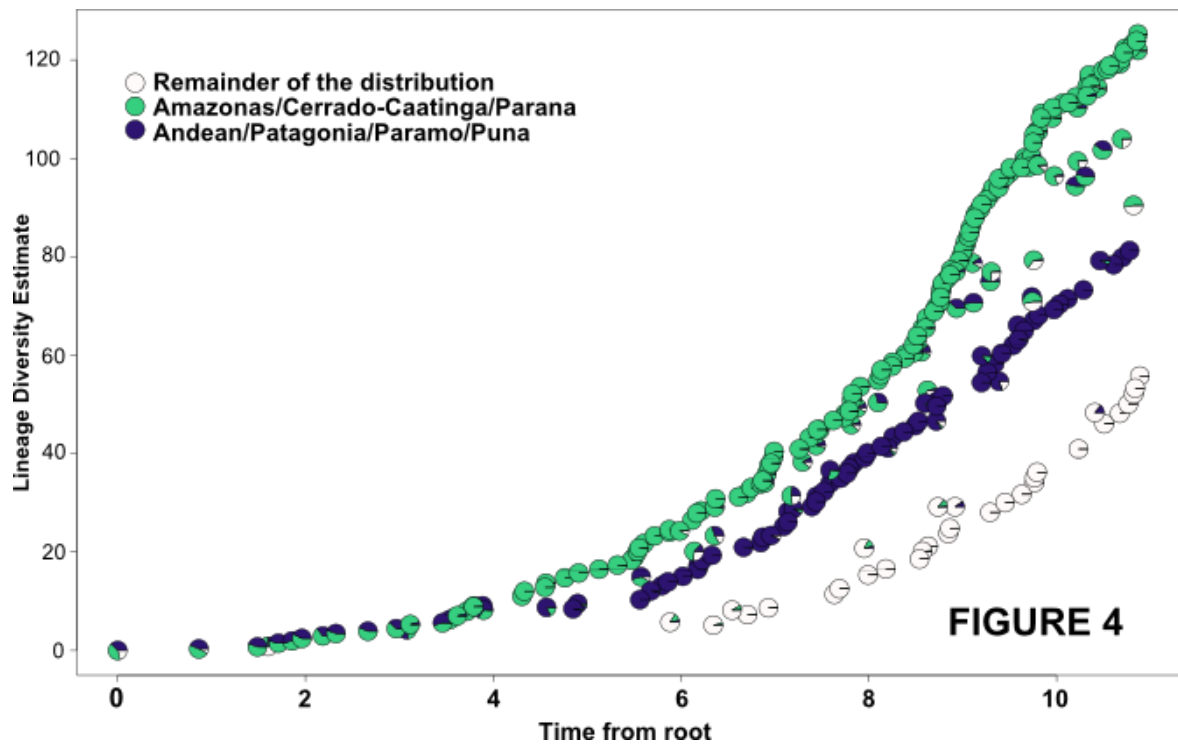


Figure 4. Lineage diversity estimates for Sigmodontine. Comparison of historical lineage diversity estimates and relative branching time estimates for each node in the MCC tree colored according to distinct geographic areas.

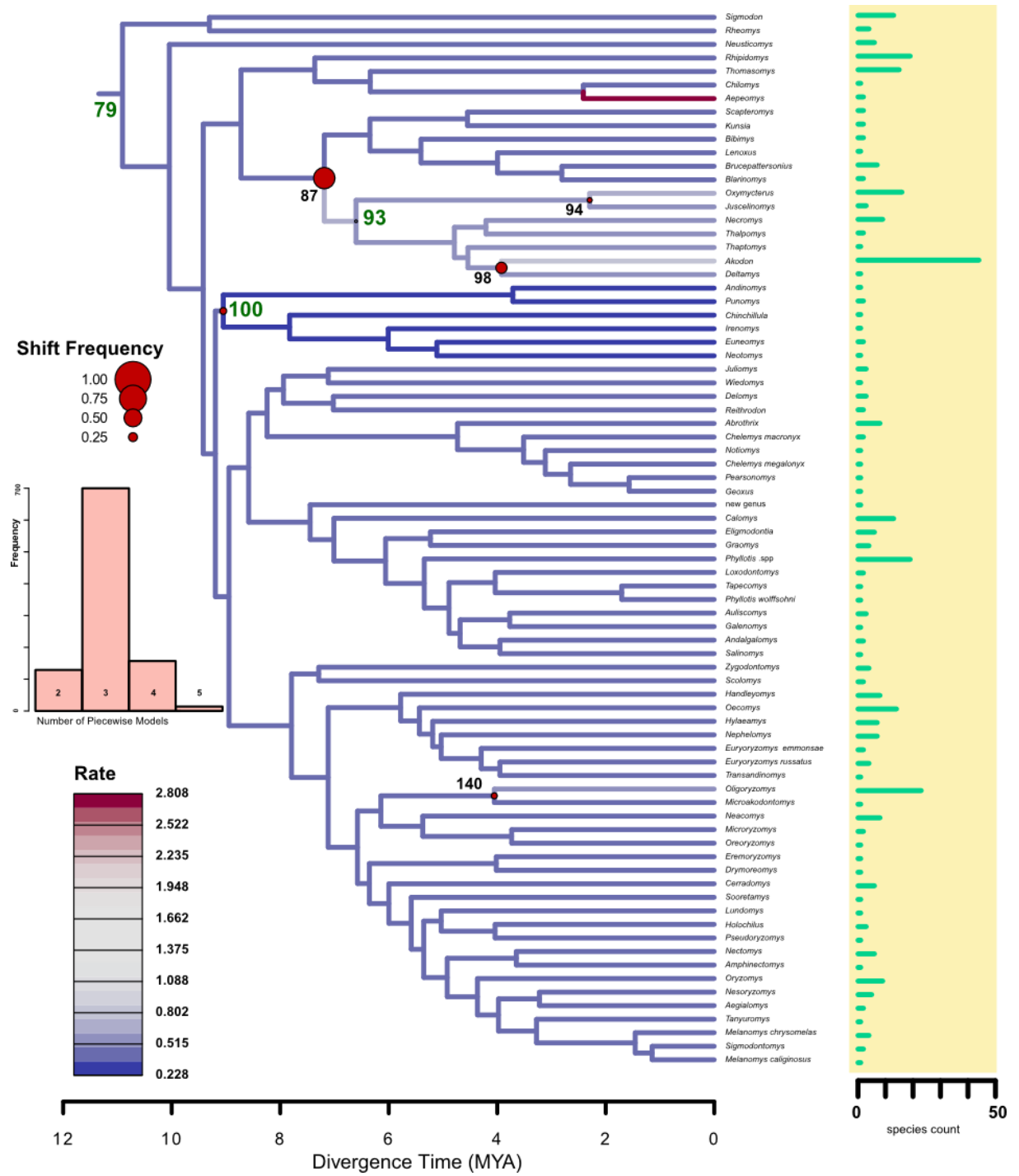


Figure 5. MCC for Sigmodontinae showing diversification rates estimated under the MEDUSA likelihood model. MEDUSA analyses were conducted using 1000 trees randomly selected from the posterior distribution in BEAST, pruned to a backbone tree representing only genera in most of the cases. Horizontal bars illustrate the species richness of each taxa. Numbers and circles close to nodes indicate the inferred positions of multiple rate shifts based on AIC scores for among-lineage rate variation (also, highlighted in green the relevant nodes when the MCC tree alone was examined). Histogram show frequency of number shifts over 1000 subsampled trees.

Supplementary Figures

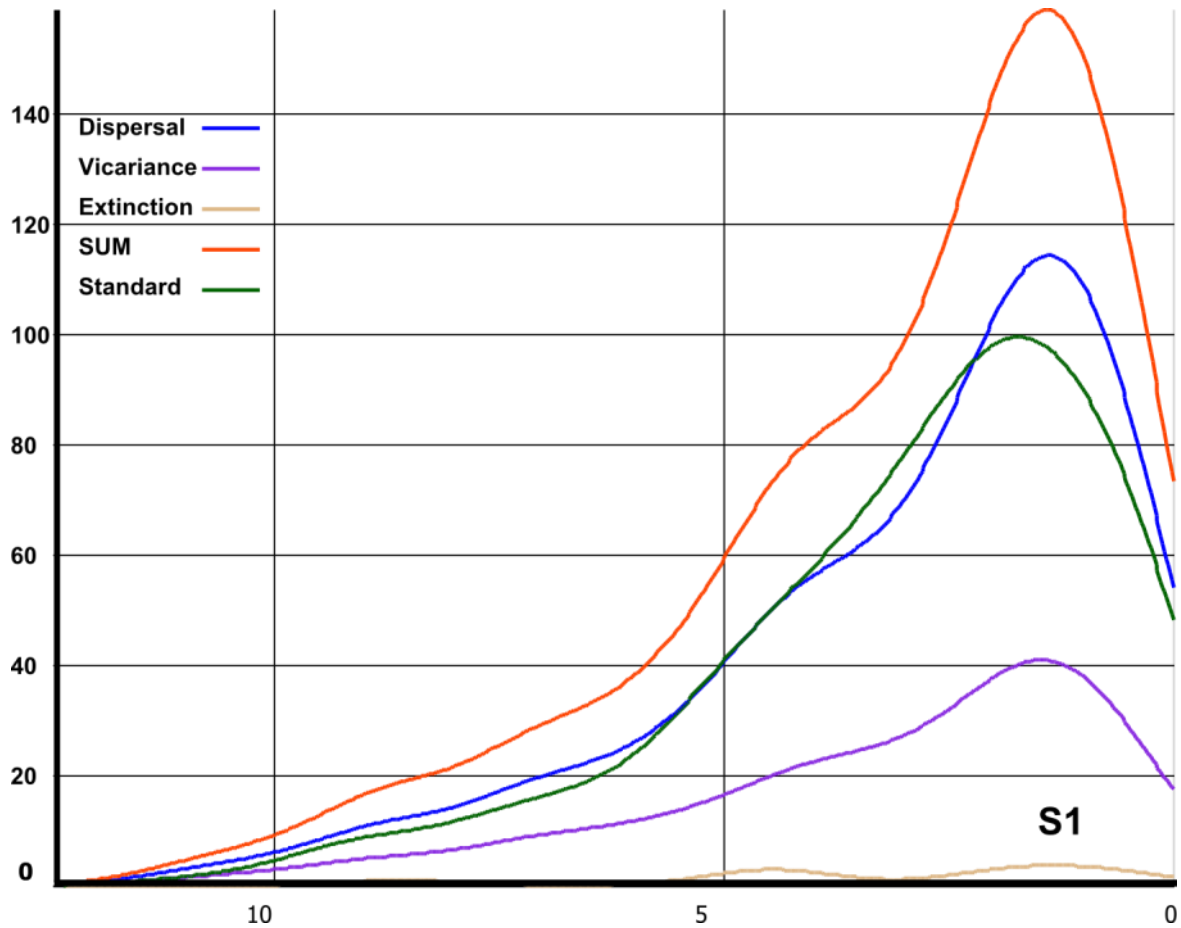


Figure S1. Time-events curve analysis for the radiation of Sigmodontinae. SUM curve is the sum of all events, including Dispersal, Vicariance and Extinction. Standard curve is a curve when each node of tree has one and only event.

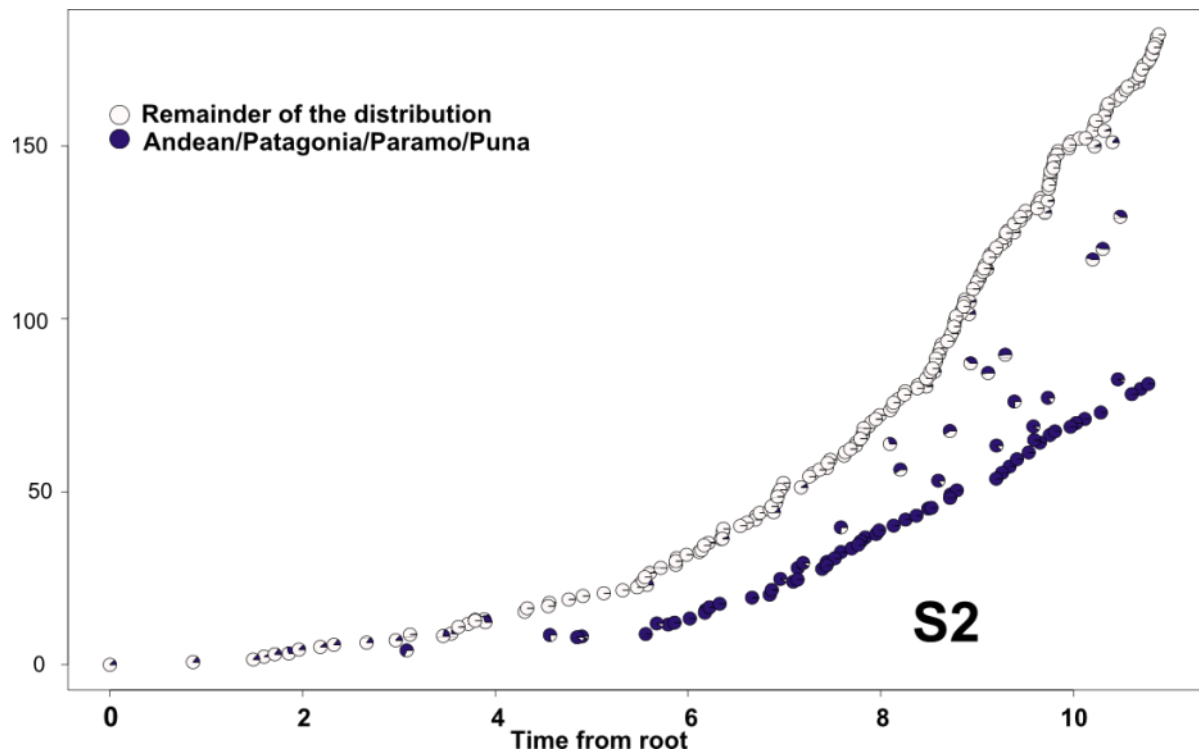


Figure S2. Lineage diversity estimates for Sigmodontine, model “Andean”. Lineage diversity present at each internal node discriminating for geographic areas. Here the comparison of a composite are of Andean+Paramo+Patagonian+Puna area versus the remainder of the distribution is shown.

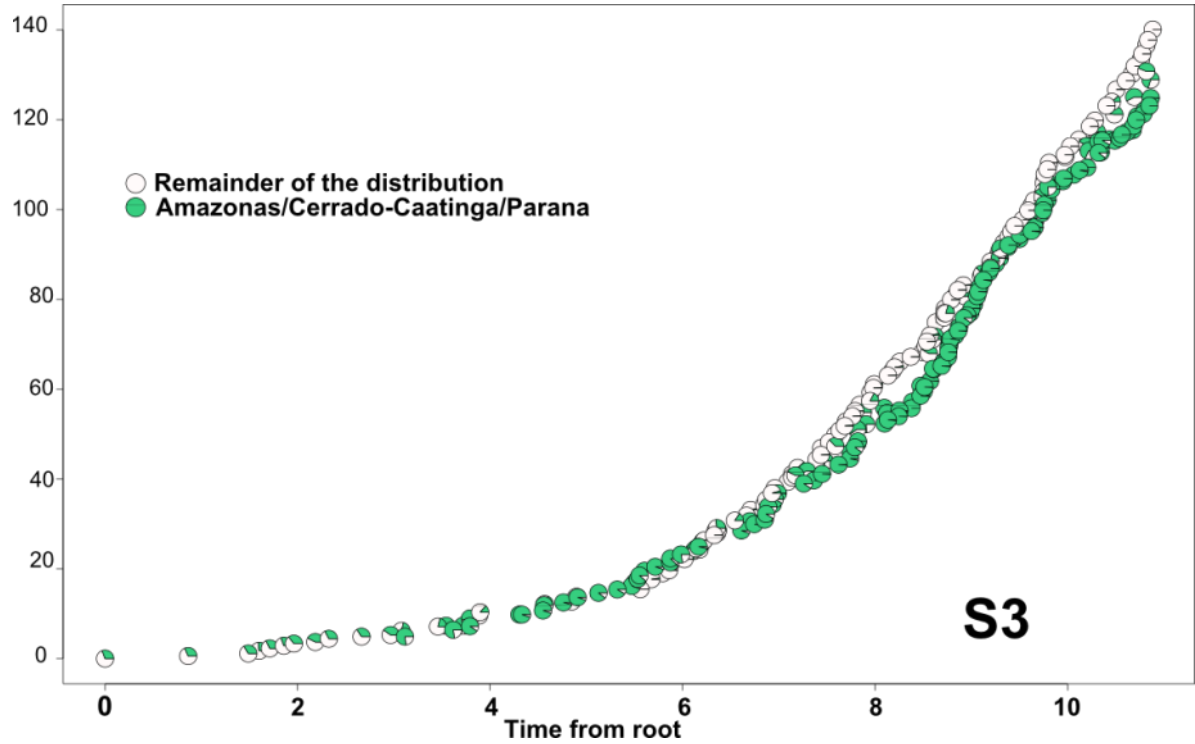


Figure S3. Lineage diversity estimates for Sigmodontine, model "Amazonas". Lineage diversity present at each internal node discriminating for geographic areas. Here the comparison of a composite area of Amazonas+Cerrado-Caatinga+Parana area versus the remainder of the distribution is shown.

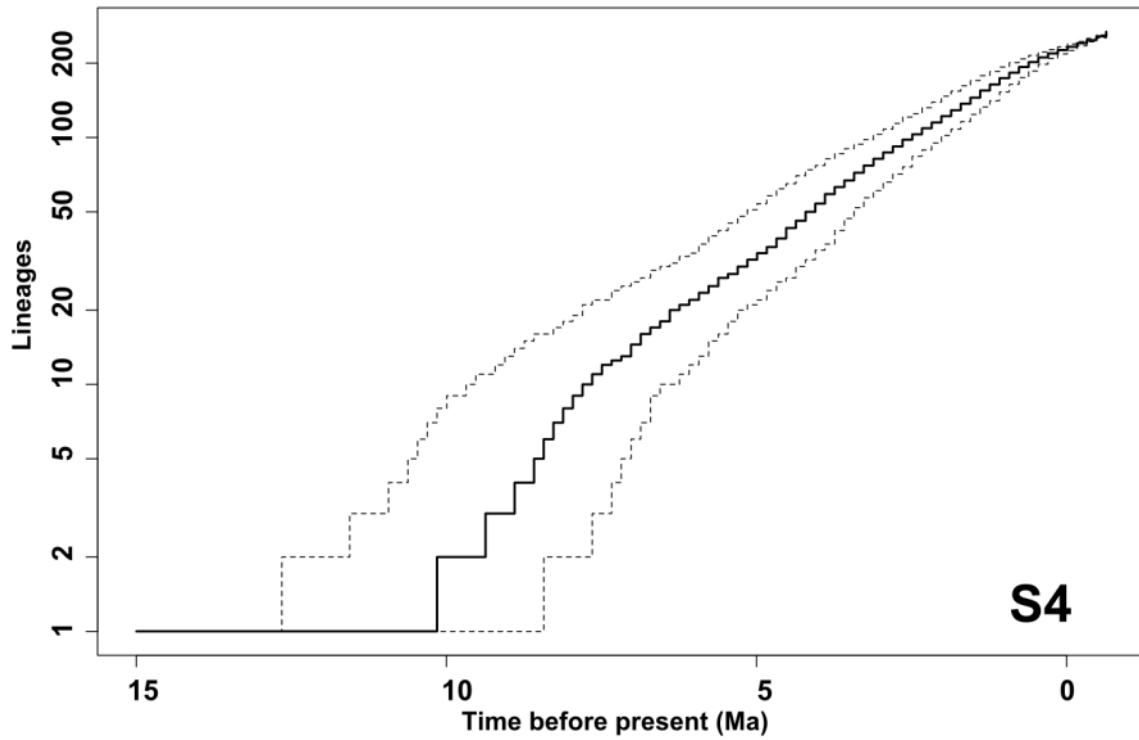


Figure S4. Lineages through time (LTT) for Sigmodontinae considering 1000 topologies. The results for LTT analysis for a subset of 1000 trees taken from the posterior distribution of the BEAST runs. Mean and 95% confidence interval are shown.



Figure S5. MCC of Sigmodontinae showing diversification rate shifts. Rate shifts inferred under the rc test (in purple) and the one recovered fitting a 2-speciation diversification model with the package geiger (in red) are shown.

Figure S6. Ancestral state reconstruction with for the altitudinal range of sigmodontine species. Altitudinal ranges are illustrated by colored bars on the tips of the branches. Pies at internal nodes represent ancestral probabilities of the traits recovered by simmap.

Figure S7. Ancestral state reconstruction for the vegetation type occupied for sigmodontine species. Habitats associated with vegetation are illustrated by colored bars on the tips of the branches. Pies at internal nodes represent ancestral probabilities of the traits recovered by simmap.

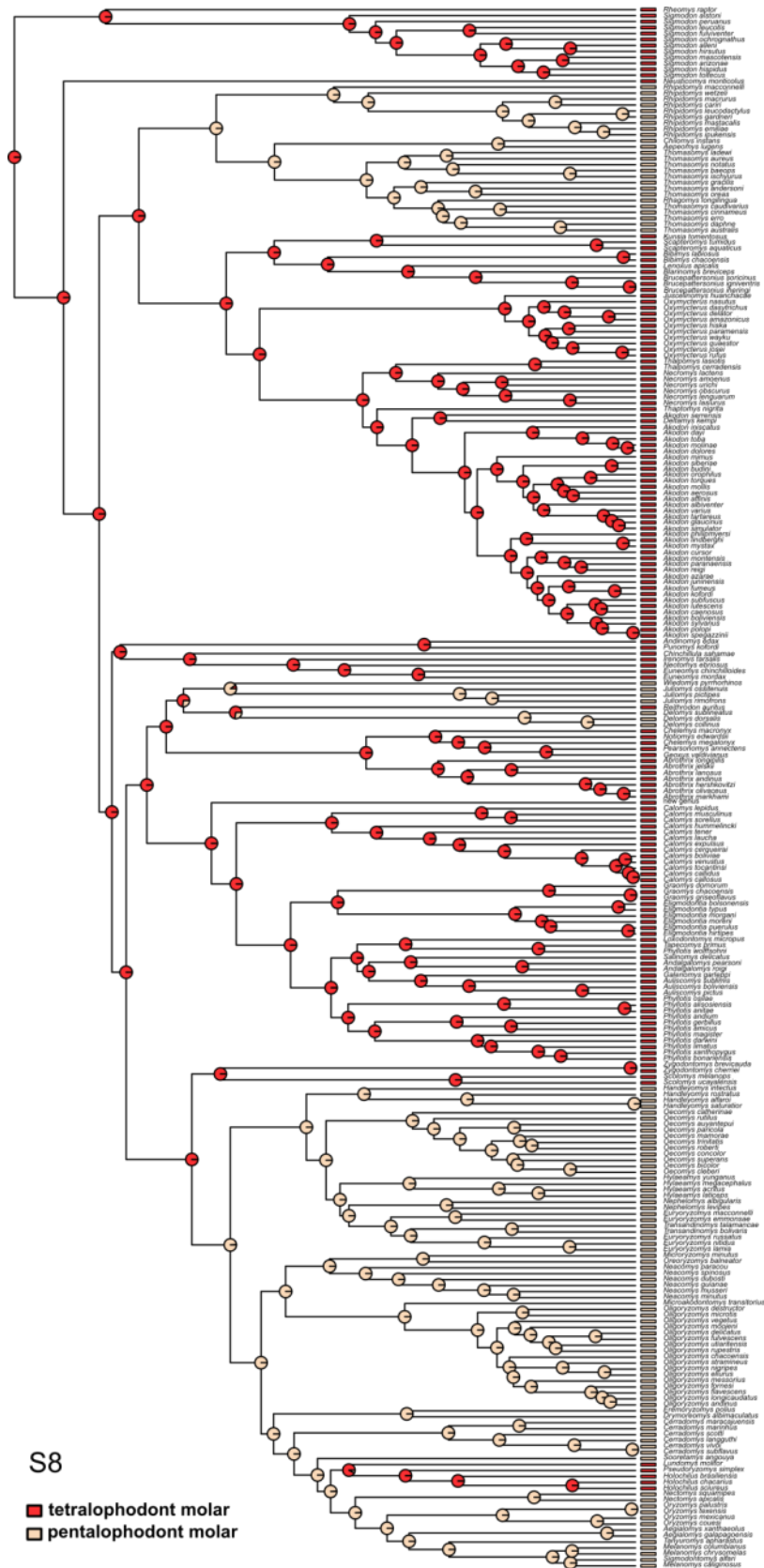


Figure S8. Ancestral state reconstruction for the molar plan of sigmodontine species. Molar morphology is illustrated by colored bars on the tips of the branches. Pies at internal nodes represent ancestral probabilities of the traits recovered by simmap.

TABLES

Clade/Nodes	Probabilities for nodal reconstructions given as percentage, for values > 9%	Age (Ma)	95% HPD
Sigmodontinae (crown)	B 67.40 Caribbean	10.92	8.91-13.06
	BE 13.62 Caribbean-Nearctic		
Sigmodontinae (stem)	-	13.95	11.17-16.79
Sigmodontini	B 64.15 Caribbean	5.03	4.37-5.9
	AB 30.35 Amazon-Caribbean		
Neusticomys-Oryzomyalia	H 43.41 Paramo	10.04	8.09-12.00
	B 29.13 Caribbean		
Oryzomyalia		9.42	7.71-11.23
	K 39.77 Puna		
	H 13.51 Paramo		
	A 12.94 Amazonian		
Oryzomyini	A 64.71 Amazonian	7.79	6.32-9.34
	I 9.99 Cerrado-Caatinga		
Phyllotini	I 56.08 Cerrado-Caatinga	7.45	6.04-8.98
	K 30.63 Puna		
Abrotrichini	G 80.81 Patagonian	4.73	3.85-5.69
Akodontini	F 61.75 Parana	7.19	5.79-8.78
	I 9.50 Cerrado-Caatinga		
MRCA Reithrodon-Juliomys	F 76.07 Parana	7.94	6.39-9.54
Clade A	K 98.00 Puna	9.05	7.45-10.98
Thomasomyini	A 52.18 Amazonian	7.36	5.85-9.00
	K 17.79 Puna		
	H 13.75 Paramo		

Table 1. Ancestral area and age estimates for key nodes. Probabilities for nodal ancestral area reconstruction with BMM method in RASP along the age estimates obtained with the BEAST analysis.

Model	constraints	lambda111	lambda112	lambda122	lambda211	lambda212	lambda222	mu1	mu2	q12	q21	lnLik	AIC
full "Andean"	none	0.3491	5.96E-08	0.0125	5.15E-06	0.0561	0.2101	2.03E-06	2.21E-06	0.0331	1.17E-06	-671.44	1362.88
constrain 1	mu1=mu2, q12=q21	0.3468	8.54E-03	0.0116	1.82E-05	0.0376	0.2219	9.67E-07	9.67E-07	0.0256	2.56E-02	-672.6165	1361.233
constrain 2	mu1=mu2	0.3491	3.74E-05	0.0125	5.27E-09	0.0561	0.2101	2.91E-06	2.91E-06	0.0331	1.16E-05	-671.4408	1360.882
constrain 3	q12=q21	0.3465	8.86E-03	0.0116	5.93E-06	0.0376	0.2218	1.28E-07	3.81E-06	0.0254	2.54E-02	-672.6165	1363.233
constrain 4	1 lambda, mu1=mu2, q12=q21	0.1108	0.1108	0.1108	0.1108	0.1108	0.1108	2.31E-05	2.31E-05	4.02E-06	4.02E-06	-729.1731	1464.346
full "Amazonas"	none	0.2267	2.12E-06	5.23E-06	3.51E-07	0.1311	0.3084	2.90E-09	1.00E-05	0.0119	1.00E-02	-699.3106	1418.621
constrain 1	mu1=mu2, q12=q21	0.2263	8.31E-07	5.78E-08	3.39E-07	0.1300	0.3091	1.26E-06	1.26E-06	0.0115	1.15E-02	-699.3117	1414.623
constrain 2	mu1=mu2	0.2266	8.12E-07	8.02E-07	1.03E-08	0.1313	0.3083	1.33E-06	1.33E-06	0.0118	9.91E-03	-699.3102	1416.62
constrain 3	q12=q21	0.2264	5.87E-07	2.71E-06	2.46E-05	0.1299	0.3092	1.15E-06	4.13E-07	0.0116	1.16E-02	-699.3121	1416.624
constrain 4	1 lambda, mu1=mu2, q12=q21	0.1107	0.1106855	0.1107	0.1107	0.1107	0.1107	1.03E-05	1.03E-05	2.46E-05	2.46E-05	-734.0252	1474.05

Table 2. Comparison of full and constrained maximum Cladogenetic State change Speciation and Extinction (ClaSSE) models evaluating the geographic range of members of Sigmodontinae. For the “Andean” Model geographic range was treated as follows: 1) present in the remainder of the distribution of Sigmodontinae 2) present in an area composed of Andean, Paramo, Puna and Patagonian regions. For the “Amazonian” Model geographic range was treated as follows: 1) present in the remainder of the distribution of Sigmodontinae 2) present in an area composed of Amazonian, Cerrado-Caatinga and Parana regions. Lambda = trait specific speciation rates; mu = traits specific extinction rates; q = transition rate parameters. Constrained models are compared using the Akaike Information Criterion (AIC). The models with the lowest AIC scores are in bold.

Model	Parameters				lnLik	AIC
Birth-Death	lambda=0.3322632	mu=2.74e-08			669.4229	- 1334.846
Pure-Birth	lambda=0.3322461				669.4229	- 1336.846
Y2R	r1=0.4016464	r2=0.1902723	st1=1.099379		682.5118	- 1359.024
Y3R	r1=0.3889555	r2=0.8188556	r3=0.1902723	st1=1.174384, st2=1.099379	685.2543	- 1360.509
SPVAR	lambda0=0.8892536	mu=0.001	k=0.1188261		678.6645	- 1351.329
EXVAR	lambda0=0.3327791	mu=0.001	z=0.9999833		669.363	- 1332.726
BOTH	lambda0=0.8883158	mu=0.001	K=0.1187328, z=0.1912819		678.665	- 1349.33
DDX	r1=1.259815	x=0.2813119			678.3511	- 1352.702
DDL	r1=0.5357646	k=413.5687			681.0322	- 1358.064

Table 3. Summary of diversification models fitted to the branching times derived from the Sigmodontinae phylogeny. (Y2R - Yule-2-rate; Y3R - Yule-3-rate; SPVAR - Speciation exponential decline; EXVAR - Extinction exponential increase; BOTH - Both rates variable; DDX - Diversity-Dependent, exponential; DDL- Diversity-Dependent, linear).

Model	constraints	lambda1	lambda2	lambda3	mu1	mu2	mu3	q12	q13	q21	q23	q31	q32	lnLik	AIC
vegetation	none	0.26	0.84	0.24	1.22E-07	2.86E-07	3.17E-08	0.1034	0.0346	0.5527	0.4411	2.53E-07	0.02	-760.3422	1544.684
constraint 1	lambda1=lambda2=lambda3, mu1=mu2=mu3, all q equal except q31=2.5e-07	0.33	0.33	0.33	4.51E-09	4.51E-09	4.51E-09	0.08	0.08	0.08	0.08	2.50E-07	0.08	-805.3374	1616.675
constraint 2	lambda1=lambda2=lambda3	0.33	0.33	0.33	3.97E-08	1.92E-08	7.85E-08	0.11	0.06	0.40	0.38	4.00E-09	0.02	-780.3107	1580.621
constraint 3	mu1=mu2=mu3, all q equal except q31=2.5e-07	0.43	0.08	0.27	2.00E-08	2.00E-08	2.00E-08	0.09	0.09	0.09	0.09	2.50E-07	0.09	-796.0721	1602.144
2 epochs	1st epoch	0.21	0.88	0.17	5.13E-08	5.29E-08	8.90E-08	0.13	0.05	0.53	0.59	8.48E-07	0.02	-745.5209	1539.042
	2nd epoch	0.48	0.10	0.53	1.84E-07	8.44E-08	1.84E-07	0.09	0.06	0.26	0.00	4.85E-06	0.00		
altitude	none	0.42	0.14	0.29	6.42E-08	3.46E-07	3.70E-07	0.17	0.06	0.23	0.04	2.57E-08	0.17	-795.5778	1615.156
constraint 1	lambda1=lambda2=lambda3, mu1=mu2=mu3, all q equal except q31=2.6e-08	0.33	0.33	0.33	1.47E-09	1.47E-09	1.47E-09	0.08	0.08	0.08	0.08	2.60E-08	0.08	-815.6657	1637.331
constraint 2	lambda1=lambda2=lambda3	0.33	0.33	0.33	0.00E+00	0.00E+00	4.22E-08	0.22	0.02	0.34	0.13	2.70E-09	0.18	-801.903	1623.806
constraint 3	mu1=mu2=mu3, all q equal except q31=2.6e-08	0.41	0.18	0.24	2.82E-08	2.82E-08	2.82E-08	0.09	0.09	0.09	0.09	2.60E-08	0.09	-806.6526	1623.305
2 epochs	1st epoch	0.33	0.25	0.12	1.13E-08	4.60E-09	1.08E-01	0.22	0.00	0.00	0.35	1.58E-07	0.14	-784.4839	1616.968
	2nd epoch	0.46	0.02	0.78	1.25E-08	2.84E-08	2.27E-01	0.02	0.03	0.00	0.01	9.05E-08	0.00		

Table 4. Comparison of full and constrained maximum Multiple State Speciation and Extinction (MuSSE) models.

Vegetation type was treated as follows: 1) open-vegetation; 2) mixed; 3) forest. Altitudinal range was treated as follows: 1) lowland; 2) middle range; 3) highlands. Lambda = trait specific speciation rates; mu = traits specific extinction rates; q = transition rate parameters. Constrained models are compared using the Akaike Information Criterion (AIC). The models with the lowest AIC scores are in bold.

Model	LH	r.1	lambda.1	LH.1	r.2	lambda.2	LH.2	eps	aic
NDR1	-320.766	0.4289	0.4289					8.25E-08	643.532
NDR2	-312.2796	0.3507	0.3507	-215.8727	0.4999	0.4999	-96.4069	8.25E-08	630.5592

Table S3. Fit of one and 2-rate diversification (NDR1 and NDR2) models to combined phylogenetic/taxonomic data. r=net diversification rate lambda-mu; lambda=speciation rate; eps=mu/lambda. Partition 1 contains the root. Partition 2 is the clade defined by node 138 see Fig S2. LH, LH1 and LH2 log log-likelihood at the maximum, partition 1 and 2 respectively.

Shift node	Proportion	mean shift	median shift	minimum shift	maximum shift	standard shift
87	0.599	0.3291	0.3269	0.2148	0.5315	0.0466
98	0.315	0.6265	0.6187	0.3873	0.8779	0.1001
100	0.191	3.7292	0.3556	-0.1748	632.401	45.7291
140	0.174	0.6156	0.6053	0.4802	0.8559	0.0822
94	0.144	0.8061	0.7962	0.5879	1.1554	0.1215
93	0.08	-0.0704	0	-0.2281	0	0.0921

Table S4. Results for the MEDUSA analyses for Sigmodontinae.

MEDUSA analyses were conducted using 1000 trees randomly selected from the posterior distribution in BEAST, pruned to a backbone tree representing only genera in most of the cases. Rate shifts were selected based on AIC scores for among-lineage rate variation.

Model	constraints	lambda0	lambda1	mu0	mu1	q01	q10	lnLik	AIC
full	none	0.3427501	0.3182811	5.87E-07	3.05E-07	9.01E-03	3.82E-03	-587.9023	1187.805
constrained	lambda0=lambda1	0.332331	0.332331	1.76E-07	2.18E-07	9.07E-03	3.75E-03	-588.0776	1186.155
2 epochs	1st epoch	0.3181414	0.2364569	6.54E-07	2.17E-08	1.05E-06	1.03E-11	-571.3639	1166.728
	2nd epoch	0.3934437	0.5726885	9.84E-08	4.90E-07	3.08E-02	1.65E-02		

Table S5. Comparison of full and constrained maximum Binary State Speciation and Extinction (BiSSE) models.

Trait 0 tetralophodont molar; Trait 1 pentalophodont molar. Lambda = trait specific speciation rates; mu = traits specific extinction rates; q = transition rate parameters. Constrained models are compared using the Akaike Information Criterion (AIC). The model with the lowest AIC scores is in bold.

CAPÍTULO 3.

Interacciones entre el context ecológico y la historia evolutiva.

Understanding adaptive radiations: the interplay between ecological context and evolutionary history.

**4. UNDERSTANDING ADAPTIVE RADIATIONS: THE INTERPLAY
BETWEEN ECOLOGICAL CONTEXT AND EVOLUTIONARY HISTORY.**

Autores: Andrés Parada, Guillermo D'Elía y Eduardo Palma.

En preparación.

Abstract

Species richness patterns have fascinated biologists since a long time. Ecologists and biogeographers have sought explanations in a variety of ecological and historical processes and these patterns can be understood as the outcome of widespread processes acting at different scales. Here, I review several explanations for species diversity patterns. Ecological opportunity (or “a wealth of evolutionarily accessible resources”) has been championed as the promoter of adaptive radiations, so its role as the promoter of diversification is considered in some detail. The nature of the signatures left by adaptive radiations are revised, as well as the state of the art methods used to evaluate these patterns. We focus on how different mechanisms or factors (unique story, local determinism, species range and ecological zone of origin) could have triggered the radiation of the Sigmodontinae subfamily (Rodentia, Cricetidae), one of the most diverse Neotropical mammalian clades (~ 400 living species and 82 genera). Ecological context seems relevant to fully understand the evolution of this group. We further consider why some clades might fail to radiate even in the presence of accessible resources and which circumstances might have slowed down the radiation in certain groups.

INTRODUCTION

“The archives of natural history are filled with ... cases of species formation exploding as a response to ecological opportunity.... Natural history becomes all the more pleasing and interesting when we look at it through the lens of evolutionary theory and search for the starbursts of adaptive radiation.” (Wilson 1992 [1], p. 112).

Patterns of species richness reflect the balance between speciation and extinction over the evolutionary history of life. These processes are in turn influenced among other things by the size and physiography of regions, environment, and certain attributes of individuals and species. Prior to ca. 1960, most hypotheses in explaining species richness patterns were derived from history and geography. According to this widespread view, diversity arises via evolutionary diversification over long periods within large areas [2], with emphasis in the relative stability of the tropics [3] and the more rapid evolution and species production in tropical environments [4]. Thereafter, community ecology focused on patterns of local diversity as the consequence of ecological sorting of species available within a region [5–7]. Moreover, since Darwin times, biologists have been fascinated by the extraordinary diversity of ecology, morphology, behavior, and species richness of some taxa. The evolutionary exuberance of such “adaptive radiations” highlights the numerous ways of natural selection to produce biological diversity, as Darwin realized [8]. “Adaptive radiation” refers here to “evolutionary divergence of members of a single phylogenetic lineage into a variety of different adaptive forms” ([9]; glossary). Iconic examples of adaptive radiation include Darwin’s finches on the Galápagos islands, the lizards of the genus *Anolis* on the Caribbean islands, and the cichlid fishes of the East African Great Lakes, among many others [10].

Besides the intrinsic value in understanding how these groups had flourished, these cases with numerous branching events tied to ecological process facilitating diversification, provide an ideal scenario to examine the interplay of natural selection and the ecological context.

The role of Ecological opportunity

Most theories of adaptive radiation, including Simpson's [11] and Schluter's [10], stated that the process begins with ecological opportunity (see also [12,13]). It must be noted that Losos [13] argued that while ecological opportunity usually triggers adaptive radiation, ecological opportunity appears as neither **necessary nor sufficient** for radiation to occur. Ecological opportunity is "*loosely defined as a wealth of evolutionarily accessible resources little used by competing taxa*" [10]. Several recent phylogenetic comparative studies emphasized the relevance of ecological opportunity: an early burst of cladogenesis is usually linked with lower rates later in history resulting from niche filling (e.g., [14–16]) or high rates of morphological diversification early in a clade's history [17–19]).

As summarized by Losos [13], there are four main different ways as resources might be accessible :

- a) Appearance of new resources.** Evolution of a clade may provide opportunities for other taxa.
- b) Extinction of species previously using resources.** Extinction events can remove ecologically dominant species, leaving more resources available to the surviving species.

c) Colonization of an area in which resources were previously not used. A new area would provide access to resources due to the lack of competitors. Moreover, the absence of predators may remove constraints on using habitats, otherwise unexploited, promoting niche shifts.

d) Evolution of a trait that permits utilization of resources in ways not previously possible. The evolution of a new feature may allow to use a resource. By these means an ancestral species would exploit resources previously unattainable and then diversify. These features are labeled as “key innovations” (e.g. [20]).

Species newly formed may encounter entirely different ecological opportunities made accessible by adaptive evolution or created by the presence of a new sister species. Some details in the chain of events on how ecological opportunity ends in an adaptive radiation remain unresolved. Even in taxa experiencing adaptive radiation, most speciation appears to be associated with geographical isolation of populations. If speciation is occurring mainly under allopatry, the role of ecological opportunity would be relegated to initiate ecological release early in the adaptive radiation and later it might increase the opportunities for reproductive isolation or reduce the probability of extinction, instead of creating new species directly [10]. Moreover, mechanisms linking resource availability and speciation rates like ecological release are not clearly understood. Upon encountering ecological opportunity, populations will experience a relaxation of selection acting on ecological traits, increasing in size due to density compensation [21], will expand their habitat use to take advantage of new resources, and will show increased variation in ecologically important traits. After initial divergence, if speciation follows, variation acquired via ecological opportunity will be

preserved in macroevolutionary time [22], and newly formed species can exploit new ecological opportunities.

Looking for the signature of an adaptive radiation

In order to understand species richness patterns in a historical context, lineages through time [23–25] and other methods for assessing the tempo of evolution has been widely used in last decade (e.g. [26]). The most common measure of “slowdowns” is the gamma statistic of Pybus & Harvey ([27]), which compares observed sets of intervals between speciation events to those expected under a uniform process of diversification. Numerous authors have recovered an apparent slowdown in the rate of lineage diversification through time (e.g.[10,14,15,17,28]). These apparent “bursts” of lineage accumulation early in the history of radiations are followed by declines in diversification rates as diversity rises and ecological niches are filled. Some caution is needed to interpret these results since diversification models with different ecological assumptions, including those models involving no ecological differences among species at all, may nevertheless generate similar patterns of diversification (e.g.[29–31]).

New methods to compare models of lineage diversification have been developed in the last decade, as summarized in [12,32]. One of the most used methods is MEDUSA [33] which uses stepwise Akaike Information Criterion. The advantage of this approach is the ability to consider the known taxonomic richness along the topologies of interest. Another recent development is the implementation of likelihood models that unites both, trait evolution and species diversification, avoiding biases that occur when the two are treated separately. The

BiSSE (binary state speciation and extinction [34]) and MuSSE (multistate extension of the former) methods, as well as others encompassing quantitative trait evolution [35,36], allow to test whether character states are associated with different rates of speciation or extinction. Comparatively, few studies have looked for an analogous slowdown in the rate of trait evolution in a comparative context. For example, Harmon et al. [17] examined body size and shape evolution in a comparative context across a large data set. The authors found no decreased rate of trait evolution. The contrast between these findings and the vast evidence of slowdown rates of lineage accumulation through time in adaptive radiations implies that adaptive divergence continues even after an adaptive radiation has reached some equilibrium level of species diversity [12]. This would imply that the evolutionary trajectories do not necessarily involve rapid diversification of both lineages and ecologically important traits (e.g. [17]). Thus, the tempo of adaptive radiation might be limited more by the emergence of new species than by the evolution of new traits ([10]).

Is niche evolution linked to radiations?

Over the course of an adaptive radiation, lineages are expected to rapidly fill vacant niche space as they diversify [28]. If this process is driven by ecological opportunity, then eventually unoccupied niches should run out, causing the rate of diversification to decrease through the course of an adaptive radiation. This process would leave a signature on the rates of lineage diversification through time, causing the apparent lineage diversification rate to decrease through time.

An adaptive radiation driven by ecological opportunity should leave a signature in comparative data. As mentioned above, adaptive radiation into new forms should be reflected as an increased rate of diversification during some time period in the history of a group. Also, as accessible niches become occupied, opportunity for ecological speciation should become increasingly limited, and rates of diversification should slow through time [11]. Ecological opportunity is neither necessary nor sufficient for adaptive radiation to occur [13]. One scenario would involve competitive replacement, in which a clade supplants another one through the fossil record [37], although a radiating clade is unlikely to replace another one solely by outcompeting them. Another case would imply some clades self-perpetuates via creating their own ecological opportunity while they are radiating. An increase in the number of species in the communities may represent more resources available. There is no evidence that adaptive radiation in the absence of ecological opportunity is a popular phenomenon (see [13]).

Historical unfolding of diversity

As part of a comprehensive framework, Ricklefs [see Table 1, [38]] provided a simple classification on the mechanisms that influence global diversity:

Influenced by “Local determinism”

- a) Limiting similarity and saturation
- b) Diversity increases resistance to invasion

Influenced by Regional/historical processes

- a) Ecological zone of origin
 - i) Age and area
 - ii) Adaptive diversification
- b) Net rate of diversification
 - i) Physiography and history promote speciation
 - ii) Climate change and catastrophe because extinction
 - iii) Diversity promotes or retards diversification

In the particular scenario of the Neotropics, several mechanisms have been postulated to explain the evolution of its biodiversity. These have been very roughly classified ([39]) into two major categories: “biotic” (e.g., soil adaptations; interactions with pollinators, dispersers and herbivores; niche conservatism; dispersal ability) and “abiotic” (e.g., time; rainfall, temperature and area; mountain uplift; hydrological changes).

A Chronology of the diversification patterns in the Neotropics.

The main Quaternary environmental shifts have been linked to the glacial–interglacial cycles whereas the Neogene (Miocene-Pliocene) has been characterized by significant tectonic and paleogeographical arrangement, generating new corridors and barriers [40].

According to the “refugia hypothesis”, Neotropical speciation was promoted by the alternation of vicariance and gene flow cycles linked to glacial contractions and interglacial expansions of lowland rainforests patches (or “refugia”), controlled by recurrent aridity–humidity oscillations. Critics to the “assumed glacial aridity” collided with this view [41].

Thus, instead of climatic cycles, diversification is explained by events occurring during Neogene rather than in the Quaternary. Some of these events include the closure of the Panama Isthmus, the Andean uplift, or hydrological changes like the draining of the Pebas system (e.g [42,43]). Alternatives to the “refugia hypothesis” include the Riverine model [44], disturbance-vicariance hypothesis [as in [45,46]] and the Gradient model [47]. Recurrent altitudinal migrations during the Pleistocene glacial cycles may have promoted diversification in the Andes. These migrations lead to connectedness or isolation of high-mountain biomes (cf. [48]). On the other hand, in the Amazon basin, low temperatures and atmospheric CO₂ depletion could have led to downward migrations of the biota of surrounding mountains. The resulting heterogeneous spatial patterns would have promoted vicariance [46,49,50]. This disturbance–vicariance hypothesis proposed a Miocene downward biotic migration from the Andes to the Amazon lowlands and subsequent migration, and speciation through the lowlands in the Pleistocene.

According to Hoorn et al. [43] mountain building creates landscape and climatic changes, ecological gradients and physical habitats that set the stage for species evolution. Depending on the context involved, mountains then serve as barriers to some taxa and corridors to others. The mountain uplift in turn influences drainage in adjacent regions which act as bridges, barriers or species pumps. Given the high diversity, dispersal into other areas, it is more probable that mountains can become species pumps that feed the rest of their continents e.g. [51]. In this way, plate tectonic dynamics unchains a domino effect that ripple through long periods of time and impacts on diversity.

Radiations in the Neotropics: a case study of sigmodontine rodents

The pervasive species plant and vertebrate species richness in the Neotropics has been subject of numerous reviews (e.g.[39,52,53]). Specifically, this region harbors about one-quarter of the world's mammal fauna, estimated by Patterson [54] in about 1145 species in 12 orders, with more than half of the species diversity in the order Rodentia, although these numbers certainly had increased in the last decades. One of the most diverse and more broadly distributed groups is the subfamily Sigmodontinae (Rodentia, Cricetidae) with about 400 living species in 85 genera [55]. These rodents have proliferated profusely occupying a variety of habitats and lifestyles.

Briefly, a recent analysis (Parada et al. submitted) has recovered evidence supporting correlates between transitions in lifestyle (altitudinal range and vegetation type occupied) and differential diversification rates. Additionally, several rate shifts were detected, particularly accelerated rates within the tribe Akodontini and a slowdown in diversification rates in a lineage inhabiting the Puna ecoregion (or “Clade A”). In the following sections the means by how a “unique history”, local determinism, species range or the “ecological zone of origin” could had influenced the radiation of Sigmodontinae are discussed. As we will see, some of these concepts are essential to understand the reasons behind the success or failure of certain clades.

Why some clades fail to radiate?

The majority of ecological opportunities might never promote an adaptive radiation. These cases of ‘failed radiation’ possess an intrinsic value that could help us to understand the complex links between resource utilization, adaptation and speciation. Even in the presence of ecological opportunity, a clade could fail to radiate in a number of ways [13] as it is mentioned next:

Lack of access to ecological opportunity. Suitable resources actually may not be accessible for some groups. Some organisms are unable to seize resources even when these abound.

Lack of speciation. Adaptive radiation requires both species production and diversification into different ecological niches. If for some reason a group does not speciate, then adaptive radiation cannot occur, even in the presence of ecological opportunity. Transient variation requires to be “locked in” as a requisite to further diversify.

Lack of ecological access. Early colonizers might have taken over existing resources, precluding diversification by later arrivals.

Lack of evolvability. “Evolvability,” the ability to evolve readily into diverse forms [10] could influence adaptive radiations. While some organisms will change more slowly or not at all, other will be able to adapt to local circumstances rapidly. Factors that are involved with evolvability such modularity, behavioral and phenotypic plasticity are usually invoked. Additionally, some organisms may have a genetic architecture that make it hard to exploit natural discontinuities in the environment or in niche space. Organisms would usually follow genetic “lines of least resistance” (Schluter, 1996), and if these lines do not match habitat or resource availability provided by ecological opportunity, diversification would decline [12,56].

Gene flow might obstruct speciation. Also, according to theory, gene flow might hamper speciation [57], which can prevent populations in novel environments from being isolated from source populations, although ecological speciation to some extent might overcome this shortcomings [58,59]. Additionally, the presence of many intermediate environments along a gradient would spur high levels of gene flow among populations and slowdown speciation rates [60]. One could argue that in some cases ecological opportunity is defined a posteriori once an exemplar case of unusual richness is found. This bias could have been hindering our perception on which are attributes of habitats, traits or resources relevant to the question at hand. Following this brief layout of the circumstances that could diminish an evolutionary radiation, I revisit four scenarios commonly invoked to explain the fate of evolutionary radiations.

Do unique history and geography prevail?

Diversification rates might be higher in regions or ecological zones of high diversity [61–63]. Certain ecological conditions, such as temperature, could promote or slowdown diversification [64]. Unique physiography or geographic configurations of regions could affect extinction so this would depend on an unique history. Unusual differences in species richness in the same habitat between regions or (“diversity anomalies” as in [38]) are then attributed to special explanations suggested by the case at hand rather than obtaining explanations emerging from the use of data and testing predictions. The sigmodontine radiation pose an intriguing case since not all lineages presumably in the presence of the same

ecological opportunity have radiated. This might be the result of an inaccurate perception of what constituted ecological opportunity for some taxa. There is consistency between certain relationships (e.g. high diversity in montane forested habitats; increasing diversity after spreading into lowlands) across the radiation. The clade associated with the Puna [or “clade A”] or the species-poor lineages of uncertain affinities in the Southeastern Brazil apparently “failed” to radiate. This pattern may be hardly explained by an “unique history” that should have affected simultaneously other sigmodontine representatives.

Local determinism

Another aspect commonly invoked to explain “diversity anomalies” is local determinism, implying that patterns of diversity reflect attributes of the physical environment that influence the species interactions. The rise of local determinism was inspired by the results of models and microcosm experiments showing that local interactions run to equilibrium quickly, generally within a few generations [65]. Larger regional processes responsible for the production of new species (speciation, immigration from elsewhere) are much slower than those acting at local scale. Thus, large-scale processes were considered too weak to influence local equilibrium achieved by local processes. Gould’s [66] metaphor in which “any replay of the tape would lead evolution down a pathway radically different from the road actually taken” highlighted that it is unlikely to obtain identical evolutionary trajectories. **First**, species are unlikely to occur in identical adaptive landscapes. As a generality, ecologically similar, but distantly related, clades are unlikely to radiate in the same geographic area. Consequently, such clades likely will not experience the same patterns of selection, because

environments in different areas likely will differ; for example, interactions with different sets of other clades are likely to produce different evolutionary outcomes. **Second**, clades that are distantly related differ in so many ways that entire evolutionary radiations are unlikely to unfold in the same way. The course of evolutionary diversification may depend on the starting point of a radiation (Gould's [67], p. 1160] "happenstance of a realized beginning"), it seems crucial to realize that the biology of the ancestral species—its natural history, phenotype, even its genetic variation— can influence subsequent evolutionary change [68]. It must be noticed that the perception of historical contingency depends on the frame of reference. A discernible effect can be observed given that among **same** ecological context (e.g. montane forest or lowlands) some lineages flourish while exploiting those ecological opportunities, whereas **within** each area history could hamper the chance to radiate.

The unpredictability of evolution by natural selection goes back to Darwin [69], who illustrated it via the analogy of an architect building an edifice with uncut stones, falling from a precipice and how the variation imposed contrivances to the process of generating new species (or the construction in the analogy). Subsequently, Simpson considered that evolution is driven principally by natural selection of chance variations in changing environments, and is therefore "opportunistic" ([70], pp. 160-186); natural selection exploits these changes by perpetuating the most favorable of the existing or new variants in the existing or new environment [70]. "Opportunistic" means that there is no plan or provision by which organisms automatically vary in ways that are adaptive. Another reason on the argument of unpredictability is there are "multiple solutions" to any environmental "problem," or multiple ways of exploiting any environmental opportunity.

To put it more clearly: *“Evolution works on the materials at hand: the groups of organisms as they exist at any given time and the mutations that happen to arise in them. The materials are the results of earlier adaptations plus random additions and the orienting factor in change is adaptation to new opportunities. If this view of evolution is correct, then we must expect to find similar opportunities exploited in different ways. The problems involved in performing certain functions should have multiple solutions.”* (Simpson, pp. 164-165). And also: *“There are two aspects of opportunism: to seize such diverse opportunities as occur, and when a single opportunity or need occurs, to meet it with what is available, even if this is not the best possible.”* (Simpson, pp. 167-168).

As hinted by Gould and Lewontin [71] when “multiple adaptive peaks” are occupied, one usually have no basis for concluding that one instance is better than another. In the case of the sigmodontine radiation, it is hard to determine if certain “unusual” historical event or local condition hampered or slowed down the diversification -for example in the Puna region-. Also, knowing how the sequence of invasion of lowlands unfolded might seem crucial to determine the success of the species-rich akodont or oryzomyine rodents. Perhaps the order of entrance into a new ecological zone or the primacy of a group over a certain zone is what determines the chances to radiate. Important changes in physiography, tectonics, climate and vegetation prevailed for most of the Miocene-Pliocene. The order and magnitude of these events in certain areas, or rather, how this sequence of events affected differentially the chances to radiate for some groups, remains undetermined.

Does Species Range Affect Speciation?

The relationship between species ranges and speciation have spurred two contradictory expectations [72]. One view that goes back to Darwin [73] holds that large range sizes should increase speciation rates. On one hand, larger ranges make more likely to encounter not only new habitats but also barriers that might isolate populations and promote speciation [72,74]. High dispersal abilities and broad niche widths would lead to species insensitive to barriers, which should damp speciation. On the other hand, factors that might produce larger ranges, (e.g. high dispersal abilities and broad niche widths) would left species impervious to barriers, which should damp speciation [75,76].

The assumption that high dispersal abilities and broad niche widths translate into large ranges requires close scrutiny. These two factors may not always promote large ranges [72]. Lester et al. [77] argued that in most cases dispersal abilities do not correlate with range sizes. Furthermore, the association of niche widths and range sizes has been questioned (e.g.,[78]). In the case under scrutiny, dispersal and the interaction between newly formed species, corridors and barriers, together with the complete disappearance of the Pebas system through Miocene, may explain the lack of success of the lineages of uncertain affinities inhabiting the lowlands which might be remnants of a more diverse past [e.g. [79]]. So far, the dynamism of the region in the past, and some contradictory interpretations about the paleoecology of the region hamper considering these assumptions.

The role of the “Ecological zone of origin”

Another view considers species richness as the result of diversification within ancestral ecological zones of origin, coupled with occasional adaptive shifts associated with invasion of

new ecological zones [80,81]. Gradients of diversity would produce in turn greater species richness in environments that are older, more widespread, or less stressful. The comparative analysis with sigmodontine data found no such correlation although apparently the “tropical lowlands” accrued more diversity over time than the remainder of the distribution. Since the Neotropics have gone through numerous changes in physiography [82,83] the pattern in species richness must be explained via a different model without invoking “stable environments”. The results also suggested that clades radiated outside of their ecological zone of origin (e.g. the Akodontini or Phyllotyini tribes proliferated in the lowlands rather than in the Puna region). Additionally, the apparent slowdown in diversification agree with the onset of glaciations, but testing the relationships between these two phenomena awaits gathering more relevant ecological data (e.g. more detailed knowledge for the species distribution), and do more critical taxonomic work. Moreover, once estimates of niche occupancy are available it would be possible to obtain a better understanding on how niche evolution is associated with the diversification of this group.

Not all “triggers” were examined

When the pattern of the sigmodontine radiation was examined, some other plausible drivers could not be considered. For example, switch in diet type has been linked to diversification rates (e.g. [84]). Considering that the physiography of certain regions or characteristics of the environment such as a tropical climate could influence speciation rate [84], the thermal performance of organisms is also an ideal candidate to further test the link

between niche and diversification patterns. Also, the pervasive chromosomal diversity (e.g. [86,87]) or the species range could have influenced speciation rates [72].

As we begin to understand the emerging patterns within the sigmodontine radiation, we should carefully examine more plausible drivers to fully understand the different scenarios that promoted or diminished the proliferation of some clades. Some of the effects of these drivers might be intertwined. Gathering more data is critical to dissect the relative contribution of these drivers to the current species diversity.

A coda on the bias of studying big trees.

Phylogeny reconstruction fosters a perceptual bias of continual increase in species richness, and the analysis of primarily large clades produces a data selection bias. Since they only include extant species, molecular phylogenies, carry the perceptual bias of increasing diversity [see also [88,89]]. Hence, models with expanding diversity with time, either under exponential growth or with some sort of diversity saturation are commonly used.

The clades we chose to examine in our study might bias the inferences we make. Large clades are preferred over smaller ones for studying diversification since there is presumption these will reflect evolutionary process in a better way. Large clades, in order to obtain their current size, are likely to have undergone a stochastically high rate of speciation early in the history of the group. Thus, stochastic bursts, which are more likely to have occurred in “unusually large” trees, may be erroneously attributed to ecological processes (e.g. adaptive radiations) [89]. As more complex models of diversification are available it is crucial to fully

understand the explanatory power and caveats of these models in order to fully understand the patterns of diversification.

CONCLUDING REMARKS

Diversity patterns of South American biota provide an ideal scenario to investigate the ecological and historical context that promotes evolutionary radiations. The radiation of Sigmodontinae is certainly impressive. Assembling the big picture of the evolution of sigmodontines would certainly require the integration of lifestyle traits that are associated with local ecological processes and also understanding the interaction of evolutionary and biogeographic processes that depend on unique features of the history and physiography of regions.

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5. CONCLUSIONES GENERALES

Esta tesis tuvo como objetivo general indagar el *tempo* y modo de evolución de los roedores neotropicales de la subfamilia Sigmodontinae, evaluando asimismo el rol de la oportunidad ecológica en la radiación del grupo. Se logró establecer una cronología para la radiación de Sigmodontinae, hipotetizándose un origen de ~12 Ma evidenciándose una profusa diversificación durante el Mioceno tardío. Esto contradice las hipótesis de un “arribo tardío” a Sudamérica por parte del ancestro común sigmodontino.

Luego de considerar el *tempo* de la radiación, se analizaron los “drivers” detrás de esta diversificación. En particular, se consideró si ha influido el área ocupada, el hábitat, la altitud o la morfología molar en la diversificación del grupo. Se realizó una reconstrucción del área ancestral para los linajes sigmodontinos, la que sugiere que varias tribus se originaron y radiaron fuera de los Andes, contrario a lo predicho por otros autores. El ancestro común más reciente de los sigmodontinos habría ocurrido en la región del Caribe y habría arribado a Sudamérica a través del mar Caribe.

Los resultados sugieren un enlentecimiento de la tasa de la diversificación en el tiempo, un patrón considerado como señal de radiación adaptativa. Se detectaron varios cambios de tasa de diversificación en varios linajes (dentro de Akodontini por ejemplo, o en el linaje asociado con la región de la Puna). Los resultados sugieren una correlación entre rango altitudinal y tipo de vegetación y las tasas de diversificación. Por otra parte, los cambios en el tipo de molar parecen no haber influido en la tasa de especiación. La reconstrucción ancestral de hábitat y de morfología molar permitió considerar cómo fue la evolución de estos rasgos dentro de cada una de las tribus.

El levantamiento Andino y los cambios en vegetación habrían generado amplia “oportunidad ecológica” durante el Mioceno/Plioceno promoviendo la diversificación de Sigmodontinae. Las tierras bajas tropicales (regiones Amazónica, Caatinga, Cerrado Paraná) parecen haber sustentado altas tasas de especiación mientras que los Andes dieron origen a varios linajes que diversificaron al dispersar fuera de los Andes.

Finalmente, se hizo una revisión de aquellos mecanismos explicativos de patrones de riqueza de especies que serían útiles para entender el acúmulo de diversidad en los roedores

sigmodontinos. Para esto se consideró cómo el acceso a recursos en los roedores sigmodontinos puede haber influido en la proliferación -o falta de ésta- en algunos grupos dentro de la Subfamilia, contextualizando los resultados obtenidos previamente.

Explicaciones como la influencia del “determinismo local” o la “zona ecológica de origen” parecen marcos promisorios para entender la radiación de este grupo.

A futuro es deseable poder explorar el patrón de radiación accediendo a un muestreo más extenso, con representantes de la Subfamilia aún no incluidos en el análisis, e incluir más datos del ambiente ocupado y morfología que sirvan de proxy de nicho para entender cómo varía la ocupación de éste a lo largo del tiempo. El empleo de las estrategias empleadas en esta tesis y nuevos modelos, hará posible, por ejemplo, poner a prueba hipótesis en distintas escalas a la considerada aquí. Por ejemplo, la influencia que tuvieron distintos cambios en el paisaje en las tasas de diversificación [en distintas regiones] en los últimos 2 Ma, y corroborar bajo qué otras circunstancias hubo enlentecimientos en la tasa de diversificación asociados con la ocupación de ciertos ambientes. Estos y otros enfoques permitirán apreciar aún más los procesos ligados a la radiación sigmodontina y entender los patrones evolutivos presentes en Sudamérica.