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A new species of *Deltamys* Thomas, 1917 (Rodentia: Cricetidae) endemic to the southern Brazilian Araucaria Forest and notes on the expanded phylogeographic scenario of *D. kempi*

FERNANDO MARQUES QUINTELA[1,2,8], FABRÍCIO BERTUOL[3], ENRIQUE MANUEL GONZÁLEZ[4], PEDRO CORDEIRO-ESTRELA[5], THALES RENATO OCHOTORENA DE FREITAS[1,6] & GISLENE LOPES GONÇALVES[6,7]

[1]*Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Biologia Animal, Av. Bento Gonçalves 9500, Porto Alegre, RS, Brazil*
[2]*Universidade Federal do Rio Grande, Programa de Pós-Graduação em Biologia de Ambientes Aquáticos Continentais,. Av. Itália km 8, Rio Grande, RS, Brazil*
[3]*Instituto Nacional de Pesquisas da Amazônia, Programa de Pós-Graduação em Genética, Conservação e Biologia Evolutiva, Av. André Araújo 2936, Manaus, AM, Brazil*
[4]*Museo Nacional de Historia Natural, 25 de mayo 582, CEP 11000, Montevideo, Uruguay*
[5]*Universidade Federal da Paraíba, Departamento de Sistemática e Ecologia, Laboratório de Mamíferos, Cidade Universitária, João Pessoa, PB, Brazil*
[6]*Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Genética e Biologia Molecular, Av. Bento Gonçalves 9500, Porto Alegre, RS, Brazil*
[7]*Departamento de Recursos Ambientales, Facultad de Ciencias Agronómicas, Universidad de Tarapacá, Arica, Chile*
[8]*Corresponding author*

Abstract

Deltamys is a monotypic sigmodontine rodent from the Pampas of South America. In addition to the formally recognized *D. kempi* that inhabits lowlands, an undescribed form *Deltamys* sp. 2n=40 was recently found in the highlands of south-eastern Brazil. In the present study, we perform a phylogeographic reassessment of *Deltamys* and describe a third form of the genus, endemic to the Brazilian Araucaria Forest. We describe this new species based on an integrative analysis, using complete cytochrome b DNA sequences, karyology and morphology. Bayesian tree recovered two allopatric clades (lowlands vs. highlands) and three lineages: (i) the lowland *D. kempi*, (ii) the highland *Deltamys* sp. 2n=40, and (iii) *Deltamys araucaria* **sp. n.** *Deltamys araucaria* **sp. n.** is karyotypically (2n=34) and morphologically distinguishable from *D. kempi* (2n=37-38), showing a tawnier dorsum/flank pelage, presence of a protostyle, M1 alveolus positioned anteriorly to the posterior margin of the zygomatic plate, and several other distinguishing characteristics. A phylogeographic assessment of *D. kempi* recovered two haplogroups with significant differences in skull measurements. This phylogeographic break seems to have been shaped by the Patos Lagoon estuarine channel. The diversification in *Deltamys* might have been triggered by dispersal of older lineages over different altitudinal ranges in the Paraná geological basin.

Key words: Akodontini, altitudinal gradient, cytochrome *b*, dispersal, evolution

Introduction

Deltamys Thomas, 1917 is a monotypic rodent genus, which for almost a century was thought to be associated only with grasslands in the Pampas biome (lowlands) (González & Pardiñas 2002; González & Martínez-Lanfranco 2010). Recently, a new allopatric lineage, referred to as *Deltamys* sp. 2n=40 (Ventura *et al*. 2011), was found on the Meridional Plateau (hereafter highlands; Fig. 1), a region originally covered over most of its area by Atlantic Forest vegetation, specifically, the Araucaria Forest (i.e. Mixed Ombrophilous Forest). The genus was thought to exhibit a particular karyotype system, due to the distinctive sex-chromosome system of *D. kempi*, in which males (2n=37/FN=38) are determined by $X_1X_2Y_1$ and females (2n=38/FN=38) by $X_1X_1X_2X_2$ (Sbalqueiro *et al.* 1984; Castro *et al.*

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1991; González & Pardiñas 2002; Ventura *et al*. 2011). *Deltamys* sp. 2n=40, in contrast, shows the common mammalian sex-determination system XY/XX (Ventura *et al*. 2011). In addition to the karyological differences, these two *Deltamys* lineages are highly divergent based on DNA sequences of the cytochrome *b* (cyt *b*) gene, showing ca. 12% genetic distance. They form a well-supported sister clade to *Akodon* (Ventura *et al.* 2011).

Deltamys kempi is the only formally recognized species in the genus, described by Thomas (1917) based on specimens from "Isla Ella", an uncertain locality in the Paraná River estuary. Similarities between *D. kempi* and small *Akodon* forms have led some authors to consider *Deltamys* a subgenus or even a synonym of *Akodon* (e.g. Ellerman 1941; Cabrera 1961; Massoia 1964; Reig 1987; Barrantes *et al.* 1993; Musser & Carleton 1993). Morphological (González & Massoia 1995), cytogenetic (Gentile de Fronza *et al*. 1981; Sbalqueiro *et al.* 1984; Castro *et al*. 1991) and later molecular data (D'Elía *et al.* 2003; Montes *et al.* 2008), however, supported the generic status of *Deltamys*.

The intraspecific genetic diversity in *D. kempi* was assessed through analysis of the cytochrome *b* (cyt *b*) and nuclear recombination activating gene 2 sequences (Montes *et al.* 2008), and in *Deltamys* sp. (2n =40) based only on cyt *b* data (Ventura *et al*. 2011). *Deltamys kempi* shows high haplotypic diversity, considering the number of localities surveyed in these studies. The first phylogeographic analysis, using 30 specimens from eight localities along the species distribution (10 haplotypes recovered) (Montes *et al.* 2008), showed an internal division of *D. kempi* into two groups: (i) restricted to the coastal plain of northern Rio Grande do Sul state (RS) plus one locality on the northeastern shore of the Patos Lagoon; and (ii) extending to inland RS, southern RS coastal plain, Uruguay and Argentina. These groups also show significant differences in a craniometric analysis (Montes *et al*. 2008). Based on these molecular and morphological divergences, Montes *et al*. (2008) suggested that *D. kempi* could encompass two taxa. In spite of these important findings, Montes *et al*. (2008) did not evaluate the genetic diversity on the central RS coastal plain (*Restinga* of São José do Norte), and sampled only one Uruguayan locality. Furthermore, the craniometric analysis in their study did not include samples from Uruguayan populations.

In this study we expanded this scenario, including a number of localities in Uruguay and also within Rio Grande do Sul state. More importantly, we explored a continuation of the distribution beyond the northern limit of the distribution range defined by Montes *et al.* (2008), considered as Torres municipality (29° 20'S; 049° 43'W). This limit is a narrow region of the coastal plain, delimited eastward by the Atlantic Ocean and westward (ca. 2 Km) by a forested mountain range. The mountain range lies within the Atlantic Forest domain and is characterized by a mosaic of grassland patches within an Araucaria Forest. Accordingly, we sampled in this region (São Francisco de Paula municipality; Fig. 1) specimens that externally resemble *D. kempi* (small size, relatively large head, short limbs and tail, soft pelage, small eyes, delicate skull; González & Pardiñas 2002), except for a tawnier pelage. Given the existence of *Deltamys* sp. (2n=40) in the Araucaria Forest (Ventura *et al*. 2011), we investigated the phylogenetic status of the SFP morphotype, and describe it here as a new species. We also re-examined the genetic structure and skull morphological differentiation in *D. kempi,* using a larger and broader sample.

Material and methods

Sample collection. We sampled 18 individuals from the São Francisco de Paula (hereafter SFP) highlands (eight were collected and 10 were released after their ear tips were clipped, as requested by the local managers) and 69 of *D. kempi* (all collected) from lowland localities in eastern and southern RS (Fig. 1; Table 1). Skin, skull and tissue samples were prepared and housed at the Genetics Department of the Universidade Federal do Rio Grande do Sul (DG-UFRGS), Museu de Ciências Naturais da Universidade Luterana do Brasil (MCNU) and Zoology Department of the Universidade Regional de Blumenau (DZ-FURB). For the morphometrics approach, we also included 55 specimens from Uruguay, housed at the American Museum of Natural History (AMNH) and the Museo Nacional de Historia Natural (EMG and PCE). All material used is listed in Appendix I. The collecting was approved by the Brazilian governmental authority ICMBio—SISBIO 29358-1.

Cytogenetic methods. Chromosome preparations were obtained from femoral marrow of one male and one female from SFP, following Ford and Hamerton (1956). Individuals were euthanized 35 minutes after a subcutaneous adminstration of 0.1% colchicine solution (1 ml/100 g of body weight) and marrow cells were extracted and treated with hypotonic citrate. Diploid number was established after analyzing at least 10 metaphases/individual. Suspensions of bone-marrow in Carnoy's fixative were deposited at DG-UFRGS.

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

Species	#	Locality	N	Haplotype	Reference
Deltamys kempi					
	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	This study
	7	AR: Buenos Aires*	-	H18	Montes *et al*. 2008; D'Elía *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
Deltamys araucaria sp. n.					
	22	BR: RS, São Francisco de Paula	18	H38, H39, H40, H41, H42	This study
Deltamys sp. (2n=40)	23	BR: RS, Esmeralda*	3	H43, H44	Ventura et al. 2011

UR: Uruguay, AR: Argentina, BR: Brazil. * Localities sampled by Montes *et al*. (2008) and Ventura *et al*. (2011), incorporated into this study from the GenBank database.

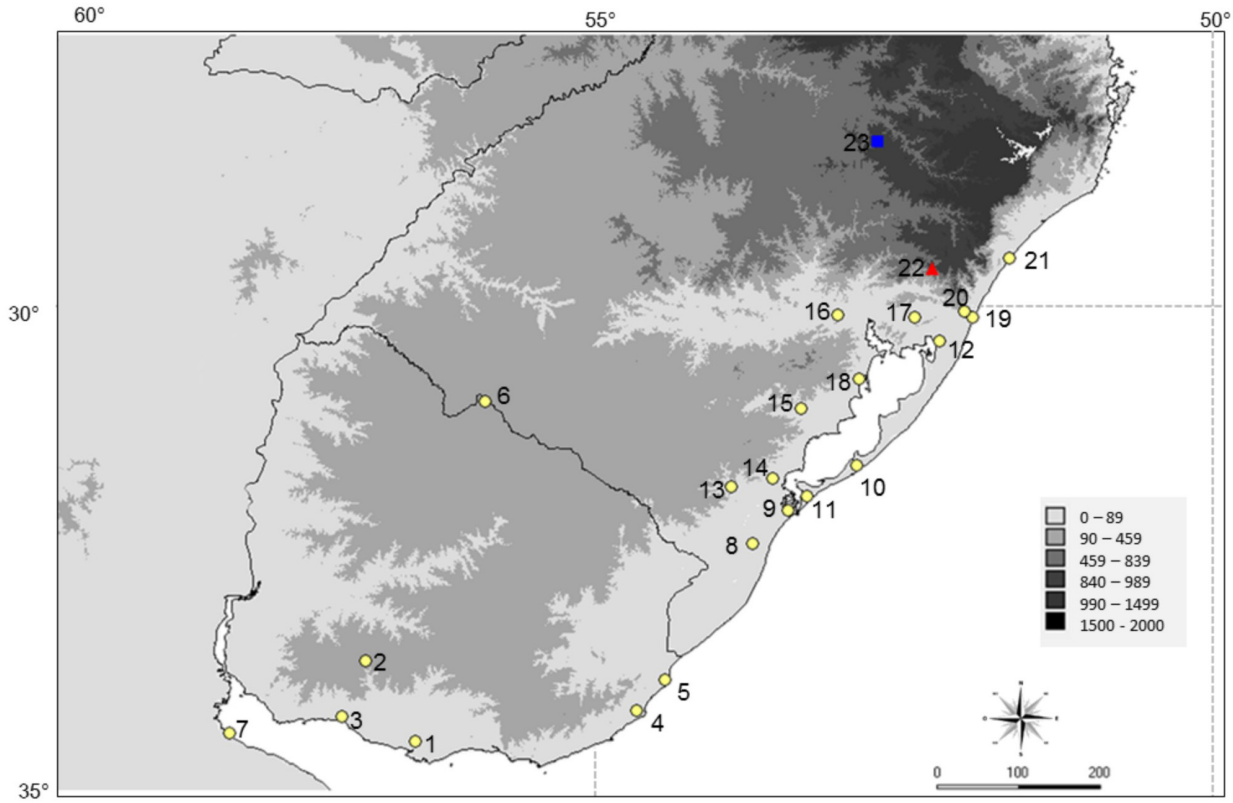

FIGURE 1. Distributional range of *Deltamys* in South America, showing the allopatry of lowland and highland populations. Collecting localities of *Deltamys kempi* are marked in yellow circles, *Deltamys araucaria* **sp. n.**. is indicated by the red triangle, and *Deltamys* sp. (2n=40) is indicated by the light-blue square. Numbers correspond to localities presented in Table 1.

Molecular methods. The majority of the DNA sequences analyzed were generated for this study, obtained according to the following protocol. Genomic DNA was isolated from 87 specimens (Table 1) using approximately 0.2 g of liver or muscle tissue, with the Cetyl Trimethyl Ammonium Bromide method (Doyle & Doyle 1984). The complete cyt *b* gene (1,143 bp) was amplified using a polymerase chain reaction (PCR) with primers MVZ05 and MVZ14 (Smith & Patton 1993). Reaction concentration included: 25 µL total volume, including 2.5 µL of 10× PCR buffer, 1.0 µL of $MgCl_2$ (50 mM), 0.5 µL of dNTP mixture (10 mM for each nucleotide), 0.3 µL of Platinum® Taq DNA polymerase (Invitrogen, Waltham, MA), 0.3 µL of each primer (10 µM), and 1 µL of DNA template (100 ng/µL). PCR thermal profiles included an initial denaturation at 94 °C (5 min), followed by 39 cycles with denaturation at 94 °C (20 s), annealing at 48 °C (30 s), polymerization at 72 °C (45 s), and a final extension at 72 °C (7 min). PCR products were purified using ExoSap-IT® (USB Corporation) and sequenced using an ABI 3730-XL automatic sequencer (Perkin Elmer, Applied Biosystems, Foster City, CA), with the above-listed primers. Electropherograms were manually checked to locate any discrepancy, and edited in MEGA 6 (Tamura *et al*. 2013). Nucleotide sequences generated from this study were deposited in GenBank under the accession numbers KM275437- KM275467.

Sequence analysis. We reconstructed a maximum likelihood (ML) tree to infer phylogenetic relationships in *Deltamys* . We used original data generated for 18 specimens from SPF and 75 specimens of *D. kempi* (32 novel haplotypes). Haplotypes of *D. kempi* (Montes et al. 2008) and three sequences of *Deltamys* sp. 2n=40 (Ventura et al. 2011) were incorporated from GenBank (Table 1). Representative akodontine species (e.g., *Oxymcterus paramensis* and *Brucepattersonius soricinus*), some of them sympatric do *Deltamys* (e.g., *Akodon paranaensis, A. reigi, A. azarae* and *A. serrensis*; *Necromys lasiurus*), were used to root the tree downloaded from Genbank database. The ML analysis was conducted using the HKY (Hasegawa et al. 1985) nucleotide substitution model that was selected by jModeltest 2 (Darriba et al. 2012). 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% cutoff after 1000 bootstrap iterations.Pairwise genetic distances (*p*-distances) among clades were estimated in MEGA 6.

We characterized mtDNA diversity for all lineages, including variable sites, definitions of haplotypes, haplotype and nucleotide diversity, and Neutrality tests (Tajima's *D*, Fu and Li's *D* and *F,* Fu's *FS*), using the program DNASP 5.0 (Librado & Rozas 2009). A median-joining haplotype network (Bandelt *et al*. 1999) was constructed in NETWORK 4.6 (http://www.fluxus-engineering.com/sharenet.htm). Levels of genetic structure among subpopulations of *D. kempi* were characterized using ϕ_{ST}, which is analogous to Wright's *F*-statistics but takes into account the genetic distance among haplotypes, using ARLEQUIN 3.5 (Excoffier & Lischer 2010).

Morphological analysis. We directly examined the skull and skin for diagnostic characters to distinguish between *Deltamys* **sp. n.** and *D. kempi* from RS and Uruguay lowlands. The skull of the *D. kempi* holotype was examined through high-definition photographs. The descriptive terminology for general external morphology follows Hershkovitz (1966) and Voss (1988). We transcribed the external measurements 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 and Diaz (2011) and Machado *et al*. (2011).

Twenty-one craniodental measurements (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]) were taken with a digital caliper accurate to the nearest 0.01 mm. Measurements were taken as illustrated by Hershkovitz (1962) and Voss (1988) and converted to decimal logarithms. Missing values (3.2%) were estimated by the method of linear regression (Gauthier *et al*.) 2003. All measurements were positively correlated to GL, which was treated as independent variable. The other measurements were treated as dependent variables and missing values estimated by the model generated for each variable (expected values). We performed two craniometric analyses. First, we examined craniometric differences between specimens of *Deltamys* **sp. n.** (n=7) and *D. kempi* (n=121) through a principal components analysis (PCA) using the first three principal components from the variance-covariance matrix generated from logarithm-converted measurements. We then tested the hypothesis of significant differences in the skull measurements between samples from *D. kempi* mitochondrial clades A (n=34) and B (n=38) previously identified in the Bayesian analysis (see Results). For this, we performed a PCA using the first three principal components from the variance-covariance matrix generated from logarithm-converted measurements. We also performed a discriminant analysis aiming to evaluate the percentage of correct classification of the specimens between mitochondrial clades A and B.. Because sexual dimorphism in the sets of cranial measurements used here was not detected previously (Montes *et al*. 2008), we pooled the sets from females and males for analysis.

Results

We identified high levels of variation in cyt *b* sequences, including 37 haplotypes in *D. kempi* (2n=37-38) from the lowlands and seven in the highlands; five in the SFP morphotype and two in *Deltamys* sp. (2n=40) (Tables 1, 2). Haplotypes found in individuals from SFP were exclusive to this lineage (Figs. 2, 3). Neutrality tests did not indicate a pattern of population expansion, except for the SFP morphotype, which showed significant negative values for most of parameters, but FS Fu's test (Table 2). The genetic divergence between *D. kempi* and either SPF or *Deltamys* sp. (2n=40) were 12% (Table 3). .Specimens from SFP formed a strongly supported bootstrap >0.95) clade, reciprocally monophyletic to the *Deltamys* sp. 2n=40 from Esmeralda (Fig. 2). The genetic distance between them was 8% (Table 3).

*Deltamy*s from SFP showed 2n=34 and FNa=34 (Fig. 4), and therefore is karyotypically distinct from *D. kempi* (2n=38) and the sister lineage from Esmeralda (2n=40). *Deltamys* from SFP presented the commom mammalian sex chromosome system XY/XX, in contrast with the exclusive system $X_1X_2Y_1/X_1X_1X_2X_2$ observed in *D. kempi. Deltamys* from SFP showed clear qualitative craniodental and pelage characters that distinguished it from *D. kempi*, which includes: (1) dorsal and flank pelage markedly tawnier (dorsal and flank pelage of *D. kempi* is grayer)

(Fig.5), (2) presence of protostyle (protostyle absent in *D. kempi*) (Fig. 6), (3) M1 anterior alveolus parallel to or above the posterior border of the zygomatic plate, in ventral view (in *D. kempi* the M1 anterior alveolus is positioned below or parallel to the posterior border of the zygomatic plate) (Fig.6), (4) hamular process short, stocky and of similar width along its length (hamular process with a long ramus and a posteriorly divergent section in *D. kempi*), (5) interparietal comparatively wider (interparietal reduced but present as a distinct losangular bony plate in *D. kempi*) (Fig.6), (6) region of the suture between the frontal and the maxillary at the border of the orbit rounded (in *D. kempi* this region is shelflike, almost forming a plate), (7) presence of enteroloph (enteroloph is present in only two of 62 *D. kempi* specimens), (8) ectolophid conspicuous (ectolophid is absent in most *D. kempi* specimens, and when present, it is vestigial, conspicuous in only a single specimen). However, in the PCA analysis the scores of this new lineage were displayed within the convex hull formed by *D. kempi* individual scores (Fig. 7). External and craniometric measurements of *Deltamys* sp. 2n=34 and *D. kempi* specimens overlapped (Table 4). The genetic divergence at specific level, the distinct karyotype and the marked morphological differences support the recognition of the SFP form as a new *Deltamys* species, therefore described.

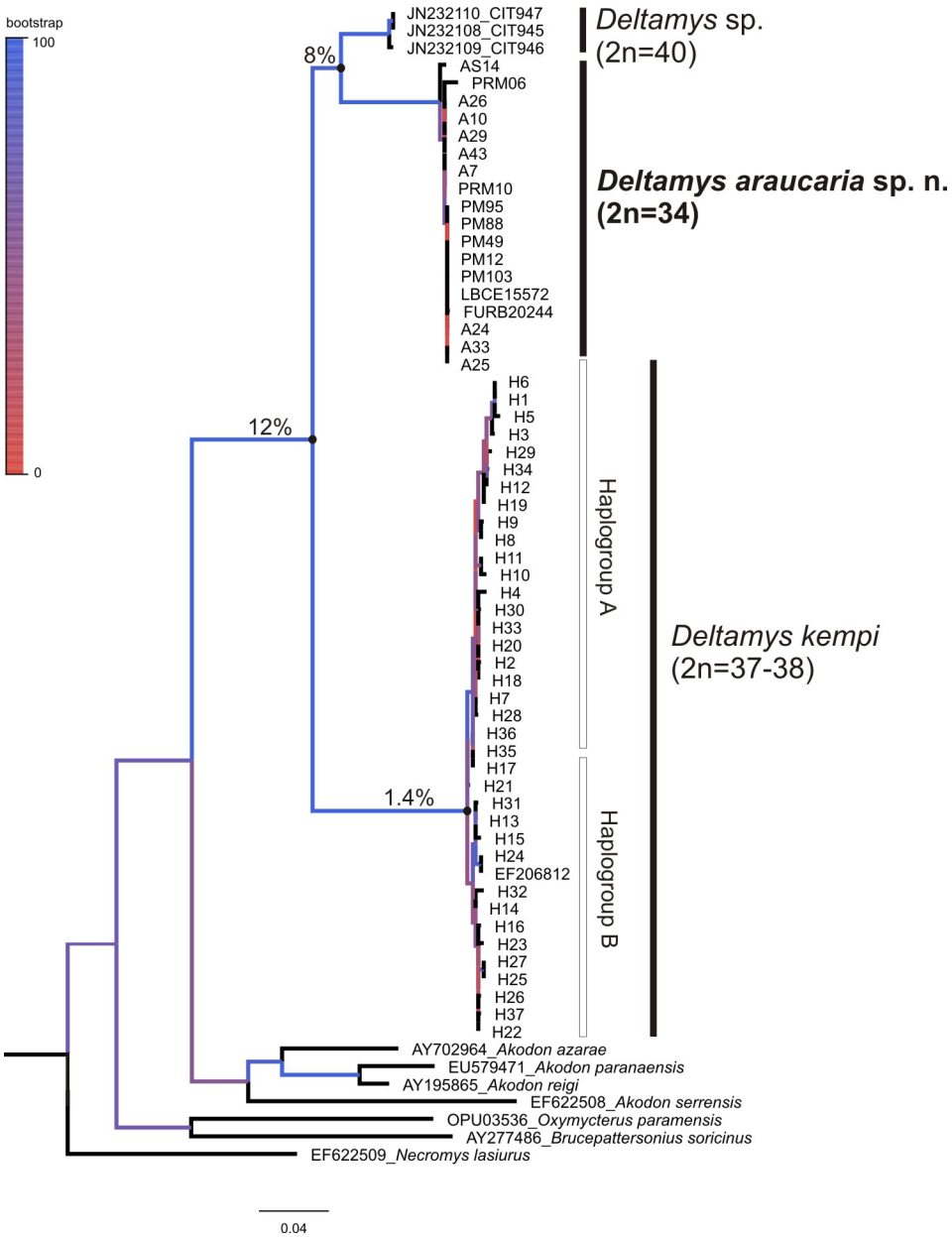

FIGURE 2. Maximum likelihood tree of *Deltamys* constructed based on cytochrome *b* complete sequences. Boostrap branch support is indicated by gradient of colors, accordingly to the legend. Numbers above branches represent the percentage of genetic divergence (*p*-distance) between clades. Bold indicate specimens of the new taxa described in this study.

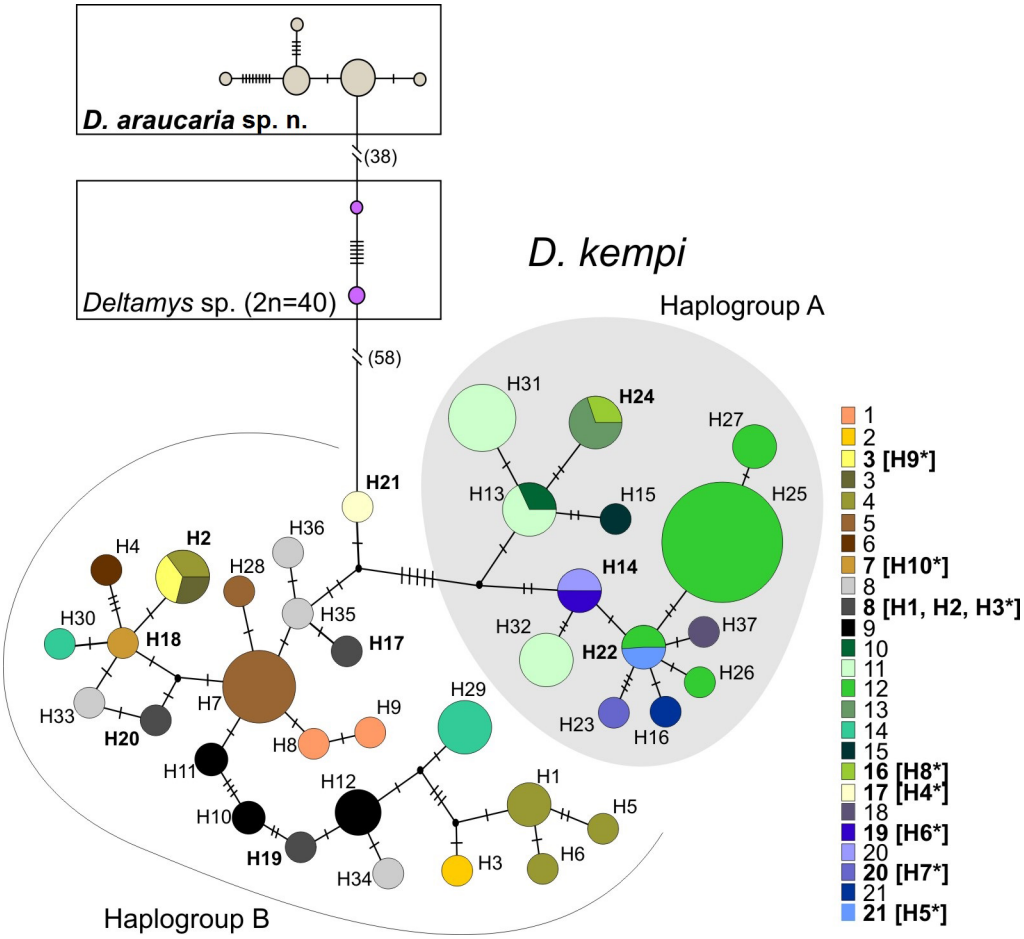

FIGURE 3. Haplotype network for *Deltamys kempi* obtained under the median-joining method. Population labels are given according to Table 1 (bold indicates haplotypes [H1-H10] found by Montes et al. [2008] in the same localities sampled in this study). The size of each circle is proportional to the haplotype frequency. Median vectors are represented by small black circles. All lines represent one mutational step. Clades represent major haplogroups (A and B) of *D. kempi* identified in the phylogenetic analysis.

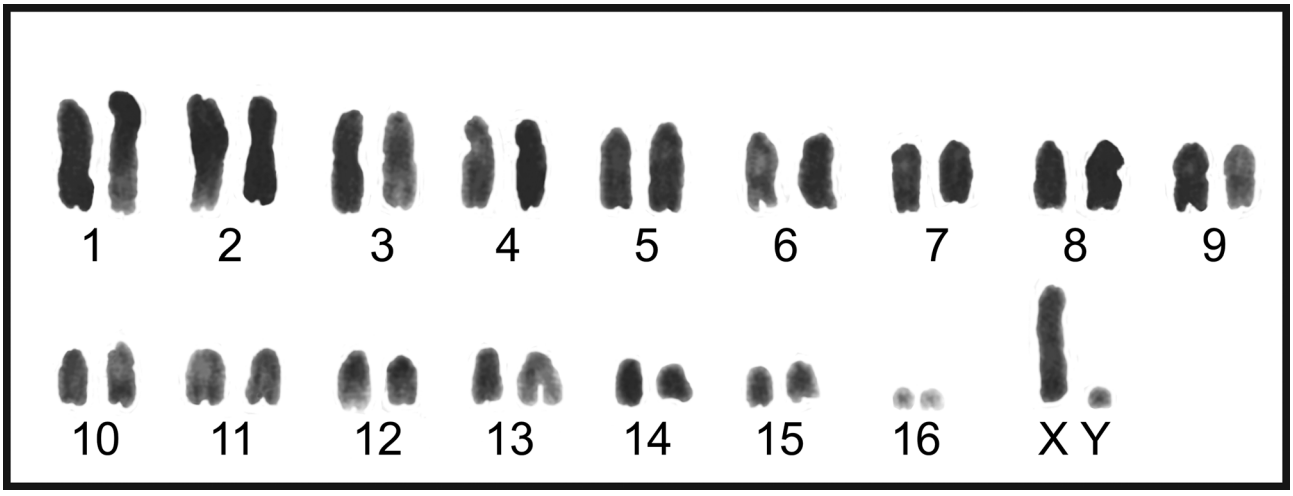

FIGURE 4. Karyotype (conventional Giemsa staining) of *Deltamys araucaria* **sp. n.** showing 2n=34 and FNa=34. The small metacentric pair corresponds to pair 16.

TABLE 2. Number of sequences (N), haplotypes (Ha), polymorphic sites (S), haplotype diversity (Hd ± standard deviation [SD] and nucleotide diversity (Pi± SD) in *Deltamys* are shown. The global neutrality tests Tajima's *D*, Fu and Li's *D* and *F* and Fu's *FS* estimated using DnaSP v5 are also given.

Species	N	H	S	Hd (±SD)	Pi (±SD)
Deltamys kempi	75	37	52 (6.4%)	0.946±0.017	0.01178±0.0004
Deltamys araucaria **sp. n.**	18	5	15 (1.3%)	0.662±0.094	0.00271±0.0012
Deltamys sp. (2n=40)	3	3	12 (1.0%)	1.000±0.272	0.00702±0.0021

continued.

	Neutrality tests			
		Fu and Li's		
Species	Tajima's *D*	D	F	Fu's *FS*
Deltamys kempi	-0.34326	-0.82542	-0.76295	-10.870
Deltamys araucaria **sp. n.**	-1.97476*	-2.97581**	-3.11006**	0.625
Deltamys sp. (2n=40)	-	-	-	0.901

* P < 0.01, ** P < 0.05**.**

TABLE 3. Evolutionary divergence estimates based on cytochrome b sequences between pairwise groups of *Deltamys* (and a sister species, *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 replications), is shown. Analyses were conducted using uncorrected *p*-distance.

	Deltamys kempi (clade A)	*Deltamys kempi* (clade B)	*Deltamys* sp. (2n=40)	*Deltamys araucaria* **sp. n.**
Deltamys kempi (clade A)	-			
Deltamys kempi (clade B)	0.01±0.00	-		
Deltamys sp. (2n=40)	0.11±0.02	0.12±0.02	-	
Deltamys araucaria **sp. n.**	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

***Deltamys araucaria* sp. n.**
Araucaria Grass Mouse
Figures 5,8, Table 4

Holotype. FURB 20296 (field number PRM 137), adult female with skull (left zygomatic arch and incisors damaged), skin, post-cranial skeleton, ethanol-preserved forefoot and hind foot, collected by F. M. Quintela on 17 August 2012. Frozen tissue sample (PRM 137) and suspension of bone-marrow in Carnoy's fixative (PRM 137) were deposited in the Department of Genetics at UFRGS (DG-UFRGS).

Type locality. São Francisco de Paula municipality (29º29'73"S, 050º13'49"W; 913 m above sea level), Rio Grande do Sul State, Brazil.

Paratypes. Seven topotypes (FURB 20220, 20240, 20244, 20297, 20330, 20954 and 20955) were collected between 1 April and 12 October 2012. A frozen tissue sample (FQ 84-86; PRM 10, 103, 104, 106, 124, 138) and a suspension of bone marrow in Carnoy's fixative (FQ 85 and 86) were deposited in the Department of Genetics at DG-UFRGS.

Distribution. Known only from shrub and herbaceous palustrine systems in São Francisco de Paula municipality (altitude ca. 913 m above sea level), Serra Geral highlands, Rio Grande do Sul state, southern Brazil. This region is within the biogeographic domains of Araucaria Forest, Atlantic Forest biome.

Diagnosis. A markedly tawny furred *Deltamys* species; 2n=34, FNa=34; protostyle present; M1 anterior alveolus parallel to or above posterior border of zygomatic plate in ventral view.

TABLE 4. External and cranial measurements (in millimeters) of *Deltamys kempi* and *Deltamys araucaria* (mean [X] ± one standard deviation [SD], the observed range [in parentheses], and the sample size [N]).

	Deltamys kempi			*Deltamys araucaria* **sp. n.**		
	X ± SD	Range	N	X ± SD	Range	N
HBL	79.77 ± 8.31	(71.7–98.03)	44	79.76 ± 11.67	(68.41–91.53)	7
LT	74.11 ± 6.82	(62.26–86.33)	44	65.92 ± 5.76	(61.85–70.11)	7
Ear	13.06 ± 1.10	(10.11–15.96)	44	14.15 ± 2.61	(12.37–16.08)	7
HF	21.65 ± 2.14	(20.17–23.22)	44	21.86 ± 2.54	(20.04–23.63)	7
IB	1.79 ± 0.47	(1.46–2.12)	114	1.65 ± 0.08	(1.59–1.71)	7
LMR	3.83 ± 0.70	(3.34–4.33)	118	4.00 ± 0.14	(3.91–4.01)	7
NL	9.26 ± 1.93	(7.89–10.63)	112	9.47 ± 1.20	(8.62–10.33)	7
BR	4.07 ± 0.98	(3.37–4.77)	119	3.83 ± 0.34	(3.59–4.08)	7
TBL	3.97 ± 0.82	(3.38–4.55)	108	4.08 ± 0.35	(3.84–4.33)	7
BB	10.46 ± 1.59	(9.33–11.58)	112	10.65 ± 0.32	(10.42–10.88)	7
BZB	2.00 ± 0.58	(1.59 -2.41)	117	1.87 ± 0.21	(1.72–2.03)	7
LIB	4.27 ± 0.61	(3.84–4.70)	119	4.37 ± 0.11	(4.29- 4.44)	7
LR	8.20 ± 1.44	(6.47–9.93)	113	8.96 ± 0.42	(8.06–9.26)	7
DFM	4.55 ± 0.39	(3.92–5.19)	118	4.73 ± 0.17	(4.61–4.86)	7
BFM	1.05 ± 0.39	(0.77–1.32)	118	1.13 ± 0.11	(1.05–1.21)	7
CIL	23.19 ± 3.92	(20.42–25.96)	108	22.82 ± 1.13	(22.02–23.63)	7
TL	24.36 ± 3.63	(21.80–26.93)	101	24.38 ± 1.01	(23.67–25.09)	7
PL	3.56 ± 0.75	(3.03–4.09)	119	3.60 ± 0.23	(3.44–3.76)	7
LD	6.15 ± 1.65	(4.98–7.32)	118	6.54 ± 0.56	(6.14–6.94)	7
OL	8.11 ± 1.33	(7.16–9.04)	114	8.04 ± 0.29	(7.83–8.25)	7
HB	9.04 ± 1.87	(7.72–10.36)	99	9.27 ± 0.44	(8.96–9.59)	7
BZ	11.67 ± 1.56	(10.56–12.77)	100	11.25 ± 0.48	(10.91–11.60)	7
IFL	5.06 ± 0.42	(4.17–5.96)	118	5.48 ± 0.56	(5.08–5.88)	6
IFW	1.61 ± 0.61	(1.18–2.04)	118	1.74 ± 0.24	(1.57–1.91)	6
DFO	6.09 ± 0.59	(5.67–6.51)	106	6.16 ± 0.24	(5.99–6.33)	7
Weight	27 ± 9	(14–41)	37	16 ± 8	(10–22)	7

Description. Pelage dense and soft, uniformly tawny brown on dorsum (Fig. 5), slightly lighter on face and cheeks; guard-hairs about 9 mm over back, all eumelanic; overhair about 7 mm on back, three-banded, eumelanic on distal tip and most of length, and pheomelanic in distal section; under-hair about 5 mm on back, all eumelanic. Ventral pelage (Fig. 5) grayish-brown, clearly demarcated from browner flanks; dichromatic overhair about 6 mm in middle chest, pheomelanic in distal segment and eumelanic in central and proximal segments; dichromatic under-hair about 3.7 mm in middle chest, with same banding pattern as ventral overhair. Presence of superciliary and inconspicuous mystacial and submental vibrissae; measurements of largest vibrissae from holotype FURB 20330: mystacial 16.3 mm, superciliary 9.8 mm, submental 6.5 mm; longest mystacial not reaching bases of pinnae when laid back along head. Ears rounded, not reaching eyes when laid forward, densely covered with 2.4 mm eumelanic thick hairs. Tail about 75% of head-body length; unicolor and dark; hair coverage barely visible; hair arranged in triplets on each scale, subequal in size, with central hair reaching two and half scales; 4-5 mm terminal pencil. Mammae eight, in pectoral, axial, abdominal and inguinal pairs.

Hind foot about 26% of head-body length; plantar surface dark; densely covered with short melanic hair; digits II, III and IV subequal in size; digit I conspicuously longer than digit V; claws grooved, about 2.5 mm long in digit III; ungueal hair most melanic, not reaching tip of claws; thenar pad about 2.5 x interdigital pad; interdigital pads subequal in size; hypothenar pad slightly shorter than interdigital pads. Forefoot sparsely covered with melanic and

bicolor hair on metapodial and digits; digits III and IV subequal in size; digits II and V subequal in size; well-developed claws, extending about 1.7 mm past extremity of digits III and IV; ungueal hair not covering claws; dark palmar surface; hypothenar pad slightly longer than thenar pad; interdigital pad 3 conspicuously longer than interdigital pads 1 and 2.

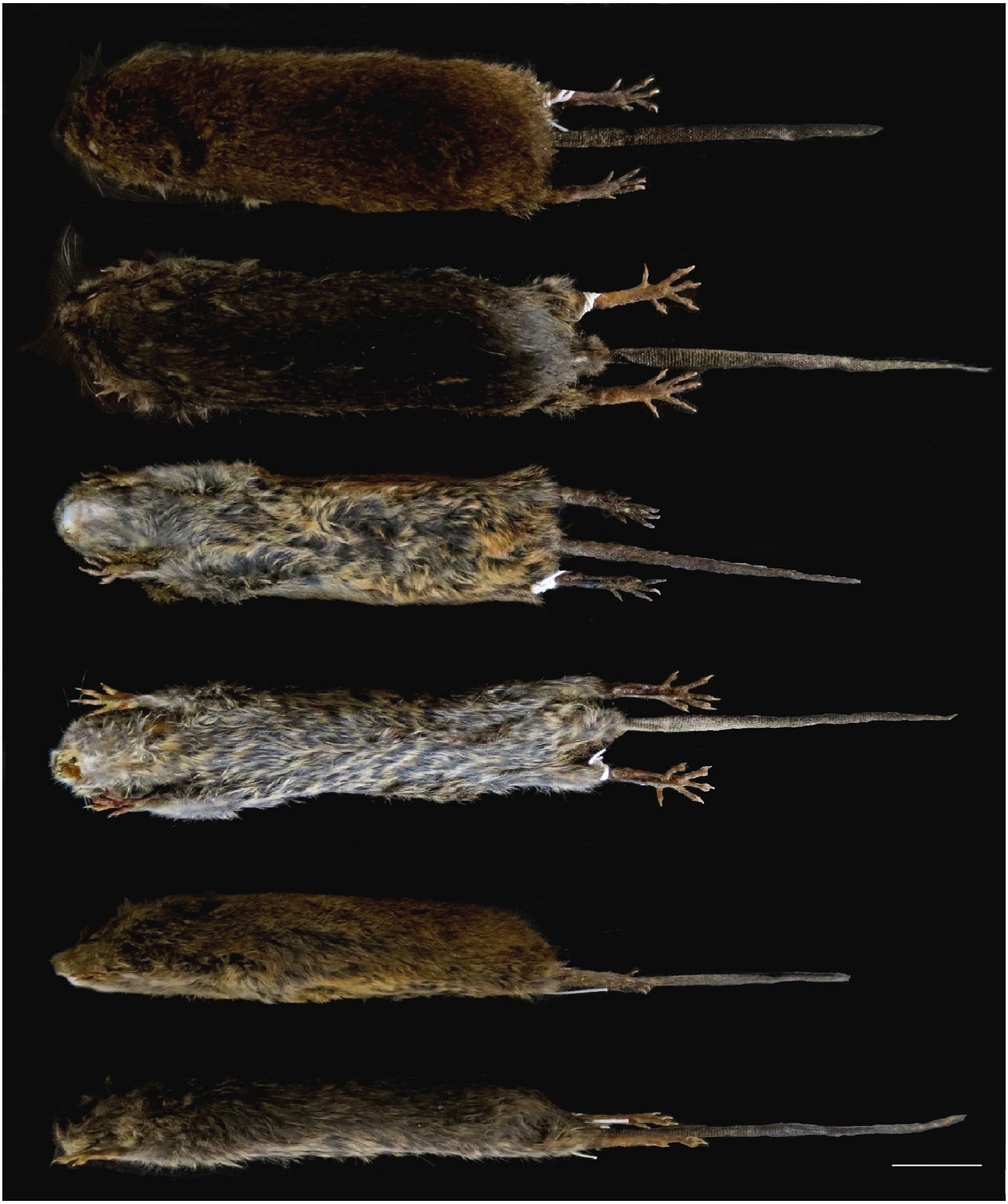

FIGURE 5. Dorsal (upper), ventral (middle) and flank (bottom) views of skins of *Deltamys araucaria* **sp. n.** (holotype, FURB 20296; above) and *Deltamys kempi* (FURB 20308). Bar = 20 mm.

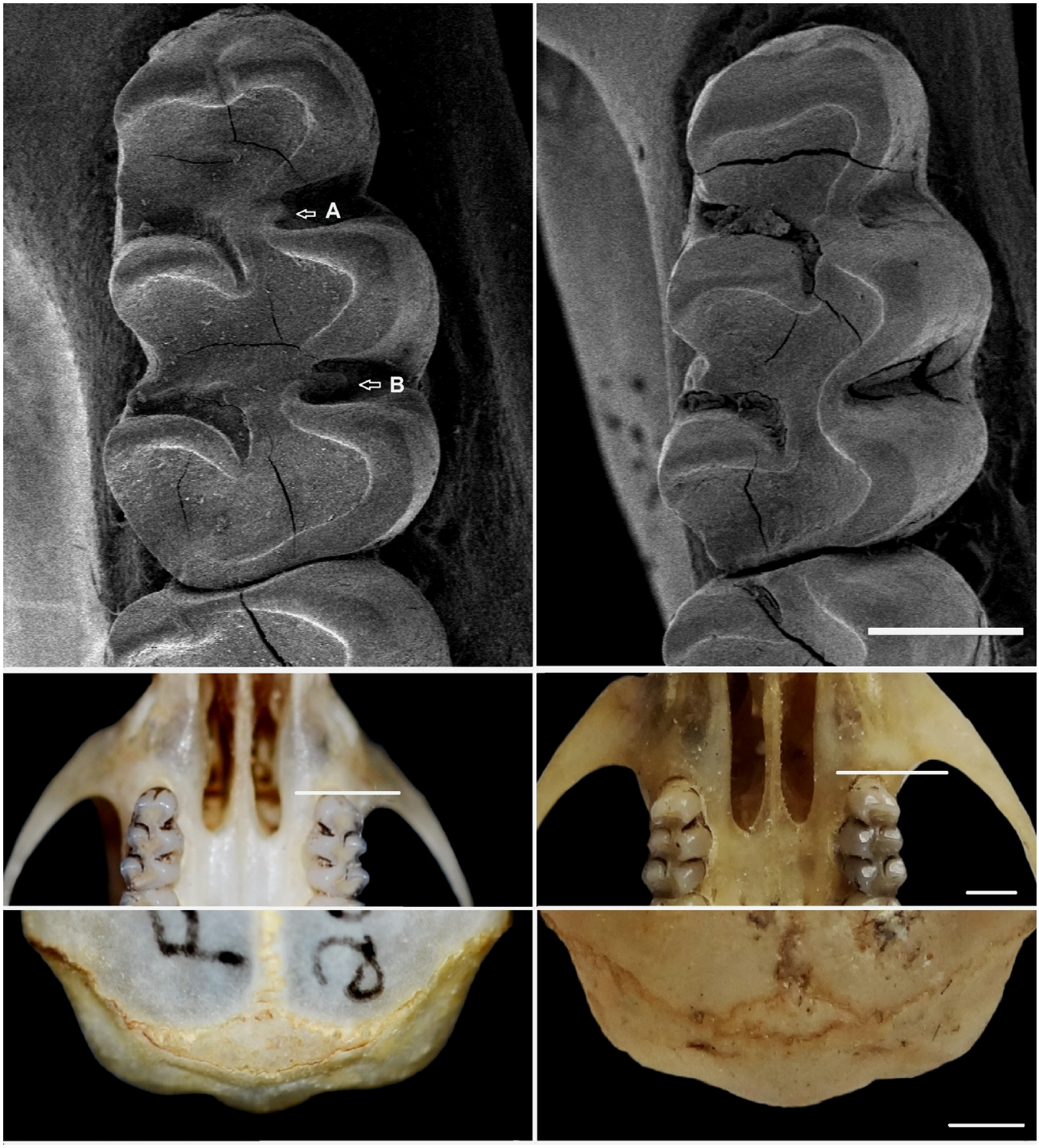

FIGURE 6. Morphological distinctive traits in *Deltamys*. **Above**, first upper molar of *Deltamys araucaria* **sp. n.** (FURB 20330; left) and *D. kempi* (TR 2136; right), showing the presence of protostyle (A) and enteroloph (B) in *D. araucaria* (bar = 0.5 mm; 30x). **Middle**, position of M1 anterior border in relation to posterior border of zygomatic plate in *D. auraucaria* **sp. n.** (left) and *D. kempi* (right) (bar=1 mm). **Bottom**, proportion of interparietal in *D. araucaria* (left) and *D. kempi* (right) (bar=2mm).

Skull (Fig. 8) delicate, with narrow elongated rostrum and narrow zygomatic arches; gnathic process little developed, not exceeding anteriormost extremity of incisor in lateral view; well-developed anterior process of premaxillary but not forming rostral tube (trumpet) with nasal extremity; slightly inflated incisive capsular projection; nasofrontal suture "V"-shaped; shallow zygomatic notches; rounded lacrimal; narrow nasolacrimal foramen, similar to M1 in size; broad zygomatic plate, directed backward, with posterior border positioned behind of anterior plane of M1; elongate incisive foramina with their broadest point around premaxilla-maxillary suture,

posterior extremity reaching protoflexus or protocone; narrow antorbital bridge; posterior palatine foramina small and ovate, located in maxillopalatine suture at height of M2 hypoflexus; hourglass-shaped interorbital region, with rounded margins and posteriorly broader; conspicuous ethmoid foramen; sphenopalatine foramen horizontally elongated, reaching from M1 metacone to M2 posterior border; large optic foramen, vertically elongated and located behind M3; large anterior lacerated foramen, similar to optic foramen in size; zygomatic arches narrow, anteriorly convergent and posteriorly slightly divergent, surpassing border of braincase in dorsal view; frontal-parietal-squamosal suture at exact point of parietal anterior process extremity or slightly behind this process; conspicuous elongated jugal; shallow glenoid fossa; mesopterygoid fossa narrow, extending from slightly behind M3 alveoli to posterior border of zygomatic arches, straight along most of its length and divergent posteriorly; presphenoid posteriorly wider; sphenopalatine vacuities elongated, similar to M1 in size, and divided by narrow presphenoid strut; basisphenoid plane; basioccipital with prominent median crest; parapterygoid fossa shallow and narrow; hamular processes of pterygoid straight and divergent; coronal suture "U"-shaped; carotid as circulatory pattern 1 of Voss (1988); large and oval stapedial foramen; large petrotympanic fissure, varying in width; little-developed stapedial spine; broad Eustachian tube; moderately inflated tympanic bulla, similar in size to superior molar row; punctual carotid canal; large jugular foramen; thin malleus; orbicular apophysis present; broad hamular process of squamosal; postglenoid foramen conspicuously larger than subsquamosal foramen; braincase moderately inflated; interparietal varying in length and width; mastoid slightly inflated, similar in size to external auditory meatus, bordered posteriorly by elongated foramen; exoccipital conspicuously trilobed, without prominent crests; occipital condyle slightly inflated; well-developed paraoccipital process; large foramen magnum.

Mandible (Fig. 8) gracile, length without incisors about 49% of greatest skull length; height about 41% of length without incisors; elongated ramus; short diastema, similar in size to molar row, anterior extremity located parallel to molar plane; conspicuous mental foramen, visible from lateral and occlusal views; little-developed masseteric ridge; conspicuous capsular projection; delicate coronoid process isolated from condyloid by shallow elongated sigmoid notch, strongly concave anteriorly; condyloid process robust, markedly thickened distally; shallow mandibular notch; little-developed angular process, distally thickened and perpendicularly projected.

Upper incisors orthodontt and orange; lower incisors much paler. Upper molars (Fig. 9) parallel, crested; lingual cusps higher than labial cusps. Main cusps (paracone, metacone, protocone, hypocone) parallel. M1 with well-developed anteromedian flexus in specimens with little-worn molars, dividing procingulum into anterolingual and anterolabial conules subequal in size; anteroloph and mesoloph present but reduced; little-developed but conspicuous protostyle and enteroloph (Fig. 6); protocone and paracone equal in size; hypocone and metacone equal in size; deep paraflexus and metaflexus, curved backward parallel to median mure; shallow protoflexus and hypoflexus; little-developed posteroloph, visible mainly in specimens with unworn dentition (e.g. FURB 20244). M2 lacking anteroconule; anteroloph visible in specimens with slight or moderate wear; protocone and paracone subequal in size; hypocone and metacone subequal in size; anteroloph and mesoloph present but reduced, visible in specimens with all degrees of wear; hypoflexus deep and straight; paraflexus deep and curved backward parallel to median mure; narrow median mure between hypoflexus and paraflexus. M3 with three visible cusps, protocone, paracone, and metacone; protocone conspicuously larger than paracone and metacone; presence of two or three fossetes in specimens with dentition little worn. Lower molars (Fig. 9) parallel and crested, with lingual cusps higher than labial ones. Main cusps slightly alternated; m1 with well-developed procingulum; shallow anteromedian flexid visible only in specimens with unworn or slightly worn molars; anterolabial conulid larger than anterolingual conulid; well-developed protostylid; anterolophid conspicuous, parallel to protostylid; protoflexid deep and straight; anteroflexid and metaflexid shallow; protoconid larger than metaconid; mesoflexid deep and straight; hypoflexid deep and upward-curved; mesoflexid and hypoflexid separated by narrow median mure; well-developed ectolophid; mesolophid fused with entoconid; shallow and straight entoflexid; well-developed posterolophid, separated from entoconid by deep and upward-curved posteroflexid. m2 lacking anteroconulids; well-developed protostylid, separated from protoconid by deep protoflexid; protoconid larger than metaconid; hypoconid larger than entoconid; mesoflexid deep and curved upward; hypoflexid deep and diagonal; mesoflexid and hypoflexid separated by narrow median mure; ectolophid visible in specimens with dentition little worn; mesolophid fused with entoconid; well-developed posterolophid, separated from entoconid by deep and upward-curved posteroflexid; m3 with six visible cusps in specimens with little-worn dentition; inconspicuous protostylid, fused with protoconid; protoconid larger than metaconid; hypoconid larger than entoconid; mesoflexid deep and upward-curved; hypoflexid deep and straight; mesoflexid and hypoflexid separated by narrow median

mure; mesolophid fused with entoconid; well-developed posterolophid, separated from entoconid by deep and diagonal posteroflexid (or fossete in specimens with moderately worn dentition).

Axial skeleton composed of 13 ribs, 7 cervical vertebrae, 13 thoracic vertebrae, 4 sacral vertebrae, 26 caudal vertebrae; presence of conspicuous neural spine on second thoracic vertebrae. Scapula wide, thin and translucent except at borders; shallow supra- and infraspinous fossae; acromion delicate, with shallow concavity; deep notch, reaching about one-third of spine length. Humerus shorter than scapula; well-developed head; conspicuous greater and lesser tubercles; well-developed and thick deltoid tuberosity. Radius longer than humerus, fused with ulna except for small segment behind trochlear notch, which forms a slightly elongated foramen. Elongated ilium, showing conspicuous iliac crest. Robust ischium, translucent at center and internal border. Large obturator foramen. Femur thin and curved; well-developed head; conspicuous median tuberosity; medial and lateral condyles little developed; shallow patellar groove.

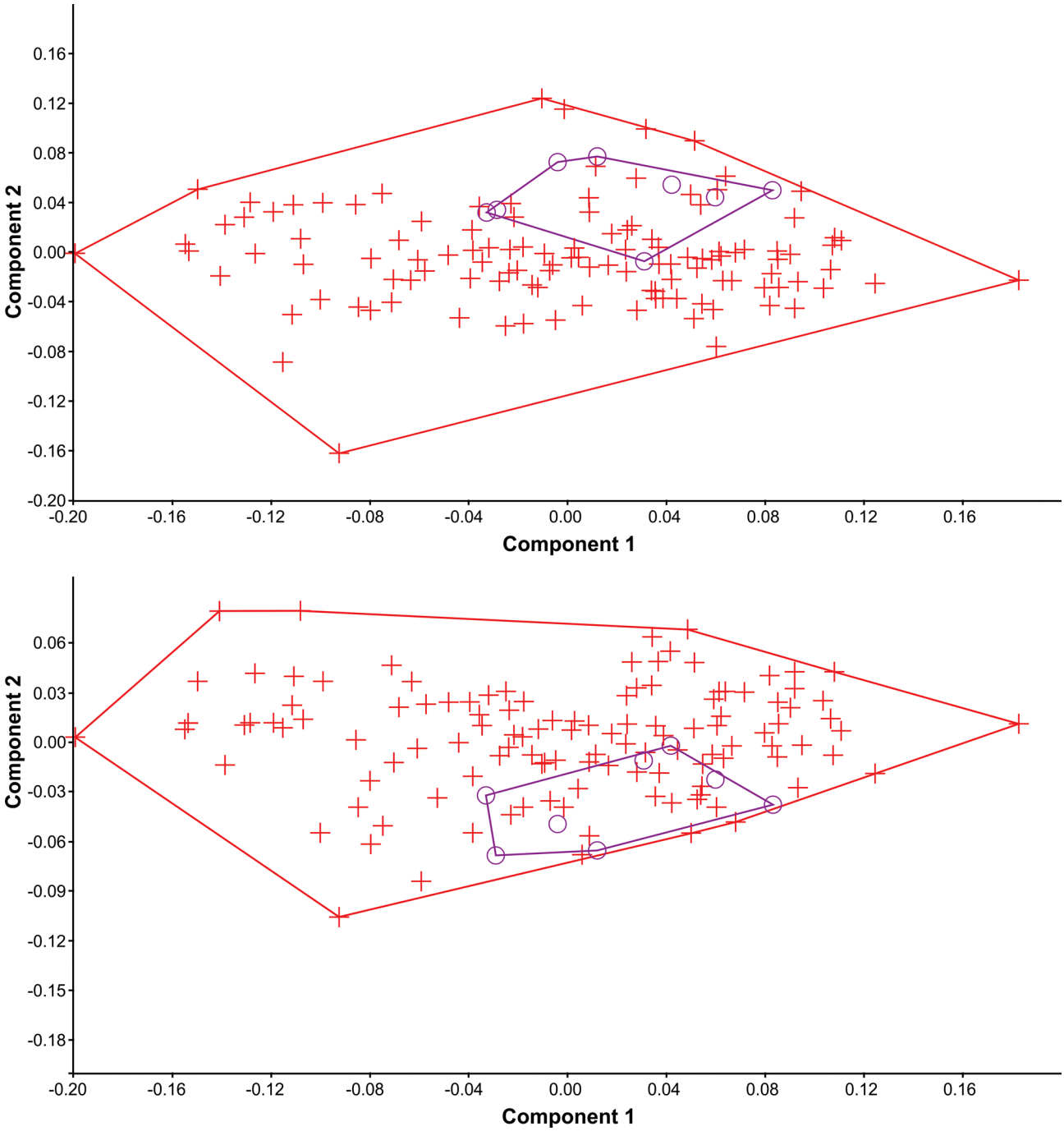

FIGURE 7. Convex hull for specimen scores of *Deltamys kempi* (crosses) and *Deltamys araucaria* **sp. n.** (circles) on the principal components 1 and 2 (above) and 1 and 3 (below) extracted from the variance-covariance matrix of 21 cranial measurements.

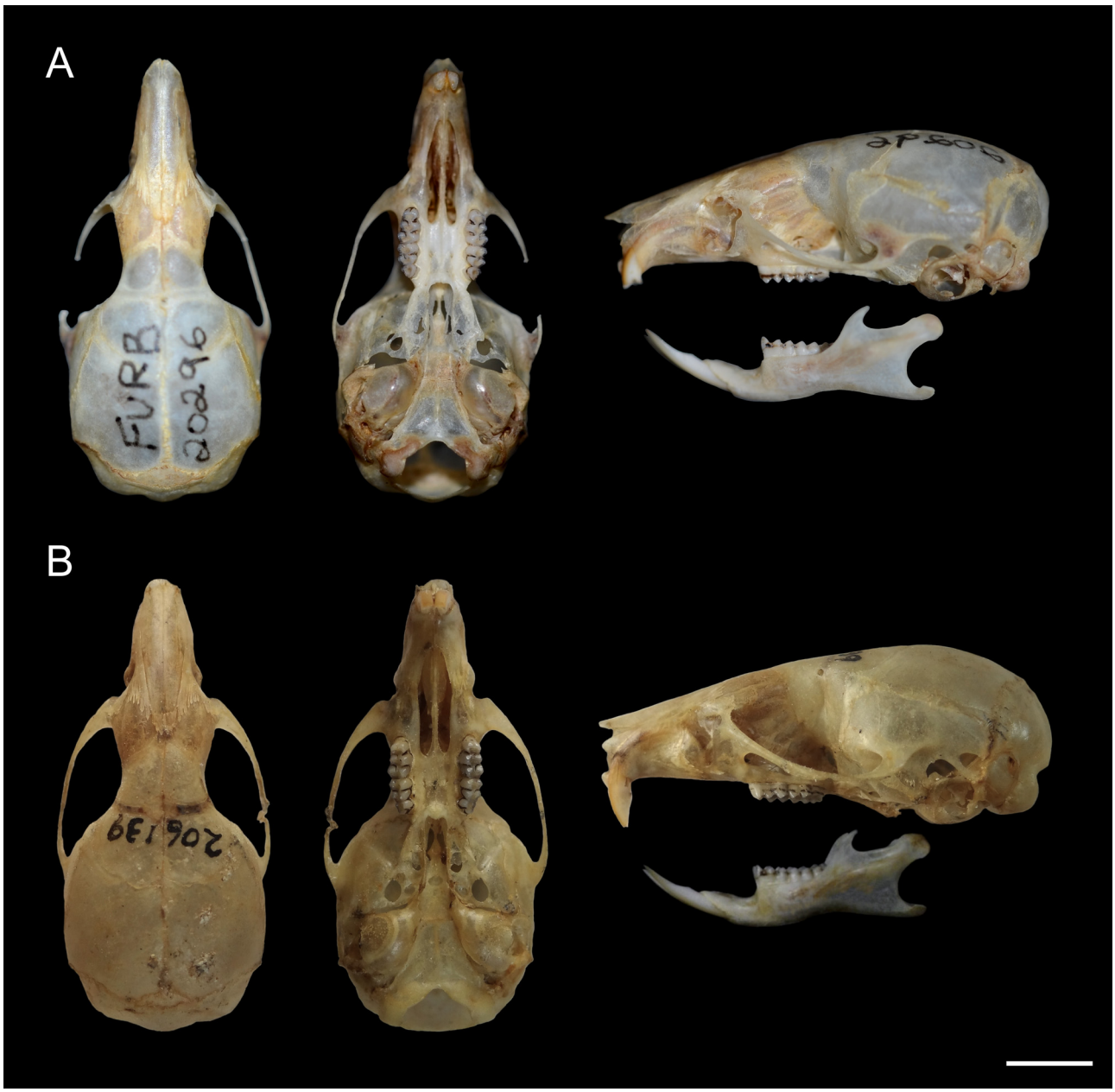

FIGURE 8. Dorsal (left), ventral (middle) and lateral (right) views of skull and labial view of mandible (bottom right) of *Deltamys araucaria* **sp. n.** (holotype, FURB 20296; above) and *Deltamys kempi* (AMNH 206139). Bar = 5 mm.

Karyotype. Diploid number (2n) of 34 chromosomes and an autosomal fundamental number (FNa) of 34 arms. Autosome complement characterized by 15 acrocentric pairs and 1 pair of small metacentric chromosomes. Sex determination system XY/XX; chromosomes X and Y acrocentric (Fig. 4).

Etymology. Refers to *Araucaria angustifolia* (Bertol) Kuntze (Pinopsida: Araucariaceae), the major arboreal element in the Araucaria Forest or Mixed Ombrophilous Forest, the vegetation domain where the type locality is inserted. Proposed as a noun in apposition.

Natural history. Specimens of *D. araucaria* were collected in palustrine savanna with a predominance of *Eryngium pandanifolium* (Apiaceae) and *Baccharis* sp. (Asteraceae) and unidentified treelets, in areas with soggy soil. The species was found in sympatry with the didelphid *Monodelphis dimidiata* Wagner and the sigmodontines *Akodon montensis* Thomas, *Oligoryzomys flavescens* Waterhouse, *Oxymycterus nasutus* Waterhouse and *Scapteromys meridionalis* Quintela *et al*. Parallel sampling in dense forest habitats of the region produced no additional individuals, which indicates that *D. araucaria* is associated with the open herbaceous/savannic palustrine systems.

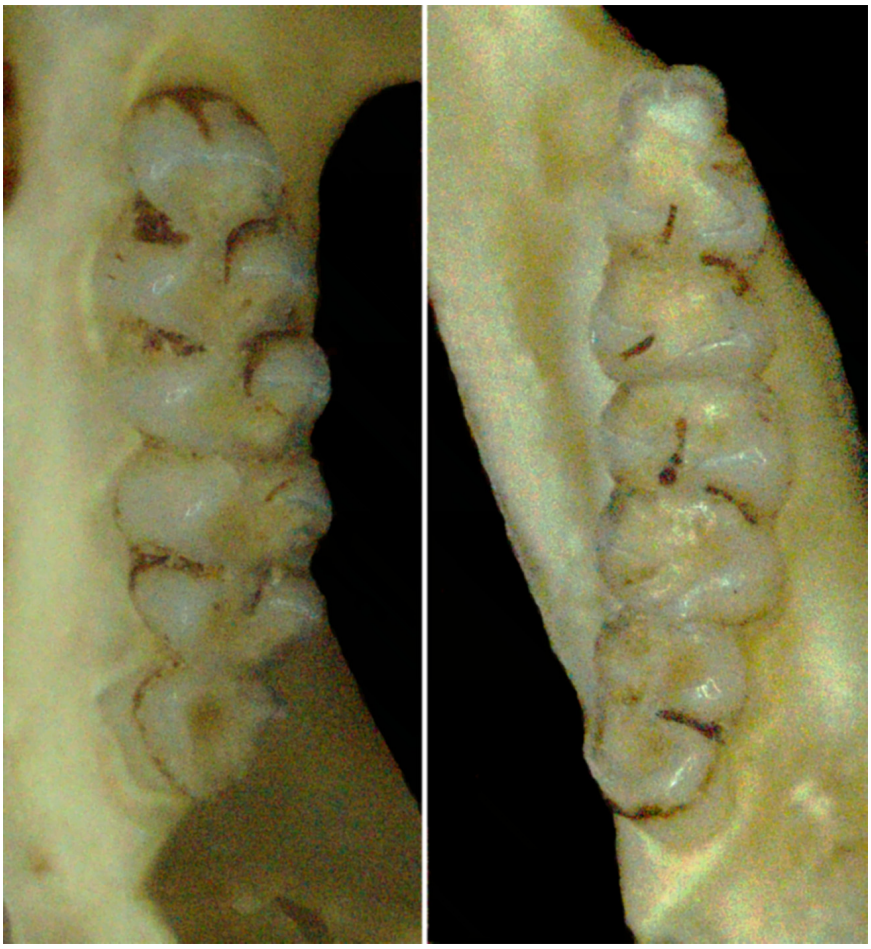

FIGURE 9. Left maxillary molars (left) and right mandibular molars (right) of *Deltamys araucaria* **sp. n.** holotype (FURB 20296) (bar=1mm).

Discussion

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

Tracing a historical biogeographic hypothesis on *Deltamys* speciation is a delicate task, partially due to the small sample and incipient data on Meridional Plateau forms. First, it is noticeable how populations from the highly divergent forms *D. kempi* and the new lineage herein reported are separated by only ca. 50 km between Torres, on the northern coastal plain, and São Francisco de Paula, at the plateau border. This stretch, however, has an altitudinal variation of more than 900 m, partially due to the presence of plateau slopes (*Serra Geral*). Thus, it is possible that the altitudinal gradient has shaped the differentiation in *Deltamys*, with one lineage dispersing across the volcanic highlands of the Meridional Plateau and differentiating into two forms, and the *D. kempi* lineage dispersing through the sedimentary lowlands of the Paraná basin and later spreading along the Cenozoic coastal plain. Mountain ranges and ridges have long been considered topographic elements associated with speciation processes in akodontine rodents (Reig 1984; Geise 2001; Gonçalves *et al*. 2007; Gonçalves & Oliveira 2014; Quintela *et al*. 2014).

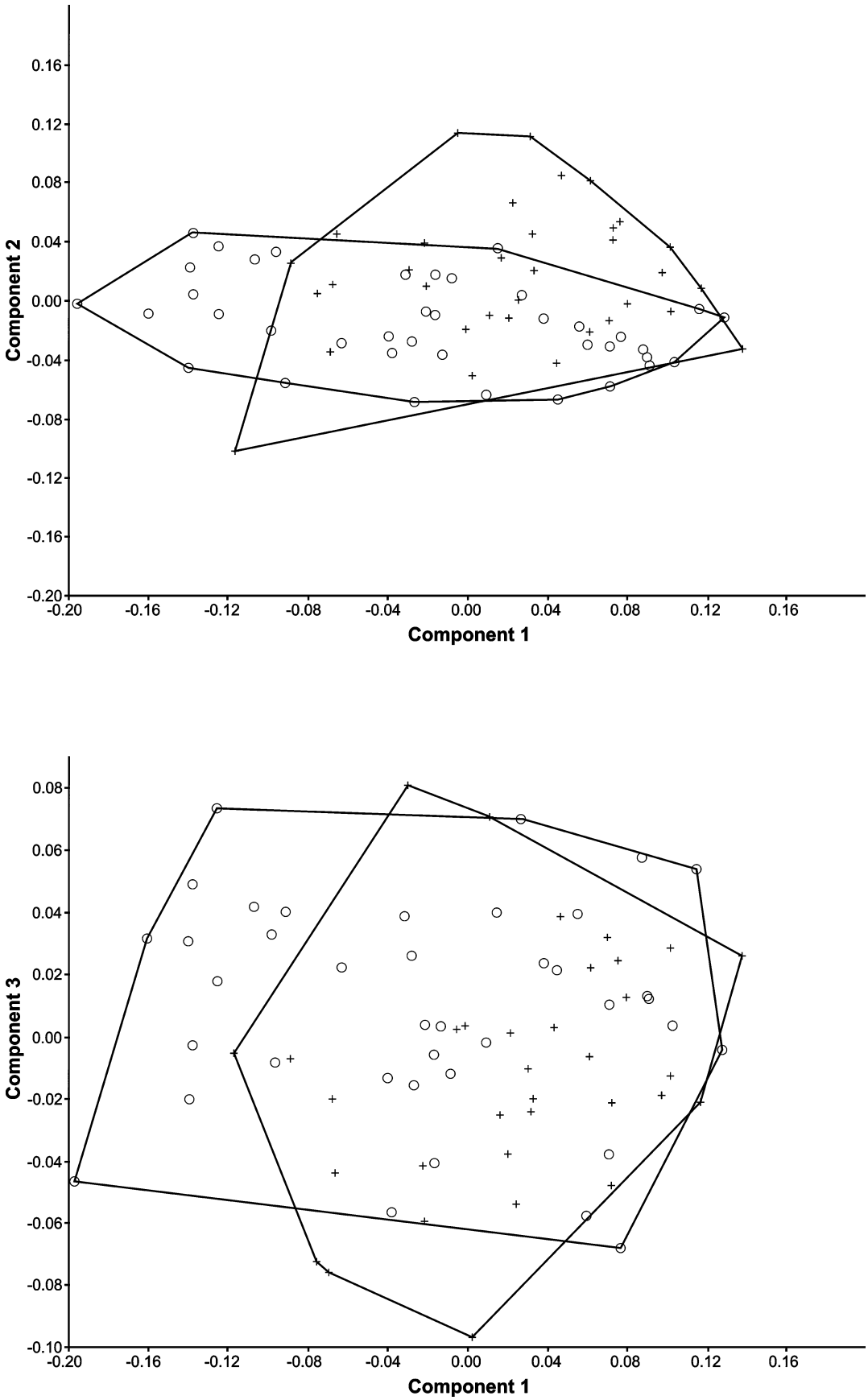

FIGURE 10. Scores for *Deltamys kempi* specimens from haplogroup A (crosses) and B (circles) on the principal components 1 and 2 (above) and 1 and 3 (below) extracted from the variance-covariance matrix of 21 cranial measurements.

The two *Deltamys* lineages from the Meridional Plateau 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 separated by about 160 km in the northeastern RS highlands. In view of the marked differentiation, it is expected that strong evolutionary processes have acted on the highlands *Deltamys.* The sparse data on these forms, however, do not permit any further inferences about these processes, but it should be noted that two major rivers of the northern Atlantic hydrographic basin (Caí and Antas rivers) (Vieira 1984) run between the localities of the so-far-known occurrence of *D. araucaria* (São Francisco de Paula) and *Deltamys* sp. (2n=40) (Esmeralda). Rivers are suggested to be geographic elements that shaped the speciation in small mammals such as didelphid marsupials and echimyid rodents (Patton & Da Silva 2000; Teta *et al*. 2009; Nascimento *et al*. 2013). The unstable forest coverage in southern Brazil during the Pleistocene climatic fluctuations (Valdez & D'Elía 2013) and the possibility of isolation of lineages in suitable open areas during interglacial forest expansions should also be considered.

Deltamys araucaria is genetically and karyotypically divergent from *D. kempi* and *Deltamys* sp. (2n=40) and is morphologically distinguishable from *D. kempi* by discrete diagnostic characters. Although an investigation of the chromosomal evolution in *Deltamys* is beyond the scope of the present study, we can observe that the highland forms *D. araucaria* and *Deltamys* sp. (2n=40), sister species according to our phylogenetic analysis, share the same sex-determination system (XY/XX), while the $X_1X_2Y_1/X_1X_1X_2X_2$ system represents an autapomorphic condition in *D. kempi*. Therefore, for a better understanding of evolution in *Deltamys*, it is crucial to examine the morphology of 2n=40 specimens (not available for this study) as well to apply banding and FISH techniques for analysis of the chromosomal homology. It is also remarkable the presence of a small metacentric pair of autosomes in *D. araucaria.* The pair of small metacentric autosomes is considered a karyotypic marker of *Akodon*, which allows distinguishing forms from this genus from the closely related *Deltamys* (Fronza *et al*. 1981; Ventura *et al*. 2011). Our finding, however, does not corroborate this statement and suggests that the presence of a small autosome pair does not represent a reliable marker for genus *Akodon.*

Specific genealogical analysis of the *D. kempi* haplotype evidenced an internal structure of two subclades (A and B): haplogroup A and haplogroup B (Fig. 3). Haplogroup A was restricted to RS, occurring on the northern and central coastal plain and west of Patos Lagoon. Haplogroup B extended from Buenos Aires to Uruguay, and in RS to regions of the southern coastal plain and west of Patos Lagoon. The haplotype network showed a pattern characterized by local diversification (i.e., markedly structured), as only five of 37 haplotypes were shared by two or three nearby localities (Fig. 3). In Haplogroup A, four of the 13 recovered haplotypes were shared by pairs of localities, each within the same physiographic unit. Haplogroup B extends over the remaining distributional area. Analysis of the genetic differentiation among subpopulations based on haplotype-frequency (ϕ_{ST}) revealed a population structure pattern (Table 5). In the PCA results, however, samples from the two clades did not segregate on the multivariate space, showing a high overlap in the convex hull on both PC1 x PC2 and PC1 x PC3 (Fig. 10). In the discriminant analysis, both clades showed a high percentage of correct classifications (Haplogroup A: 94.2%; Haplogroup B: 92.2%).

The analysis of the spatial distribution of *D. kempi* cyt *b* sequences revealed a population structure that is characterized by marked local diversification. This pattern may reflect some presumably ecological-behavioral aspects of the species. Despite the lack of data on time-scaled movements or home-range, it is expected that *D. kempi* has a limited dispersal capacity, considering its small size and proportionally short forefeet and limbs (González & Pardiñas 2002). Individuals observed in a large terrarium moved slowly and over short distances (during both day and night), generally limited to short movements beneath the litter. The other aspects that may contribute to the observed structure pattern are that *D. kempi* seems to be naturally rare in many parts of its distribution (Massoia 1964; González & Pardiñas 2002; Montes *et al*. 2008; Quintela *et al*. 2012; 2013), and populations seem to occur generally associated with scrub vegetation composed of specific palustrine herbaceous species (Massoia 1964; present study), which probably impedes gene flow.

Our expanded data on the *D. kempi* phylogeographic structure supported the previous scenario of a subdivision into two major phylogroups that are markedly differentiated in both cyt *b* and the craniometric characters, in which one group is restricted to northern areas and the other is 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 newly sampled populations. The 'northern' group recovered by Montes *et al*. (2008) ('Tramandaí group') included populations from Torres, Tramandaí and Osório, on the northern RS coastal plain, and Tapes, northwest of the Patos Lagoon. In our analysis, these samples grouped in Clade A with haplotypes from four other localities on the

TABLE 5. Population pairwise Φ*ST*s for comparisons between populations of *Deltamys kempi* from the Pampas. Abbreviations for locations are defined in Fig. 1 and Table 1.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	0.00																			
2	0.88	0.00																		
3	0.87	1.00	0.00																	
4	0.55	-0.15	0.44	0.00																
5	0.91	1.00	1.00	0.54	0.00															
6	0.83	0.95	0.94	0.60	0.91	0.00														
7	0.78	1.00	1.00	0.39	1.00	0.85	0.00													
8	0.19	0.31	0.24	0.33	0.26	0.15	-0.17	0.00												
9	0.33	0.38	0.50	0.40	0.59	0.57	0.33	0.11	0.00											
10	**0.91**	**1.00**	**1.00**	**0.68**	**1.00**	**0.96**	**1.00**	**0.54**	**0.69**	0.00										
11	0.77	0.80	0.76	0.76	0.76	0.80	0.69	0.69	0.76	**0.46**	0.00									
12	**0.95**	**0.96**	**0.96**	**0.92**	**0.95**	**0.95**	**0.95**	**0.88**	**0.93**	**0.88**	0.74	0.00								
13	**0.96**	**1.00**	**1.00**	**0.78**	**1.00**	**0.98**	**1.00**	**0.69**	**0.80**	**1.00**	0.38	0.92	0.00							
14	0.45	0.30	0.36	0.31	0.47	0.43	0.25	0.07	0.14	0.66	**0.74**	**0.91**	**0.77**	0.00						
15	**0.93**	**1.00**	**1.00**	**0.72**	**1.00**	**0.97**	**1.00**	**0.61**	**0.73**	**1.00**	0.22	0.91	1.00	**0.71**	0.00					
16	**0.85**	**1.00**	**1.00**	**0.52**	**1.00**	**0.92**	**1.00**	**0.20**	**0.50**	**1.00**	0.62	0.92	1.00	**0.45**	1.00	0.00				
17	**0.93**	**1.00**	**1.00**	**0.73**	**1.00**	**0.97**	**1.00**	**0.64**	**0.75**	**1.00**	0.39	0.76	1.00	**0.73**	1.00	1.00	0.00			
18	**0.33**	**0.12**	**-0.29**	**0.46**	**0.15**	**0.59**	**-0.47**	**0.21**	**0.42**	**1.00**	0.33	0.85	0.00	**0.36**	-0.47	-0.47	-0.16	0.00		
19	**0.92**	**1.00**	**1.00**	**0.70**	**1.00**	**0.97**	**1.00**	**0.58**	**0.71**	**1.00**	0.04	0.76	1.00	**0.68**	1.00	1.00	1.00	-0.47	0.00	
20	**0.83**	**0.78**	**0.74**	**0.74**	**0.85**	**0.92**	**0.67**	**0.66**	**0.75**	**0.20**	0.36	0.73	0.72	**0.73**	0.43	0.56	-0.33	0.21	1.00	0.00
21	**0.93**	**0.94**	**0.93**	**0.78**	**0.96**	**0.96**	**0.91**	**0.69**	**0.78**	**0.78**	0.42	0.70	0.92	**0.77**	0.85	0.87	0.33	0.33	0.33	0.00

northern and central coastal plain and two localities at the border of the Precambrian Sul-Riograndense Shield. The northern clade of *D. kempi*, however, is not restricted to the recent Cenozoic deposits as previously found by Montes *et al*. (2008), but also spreads over the Shield. Clade A also encompassed samples from the central RS coastal plain, a region not sampled in previous molecular surveys (D'Elía *et al*. 2003; Montes *et al*. 2008; Ventura *et al*. 2011). This region corresponds to a long narrow sedimentary deposit formed by the last two marine transgressions, which occurred at 120 and five kya (Tomazelli & Villwock 2000). At these times, the sea covered a large area of the continental shelf, forming the Patos Lagoon, the largest lagoon system in South America (Vieira 1984). The phylogeographic break observed in *D. kempi* seems to be related to the evolution of this lagoon system, where the estuarine channel may constitute a geographical barrier to the historical gene flow. This pattern of internal differentiation was also observed for the sympatric akodont *Scapteromys tumidus* (Quintela *et al*. 2015).

Similarly to 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 binomial or a subspecific trinomial classification. A first attempt at a *D. kempi* subspecific arrangement was proposed by González & Massoia (1995), who assigned populations from Argentina to the nominal subspecies, and populations from Uruguay and Brazil to *D. kempi langguthi.* This classification, however, was based only on morphological features and was subsequently contested by Montes *et al.* (2008), who in turn found a genetic/craniometric-based internal division, but with a spatial coverage of clades markedly different from the geographical context of the subspecific proposal of González & Massoia (1995). The internal clades recovered herein and in the analyses of Montes *et al*. (2008) and Ventura *et al*. (2011) are geographically congruent, strongly indicating that *D. kempi* is passing through a process of differentiation. The significant craniometric differences found here and by Montes *et al*. (2008) support this supposition of an internal differentiation process. 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 degree of overlap of convex hulls in the PCA), in our view, is not consistent with a formal taxonomic division within *D. kempi*. The other fact is that "northern" and "southern" clades are apparently sympatric west of the Patos-Mirim complex, which disagrees with the formal concept of subspecies (see Mallet 2007 for further details). In this case, the most appropriate procedure could be to treat each clade as independent ESUs (Evolutionary Significant Unit; see Moritz 1994), considering the divergence in sequences of the nuclear recombination activating gene 2 between the clades previously found by Montes *et al*. (2008). Moreover, there is no clear objection for sympatry conditions in the definition of ESUs, which also does not consider the level of divergence an arbitrary criterion, since the monophyly of both the nuclear and mitochondrial markers was confirmed (Moritz 1994).

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References

Bandelt, H.J., Foster, P. & Röhl, A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, 16, 37–48.
https://doi.org/10.1093/oxfordjournals.molbev.a026036

Barrantes, G.E, Ortells, M.O & Reig, A.O. (1993) New studies on allozyme genetic distance and variability in akodontine rodents (Cricetidae) and their systematic implications. *Biological Journal of the Linnean Society*, 48, 283–298. https://doi.org/10.1111/j.1095-8312.1993.tb02092.x

Bianchini, J. & Delupi, L. (1994) Consideraciones sobre el estado sistemático de *Deltamys kempi* Thomas, 1917 (Cricetidae: Sigmodontinae). *Physis*, 49, 27–35.

Cabrera, A. (1961) Catálogo de los mamíferos de América del Sur. *Revista del Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Ciencias Zoologicas*, 4, 309–732.

Carrizo, L.V. & Diaz, M. (2011) Descripción del postcráneo de *Rhipidomys austrinus* y *Graomys griseoflavus* (Rodentia, Cricetidae, Sigmodontinae). *Iheringia, Série Zoologia*, 101, 107–129. https://doi.org/10.1590/S0073-47212011000200008

Castro, E.C., Mattevi, M.S., Maluf, S.W. & Oliveira, L.F.B. (1991) Distinct centric fusions in different populations of *Deltamys kempi* (Rodentia, Cricetidae) from South America. *Cytobios*, 68, 153–159.

D'Elía, G., González, E.M. & Pardiñas, U.F.J. (2003) Phylogenetic analysis of sigmodontine rodents (Muroidea), with special reference to the akodont genus *Deltamys. Mammalian Biology*, 68, 351–364. https://doi.org/10.1078/1616-5047-00104

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9, 772. https://doi.org/10.1038/nmeth.2109

Doyle, J.J. & Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19, 11–15.

Ellerman, J.R. (1941) *The families and genera of living rodents. Vol. 2. Family Muridae*. British Museum of Natural History, London, 690 pp.

Excoffier, L. & Lischer, H.E.L. (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analysis under Linux and Windows. *Molecular Ecology Resources*, 10, 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39, 783–791. https://doi.org/10.2307/2408678

Ford, C.E. & Hamerton, J.L. (1956) A colchicine hypotonic citrate squash sequence for mammalian chromosomes. Stain Technology, 31, 247–251. https://doi.org/10.3109/10520295609113814

Fronza, M., Wainberg, R. & Cataleo, G. (1981) Multiple sex chromosomes in *Deltamys kempi* (Rodentia: Cricetidae): Preliminary steps towards the establishment of the XY1Y2/XX system. *Caryologia*, 34, 457–466. https://doi.org/10.1080/00087114.1981.10796914

Guathier, O., Landry, P. A. & Lapoint, F.J. (2001) Missing data in craniometrics: a simulation study. *Acta Theriologica*, 48, 25–34. https://doi.org/10.1007/BF03194263

Geise, L., Smith, M.F. & Patton, J.L. (2001) Diversification in the genus *Akodon* (Rodentia: Sigmodontinae) in southeastern South America: mitochondrial DNA sequence analysis. *Journal of Mammalogy*, 82, 92–101. https://doi.org/10.1644/1545-1542(2001)082%3C0092:DITGAR%3E2.0.CO;2

Gonçalves, P.R. & Oliveira, J.A. (2014) An integrative appraisal of the diversification in the Atlantic forest genus *Delomys* (Rodentia: Cricetidae: Sigmodontinae) with the description of a new species. *Zootaxa*, 3760 (1), 1–38. https://doi.org/10.11646/zootaxa.3760.1.1

Gonçalves, P.R., Myers, P., Vilela, J.F. & Oliveira, J.A. (2007) Systematics of species of the genus *Akodon* (Rodentia: Sigmodontinae) in southeastern Brazil and implications for the biogeography of the Campos de Altitude. *Miscellaneous Publications, Museum of Zoology, University of Michigan*, 197, 1–24.

González, E.M. (2006) Mamíferos terrestres no voladores de la zona costera uruguaya. *In*: Menafra, R., Rodríguez-Gallego, L., Scarabino, F. & Conde, D. (Eds.), *Bases para la conservación y el manejo de la costa uruguaya.* Vida Silvestre Uruguay, Montevideo, pp. 329–341.

González, E.M. & Martínez-Lanfranco, J.A. (2010) *Mamíferos de Uruguay. Guía de campo e introducción a su estúdio y conservación*. Banda Oriental, Museo Nacional de Historia Natural, Vida Silvestre Uruguay, Montevideo, 463 pp.

González, E.M. & Massoia, E. (1995) Revalidación del género *Deltamys* Thomas, 1917, con la descripción de una nueva subespecie de Uruguay y Sur del Brasil (Mammalia, Rodentia: Cricetidae). *Comunicaciones Zoológicas del Museo de Historia Natural de Montevideo*, 12, 1–8.

González, E.M. & Pardiñas, U.F.J. (2002) *Deltamys kempi*. *Mammal Species*, 771, 1–4. https://doi.org/10.1644/1545-1410(2002)711%3C0001:DK%3E2.0.CO;2

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology*, 59, 307–321. https://doi.org/10.1093/sysbio/syq010

Hasegawa, M., Kishino, H. & Yano, T. (1985) Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, 22, 160–174. https://doi.org/10.1007/BF02101694

Hershkovitz, P. (1962) Evolution of Neotropical cricetine rodents (Muridae) with special reference to the phyllotine group. *Fieldiana, Zoology*, 46, 1–524.
https://doi.org/10.5962/bhl.title.2781

Librado, P. & Rozas, J. (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25, 1451–1452.
https://doi.org/10.1093/bioinformatics/btp187

Machado, L.F., Paresque, R. & Christoff, A.U. (2011) Anatomia comparada e morfometria de *Oligoryzomys nigripes* e *O. flavescens* (Rodentia, Sigmodontinae) no Rio Grande do Sul, Brasil. *Papéis Avulsos de Zoologia*, 51, 29–47.
https://doi.org/10.1590/s0031-10492011000300001

Mallet, J. (2007) Subspecies, semispecies, superspecies. *In*: Levin, S.A. (Ed.), *Encyclopedia of Biodiversity.* Elsevier, Oxford, pp. 1–5.
https://doi.org/10.1016/b0-12-226865-2/00261-3

Massoia, E. (1964) Sistemática, distribución geográfica y rasgos etoecológicos de *Akodon* (*Deltamys*) *kempi* (Rodentia, Cricetidae). *Physis*, 24, 299–305.

Montes, M.A., Oliveira, L.F.B., Bonatto, S.L., Callegari-Jacques, S.M. & Mattevi, M.S. (2008) DNA sequence analysis and the phylogeographical history of the rodent *Deltamys kempi* (Sigmodontinae, Cricetidae) on the Atlantic Coastal Plain of south of Brazil. *Journal of Evolutionary Biology*, 26, 1823–1835.
https://doi.org/10.1111/j.1420-9101.2008.01586.x

Moritz, C. (1994) Defining 'Evolutionary Significant Units' for conservation. *Trends in Ecology and Evolution*, 9, 373–375.
https://doi.org/10.1016/0169-5347(94)90057-4

Musser, G.G. & Carleton, M.D. (1993) Family Muridae. *In*: Wilson, D.E. & Reeder, D.M. (Eds.), *Mammal species of the world: a taxonomic and geographic reference.* Smithsonian Institution Press, Washington, D.C., pp. 501–756.

Nascimento, F.F, Lazar, A., Menezer, A.N., Durans, A.M., Moreira, J.C., Salazar-Bravo, J., D'Andrea, P.S.D. & Bonvicino, C.R. (2013) The role of historical barriers in the diversification processes in open vegetation formations during the Miocene/Pliocene using an ancient rodent lineage as a model. *PLoS ONE*, 8, e61924.
https://doi.org/10.1371/journal.pone.0061924

Quintela, F.M., Gonçalves, B.I., Trindade, G.E., Santos, M.B. & Tozetti, A.M. (2013) Pequenos mamíferos não-voadores (Didelphimorphia, Rodentia) em campos litorâneos do extremo sul do Brasil. *Biota Neotropica*, 13, 284–289.
https://doi.org/10.1590/S1676-06032013000400025

Quintela, F.M., Gonçalves, G.L., Althoff, S.L., Sbalqueiro, I.J., Oliveira, L.F.B. & Freitas, T.R.O. (2014) A new species of swamp rat of the genus *Scapteromys* Waterhouse, 1837 (Rodentia: Sigmodontinae) endemic to *Araucaria angustifolia* Forest in Southern Brazil. *Zootaxa*, 3811 (2), 207–225.
https://doi.org/10.11646/zootaxa.3811.2.3

Quintela, F.M., Gonçalves, G.L., Bertuol, F., González, E.M. & Freitas, T.R.O. (2015) Genetic diversity of the swamp rat in South America: Population expansion after transgressive-regressive marine events in the Late Quaternary. *Mammalian Biology*, 80, 510–517.
https://doi.org/10.1016/j.mambio.2015.08.003

Quintela, F.M., Santos, M.B., Christoff, A.U. & Gava, A. (2012) Pequenos mamíferos não-voadores (Didelphimorphia, Rodentia) em dois fragmentos de mata de restinga de Rio Grande, Planície Costeira do Rio Grande do Sul. *Biota Neotropica*, 12, 261–266.
https://doi.org/10.1590/S1676-06032012000100021

Reig, O.A. (1984) Distribuição geográfica e história evolutiva dos roedores muroideos sulamericanos (Cricetidae: Sigmodontinae). *Revista Brasileira de Genética*, 7, 333–365.

Sbalqueiro, I.J., Mattevi, M.S. & Oliveira, L.F.B. (1984) An $X_1X_1X_2X_2/X_1X_2Y$ mechanism of sex determination in a South American rodent, *Deltamys kempi* (Rodentia: Cricetidae). *Cytogenetics and Cell Genetics*, 38, 50–55.
https://doi.org/10.1159/000132029

Smith, M.F. & Patton, J.L. (1993) The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. *Biological Journal of the Linnean Society*, 50, 149–177.
https://doi.org/10.1111/j.1095-8312.1993.tb00924.x

Tamura, K., Stecher, G., Peterson, D. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics analysis version 6.0. *Molecular Biology and Evolution*, 30, 2725–2729.
https://doi.org/10.1093/molbev/mst197

Teta, P., D'Elía, G., Flores, D. & La Sancha, N. (2009) Diversity and distribution of the mouse opossum of the genus *Thylamys* (Didelphimorphia, Didelphidae) in north-eastern and central Argentina. *Gayana*, 73, 180–199.
https://doi.org/10.4067/S0717-65382009000200003

Thomas, O. (1917) On small mammals from the Delta del Paraná. *Annual Magazine of Natural History*, 8, 95–100.
https://doi.org/10.1080/00222931709486975

Tomazelli, L.J. & Villwock, J.A. (2000) O Cenozóico no Rio Grande do Sul: Geologia da Planície Costeira. *In*: Holz, M. & De Ros, L.F. (Eds.), *Geologia do Rio Grande do Sul*. CIGO/UFRGS, Porto Alegre, pp. 375–406.

Udrizar Sauthier, D.E., Abba, A.M., Pagano, L.G. & Pardiñas, U.F.J. (2005) Ingreso de micromamíferos brasílicos en la província de Buenos Aires, Argentina. *Mastozoologia Neotropical*, 12, 91–95.

Valdez, L. & D'Elía, G. (2013) Differentiation in the Atlantic Forest: phylogeography of *Akodon montensis* (Rodentia, Sigmodontinae) and the Carnaval-Moritz model of Pleistocene refugia. *Journal of Mammalogy*, 94, 911–922. https://doi.org/10.1644/12-MAMM-A-227.1

Ventura, K., Fagundes, V., D'Elía, G., Christoff, A.U. & Yonenaga-Yassuda, Y. (2011) A new allopatric lineage of the rodent *Deltamys* (Rodentia: Sigmodontinae) and the chromosomal evolution in *Deltamys kempi* and *Deltamys* sp. *Cytogenetics and Genome Research*, 135, 126–134. https://doi.org/10.1159/000331584

Vieira, E.F. (1984) *Rio Grande do Sul: Geografia Física e Vegetação*. Sagra, Porto Alegre, 184 pp.

Voss, R.S. (1988) Systematics and ecology of ichthyomyine rodents (Muroidea): Patterns of morphological evolution in a small adaptive radiation. *Bulletin of the American Museum of Natural History*, 188, 260–493.

APPENDIX

Specimens used in molecular ([a]) and skull morphometric ([b]) analyses are 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 araucaria (23) BRAZIL: Rio Grande do Sul: São Francisco de Paula (FURB 20220[ab], 20240[ab], 20244[ab], 20296[ab], 20297[ab], 20330[ab], 20954[ab], 20955[ab]; A7[a], A10[a], A24[a], A25[a], A26[a], A29[a], A33[a], A43[a], AS14[a], LBCE15572[a]).

***Deltamys* sp. (2n=40)** (3) BRAZIL: Rio Grande do Sul: Esmeralda (CIT 945/JN232108*, CIT 946/JN232109*, CIT 947/JN232110*).

***Deltamys kempi*—**BRAZIL (119): Rio Grande do Sul: Torres: Parque Estadual de Itapeva (TR 2159[a], EF206809*), Osório (TR 2137[a], MN42081/EF206811*), Tramandaí (EF206809*, EF206810*), 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]), Charqueadas (EF206808*), Tapes (MCNU 1813[a], EF206812*), Viamão (TR 2145[a]), Cristal (TR 2125[a]), 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]), 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], EF206805*, EF206806*, EF206807*), Santa Vitória do Palmar—Lagoa Mangueira (FURB 20308). URUGUAY: Rivera, Cofusa (EMG 1099[a]), Rocha—Parque Santa Teresa (EMG 1808[a]; PCE 05[ab], 06[a], 11[a], 12[a], 13[a]), Refúgio 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], MNHN4151/AY195862*). ARGENTINA: Buenos Aires—La Balandra (UP42/AY195861*).