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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 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.

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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). One of these cases is that of the subfamily Sigmodontinae (family Cricetidae); aspects that remain unclear include, the number of sigmodontine lineages that invaded South America and the timing of entrance into South America of this or these lineage/s. 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). Contro-

versies concerning the sigmodontine radiation have focused on the geographic placement of the basal diversification, and the timing that ancestral forms entered into South America (reviewed in D'Elía (2000) and 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, Steppan et al. (2004) considered as the most

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plausible scenario the dispersal of a single lineage into South America followed by an adaptive radiation.

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 sigmodontines of the tribe Phyllotini 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 (Steppan et al., 2004). This study, focused 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 Oryzomyia (defined as the clade containing the most recent common ancestor of the tribes Akodontini, Oryzomyini, Phyllotini, Thomasomyini, and Reithrodontini and all of its descendants, excluding Sigmodontini; cf. Steppan et al., 2004: 547). The origin of Sigmodontinae was estimated between 10.6 and 14.5 Ma and the origin of Oryzomyia 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'Elia, 2003b; D'Elia 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'Elia

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, which included the species here analyzed, have shown that these genera are monophyletic (e.g., D'Elia et al., 2003, 2005; D'Elia 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; Steppan 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 Steppan 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 four 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. Conver-

gence 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 Pardifias 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-HPC (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 was 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, 2011). 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). Seven broad distributional areas were used: Central America, North America, South America, Galapagos, Africa, Eurasia, and Oceania. Terminals present in more than one of the considered area were coded accordingly. 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. 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 two 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 for publication; 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 inferred from the IRBP solo matrix is similar to that gathered from 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 Oryzomyia (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, Serravalian 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 (gray line in Fig. 2); meanwhile the crown

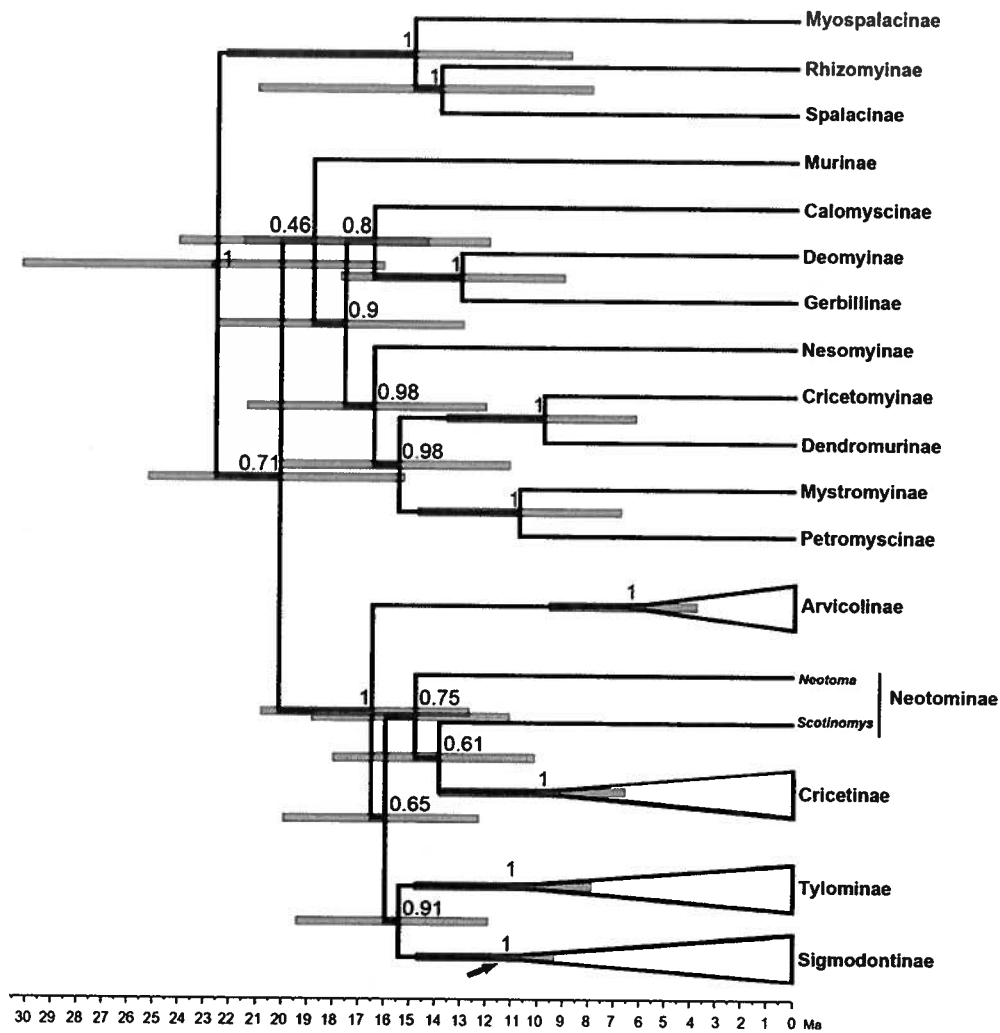


Fig. 1. Chronogram illustrating the diversification of Muroidea obtained from the Bayesian analysis of a matrix combining IRBP and cyt b gene sequences. Ages are represented as mean node height for a maximum clade credibility tree compiled from post-burnin trees topologies from 3 independent runs (9.0×10^7 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).

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 Fig. 3. 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 was recovered as the most probable ancestral re-

gion (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, Fig. 4.

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'Elia (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

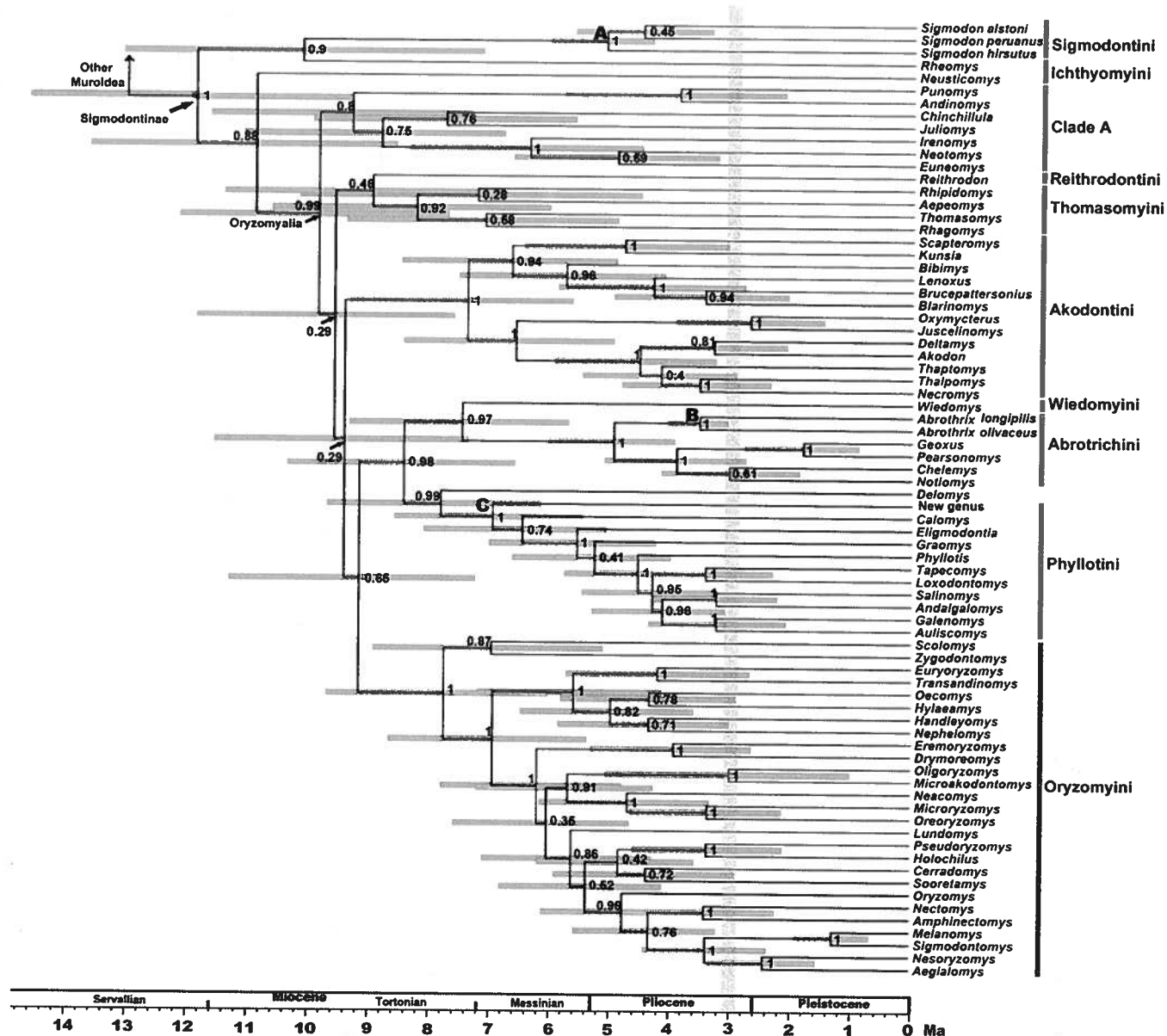


Fig. 2. Chronogram illustrating the diversification of Sigmodontinae obtained from the Bayesian analysis of a matrix combining IRBP and cyt b gene sequences. Ages are represented as mean node height for a maximum clade credibility tree compiled from post-burnin trees topologies from 3 independent runs (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.

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).

Lineage	IRBP/cyt b			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
Abrotrichini	4.92	5.40E-03	3.93–6.02	4.91	5.70E-03	3.66–6.33
Akodontini	7.36	1.30E-02	5.62–9.29	6.98	1.40E-02	4.91–9.34
Oryzomyia	9.81	1.60E-02	7.68–12.08	10.71	1.80E-02	8.10–13.67
Oryzomyini	7.72	1.30E-02	6.01–9.64	8.51	1.70E-02	6.18–11.00
Phyllotini	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

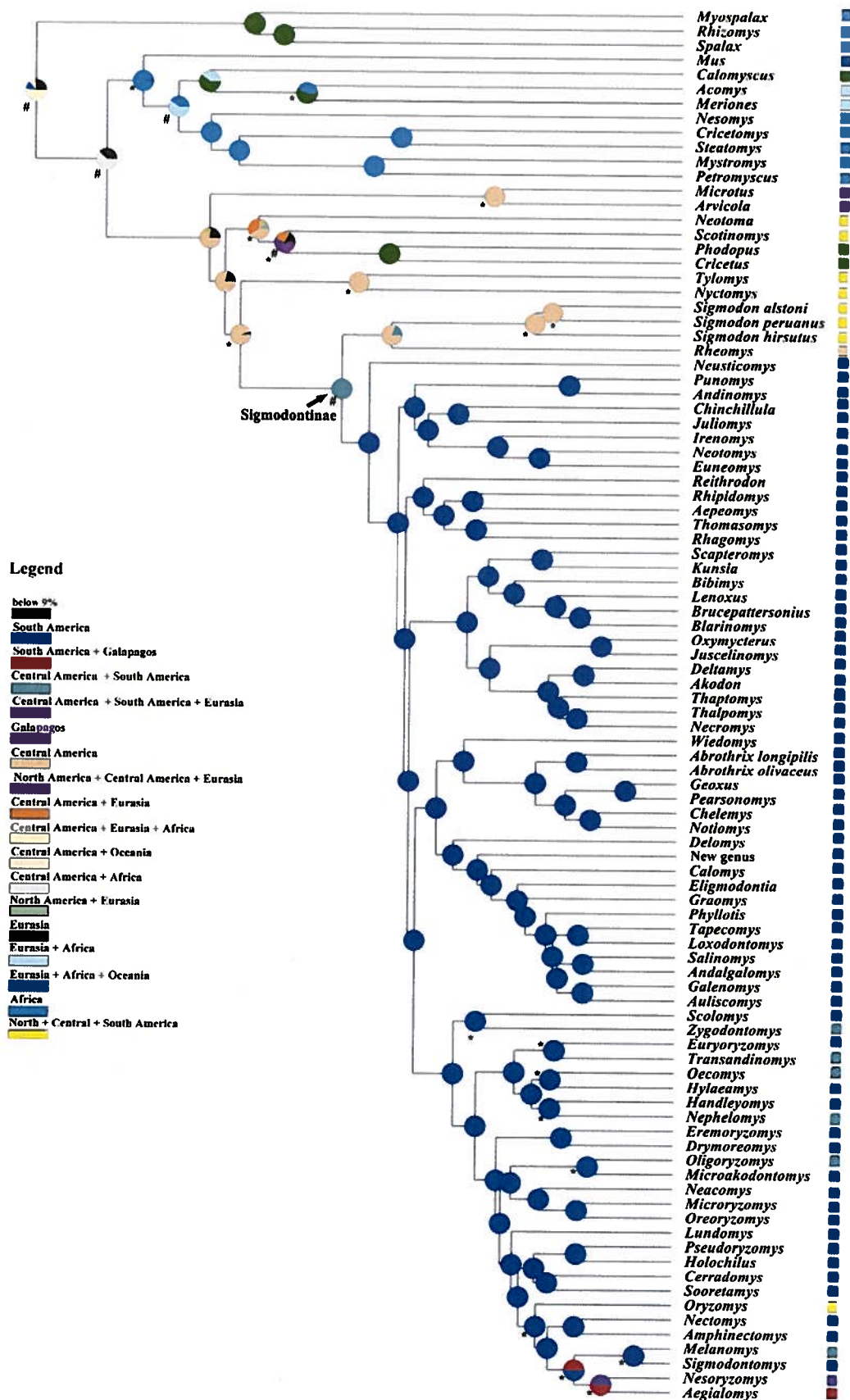


Fig. 3. Historical biogeographic analysis of Sigmodontinae. Ancestral distributions were reconstructed with Dispersal-Vicariance Analysis (S-DIVA) in RASP (Reconstruct Ancestral State in Phylogenies) using the maximum clade credibility tree recovered with BEAST for the combined dataset (keeping three "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.

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 Oryzomyia (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 Oryzomyia 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 3 Ma (Bartoli et al., 2005, see gray line in Fig. 2). 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. 3). Given that Central and South America were not connected (Kirby et al., 2008) at the time that of origin of the sigmodontine lineage, the precise geographic distribution of the sigmodontine ancestor as well as the number 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., Oryzomyia, 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 Oryzomyia 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. 3). 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. Paleogeographic 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 incursions 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 de "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 Blancas (Peláez-Campomanes and Martín, 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-Campanones and Martin, 2005), has not been properly assessed, and as such, the North American history of the sigmodontines may be much shorter than commonly assumed (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 originate 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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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2012.12.001>.

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