



## Short Communication

# Genetic diversity of the swamp rat in South America: Population expansion after transgressive-regressive marine events in the Late Quaternary

Fernando M. Quintela<sup>a,\*</sup>, Gislene L. Gonçalves<sup>b,c</sup>, Fabrício Bertuol<sup>d</sup>, Enrique M. González<sup>e</sup>, Thales R.O. Freitas<sup>a,b</sup>

<sup>a</sup> Programa de Pós-Graduação em Biologia Animal, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, Prédio 43435, CEP 91501-970, Porto Alegre, RS, Brazil

<sup>b</sup> Programa de Pós-Graduação em Genética e Biologia Molecular, Departamento de Genética, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, Prédio 43323, CEP 91501-970, Porto Alegre, RS, Brazil

<sup>c</sup> Instituto de Alta Investigación, Universidad de Tarapacá, Antofagasta 1520, Arica, Chile

<sup>d</sup> Programa de Pós-Graduação em Genética, Conservação e Biologia Evolutiva, Instituto Nacional de Pesquisas da Amazônia, Avenida André Araújo, 2936, CEP 69080-971, Manaus, AM, Brazil

<sup>e</sup> Museo Nacional de Historia Natural, 25 de mayo 582, CEP 11000, Montevideo, Uruguay

## ARTICLE INFO

## Article history:

Received 6 December 2014

Accepted 20 August 2015

Handled by Paul Grobler

Available online 29 August 2015

## Keywords:

Genetic differentiation

Geological evolution

Pampas

Phylogeography

*Scapteromys tumidus*

## ABSTRACT

We examined the phylogeography of the South American swamp rat *Scapteromys tumidus* using complete mitochondrial DNA cytochrome b sequences. This species is endemic to the Pampas biome and lives near the coastal plain but also in inland continental areas. The coastal domain of such region experienced a highly dynamic geological history. The inland fraction is part of Precambrian and Mesozoic spills and sedimentary basins, while most of the coastal plain was shaped by transgressive-regressive marine events in the Late Quaternary. As fluctuations in the sea level during this period produced large lateral displacements of the shoreline, originating four barrier-lagoon systems that shaped the present coastline, continental area significantly increased from this time. We hypothesized that the *S. tumidus* have expanded its range from that period, ramped by its adaptive ability to humid areas. Thus, we examined whether historical Pleistocene events had affected genetic variation of this species along its distributional range. Bayesian phylogenetic analysis and the haplotype network inferred two major genetic clusters along the distribution of *S. tumidus*. Neutrality tests suggest that populations experienced demographic changes and Bayesian skyline plot evidenced a marked recent demographic expansion pattern, intensified from 350 to 300 kya. Such date coincides with the formation of the lagunar barrier II (dated to ca. 325 kya) and the concurrent initial formation of the coastal plain and increase in continental area.

© 2015 Deutsche Gesellschaft für Säugetierkunde. Published by Elsevier GmbH. All rights reserved.

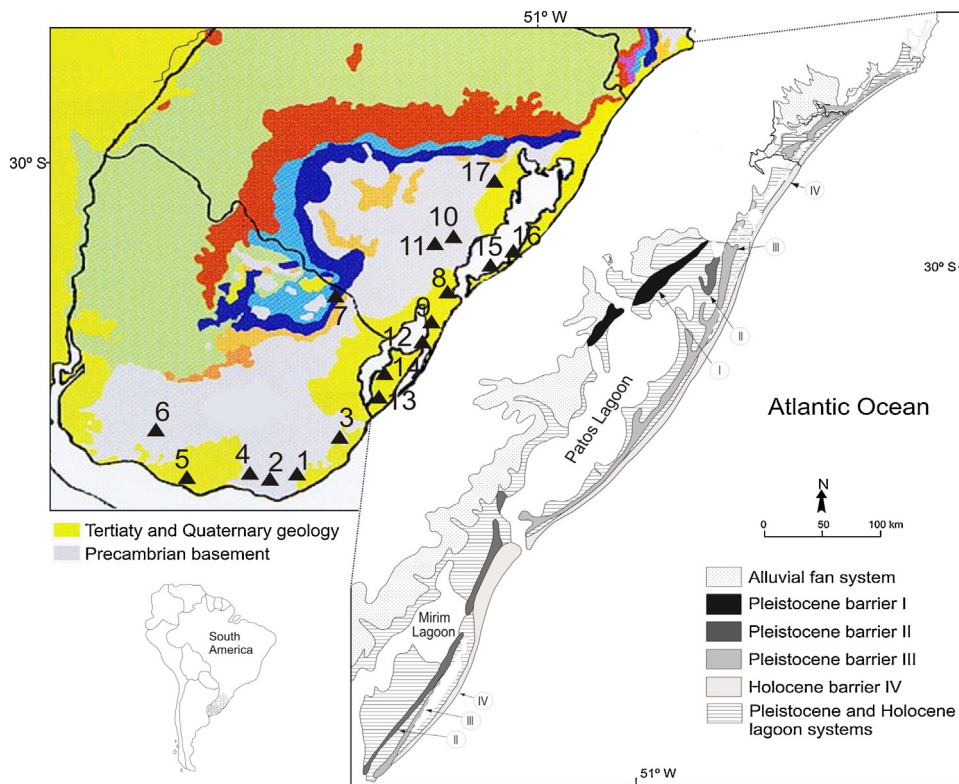
The influence of Quaternary climate and geological changes on the extant diversity of Neotropical species has been recurrently studied in South America organisms from a tropical systems perspective, in which a retraction into refuges is commonly proposed (Carnaval and Moritz, 2008; Carnaval et al., 2009; Haffer, 1969; Porto et al., 2013; Vanzolini and Williams, 1981). In contrast, open areas such as the Pampas overall present a distinct model in relation to tropical systems, such as an expansion due

to low temperature and dry conditions during the Pleistocene (see Turchetto-Zolet et al., 2013). Phylogeographic studies in this system, dealing with different organisms (e.g. rodents [Lopes et al., 2013; Mapelli et al., 2012; Montes et al., 2008; Mora et al., 2013; Palma et al., 2012], plants [Fregonezi et al., 2013; Longo et al., 2014; Mäder et al., 2013], lizards [Felappi et al., 2015] and Patagonian fauna and flora [Sérsic et al., 2011]), unravel contrasting patterns in regional and local scales.

Population genetic structure is determined not only by micro evolutionary processes (as genetic drift and natural selection), but also via its complex interaction with ecological parameters, including population size and migration (Allendorf and Luikart, 2012). Thus, explore genetic variation in species presenting conspicuous ecological traits, such as habitat preference, is suitable to infer

\* Corresponding author.

E-mail addresses: [fmquintela@yahoo.com.br](mailto:fmquintela@yahoo.com.br) (F.M. Quintela), [lopes.goncalves@ufrgs.br](mailto:lopes.goncalves@ufrgs.br) (G.L. Gonçalves), [fabricao.bertuol@gmail.com](mailto:fabricao.bertuol@gmail.com) (F. Bertuol), [emgonzalezuy@gmail.com](mailto:emgonzalezuy@gmail.com) (E.M. González), [thales.freitas@ufrgs.br](mailto:thales.freitas@ufrgs.br) (T.R.O. Freitas).



**Fig. 1.** Collecting localities of *Scapteromys tumidus*. Numbers correspond to sites showed in Table 1. Coastal plain is shown in detail to depict the four barrier stages.

demographic changes during glacial and interglacial cycles in the Neotropics.

The swamp rat *Scapteromys tumidus* (Waterhouse 1837) is endemic to the Pampas grassland biome (Overbeck et al., 2007), distributed in the southernmost Brazil (Rio Grande do Sul State [RS]) and entire Uruguay (D'Elía and Pardiñas, 2004; Freitas et al., 1984; Musser and Carleton, 2005). This species occupies habitats near wetland vegetation (wet meadows, reeds, *Typha* and *Eryngium* stands) (Barlow, 1969; González and Martínez-Lanfranco, 2010) and watercourses, such as swamps, ponds, small creeks, and large rivers (D'Elía and Pardiñas, 2004). *Scapteromys tumidus* is semi-aquatic, as it presents morphological traits to swim (Massoia and Fornes, 1964), as well as to climb trees, a behavior reported as an adaptation to live in flooded areas (Barlow, 1969; Sierra de Soriano, 1969). Currently, this species occupies both coastal and inland areas, along a wide range.

Demographic history and biogeography might have had an essential role in outline the genetic variation of populations (D'Elía and Pardiñas, 2004). The coastal domain of such region experienced a highly dynamic geological history. The inland fraction is part of Precambrian and Mesozoic spills and sedimentary basins, while most of the coastal plain was shaped by transgressive-regressive marine events in the Late Quaternary. As fluctuations in the sea level during this period produced large lateral displacements of the shoreline, originating four barrier–lagoon systems that shaped the present coastline (Fig. 1), continental area significantly increased from this time. We thus hypothesized that the swamp rat might have expanded its range by colonizing new available areas, increasing effective population size from the Late Quaternary. Long periods of population stability, or, alternatively, the recent colonization of new habitats have contrasting effects on the pattern of spatial apportionment of genetic variation at local and regional levels (Matocq et al., 2000; Slatkin, 1993; Wlasiuk et al., 2003). Thus, herein we characterize patterns of genetic variation in populations of *S. tumidus* across its distributional range in the Pampas biome, in

order to evaluate the phylogeographic structuring of populations. Based on complete mitochondrial cytochrome b sequence haplotypes we inferred past demographic changes and addressed to what extent geological and historical factors have shaped the current distribution of the swamp rat, and might have influenced intraspecific patterns of variations.

A total of 131 specimens of *S. tumidus* (114 field-collected from 2010 through 2012, and 17 incorporated from database), from 17 localities, covering the whole species distribution, were surveyed (Fig. 1 and Table 1). Genomic DNA was isolated from tissue samples (liver and/or muscle) using the CTAB method modified from Doyle and Doyle (1987). The complete *cyt b* (1146 bp) gene was amplified through Polymerase Chain Reaction (PCR) using primers MVZ05 and MVZ14, following conditions described by Smith and Patton (1993). PCR aliquots were checked on 1% agarose gel stained with GelRed (Biotium Inc., Hayward, CA, USA). The remaining products were purified with Exonuclease and Shrimp Alkaline Phosphatase (GIBCO-BRL Life Sciences/Invitrogen, Carlsbad, CA, USA), and sequenced at Macrogen, Inc., Seoul, Republic of Korea, with the same primers used in the PCR. Forward and reverse sequences were aligned and cross-checked to resolve ambiguities. Fifteen new haplotypes were deposited in GenBank under the accession numbers KP233846–60 (Table 1).

Multiple sequence alignments were performed using Codon Code Aligner (CodonCode Corp., USA), and inspected manually. Measurements of mtDNA diversity, including the mean number of pairwise differences (Nei, 1987), definitions of haplotypes, and haplotype diversity, and Neutrality tests (Tajima's *D*, Fu and Li's *D* and *F*, Fu's *FS*) were calculated in the program DNASP 5.0 (Librado and Rozas, 2009). A median joining haplotype network (Bandelt et al., 1999) was constructed in NETWORK 4.6 (<http://www.fluxus-engineering.com/sharenetwork.htm>). Levels of genetic structure among subpopulations was characterized using  $\phi_{ST}$ , which is analogous to Wright's *F*-statistics but takes into account the genetic distance among haplotypes using ARLEQUIN 3.5 (Excoffier and

**Table 1**  
Sample sites of *Scapteromys tumidus* presented in the map (Fig. 1), with locality information, latitude (Lat.) and longitude (Long.), number of individuals collected (N), specimens ID and Genbank Accession Number. Bold indicate data obtained from literature.

Map	Abbreviation	Locality <sup>a</sup>	Lat. (S)	Long. (W)	N	Sample ID <sup>b</sup>	Genbank Accession
<b>1</b>	<b>MLD</b>	<b>UY: Maldonado; Arroyo El Renegado</b>	34°47'00"	55°16'00"	<b>2</b>	<b>CA 682</b>	<b>AY445556</b>
		<b>UY: Maldonado; Las Flores</b>	–	–	<b>3</b>	<b>MNHN 3844</b> <b>MNHN 4287; MNHN 4288</b>	<b>AY445555</b> <b>AY445553;</b> <b>AY445552</b>
<b>2</b>	<b>SJS</b>	<b>UY: San Jose; Kiyu</b>	34°39'00"	56°45'00"	<b>1</b>	<b>MVZ 183268</b> <b>GD 326</b>	<b>AF108669</b> <b>AY445558</b>
3	CAS	UY: Rocha; Refugio de Fauna Laguna do Castillos	–	–	7	EMG 1957	KP233846
<b>4</b>	<b>PAL</b>	<b>UR: Rocha, La Paloma; Arroyo La Palma</b>	<b>34°35'18"</b>	<b>54°10'71"</b>	<b>5</b>	EMG 1998 EMG 1994, 1999, SVC 089, 099, 101	KP233856 KP233857
						<b>CA 628</b>	<b>AY445559</b>
5	CNL	UY: Canelones; Rincon Del Colorado	–	–	9	<b>MNHN 4266</b> <b>MNHN 4264</b> <b>MNHN 4263</b> <b>MNHN 4269</b>	<b>AY445560</b> <b>AY445561</b> <b>AY445562</b> <b>AY445563</b>
						EMG 1223, 1245, 1252, 1258 EMG 1236, 1238, 1243, 1257 EMG 1772 EMG 1762 EMG 1763–66, 1768, 1773, 1775, 1783	KP233846 KP233848 KP233853 KP233852 KP233853
<b>6</b>	FLO	UY: Flores; Rio São José	–	–	9	<b>GD 638</b>	<b>AY445564</b>
<b>7</b>	<b>RIV</b>	<b>UY: Rivera; Estancia La Quemada 2</b>	<b>32°01'83"</b>	<b>54°37'04"</b>	<b>3</b>	<b>GD 649</b> <b>GD 650</b> <b>GD 639</b> <b>GD 640</b>	<b>AY445569</b> <b>AY445570</b> <b>AY445565</b> <b>AY445566</b>
		<b>UY: Rivera; Estancia La Quemada 1</b>	<b>32°01'20"</b>	<b>54°34'22"</b>	<b>4</b>	<b>GD 643</b> <b>GD 644</b>	<b>AY445567</b> <b>AY445568</b>
8	APA	BR: Rio Grande do Sul, Rio Grande; APA Lagoa Verde	32°09'21"	52°11'19"	15	MCNU 3390; 3392–3402	KP233847
9	TAI	BR: Rio Grande do Sul, Rio Grande; Taim	32°33'26"	52°30'28"	1	MCNU 3391 TAI61	KP233846 KP233846
10	PEL	BR: Rio Grande do Sul, Capão do Leão; Horto BITL	31°48'00"	52°25'00"	8	PL01, 03	KP233847
11	POS	BR: Rio Grande do Sul, Pedro Osório	31°56'46"	52°45'37"	6	PL02, 06 MCNU 3369, PL 13, 47 PL 37	KP233846 KP233858 KP233859
						MCNU 2959, MCNU 2966	KP233846
12	MAN	BR: Rio Grande do Sul, St. Vitória do Palmar; Lagoa Mangueira	32°58'51"	52°43'34"	7	MCNU 3018, MCNU 3019, MCNU 3020 MCNU 3021	KP233847 KP233860
						MCNU 2917, 2948, 2965, 2969, 2973, 2975, 3017	KP233847
13	BOT	BR: Rio Grande do Sul, St. Vitória do Palmar; Botafogo	33°27'00"	53°16'05"	5	MCNU 3406, 3407, 3408, 3409, MCNU 3410	KP233848 KP233849
14	JOS	BR: Rio Grande do Sul, St. Vitória do Palmar; Josapar	33°08'50"	52°58'14"	17	MCNU 2747–57, 2762–65, 3404–5	KP233848
15	BUJ	BR: Rio Grande do Sul, São José do Norte; Bujuru	31°36'37"	51°23'34"	11	MCNU 3370–77; TRO 2103, 2104, 2105	KP233850
16	SJN	BR: Rio Grande do Sul, São José do Norte; 14 Km N	31°54'42"	51°56'08"	12	MCNU 3378, 3379, 3381–84, 3386–88, TRO 2100–02	KP233850
17	CAM	BR: Rio Grande do Sul, Camaquã	30°55'09"	51°53'45"	3	MCNU 2968, 2970–71	KP233851

<sup>a</sup> UY – Uruguay; BR – Brazil.

<sup>b</sup> CA – Biologia Animal, Universidad de la Republica, Montevideo; UY – MNHN, Museo Nacional de Historia Natural, Montevideo, UY; MVZ – Museum of Vertebrate Zoology, University of California, Berkeley; EMG – Enrique M. Gonzales, Museo Nacional de Historia Natural, Montevideo, UY; GD – Guillermo D'Elía, Universidad de la Republica, Montevideo, UY; MCNU – Museu de Ciências Naturais da Universidade Luterana do Brasil, BR; TAI – Fernando M. Quintela, Universidade Federal do Rio Grande do Sul, BR; PL – Patrícia Langone, Universidade Federal do Rio Grande do Sul; TRO – Thales Renato Ochotorena, Universidade Federal do Rio Grande do Sul.

**Table 2**

Summary of cytochrome b sequence variability found in *Scapteromys tumidus*, including the number of individuals (N), haplotypes (H), variable sites (S), haplotype diversity  $\pm$  standard error (Hd  $\pm$  SD), nucleotide diversity ( $\pi$ ) and Neutrality tests (Tajima's D, Fu and Li's D and F and Fu's FS). Analysis was also separated by haplogroups (GI and GII) previously defined in the Bayesian phylogenetic approach and the whole dataset. Neutrality tests were not significant ( $p > 0.05$ ).

	N	S	H	Hd $\pm$ SD	$\pi$	Neutrality test			
						Tajima's D	Fu and Li's		Fu's FS
							D	F	
Population									
MLD	4	3	3	0.833 $\pm$ 0.222	0.0023	1.082	1.082	0.971	0.013
SJS	1	–	–	–	–	–	–	–	–
CAS	7	4	4	0.821 $\pm$ 0.101	0.0015	0.582	0.563	0.621	–0.113
PAL	5	1	2	0.600 $\pm$ 0.175	0.0007	1.223	1.222	1.152	0.624
CNL	9	5	5	0.861 $\pm$ 0.087	0.0015	–0.272	–0.593	–0.571	–1.185
FLO	9	8	6	0.833 $\pm$ 0.127	0.0019	–1.021	–0.732	–0.892	–1.833
RIV	7	0	1	0.000 $\pm$ 0.000	0.0000	–	–	–	–
APA	15	4	4	0.371 $\pm$ 0.153	0.0005	–1.512	–1.473	–1.693	–1.282
TAI	1	–	–	–	–	–	–	–	–
PEL	8	4	4	0.821 $\pm$ 0.101	0.0001	–0.123	–0.172	–0.172	–0.423
POS	6	3	3	0.667 $\pm$ 0.160	0.0012	–0.754	–0.389	–0.507	–0.668
MAN	7	0	1	0.000 $\pm$ 0.000	0.0000	–	–	–	–
BOT	5	4	2	0.400 $\pm$ 0.237	0.0014	–1.093	–1.093	–1.113	–2.202
JOS	17	0	1	0.000 $\pm$ 0.000	0.0000	–	–	–	–
BUJ	11	0	1	0.000 $\pm$ 0.000	0.0000	–	–	–	–
SJN	12	0	1	0.000 $\pm$ 0.000	0.0000	–	–	–	–
CAM	3	0	1	0.000 $\pm$ 0.000	0.0000	–	–	–	–
Haplogroup									
GI	26	1	2	0.212 $\pm$ 0.097	0.0019	–0.310	0.612	0.414	0.162
GII	105	16	15	0.842 $\pm$ 0.018	0.0003	–0.609	–1.000	–1.022	–3.292
Total	131	17	22	0.869 $\pm$ 0.012	0.0056	–0.277	–0.277	–0.173	–0.457

Lischer, 2010). Bayesian phylogenetic analyses were performed using Markov chain Monte Carlo (MCMC) sampling as implemented in BEAST 2 (Bouckaert et al., 2014) using all haplotypes of *S. tumidus* recovered in this study. Individuals of *S. tumidus* from previous studies deposited in GenBank were also incorporated (Table 1), as well as *S. aquaticus* used to root the tree (GenBank Accession Number: AY275132). We employed the GTR model of nucleotide substitution, estimated in jModelTest (Posada, 2008). Uniform interval priors were assumed for all parameters except base composition, for which we assumed a Dirichlet prior. We performed four independent runs of 25 million generations each with a 5000-step thinning. All analyses were checked for convergence in Tracer 1.5 (Rambaut and Drummond, 2007) by plotting the log-likelihood values against generation time for each run, and the first five million generations were discarded as burn-in. All posterior parameter estimates had effective sample sizes (ESS) above 200 and the remaining trees were used to calculate posterior probabilities for each node. We estimated divergence times using a relaxed molecular clock framework that allows evolutionary rates to vary along the branches under an uncorrelated lognormal relaxed-clock model (Drummond et al., 2006), as implemented in BEAST. We used lognormal prior distributions to constrain the main node (A, Fig. 2A) with fossil calibration (Ho and Phillips, 2009). Prior information from the fossil record was incorporated based on the species *Scapteromys hershkovitzi*, a paleontological evidence of their clade membership and taxonomic status, with an age estimated in 2.2 mya (Quintana, 2002). Average genetic distances (Kimura 2-parameters) between major clades and among pairs of populations were calculated with MEGA 5.1 (Tamura et al., 2011).

Demographic history of the swamp rat was assessed using a Bayesian framework in the program BEAST 2, which incorporates the uncertainty in the genealogy through MCMC integration under a coalescent model (Drummond et al., 2005). Bayesian analysis was run for 50 million iterations and sampled every 5000 steps under a strict molecular clock with substitution rates equals to 0.006 ( $\pm 1.79e^{-5}$ ) per million years as normal priors (calculated in this study). The first 10% of the iterations were discarded to allow for

burn-in. The best-fit substitution model for the data was estimated in jModelTest (Posada, 2008). To assess the robustness of parameter estimates, 4 independent chains were run with identical settings. Log-files were analyzed in Tracer 1.5, and effective sample sizes were used to evaluate MCMC convergence within chains.

Mitochondrial sequence analysis resulted in 30 variable sites, which yield 17 haplotypes (Table 2). Bayesian inference (BI) revealed an internal division into three clades, but supports the major split into two groups, defined as Haplogroup I (GI) and Haplogroup II (GII) (Fig. 2A). Group I was formed by localities on the Rio Grande do Sul State (RS) central coastal plain (Bujuru and São José do Norte) and Camaquã, west of Patos Lagoon, in the Rio Grande do Sul State. Group II included two clades: (1) formed by haplotypes of Flores and Rivera, in south-central and southwestern Uruguay and (2) all remaining Uruguayan localities, southern RS coastal plain and Pelotas and Pedro Osório (RS), west of the São Gonçalo Channel and north of Mirim Lagoon. Haplotype network indicated that GI was separated from GII by five mutational events (Fig. 2B). Genetic divergence between haplogroups GI and GII was 1.2%. Strong genetic structure was found based on the  $\phi_{ST}$  values (Table 3), which ranged from 0 (BUJ vs. SJN) to 1 (several pairwise comparisons). Populations from the GI vs. those of GII presented pairwise  $\phi_{ST} > 0.39$ .

In the Bayesian time-calibrated tree, the most basal internal divergence in *S. tumidus* was observed between GI and the other two clades (Fig. 2A, Node A), estimated at ca. 1 mya (Table 4). Within this clade, the basal dichotomy was related to the divergence between the clade formed by H7 + H8 + H9 and all the remaining haplotypes (Fig. 2A, Node B), and was estimated at ca. 650 kya. Haplotypes of Group I showed a single shallow differentiation, estimated to have diversified ca. 200 kya. Within the third clade (Fig. 2A, Node C), the divergences between the haplotypes were estimated at ca. 330 and 170 kya. The third clade showed a basal dichotomy, estimated to begin diversification at ca. 420 kya, which resulted in two clades, formed by two and seven haplotypes respectively. The divergence times between the haplotypes within these clades were estimated at between 330 and 10 kya.

**Table 3**

Genetic structure among populations of *Scapteromys tumidus* based on pairwise  $\phi_{ST}$ . Bold indicates significant values ( $p < 0.05$ ). Shadow represent the Haplogroup I. Populations are listed in Table 1.

Pop.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.
1. MLD																
2. SJS	-0.95															
3. CAS	0.18	0.60														
4. PAL	0.00	0.01	<b>0.59</b>													
5. CNL	<b>0.22</b>	0.45	<b>0.44</b>	<b>0.62</b>												
6. FLO	<b>0.25</b>	0.39	<b>0.73</b>	<b>0.62</b>	<b>0.48</b>											
7. RIV	<b>0.21</b>	1.00	<b>0.88</b>	<b>0.58</b>	<b>0.72</b>	0.02										
8. APA	<b>0.37</b>	0.86	<b>0.80</b>	<b>0.72</b>	<b>0.61</b>	<b>0.81</b>	<b>0.96</b>									
9. TAI	-0.99	1.00	<b>0.30</b>	<b>0.23</b>	0.23	0.59	<b>1.00</b>	0.85								
10. PEL	<b>0.19</b>	0.29	<b>0.55</b>	<b>0.59</b>	<b>0.35</b>	<b>0.65</b>	0.82	<b>0.24</b>	0.14							
11. POS	<b>0.17</b>	0.68	<b>0.73</b>	<b>0.57</b>	<b>0.55</b>	<b>0.74</b>	<b>0.92</b>	<b>0.41</b>	0.68	0.01						
12. MAN	<b>0.17</b>	1.00	<b>0.79</b>	<b>0.57</b>	<b>0.58</b>	<b>0.78</b>	<b>1.00</b>	0.06	1.00	<b>0.2</b>	0.40					
13. BOT	0.07	1.00	<b>0.57</b>	<b>0.50</b>	0.01	0.53	<b>0.82</b>	<b>0.73</b>	0.20	<b>0.41</b>	<b>0.62</b>	<b>0.72</b>				
14. JOS	<b>0.41</b>	0.00	<b>0.87</b>	<b>0.74</b>	<b>0.39</b>	<b>0.77</b>	<b>1.00</b>	<b>0.93</b>	1.00	<b>0.75</b>	<b>0.90</b>	<b>1.00</b>	0.27			
15. BUJ	<b>0.39</b>	<b>1.00</b>	<b>0.95</b>	<b>0.68</b>	<b>0.92</b>	<b>0.91</b>	<b>1.00</b>	<b>0.98</b>	1.00	<b>0.93</b>	<b>0.97</b>	<b>1.00</b>	<b>0.95</b>	<b>1.00</b>		
16. SJN	<b>0.42</b>	<b>1.00</b>	<b>0.96</b>	<b>0.69</b>	<b>0.92</b>	<b>0.91</b>	<b>1.00</b>	<b>0.98</b>	1.00	<b>0.94</b>	<b>0.97</b>	<b>1.00</b>	<b>0.95</b>	<b>1.00</b>	0.00	
17. CAM	<b>0.41</b>	<b>1.00</b>	<b>0.91</b>	<b>0.41</b>	<b>0.85</b>	<b>0.83</b>	<b>1.00</b>	<b>0.97</b>	1.00	<b>0.87</b>	<b>0.94</b>	<b>1.00</b>	<b>0.88</b>	<b>1.00</b>	<b>1.00</b>	<b>1.00</b>

The haplotype network indicated a pattern of population expansion (Fig. 2B). Bayesian skyline plot analysis showed a long period of demographic stability that extends from 1.1 mya to 650 kya, followed by an event of recent expansion (Fig. 3). This expansion intensified from 350 to 300 kya, coinciding with the formation of the lagunar barrier II, dated to ca. 325 kya (Tomazelli and Villwock, 1996, 2000). The lagunar barrier II was the first depositional system that shaped the southern and central RS coastal plain, during a maximum marine transgression in the Middle Pleistocene (Tomazelli and Villwock, 1996, 2000). The formation of the coastal plain was followed by depositional events that formed barriers III (Pleistocene) and IV (Holocene) and Holocene marine-lagunar-alluvial sediments (Vieira, 1984; Tomazelli and Villwock, 1996, 2000). Thus, it is possible that the demographic expansion detected in *S. tumidus* could be related to the increase in continental area resulting mainly from the last three depositional events (barriers II, III and IV; Tomazelli and Villwock, 1996, 2000). *Scapteromys tumidus* is commonly trapped in pioneer wetland vegetation (wet meadows, reeds, *Typha* and *Eryngium* stands) (Barlow, 1969; González and Martínez-Lanfranco, 2010; present study) and

**Table 4**

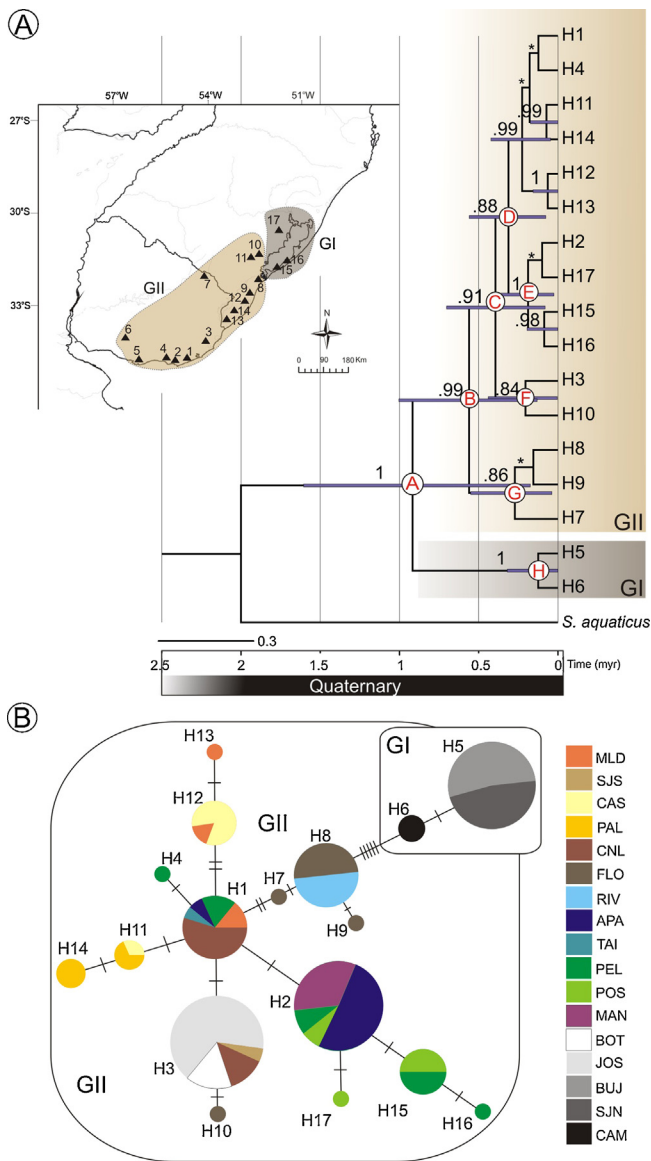
Divergence time estimates in populations of *Scapteromys tumidus*. Mean time to the most recent common ancestor (tMRCA) and 95% credibility intervals (95% CI) in millions of years, calculated using a relaxed molecular clock on complete mitochondrial DNA sequences. Node letters correspond to those in Bayesian tree depicted in Fig. 2A.

Node	Divergence time	
	Mean	95% HPD
A	1.0	1.76–0.003
B	0.65	1.16–0.0023
C	0.42	0.78–0.0013
D	0.33	0.60–0.001
E	0.19	0.47–0.001
F	0.17	0.45–0.001
G	0.33	0.66–0.0012
H	0.20	0.53–0.0001

seems not to require complexly structured habitats for its occurrence. This ecological feature may have benefited the species' demographic expansion over the pioneer formations in each sedimentary barrier. Historical demographical expansion associated with the Pleistocene-Holocene climate and sea-level oscillations and sedimentary deposition in the subtropical Atlantic coastal zone were found for the ctenomyid rodent *C. talarum* (Mora et al., 2013) and the solanacean plant *C. heterophylla* (Mäder et al., 2013). On the other hand, a pattern of recent population retraction was found in the ctenomyid *C. minutus* (Lopes et al., 2013), which occurs in sympatry with *S. tumidus* on the RS central coastal plain, reinforcing that particular life history traits play a central role in shape patterns observed in different organisms, even within the same taxonomic group (such as rodents), which highlights the significance of descriptive studies on genetic variation.

Of the 17 identified haplotypes, 10 occurred in Uruguayan territory, and eight were exclusive to Uruguayan localities. Samples from southwestern Precambrian Uruguay in Flores Department (Bossi and Navarro, 1988) showed four haplotypes, the highest local diversity observed. Patterns of populations originating inland that expanded to the Quaternary subtropical South American coastal plains were found for the akodontine *D. kempii* (Montes et al., 2008), the ctenomyid *Ctenomys talarum* (Mora et al., 2013) and the solanacean plant *Calibrachoa heterophylla* (Mäder et al., 2013), which might be also the case for the swamp rat.

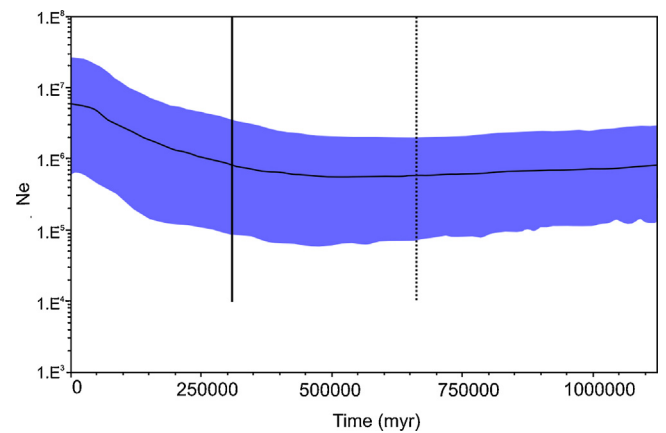
The analysis of *S. tumidus* cyt *b* haplotypes showed an internal structure, where two major supported groups were identified. The genetic divergences between GI and GII (1.2%) were similar to the distances found between haplogroups within the sympatric *Deltamys kempii* (Montes et al., 2008) and lower than distances found for the akodontine species *Akodon montensis* (Valdez and D'Elía, 2013), *Blarinomys breviceps* (Ventura et al., 2012) and *Thaptomys nigrata* (Ventura et al., 2010). D'Elía and Pardiñas (2004) found a range of distance from 3.6% to 4.7% between populations assigned to *S. aquaticus* and *S. tumidus*, while the highest intraspecific divergence was 1%. In relation to *S. meridionalis*, Quintela et al. (2014) found ca. 5% of mean divergence to both *S. tumidus* and *S. aquaticus*,



**Fig. 2.** Phylogenetic analysis of *Scapteromys tumidus* haplotypes. (A) Bayesian consensus time-tree based on 17 haplotypes of the complete cytochrome *b* gene. Posterior probabilities are indicated above each branch. Groups I and II represent haplogroups identified in Bayesian phylogenetic analysis. Internal nodes are shown and ages are detailed in Table 4. Bars represent 95% highest posterior density (HPD) node age estimate. (B) Haplotype network reconstructed based on median-joining analysis. Colors represent populations listed in Table 1. Circle sizes are proportional to the frequency of occurrence of the respective haplotype. Small bars crossing branches represent substitution events. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

and 2.8% of intraspecific variation. This comparative assessment suggests that the divergence between haplogroups in our study is closer to an intraspecific pattern rather than an interspecific divergence level previously determined for *Scapteromys* (D’Elia and Pardiñas, 2004; Quintela et al., 2014). Because any external morphological character and/or craniodental pattern were found fixed among specimens of the haplogroups we are cautious to consider GI and GII as separated biological entities and suggest a currently ongoing diversification process of *S. tumidus* lineages. Although the differences accumulated so far are not enough to represent distinct species yet but rather may be considered distinct subspecies of *S. tumidus*.

Haplogroup I, found on the border of the RS Precambrian Shield and the RS Quaternary central coastal plain, consisted of a deep



**Fig. 3.** Bayesian skyline plot showing the complete reconstruction of the female effective population size fluctuations through time in *Scapteromys tumidus* in the Quaternary. Solid curve represents the mean estimates and shaded area indicates the 95% highest posterior density (HPD) limits. Past time (in years) is indicated in the horizontal axis and effective population size in the vertical axis. Solid and dotted lines are the lower and the median, respectively, estimated tMRCA, projected on the time line.

divergent unit. It is remarkable that this lineage is currently distributed over a recently formed geological region of the species’ distribution, the RS central coastal plain. This region consists of sedimentary deposits formed during the Pleistocene-Holocene maximum marine transgressions, estimated to have occurred since 120 kya (Tomazelli and Villwock, 1996, 2000). We estimated the divergence time for the *S. tumidus* Group I lineage of the node with the common ancestral at 1 myr but the origin of this group is likely more recent (<500 kya). A dispersive lineage of *S. tumidus* was recovered in the derived haplotypes H5 and H6, with the former restricted to Camaquã, on the border of the RS Precambrian Shield, and the latter to the RS central coastal plain, Pleistocene Barrier III, estimated to have diverged around 200 kya, probably at the eastern Sul-rio-grandense Shield (Pelotas Batholith; Philipp et al., 2000). From this region, the species dispersed farther north along the Pelotas Batholith and the Paleogene alluvial fan system (Tomazelli and Villwock, 2000). The colonization eastward and to the geomorphological unit of the RS central sedimentary coastal plain (also known as São José do Norte restinga; Vieira, 1984) occurred successively to Pleistocene-Holocene marine transgression-regression events. Contrary to expectations, populations from the central (Haplogroup I) and southern (Haplogroup II) segments of the RS coastal plain, separated by only ca. 20 km (which includes the Patos Lagoon estuary mouth), do not share haplotypes. Considering the large sample size for both segments, it is possible that there was no historical gene flow between the populations separated by the Patos Lagoon estuary, and this might represent a geographic barrier to gene flow. This phylogeographic break pattern observed in *S. tumidus*, therefore, was apparently shaped by the evolution of the Quaternary RS coastal plain and by the species’ dispersal capacity and its limitations in crossing the Patos Lagoon estuary. Large water bodies have also influenced genetic divergence in other rodents, such as echimyids (Silva and Patton, 1993), ctenomyids (Mora et al., 2013), murids (Nicolas et al., 2012) and sigmodontines (Costa, 2003; Costa et al., 2000; Ventura et al., 2012). In addition, karyotype differences of *S. tumidus* were recently observed in individuals from north of the estuary mouth (I. Haas, personal communication). Thus, a recent process of differentiation into two lineages might be occurring in the swamp rat of the Pampas, similar to findings in the *Araucaria angustifolia* Forest in Southern Brazil for *S. meridionalis* (2n=34-36) (Quintela et al., 2014). In this case, besides the phylogeographic barrier (estuary mouth), a karyotypic isolation could be underlying the restrict gene flow between

major haplogroups. A detailed karyotype analysis of representative populations from the haplogroups GI and GII remains to be investigated.

## Acknowledgements

We are grateful to Alexandre U. Christoff and Eduardo Coelho, Museu de Ciências Naturais, Universidade Luterana do Brasil, Robert S. Voss and Eileen Westwig, American Museum of Natural History, for allow us access to the *Scapteromys* material. We are also thankful to Graziela Iob (UFRGS) for helping in the fieldwork and Patricia Langone (UFRGS) for providing tissue samples. Janet W. Reid edited the text. This research was financially supported by the Coordenadoria de Aperfeiçoamento Pessoal (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS).

## References

- Allendorf, F.W., Luikart, G.H., 2012. *Conservation and the Genetics of Populations*, 2nd ed. Wiley-Blackwell, 624 pp.
- Bandelt, H.J., Foster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* 16, 37–48.
- Barlow, J.C., 1969. Observations on the biology of rodents in Uruguay. *Life Sci. Contrib. R. Ont. Mus.* 75, 1–59.
- Bossi, J., Navarro, R., 1988. *Geología del Uruguay*. Universidad de la República, Montevideo.
- Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M.A., Rambaut, A., Drummond, A.J., 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* 10 (4), e1003537.
- Carnaval, A.C.O.Q., Hickerson, M.J., Haddad, C.F.B., Rodrigues, M.T., Moritz, C., 2009. Stability predicts genetic diversity in the Brazilian Atlantic Forest hotspot. *Science* 323, 785–789.
- Carnaval, A.C., Moritz, C., 2008. Historical climate modelling predicts patterns of current biodiversity in the Brazilian Atlantic forest. *J. Biogeogr.* 35, 1187–1201.
- Costa, L.P., 2003. The historical bridge between the Amazon and the Atlantic Forest of Brazil: a study of molecular phylogeography with small mammals. *J. Biogeogr.* 30, 71–86.
- Costa, L.P., Leite, Y.L., Fonseca, G.A., Fonseca, M.T., 2000. Biogeography of South American forest mammals: endemism and diversity in the Atlantic Forest. *Biotropica* 32, 872–881.
- D'Elia, G., Pardiñas, U.F.J., 2004. Systematics of Argentinean, Paraguayan, and Uruguayan swamp rats of the genus *Scapteromys* (Rodentia, Cricetidae, Sigmodontinae). *J. Mammal.* 85, 897–910.
- Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19, 11–15.
- Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biol.* 4, e88.
- Drummond, A.J., Rambaut, A., Shapiro, B., Pybus, O.G., 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. *Mol. Biol. Evol.* 22, 1185–1192.
- Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analysis under Linux and Windows. *Mol. Ecol. Resour.* 10, 564–567.
- Felappi, J.F., Vieira, R.C., Fagundes, N.J.R., Verrastro, L.V., 2015. So far away, yet so close: strong genetic structure in *Homonota uruguayensis* (Squamata, Phyllodactylidae), a species with restricted geographic distribution in the Brazilian and Uruguayan Pampas. *PLOS ONE* 10 (2), e0118162.
- Fregonezi, J.N., Turchetto, C., Bonatto, S.L., Freitas, L.B., 2013. Biogeographical history and diversification of *Petunia* and *Calibrachoa* (Solanaceae) in the Neotropical Pampas grassland. *Bot. J. Linn. Soc.* 171, 140–153.
- Freitas, T.R.O., Mattevi, M., Oliveira, L.F.B., 1984. Unusual C-band in three karyotypically rearranged forms of *Scapteromys* (Rodentia, Cricetidae) from Brazil. *Cytogenet. Cell Genet.* 38, 39–44.
- González, E.M., Martínez-Lanfranco, J.A., 2010. *Mamíferos de Uruguay. Guía de campo e introducción a su estudio y conservación*. Banda Oriental, Museo Nacional de Historia Natural, Vida Silvestre, Montevideo.
- Haffer, J., 1969. Speciation in Amazonian forest birds. *Science* 165, 131–137.
- Ho, S.Y.W., Phillips, M.J., 2009. Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. *Syst. Biol.* 58, 367–380.
- Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25, 1451–1452.
- Longo, D., Lorenz-Lemke, A.P., Mäder, G., Bonatto, S.L., Freitas, L.B., 2014. The phylogeography of the *Petunia integrifolia* complex in southern Brazil. *Bot. J. Linn. Soc.* 174, 199–213.
- Lopes, C.M., Ximenes, S.S.F., Gava, A., Freitas, T.R.O., 2013. The role of chromosomal rearrangements and geographical barriers in the divergence of lineages in a South American subterranean rodent (Rodentia: Ctenomyidae: *Ctenomys minutus*). *Heredity* 111, 293–305.
- Mäder, G., Fregonezi, J.N., Lorenz-Lemke, A.P., Bonatto, S.L., Freitas, L.B., 2013. Geological and climatic changes in Quaternary shaped the evolutionary history of *Calibrachoa heterophylla*, an endemic South-Atlantic species of petunia. *BMC Evol. Biol.* 13, 178.
- Mapelli, F.J., Mora, M.S., Mirol, P.M., Kittlein, M.J., 2012. Population structure and landscape genetics in the endangered subterranean rodent *Ctenomys porteus*. *Conserv. Genet.* 13, 165–181.
- Massoia, E., Fornes, A., 1964. Notas sobre el genero *Scapteromys* (Rodentia-Cricetidae). I. Sistemática, distribución geográfica y rasgos etoecológicos de *Scapteromys tumidus* (Waterhouse). *Physis* 24, 279–297.
- Matocq, M.D., Patton, J.L., da Silva, M.N.F., 2000. Population genetic structure of two ecologically distinct Amazonian spiny rats: separating history and current ecology. *Evolution* 54, 1423–1432.
- Montes, M.A., Oliveira, L.F.B., Bonatto, S.L., Callegari-Jacques, S., Mattevi, M.S., 2008. DNA sequence analysis and the phylogeographical history of the rodent *Deltamys kempi* (Sigmodontinae, Cricetidae) on the Atlantic Coastal Plain of south of Brazil. *J. Evol. Biol.* 21, 1823–1835.
- Mora, M.S., Cutrera, A.P., Lessa, E.P., Vassallo, A.L., D'Anatro, A., Mapelli, F.J., 2013. Phylogeography and population genetic structure of the Talas tuco-tuco (*Ctenomys talarum*): integrating demographic and habitat histories. *J. Mammal.* 94, 459–476.
- Musser, G.G., Cleton, M.D., 2005. Superfamily Muroidea. In: Wilson, D.E., Reeder, D.M. (Eds.), *Mammal Species of the World: A Taxonomic and Geographic Reference*. The Johns Hopkins University Press, Baltimore, pp. 894–1531.
- Nei, M., 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Nicolas, V., Missou, P., Colyn, M., Cruaud, C., Denys, C., 2012. West-Central African Pleistocene lowland forest evolution revealed by the phylogeography of *Misonne's* soft-furred mouse. *Afr. Zool.* 47, 100–112.
- Overbeck, G.E., Müller, S.C., Fedelis, A., Pfadenhauer, J., Pillar, V.D., Blanco, C.C., Boldrini, I.L., Both, R., Forneck, E.D., 2007. Brazil's neglected biome: the South Brazilian Campos. *Perspect. Plant Ecol. Evol. Syst.* 9, 101–116.
- Palma, R.E., Boric-Bargetto, D., Torres-Pérez, F., Hernández, C.E., Yates, T.L., 2012. Glaciation effects on the phylogeographic structure of *Oligoryzomys longicaudatus* (Rodentia: Sigmodontinae) in the Southern Andes. *PLoS ONE* 7, e32206.
- Philipp, R.P., Nardi, L.V.S., Bitencourt, M.F., 2000. O Batólito Pelotas no Rio Grande do Sul. In: Holz, M., De Ros, L.F. (Eds.), *Geologia do Rio Grande do Sul*. CIGO/UFRGS, Porto Alegre, pp. 133–160.
- Porto, T.J., Carnaval, A.C., da Rocha, P.L.B., 2013. Evaluating Forest refuges models using species distribution models, model filling and inclusion: a case study with 14 Brazilian species. *Divers. Distrib.* 19, 330–340.
- Posada, D., 2008. jModelTest: phylogenetic model averaging. *Mol. Biol. Evol.* 25, 1253–1256.
- Quintana, C.A., 2002. Roedores cricétidos del Sanandresense (Plioceno tardío) de la provincia de Buenos Aires Argentina. *Mastozool. Neotrop.* 9, 263–275.
- Quintela, F.M., Gonçalves, G.L., Althoff, S.L., Sbalqueiro, I.J., Oliveira, L.F.B., Freitas, T.R.O., 2014. A new species of swamp rat of the genus *Scapteromys* Waterhouse, 1837 (Rodentia: Sigmodontinae) endemic to *Araucaria angustifolia* Forest in Southern Brazil. *Zootaxa* 3811, 207–225.
- Rambaut, A., Drummond, A.J., 2007. <http://beast.bio.ed.ac.uk/Tracer> (accessed 03.05.13).
- Sérsic, A.N., Cosacov, A., Cocucci, A.A., Johnson, L.A., Pozner, R., Avila, L.J., Sites Jr., J.W., Morando, M., 2011. Emerging phylogeographical patterns of plants and terrestrial vertebrates from Patagonia. *Biol. J. Linn. Soc.* 103, 475–494.
- Sierra de Soriano, B., 1969. Algunos caracteres externos de cricetinos y su relación con el grado de adaptación a la vida acuática (Rodentia). *Physis* 28, 471–486.
- Silva, M.N.F., Patton, J.L., 1993. Amazonian phylogeography: mtDNA sequence variation in arboreal echimyid rodents (Caviomorpha). *Mol. Phylogenet. Evol.* 2, 243–255.
- Slatkin, M., 1993. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* 47, 264–279.
- Smith, M.F., Patton, J.L., 1993. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. *Biol. J. Linn. Soc.* 50, 149–177.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28, 2731–2739.
- Tomazelli, L.J., Villwock, J.A., 1996. Quaternary geological evolution of Rio Grande do Sul Coastal Plain, southern Brazil. *An. Acad. Bras. Ciênc.* 68, 373–382.
- Tomazelli, L.J., Villwock, J.A., 2000. O Cenozóico no Rio Grande do Sul: geologia da Planície Costeira. In: Holz, M., De Ros, L.F. (Eds.), *Geologia do Rio Grande do Sul*. CIGO/UFRGS, Porto Alegre, pp. 375–406.
- Turchetto-Zolet, A.C., Pinheiro, F., Salgueiro, F., Palma-Silva, C., 2013. Phylogeographical patterns shed light on evolutionary process in South America. *Mol. Ecol.* 22, 1193–1213.
- Valdez, L., D'Elia, G., 2013. Differentiation in the Atlantic Forest: phylogeography of *Akodon montensis* (Rodentia, Sigmodontinae) and the Carnaval-Moritz model of Pleistocene refugia. *J. Mammal.* 94, 911–922.
- Vanzolini, P.E., Williams, E.E., 1981. The vanishing refuge: a mechanism for ecogeographical speciation. *Papéis Avulsos Zool.* 34, 251–255.
- Ventura, K., Sato-Kuwabara, Y., Fagundes, V., Geise, L., Leite, Y.L.R., Costa, L.P., Silva, M.J.J., Yonene-ga-Yassuda, Y., Rodrigues, M.T., 2012. Phylogeographic structure

- and karyotypic diversity of the Brazilian Shrew Mouse (*Blarinomys breviceps*, Sigmodontinae) in the Atlantic Forest. *Cytogenet. Genome Res.* 138, 19–30.
- Ventura, K., Silva, M.J.J., Yonenaga-Yassuda, Y., 2010. *Thaptomys* Thomas 1915 (Rodentia, Sigmodontini, Akodontini) with karyotypes  $2n = 50$ ,  $FN = 48$ , and  $2n = 52$ ,  $FN = 52$ : two monophyletic lineages recovered by molecular phylogeny. *Genet. Mol. Biol.* 33, 256–261.
- Vieira, E.F., 1984. *Rio Grande do Sul: Geografia Física e Vegetação*. Sagra, Porto Alegre.
- Wlasiuk, G., Garza, J.C., Lessa, E.P., 2003. Genetic and geographic differentiation in the Rio Negro tuco-tuco (*Ctenomys rionegrensis*) inferring the roles of migration and drift from multiple genetic markers. *Evolution* 57, 913–926.