FERNANDO MARQUES QUINTELA

ASPECTOS FILOGEOGRÁFICOS E TAXONÔMICOS DE *DELTAMYS* E *SCAPTEROMYS* (CRICETIDAE: SIGMODONTINAE)

Tese apresentada ao Programa de Pós-Graduação em Biologia Animal, Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do título de Doutor em Biologia Animal.

Area de concentração: Zoologia

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ASPECTOS FILOGEOGRÁFICOS E TAXONÔMICOS DE *DELTAMYS* E *SCAPTEROMYS* (CRICETIDAE: SIGMODONTINAE)

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"Only when the last tree has been felt

The last fish caught

The last river poisoned

Will you know

That men cannot eat money”

Música: Uaschitschun

Album: Port Royal

Artista: Running Wild

Gênero: Power Metal

Ano: 1988

Aos meus pais, Alírio e Nadir.

À minha esposa, Josiane Alves.

À Chyntia Ibarra (in memorian).

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SUMÁRIO

LISTA DE TABELAS

LISTA DE FIGURAS

Capítulo II

Figure 9. Occlusal views of upper and lower molar series of *Scapteromys* sp. n. holotype (FURB 20253). Bar = 1 mm.

Figure 10. Biogeographic regions and provinces in South America (A), with details of the study area (in dark blue) and sampling sites of *Scapteromys* sp. n. [For details see Material and Methods] (B); and *Araucaria angustifolia* Forest habitat (C). Sources: A, adapted from Morrone (2006); C, Photography of *Araucaria angustifolia* Forest: G.L. Gonçalves.

Capítulo III

Fig. 1.— Collecting localities of *Scapteromys tumidus*, indicating samples utilized in both molecular and geometric analysis (black circles), molecular analysis only (white circles) and geometric morphometrics only (gray circles). Numbers correspond to localities of haplotypes showed in Table 1. Other localities are listed in Appendix. Ellipses and abbreviations indicate the geographic clusters defined by skull geometric morphometric analysis (see Material and methods). Coastal plain is shown in detail to depict the four barrier stages.

Fig. 2.— Landmark locations on dorsal (A), ventral (b) and lateral (C) views of skull of *Scapteromys tumidus*. Detailed description of the location of landmarks is given in Appendix II.

Fig. 3.— Scatter plot of first two axes of Canonical Variate Analysis (CVA) for *Scapteromys tumidus* skull shape configurations of samples from eight geographic clusters in Uruguay and southern Brazil and for dorsal (A), ventral (B) and lateral (C) views. CV1 represent skull shape variation on the first axis and CV2 represent skull shape variation on the second axis. Positive scores in solid lines and negative scores in dotted lines on the shape difference representations.

Fig. 4.— Neighbor-joining phenograms generated from Mahalanobis distances for dorsal (A), ventral (B) and lateral (C) views of the skull of *Scapteromys tumidus*. Acronyms correspond to geographic clusters.

Fig. 5.— Intraspecific analysis of *Scapteromys tumidus*. Bayesian phylogenetic tree based on 14 haplotypes of the cytochrome *b* gene. Posterior probabilities and estimated time of divergence are indicated above each branch. Groups I, II and III represent major haplogroups identified. Internal nodes A, B and C are discussed in the main text.

Fig. 6.— Haplotype network reconstructed based on median-joining analysis. Colors (and symbols in B&W version) represent sampling localities listed in the inserted legend. Circle sizes are proportional to the frequency of occurrence of the respective haplotype. Small bars crossing branches represent substitution events. Groups I, II and II represent major haplogroups identified in Bayesian phylogenetic analysis.

Fig. 7 .— 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. Panel above: solid and dotted lines are the lower and the median, respectively,

estimated tMRCA, projected on the time line. Panel below indicates the last 400.000 years in detail; dashed lines indicate each of the four marine transgressive-regressive events in the Coastal Plain of Rio Grande do Sul, Southern Brazil (see Figure1 for details).

Capítulo IV

Fig.1. Hypsometry of South America (left), with detail on the study area (right). Narrow indicates the possible route of *Deltamys* basal dispersion, discussed in the main text.

Fig. 2. Collecting localities of *Deltamys kempi* (red circles) and the new lineage of *Deltamys* from Meridional Plateau (blue circle). Numbers correspond to localities showed in Table 1.

Fig. 3. Bayesian phylogeny tree based on 43 cytochrome *b* haplotypes of *Deltamys* and one haplotype of each Akodontini genus. Numbers indicate the mean estimated time of divergence of the main nodes. Blue bars indicate the range of the estimated time.

Fig. 4. Median-joining network reconstructed based on 37 haplotypes of *Deltamys kempi*. Colors represent sampling localities listed in the inserted legend. Circle sizes are proportional to the frequency of occurrence of the respective haplotype. Small bars crossing branches represent substitution events. Clades A and B represent major haplogroups identified in Bayesian phylogenetic analysis.

Fig. 5. Scores of *Deltamys kempi* specimens from clades A (crosses) and B (circles) on the principal components 1 and 2 (above) and 1 and 3 (below) extracted from the variance-covariance matrix of 21 cranial measurements.

RESUMO

A tribo Akodontini corresponde a segunda maior tribo de roedores sigmodontíneos, compreendendo 13 gêneros e mais de 100 espécies reconhecidas. Os gêneros *Deltamys* e *Scapteromys* abrigam akodontes distribuídos em áreas úmidas do Brasil meridional, Uruguai e nordeste da Argentina. Este estudo objetivou investigar a variabilidade genética e morfológica, padrões filogeográficos e eventos demográficos históricos em *Scapteromys* no Brasil e Uruguai e *Deltamys* em toda a sua distribuição. Análises de seqüências do gene mitocondrial citocromo b (cit *b*) revelaram que os cariomorfos 2n=34 e 2n=36 de *Scapteromys* procedentes do Planalto Meridional correspondem a uma única espécie, nova e aqui descrita, internamente subestruturada. Essa estruturação, no entanto, não corresponde aos cariótipos. *Scapteromys meridionalis* sp.n. possui pelagem comparativamente escurecida e menores valores na maioria das medidas externas e craniométricas. Também é distinguível de *S. tumidus* pela forma do processo hamular do pterigóide e morfologia plantar do *manus*. A análise filogeográfica de *S. tumidus* revelou a estruturação deste táxon em três haplogrupos, sendo um destes, ocorrente no extremo norte da distribuição da espécie e porção central da planície costeira do Rio Grande do Sul (RS), marcadamente divergente em seqüências de cit *b* e na forma do crânio. Foi observado um evento de expansão demográfica temporalmente coincidente com o início da formação da planície costeira do RS. *Deltamys kempi* apresentou um padrão filogeográfico bem estruturado, caracterizado por diversas diferenciações locais e poucos haplótipos compartilhados entre localidades. Assim como em estudos precedentes, foram identificados dois clados mitocondriais, apoiados por diferenças craniométricas. Nossos resultados indicam que a quebra filogeográfica observada tanto em *D. kempi* quanto em *S. tumidus* pode estar relacionada à evolução geológica da planície costeira do RS e conseqüente formação do canal do estuário da Lagoa dos Patos, que

pode representar uma barreira geográfica para o fluxo gênico histórico. Similarmente a *S. meridinalis*, uma nova linhagem de *Deltamys* divergente a nível específico é reconhecida e apresentada, porém ainda não descrita. Esta linhagem é encontrada em uma única localidade no Planalto Meridional do RS e mostrou-se, segundo análises de sequências de cit *b*, irmã de *Deltamys* sp. (2n=40), outra linhagem também ocorrente no Planalto Meridional e ainda não formalmente descrita. É possível que a diferenciação no gênero *Deltamys* tenha sido direcionada por eventos de dispersão e isolamento em diferentes gradientes altitudinais na porção leste rio-grandense da bacia geológica do Paraná. Dada a existencia de um maior esforço em análises filogeográficas de espécies e gêneros de sigmodontíneos em sistemas tropicais, este estudo representa uma significativa contribuição aos processos evolutivos na região subtropical, ainda pouco explorado também para outras espécies. Os padrões encontrados podem ser contrastados em futuras abordagens com organismos diversos.

ABSTRACT

The tribe Akodontini represents the second largest tribe of sigmodontine rodents, comprising 13 genera and more than 100 recognized species. The genera *Deltamys* and *Scapteromys* compass akodonts distributed in wetlands of southern Brazil, Uruguay and northeastern Argentina. The present study aimed to investigate the genetic and morphological variability, phylogeographic patterns and historical demographic events in *Scapteromys* from Brazil and Uruguay and *Deltamys* along its whole distribution. Analysis of sequences of the mitochondrial gene citochromo b (cyt *b*) revealed that the karyomorphs 2n=34 and 2n=36 of Meridional Plateau *Scapteromys* correspond to a single species, internally substructured. This structure, therefore, does not correspond to the karyotypes. *Scapteromys meridionalis* sp.n. has comparatively darker pelage and smaller values of most external and craniometric measurements. It is also distinguishable from *S. tumidus* by the shape of hamular process of pterygoid and morphology of *manus* surface. The phylogeographic analysis of *S. tumidus* revealed that this taxon is structured in three haplogroups, one of them occurring in the extreme north of the species distribution and Central Rio Grande do Sul (RS) coastal plain markedly divergent in cyt *b* sequences and skull shape. We observed a demographic expansion event temporally coincident with the beginning of the formation of RS coastal plain. *Deltamys kempi* presented a well structured phylogeographic pattern, characterized by several local differentiations and few haplotypes shared by sampled localities. As in previous studies, two mitochondrial clades were identified, supported by craniometrical differences. Our results indicate that the phylogeographic break observed both in *D. kempi* and *S. tumidus* can be related to the geological evolution of RS coastal plain and consequent formation of the Patos lagoon

estuarine channel, which may represent a geographic barrier to the historical gene flow. Finally, a new *Deltamys* lineage divergent at specific level is presented. This lineage is known from a single locality in RS Meridional Plateau and appears in cyt *b* sequences analysis as sister of *Deltamys* sp. (2n=40), another undescribed form from Meridional Plateau. It is possible that the differentiation in *Deltamys* genus have been driven by events of dispersion and isolation along distinct altitudinal gradients in RS eastern portion of the Paraná geological basin. Given the existence of a larger effort in phylogeographic analyzes of species and genera of sigmodontines in tropical systems, this study represents a significant contribution to the evolutionary processes in the subtropical region, yet little investigated also for other species. Patterns found herein can be also contrasted to other organisms in further studies.

CAPITULO I

Introdução geral

1. OS ROEDORES SIGMODONTÍNEOS

Os roedores (ordem Rodentia) compreendem a maior ordem de mamíferos viventes, incluindo mais de 2.200 espécies descritas, o que corresponde a 42% de toda a diversidade mastofaunística mundial (Carleton & Musser 2005). Análises filogenéticas baseadas em sequências de 11 genes mitocondriais e nucleares de 81% dos gêneros e 56% das espécies reconhecidas sustentam a divisão desta ordem em quatro clados: Ctenohystrica, *squirrel-related clade*, Castorimorpha e "Myodonta + Anomaluroidea" (Fabre *et al*. 2012). O clado "Myodonta + Anomaluroidea" ou *mouse-related clade*, por sua vez, abrange o grande grupo Muroidea (Fabre *et al*. 2012). Tradicionalmente tratado como uma superfamília da subordem Myomorpha (ver Musser & Carleton 2005), Muroidea abrange mais de 300 gêneros e 1500 espécies (Fabre *et al*. 2012), sendo considerado o mais diverso grupo de roedores (Catzeflis *et al.* 2002).

Dentre as seis famílias reconhecidas de roedores muróideos, Cricetidae representa a segunda em diversidade, com mais de 580 espécies (Musser & Carleton 2005). Os roedores cricetídeos estão distribuídos na Eurásia, África e Américas, sendo que a maior diversidade de espécies ocorre na Ásia e Américas (Musser & Carleton, 2005). Estão divididos em seis subfamílias, assim distribuídas: Arvicolinae: Eurásia e América do Norte; Cricetinae: Eurásia; Lophiomyinae: África; Neotominae: Américas do Norte e Central; Sigmodontinae: Américas do Sul, Central e do Norte; Tylomiinae: América do Norte (México), América Central e Norte da América do Sul (Musser & Carleton 2005).

A subfamília Sigmodontinae compreende um diversificado grupo de cricetídeos distribuídos principalmente na América do Sul, com espécies ocorrentes nas Américas Central e do Norte e um gênero endêmico das Ilhas Galápagos (D'Elía 2003). São reconhecidas cerca de 400 espécies viventes, distribuídas em 82 gêneros (Parada *et al*. 2013). O contexto biogeográfico da origem e diversificação dos roedores sigmodontíneos é um enigma que vem recebendo a atenção de estudiosos da mastofauna Neotropical há mais de 60 anos. As hipóteses *late-arrival* sugerem que os ancestrais de sigmodontíneos da America do Sul atingiram este continente através da passagem terrestre formada após o soerguimento do istmo do Panamá (Smith & Patton 1999). Nesta linha, Simpson (1950) sugere uma rápida radiação na América do Sul, enquanto que Patterson & Pascual (1972) e Baskin (1978) assumem que muitos dos gêneros de Sigmodontinae já haviam se diversificado nas Américas do Norte e Central anteriormente ao surgimento do istmo. Por outro lado, a hipótese *early-arrival* defendida por Hershkovitz (1966a, 1972), Marshall (1979) e Reig (1980, 1984), sugere que as formas ancestrais atingiram a América do Sul anteriormente ao soerguimento do istmo do Panamá, através de dispersão oceânica.

Fósseis de sigmodontíneos na América do Sul são escassos. O registro mais antigo do grupo no subcontinente é um fragmento molar procedente do Mioceno tardio (7,14 milhões de anos atrás [m.a.a.]) do noroeste argentino (Nasif *et al.* 2009), cuja morfologia se assemelha ao padrão sigmodonte de Phyllotini viventes. Outros registros fósseis apontam a presença de ao menos quatro tribos (Abrothrichini, Akodontini, Phyllotini e Reithrodontini) na província de Buenos Aires, Argentina, em idades anteriores ao estabelecimento do istmo do Panamá (Pardiñas & Tonni 1998; Pardiñas *et al.* 2002; Nasif *et al.* 2009). Em sustentação a estes dados, datações moleculares estimam que as tribos formalmente reconhecidas diversificaram-se entre 4,9 e 9,4

m.a.a. (Parada *et al*. 2013), período portanto anterior ao soerguimento do istmo. No entanto, apesar dos avanços sobre o conhecimento acerca a biogeografia histórica dos sigmodontíneos, aspectos tais como a área geográfica onde se deu a diversificação entre as principais linhagens, permanecem ainda sem elucidação (Smith & Patton 1999; Pardiñas 2000; Parada *et al*. 2013).

2. A TRIBO AKODONTINI

Visto a grande diversidade do grupo, muito esforço tem se empregado para o conhecimento sobre as relações filogenéticas e consequente classificação dos roedores sigmodontíneos. Tradicionalmente, os sigmodontíneos têm sido arranjados em grupos de gêneros baseados em similaridades morfológicas, sendo que alguns destes grupos receberem o status formal de tribo (ver Vorontzov 1959; Smith & Patton 1999; D'Elía *et al*. 2005). No entanto, investigações sobre o status filogenético dos diversos gêneros, utilizando-se diferentes marcadores moleculares, têm resultado em rearranjos dos clados supragenéricos (Smith & Patton 1999; D'Elía 2003; D'Elía *et al*. 2005). Atualmente nove tribos (Abrotrichini, Akodontini, Ichthyomyini, Oryzomyini, Phyllotini, Reithrodontini, Sigmodontini, Thomasomyini, Wiedomyini) são reconhecidas, sendo que 11 gêneros permanecem como Sigmodontinae *incertae sedis* (D'Elía *et al*. 2007).

A tribo Akodontini corresponde a segunda maior tribo em diversidade e atualmente abrange 13 gêneros (*Akodon*, *Bibimys*, *Blarinomys*, *Brucepattersonius*, *Deltamys*, *Kunsia*, *Juscelinomys*, *Necromys*, *Oxymycterus*, *Podoxymys*, *Scapteromys*, *Thalpomys* e *Thaptomys*) e mais de 100 espécies (D'Elía *et al*. 2007; Ventura *et al*. 2012). Este tribo foi originalmente estabelecida por Vorontzov (1959) para agrupar os gêneros *Akodon*, *Zygodontomys*, *Microxus* (= *Akodon*), *Podoxymys*, *Lenoxus*, *Oxymycterus*, *Blarinomys* and *Notiomys*. No entanto, Thomas

(1916) já havia agrupado em sete gêneros as formas relacionadas à *Akodon,* referindo-os como "akodont genera". Desde então, análises morfológicas (*e.g*. Osgood 1925; Anthony 1929; Gyldenstolpe 1932; Tate 1932; Hooper & Musser 1964; Hershkovitz 1966; Reig 1987), citogenéticas (*e.g*. Fagundes et al. 1998; Hass *et al.* 2011; Ventura *et al.* 2012) e moleculares (*e.g*. Smith & Patton 1999; D'Elía 2003; D'Elía *et al*. 2003, 2005; Parada *et al*. 2013) vêm buscando compreender as relações inter e intragenéricas das formas akodontes.

3. O GÊNERO *SCAPTEROMYS*

O gênero akodonte *Scapteromys* compreende atualmente três espécies. *Scapteromys tumidus* Waterhouse 1837 (número diplóide [2n]=24, número de braços autossômicos [NA]=40) distribui-se no Uruguai e nas porções sul e leste do Estado do Rio Grande do Sul (RS) (D'Elía & Pardiñas 2004). *Scapteromys aquaticus* Thomas 1920 (2n=32, NA=40) ocorre no sul do Paraguai e nordeste da Argentina (D'Elía & Pardiñas 2004), além de registros pontuais no oeste do Uruguai (localidade Las Cañas) (D'Elía & Pardiñas 2004; Bonvicino *et al.* 2013) e oeste do RS (localidade São Borja) (Bonvicino *et al.* 2013). *Scapteromys meridionalis* (Quintela *et al.* 2014) foi recentemente descrito como parte desta tese e possui distribuição restrita a sistemas palustres em meio à formação Floresta Ombrófila Mixta ou Floresta com Araucária, nos estados brasileiros do Paraná, Santa Catarina e RS.

Scapteromys foi originalmente estabelecido como subgênero por Waterhouse (1837) para alocar *Mus tumidus*, descrito a partir de espécimes coletados por Charles Darwin em Maldonado, Uruguai (D'Elía & Pardiñas 2004). Posteriormente, Waterhouse (1839) incluiu *tumidus* no novo gênero *Hesperomys*, dissolvendo *Scapteromys*. Peters (1860) incluiu *tomentosus* (originalmente *Mus tomentosus* Lichtenstein, 1830) no subgênero *Scapteromys*. Fitzinger (1867) reconsiderou

Scapteromys e o elevou à categoria de gênero. Thomas (1920) descreveu *Scapteromys aquaticus* a partir de espécimes coletados em Isla Ella, Argentina. Gyldenstope (1932) redescreveu o gênero *Scapteromys* e listou cinco espécies (*S. aquaticus*, *S. fronto*, *S. gnambiquarae*, *S. tomentosus*, and *S. tumidus*). Massoia & Fornes (1964) analisaram populações de *Scapteromys* do Uruguai e Argentina e consideraram *aquaticus* uma subespécie de *S. tumidus*. Hershkovitz (1966) revisou o "grupo Scapteromyine" e estabeleceu o gênero *Kunsia*, alocando *tomentosus* e *fronto* neste táxon e desconsiderando *gnambiquarae* e a distinção subespecífica em *tumidus.* Posteriormente, análises citogenéticas de *Scapteromys* procedentes da Argentina (2n=32) e Uruguai (2n=24) (Brum-Zorrilla *et al.*1972, 1986; Fronza *et al.* 1976) forneceram suporte para o reconhecimento de *tumidus* e *aquaticus* como espécies distintas. Novos dados citogenéticos de espécimes procedentes do Planalto Meridional, sul do Brasil, revelaram os citótipos 2n=34 e 2n=36, que foram considerados espécies distintas (Freitas *et al*., 1984).

A aplicação de técnicas de análises moleculares trouxe novos *insights* sobre as relações intra e supragenéricas em *Scapteromys*. Com base em similaridades morfológicas, Massoia (1979) havia agrupado os gêneros *Scapteromys*, *Kunsia* e *Bibimys* na tribo Scapteromyini. No entanto, a análise de sequências do gene mitocondrial citocromo b (cyt *b*) (Smith & Patton 1999) revelaram que *Scapteromys* e *Kunsia* formam um clado juntamente com *Akodon* e outros seis gêneros, podendo ser considerados como pertencentes à tribo Akodontini. Adicionalmente, *Scapteromys*, *Kunsia* e *Bibimys* não formam um grupo monofilético em análise filogenética de sequências de cit *b* (D'Elía 2003; D'Elía *et al.* 2005), o que levou à desconsideração da tribo Scapteromyini. Quanto aos limites entre espécies, D'Elía & Pardiñas (2004) verificaram através de sequências de cit *b* que as formas do Paraguai e Argentina e do Uruguai representam espécies distintas, correspondendo, respectivamente a *S. aquaticus* e *S. tumidus*. Análises de sequências

de cit *b* também revelaram que os espécimes procedentes do Planalto Meridional representam uma espécie distinta, denominada *S. meridionalis* (Quintela *et al.* 2014; presente estudo).

As espécies de *Scapteromys* habitam principalmente bordas de sistemas palustres com predominância de vegetação herbácea (Hershkovitz 1966; Barlow 1969; Quintela *et al*. 2014). Possuem capacidade escansorial, como uma adaptação à vida em ambientes alagados (D'Elía & Pardiñas 2004). Análises de conteúdo estomacal de espécimes de *S. tumidus* e *S. aquaticus* indicam hábitos alimentares onívoros, sendo encontrados restos vegetais, insetos, oligoquetos e hirudíneas (Massoia & Fornes 1964; Barlow 1969; González & Lanfranco 2010).

4. O GÊNERO *DELTAMYS*

O gênero *Deltamys* compreende duas linhagens divergentes a nível específico, baseado em dados moleculares e citogenéticos (Ventura *et al*. 2011). A única espécie formalmente descrita, *Deltamys kempi* Thomas,1917, distribui-se do norte da planície costeira do RS até uma estreita faixa costeira da Província de Buenos Aires, Argentina (González & Pardiñas 2002). *Deltamys kempi* apresenta cariótipo 2n=37 em machos e 2n=38 em fêmeas, NA=38 em ambos os sexos e sistema único de determinação sexual do tipo $X_1X_1X_2X_2$/ X_1X_2Y (Sbalqueiro *et al.* 1984). Baseados em diferenças craniométricas sutis e na coloração da pelagem, González & Massoia (1995) propuseram uma distinção subsepecífica, sendo as populações argentinas correspondentes à forma nominal (*D. k. kempi*) e as populações uruguaias e brasileiras designadas como *D. k. langguthi*. Uma segunda linhagem ainda não nominada (*Deltamys* sp. em Ventura *et al*. [2011]) apresenta 12% de divergência genética (cyt *b*) em relação à espécie irmã *D. kempi*, além de cariótipo caracterizado por 2n=40, NA=40 e sistema sexual do tipo XX/XY. Esta linhagem é

conhecida para apenas uma localidade (Esmeralda) em meio à formação Floresta Ombrófila Mista (Floresta com Araucária) no norte do RS (Ventura *et al*. 2011).

Deltamys se assemelha às menores formas de *Akodon*, o que já havia sido observado por Thomas (1918). Tais semelhanças morfológicas, portanto, fizeram com que alguns autores considerassem *Deltamys* como um subgênero ou mesmo sinônimo de *Akodon* (e.g. Ellerman 1941, Cabrera 1961, Massoia 1964, Reig 1987, Musser & Carleton, 1993). No entanto, reavaliação de caracteres morfológicos (González & Massoia 1995) e dados citogenéticos (Gentile de Fronza *et al*. 1981; Sbalqueiro *et al* 1984; Castro *et al*. 1991) e moleculares (cyt *b*) (D'Elía *et al*. 2003) dão suporte ao status genérico de *Deltamys*.

Análises filogenéticas de sequências de cyt *b* revelaram *Deltamys* como grupo irmão de *Akodon* (D'Elía *et al*. 2003), confirmando as suposições com bases morfológicas sobre sua condição de akodonte (e.g. Thomas 1918, Ellerman 1941, Cabrera 1961, Massoia 1964, Reig 1987). D'Elía et al. (2003), no entanto, não encontraram divergências genéticas favoráveis a uma distinção subespecífica em *D. kempi*, sugerindo uma reavaliação de seu conteúdo taxonômico. Análises filogeográficas com base em sequências de cyt *b* e do gene nuclear RAG2 (recombination-activing gene 2) (Montes *et al*. 2008), entretanto, revelaram uma estruturação em *D. kempi*, sendo um clado restrito à porção norte da planície costeira do RS (extremo norte da distribuição geográfica) e outro clado correspondente ao restante da distribuição da espécie. Espécimes procedentes dos dois clados são também craniometricamente diferenciados. Montes *et al*. (2008), no entanto, não apresentam nenhuma proposta taxonômica para divisão interna em *D. kempi*.

Deltamys kempi é uma espécie associada a ambientes pantanosos principalmente de áreas abertas. Possui hábitos alimentares onívoros, sendo encontradas folhas, sementes e insetos no

conteúdo estomacal de exemplares analisados (Massoia 1964; González & Pardiñas 2002). *Deltamys* sp. (Ventura *et al*. 2011) carece de dados ecológicos.

5. FILOGEOGRAFIA E ANÁLISES MORFOLÓGICAS ASSOCIADAS

A filogeografia é definida como o estudo dos princípios e processos que determinam a distribuição das linhagens genealógicas intraespecíficas ou entre espécies proximamente relacionadas (Avise 1994, 2000). Baseia-se na análise dos processos históricos responsáveis pela distribuição espacial das linhagens evolutivas, integrando informações geológicas, paleontológicas, etológicas, demográficas e filogenéticas. (Avise 2000). Através da análise de marcadores moleculares, busca-se estabelecer as relações filogenéticas entre os haplótipos do táxon (a) em questão e associar esta informação ao espaço geográfico, integrando desta forma genealogia e biogeografia (Avise 2000).

Os marcadores moleculares possuem a capacidade de fornecer informações sobre o fluxo gênico entre populações (Irwin 2002) e detectar eventos históricos demográficos como expansões e retrações populacionais (Bertorelle *et al.* 2010; Lopes *et al*. 2013). Os marcadores utilizados em análises filogeográficas são genes mitocondriais, nucleares, microsatélites e alozimas, havendo estudos que se utilizam mais de um marcador (Avise 2000, Beheregaray 2008). Os marcadores mais utilizados, no entanto, são os genes mitocondriais (Behegaray 2008). O DNA mitocondrial apresenta alta taxa de evolução (mtDNA), sendo capaz de caracterizar a variabilidade intra específica e identificar populações, além de apresentar ausência de recombinação (Avise 1994, 2000).

Associadas aos dados moleculares, alguns estudos filogeográficos utilizam-se de análises sobre a variação morfológica do táxon ou taxa investigados (Garnier 2005, Montes *et al*. 2008;

Costa *et al.* 2011; Santamaria *et al*. 2013). Essa variação pode ser detectada através da utilização de métodos de morfometria tradicional ou morfometria geométrica (Rohlf & Marcus 1993; Fruciano *et al.* 2011). Os métodos tradicionais são baseados em análises de distâncias (comprimentos, larguras), ângulos e proporções, enquanto que as técnicas de morfometria geométrica capturam a forma das estruturas através de marcos anatômicos em duas ou três dimensões (Rohlf & Marcus 1993). Portanto, a morfometria geométrica representa um avanço em relação aos métodos tradicionais, uma vez que a variação na forma é possível através de representações gráficas (Rohlf & Marcus 1993; Monteiro & dos Reis 1999).

Estudos sobre aspectos filogeográficos de roedores neotropicais são ainda escassos, considerando-se a grande diversidade dos grupos. A maior parte destes estudos abordam espécies ou gêneros de Echimyidae e Sigmodontinae característicos de sistemas florestais. Por exemplo, Da Silva & Patton (1993, 1999) analisaram a estrutura filogeográfica de equimídeos (*Echimys*, *Dactylomys*, *Makalata*, *Mesomys*, *Isothrix*, *Proechimys*) e orizomíneos (*Neacomys, Oecomys, Oryzomys, Scolomys*) na Amazônia. Costa (2003) analisou orizomíneos (*Oryzomys*, *Oecomys*) e thomasomíneos (*Rhipidomys*) na Amazônia e Mata Atlântica. O gênero *Rhipidomys* foi também avaliado em toda a sua distribuição (Costa *et al*. 2011), o que revelou duas novas espécies. Ventura *et al*. (2012) descreveu padrões filogeográficos e cariotípicos do akodonte *Blarinomys breviceps*, endêmico da Mata Atlântica. Outro akodonte endêmico da Mata Atlântica, *Akodon montensis*, foi analisado por Valdez & D'Elía (2013), sugerindo a existência de um refúgio pleistocênico de Mata Atlântica subtropical. Em florestas temperadas da Patagônia e Andes ocidental, Palma *et al*. (2005) analisaram *Oligoryzomys longicaudatus* e encontraram resultados que refutam uma distinção subespecífica neste táxon. Cañón *et al*. (2010) avaliaram a estrutura genética em *Loxodontomys micropus* em florestas temperadas andinas, o que resultou na

sinonimização de *L. pikumche* em *L. micropus*. Já em fisionomias abertas neotropicais observa-se a predominância de investigações sobre padrões filogeográficos em histricognatos ctenomídeos (Mora *et al*. 2006, 2007; Lopes & Freitas, 2012; Mapelli *et al*. 2012; Fasanella *et al*. 2013; Lopes *et al*. 2013) e octodontídeos (Opazo *et al*. 2008; Ojeda 2010; Gallardo *et al*., 2013), sendo raros os trabalhos sobre sigmodontíneos. Nascimento *et al*. (2011) testaram o efeito de rios como barreiras sobre a estruturação populacional no filotíneo *Calomys tener* nos Biomas Cerrado e Caatinga. No bioma Pampa, Montes *et al*. (2008) relacionou a evolução geológica da planície costeira do RS à diferenciação genética e craniométrica em *Deltamys kempi*. Portanto, como já observado por Turchetto-Zolet *et al*. (2013), existe um maior esforço empregado em análises filogeográficas de espécies e gêneros de sigmodontíneos em sistemas tropicais, enquanto que os processos evolutivos nos taxa de distribuição subtropical permanecem pouco explorados.

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OBJETIVOS

1. OBJETIVO GERAL

Caracterizar a variabilidade morfológica e genética e inferir padrões filogeográficos e de demografia histórica em *Scapteromys* no Brasil e Uruguai, e *Deltamys* em toda distribuição.

2. OBJETIVOS ESPECÍFICOS

1. Investigar a divergência morfológica e genética entre as formas de *Scapteromys* do Planalto Meridional 2n=34 e 2n=36, comparativamente às espécies reconhecidas no gênero, fazendo inferências taxonômicas a partir destes resultados;

2. Caracterizar padrões filogeográficos em *Scapteromys tumidus* através da análise de sequências do gene citocromo *b* e inferir possíveis fatores associados;

3. Descrever a variação na forma do crânio em *S. tumidus* através de técnicas de morfometria geométrica e testar a congruência geográfica entre divergência morfológica e diferenciação genética;

4. Caracterizar padrões filogeográficos em *Deltamys kempi* através da análise de sequências do gene citocromo *b* e craniometria e inferir possíveis fatores associados;

5. Estabelecer a posição filogenética de uma forma morfologicamente semelhante a *Deltamys* procedente do Planalto Meridional do Rio Grande do Sul.

CAPITULO II

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A new species of swamp rat of the genus *Scapteromys* Waterhouse, 1837 (Rodentia: Sigmodontinae) endemic to *Araucaria angustifolia* Forest in Southern Brazil

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Abstract

A new species of swamp rat of the genus *Scapteromys* from the Meridional Plateau of Southern Brazil is described. Morphological, molecular, and karyological analysis support the recognition of the new species, distinct from *S. aquaticus* and *S. tumidus*. *Scapteromys* sp. nov. is significantly smaller than the congeneric taxa considering most of the external and craniometric measurements and the pelage is conspicuously grayer and darker. It can be distinguished from *S. tumidus* by the laterally extended thenar pad of the manus and the parallel edges of the hamular process of the pterygoid, and from *S. aquaticus* by a grayer and darker pelage and smaller values of most external and craniometric measurements. Karyological analysis indicated a difference in chromosome numbers across the distributional range: 2n=34 and 2n=36. A total of 11 haplotypes were found along the range of the new species within the biogeographic province of *Araucaria angustifolia* Forest. Strongly supported substructure was found within the new taxon, resulting in two reciprocally monophyletic clades.

Key words: biodiversity, cytochrome *b*, molecular phylogeny, morphology, new taxon, time-calibrated tree

Introduction

Scapteromys Waterhouse, 1837 comprises two recognized species of medium sized rats associated to areas bordering rivers, swamps, lagoons and small creeks (D'Elía & Pardinãs 2004; González & Martinez-Lanfranco 2010). Analysis of the mitochondrial cytochrome *b* (cyt *b*) gene

and the nuclear interphotoreceptor retinoid binding protein (IRBP) gene allocated this sigmodontine genus to the tribe Akodontini (Smith & Patton 1999; D'Elía 2003). *Scapteromys* has received some attention by researchers, which resulted in taxonomic and systematic reevaluations. Gyldenstolpe (1932) redescribed the genus, providing new morphological observations and listed five species (*S. aquaticus*, *S. fronto*, *S. gnambiquarae*, *S. tomentosus*, and *S. tumidus*). Ellerman (1941) provided generic external and craniodental anatomical definitions and listed the five named forms recognized by Gyldenstolpe (1932). Massoia & Fornes (1964) compared qualitative (coat-color, fronto-parietal suture and mesopterygoid fossa shape) and quantitative (external, cranial, penile and bacular) characters between Argentinean and Uruguayan samples, assuming a subspecific classification (*S. t. tumidus* for Uruguay and *S. t. aquaticus* for Argentina). Hershkovitz (1966) presented external, craniodental, penile and bacular anatomical descriptions and some external, cranial and bacular measures and did not consider the Argentinean and Uruguayan forms as distinct subspecies. Later, cytogenetic studies of *Scapteromys* from Argentina and Uruguay (Brum-Zorrilla *et al.*1972; 1986; Fronza *et al.* 1976) supported the specific distinction.

The most recent investigation, based on cyt-*b* sequences and morphological data (D'Elía & Pardinãs 2004), sustained the existence of two species: (i) *Scapteromys aquaticus* (Thomas, 1920) (2n=32), ranging from southern Paraguay to east-central Argentina, with records in Las Cañas, southeastern Uruguay, and São Borja, the western part of Rio Grande do Sul state (RS) in southern Brazil (Bonvicino *et al*. 2013), and (ii) *Scapteromys tumidus* (Waterhouse, 1837) (2n=24) widespread across Uruguay and RS coastal plain, but not in highlands (Freitas *et al*. 1984; D'Elía & Pardinãs 2004).

In addition to the formally recognized species, two *Scapteromys* karyomorphs were found by Freitas *et al*. (1984) in highlands of Southern Brazil: 2n=34 occurred in northern RS and 2n=36 in eastern Paraná state. Data on constitutive heterochromatin (C-bands) from the referenced work revealed that Robertsonian translocations occurred in two independent pathways in *Scapteromys* karyomorphs. The 2n=24 form (*S. tumidus*) was derived by centric fusions from 2n=32 (*S. aquaticus*) while 2n=34 has the same bi-armed elements of 2n=36, differing only by one pair. Brum-Zorrilla *et al*. (1986), analyzing the G-band patterns, also stated that the form 2n=24 is chromosomally more closely related to the 2n=32 form.

Recently, as part of an ongoing study on the diversity of small mammals from the biogeographic provinces of Paraná and *Araucaria angustifolia* Forests (= Meridional Plateau; Morrone 2006) in southern Brazil, we collected specimens of *Scapteromys* with the karyotypes as the 2n=34 and 2n=36 karyomorphs of Freitas *et al*. (1984). Here, we described this form as a new *Scapteromys* species, which is morphologically, karyotypically and genetically distinguishable from its congeneric forms, in addition to occurring in allopatry. In addition, a population analysis was carried out across the geographical range of the new taxon in order to characterize patterns of variation, and a molecular phylogeny of these specimens was reconstructed based on cyt-*b* sequences.

Material and methods

Specimens examined. Five specimens were collected in the Meridional Plateau with the permission of Sisbio-ICMBio (Brazilian Environmental Protection Agency). Skin, skull, and tissue samples were prepared and housed at the Fundação Universidade Regional de Blumenau

(FURB). Also, we incorporated 18 specimens from Santa Catarina and Paraná States deposited in FURB and Universidade Federal do Paraná (UFPR) with skin, skull, tissue and karyotype data (when available), covering the entire distribution currently recognized (Fig.1). In order to compare specimens from the highlands to the recognized *Scapteromys* species, we accessed skulls and skins of 42 specimens of *S. aquaticus* housed in Museo Municipal de Ciencias Naturales Lorenzo Scaglia, Mar de Plata, Argentina (FCM), and 244 specimens of *S. tumidus* housed in the American Museum of Natural History, New York (AMNH; topotypes 206243-206250, 206252-206258) and the Museu de Ciências Naturais from Universidade Luterana do Brasil, Canoas, Brazil (MCNU). The holotypes (skulls) of *S. aquaticus* and *S. tumidus* were examined by high definition photographs. All material used is listed in the Appendix.

Morphological data analysis. Skulls and skins were directly examined for diagnostic characters. We also evaluated the distribution of the types of fronto-parietal suture and mesopterygoid fossa and the presence/absence of the palatal process, which are considered to be consistent characters for the diagnosis of *Scapteromys* species (Massoia & Fornes 1964; D'Elía & Pardinãs 2004). Descriptive terminology of general external morphology follows Hershkovitz (1966) and Voss (1988). We transcribed the external measures of total length (TL), head-body length (HB), length of tail (LT), height of ear (Ear), and hindfoot length from specimen tags and field notes. When TL was available instead of HB, we calculated HB by subtracting LT from TL. External measurements are given to the nearest millimeter (mm).

Cranial terminology follows Hershkovitz (1962) and Voss (1988), and molar terminology follows Reig (1977). Postcranial terminology follows Voss (1988), Carrizo & Diaz (2011) and Machado *et al*. (2011). Stomach terminology follows Carleton (1973). Craniodental

measurements were taken with a digital caliper accurate to the nearest 0.01 mm. The following measurements were taken as illustrated by Hershkovitz (1962) and Voss (1988): breadth of incisors (BI), length of molar row (LMR), length of nasal (LN), length of rostrum (LR), length of tympanic bulla (LTB), breadth of braincase (BB), breadth of zygomatic plate (BZB), least interorbital length (LIB), length of rostrum (LR), distance between first molars (DFM), breadth of first molar (BFM), condylo-incisive length (CIL), greatest length (GL), palatal length (PL), length of diastema (LD), orbital length (OL), height of braincase (HB), breadth of zygomatic (BZ), length of incisive foramina (LIF), breadth of incisive foramina (BIF), breadth of occipital condyles (BOC). Differences in means of each measurement between the specimens from the Meridional Plateau and specimens from the Argentinean provinces of Buenos Aires and Entre Rios (*S. aquaticus*), and from Maldonado, Uruguay (*S. tumidus* topotypes) were tested with student *t* test. For this, we used measurements only from subadults and adults specimens based on the age classification proposed by Barlow (1969). We also performed a principal component analysis (PCA) from the variance-covariance matrix generated from logarithm converted measurements. Missing values (5.4%) were estimated by regression.

Molecular data. Molecular analysis was based on the first 801 base pairs (bp) of the cytochrome b (cyt-*b*) gene including 29 specimens of *Scapteromys* from eight populations in the Meridional Plateau (Fig. 1; Table 1). Several sequences of *S. tumidus* and *S. aquaticus* as well as of the sister lineage *Kunsia* (Smith & Patton 1999), were obtained from Genbank (Table 1). The cyt-*b* gene obtained from specimens of this study was amplified and sequenced using primers MVZ05 and MVZ16 (da Silva & Patton 1993). The thermocycler conditions for amplification by polymerase chain reaction (PCR) were as follows: 2 min of initial denaturation, 30 cycles of 15 s of

denaturation at 95°C, 25 s of annealing at 50°C, and 30 s of extension at 72°C followed by 7 min of final extension at 72°C. PCR products were purified using Exonuclease I (GE Healthcare) and Shrimp alkaline phosphatase, sequenced with the same PCR primers and labeled with BigDye (Applied Biosystems) Terminator v.3.1 Cycle Sequencing Kit DNA analyzer at Macrogen Inc. (Seoul, Republic of Korea). Sequences generated were deposited in Genbank under the accession numbers KF536928-KF536939 (Table 1).

We visually inspected and aligned sequences using the program Clustal X in MEGA version 5 (Tamura *et al*. 2011) running in full mode with no manual adjustment. A phylogenetic analysis of *Scapteromys* was carried out based on haplotypes of specimens sequenced in this study, in addition to *S. tumidus* and *S. aquaticus* (the sister lineages), using maximum likelihood (ML) method. The ML analysis was conducted using the HKY (Hasegawa *et al*. 1985) nucleotide substitution model that was selected by jModeltest (Posada 2008). Searches were conducted in PHYML 3.0 (Guindon et al. 2010) through 1000 replicates of heuristic search with random addition of sequences and TBR. Monophyly-confidence limits were assessed with the bootstrap method (Felsenstein 1985) at a 50% cut-off after 1000 bootstrap iterations. We also analyzed inter- and intra-specific genetic distance using the Kimura 2-parameters model (Kimura 1980) procedure, with 1000 bootstrap replications, through the software MEGA 5.0.

Hierarchical analysis of the distribution of genetic diversity was conducted as an analysis of molecular variance (AMOVA—Excoffier *et al*. 1992) using Arlequin version 3.11 (Excoffier & Schneider 2005). Hierarchical levels were defined on the basis of major clades found in the ML analysis. Median-joining (MJ) network (Bandelt *et al*. 1999) reconstruction in the program Network version 4.5.1.6 (http://www.fluxus-engineering.com) was used to infer intraspecific genealogy. MJ was calculated using only variable sites.

Results

Sequencing of 801 base pairs of the cyt-*b* gene revealed 36 polymorphic sites that provided 11 haplotypes for the 29 examined specimens of *Scapteromys* from the Meridional Plateau (Table 1). The ML consensus tree strongly supports a monophyletic status based on all haplotypes observed in *Scapteromys* sp. n. The other two species clustered as reciprocally monophyletic sister lineages and a sister clade of *Scapteromys* sp. n. (Fig. 2A). Thus, in agreement with our hypothesis, *Scapteromys* sp. n. represents a new monophyletic lineage, conspicuously divergent from other *Scapteromys* taxa (genetic distance ca. 5%) (Fig. 2A).

Specimens of *Scapteromys* sp. n. present a marked intraspecific structure, split into 2 strongly supported, reciprocally monophyletic groups (Clade 1 and Clade 2) (Fig. 2B). Genetic divergence between these clades was estimated as 2.8% (Fig.2A). Intraspecific genealogy based on 11 haplotypes of *Scapteromys* sp. n. supported these 2 groups separated by at least 12 mutational steps (Fig. 2B). Three unique haplotypes were found within Clade 1, including specimens from northeastern Rio Grande do Sul and southern Santa Catarina states. The remaining 8 haplotypes were observed exclusively in five different localities of northern Santa Catarina and southern Paraná states belonging to Clade 2. AMOVA indicated that total genetic variation was mainly explained by differences within groups (87.8%). Divergence between groups accounts for only 12.2% of the genetic variation. Parameters of variability, such as haplotype (Hd) and nucleotide (π) diversity observed were 0.80 ± 0.01 and 0.002, respectively, for Clade 1. In the clade 2, we found 0.68 ± 0.01 (Hd) and 0.003 (π).

Scapteromys sp. n. (2n=34-36, FNa=40) differs from *S. aquaticus* (2n=32, FNa=40) and *S. tumidus* (2n=24, FNa=40) (Fig.3). Individuals of the new species had diploid number (2n) of

34/36 chromosomes and autosomal fundamental number (FNa) of 40 arms; 1st two submetacentric pairs were large, submetacentric medium-sized pairs and 12/14 acrocentric pairs with various sizes. The sexual pair in males is formed by a large X-acrocentric and small Y-acrocentric chromosome (Fig.3). The karyotype 2n=34 and FNa=40 was found in the states of Rio Grande do Sul (locality of São Francisco de Paula) and Santa Catarina (localities of Água Doce and São Domingos). The karyotype 2n=36 and FNa=40 was found in Paraná state (localities of São José dos Pinhais and São Mateus do Sul). Despite its allopatry (see Fig. 1), karyomorphs distribution showed no correspondence with the clade subdivision of cyt-*b* haplotypes. Clade 2 included specimens with both 2n=34 (Água Doce, São Domingos) and 2n=36 (São José dos Pinhais).

The new taxon has smaller dimensions than its congenerics, according to most of the external and cranial measurements (Table 2). The first three principal components (PCs) explained 83.5% of the total craniometric variation (PC1: 65.9%, PC2: 13.7%, PC3: 3.9%). In PC1 × PC2 (Fig. 4A), the convex hull of *Scapteromys* sp. n. was almost completely discriminated along the negative end of PC1, showing no overlap with *S. aquaticus* or *S. tumidus.* In PC1 × PC3 (Fig. 4B), the convex hull of *Scapteromys* sp. n. was almost completely discriminated along the negative end of PC1, only slightly overlapping with *S. aquaticus* and *S. tumidus.* These areas of overlap, however, were visually smaller than those between *S. aquaticus* and *S. tumidus*. All of the 21 cranial distances were positively related to PC1, which can be interpreted as a general size factor. The main cranial distances positively related to PC2 were BIF, LR, LD, LTB and LN. The distances of LIF, BZB, OL, LMR and BOC were the main ones positively related to PC3.

***Scapteromys meridionalis* sp. n.**

Plateau Swamp Rat

Figures 5 -10; table 2

Holotype. Adult male, FURB 20253 (original number FQ 82), with skin, skull, post-cranial skeleton and fluid-preserved left forefoot and hindfoot, collected by F. M. Quintela on 16 October 2012. Frozen tissue samples (FQ 82) deposited in Department of Genetics at UFRGS; suspension of bone-marrow in Carnoy's fixative (number FQ 82) was deposited in Department of Genetics at UFPR. Karyotype 2n=34, FNa=40.

Type locality. São Francisco de Paula municipality (29º29'73"S, 50º13'49"W; 913 m above sea level), Rio Grande do Sul State, Brazil.

Paratypes. Four topotypes (FURB 20156, FURB 20252, FURB 20285 and FURB 20286) were collected between April 2012 and October 2012. Eighteen specimens collected between 2005 and 2012 in the following localities in southern Brazil: Santa Catarina State – Água Doce (FURB 9972), Campo Alegre (FURB 12658), Campo Belo do Sul (FURB 15129, 15166), Doutor Pedrinho (FURB 18676, 18993, 18996, 18999, 20044), Passos Maia (FURB 18908), São Domingos (FURB 12309); Paraná State – São Mateus do Sul (FURB 15364), Candói (FURB 15979), São José dos Pinhais (UFPR 985, 1036, 1052, 1059, 1063) (Fig.1). Skins, skulls (FURB 9266 and FURB 15364 with missing skulls), fluid-preserved carcass and frozen tissue samples are housed at FURB.

Distribution. Known from shrub and herbaceous palustrine systems in the biogeographic domains of Paraná and *Araucaria angustifolia* Forest, from southern Paraná to Northern Rio

Grande do Sul states (a highland region called "*Serra Geral"*), in altitudes between 530 and 1017 m.

Diagnosis. A small-sized, darker furred *Scapteromys* species; 2n = 34/36; tail bicolored mainly in the proximal half; quadrate mesopterygoid fossa; median palatine process present; hamular processes of pterygoid with straight borders; thenar of manus laterally extended.

Description. Pelage dense and soft, tawny gray in dorsum (Fig. 5), darker on the top of the head and on the middle and posterior central regions, but without forming a clear banding pattern; guard-hairs about 14 mm over the back, all eumelanic; overhair about 10 mm over the back, dichromatic, with a distal feomelanic band and most of its length eumelanic, including the distal tip; underhair about 7 mm over the back, with the same banding pattern of the overhair. Ventral pelage (Fig. 5) whitish gray, without clear limit with darker flank parts, except by the middle abdomen. Ventral pelage is composed by over- and underhair; overhairs about 10 mm in middle chest, eumelanic in the basis and the mid region and feomelanic in the distal region; underhair about 5.5 mm in middle chest with the same banding pattern of overhairs. Presence of superciliary, mystacial, genal and inconspicuous submental vibrissae; measurements of largest vibrissae from holotype: mystacial - 31.8 mm, superciliary - 23.4 mm, genal -26.30 mm, submental - 10.04 mm; longest mystacial not reaching the bases of pinnae when laid back alongside head. Rounded ears, densely covered by 3 mm long thick hairs, eumelanic in most of its length and feomelanic in the distal tip. Tail about 92% of the head-body length; densely hair covered; bicolored in the proximal half, with the dorsal region dark brown and the ventral region tawny, dark brown in the distal half; tail uniform dark in some paratypes; 6-10 mm terminal pencil; hair arranged in triads in each scale, with the central slightly longer than the laterals; eumelanic hair predominate in dorsal and ventral distal half while eumelanic hair cover the

ventral proximal half; hairs in ventral proximal half (about 7 mm) are longer than in other regions (about 3 mm), forming a kind o fringe. Mammae eight in pectoral, axial, abdominal and inguinal pairs.

Hind foot about 25% of head-body length; digits II, III and IV subequal in size; undeveloped interdigital webs, reaching about 18% of the height between digits II and III and 34% of the height between digits III and IV; densely covered by hair (eumelanic in proximal half and feomelanic in distal half) in metapodial and digits; most distal hair reaching until about the first third of claws; grooved claws; thenar pad about twice the size of the largest digital pad, laterally extended (Fig. 6); interdigital pads 2 and 3 subequal in size, followed by interdigital 4, interdigital 1 and hypothenar in descending order of size. Forefoot densely covered by feomelanic hair in metapodial and digits, not covering the claws; digits II, III and IV subequal in size; well-developed claws, reaching about 68% of the length of digits I-IV; well-developed claw in vestigial pollex, surpassing its extremity; five pads in plantar forefoot, thenar, hypothenar, and interdigitals 2, 3 and 4; interdigital pads subequal in size; hypothenar about 1.5 x thenar size and 4 x interdigital pad size.

Skull (Fig. 7A) characterized by elongate rostrum; gnathic process varying in size; well-developed anterior process of premaxillary but not forming a rostral tube (trumpet) with nasal extremity; slightly inflated incisive capsular projection; nasofrontal suture V shaped; deep zygomatic notches; diagonally shaped lacrimal; horizontally elongated nasolacrimal foramen; broad zygomatic plate, leaning forward, with an anterior rounded projection; elongate incisive foramina with their broadest point posterior to the transversal midline, posterior extremity reaching from anterolingual conule to hypoflexus; narrow antorbital bridge; well-developed tubercle in the anterior basis of zygomatic plate; median palatine process present; elongated

anterior palatine foramen, followed by three punctual foramina in posterior palatine; hourglass-shaped interorbital region, with rounded margins and well-developed parietal dorsal anterolateral process; small frontal foramen; sphenopalatine foramen reduced and horizontally elongated; punctual ethmoid foramen localized in frontal, behind maxillary-frontal suture; optic foramen similar in size to M2; narrow zygomatic arch, anterior and posteriorly convergent and not surpassing the border of braincase in dorsal view; frontal-parietal-squamosal suture in the exact point of parietal anterior process extremity or little behind this process; presence of a tubercle in squamosal border, anteriorly to squamosal root of zygomatic; visible and elongated jugal; mesopterygoid fossae narrow, slightly divergent, extending anteriorly behind or between posterior M3 alveoli; sphenopalatine vacuities divided by a narrow strut; parapterygoid fossae shallow and subequal in size with quadrate mesopterygoid fossae; hamular processes of pterygoid straight shaped and slightly divergent; alisphenoid strut present, separating buccinator-masticatory foramen and foramen ovale accessorius; large and broad petrotympanic fissure, with stapedial spine varying in size; carotid circulatory pattern 1 of Voss (1988); small carotid canal and stapedial foramen; moderately inflated tympanic bulla with size similar to superior molar row; thin malleus; orbicular apophisis present; hamular process of squamosal with straight borders (Fig. 8); postglenoid foramen larger or subequal in size with subsquamosal foramen; braincase not inflated in adults; basioccipital with undeveloped median crest; inflated occipital condyle, surpassing the braincase borders in ventral view; paroccipital process well-developed; interparietal varying in length and width; inflated mastoid with a large posterior foramen in posterior border; exoccipital inflated with conspicuous longitudinal and transversal crests.

Mandible (Fig. 7A) short and delicate, with height about 45% of length without incisors; elongate and narrow ramus; anterior extremity of diastema located slightly above molar plane;

conspicuous mental foramen, visible from lateral and occlusal views and subdivided in an anterior shorter and a posterior larger foramen; undeveloped masseteric ridges; superior masseteric ridge forming an undeveloped tubercle under m1; undeveloped capsular projection under coronoid ramus; delicate coronoid process isolated from condyloid by a shallow but strongly concave sigmoid notch; coronoid process broad, isolated from a less broad angular process by a shallow mandibular notch.

Upper incisors orthodont and orange; lower incisors grooved and much paler. Upper molars (Fig. 9) parallel, crested, with labial cusps higher than lingual cusps. Main cusps alternate. M1 with well-developed anteromedian flexus in specimens with little-worn molars, clearly dividing the procingulum in anterolingual and anterolabial conules, which are subequal in size; well-developed anteroloph, parallel to anterolabial conule and surpassing its outer border; anteroloph proximally isolated from anterolabial conule by anteroflexus in specimens with few-worn molars; anteroloph coalescent with anterolabial conule, surpassing the anterolabial outer border in specimens with well-worn molars; paraflexus and metaflexus substantially longer than protoflexus and hypoflexus and curved backward in its proximal half; protoflexus and hypoflexus straight; protocone and hypocone subequal in size and larger than paracone and metacone; paracone and metacone subequal in size; well-developed mesoloph, distally separated from paracone by a shallow mesoflexus and surpassing the paracone outer border; posteroloph conspicuous only in specimens with unworn molars; posteroloph fused with metacone in specimens with moderate or advanced molar wear. M2 lacking conules; well developed anteroloph isolated from paraflexus by deep and backward-curved anteroflexus; paracone subequal in size with metacone; Mesoloph separated from paracone by a shallow mesoflexus and not surpassing the paracone outer border; paraflexus and metaflexus deep and curved backward;

hypocone visually larger than protocone; posteroloph conspicuous in specimens with unworn molars and fused with metacone in specimens with worn molars; M3 with anteroloph separated from metacone by a shallow anteroflexus in specimens with few-worn molars; paracone well-developed connected to mesoloph by the paralophule; mesoloph connected to paracone; protocone larger than hypocone; shallow hypoflexus; posteroloph absent; in worn molars the only evident cusps are paracone, metacone and protocone. Lower molars (Fig. 9) parallel and crested, with lingual cusps higher than labial ones; main cusps alternate; m1 with shallow anteromedian flexid only in specimens with unworn molars; metaconid and entoconid subequal in size; hypoconid slightly larger than protoconid; well-developed protostylid, separated from protoconid by a shallow protoflexid; hypoflexid comparatively shallow and straight; metaflexid deep and strongly frontward in its proximal half; well-developed mesolophid, separated from metaconid by a shallow entoflexid; posteroflexid deep and slightly frontward; well-developed posterolophid; m2 lacking conulids; metaconid slightly larger than entoconid; protoconid and hypoconid subequal in size; undeveloped protostylid, separated from protoconid by a shallow protoflexid; hypoflexid relatively shallow and straight; mesoflexid deep and frontward; well-developed mesolophid separated from entoconid by a shallow entoflexid; posteroflexid deep and straight; well-developed posterolophid; m3 lacking conulids; protostylid distinguishable only in specimens with unworn molars; metaconid larger than entoconid; protoconid larger than hypoconid; mesolophid fused with entoconid; mesoflexid deep and frontward; hypoflexid deep and straight; well developed posterolophid, distally connected to entoconid and characterizing the posteroflexid as a fossette.

Axial skeleton composed by thirteen ribs, 19 thoracicolumbar vertebrae, 5 sacral vertebrae, 30 caudal vertebrae; presence of a conspicuous neural spine in the second thoracic vertebrae.

Scapula thin and translucent, except by borders; acromion with conspicuous concavity; deep notch, reaching about a quarter of the spine length. Humerus robust; well-developed head; lesser tubercle more prominent than greater tubercle; less-developed lateral epicondylar crest; well-developed deltopectoral crest, with slight concavity in inferior border; supratrochlear foramen present. Ulna fused to radius except by a small segment behind trochlear notch, forming a small and elongated fissure. Elongated ilium and gluteal fossa; conspicuous femoralis tuberosity; narrow pelvic symphysis. Elongated tibia, with well developed lateral and medial crests. Fibula thin, attached to tibia behind the distal half.

Stomach bilocular-discoglandular (Carleton 1973); shallow incisura angularis, surpassing slightly the esophageal opening and giving a bipartite pattern softly marked.

Comparisons. *Scapteromys meridionalis* differs from *S. aquaticus* by: (1) diploid number of 34 or 36 chromosomes (versus 2n=32 in *aquaticus*); (2) smaller size, including head-body length, tail length, and 15 craniodental measurements (Table 2); darker and grayer dorsal pelage (versus more tawny in *S. aquaticus*) (Fig. 5). *Scapteromys meridionalis* differs from *S. tumidus* by: (1) diploid number of 34 or 36 chromosomes (versus 2n=24 in *S. tumidus*); (2) smaller size (on average), including all external measurements, weight and craniodental measurements except length of tympanic bulla and breadth of occipital condyles (Table 2); (3) darker and grayer dorsal pelage (versus more tawny in *S. tumidus*) (Fig. 5); (4) laterally extended thenar pad of manus, reaching almost the height of superior proximal border of pollex nail in lateral view (versus ventral thenar pad of manus in *S. tumidus*) (Fig. 6). This distinction was evident only in fluid preserved forefeet, which included the holotype and one paratype of *Scapteromys meridionalis* and 39 specimens of *S. tumidus* in our analysis; (5) parallel edges of hamular process of pterygoid (versus edges with inner concavity in *S. tumidus*) (Fig 8). This is a consistent cranial

character; parallel edges were present in all *Scapteromys meridionalis* and a single *S. tumidus* specimen while 119 specimens of *S. tumidus* showed edges with inner concavity. Auxiliary character: proximal dark median ventral stripe was absent in the tail of specimens with a bicolored tail. Forty-nine of 55 examined specimens of *S. tumidus* with a bicolored tail showed a proximal dark median ventral stripe, while the other 12 specimens had a uniform dark tail; *S. aquaticus* showed less conspicuous bicolored pattern, but the median ventral stripe was evident in all adults. Observation: young specimens of *S. aquaticus* and *tumidus* are darker and grayer than subadults and adults, resembling the color pattern of *S. meridionalis*. However, all subadults and adults of *S. tumidus* specimens examined had a conspicuous tawny-gray pattern, clearly distinguishable from *S. meridionalis*. Some adult specimens of *S. aquaticus* were grayer than *S. tumidus* but conspicuously tawnier than the taxon described.

Karyotype. Individuals of the new species had a diploid number (2n) of 34/36 chromosomes and an autosomal fundamental number (FNa) of 40 arms. The holotype showed 2n=34 and FNa=40. See above for karyotype description.

Etymology. Reference to Meridional Plateau, the geological formation where the specimens of the type series were collected.

Natural history. Specimens of *S. meridionalis* were collected in palustrine open areas (swamps and flooded grasslands) of the Meridional Plateau, Atlantic Forest domain, mainly in soggy soils with predominance of *Eryngium pandanifolium* (Apiaceae) and *Baccharis* sp. (Asteraceae) (Fig.10). The species was found sympatric with the didelphid *Monodelphis dimidiata* Wagner and the sigmodontines *Akodon montensis* Thomas, *Holochilus brasiliensis* Desmarest, *Oligoryzomys flavescens* Waterhouse, *Oxymycterus nasutus* Waterhouse and an undescribed form of *Deltamys*. Despite its distribution across the Atlantic Forest domain, this

new species seems to be an inhabitant of open palustrine systems, which is characteristic of this genus.

Discussion

Scapteromys meridionalis is genetically and morphologically distinct from its congenerics. Moreover, the lineage is restricted to the biogeographic region of *Araucaria angustifolia* Forest. Levels of evolutionary divergence fall within the range reported for variation within a mammal genus (Bradley & Baker 2006), comparable to values between recognized congeneric species of sigmodontine rodents (e.g. D'Elía *et al*. 2008; Palma *et al*. 2010; Costa *et al*. 2011).

Reciprocal monophyletic clades were found, which led us to consider that it might be correspondent to karyomorphs 2n=34 (Clade 1) and 2n=36 (Clade 2). However, complete correspondence was ruled out, because Clade 2 included specimens with both 2n=34 (Água Doce, São Domingos) and 2n=36 (São José dos Pinhais). Additional information regarding diploid number from some highland specimens were registered by collectors, including those with haplotypes H2, H3 and H5, which were of the karyomorph 2n=34. In addition, some specimens of Paraná (e.g. São José dos Pinhais) were of the karyotype 2n=36 (unpublished data). D'Elía & Pardiñas (2004) pointed out that karyomorphs do not match species boundaries and therefore should not be used to diagnose *Scapteromys* species. Thus, the different karyotypes found in *S. meridionalis* (divergent only by a single centric fusion in 2n=34) likely represent a simple intraspecific polymorphism, which was already described in other sigmodontine rodents (e.g. Nachman 1992; Fagundes *et al*. 1998; Weksler & Bonvicino 2005).

Evolutionary distance between Clade 1 and Clade 2 was 2.8%, i.e. almost half of the distance between the new taxon and either *S. aquaticus* or *S. tumidus* found in our analysis (ca.

5%). D'Elía & Pardiñas (2004) found a range of nucleotide substitutions from 3.6% to 4.7% between populations of the two main recovered clades that were attributed to *S. aquaticus* and *S. tumidus*, while the highest observed substitutions within the clades (intraspecific divergence) was 1%. Considering the use of the same molecular marker (cyt-*b*), it is noticeable that the divergence between *S. meridionalis* sub-clades in our study is closer to an intra-specific pattern rather than an interspecific divergence level previously determined for *Scapteromys* (D'Elía & Pardiñas 2004). Moreover, we did not find any morphological difference fixed among specimens of the sub-clades. Thus, we are cautious to consider these groups as an evolutionary single unit and suggest a currently ongoing diversification process of *S. meridionalis* lineages. However, the differences accumulated so far are not enough to represent distinct species yet but rather may be considered distinct subspecies of *S. meridionalis*.

***Scapteromys* history.** Data from the present work provide additional insights to the evolutionary history of this member of the tribe Akodontini. The cyt-*b* tree reveals a *Scapteromys* clade showing a dichotomy between the sister species *S. aquaticus* and *S. tumidus* on the one hand and *S. meridionalis* on the other hand. Freitas *et al*. (1984) based on G-band analysis, also suggested two independent evolutionary routes for *Scapteromys* diversification. These authors established four karyotypic forms (2n=36, 34, 32 and 24), all of them with 40 autosomal arms. The highland forms (2n=34 and 36), herein assigned to *S. meridionalis* (considering the presence of karyotyped specimens showing 2n=34 and 36 in the type-series) share three biarmed pairs, while a centric fusion produced an additional pair in 2n=34. The 2n=24 karyomorph (*S. tumidus*) is marked by rearrangements and biarmed elements distinct from those present in the 2n=34 and 36 forms. This karyomorph is probably derived from 2n=32 (*S. aquaticus*) by four centric fusions (Freitas *et al*. 1984). The cyt-*b* tree topology presented

herein supports the previous hypothesis for *Scapteromys* diversification based on cytogenetic data.

Morphological diagnosis. The pelage coloration seems to be a useful character to distinguish between *S. meridionalis* and its congenerics. Young specimens are dark gray in the three species, whereas adults have distinct patterns of pelage coloration, with *S. aquaticus* and *tumidus* markedly more tawny and *S. meridionalis* grayer and darker on the dorsum of all examined specimens. Another external character, the thenar pad of the manus, was markedly extended laterally in the fluid-preserved forefeet of two *S. meridionalis* types, compared to the ventral thenar pads of 39 *S. tumidus* fluid-preserved specimens. This character, however, is applicable only in fresh and forefoot fluid preserved specimens, once the dissecting effect of skin preparation can mischaracterize the plantar surfaces. The state of this character was underdetermined in *S. aquaticus* due to the lack of fluid preserved structures in the examined collection series. The frequency of occurrence of the hamular process types of the pterygoid was also consistent in its distribution between the new taxon and *S. tumidus*, and can be useful in species identification by skull analysis. However, the parallel condition of hamular process borders is shared with *S. aquaticus*. Other characters, more variable in their frequencies, can be used in complementary comparisons, but not as single diagnostic characters. The U-shaped fronto-parietal suture (illustrated in Massoia & Fornes 1964 and D'Elía & Pardiñas 2004) was present in 13 *S. meridionalis* types (including the holotype), while two specimens had a W-shaped pattern and three others had irregular forms. Despite the U-shaped suture being most frequent in *S. meridionalis*, this pattern was also present in 68 of 188 (36%) of *S. tumidus* specimens analyzed for this character. Nevertheless, all analyzed topotypes of *S. tumidus* showed the W-shaped pattern. This character was also variable in *S. aquaticus*, in which U-shaped

pattern is predominant (D'Elía & Pardiñas 2004; present study). The quadrate shape of the mesopterygoid fossa and the presence of a median palatine process cannot be considered diagnostic characters. Although predominant frequencies of these character states were found in *S. aquaticus* and *S. tumidus*, they are still highly polymorphic (D'Elía & Pardiñas 2004; present study) and cannot provide a reliable morphological discrimination. Tail coloration is also a polymorphic character (see *comparisons* in Results section) and should be used as auxiliary information on species diagnostics.

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APPENDIX

Specimens examined. Abbreviations of institutions are as follows: American Museum of Natural History, New York, United States (AMNH); Municipal de Ciencias Naturales Lorenzo Scaglia, Mar de Plata, Argentina (FCM); Fundação Universidade Regional de Blumenau, Blumenau, Brazil (FURB); and Museu de Ciências Naturais, Universidade Luterana do Brasil, Canoas, Brasil (MCNU), Universidade Federal do Paraná, Curitiba, Brasil (UFPR).

Scapteromys aquaticus (42) ARGENTINA: Buenos Aires: Paraná River Delta: Punta Lara - Ensenada (FCM 33, 34, 36, 183, 184, 186, 315, 342, 344, 347, 354, 356, 358, 359, 361, 381, 478, 489-491, 494- 497, 500, 556, 558, 596, 1144), Palaje Talaveja (FCM 411, 412, 414, 472, 474), Arroyo San Felipe (FCM 416), Zarate - Arroyo Pesqueria (FCM 330, 331), locality not specified (FCM 214). Santa Fe: La Matilde: San Javier- Alejandra (FCM 1511). Entre Rios: Arroyo Sagastume (FCM 27), Medanos (FCM 916).

***Scapteromys meridionalis*.** (23). BRAZIL: Rio Grande do Sul: São Francisco de Paula (FURB 20156, 20252 [paratypes], 20253 [holotype], 20285, 20286 [paratypes]). Santa Catarina: Água Doce (FURB 9972 [paratype]), Campo Alegre (FURB 12658 [paratype]), Campo Belo do Sul (FURB 15129, 1516627 [paratypes]), Doutor Pedrinho (FURB 18676, 18993, 18996, 18999, 20044 [paratypes]), Passos Maia (FURB 18908 [paratype]), São Domingos (FURB 12309 [paratype]). Paraná State: Candói (FURB 15979 [paratype]), São José dos Pinhais (UFPR 985, 1036, 1052, 1059, 1063 [paratypes]), São Mateus do Sul (FURB 15364 [paratype]).

Scapteromys tumidus (244). BRAZIL: Rio Grande do Sul: Nova Santa Rita (MCNU 2760), Ilha do Pavão (MCNU 842, 843), Camaquã (MCNU 2968, 2970, 2971), Dom Pedrito (MCNU

780, 1981, 1989, 1990, 1992-1994, 1999-2004), Bujuru (MCNU 3370-3377), Capão do Leão (MCNU 2991, 2997, 3023, 3369), 14 km N São José do Norte (MCNU 3378-3389, 3403), Pedro Osório (MCNU 2959-2961, 2966, 2972, 2974, 3018-3020), Mata da Estrada Velha (MCNU 598, 625, 931, 932, 1485, 1486, 1525, 1781-1792, 1794, 2475), Quinta (AMNH 235430-235434), Lagoa Verde (MCNU 3390-3402), Arroio Grande (MCNU 700-703, 705, 706, 1507, 2479), Jaguarão (MCNU 1954, 2097, 2115, 2150, 2151), Lagoa Mangueira (FURB 20287, 20288, 20289, 20290, MCNU 2948, 2965, 2969, 2973, 2975, 3017, 3022), Josapar (MCNU 2748-2757, 2763, 2764, 3404, 3405), Botafogo (MCNU 467, 683, 684, 686, 1507, 3406-3410). URUGUAY: Treinta y Tres: 16 km SW Tacuari River (AMNH 206313-206323, 206327-206330, 232503). Rocha: 22 km SE Lascano (AMNH 206266-206267). Soriano: 3 km E Cardona (AMNH 206268-206289, 206291, 206292, 206295, 206296, 206298-206302, 206310-206312). Canelones: mouth of Del Bagre stream (AMNH 232501, 232502), 36 km E Montevideo by Interbalnearia Highway (AMNH 206208-206210, 206216-206225, 206230-206235, 206239, 206240, 232504), mouth of Tropa Vieja stream (AMNH 232503). Montevideo: Santa Lucia River (AMNH 188784, 206259-206261, 206263, 206264). Maldonado: Maldonado River mouth (AMNH 206243-206250, 206252-206258).

TABLE 1. Specimens used in the molecular analysis.

Taxa	Collection site	N	Map #	Voucher	Haplotype	Genbank Accession Number	Reference
Ingroup							
Scapteromys meridionalis							
	BR: Rio Grande do Sul, São Francisco de Paula	5	1	FURB6640, 20252, 20253; 20156, 20285	H5	KF536932	This study
		1		FURB 20286	H2	KF536929	This study
	BR: Santa Catarina, Campo Belo do Sul	1	2	FURB15166	H3	KF536930	This study
		1		FURB15129	H2	KF536929	This study
	BR: Santa Catarina, Água Doce	1	3	FURB 9972	H11	KF536938	This study
	BR: Santa Catarina, São Domingos	1	6	FURB12309	H1	KF536928	This study
		1		FURB6347	H9	KF536936	This study
	BR: Santa Catarina Doutor Pedrinho	4	4	FURB18676; 18993; 18996; 20044;	H4	KF536931	This study
		1		FURB 18999	H8	KF536935	This study
	BR: Paraná, Candói	1	10	FURB15979	H10	KF536937	This study
	BR: Santa Catarina Campo Alegre	1	5	FURB12658	H6	KF536933	This study
	BR: Paraná; São José dos Pinhais	11	8	UFPR1007; 1036; 957; 958; 959; 972; 985; 1052; 1057; 1059; 981	H6	KF536933	This study
		1		UFPR 1063	H7	KF536934	This study
Scapteromys aquaticus							
		1		LBCE12350			Bonvicino et al. (2013)
		2				AY275131-32	D'Elía (2003)
		18				AY445534- 51	D'Elía & Pardiñas (2004)
Scapteromys tumidus							
		1				AF108669	Smith & Patton (1999)
		1				AY275133	D'Elía (2003)
		16				AY445552-53; 55-62; 64-68; 70	D'Elía & Pardiñas (2004)
Outgroup							
Kunsia tomentosus		1				AY275120	D'Elia (2003)

TABLE 2. External and cranial measurements (in millimeters) of *Scapteromys* sp. n**.,** *Scapteromys aquaticus* and *Scapteromys tumidus* (mean [X] ± one standard deviation [SD], the observed range [in parenthesis], and the sample size [n]). *P* indicates significance levels of differences obtained by *t*-test (*Scapteromys* sp. n**.** x *S. aquaticus* / *Scapteromys* sp. n**.** x *S. tumidus*; matrices composed by all *Scapteromys* . sp. n**.**, *S. aquaticus* listed in Appendix (*n* varying from 9 to 42; weight not included) and *S. tumidus* topotypes [AMNH 206243-20658; *n* varying from 12 to 16], *p < 0.05, **p < 0.01, ***p < 0.001, ns = not significant.

	Scapteromys sp. nov.			*Scapteromys aquaticus*			*Scapteromys tumidus*			*P*
	X ± SD	Range	N	X ± SD	Range	n	X ± SD	Range	n	
HBL	135 ± 13	(114 - 135)	15	159 ± 12	(111 - 188)	42	159 ± 14	(126 - 208)	101	***/***
LT	114 ± 9	(101 - 125)	15	136 ± 16	(94 -165)	42	140 ± 10	(110 - 161)	94	***/***
Ear	22 ± 3	(13 - 26)	15	21 ± 2	(17 - 24)	41	23 ± 2	(17 - 29)	98	ns/**
HF	34 ± 2	(32 - 37)	15	35 ± 2	(32 - 39)	42	40 ± 2	(36 - 45)	101	ns/***
BI	2.57 ± 0.18	(2.28 - 3.21)	23	3.19 ± 0.38	(2.42 - 4.31)	16	3.33 ± 0.31	(2.27 - 4.29)	215	***/***
LMR	5.62 ± 0.21	(5.17 - 5.87)	23	6.48 ± 0.30	(6.13 - 7.02)	16	6.59 ± 0.28	(5.91 - 7.29)	209	***/***
LN	13.88 ± 0.81	(12.56 - 15.88)	23	14.62 ± 1.53	(12.29 - 18.56)	16	15.45 ± 1.11	(12.33 - 17.75)	215	ns/**
BR	5.67 ± 0.40	(5.18 - 7.12)	23	6.93 ± 1.19	(5.85 -10.87)	16	6.74 ± 0.36	(5.33 - 7.88)	222	***/***
LTB	5.77 ± 0.37	(4.51 - 6.45)	23	5.90 ± 0.52	(5.36 - 6.92)	10	5.77 ± 0.31	(4.99 - 6.42)	195	ns/ns
BB	15.11 ± 0.37	(13.76 - 15.82)	23	15.64 ± 1.22	(12.19 - 17.13)	11	15.89 ± 0.54	(14.53 - 17.38)	200	*/***
BZB	3.27 ± 0.33	(2.64 - 3.86)	23	3.72 ± 0.37	(2.76 - 4.62)	16	3.77 ± 0.31	(2.82 - 4.75)	224	***/***
LIB	5.18 ± 0.21	(4.82 - 5.49)	23	5.76 ± 0.32	(5.26 - 6.44)	15	5.46 ± 0.23	(4.91 - 6.24)	224	***/***
LR	13.22 ± 0.91	(11.23 - 15.19)	23	13.17 ± 1.37	(10.93 - 15.64)	14	13.56 ± 1.11	(9.37 - 16.07)	213	ns/ns
DFM	6.89 ± 0.47	(5.56 - 7.73)	23	7.45 ± 0.62	(6.62 - 8.78)	15	7.63 ± 0.52	(6.42 - 8.76)	206	***/**
BFM	1.77 ± 0.22	(1.46 - 2.11)	23	1.89 ± 0.23	(1.56 - 2.24)	16	2.01 ± 0.22	(1.73 - 2.58)	215	**/***
CIL	33.26 ± 1.68	(29.62 - 36.27)	23	35.13 ± 3.11	(30.07- 40.01)	9	36.82 ± 2.09	(30.42 - 42.08)	199	*/***
TL	35.74 ± 1.56	(32.92 - 39.07)	23	38.09 ± 3.11	(32.89 - 43.43)	9	38.77 ± 2.03	(32.44 - 43.57)	197	**/**
PL	5.57 ± 0.42	(4.82 - 6.46)	23	6.52 ± 0.77	(5.5 - 8.01)	19	6.5 ± 0.5	(5.4 - 8.0)	219	***/***
LD	8.73 ± 0.71	(7.54 - 10.03)	23	8.83 ± 0.89	(7.11 - 10.46)	15	9.42 ± 0.79	(6.91 - 11.18)	219	ns/ns
OL	11.33 ± 0.37	(10.13- 12.02)	23	11.92 ± 0.77	(10.64 - 13.08)	10	12.47 ± 0.62	(10.44 - 14.59)	220	**/***
HB	12.29 ± 0.42	(11.12 - 12.97)	23	12.93 ± 0.44	(12.18 - 13.61)	10	13.08 ± 0.43	(11.89 - 14.12)	186	***/***
BZ	17.43 ± 0.48	(16.31 - 18.13)	22	18.71 ± 1.29	(16.72 - 21.42)	11	19.18 ± 0.93	(16.17 - 21.52)	204	***/***

LIF	7.44 ± 0.56	(5.81 - 8.53)	23	7.71 ± 0.69	(6.41 - 9.07)	15	8.18 ± 0.62	(6.02 - 9.93)	220	ns/**
BIF	2.63 ± 0.31	(2.02 - 3.13)	23	2.54 ± 0.24	(2.18 - 2.86)	15	2.25 ± 0.23	(1.65 - 2.84)	223	ns/***
BOC	8.01 ± 0.33	(7.54 - 8.43)	22	8.86 ± 0.32	(8.48 - 9.62)	9	8.91 ± 0.33	(8.02 - 9.73)	200	***/***
Weight	77 ± 12	(50 - 91)	15		211	1	118 ± 28	(50 - 165)	61	***

continuação da tabela 2

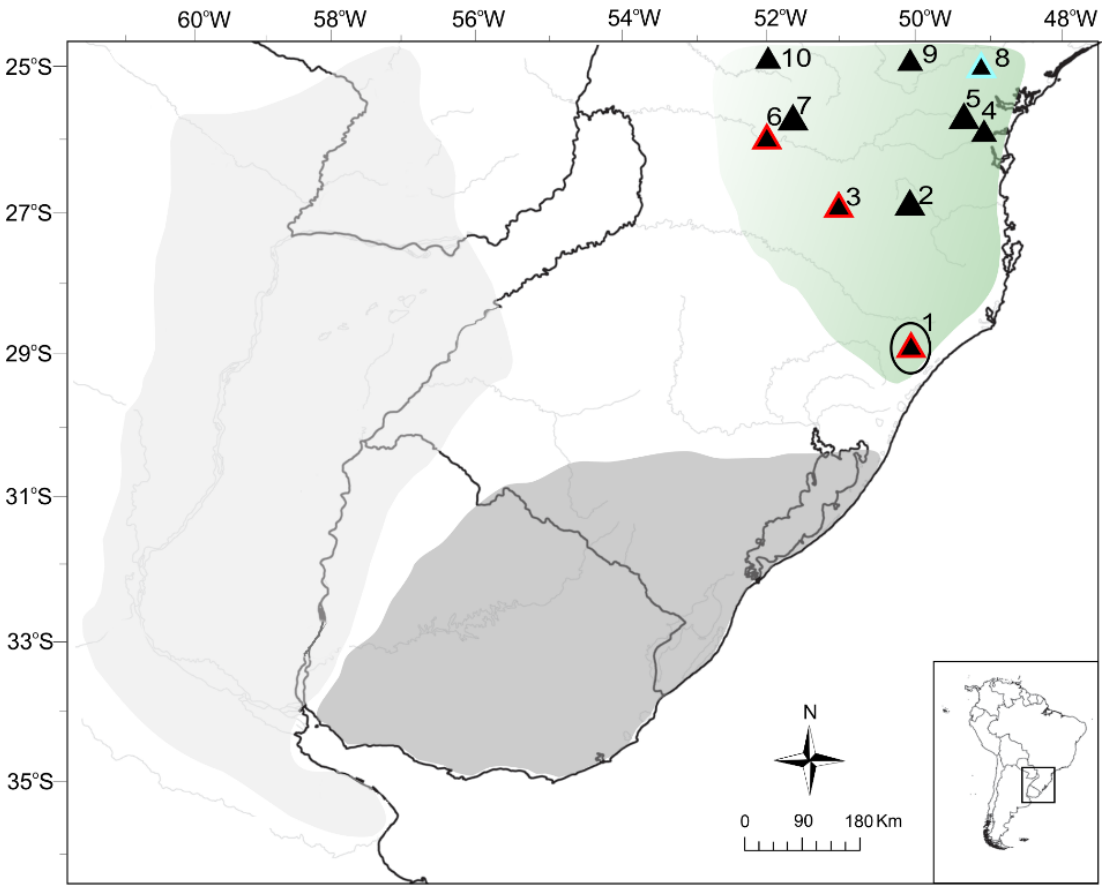

FIGURE 1. Distributional range of the three *Scapteromys* species (Green, *Scapteromys* **sp. n**.; light gray, *S. aquaticus*; dark gray, *S. tumidus*) and collecting sites of *Scapteromys* sp. n**.** (Brazil: 1 – Rio Grande do Sul: São Francisco de Paula , 2 – Santa Catarina: Campo Belo do Sul, 3 – Santa Catarina: Água Doce, 4 – Santa Catarina: Doutor Pedrinho, 5 – Santa Catarina: Campo Alegre, 6 – Santa Catarina: São Domingos, 7 – Santa Catarina: Passos Maia, 8 – Paraná: São José dos Pinhais, 9 – Paraná: São Mateus do Sul, 10 – Paraná: Candói). Red open triangles represent localities with presence of 2n=34, and blue open triangles indicate the presence of 2n=36. The circled triangle indicates the type locality.

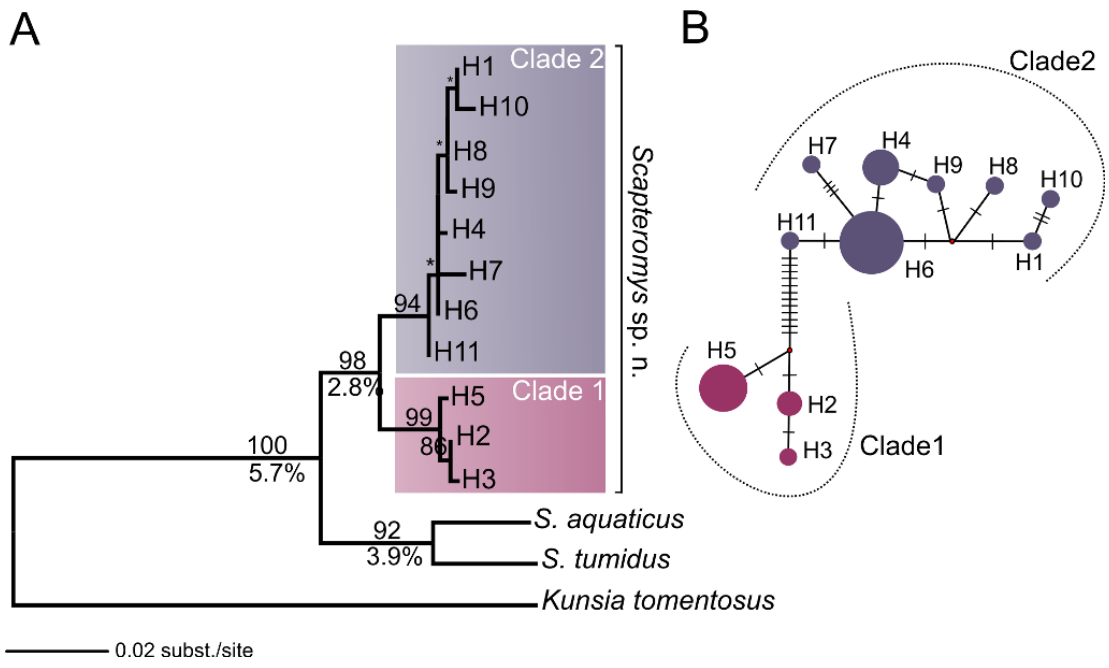

FIGURE 2. Phylogenetic relationships of *Scapteromys*. A)ML tree of *Scapteromys* sp. n. haplotypes and relationships within *Scapteromys* reconstructed using 801 base pairs of cyt-*b* sequences and rooted with *Kunsia tomentosus*. Numbers above branches represented bootstrap support (asterisk indicates values < 50%) and below indicate genetic divergence based on K2P distances B) Median joining network of the 11 cyt-*b* haplotypes of *Scapteromys* sp. n**.** Areas are proportional to haplotype frequencies. Colors indicate major clades identified from the phylogenetic tree. Small black bars represent nucleotide differences between haplotypes. Small red circles represent missing haplotypes.

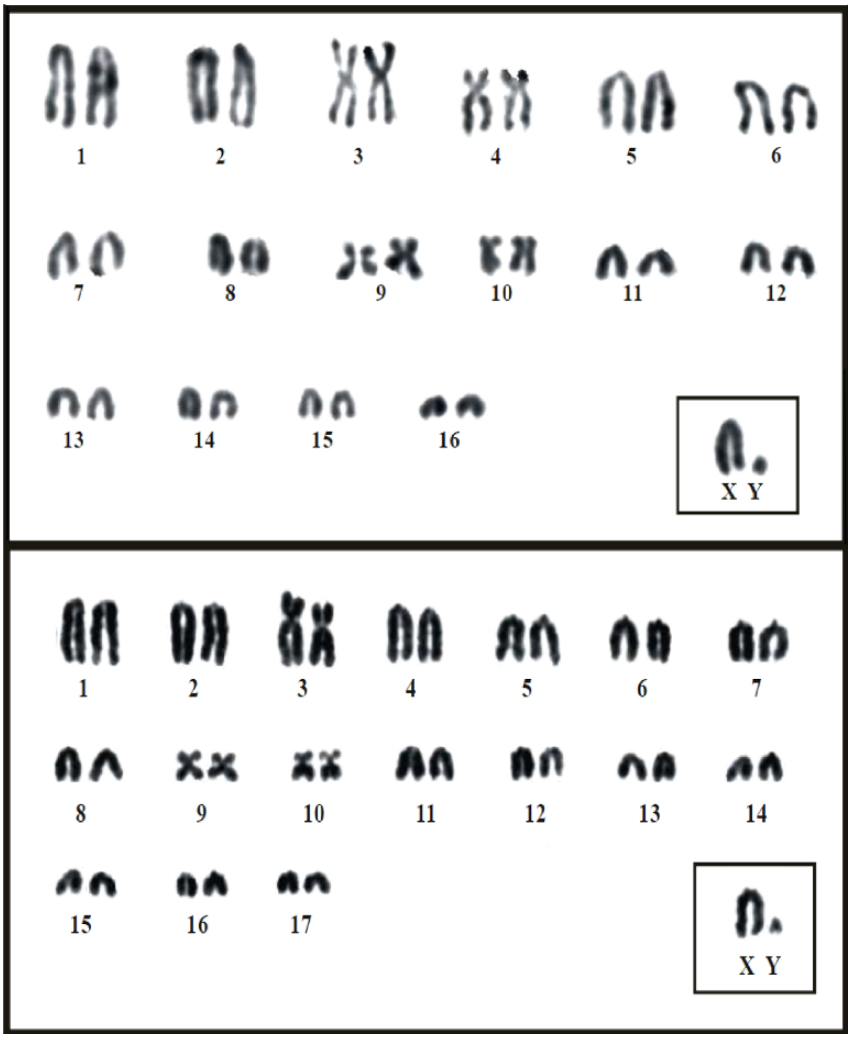

FIGURE 3. Karyotype (conventional Giemsa standing) of *Scapteromys* sp. n. Above: 2n=34, FNa=40; below: 2n=36, FNa=40.

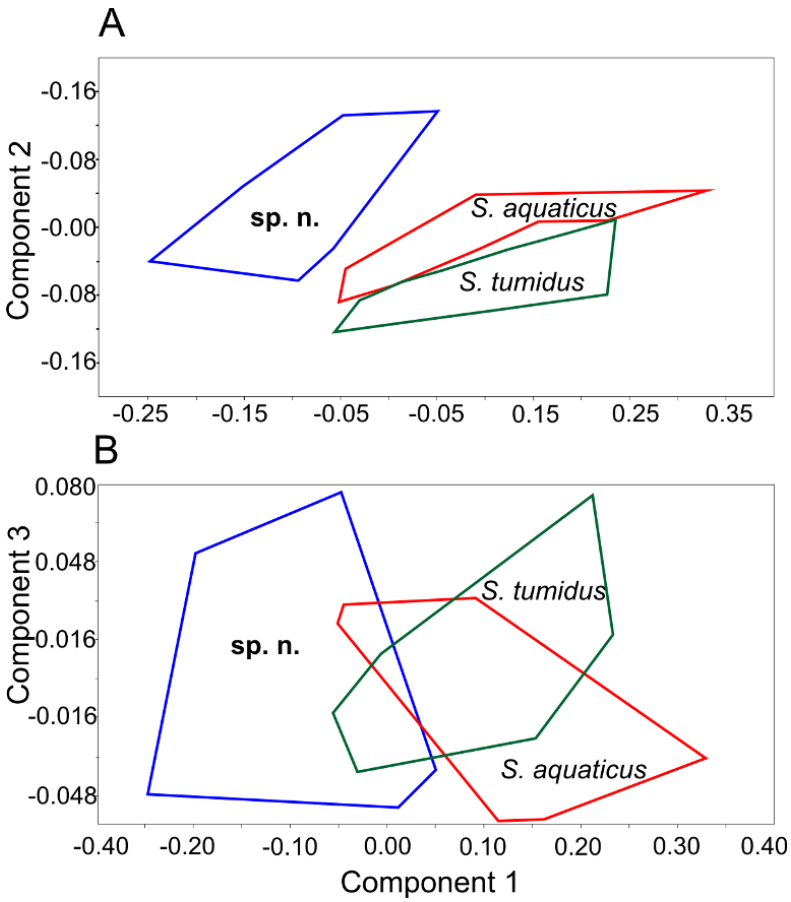

FIGURE 4. Convex hull for specimens scores of *Scapteromys* species on the principal components 1 and 2 (A) and 1 and 3 (B) extracted from the variance-covariance matrix of 21 cranial measurements.

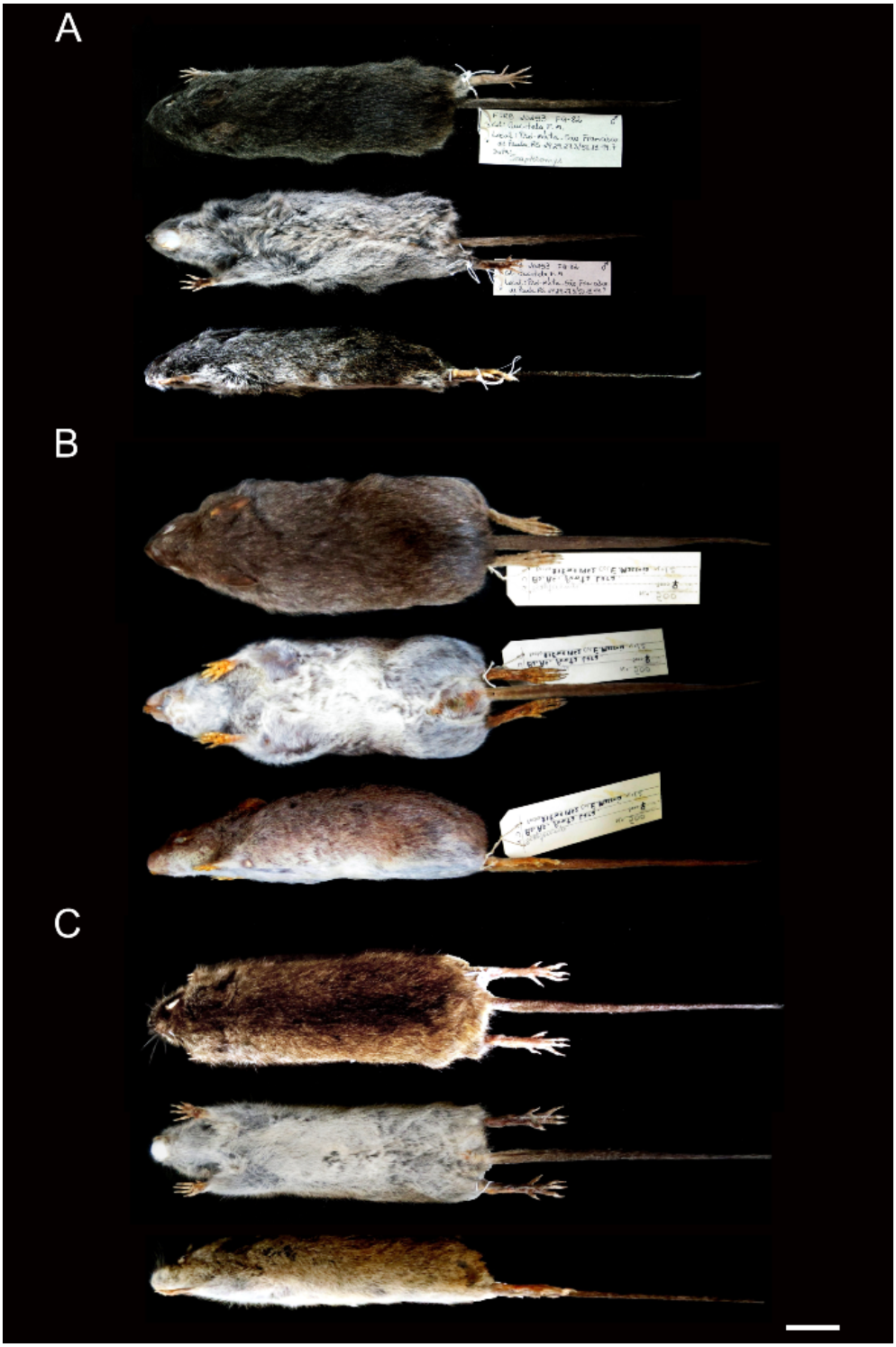

FIGURE 5. Dorsal (upper), ventral (middle) and bottom (bottom) views of skins of (A) *Scapteromys* sp. n. (holotype, FURB 20253), (B) *S. aquaticus* (FCM 500) and (C) *S. tumidus* (FURB 20290). Bar = 10 mm.

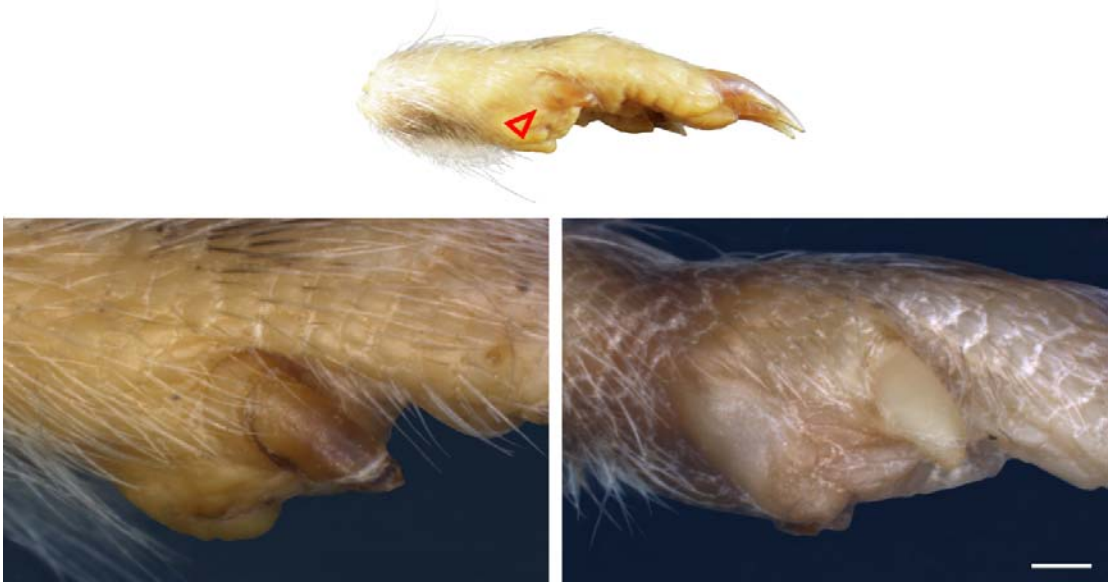

FIGURE 6. Thenar pad of manus (indicated by the red arrow) and its respective morphology in *Scapteromys* sp. n. (holotype, FURB 20253) (right) and *S. tumidus* (FURB 20290) (left). Bar = 1 mm.

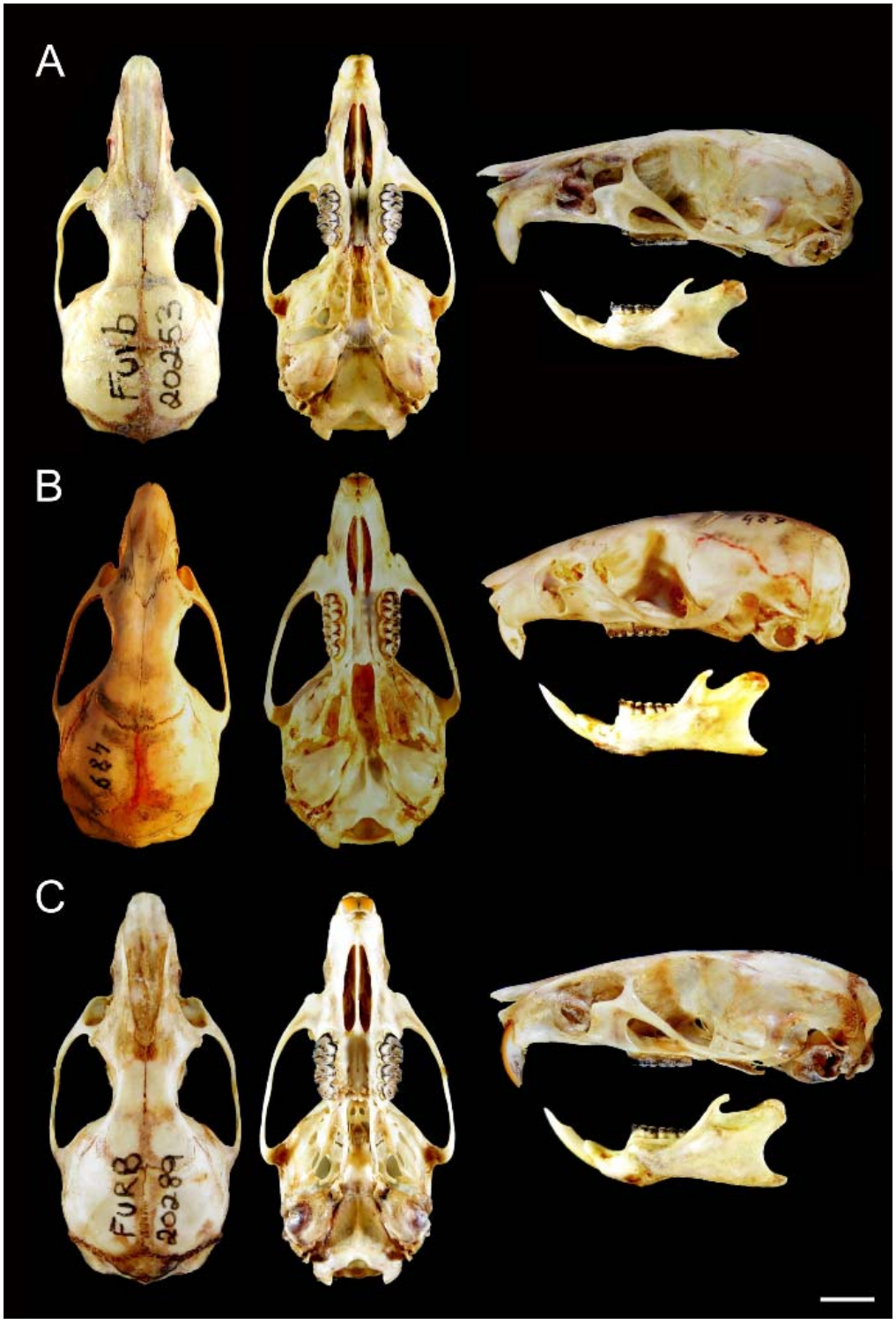

FIGURE 7. (A) Dorsal (left), ventral (middle) and lateral (right) views of skull and labial view of mandible (bottom right) of (A) *Scapteromys* sp. n. holotype (FURB 20253). (B) *S. aquaticus* (FCM 489) and (C) *S. tumidus* (FURB 20289). Bar = 5 mm.

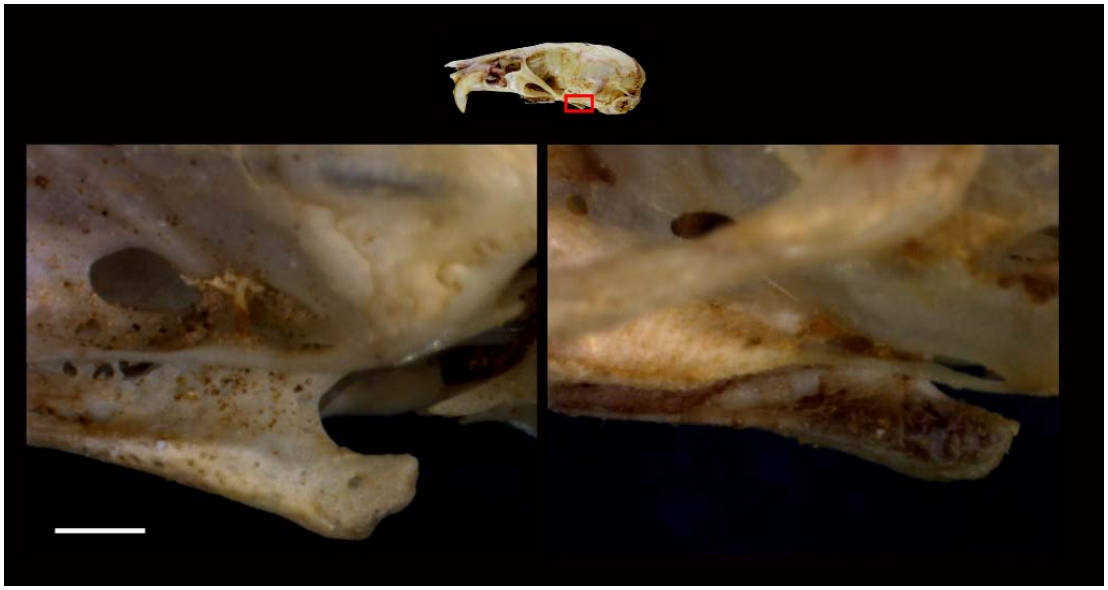

FIGURE 8. Hamular process of pterygoid (red square) and its respective shapes in *S.* sp. n. (holotype, FURB 20253) (left) and *S. tumidus* (MCNU 3378) (right).Bar = 1 mm.

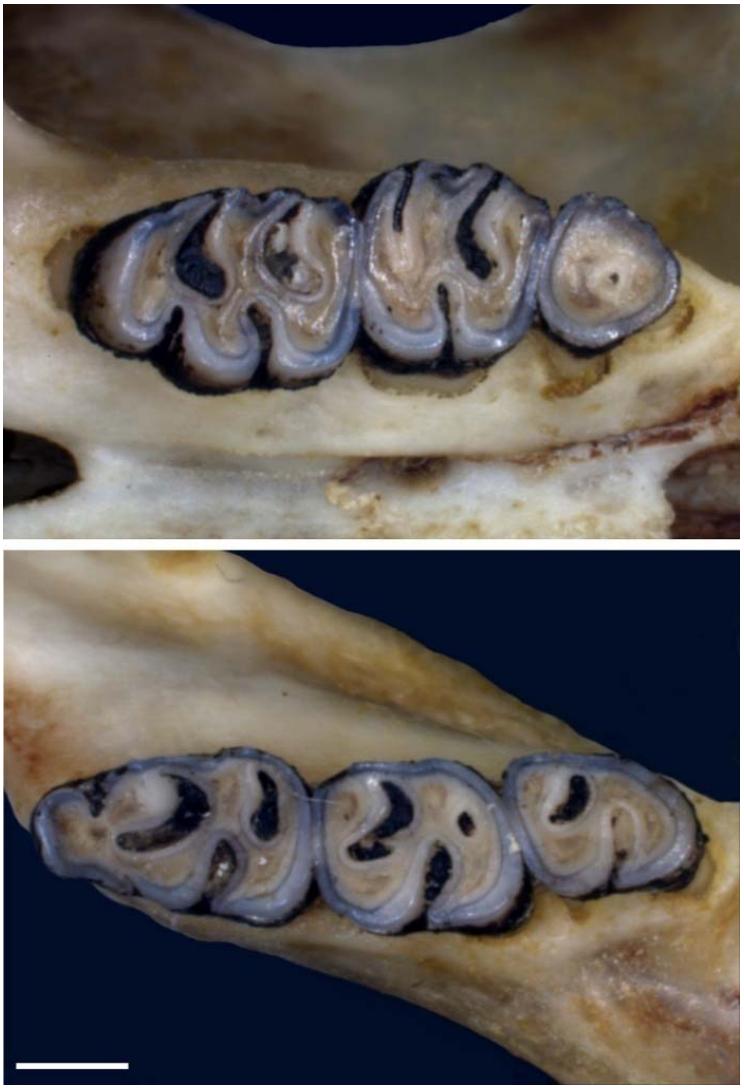

FIGURE 9. Occlusal views of upper and lower molar series of *Scapteromys* sp. n. holotype (FURB 20253). Bar = 1 mm.

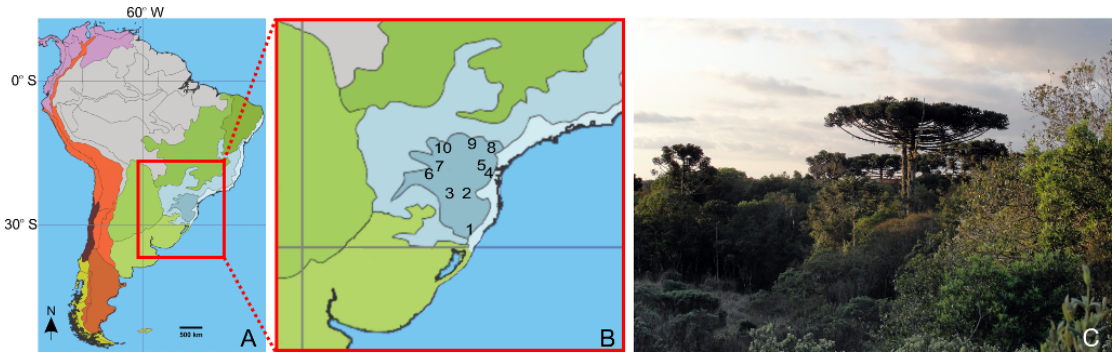

FIGURE 10. Biogeographic regions and provinces in South America (A), with details of the study area (in dark blue) and sampling sites of *Scapteromys* sp. n. [For details see Material and Methods] (B); and *Araucaria angustifolia* Forest habitat (C). Sources: A, adapted from Morrone (2006); C, Photography of *Araucaria angustifolia* Forest: G.L. Gonçalves.

CAPÍTULO III

Artigo submetido: *Journal of Mammalogy*

Running heading: Phylogeography of the swamp rat

Geometric morphometrics of skull shape combined with Bayesian DNA sequence analysis reveals intraspecific patterns in the swamp rat *Scapteromys tumidus* (Cricetidae: Sigmodontinae)

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Microevolution is a process of small evolutionary changes within populations. In geologically dynamic regions, such as the northern portion of the South American Pampas biome, this process can be driven by dispersal and vicariant events. We examined the morphological and genetic variation in the pampean rodent *Scapteromys tumidus* through skull geometric morphometrics and analyses of cytochrome *b* (cyt *b*) sequences. The geometric descriptors showed no significant differences in skull size between geographic clusters, whereas the differences in shape were highly significant. Fourteen haplotypes were identified and three main haplogroups were recovered, with the *p*-distances between them ranging from 0.5 to 1.2 %. We found a congruence between morphological and genetic divergence; samples from the Rio Grande do Sul (RS) central coastal plain were the most differentiated, in both the morphological and the genetic analyses. Haplotypes from these samples plus one locality on the border of the RS Precambrian Shield comprised the most basal mitochondrial clade, estimated to have diverged about one million years ago. The Patos Lagoon estuary seems to represent a geographic barrier to historical gene flow, because no haplotype was shared between localities in the central and southern segments of the RS coastal plain. We found significant although moderate correlations between geographic and morphological distances, supporting the isolation-by-distance model. Because these correlations were not strong, it is possible that other factors (environmental heterogeneity and/or geographic barriers) may have affected *S. tumidus* skull differentiation. Bayesian analyses

detected a demographic expansion event, which coincided with the formation of the RS coastal plain. The geological evolution of the RS coastal plain may have shaped the phylogeographic break, morphological differentiation and demographic events in *S. tumidus.*

Keywords: genetic differentiation, geological evolution, microevolution, Pampas, phylogeography, skull shape.

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Small evolutionary changes within populations are the basis of the formation of new species, as first proposed by Charles Darwin in 1859. Accordingly, marked genotypic and phenotypic variation characterize different evolutionary units over a geographic area (dos Reis et al. 2002a), which can result in speciation (Avise 2000). Description of patterns of variation in genetic and morphological characters within and among populations is an essential basis for defining the boundaries of independent evolutionary units in nature (Fernandes et al. 2009). However, contrary to expectations, a few examples exist of small differences in both morphological (especially considering presumed conserved traits) and genetics within natural populations (Marchiori et al. 2014).

Particularly in rodents, most studies have focused on closely related (Fadda and Corti 1998, 2000, 2001; Beolchini and Corti 2004; Mullin et al. 2004a, 2004b; Barčiová and Macholán 2006), or genus-level taxa, regarding morphology. Only a small number of studies have explored intraspecific variation in morphological characters (dos Reis et al. 2002a, b; Fornel et al. 2010;

Yazdi et al. 2011). Surprisingly, such studies have repeatedly found that variations correspond to distinct geographic units, concordant with an isolation-by-distance model, leading to differentiation (Wright 1943). Despite this, integrative studies examining geographic variation in morphology and genetics within populations are few, particularly investigations of phylogeographic patterns at a regional scale.

A marked example of an assumed conserved morphology is found in the water rat *Scapteromys tumidus* (Waterhouse 1837), endemic to the *Campos* grassland biome (Overbeck et al. 2007) in southern Brazil (RS) and all of Uruguay (Freitas 1984; D'Elía and Pardinãs 2004; Musser and Carleton 2005). The systematics of *S. tumidus* has been the focus of several studies, mainly to evaluate its relationships with the Argentinean form originally described as *S. aquaticus* by Thomas (1920) (Massoia and Fornes 1964; Hershkovitz 1966; Freitas et al. 1984; D´Elia 2003; D´Elia and Pardiñas 2004). However, no integrative data exist for this species, considering the lack of information on morphological and genetic characters from populations across the broad distributional range in the *Campos* biome which is essential to infer the evolutionary history of this taxon and to evaluate the existence of ongoing speciation processes. Elucidation of microevolution and regional patterns in *S. tumidus* is particularly interesting because populations on the RS coastal plain (northernmost *Campos*) are settled in a region with an especially dynamic geological history. This situation is particularly suitable for the investigation of dispersal events and other historical demographic processes that might affect the way in which this variation is allocated within the species, as demonstrated in other taxa (Montes et al. 2008; Mader et al. 2013; Fregonezi et al. 2013; Lopes et al. 2013; Longo et al. 2014).

The *Campos* biome is composed of grasslands and mosaics of grassland/forest that have likely covered central and southern South America since the Eocene (ca. 35 mya) (Bauermann et

al. 2011). In some regions of the continent, according to palynological analyses (Strömberg 2011) these open landscapes have predominated since the Miocene-Pliocene transition (ca. 5 mya). The long period of existence of these open physiognomies enabled the unfolding of evolutionary events that culminated in speciation and endemism (Bencke et al. 2009; Boldrini et al. 2009; Bauermann et al. 2011).

Despite the lower biological richness of the *Campos* in relation to other South American biomes (Amazon Forest, Atlantic Forest, Cerrado) (Cabrera and Willink 1980; Overbeck et al. 2007), the region hosts a high level of biodiversity and endemism (Overbeck et al. 2007; Bencke 2009). The northern *Campos* (southern region of RS and all of Uruguay) experienced a dynamic geological history, which shaped conspicuous hydrographical and geomorphological elements (Windhausen 1931; Bossi and Navarro 1988; Tomazelli and Villwock 2000). The inland region is part of Pre-Cambrian and Mesozoic spills and sedimentary basins (Bossi and Navarro 1988; Chemale-Jr. 2000; Phillip et al. 2000), while most of the coastal region was shaped by transgressive-regressive marine events during the Quaternary (Tomazelli and Villwock 2000). These events are related to Pleistocene-Holocene climatic oscillations and deglaciations; each transgressive maximum corresponded to a maximum deglaciation. In RS, the last three transgressive-regressive events produced four depositional systems, which originated Patos-Mirim, the largest lagunar complex in South America (Vieira 1984). This geological continuity between Precambrian and Paleo-Mesozoic elements and the "recently" shaped elements of the Cenozoic coastal zone (and its role as a potential barrier to dispersal of some organisms) makes the northern area particularly appropriate for investigation of the effects of evolutionary and past demographic processes on natural populations.

In the present study we characterized the patterns of morphological and genetic variation in populations of *S. tumidus* across the *Campos* biome, in order to evaluate the genetic structure and infer the evolutionary history of this species. Geometric morphometrics of skull shape and size was used to detect small variations. Sequences of the cytochrome *b* (cyt *b*) gene from mitochondrial DNA (mtDNA) were used to recover the matrilineal phylogeographic history. We address to what extent the geological or other historical factors have shaped the current distribution of the water rat, and might have influenced intraspecific patterns of variations. Also, isolation-by-distance likely had a significant effect on differentiation, particularly regarding morphology, and we therefore evaluated it as well.

MATERIAL AND METHODS

Study area and specimens.— The sampling localities were distributed across the entire range of *S. tumidus* in the *Campos* biome in Uruguay and southern Brazil (RS) (Fig. 1). Nearby localities (separated by distances ≤ 5 km) were grouped, which resulted in 19 collection sites or geographic operational units. A total of 180 samples of *S. tumidus* from 18 localities were included in the geometric morphometric analysis (Appendix I), and 107 specimens from 13 localities were used in the molecular approach (Table 1; Appendix I). Tissue samples are deposited at the Genetics Department of the Universidade Federal do Rio Grande do Sul, Porto Alegre (TRO) and the Museo Nacional de Historia Natural, Montevideo (EMG and SVC). Skulls, skins and fluid-preserved carcasses are stored in the Museu de Ciências Naturais at the Universidade Luterana do Brasil, Canoas (MCNU) and the Museo Nacional de Historia Natural, Montevideo.

Geometric morphometrics.— Variations in skull size and shape were analyzed in 180 specimens (107 males and 73 females) for the dorsal view, 172 specimens (102 males and 70 females) for the ventral view, and 167 specimens (101 males and 66 females) for the lateral view. All specimens were adults according to criteria defined by Barlow (1969), with the third molar fully erupted and showing signs of wear. Undamaged skulls were photographed in the dorsal, ventral and left lateral views. Damaged skulls were photographed only in the view(s) with the landmark regions intact. The photographs were taken with a digital camera with 10 megapixels (4608 × 3456) of resolution, zoom of 2.1 × and external accessory illumination. A total of 22 two-dimensional landmarks were collected in the dorsal view, 23 in the ventral view and 22 in the lateral view of the skull (Fig. 2; Appendix II). Coordinates of landmarks were obtained using the software TPSDig2, version 2.16 (Rohlf 2010). Coordinates were superimposed using a generalized Procrustes analysis (GPA) algorithm (Dryden and Mardia 1998). The size of each skull in each view was estimated from its centroid size, which is defined as the square root of the sum of the squares of the distances of each landmark from the centroid (Bookstein 1991). Because we used different distances in the skull photographs, the images were corrected for size by IMP CoordGen6f (Sheets 2001), using a scale factor.

In order to improve the statistical analyses and graphical visualization, neighboring localities without a potential barrier to gene flow between them (large rivers, lagoons or estuary mouths) were grouped in 8 geographic clusters, named: Campanha Gaucha (CG), West shore of Mirim Lagoon (WM), São José do Norte *restinga* (SJN), Rio Grande *restinga* (RG), Soriano (SO), Maldonado (MA), South of Mirim Lagoon (SM) and Canelones/Montevideo (CM) (Fig. 1). Sexual dimorphism was evaluated using two groups, formed by the set of male or female

specimens of all populations. Size differences between sexes for each view were tested with a Student's *t*-test on log-transformed centroid size. The ANOVAs were used to compare the log centroid size for geographic clusters, and Tukey's test was used for multiple comparisons. The difference in shape between sexes for each view was tested with a multivariate analysis of variance (MANOVA) on shape variables. Because the MANOVA detected no significant differences in shape, and small significant differences in size only for the dorsal view between sexes (see results), males and females were pooled for the analysis of geographic variation. A consensus configuration of landmark configurations of all clusters was computed for the dorsal, ventral and lateral views, based on the orthogonal generalized least-squares Procrustes method (Rohlf and Slice 1990). The hypothesis of existence of significant differences in skull shape among geographic clusters was tested with the MANOVA method for each view. Then, we performed a pairwise MANOVA to identify pairs of clusters that showed significant differences in shape. Linear discriminant analysis (LDA) was performed, calculated on a subset of PCs to compute the leave-one-out cross-validation to calculate the percentages of the correct classification for each cluster defined a priori (Baylac and Friess 2005). A Canonical Variate Analysis (CVA) was carried out as a discriminant analysis, with all clusters pooled and the distribution of clusters visualized in the multivariate space of the first two canonical axes.

The hypothesis that the isolation-by-distance model underlies the observed intraspecific variation was tested in two ways. First, we used morphometric data to calculate the Mahalanobis distance matrix (morphological distances) between pairwise clusters. The morphological similarities and dissimilarities between clusters were visualized in a phenogram resulting from the Neighbor-joining method, using Mahalanobis distances for each view. Second, a Mantel test was performed, which analyzed the correlation between the two matrices, geographic distances

and Mahalanobis morphological distances, using 10 000 random permutations. The geographic distance matrix was based on linear distances of each locality, constructed by the software Geographic Distance Matrix Generator, version 1.2.3 (Ersts 2009). For the statistical analyses and to generate the graphs, we used the "R" language and environment for statistical computing, version 2.14.1 (R development Core Team; http://www.r-project.org) and the libraries "ape" (Paradis et al. 2004), "ade4" (Dray and Dufour 2007), "MASS" (Venables and Ripley 2002), and "stats" (R development Core Team). For geometric morphometrics, the procedures were carried out using the "Rmorph" library for R (Baylac 2008).

Molecular analysis.— Tissue samples (liver and muscle) were obtained from specimens collected in the field from 2010 through 2012, according to the guidelines of the American Society of Mammalogists (Sikes et al. 2011). Genomic DNA was extracted from samples (preserved in 96% ethanol at -20ºC) using the CTAB method modified from the protocol of Doyle and Doyle (1987). The cyt *b* (801 bp) gene was amplified by PCR using primers MVZ05 and MVZ14 and conditions as described by Smith and Patton (1993). 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. Haplotypes were deposited in GenBank under the accession numbers KJ622177-KJ622190. Electropherograms were inspected and aligned using Codon Code Aligner (CodonCode Corp., USA). Measurements of mtDNA diversity, including the mean number of pairwise differences (Nei 1987), definitions of haplotypes, and haplotype

diversity, 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/sharenet.htm). Pairwise estimates of gene flow (Hudson et al., 1992) were calculated in Arlequin 3.5 (Excoffier and Lischer 2010). In order to infer hierarchical population structure, analyses of molecular variance (AMOVA) were performed considering both genetic distances between haplotypes and frequencies, using Arlequin 3.5 (Weir and Cockerham 1984). An AMOVA was performed across groups (identified in the Bayesian analysis), to test the effect of geographical proximity on the partitioning of the genetic variance. We constructed a Bayesian phylogenetic tree using BEAST 2.0.1 (Drummond and Rambaut 2007) based on all haplotypes observed in *S. tumidus*. Individuals of *S. tumidus* and *S. aquaticus* from GenBank were also incorporated, and the tree was rooted with *Kunsia tomentosus*, likewise obtained from this public database (Appendix I). The substitution model used was GTR with 4 gamma categories. We carried out a Yule branching rate prior, with rate variation across branches assumed to be uncorrelated and log-normally distributed (Drummond et al. 2006). We calibrated one prior based on node ages estimated by Parada et al. (2013) for akodontines, particularly the tMRCA of *Scapteromys* + *Kunsia* (ca. 5 mya). Each Markov chain Monte Carlo was run for 10^7 iterations (burn-in 20,000), with parameters sampled every 1,000 steps. Examination of the Markov chain Monte Carlo samples using Tracer 1.4.8 (Rambaut and Drummond 2007) suggested that the independent chains were each adequately sampling the same probability distribution, and the effective sample sizes for all parameters of interest were greater than 200.

In order to examine the effect of past population dynamics on genetic structure, we estimated the time of the most recent common ancestor (tMRCA) for haplogroups previously

identified in the phylogenetic analysis, by applying the Bayesian skyline approach in the program Beast 1.7.2. The molecular model of evolution (HKY) was provided by MrModeltest 2.2 (http://www.abc.se/~nylander). We used a strict molecular clock with the substitution rates = 0.006 (SE $1.79e^{-5}$) substitutions per site per million years as normal priors (calculated in this study), with 100 000 000 Markov chain Monte Carlo (MCMC) simulations sampled every 1000 chains. The first 20% 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 1.4.8 (Drummond and Rambaut 2007), and effective sample sizes (>200) were used to evaluate MCMC convergence within chains.

RESULTS

Variation in skull shape and size.— Student's *t*-test showed significant differences in centroid size between sexes for the dorsal view (t = -2.172, d.f. = 1, $P = 0.031$), while the differences were non-significant for the ventral (t = -0.1753, d.f. = 1, $P = 0.082$) and lateral (t = -1.205, d.f. = 1, $P = 0.23$) views. MANOVA results indicated no significant differences in shape between males and females for any view (dorsal: $\lambda_{Wilks} = 0.735$; $F_{(1, 178)} = 1.101$; $P = 0.330$; ventral: $\lambda_{Wilks} = 0.675$; $F_{(1, 170)} = 1.306$; $P = 0.124$; lateral: $\lambda_{Wilks} = 0.706$; $F_{(1, 165)} = 1.306$; $P = 0.272$). ANOVA showed no significant differences in centroid size among geographic clusters (dorsal: $F_{(7, 172)} = 1.583$; $P = 0.143$; ventral: $F_{(7, 164)} = 1.79$; $P = 0.092$; lateral: $F_{(7, 159)} = 1.362$; $P = 0.225$). The MANOVA tests detected significant differences in skull shape among geographic clusters for all views (dorsal: $\lambda_{Wilks} = 0.0044$; $F_{(7, 172)} = 3.482$; $P = 2.2 \times 10^{-16}$; ventral: λ_{Wilks}=

0.0063; $F_{(7, 164)} = 2.793$; $P = 2.2 \times 10^{-16}$; lateral: $\lambda_{Wilks} = 0.0053$; $F_{(7, 159)} = 2.988$; $P = 2.2 \times 10^{-16}$). The pairwise MANOVA showed significant differences in 22 of 28 comparisons between geographic clusters. The *F* values and significance levels are shown in Table 2.

For the dorsal view, the first two canonical variation axes (CV1 and CV2) explained 45.9% (26.6% and 19.3%, respectively) of the differences in shape among the geographic clusters. The projection of individual scores showed an overlap of the clusters, except for cluster SJN, which was completely separated along negative CV1 (Fig. 3a). Cluster MA was discriminated in the positive CV1, with a small overlap with the geographically closest cluster CM. Specimens from cluster SJN showed a slender rostrum, a broader zygomatic arch (with anteriorly positioned squamosal root) and a less-inflated braincase. Specimens in cluster MA showed a shorter rostrum, a narrower zygomatic arch (posteriorly positioned squamosal root) and a more-inflated braincase (Fig. 3a). For the ventral view, the first two canonical variate axes explained 48.7% of the shape variation (CV1: 26.4%; CV2: 22.3%). We observed a marked overlap of the geographic clusters, with a clear discrimination of cluster SJN along the positive CV2 (Fig. 3b). Cluster SJN specimens were associated with a posteriorly positioned squamosal zygomatic root and slightly more-anterior tympanic bulla (Fig. 3b). In lateral view, the first two CV axes explained 42.8% of the shape variation (CV1: 25.4%; CV2: 17.4%). The cluster SJN sample was discriminated on the negative CV1, while the Uruguayan clusters SO, MA and SM grouped on the positive CV1 (Fig. 3c). Cluster SJN specimens tended to have an anteriorly extended premaxillary, posterior squamosal zygomatic root, and broader braincase and tympanic bulla. The Uruguayan clusters on the positive CV1 tended to have a shorter anterior premaxillary region, anterior squamosal zygomatic root, and less-robust braincase and tympanic bulla (Fig. 3c). The percentages of correct classification for each view of the skull are shown in Table 3.

The Mantel test showed significant correlations between the morphological and geographic matrices for the dorsal ($R = 0.55$, $P = 0.011$), ventral ($R = 0.51$, $P = 0.015$) and lateral ($R = 0.47$, $P = 0.021$) views of the skull. These results suggest that there are regular and significant associations between skull shape and linear geographical distances among the groups. For the dorsal view, the greatest Mahalanobis distance was found between clusters SJN (RS central coastal plain) and MA (Maldonado; type locality) (Fig. 4a). The phenogram topology showed a clear division of two groups, one formed by the Uruguayan cluster branches and the other formed by the RS cluster branches. For the ventral view, the greatest Mahalanobis distance was found between clusters SJN and SO (Fig. 4b). The phenogram topology did not show the geographic structure recovered from the dorsal view, with some Uruguayan and RS cluster branches in intercalated positions. For the lateral view, the greatest Mahalanobis distance was also found between clusters SJN and MA (Fig. 4c). The phenogram also showed the topology of a division into two groups, one formed by the Uruguayan (South) and other by the RS (North) cluster branches, but with differences in the relative positions of some clusters in relation to the dorsal phenogram.

Phylogeny, haplogroups and Bayesian skyline plot.— A total of 14 haplotypes were identified from 124 sequences of *S. tumidus* (Table 1). Bayesian analysis (BA) recovered the monophyly of the species (Bayesian posterior probability [BPP] =1) (Fig. 5), and an internal division into three haplogroups (Group I [GI], Group II [GII] and Group III [GIII]), which was strongly supported. Group I (BPP=1) was formed by localities on the RS central coastal plain (Bujuru and São José do Norte) and Camaquã, west of Patos Lagoon, RS. Group II (BPP=1) was restricted to Flores and Rivera, in south-central and southwestern Uruguay, respectively. Group

III (BPP=0.83) was formed by 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 respectively. Group I was separated from GII by five mutational events. Group II was separated from GIII by two mutational events. Genetic divergence (p-distance and F_{ST}) between haplogroups was low (Table 2).

In the Bayesian time-calibrated phylogenetic analysis, the divergence between *S. tumidus* and its sister species, *S. aquaticus*, was estimated to have occurred around 2.38 mya (Fig. 5). The most basal internal divergence, between GI and the clade formed by GII + GIII (Fig. 5, Node A), was estimated at ca. 1 mya. Within this clade, the most basal dichotomy was related to the divergence between GII and GIII (Fig. 5, Node B), and was estimated at ca. 650 kya. Group I showed a single shallow differentiation, estimated to have occurred ca. 200 kya. Within GIII, the divergences between the haplotypes were estimated at ca. 330 and 170 kya. Group III showed a basal dichotomy (Fig. 5, Node C), estimated at ca. 420 kya, and which resulted in two clades, formed by two and seven haplotypes respectively. The divergence times between the haplotypes within these clades were estimated as between 330 and 10 kya.

The haplotype network indicated a pattern of population expansion. One central haplotype (H3) was widely distributed, which is present in eight localities through southern Uruguay, the southern RS coastal plain, and west of São Gonçalo Channel (Fig. 6). Haplotype 2 was present in three localities in the southern RS coastal plain and one locality on the border of the RS Precambrian Basement, west of Mirim Lagoon. Haplotype 5 occurred in two localities in southern Uruguay and two localities on the southern RS coastal plain. Haplotype 6 occurred in two localities in the southeastern Uruguayan coastal zone. Haplotype 4 was present in two localities in southern and southeastern coastal Uruguay. Haplotype 14 occurred in one locality

west of the São Gonçalo Channel and another locality on the southern RS coastal plain. Haplotype 8 occurred in two Uruguayan localities, one in the north-central and the other in the southwest. Haplotype 9 was found in two neighboring localities of the Quaternary central RS coastal plain. Three haplotypes (H11, H12 and H13) occurred exclusively in southwestern Uruguay. Haplotype 10 was found at only one locality on the border of the RS Precambrian Basement west of Patos Lagoon. Haplotype 1 was restricted to one locality in southern Uruguay (Fig. 6).

The AMOVA showed that differentiation among haplogroups corresponded to 75.6 % of the total variation, while 24.4 % of the total variation was allocated within haplogroups ($p<0.001$).

The Bayesian skyline plot (Fig. 7) showed a long period of slow demographic decline, which extends from 1.1 mya to 650 kya. After that, demographic stability persisted until 350 kya, followed by an accentuated expansion that continued until around 50 kya. The last 50 thousand years were marked by a slower demographic expansion.

DISCUSSION

Phylogeographic history of S. tumidus.— Of the 14 identified haplotypes, 10 occurred in Uruguayan territory, and 7 were exclusive to Uruguayan localities. Flores Department, located in southwestern Precambrian Uruguay (Bossi and Navarro 1988), showed four haplotypes, the highest local diversity observed. These data, together with the estimated time of divergence between the main haplogroups and available information on the geological age of the formations in the sampling area (Bossi and Navarro 1988; Philipp et al. 2000; Tomazelli and Villwock 1996,

2000), suggest that the center of diversification of *S. tumidus* occurred in the Precambrian-Mesozoic, in central-western Uruguay. Then, from this region, the species dispersed eastward, colonizing eastern Uruguay and southeastern RS, including the new habitats of the Quaternary depositional systems in coastal zones. Similar patterns of populations originating inland that expanded to the Quaternary subtropical South American coastal plains were found for the akodontine *D. kempi* (Montes et al. 2008), the ctenomyid *Ctenomys talarum* (Mora et al. 2013) and the solanacean plant *Calibrachoa heterophylla* (Mäder et al. 2013).

The analysis of *S. tumidus* cyt *b* haplotypes showed an internal structure, where three major supported groups were identified. The genetic divergences between these haplogroup pairs were similar to the distances found between haplogroups within the sympatric *Deltamys kempi* (Montes et al. 2008) and lower than distances found for the akodontine species *Akodon montensis* (Valdez and D'Elía 2013), *Blarynomys breviceps* (Ventura et al. 2012), *Thaptomys nigrita* (Ventura et al. 2010) and *Necromys* (D'Elía et al. 2008). Haplogroup I, found on the border of the RS Precambrian Shield and the RS Quaternary central coastal plain, consisted of a deep divergent unit in the molecular analysis. In the geometric morphometric approaches, samples from the RS central coastal plain (cluster SJN) segregated completely from other clusters in the dorsal-view CVA. Pairwise MANOVA including cluster SJN also gave high *F* values, indicating a differentiation in skull shape of these samples. The analysis of the geographic distribution of haplogroup I, together with its position in the BA tree, revealed an interesting pattern. It is remarkable that haplotype H9, derived from this most ancestral recovered 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). In contrast, the divergence time for the *S. tumidus* Group I lineage was estimated at 1 mya. This pattern suggests the occurrence of an early event of east-northeastward dispersal in *S. tumidus* evolutionary history. This dispersive lineage was recovered in the derived haplotypes H9 and H10, 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 III) 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 from both segments, it is possible that there was no historical gene flow between the populations separated by the Patos Lagoon estuary, and this may 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 et al. 2000; Costa 2003; Ventura et al. 2012).

Haplogroup II is restricted to central Precambrian-Mesozoic Uruguay (Bossi and Navarro 1988). Haplotype H8 showed a historical gene flow along the sampling localities, while local diversification events apparently occurred in Flores Department, represented by haplotypes H11 and H12. As mentioned above, Precambrian-Mesozoic Uruguay may represent the center of *S. tumidus* diversification, and hosted the genetic ancestral pool from which all of the haplotypes recovered here were derived.

Haplogroup III is spread over Uruguay and the southern RS coastal plain (Rio Grande *restinga*), and diverged around 650 kya, in the Middle Pleistocene. The occurrence of sympatric lineages in this group indicates a high historical gene flow (Avise 2000) between Uruguay and the southern coastal plain and the border of the Precambrian Shield in RS. In this case, the major hydrographic elements along these formations (coastal streams, lagoons, channels and paleochannels; Vieira 1983, 1984; Bossi and Navarro 1988; Weschenfelder et al. 2008) seem not to have represented geographic barriers to gene flow, unlike, apparently, the Patos Lagoon estuary. Within haplogroup III, the widely distributed haplotypes H3 and H5 probably evolved on the Uruguayan Shield, and these lineages later spread to sedimentary barriers of the southern RS coastal plain. Haplotype H2, occurring on the RS southern coastal plain and the border of the Precambrian Shield, could have evolved on any of these geological formations, considering its time of divergence (100 kya). The occurrence of haplotypes exclusive to one (H1, H7, H13) or two (H4, H6, H14) nearby localities also indicates local diversifications of these haplogroups.

Past demographic inferences.— The Bayesian analysis showed a long period of slow demographic decline, followed by an event of recent expansion. This expansion intensified from 350-300 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 Martinez-Lanfranco 2010; present study) and 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.

Isolation by distance.— The geometric morphometrics analysis detected marked geographic variation in the shape of the *S. tumidus* skull. The geographic and morphological distances were significantly correlated, as predicted by the isolation-by-distance model. This model for population genetics predicts that the gene flow is inversely related to the increase in

geographic distance, enhancing the genetic divergence between "subgroups" (Wright 1943). Interpopulational variation in skull morphology correlated with geographic distance has been found previously in echimyid (dos Reis et al. 2002a, b), murid (Fadda and Corti 1998, 2000; Yazdi et al. 2011) and ctenomyid (Fornel et al. 2010) rodents.

The phenogram generated from the dorsal-view data (the most informative on geographic variation) showed spatially structured morphological relationships, where the greatest distance was between the samples from the Rio Grande do Sul central coastal plain (cluster SJN) and the samples from Maldonado, Uruguay (cluster MA), in the far northeast and extreme southern parts of the species' range, respectively. This geographical structure was also recovered from the lateral-view data. Since geographic distance limits dispersal, the rate of migration is higher between neighboring populations than between distant populations (Wright 1943; Bradburd et al. 2013). This pattern was apparent in our data from the dorsal and lateral views, where the smallest morphological distances were found between nearby localities.

Cluster SJN was segregated from all other clusters in the canonical analysis for the dorsal-view data, indicating greater differentiation of these populations. Since the geographic pattern of haplotype richness indicates that *S. tumidus* may have originated on the Uruguayan Shield, the populations from the Rio Grande do Sul central coastal plain, far from the species' center of origin, may have resulted from long-range dispersal events and undergone morphological differentiation due to reduced gene flow and genetic drift. According to Wright (1943), in "long range populations" or "subdivisions", there is a tendency toward the fixation of certain alleles, so that the differentiation within subdivisions is slight, whereas the differentiation between subdivisions is marked, forming a pattern of "local differentiations" resulting from the fixation of different alleles among populations. Additionally, in populations that disperse over long

distances, the fixation of exclusive mutant alleles also enhances local differentiation. Therefore, it is possible that genetic traits unique to the RS central coastal-plain populations have enhanced the morphological differentiation. Nevertheless, because the morphological and geographic distances were not strongly correlated, it is expected that other factors were also associated with the geographic variation in *S. tumidus* skull shape. In addition to the geographic distance, the existence of possible geographic barriers, as discussed above, can also hamper the gene flow (Fernandes et al. 2009; Fornel et al. 2010). The influence of the spatial heterogeneity of the area covered by the study should not be ruled out. The skull structure is presumably under high environmental pressure, as it is related to feeding and sensory functions (Voss 1990). In heterogeneous landscapes, the genetic responses of populations occur directly, via selective processes, or indirectly, via demographic (effective sizes, subdivisions) and stochastic events. These responses produce the genetic variation in species (Pamilo 1988). Subsequent phenotypic variation among populations results from selection and genetic variation, plus phenotypic plasticity (Adkinson 1995). Phenotypic plasticity comes about when, under different environmental conditions, more than one phenotype is expressed from the same genotype (Pigliucci 2001). This last factor, therefore, is also associated with spatial heterogeneity. Morphological differentiation in skulls of the murids *Gerbillus* (*Dipodillus*) (Abiadh et al. 2010), *Mastomys* (Lalis et al. 2009) and *Taterillus* (Dobigny et al. 2002) Muridae), in the ctenomyid *Ctenomys minutus* (Fornel et al. 2010) and in the Japanese shrew-mole *Urotrichus talpoides* (Wilson 2013) was attributed, among other factors, to environmental heterogeneity. An analysis of the shape configurations (Fig. 3) showed that variable structures in the *S. tumidus* skull comprise the zygomatic arch, the squamosal root of zygomatic arch and the braincase. The zygomatic arch is the site of attachment of some of the masticatory muscles (mandibular

adductors): the masseter complex is connected along the maxillary root and median section of the arch; the anterior part of the zygomatic-mandibularis is attached to the maxillary root, while the posterior part of this complex is connected from the squamosal root to the glenoid fossa. Another muscle complex involved in mastication is the temporalis. The anterior and posterior temporalis are large muscles attached to the lateral braincase and mandible coronoid process, with the anterior temporalis passing through the posterior orbital fossa (Voss 1988). The visually variable regions in the *S. tumidus* skull, however, are associated with attachment of the muscles involved in mastication. Thus, together with isolation-by-distance effects, it is possible that local variations in components of the habitat and natural history (e.g., soil type, feeding habits) may also have affected *S. tumidus* skull differentiation. The effects of this and other ecological variables associated with environmental heterogeneity remain to be investigated.

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Resumo: Microevolução é um processo de pequenas modificações evolutivas dentro de populações. Em regiões geologicamente dinâmicas, tal como a porção norte do bioma sul-americano Pampas, este processo pode guiado por eventos de dispersão e vicariância. Nós examinamos a variação morfológica e genética no roedor pampeano *Scapteromys tumidus* através de análises da morfometria geométrica craniana e análises e seqüências de citocromo *b* (cyt *b*). Os descritores geométricos não mostraram diferenças significativas no tamanho do crânio entre os clusters geográficos, enquanto que diferenças na forma foram altamente significativas. Quatorze haplótipos e três principais clados foram identificados, com distâncias *p* variando entre 0.5 e 1.2 %. Nós encontramos congruência entre divergência morfológica e genética: amostras da porção central da planície costeira do Rio Grande do Sul (RS) foram as mais diferenciadas em ambas análises morfológica e genética. Haplótipos destas amostras e de mais uma localidade na borda do escudo pré-cambriano do RS compreenderam o clado mitocondrial mais basal, cuja divergência foi estimada em cerca de um milhão de anos atrás. O estuário da lagoa dos Patos parece representar uma barreira geográfica ao fluxo gênico histórico, uma vez que nenhum haplótipo foi compartilhado entre localidades nas porções medianas e sul da planície costeira do RS. Nós encontramos correlação significativa e moderada entre distâncias geográficas e morfológicas, corroborando o modelo de isolamento-por-distância. No entanto, uma vez que esta correlação não foi alta, é possível que outros fatores (heterogeneidade ambiental e/ou barreiras geográficas) estejam também relacionados à diferenciação craniana em *S. tumidus*. Análises bayesianas detectaram um evento de expansão demográfica coincidente com a formação da planície costeira do RS. A evolução geológica da planície costeira do RS, portanto, pode ter moldado a quebra filogeográfica, a diferenciação morfológica e eventos demográficos em *S. tumidus*.

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Appendix I.— Specimens used in molecular ([a]) and skull geometric morphometric ([b]) analyses and housed in: American Museum of Natural History, New York, United States (AMNH); Museu de Ciências Naturais, Universidade Luterana do Brasil, Canoas, Brazil (MCNU); Departamento de Genética, Universidade Federal do Rio Grande Sul, Porto Alegre, Brazil (TRO); Museo Nacional de Historia Natural, Montevideo, Uruguay (EMG and SVC). Sequences obtained from GenBank (*) are presented as voucher number/GenBank access number.

Kunsia tomentosus – (LHE 1620/AY275121)

Scapteromys aquaticus – URUGUAY: Río Negro Department, Las Cañas (GD609/AY445551)

Scapteromys tumidus - BRAZIL: Rio Grande do Sul: Camaquã (MCNU 2968[a], 2970[a], 2971[a]). Dom Pedrito, Fazenda São Demétrio: MCNU 1989[b], 1990[b], 1992[b], 1993[b], 1994[b], 1997[b], 1998[b], 1999[b], 2000[b], 2001[b], 2002[b], 2003[b], 2004[b]. São José do Norte: Bujuru (MCNU 3370[ab], 3371[b], 3372[ab], 3373[ab], 3374[ab], 3375[ab], 3376[a], 3377[a]; TRO 2103[a], TRO 2104[a], TRO 2105[a]), 14 km N São José do Norte (MCNU 3378[ab], 3379[ab], 3380[b], 3381[ab], 3382[ab], 3383[ab], 3384[a], 3385[b], 3386[ab], 3387[a], 3388[ab], 3389[b]; TRO 2100[a], TRO 2101[a],TRO 2102[a]). Arroio Grande (MCNU 700[b], 701[b], 702[b], 705[b], 2479[b]). Jaguarão (MCNU 1954[b], 2097[b], 2115[b], 2150[b], 2151[b]). Capão do Leão (MCNU 2967[b], 2991[b], 3023[b], 3369[ab]). Pedro Osório (MCNU 2959[a], 2960[b], 2961[b], 2966[ab], 2972[b], 2974[b], 3018[ab], 3019[ab], 3020[a], 3021[a]). Rio Grande: Mata da Estrada Velha (MCNU 598[b], 625[b], 931[b], 932[b], 1485[b], 1486[b], 1525[b], 1781[b], 1782[b], 1783[b], 1785[b], 1786[b], 1787[b], 1788[b], 1789[b], 1790[b], 1791[b], 1792[b], 1794[b]), Quinta (AMNH 235431[b], 235433[b], 235434[b], 235452[b]), Lagoa Verde (MCNU 3390[ab], 3391[ab], 3392[ab], 3393[ab], 3394[ab], 3395[ab], 3396[ab], 3397[a], 3398[ab], 3399[ab], 3400[ab], 3401[b], 3402[ab] 3403[ab], 3408[a]; 3474[b]; TRO 2106[a]). Santa Vitória do Palmar: Lagoa Mangueira (MCNU 2917[a], 2948[ab], 2965[ab], 2969[ab], 2973[ab], 2975[ab], 3017[ab], 3022[ab]), Josapar (MCNU 2747[ab],

2748[ab], 2749[ab], 2750[ab], 2751[ab], 2752[ab], 2753[ab], 2754[ab], 2755[ab], 2756[ab], 2757[ab], 2762[ab], 2763[ab], 2764[ab], 2765[ab]; 3404[ab], 3405[a]), Botafogo (MCNU 467[b], 683[b], 684[b], 686[b], 1507[b], 3406[ab], 3407[ab], 3408[ab], 3409[ab], 3410[ab]). URUGUAY: Rivera: Estancia La Quemada 1 (*GD 639/AY445565[a], *GD 640/AY445566[a], *GD 643/AY445567[a], *GD 644/AY445568[a]), Estancia La Quemada 2 (*GD 638/AY445564[a], *GD 649/AY445569[a], *GD 650/AY445570[a]). Treinta y Tres, 16 km SW Tacuari River mouth (AMNH 206314[b], 206317[b], 206318[b], 206319[b], 206320[b], 206322[b], 206327[b], 206328[b], 206330[b]). Rocha: Refugio de Fauna Laguna de Castillos (EMG 1957[a], 1994[a], 1988[a], 1999[a]; SVC[a] 089[a], 099[a], 101[a]), La Paloma, Arroyo La Palma Ruta 15 km 10 (*CA 628/AY445559[a], *MNHN 4269/AY445560[a], *MNHN 4266/AY445561[a], *MNHN 4264/AY445562[a], *MNHN 4263/AY445563[a]). Soriano: Cardona, 3 km E Cardona (AMNH 206269[b], 206272[b], 206273[b], 206274[b], 206275[b], 206276[b], 206278[b], 206280[b], 206282[b], 206283[b], 206285[b], 206287[b], 206288[b], 206292[b], 206296[b], 206298[b], 206299[b], 206300[b], 206301[b], 206302[b], 206312[b]). Canelones: mouth of Del Bagre stream (AMNH 232501[b]), Pando, mouth of Tropa Vieja stream (AMNH 232503[b]), 36 km E Montevideo by Interbalnearia Highway (AMNH 206209[b], 206216[b], 206218[b], 206219[b], 206220[b], 206221[b], 206223[b], 206230[b], 206231[b], 206233[b], 206234[b], 206240[b]), Rincón del Colorado (EMG 1223[a], 1236[a], 1238[a], 1243[a], 1245[a], 1252[a], 1257[a], 1258[a], 1772[a]). Montevideo: Santa Lucía River (AMNH 206259[b], 206260[b], 206261[b], 206262[b], 206263[b], 206264[b]). Maldonado: Maldonado River mouth (AMNH 206243[b], 206244[b], 206247[b], 206248[b], 206249[b], 206252[b], 206254[b], 206256[b], 206257[b], 206258[b]), Arroyo El Renegado, 3 km W Pan de Azucar (*CA 682/AY445556[a], *MNHN 3844/AY445555[a]), Las Flores (*MNHN 4288/AY445552[a], *MVZ 183268/AF108669[a]). San José: Kiyu (*GD 329/AY445558[a]), Flores: Río San José (EMG 1763[a], 1764[a], 1765[a], 1766[a], 1768[a], 1773[a]).

Appendix II.— Definition of landmarks for dorsal, ventral and lateral views of the skull of *Scapteromys tumidus* (represented in Fig. 2):

Dorsal view: 1. anteriormost point of suture between nasals; 2. anteriormost point of suture between nasal and premaxilla; 3. superiormost point of nasolachrymal capsule; 4. anteriormost point of zygomatic plate; 5. suture between nasal, premaxilla and frontal; 6. suture between nasals and frontals; 7. posteriormost margin of maximum constriction of antorbital bridge; 8. posteriormost point of suture between antorbital bridge (maxillary), lachrymal and frontal; 9. posteriormost margin of antorbital bridge process (lachrymal); 10. superiormost margin of zygomatic arch; 11. anteriormost margin of maximum constriction of squamosal root of zygomatic arch; 12. margin of maximum constriction of interorbital region (frontal); 13. anteriormost point of anterolateral process of parietal; 14. suture between frontal, squamosal and parietal; 15. suture between frontals and parietals; 16. superiormost point of braincase curvature; 17. superiormost point of suture between parietal and occipital; 18. suture between parietal, interparietal and occipital; 19. suture between parietals and interparietal; 20. posteriormost point of suture between interparietal and occipital; 21. posteriormost point of occipital margin; 22. midpoint of linear distance between landmarks 7 and 11 projected to zygomatic arch.

Ventral view: 1. anteriormost point of suture between nasals; 2. superiormost point of incisor alveolus; 3. posteriormost point of incisor alveolus; 4. anteriormost margin of incisive foramen; 5. superiormost point of nasolachrymal capsule; 6. posteriormost point of suture between premaxilla and maxilla; 7. anteriormost margin of zygomatic plate; 8. anteriormost margin of maximum zygomatic plate posterior constriction; 9. anteriormost margin of first molar alveolus; 10. posteriormost margin of incisive foramen; 11. posteriormost margin of third molar; 12. posteriormost point of suture between palatines; 13. superiormost margin of zygomatic arch; 14.

posteriormost margin of maximum anterior constriction of squamosal root of zygomatic arch; 15. posteriormost point of suture between squamosal and alisphenoid; 16. anteriormost margin of tympanic bulla (ectotympanic); 17. superiormost margin of tympanic bulla (ectotympanic); 18. posteriormost margin of tympanic bulla (ectotympanic); 19. midpoint of suture between basisphenoid and basioccipital; 20. anteriormost point of inferior margin of foramen magnum; 21. posteriormost margin of occipital condyle; 22. posteriormost point of superior margin of foramen magnum; 23. midpoint of linear distance between landmarks 8 and 14 projected to zygomatic arch.

Lateral view: 1. anteriormost point of nasal; 2. anteriormost point of suture between nasal and premaxilla; 3. posteriormost point of incisor alveolus; 4. inferiormost point of incisor alveolus; 5. posteriormost point of suture between nasal and premaxilla; 6. inferiormost point of suture between premaxilla and maxilla; 7. anteriormost point of zygomatic plate; 8. suture between frontal, antorbital bridge (maxillary) and lachrymal; 9. point of maximum posterior constriction of antorbital bridge; 10. point of maximum posterior constriction of maxillary root of zygomatic arch; 11. point of maximum anterior constriction of squamosal root of zygomatic arch; 12. suture between frontal, parietal and squamosal; 13. inferiormostmost point of zygomatic arch; 14. suture between parietal, squamosal and occipital; 15. suture between squamosal, alisphenoid and tympanic bulla; 16. superiormost point of tympanic bulla; 17. posteriormost point of tympanic bulla; 18. superiormost point of suture between frontal and parietal; 19. superiormostmost point of suture between parietal and interparietal; 20. posteriormost point of occipital; 21. posteriormost point of occipital condyle; 22. midpoint of linear distance between landmarks 5 and 18 projected to supraorbital (frontal) margin.

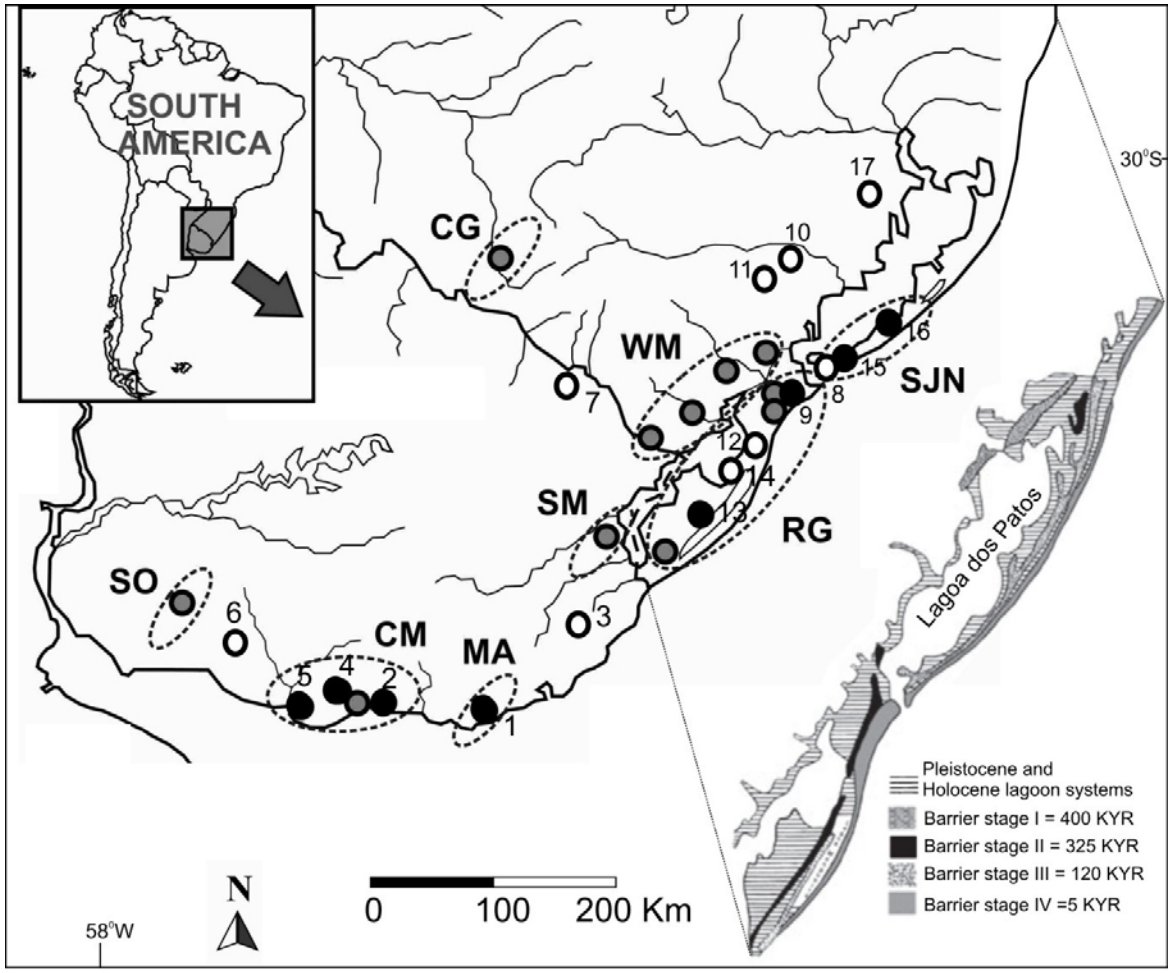

Fig. 1.— Collecting localities of *Scapteromys tumidus*, indicating samples utilized in both molecular and geometric analysis (black circles), molecular analysis only (white circles) and geometric morphometrics only (gray circles). Numbers correspond to localities of haplotypes showed in Table 1. Other localities are listed in Appendix. Ellipses and abbreviations indicate the geographic clusters defined by skull geometric morphometric analysis (see Material and methods). Coastal plain is shown in detail to depict the four barrier stages.

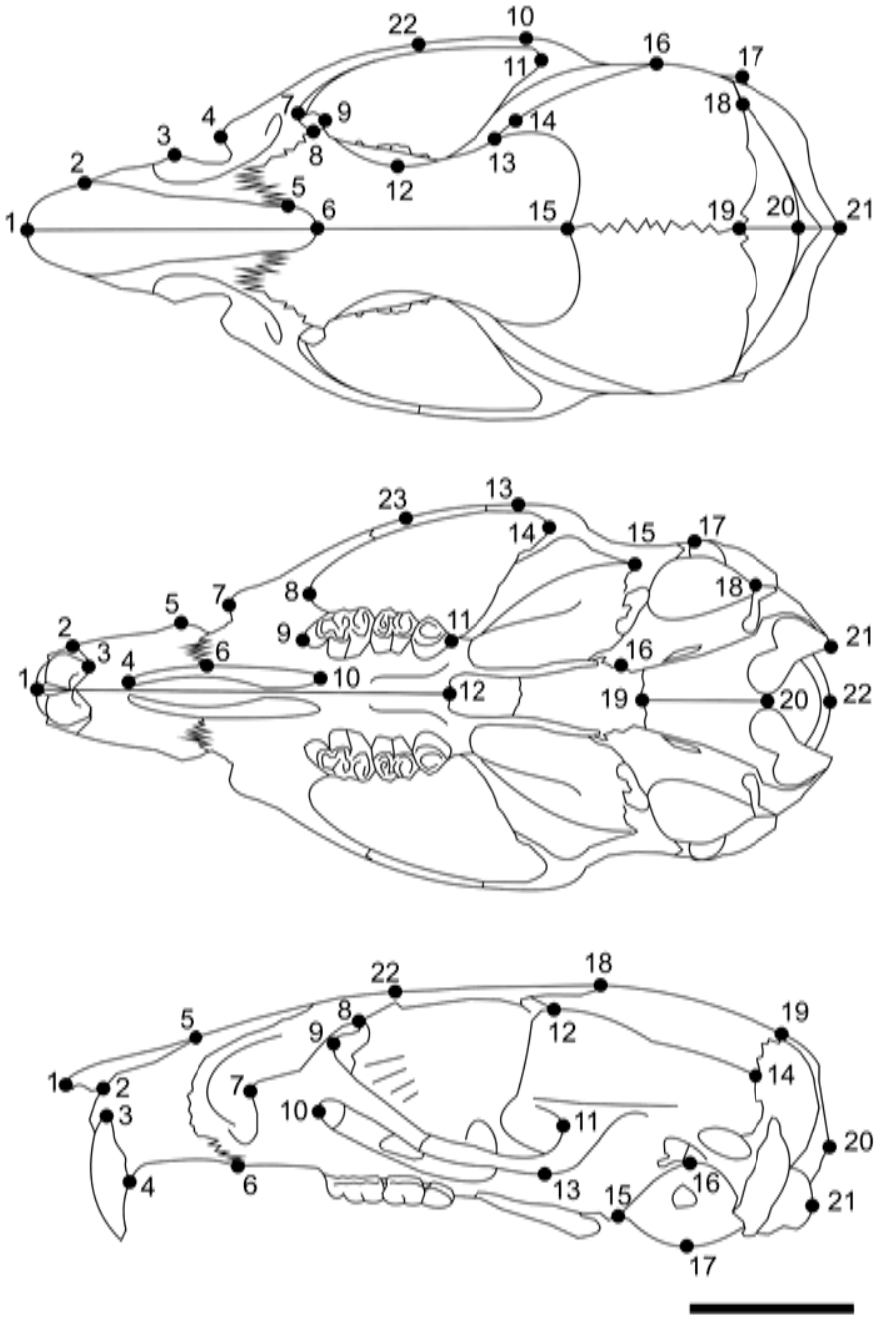

Fig. 2.— Landmark locations on dorsal (A), ventral (b) and lateral (C) views of skull of *Scapteromys tumidus*. Detailed description of the location of landmarks is given in Appendix II.

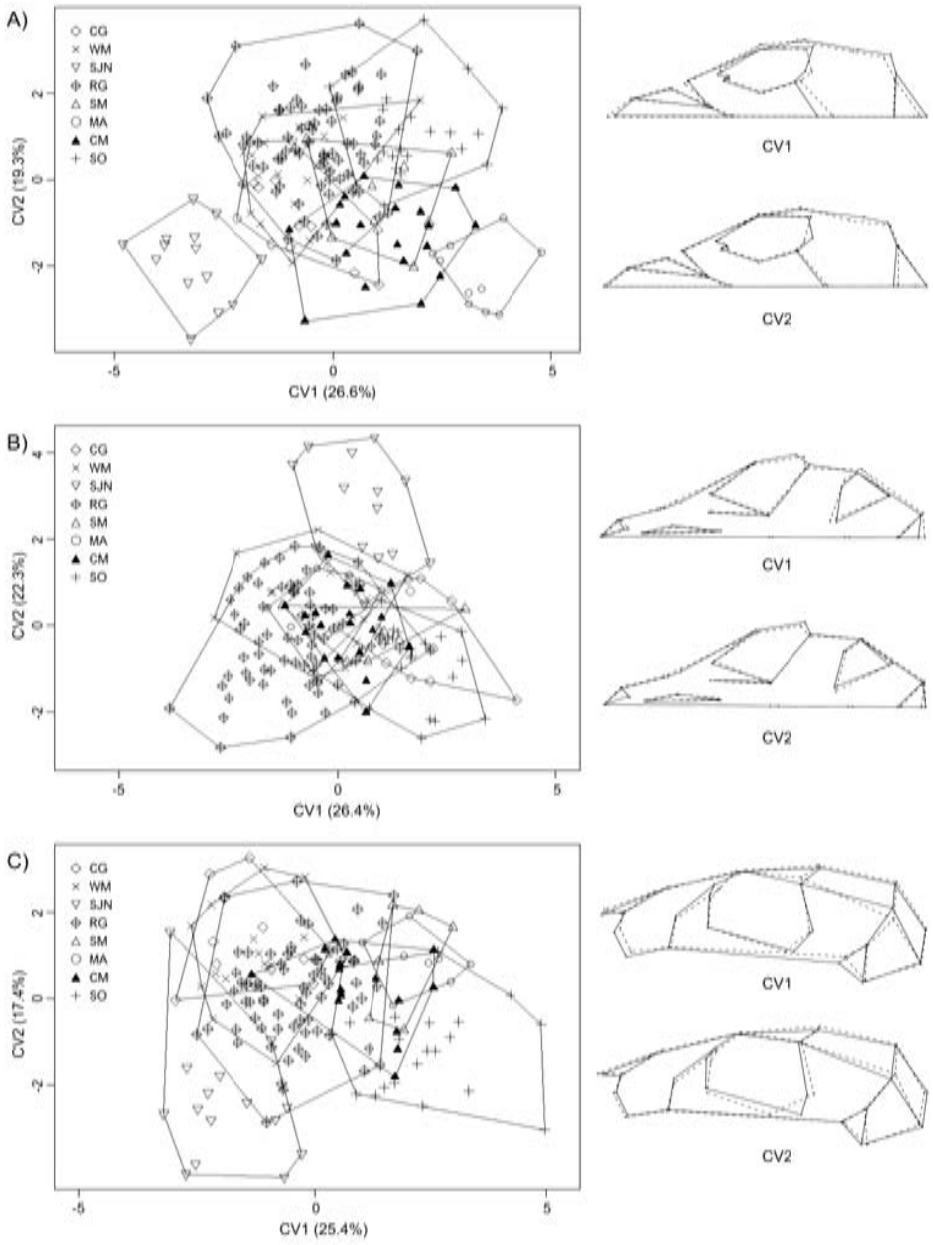

Fig. 3.— Scatter plot of first two axes of Canonical Variate Analysis (CVA) for *Scapteromys tumidus* skull shape configurations of samples from eight geographic clusters in Uruguay and southern Brazil and for dorsal (A), ventral (B) and lateral (C) views. CV1 represent skull shape variation on the first axis and CV2 represent skull shape variation on the second axis. Positive scores in solid lines and negative scores in dotted lines on the shape difference representations.

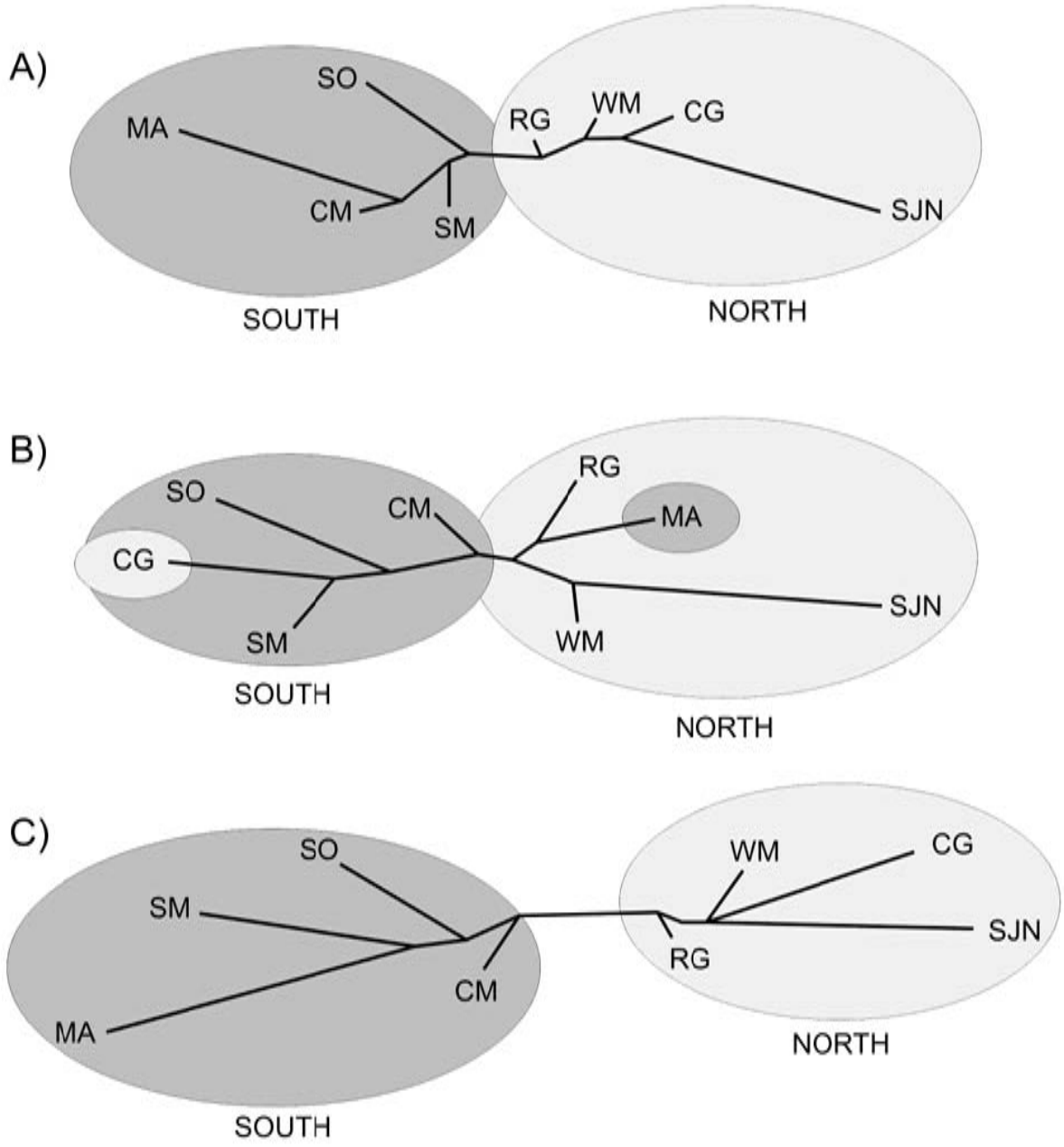

Fig. 4.— Neighbor-joining phenograms generated from Mahalanobis distances for dorsal (A), ventral (B) and lateral (C) views of the skull of *Scapteromys tumidus*. Acronyms correspond to geographic clusters.

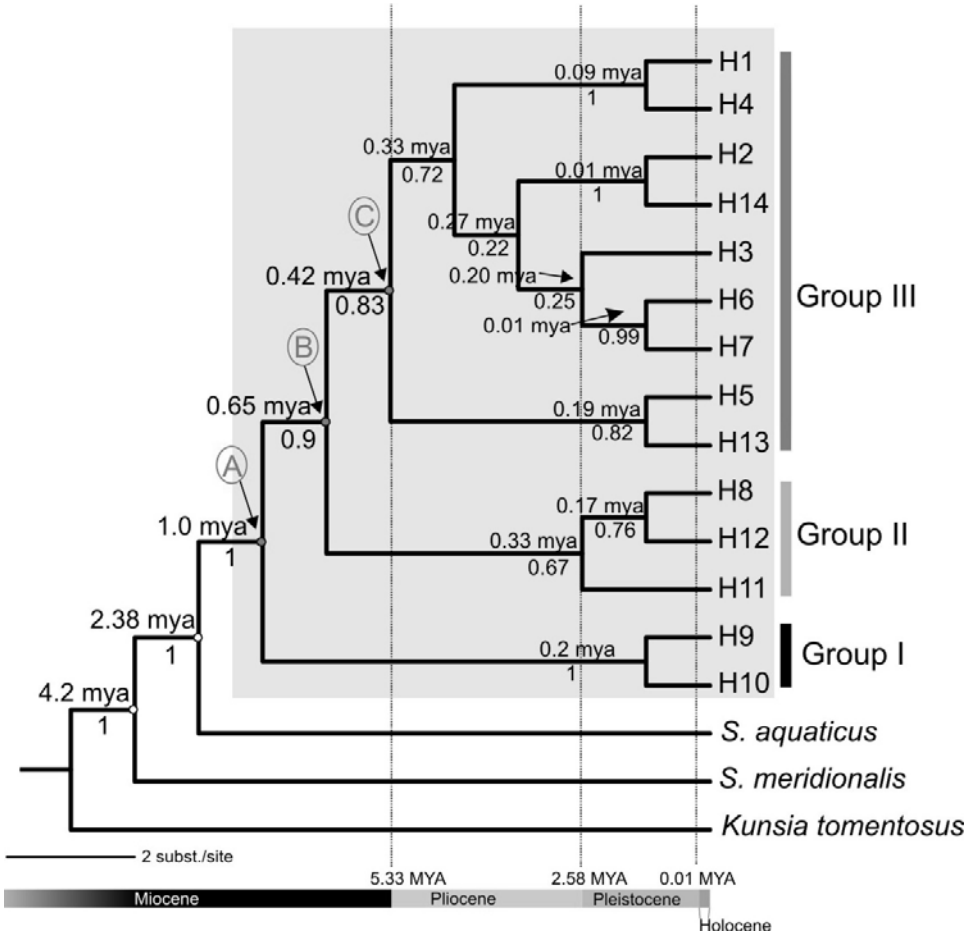

Fig. 5.— Intraspecific analysis of *Scapteromys tumidus*. Bayesian phylogenetic tree based on 14 haplotypes of the cytochrome *b* gene. Posterior probabilities and estimated time of divergence are indicated above each branch. Groups I, II and III represent major haplogroups identified. Internal nodes A, B and C are discussed in the main text.

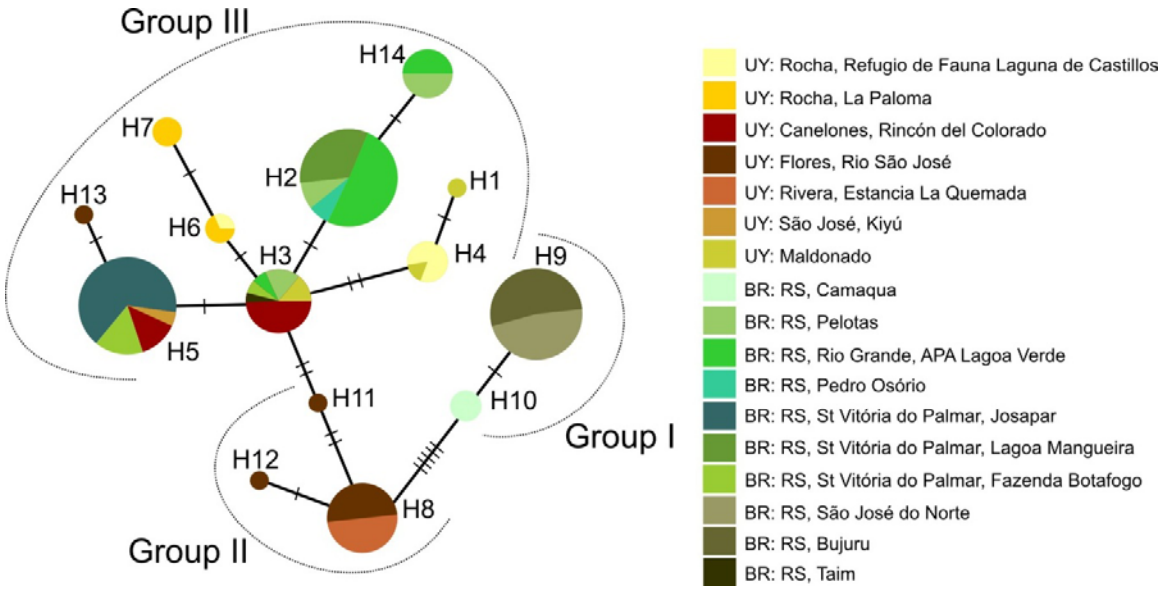

Fig. 6.— Haplotype network reconstructed based on median-joining analysis. Colors (and symbols in B&W version) represent sampling localities listed in the inserted legend. Circle sizes are proportional to the frequency of occurrence of the respective haplotype. Small bars crossing branches represent substitution events. Groups I, II and II represent major haplogroups identified in Bayesian phylogenetic analysis.

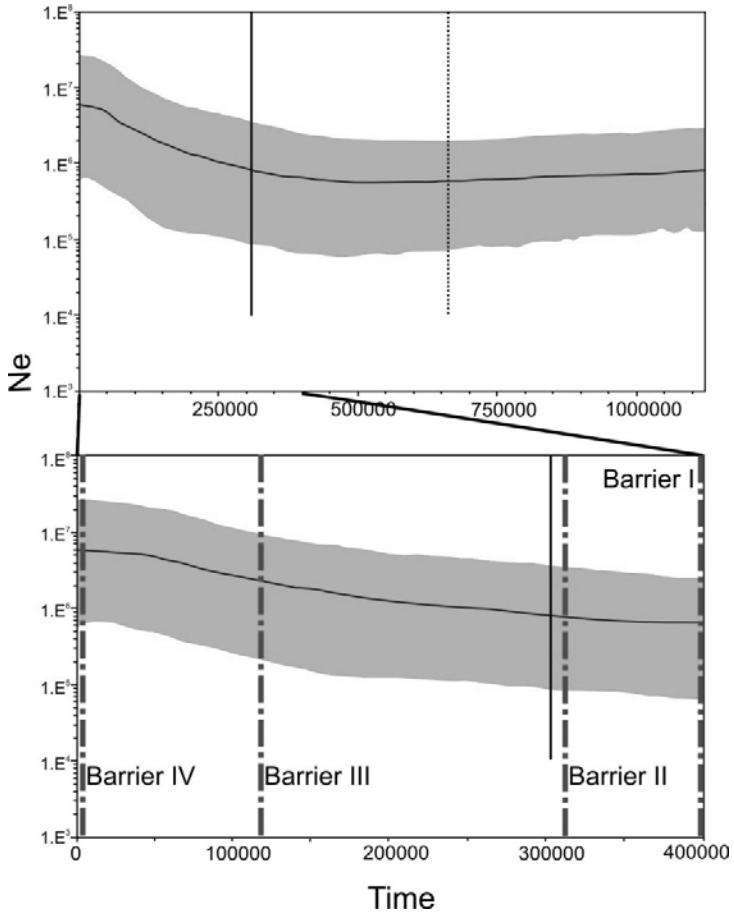

Fig. 7 .— Bayesian skyline plot showing the complete reconstruction of the female effective population size fluctuations through time in *Scapteromys tumidus* in the Quaternary. Solid curve represents the mean estimates and shaded area indicates the 95% highest posterior density (HPD) limits. Past time (in years) is indicated in the horizontal axis and effective population size in the vertical axis. Panel above: solid and dotted lines are the lower and the median, respectively, estimated tMRCA, projected on the time line. Panel below indicates the last 400.000 years in detail; dashed lines indicate each of the four marine transgressive-regressive events in the Coastal Plain of Rio Grande do Sul, Southern Brazil (see Figure1 for details).

Table 1.— Samples used in the molecular analysis. **N**, number of specimens; Haplotype, cytochrome b haplotype unambiguous found. Haplogroups identified in this study (I, II and III) are described in Figure 3.

Map	Collection site *	N	Haplotype	Haplogroup
1	UY: Maldonado, Arroyo El Renegado	2	H1, H4	III
	UY: Maldonado, Las Flores	2	H1, H4	III
2	UY: San José, Kiyu	1	H5	III
3	UY: Rocha, Refugio de Fauna Laguna de Castillos	7	H4, H6	III
4	UY: Rocha, La Paloma	5	H6, H7	III
5	UY: Canelones, Rincón del Colorado	10	H3, H5	III
6	UY: Flores, Río San José	9	H8, H11, H12	II
7	UY: Rivera Estancia La Quemada	7	H8	II
8	BR: Rio Grande do Sul, Rio Grande, APA Lagoa Verde	15	H2, H3, H14	III
9	BR: Rio Grande do Sul, Rio Grande, Taim	1	H3	III
10	BR: Rio Grande do Sul, Pelotas	7	H3	III
11	BR: Rio Grande do Sul, Pedro Osório	6	H2	III
12	BR: Rio Grande do Sul, St. Vitória do Palmar, Lagoa Mangueira	7	H2	III
13	BR: Rio Grande do Sul, St. Vitória do Palmar, Lagoa Botafogo	5	H5	III
14	BR: Rio Grande do Sul, St. Vitória do Palmar, Josapar	17	H5	III
15	BR: Rio Grande do Sul, São José do Norte, Bujuru	11	H9	I
16	BR: Rio Grande do Sul, São José do Norte	12	H9	I
17	BR: Rio Grande do Sul, Camaquã	3	H10	I

* UY: Uruguay, BR: Brazil

Table 2.— Genetic differentiation between the three major haplogroups (I, II and III) of *Scapteromys tumidus.* Above diagonal numbers indicate pairwise $F_{ST,}$ and below diagonal mean ± standard error (after 1000 bootstrap replications) of *p*-distance over sequence pairs.

	1.	2.	3.
1. Haplogroup I	-	0.743	0.768
2. Haplogroup II	0.009 ± 0.003	-	0.704
3. Haplogroup III	0.012 ± 0.003	0.005 ± 0.002	-

Table 3.— F values and significance levels of pairwise MANOVA analyses of variation in *Scapteromys tumidus* skull shape among eight geographic clusters in Uruguay and southern Brazil, for dorsal, ventral and lateral views (*P< 0.05; **P< 0.01; ***P< 0.001).

Dorsal view

	WM	**SJN**	**RG**	**CG**	**SO**	**MA**	**SM**
SJN	10.2***						
RG	1.6	6.6***					
CG	2.0	24.8***	3.1**				
SO	4.4**	22.3***	4.0***	8.6***			
MA	24.9***	16.1***	4.5***	17.2***	19.8***		
SM	3.9*	15.7***	2.6*	4.2	6.4**	4.9	
CM	2.9	12.7***	2.9**	4.1*	5.5*	5.6**	2.1

Ventral view

	WM	**SJN**	**RG**	**CG**	**SO**	**MA**	**SM**
SJN	8.9***						
RG	1.1	6.1***					
CG	3.7	12.1***	3.4**				
SO	9.4***	13.7***	3.7***	8.9*			
MA	4.2	6.8*	1.9	3.9	8.7***		
SM	3.0	7.5*	1.6	1.6	4.1	2.8	
CM	4.2*	9.0**	2.4	3.5	6.2	4.4	1.4

Lateral view

	WM	**SJN**	**RG**	**CG**	**SO**	**MA**	**SM**
SJN	4.4*						
RG	1.2	2.9**					
CG	3.2	10.1***	3.3**				
SO	8.0***	12.3***	3.4***	5.3***			
MA	6.8**	13.9***	4.5***	5.2	4.8*		
SM	3.3	5.8	1.9	4.5	2.1	5.5	
CM	5.2**	9.8***	3.6**	2.9*	2.2	3.9	1.4

Table 4.— Percentage of correct classification from discriminant analysis for *Scapteromys tumidus* skull shape from eight geographic clusters in Uruguay and southern Brazil.

	WM	**SJN**	**RG**	**CG**	**SO**	**MA**	**SM**	**CM**
Dorsal	63.6	100	87.1	92.3	95.4	90.0	77.8	70.0
Ventral	45.0	80.0	82.6	76.9	81.8	37.5	66.7	47.3
Lateral	73.7	93.3	86.9	61.5	80.9	100	83.3	75.0

CAPÍTULO IV

Artigo em preparação

(*Mammalian Biology*)

Ongoing speciation process from lowlands to highlands in South Brazil: evidence of a distinct lineage and novel patterns on the phylogeography of *Deltamys kempi* Thomas, 1920 (Rodentia: Sigmodontinae)

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Running title: New *Deltamys* lineage in Brazilian highlands

6,937 words.

Abstract: The sigmodont genus *Deltamys* comprises the single formally described *Deltamys kempi* and an undescribed form (diploid number 2n=40) from southern Brazilian highlands (Meridional Plateau). Herein we report the discovery of a second specific-level divergent form of *Deltamys* from Meridional Plateau in Rio Grande do Sul state (RS), besides an expanded scenario on *D. kempi* phylogeography. Bayesian analyses of cythocrome *b* (cyt *b*) sequences revealed that this *Deltamys* new lineage is sister to *Deltamys* sp. 2n=40, exhibiting divergence (p-distances) of 8% from this last form and 12% from *D. kempi*. The analysis of 76 sequences of *D. kempi* showed a structured pattern where 31 from a total of 37 haplotypes were found in single localities, and the other six were shared between nearby localities. Two clades were recovered, one restricted to the north of the species distribution and the other widely spread. Specimens from these clades also exhibited significant differences in skull measurements. The phylogeographic break observed in *D. kempi* seems to have been shaped by the Patos lagoon estuarine channel, once no haplotypes were shared by northern-central and southern segments of RS coastal plain. Finally, we hypothesized that the differentiation in *Deltamys* may have been triggered by dispersion of basal lineages over distinct altitudinal ranges in Paraná geological basin in RS.

Key words: Akodontini, altitudinal segregation, cytochrome *b*, dispersion, evolution, phylogeography.

Introduction

Deltamys Thomas 1917 comprises a sigmodontine genus that includes two allopatric forms (Ventura et al., 2011). The single formally recognized species, *D. kempi* Thomas 1917 is characterized by diploid chromosomal number 2n=37 in males and 2n=38 in females and fundamental number FN=38 in both sexes (Sbalqueiro et al., 1984; Castro et al., 1991; Ventura et al., 2011). This species ranges from Buenos Aires and Entre Rios Argentinean provinces to southern, central and eastern Uruguay and southern Brazil, in Rio Grande do Sul (RS) coastal plain and more inland along the western margin of Patos-Mirim lagunar complex. *Deltamys kempi* is omnivorous and associated to wetlands, mainly in open areas (Gonzalez and Pardiñas, 2002; Gonzalez and Lanfranco-Martinez, 2010). The unnamed form, referred as *Deltamys* sp. by Ventura et al. (2011), is characterized by 2N=40, FN=40 and is known from a single locality in northern RS (=Esmeralda), in the domains of Mixed Ombrophilous Forest or Araucaria Forest, a phytophysiognomy of the Atlantic Forest biome.

Deltamys kempi has a unique sexual chromosomal system, in which males are determined by $X_1X_2Y_1$ and females by $X_1X_1X_2X_2$ (Sbalqueiro et al., 1984; Castro et al., 1991; Gonzalez and Pardiñas, 2002). In *Deltamys* sp., by contrast, the sex determination is the mammalian most common system XY/XX (Ventura et al., 2011). Beside the karyological differences, the two *Deltamys* forms are highly divergent (12%) for the mitochondrial gene cytochrome *b* (cyt *b*). These lineages also appear as sister species in cyt *b* phylogenetic analysis, forming a well supported clade sister to *Akodon* (Ventura et al., 2011). The phylogenetic relationships of *Deltamys*, however, were not ever clearly understood. *Deltamys* (type species *D. kempi*) was originally described by Thomas (1917) based in specimens from "Isla Ella", an uncertain locality

in Paraná River estuary. Since its establishment, *Deltamys* have been considered a full genus (Gyldenstope, 1932; Massoia 1980; Sbalqueiro et al., 1984; Bianchini and Delupi, 1994; Gonzalez and Pardiñas, 2002; D'Elía et al., 2003; Musser and Carleton, 2005) or a synonym or a subgenus of *Akodon* (Ellerman 1941; Cabrera 1961; Massoia 1964; Reig 1984; Barrantes et al. 1993). Recent phylogenetic investigations using molecular markers (D'Elía et al., 2003), however, are concordant with the status of *Deltamys* as a distinct genus within the Akodontini tribe.

The intraspecific genetic diversity in *D. kempi* was accessed through analysis of sequences of cyt b and the nuclear recombination activating gene 2 (RAG2) (Montes et al., 2008) and in *Deltamys* sp. 2n =40 using only cyt b data (Ventura et al. 2011). *Deltamys kempi* presented high haplotypic richness, considering the number of survey localities in these studies. A phylogeographic analysis of 30 specimens sequences from eight localities along the species distribution (10 haplotypes recovered) (Montes et al., 2008) showed an internal division of *D. kempi* in two groups. One phylogroup is restricted to northern RS coastal plain plus one locality in northeastern margin of Patos Lagoon while the other spreads to inland RS, southern RS coastal plain, Uruguay and Argentina. These groups also presented significant differences in craniometric analysis (Montes et al., 2008). Based on these molecular and morphological divergences, Montes et al. (2008) suggested that *D. kempi* can encompass two taxa. Despite of these relevant findings, the study of Montes et al. (2008) did not evaluated the genetic diversity in RS central coastal plain (*Restinga* of São José do Norte), while only one Uruguayan locality was sampled. Furthermore, the craniometric analysis in the refereed study did not include any sample from Uruguayan populations.

Accordingly, in this study we expanded such scenario, including a number of localities in Uruguay, and within RS. In addition, we decided to explore a continuum of the distribution in our field-study beyond the north limit of the range defined by Montes at al. (2008), in Torres municipality (S29° 20'; W49° 43'). This is a marked region at the sea level characterized at on one side by the Atlantic Ocean and at the other side (ca. 2 Km) by forested mountains that reach an uphill of 1000m of altitudinal gradient. The above side is defined as part of the northeastern highlands of RS (Fig.1). There is a conspicuous shift in the physiognomies along this gradient (Rambo 1942). Morrone (2006) defined the uphill as the biogeographic provinces of Paraná and *Araucaria angustifolia* Forests, in the Meridional Plateau (MP) of southern Brazil. Accordingly, we had sampled in such region (São Francisco de Paula Municipality), specimens that resembles *D. kempi* (small size, relatively large head, short limbs and tail, soft pelage, small eyes, delicate skull [Gonzalez and Pardiñas, 2002]). Given the existence of an undescribed lineage in the *Araucaria angustifolia* Forest (Esmeralda locality), herein we investigate the phylogenetic position of this morphotype, which was first hypothesized as a continuum of intraspecific variation of *D. kempi*, due to its short distance to Torres (ca. 15 Km) We also reexamined the genetic structure and skull morphological differentiation in *D. kempi* using a larger and broader sample, aiming to unfold the evolutionary events related to the new covered geological formations along the species distribution.

Material and methods

Specimens and study area. —A total of 68 specimens of *D. kempi* from 16 localities in lowlands of RS and Uruguay and 2 specimens from highlands in São Francisco de Paula, RS (hereafter MP *Deltamys*-like) were field collected accordingly to the guidelines of the American Society of

Mammalogists (Sikes et al. 2011) (Table 1; Fig. 2). Tissue samples (liver and muscles) were obtained and preserved in ethanol at -20°C. Tissue samples and specimens of *D. kempi* and tissues of MP *Deltamys*-like are deposited at the Genetic Department of Federal University of Rio Grande do Sul, Porto Alegre (TR) and the National Museum of Natural History, Montevideo (EMG). Specimens of MP *Deltamys*-like are deposited at Fundação Universidade Regional de Blumenau (FURB), Blumenau.

Molecular analysis. — High-quality DNA was purified from muscle using the organic method of Cetyl Trimethyl Ammonium Bromide (CTAB). The DNA samples were amplified through a polymerase chain reaction (PCR) for either entire (1140 base pairs [bp]) cytochrome b (cyt *b*) gene using MVZ05 and MVZ14 primer pair (Smith and Patton 1993). The PCR reaction included 1 U of Taq polymerase (Invitrogen) and 1.2 mM MgCl2, at 50°C of annealing temperature in a 20-µl reaction volume. PCR products were cleaned through the ExoSap (GE Healthcare) enzymatic method, sequenced with and analyzed on an ABI3730XL (Applied Biosystems) at Macrogen® (Republic of Korea). Sequences were aligned and visually inspected using Clustal X in MEGA version 6 (Tamura et al., 2013) running in full mode with no manual adjustment. Standard diversity measurements, such as the number of variable sites (*S*), haplotype diversity (Hd) and nucleotide diversity (π) were calculated from the whole dataset and grouped by localities using MEGA 6 (Tamura et al. 2013). Also, genetic divergence across localities was estimated using *p*-distance parameter, with 1000 bootstrap replications. Intraspecific patterns of variation were estimated within all specimens of *D. kempi* though haplotypes of the cyt *b* gene directly inferred from polymorphic sequences, using DNASP 5 (Librado & Rozas, 2009) software. We used these haplotypes (total of 31 found, 27 of which were novel) and incorporated

six already described by Montes et al. (2008), and one from D'Elía et al. (2003) deposited in GenBank (Table 1), to perform a Bayesian phylogenetic reconstruction in order to i) evaluate the position of individuals collected in highlands, ii) estimate node ages of internal relationships. Three sequences of *Deltamys* sp. 2n=40 (Ventura et al. 2011) were also included in de dataset Appendix I). Representative species of akodontines genus (*Akodon, Thalpomys, Thaptomys, Brucepattersonius, Blarinomys, Lenoxus, Kunsia, Bibimys Jucelinomys and Oxymycterus*) were used as outgroup, according to relationships based on sequences of cyt *b* obtained by D'Elia (2003) . The divergence times and topology was estimated simultaneously in BEAST 2 (Bouckaert et al. 2014). The tree prior was set to the Yule calibrated process, using the GTR model (defined by MEGA 6 [Tamura et al. 2013]). The branch lengths were allowed to vary under a relaxed clock model with an uncorrelated log-normal distribution (Drummond et al., 2006). The analyses were run for 40 million generations, with every 4000th generation sampled. One prior was specified in the form of a calibration point for Akodontine was 7.36 mya (95% confidence interval: 5.62–9.29 mya; Parada et al. 2013). Convergence, effective sample sizes, and divergence times with upper and lower 95% highest posterior density (HPD) bounds were assessed in Tracer 1.4.8 (Rambaut & Drummond 2007). TreeAnnotator was used for all analyses, with 20% of the samples removed for burn-in to generate a tree that was then visualized and edited in FigTree v1.3.1 (Rambaut 2009). The haplotype sequences generated from the present study were deposited in GenBank, accession numbers KM275437- KM275467.

Skull morphometrics. — We examined the morphological variation between samples from the two *D. kempi* mitochondrial clades previously identified in the Bayesian analysis through skull linear morphometric approach. Specimens consisted of samples sequenced in the present study in

addition to individuals from localities represented in Bayesian tree clades (obtained from the American Museum of Natural History [AMNH] and MCNU, see Appendix). The blocks Clade A and Clade B consisted in 34 and 38 specimens respectively, all of them with the third molar erupted and functional. Craniodental measurements were taken with a digital caliper accurate to the nearest 0.01 mm and herein given as values rounded to the nearest 0.1 mm. The following measurements were taken as illustrated by Hershkovitz (1962), Voss (1988) and Montes et al. (2008): breadth of incisors (BI), length of molar row (LMR), length of nasal (LN), length of rostrum (LR), length of tympanic bulla (LTB), breadth of braincase (BB), breadth of zygomatic plate (BZB), least interorbital length (LIB), length of rostrum (LR), distance between first molars (DFM), breadth of first molar (BFM), condylo-incisive length (CIL), greatest length (GL), palatal length (PL), length of diastema (LD), orbital length (OL), height of braincase (HB), breadth of zygomatic (BZ), length of incisive foramina (LIF), breadth of incisive foramina (BIF), breadth of occipital condyles (BOC).

All measurements were converted to logarithms. Missing values (3.2%) were estimated by regression method. Once sexual dimorphism in the cranial measurements herein used were not detected previously (Montes et al., 2008), we pooled females and males for analysis. The hypothesis of significant differences in the skull measurements between samples from Clades A and B was tested with an analysis of variance (ANOVA). We also performed a principal component analysis (PCA) using the first three principal components (PC) and a discriminant analysis aiming to exam craniometric differentiation between the clades.

Results

Molecular phylogeny of Deltamys

A total of 145 variable sites were identified in 1140 bp of cyt *b* sequences, which included 37 haplotypes in *D. kempi* from lowlands and 2 in MP *Deltamys*-like from highlands (Table 1). The haplotypes of MP *Deltamys*-like was markedly divergent to those 37 found in individuals of *D. kempi* from lowlands, which formed a well-supported clade (BPP =1). The estimated level of genetic divergence (*p*-distance) between both clades was estimated in 0.12 (±0.02). Thus, haplotypes from highlands, or the *Araucaria angustifolia* Forest, formed a strongly supported (BPP>0.95) and reciprocally monophyletic clade to *Deltamys* sp. 2n=40 (Esmeralda). Evolutionary distance between them was calculated as 0.08 (±0.01). The three *Deltamys* lineages (*D. kempi*, *Deltamys* sp. [2n=40] and MP *Deltamys*-like) were recovered as monophyletic, and the group retrieved as sister to *Akodon boliviensis*, and closely related to *Thaptomys* + *Thalpomys* lineages (Fig. 3).

Deltamys kempi phylogeographic novel patterns

Deltamys kempi emerged as a monophyletic clade in Bayesian analysis (BPP=1), showing an internal structure divided into two clades, entitled here as haplogroups (Fig. 3). One haplogroup (Clade A) was restricted to RS, occurring in northern and central coastal plain and west of Patos Lagoon. Clade B spreads from Buenos Aires to Uruguay and RS regions of southern coastal plain and west of Patos Lagoon.

Haplotype networks showed a pattern characterized by local diversification (i.e., markedly structured), as only five from 37 haplotypes where shared by two or three nearby localities (Fig. 4). In Clade A four from the 13 recovered haplotypes were shared by pairs of localities, each one inserted in the same physiographic unit.

Haplotype H24 was shared by samples from Pedro Osório and Tapes, localities distant each other until about 180 km at the west of Patos Lagoon. Haplotype H22 was found in Palmares do Sul and Torres, separated by around 120 km in northern coastal plain. Haplotype H14 occurred in two neighboring localities (Osório and Tramandaí) also in northern coastal plain. Haplotype H13 was shared by two localities separated around 60 km in central coastal plain. The other nine haplotypes within this clade were found distributed exclusively among six localities, with Palmares do Sul and São José do Norte compassing three and two haplotypes, respectively. Clade B is spread in the remainder *D. kempi* distribution. A single haplotype (H2) occurred in more than one locality, which encompasses two sampling sites in San José and one site in Rocha, both in southern Uruguay. Other five haplotypes (H1, H5, H6, H7 and H28) occurred exclusively in Rocha department. Samples from Canelones in southern Uruguay exhibited three exclusive haplotypes (H3, H8 and H9) and a unique haplotype was found in Rivera, central-north Uruguay. The haplotype H18 was found exclusively in Buenos Aires, Argentina. In west of Patos Lagon, RS, exclusive haplotypes were found in Charqueadas (H21) and Pelotas (H29 and H30). Unique haplotypes also occurred in RS southern coastal plain localities of Rio Grande (H10, H11, H12) and Taim (H17, H20, H33, H34, H35 and H36).

The ANOVA results showed significant differences in craniometric measurements between the two *D. kempi* mitochondrial clades ($p<0.001$). In PCA results, however, samples from the two clades did not segregated on the multivariate space, presenting a high overlap in the convex hull on both PC1 x PC2 and PC1 x PC3 (Fig. 5). The first principal component was positively related to all measurements except BFM, and can be considered a factor size. PC2 was positively related mainly to IFW, BFM, LD, and LIF. PC3 was positively related to BFM, LR, BR, LMR

and IFW. In the discriminant analysis, both clades showed a high percentage of correct classification (Clade A: 94.2%; Clade B: 92.2%).

Discussion

Diversity of genus Deltamys

For a long time since its description, *Deltamys* was captured exclusively in lowland Pampean zones (for details of habitats see Massoia 1964; González and Pardiñas 2002, Bianchini and Delupi, 1994; Udrizar Sauthier et al. 2005; González 2006). The recent findings of Ventura et al. (2011), however, included highlands of MP in the biogeography of the genus. Herein we evidenced a second form divergent at a specific level in MP highlands, revealing that, within the small radiation of the genus, we can observe a higher diversification in this geological formation when compared to lowlands of northern Pampean domain, inhabited exclusively by *D. kempi*.

Meridional Plateau is the nomination given to the highland formations of the central-eastern segment of Paraná geological basin. Paraná basin extends over 1.5 million km^2 of Brazil (from Mato Grosso to RS states), east of Paraguay and North of Argentina and Uruguay, and was shaped by successive events of sedimentation and spill occurred from Ordovician to Cretaceous (Milani, 2000; Pereira et al., 2012). On most of its extension, MP is covered by the Atlantic Forest vegetation domain, which in its subtropical inland portion is composed mainly by the Mixed Ombrophilous Forest (or *Araucaria angustifolia* Forest) and the Dense Ombrophilous Forest (Silva and Casteleti, 2005). Atlantic Forest is known to hold one of the greatest biological diversities in the world (Myers et al., 2000; Tabarelli et al., 2010). About a quarter of the known South American mammals occur in Atlantic Forest, of which 32% represent endemic marsupials, primates and rodents (Costa et al., 2000). Regarding to akodontines, a list of the species endemic

to Atlantic Forest could include at least *Abrawayomys ruschi*, *Akodon montensis*, *A. paranaensis*, *A. sanctipaulensis*, *Blarinomys breviceps*, *Brucepatersonius igniventris*, *B. griserufescens*, *B. soricinus, B. paradisus*, *B. misionensis, B. guarani*, *Oxymycterus judex*, *O. caparaoe*, *O. quaestor*, *Scapteromys meridionalis* and *Thaptomys nigrita* (Mares and Braun, 2000; Bonvicino et al., 2008; Quintela et al., 2014). It is remarkable that nine of these species were described within the last 20 years (Hershkovitz, 1998; Christoff et al., 2000, Mares and Braun, 2000; Quintela et al., 2014). Thus, it is expected that the increasing of accumulated sampling effort and the exploration of areas never sampled before might unfold previously unknown forms in southern Atlantic Forest, as the case of *Deltamys*. It is also expected that sampling of new areas and the reevaluation and sequencing of collection specimens (in view of the external similarity with juvenile *Akodon*) may expand the known distribution of MP *Deltamys* forms.

Deltamys kempi was considered a few representative taxon in mammalian collections (Montes et al., 2008). Regarding *Deltamys* MP material, the only known specimens are those analyzed by Ventura et al. (2011) and the sample herein presented. Considering the period of over a century of mammalian sampling in Araucaria and Dense Ombrophilous Forest regions of southern Brazil (e.g. Thomas, 1909; Ihering, 1911; Cherem and Perez, 1996; Cademartori et al. 2002, 2004; Dalmagro and Vieira, 2005; Iob and Vieira, 2008; Lima et al. 2010), it is supposable that *Deltamys* from MP highlands represent rare forms, naturally scarse.

Deltamys evolution

This one can be considered the fourth molecular based study concerning aspects of *Deltamys* evolution (previous: D'Elia et al., 2003; Montes et al., 2003; Ventura et al., 2003) and one question that we arise here is: where did place the differentiation (split) within *Deltamys*

genus? There is no fossil record assigned to *Deltamys* (González and Pardiñas, 2002). The data available for any historical biogeography hypothesis are the cyt *b* genealogy, the geographical patterns of haplotype distribution and the geo-chronological information on the known distribution of the genus. Montes et al. (2008) hypothesized that *D. kempi* centre of dispersion could be situated in central-eastern RS. This speculation was based in the closest distance between a hoplotype from a locality in this region (Charqueadas) and the used outgroup and the geological age consistent with the estimated time of the split node. In the Bayesian tree generated in our analysis, the same haplotype from Charqueadas remained as the most ancestral, distant only one node from the basal node of *D. kempi.* It is important to highlight that our analyses included samples from 13 new localities, which originated 26 additional haplotypes in relation to Montes et al. (2008) data, and this same historical information was herein recovered. Parallelly, the Charqueadas haplotype, included in Clade B, arise as the closest to Clade A in MJ reconstruction (see Fig. 4). In view of the geo-chronological context of Charqueadas region (region of contact between the Precambrian shield (Pelotas Batolith) and the Permian-Jurassic sedimentary segment of Paraná basin [Milani, 2000; Philipp et al., 2000]) and the phylogenetic results found by Montes et al. (2008) and our analysis, it is conceivable the hypothesis of an origin of *D. kempi* placed in central-eastern RS.

Tracing back even more in *Deltamys* historical biogeography is a delicate task, partially due to small sample and incipient data on MP forms. Nevertheless, an analysis of the current distribution of the three forms and the geological-geomorphological scenario of this distribution allow us some interpretation and supposition. First, it is noticeable how populations from the highly divergent forms *D. kempi* and the new lineage herein reported are separated only by ca. 50 km between Torres, in the northern coastal plain, and São Francisco de Paula, in MP border. This

stretch, however, presents an altitudinal variation of more than 900m, partially due to the presence of MP slopes (*Serra Geral*). The Paraná basin in RS is the only formation occupied by all *Deltamys* lineages, where MP forms are restricted to the northern Cretaceous vulcanic highlands and *D. kempi* occupies the lowland Permian-Jurassic sedimentary deposits. These deposits extends over the geomorphological region called RS Peripheral Depression, characterized by lower altitudes (under 100m) and flattened relief (Wildner & Lopes, 2010), and connect to the northern coastal plain (northernmost of *D. kempi* distribution) on its eastern portion. Thus, it is possible that altitudinal gradient along central-eastern RS have shaped the differentiation in *Deltamys.* From an ancestral form probably inhabitant of this region, one lineage dispersed across the volcanic highlands and gave origin to the MP forms, while other lineage dispersed through the sedimentary lowlands, differentiating in *D. kempi* and latter spreading along the Cenozoic coastal plain. This hypothesis of dispersion followed by consequent isolation and allopatric speciation along distinct altitudinal ranges in Paraná basin is even supported by the already discussed spatial context of *D. kempi* possible origin in central-eastern RS, which represent the same spatial scenario for the split between the MP forms clade (see Fig. 3) and *D. kempi* discussed here. Mountain ranges and ridges are long considered topographic elements associated to small rodent speciation processes (Reig, 1984; Bofarull et al., 2008; Clausnitzer and Kityo, 2012; Montgelard and Matthee, 2012).

The two *Deltamys* lineages from MP are highly divergent (8%) considering the range of cyt *b* sequence divergence found among congeneric akodont species (D'Elía 2003). Both forms are known from single localities distant about 160 km in northeastern RS highlands. In view of the marked differentiation, it is expected that strong evolutionary processes have acted in highlands *Deltamys.* The few data on these forms, however, do not allow any further insight on

these processes, but it should be noted that two major rivers of northern Atlantic hydrographic basin (Caí and Antas rivers) (Vieira, 1984) runs between the localities of the so far known occurrence of *Deltamys* sp. (2n=40) (Esmeralda) and *Deltamys* "new lineage" (São Francisco de Paula). Rivers are suggested as geographic elements that shaped the speciation in small mammals such as didelphid marsupials and echimyid rodents (Patton and Da Silva, 2000; Teta et al., 2009; Nascimento et al., 2013).

Deltamys kempi expanded phylogeography

The analysis of the spatial distribution of *D. kempi* cyt *b* sequences revealed a structured pattern, markedly characterized by local diversifications (see fig. 4). This pattern may reflect some presumable ecological-behavioral aspects of the species. Despite of the lack of data on time-scaled movements or home-range, it is expected that *D. kempi* present limited capacity of dispersion, considering its small size and proportionally short forefeet and limbs (González and Pardiñas, 2002). Individuals observed in large terrarium showed slow and short movements (at day and night), generally limited to short displacements under the litter. The other aspects that may contribute to the structured pattern observed is the fact that *D. kempi* seems to be naturally rare in many parts of its distribution (Massoia, 1964; González and Pardiñas, 2002; Montes et al., 2008; Quintela et al., 2012; 2013) and populations seems to occur generally associated to scrubs of some specific palustrine herbaceous (Massoia, 1964; present study), which probably hamper the gene flow.

Our expanded data on *Deltamys kempi* phylogeographic structure supported the previous scenario of a subdivision in two major phylogroups markedly differentiated in both cyt *b* and craniometric characters, being one group restricted to north and the other widely spread in the

species distribution (Montes et al., 2008). However, the geographic coverage of the 'northern' group was substantially enlarged with the addition of new sampled populations. The 'northern' group recovered by Montes et al. (2008) ("Tramandaí group") included populations from Torres, Tramandaí and Osório, in northern RS coastal plain, and Tapes, at northwest of Patos lagoon. In our analysis, these samples grouped in Clade A with haplotypes from other four localities in northern and central coastal plain and two localities in border of RS shield (Cristal and Pedro Osório). The northern clade of *D. kempi*, however, is not restricted to the recent Cenozoic deposits as previously found by Montes et al. (2008), but spreads also over ancient geological formations of the Precambrian RS shield. Clade A also encompassed samples from central RS coastal plain, a region not sampled in previous molecular surveys (D'Elia et al., 2003; Montes et al., 2008; Ventura et al., 2011). This region corresponds to a narrow and elongated sedimentary deposit shaped by the last two marine transgressions occurred at 120 and five kya (Tomazelli and Villwock, 2000), which enclosed a large area of the continental shelf giving origin to Patos Lagoon, the largest lagunar system in South America (Vieira, 1984). The phylogeographic break observed in *D. kempi* seems to be related with the evolution of this lagunar system, where the estuarine channel may represent a geographical barrier to the historical gene flow. Clade A and B showed a large superimposition area on RS shield and sedimentary Paraná basin at west of Patos-Mirim system (between Charqueadas and Pedro Osório localities) whereas a complete geographic segregation is observed at the east of the system, where populations from distinct clades inhabiting the central (Clade A) and southern (Clade B) segments of RS coastal plain, which is separated by the estuarine channel. Clade A lineage, probably differentiated in RS shield or Paraná basin, later spread through north and central coastal plain following the successive deposition of lagunar barriers as hypothesized by Montes et al. (2008), but apparently

did not trespass the Patos lagoon estuary channel southwards. Populations from the southern segment of RS coastal plain, however, are genetically closer to populations from other physiographic regions at south and east of the species range than populations from central and northern segments of the same Pleistocene-Holocene sedimentary formation.

In spite of Patos lagoon estuary channel may represent a major hydrographic element that shaped the phylogeographic break in *D. kempi*, other expressive water bodies such as Mirim lagoon, São Gonçalo channel and rivers (e.g. Jacuí,Camaquã, Jaguarão, Uruguay) may also have played a role in local diversification, limiting the gene flow among populations. Large rivers and other water bodies have been demonstrated to act as potential barrier to gene flow among populations of small rodents (Colombi et al., 2010; Nicolas et al., 2014; Valdez and D'Elía, 2013).

Taxonomic perspectives

Contrary to our hypothesis of an intraspecific variation of *D. kempi*, we found evidences of a second form of *Deltamys* from South Brazilian highlands, increasing to three the number of known lineages divergent at a specific level within the genus. Thus, an ongoing speciation process is likely occurring from lowlands to highlands. Similarly, the akodontine species *Scapteromys meridionalis*, which is endemic to the MP highlands, was recently identified as diverged from *S. tumidus* that is widespread in the lowlands (Quintela et al. 2014). Accordingly, *Deltamys* considered monotypic until the findings of Ventura et al. (2011), currently compasses two undescribed species. Further morphological analysis of the new form will soon provide formal descriptions and binominals.

As in previous investigations using molecular markers (Montes et al., 2008; Ventura et al., 2011), our analysis showed an internal division in *D. kempi*. The question is whether this subdivision is consistent with a subspecific trinominal classification. A first attempt on *D. kempi* subspecific arrangement was proposed by González and Massoia (1995), who assigned populations from Argentinean populations to the nominal subspecies and populations from Uruguay and Brazil to *D. kempi langguthi.* This classification, however, was based only in morphological features and was posteriorly contested by Montes et al. (2008), which in turn found a genetic/craniometric-based internal division but with a spatial coverage of clades markedly distinct than the geographical context of the subspecific proposal of González and Massoia (1995). The internal clades recovered herein and in Montes et al. (2008) and Ventura et al. (2011) analyses are geographically congruent, strongly indicating that *D. kempi* is passing through a process of differentiation. The significative craniometric differences found herein and in Montes et al. (2008) study also support this process of internal differentiation. Nevertheless, the low level of genetic (distances between 1 and 2% [Montes et al., 2008; Ventura et al. 2011; present study]) and craniometric divergences (see the high superimposition of convex hulls in PCA), in our view, is not consisted with a formal taxonomic division within *D. kempi*. The other fact is that "northern" and "southern" clades are apparently sympatric at west of Patos-Mirim complex, which is a condition in disagreement with the formal concepts of subspecies (see [Mallet, 2007] for further details). In this case, the most appropriated proceeding could be to treat each clade as independent ESU's (Evolutionary Significant Unit; see [Moritz, 1994]), considering the divergence on sequences of the nuclear recombination activing gene 2 between the clades previously found by Montes et al. (2008). Moreover, there is no clear subjection for sympatry conditions in ESU's definition, which also do not consider the level of divergence an

arbitrary criteria since the monophyly of both nuclear and mitochondrial markers were attested (Moritz, 1994).

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Table 1. Localities identified on the map (#; Figure 1), indicating the number of specimens sampled (N) and haplotypes characterized in *Deltamys kempi* based on sequences of the cytochrome *b* gene.

#	Locality	N	Haplotype	Reference
1	UR: Canelones, Rincón Del Colorado	2	H8, H9	This study
2	UR: Flores, Rio San José	1	H3	This study
3	UR: San José, Arroio Cufré	2	H2	This study
	UR: San José*		H2	This study
4	UR: Rocha, Refugio de Fauna Laguna de Castillos	5	H1, H2, H5, H6	This study
5	UR: Rocha, Parque Santa Teresa	6	H7, H28	This study
6	UR: Rivera, Cofusa	1	H4	
7	AR: Buenos Aires*	-	H18	Montes et al. 2008; D'Elia et al. 2003
8	BR: Rio Grande, Reserva Ecológica do Taim	4	H33, H34, H35, H36	This study
	BR: Rio Grande, Reserva Ecológica do Taim*	-	H17, H19, H20	Montes et al. 2008
9	BR: RS, Rio Grande, APA Lagoa Verde	4	H10, H11, H12	This study
10	BR: RS, Bujuru	1	H13	This study
11	BR: RS, São José do Norte	12	H13, H31, H32	This study
12	BR: RS, Palmares do Sul	19	H25, H26,H27	This study
13	BR: RS, Pedro Osório	3	H22	This study
14	BR: RS, Pelotas	5	H29, H30	This study
15	BR: RS, Cristal	1	H15	This study
16	BR: RS, Charqueadas*	-	H21	Montes et al. 2008
17	BR: RS. Viamão, Banhado Grande	1	H37	This study
18	BR: RS, Tapes*	-	H24	Montes et al. 2008
19	BR: RS, Tramandaí*	-	H14	Montes et al. 2008

20	BR: RS, Osório	1	H14	This study
	BR: RS, Osório*	-	H23	Montes et al. 2008
21	BR: RS, Torres, Parque Estadual de Itapeva	1	H16	This study
	BR: RS, Torres*	-	H22	Montes et al. 2008
22	BR: RS, São Francisco de Paula	2	H38, H39	This study

UR: Uruguay, AR: Argentina, BR: Brazil. * Localities sampled by Montes et al. (2008) and incorporated into this study from the GenBank database.

Table 2. Evolutionary divergence estimates based on cytochrome b sequences between pairwise groups of *Deltamys* (and an outgroup [*Akodon boliviensis*] see material and methods). The number of base substitutions per site from averaging over all sequence pairs between groups, ± standard error (after 1000 bootstrap replication), is shown. Analyses were conducted using p-distance.

	D. kempi (clade B)	*D. kempi* (clade A)	*Deltamys* sp. 2n=40	MP *Deltamys*-like
D. kempi (clade B)	-			
D. kempi (clade A)	0.01±0.00	-		
Deltamys sp. 2n=40	0.12±0.02	0.11±0.02	-	
MP *Deltamys*-like	0.12±0.02	0.12±0.02	0.08±0.01	-
Akodon boliviensis	0.17±0.02	0.17±0.02	0.15±0.02	0.17±0.02

Appendix I.— Specimens used in molecular ([a]) and skull morphometric ([b]) analyses and housed in: American Museum of Natural History, New York, United States (AMNH); Museu de Ciências Naturais, Universidade Luterana do Brasil, Canoas, Brazil (MCNU); Departamento de Genética, Universidade Federal do Rio Grande Sul, Porto Alegre, Brazil (TR); Museo Nacional de Historia Natural, Montevideo, Uruguay (EMG and PCE) . Sequences obtained from GenBank (*) are presented as voucher number (when available)/GenBank access number.

Deltamys new lineage - BRAZIL: Rio Grande do Sul: São Francisco de Paula (PRM06, PRM10).

Deltamys sp. (2n=40) - BRAZIL: Rio Grande do Sul: Esmeralda; CIT 945/JN232108, CIT 946/JN232109), CIT 947/JN232110).

Deltamys kempi - BRAZIL: Rio Grande do Sul: Torres: Parque Estadual de Itapeva (TR 2159[a]). Osório: (TR 2137[a]). Palmares do Sul: (MCNU 2766[ab], 2767[ab], 2768[ab], 4007[ab] TR 2107[ab], 2108[ab], 2109 [ab], 2110[ab], 2111[ab], 2112[ab], 2113[ab], 2114[ab], 2115[ab], 2116[ab], 2117[b], 2118[ab], 2119[a], 2120[a], 2121[a], 2122[a], 2123[a], 2124[a], 2156[a], 2157[a]). Tapes: (MCNU 1813[a]). Viamão: Banhado Grande (TR 2145[a]). Cristal: (TR 2125[a]). São José do Norte: Bujuru (TR 2126[ab]), 14 km N São José do Norte (MCNU 2937[b], 2939[b], 2940[b], 2941[b]; TR 2127[ab], 2128[ab], 2129[ab], 2130[ab], 2131[ab], 2132[ab], 2133[ab], 2134[ab], 2135[ab], 2136[a]). Pedro Osório (MCNU 4002[b], 4004[ab]; TR 2138[a]). Pelotas (TR 2139[ab], 2140[ab], 2141[b], 2142[b,] 2143[a], 2144[a]; 2158[ab]). Rio Grande, Lagoa Verde (MCNU 1487[b], 1488[b], 1494[b]1501[b], 1733[b], 1734[b], 1735[b]; TR 2146[ab], 2147[ab], 2148[b], 2149[b], 2150[b], 2151[a], 2152[b]), Mata da Estrada Velha (MCNU 948[b], 949[b]), Taim (MCNU 4003[b], 4006[ab], 4008[ab], 4009[b], 4022[b]; TR 2153[a], 2154[a]). URUGUAY: Rivera, Cofusa (EMG 1099[a]). Rocha: Parque Santa Teresa (EMG

1808[a]; PCE 05[a], 06[a], 11[a], 12[a], 13[a]), Refugio de Fauna Laguna de Castillos (EMG 1926[a], 1929[a], 1930[a], 1957[a], 2003[a]), Lascano (AMNH 206146-206149 [b], 206134-206142 [b]). Canelones: Rincón del Colorado (EMG 1440[a], 1495[a]), not specified (AMNH 206097[b]). Flores: Rio San José (EMG 1762[a]). San José: Arroyo Cufré (EMG 999[a]).

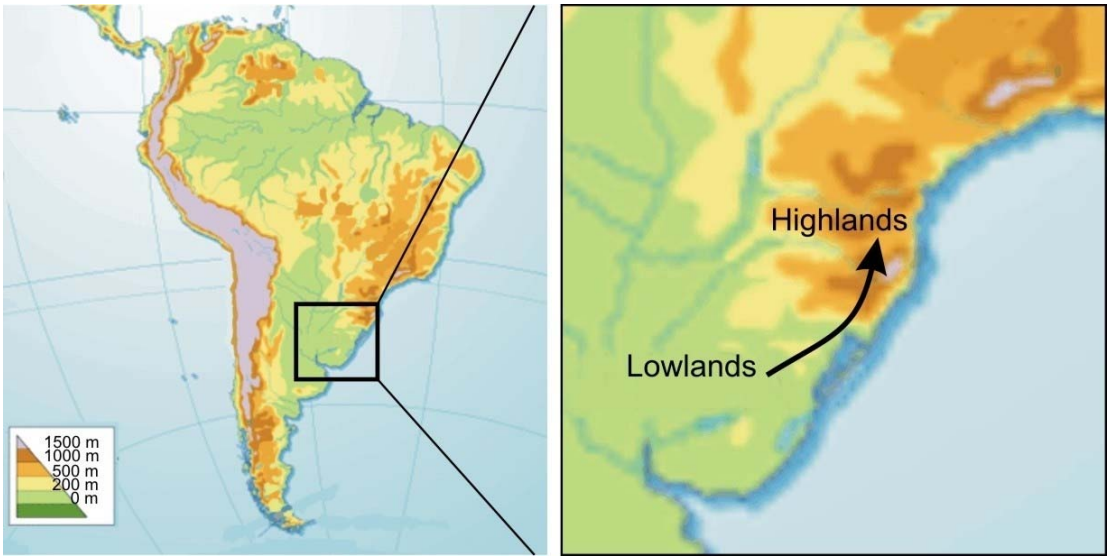

Fig.1. Hypsometry of South America (left), with detail on the study area (right). Narrow indicates the possible route of *Deltamys* basal dispersion, discussed in the main text.

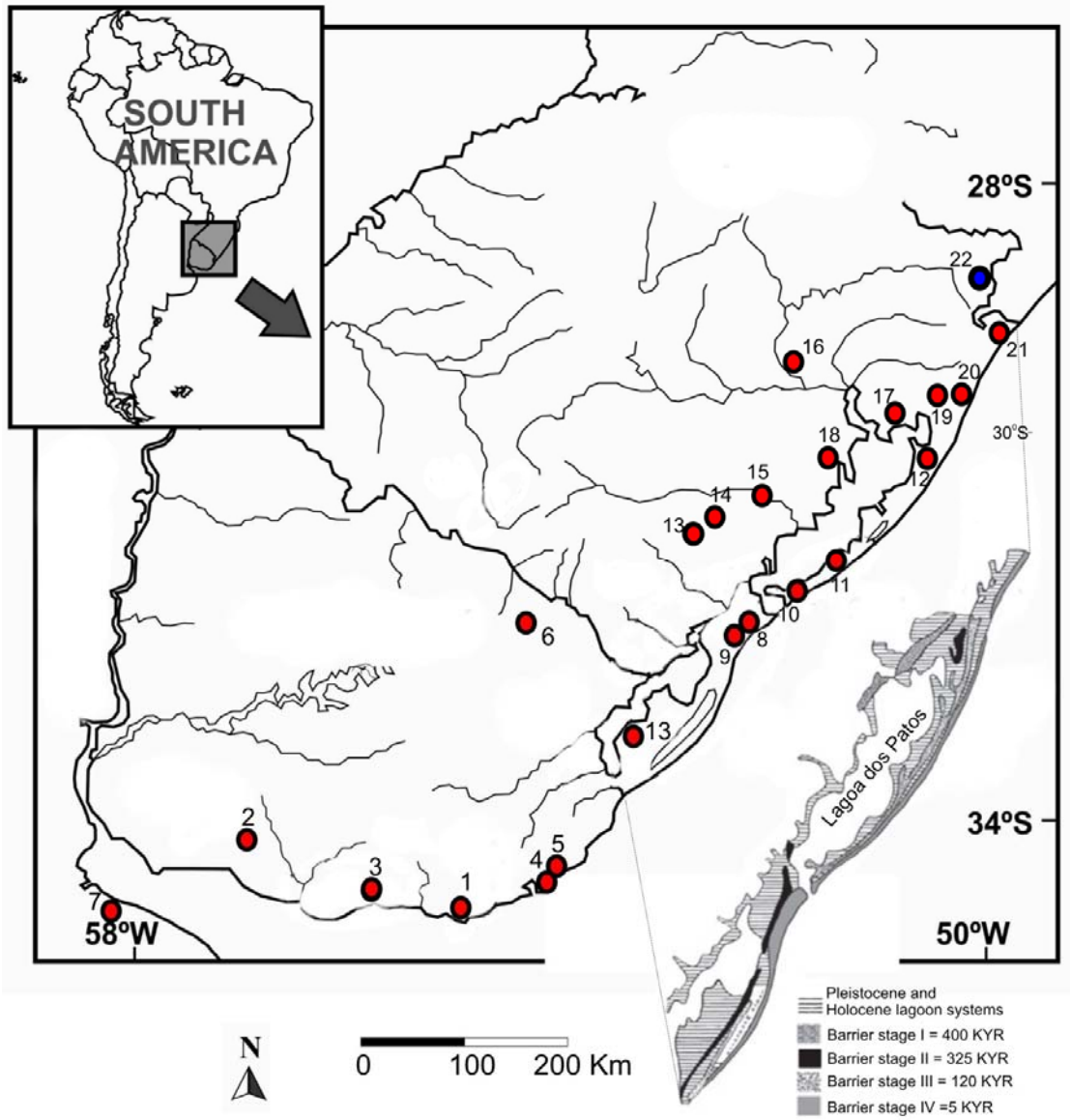

Fig. 2. Collecting localities of *Deltamys kempi* (red circles) and the new lineage of *Deltamys* from Meridional Plateau (blue circle). Numbers correspond to localities showed in Table 1.

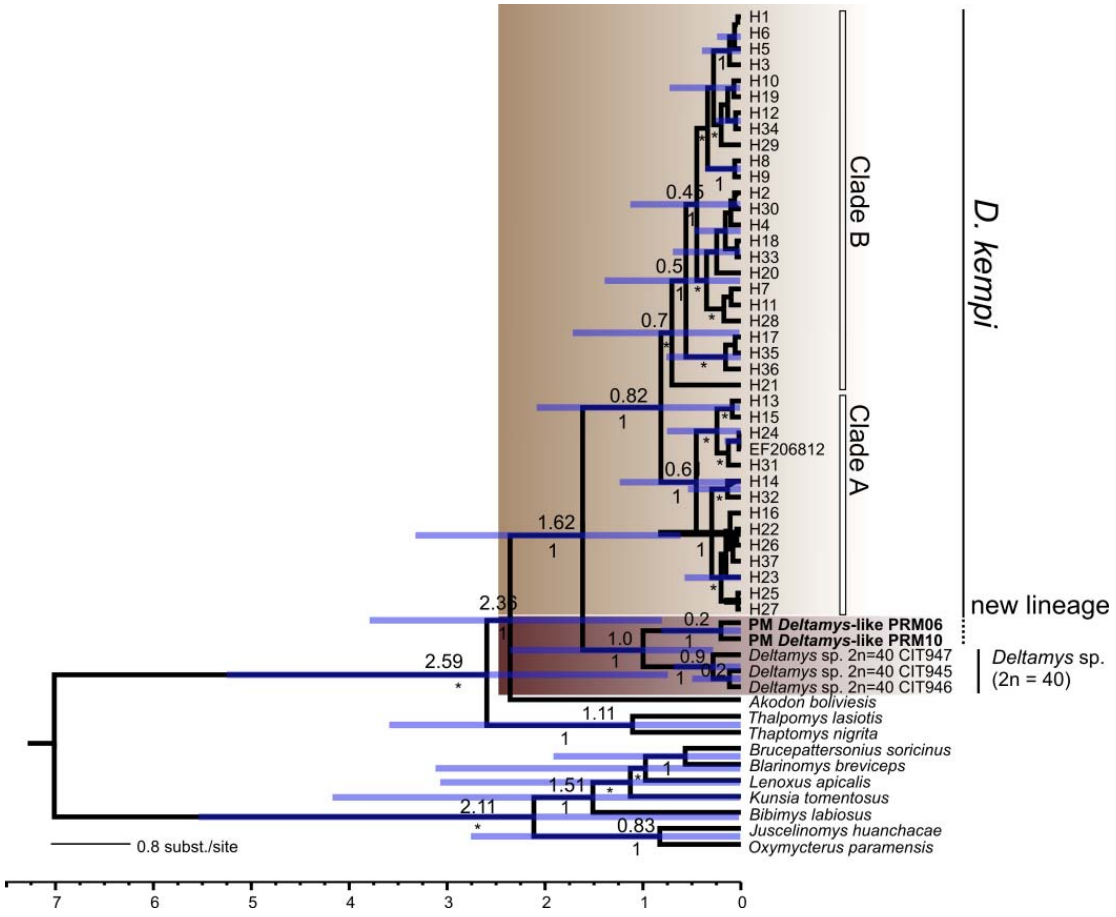

Fig. 3. Bayesian phylogeny tree based on 43 cytochrome *b* haplotypes of *Deltamys* and one haplotype of each Akodontini genus. Numbers above branches indicate the mean estimated time of divergence of the main nodes. Blue bars indicate the range of the estimated time. Numbers below branches represent Bayesian posterior probability (BPP) node support. Asterisk indicates BPP < .95.

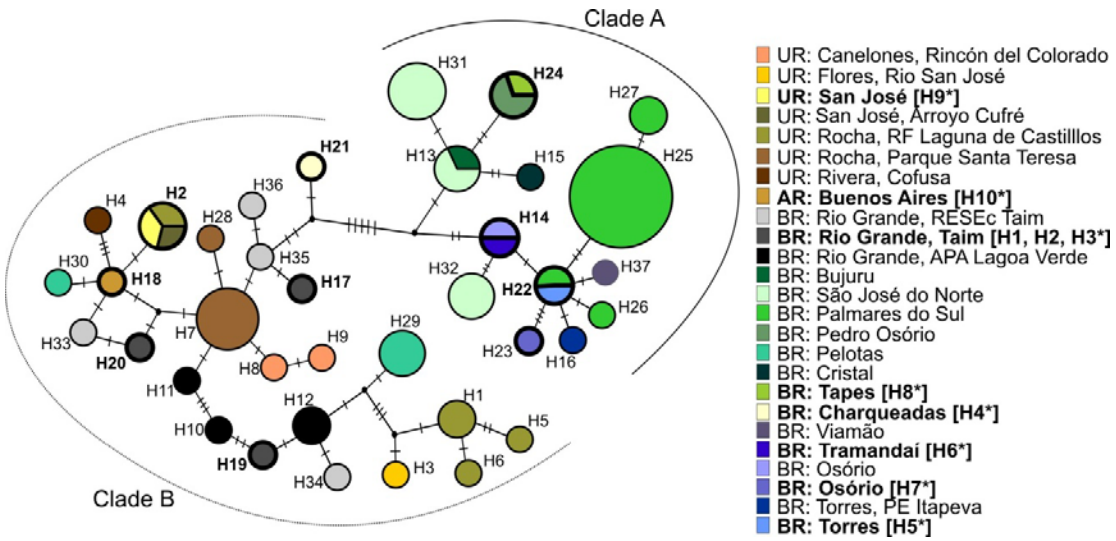

Fig. 4. Median-joining network reconstructed based on 37 haplotypes of *Deltamys kempi*. Colors represent sampling localities listed in the inserted legend (bold indicate haplotypes [H1-H10] found by Montes et al. [2008] in the same localities sampled in this study). Circle sizes are proportional to the frequency of occurrence of the respective haplotype. Small bars crossing branches represent substitution events. Clades A and B represent major haplogroups identified in Bayesian phylogenetic analysis.

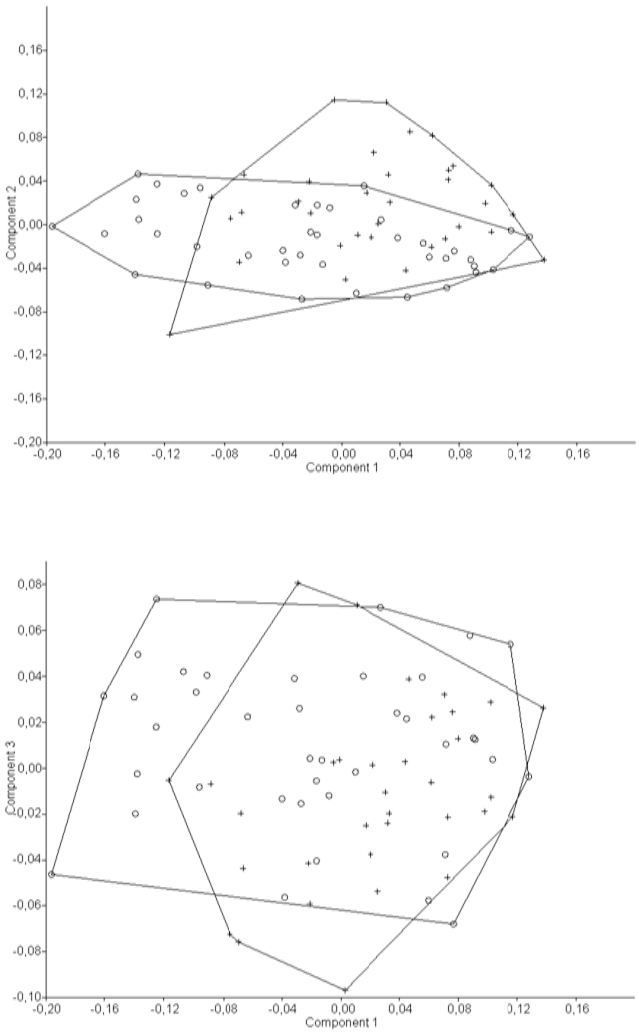

Fig. 5. Scores of *Deltamys kempi* specimens from clades A (crosses) and B (circles) on the principal components 1 and 2 (above) and 1 and 3 (below) extracted from the variance-covariance matrix of 21 cranial measurements.

CONSIDERAÇÕES FINAIS E PERSPECTIVAS

No presente trabalho foi possível descrever e nomear a forma de *Scapteromys* habitante do Planalto Meridional, conhecida a 30 anos como cariomorfos (Freitas, 1984), mas ainda carente de aplicações dos procedimentos formais da Taxonomia Zoológica. As relações filogenéticas entre este novo táxon e seus congêneres, obtida a partir de dados moleculares, concorda com hipóteses prévias sobre a evolução do gênero *Scapteromys*, baseadas em análises citogenéticas. O gênero *Scapteromys*, portanto, passa a conter três espécies formalmente descritas e reconhecidas.

Esta tese também se dedicou à descrição do padrão filogeográfico em *Scapteromys tumidus* e à reavaliação do padrão filogeográfico previamente descrito para *Deltamys kempi* (Montes et al., 2008). Observou-se que a evolução geológica da planície costeira do Rio Grande do Sul e consequente formação do canal do estuário da Lagoa dos Patos estão relacionadas à quebra filogeográfica em ambas as espécies, podendo constituir uma barreira geográfica para o fluxo gênico histórico. Comparativamente, *D. kempi* apresentou um padrão mais estruturado, caracterizado por diversas mutações locais e poucos haplótipos geograficamente compartilhados, enquanto que alguns haplótipos mais amplamente distribuídos em *S. tumidus* indicaram um maior fluxo gênico histórico entre as populações analisadas. *Scapteromys tumidus* apresentou ainda um haplogrupo marcadamente divergente em ambas as análises de sequências de cyt *b* e morfometria geométrica craniana. Apesar da diferença na forma craniana, não foram encontradas dissimilaridades qualitativas, e as distâncias genéticas encontram-se dentro da variação intraespecífica geralmente observada em Akodontini (< 2%;D'Elía, 2003). Análises de marcadores nucleares neste grupo, no entanto, poderão categorizá-lo como uma ESU (evolutionary significant unit) (Moritz, 1994), caso seja atestada uma monofilia recíproca.

Quanto à *D. kempi*, nossas análises de seqüências de cit *b* e medidas craniométricas corroboram a divisão interna deste táxon em dois clados "norte" e "sul", como previamente determinado por Montes et al. (2008) e Ventura et al. (2011). No entanto, o clado "norte" foi substancialmente estendido em direção ao sul, ao longo do escudo rio-grandense. Este novo contexto indica uma área de simpatria entre os clados à oeste do complexo Patos-Mirim. Uma vez que não há objeções claras sob condições de simpatria (Moritz, 1994) e a monofilia dos clados é conhecida para um marcador nuclear (Montes et al., 2008), é proposto a categorização de ESUs para os clados de *D. kempi*.

Ainda, uma nova linhagem de *Deltamys* divergente a nível específico e procedente do Planalto Meridional é apresentada, aumentando para três o número de linhagens específicas dentro do gênero. Espécimes desta nova linhagem estão em processo de comparação, análise e descrição morfológica. Novas expedições para a coleta de mais espécimes para análises citogenéticas já estão programadas. Espera-se em breve apresentar uma revisão do gênero *Deltamys*, onde, além de outros aspectos, serão nomeadas as formas do Planalto Meridional.

Por fim, a identificação de dois novos táxons neste estudo dentro de um contexto de possível especiação das terras baixas em relação ao Planalto Meridional é uma contribuição significativa para estudos futuros, tanto em termos evolutivos, como taxonomicos, envolvendo outros organismos. Os padrões aqui encontrados e descritos, de linhagens altamente divergentes entre planície e planalto, provavelmente sejam também verificados em outras espécies, tanto de plantas como animais, e os resultados poderão ser contrastados em estudos filogeográficos, uma vez que são escassos os estudos na região subtropical.

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