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NOTES ON GEOGRAPHIC DISTRIBUTION

New distribution records of Serra do Mar Grass Mouse *Akodon serrensis* Thomas, 1902 (Mammalia: Rodentia: Sigmodontinae) in the southernmost Brazil

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ABSTRACT: Geographic distribution is critical information for conservation of the species. In this note we report the southernmost record of *Akodon serrensis*, a Neotropical terrestrial rodent endemic of the Brazilian Atlantic Forest, but yet with undefined distribution limits. Two specimens surveyed in Rio Grande do Sul state were identified as *A. serrensis*, one by DNA analysis and other by external and cranial morphology. These records represent an expansion of nearly 177 km from the previous known limit of its southern distribution. Moreover, these new records demonstrated the importance of sampling effort in order to establish the real distribution limits of a species.

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Akodon is one of the most diverse genus of Neotropical small mammal composed by at least 41 currently known species (Musser and Carleton 2005), with new species recently described (*e.g.* Pardiñas *et al.* 2005; Braun *et al.* 2010), being 10 species recorded in the Brazilian territory (Bonvicino *et al.* 2008; Paglia *et al.* 2012). The taxonomy of *Akodon* species is confusing and complex due to the morphological similarities among them. The identifications are mainly based on skull morphology and attributes of molar teeth (Reig 1987). Some authors have informally divided the genus in species groups, but these do not represent monophyletic lineages (*e.g.* Myers 1989; Myers and Patton 1989; Myers *et al.* 1990; Hershkovitz 1990). Species from eastern Brazil had been successfully recognized using karyotypic analysis as well as external and internal morphology (Christoff *et al.* 2000; Gonçalves *et al.* 2007). The *Akodon* rodents have wide distribution in the Brazilian Atlantic forest, ranging from the northeastern to southernmost, but for some species the distribution limits are still undefined (Pardiñas *et al.* 2008).

Akodon serrensis Thomas, 1902 occurs in the eastern portion of Brazil, between the States of Espírito Santo and Santa Catarina (Pardiñas *et al.* 2008; Gonçalves *et al.* 2007; Testoni *et al.* 2012). Pereira *et al.* (2005) also recorded this rodent for Misiones province, northern Argentina (25°59′00″ S, 54°05′00″ W), but Pardiñas *et al.* (2008) considered the specimens found in Argentina to represent *A. paranaensis*. In this sense, the presence of this species in Argentine territories is now questionable. *A. serrensis* is considered endemic to the Brazilian Atlantic Forest biome (Paglia *et al.* 2012). The species have been captured in Highland fields (high-altitude grasslands), Montane and Submontane Atlantic Ombrophilous Dense Forest, and Ombrophilous Mixed Forest (for classification see IBGE 2012), between 920 m to more than 2,000 m above the sea level (Geise *et al.* 2004; Modesto *et al.* 2008; Pardiñas *et al.* 2008; Testoni *et al.* 2012). The type locality of the species is the region of Serra do Mar, Paraná State, Brazil (Thomas 1902), which is above 2,000 m of altitude, and the species karyotype is characterized by 2n = 46 and FN = 46 (Christoff *et al.* 2000: 849; Ventura *et al.* 2006). The

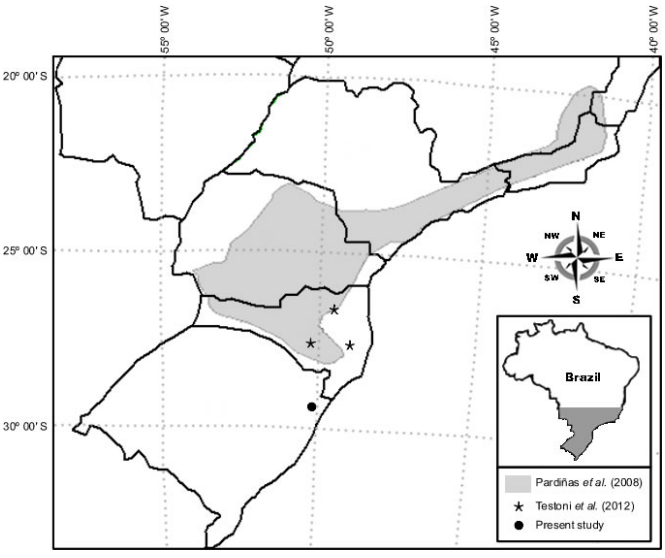

FIGURE 1. Distribution of *A. serrensis* in Brazil according to Pardiñas et al. (2008) and Testoni et al. (2012) (black stars), including the new records reported here (black circle).

external and skull morphology and measurements are significantly different from *A. paranaensis* (Christoff *et al.* 2000), a congeneric species that overlaps distribution with *A. serrensis* (Bonvicino *et al.* 2008). *A. serrensis* is considered as "least concern" in the IUCN Red List, but some populations of the species are threatened by fragmentation and habitat loss in the Atlantic Forest biome (Pardiñas *et al.*, 2008).

Here, we report the southernmost record of *A. serrensis* based on the capture and analysis of two specimens from the northeastern Rio Grande do Sul State, southern Brazil.

The specimens were identified by genetic analyses, and external and craniodental morphology. Genomic DNA was extracted from muscle by organic method (phenol-chloroform/proteinase k) (Sambrook *et al.* 1989). PCR reactions were run in a 25 µL volume using 1 µL of DNA (100 ng/µl) to amplify the *Cytochrome b* gene (*Cyt b*), with 12.5 µL of the PCR Master Mix 2X (Fermentas, Lithuania)

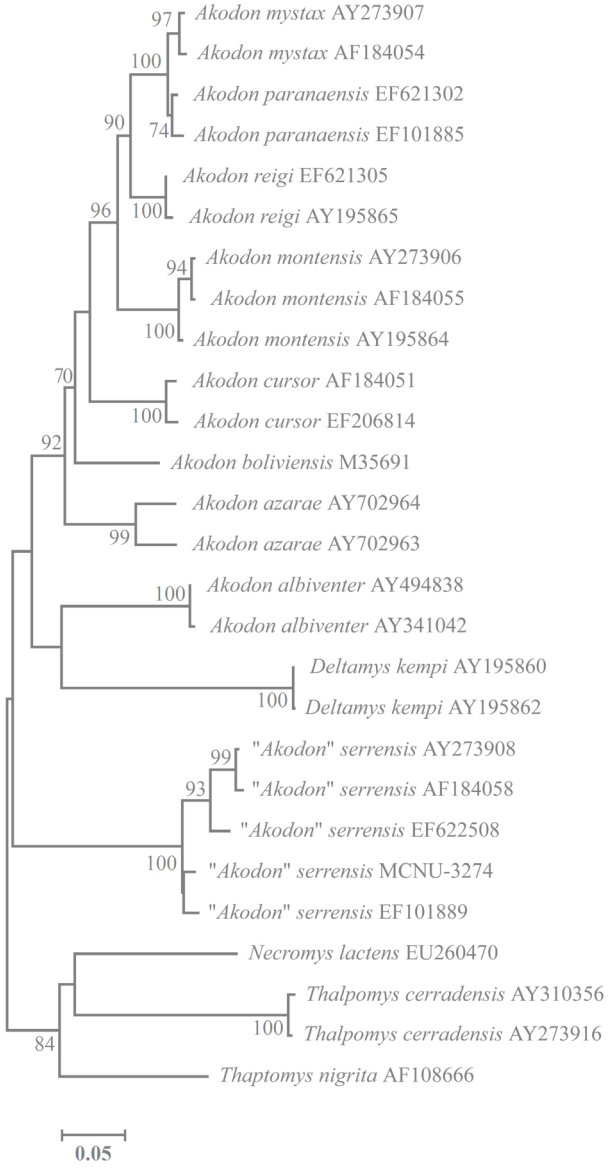

Figure 2. Phylogenetic reconstruction showing the position of the sample (MCNU 3274 adult female – *Akodon serrensis*) in the same clade of other specimens of *A. serrensis* deposited in the GenBank based on sequences of approximately 810 bp corresponding to the *Cyt b* gene. The trees were constructed using Maximum-likelihood. The values shown in the branches represent bootstrap support values with 1000 replications for each clade as well as the numbers of access to the data bank. Only bootstrap values greater than 70% are shown.

(0.05 U/µL Taq DNA polymerase, 10X buffer, 2 mM MgCl2, 0.4 mM of each dNTP), 1 µL of each primer (20 µM each) and ultrapure water to the final volume. The primers used (MVZ05 and MVZ16) and PCR conditions followed Smith and Patton (1993). Comparisons of similarity values between the sequences obtained in the present study and those deposited in the GenBank (Table 1) were done using the BLASTn program (NCBI, available online) in order to obtain the closest matches with previously published data. Moreover, throughout the phylogenetic reconstruction we assume that if the analyzed specimen forms a monophyletic clade with other sequences of *A. serrensis* from GenBank it will be considered the target species of this paper. Phylogenetic relationships were performed by Maximum Likelihood using PHYML 2.4.4 (Guindon and Gascuel 2003) incorporating evolutionary model (GTR+I+G) (Rodríguez *et al.* 1990), as selected by Modeltest 3.06 (Posada and Crandal 1998) using Akaike information criterion (AIC – Akaike 1974), with the proportion of invariable sites and gamma shape parameter as 1.6481. The confidence values of each clade for the maximum likelihood analyses were measured by the bootstrap test (Felsenstein 1985) with 1000 replications.

For the external and craniodental morphology we distinguished this species by a unique combination of morphological traits including: dorsal fur soft, with reddish coloration at dorsum (dark brown) and ventral (cinnamon) surfaces, similar at head; tail length shorter than head and body length combined, slightly bicolor, with ventral surface lighter; skull large; interorbital breadth large [mean, ± standard deviation, range in parenthesis and sample size]: [5.38 ± 0.15 (5.14 – 5.70), 16] with hourglass shape; cephalic arterial circulation primitive (Voss 1988); large molar row [4.96 ± 0.20 (4.65 – 5.31), 16] at alveolar length; large palatal bridge [4.12 ± 0.18 (3.81 – 4.41), 15]; robust molars; molars with flexus/id absent.

The first specimen of *A. serrensis* (MCNU 3274 in Figure 2) was collected on 21 September 2010, in the Center for Research and Nature Conservation Pró-Mata ("Centro de Pesquisas e Conservação da Natureza Pró-Mata" – CPCN Pró-Mata), São Francisco de Paula, Rio Grande do Sul (29°29′08.9″ S, 50°12′38.7″ W; Figure 1), on the ground level by a *Tomahawk*® live trap (IBAMA license number: 23791-1). The rodent was an adult female identified by DNA analysis, using phylogenetic reconstruction (see Figure 2). The sequence of this specimen was closely related to four specimens of *A. serrensis*, forming a unique and divergent clade highly supported by a bootstrap value of 100% in Maximum Likelihood reconstruction. This specimen presents 5.34 mm of molar row length, and 5.05 mm of interorbital breadth.

The second specimen (MCNU 3106) was captured and collected on 28 May 2011, also in the CPCN Pró-Mata, at 29°29′12.1″ S, 50°12′36.8″ W (Figure 1), by a *Sherman*® live trap installed in the ground level (IBAMA license number: 23791-1). This rodent was an adult male that was identified by cranial and external morphology (Figure 3). This specimen had large molar row length (5.52 mm) and large interorbital breadth (5.36 mm). The color pattern of fur of the two *A. serrensis* specimens (MCNU 3274 and MCNU 3106) is similar to the typical species coloration (*sensu* Christoff *et al.* 2000). First upper

molar (M1) anterocone not divided into labial and lingual conules (anteromedian flexus absent). These rodents were deposited in the MCNU Mammal Collection ("Museu de Ciências Naturais da Universidade Luterana do Brasil"), Canoas, Rio Grande do Sul State, Brazil.

Both specimens were recorded on the Ombrophilous Mixed Forest (Araucaria Forest). The area is located between 600 to 900 m above sea level, and the climate is markedly seasonal and mesothermic, with average annual temperature of ca 14.5°C and rainfall of 2,252 mm. For a detailed description of the study area see Iob and Vieira (2008).

The occurrence of *A. serrensis* reported here in the area represents the southernmost record of the species. Considering the recent report from Testoni *et al.* (2012), this record represents an expansion of 177 Km to the south of the currently known limit of its distribution. Bonvicino *et al.* (2008) reported this rodent to be present in the northern edge of Rio Grande do Sul State, but the authors did not provide voucher specimens nor localities in the distributional map given (Bonvicino *et al*. 2008: 25). Our findings confirm this approach, and expand the species

Figure 3. Ventral, dorsal, and lateral view of the skull, and lateral view of the jaws of MCNU 3106, adult male – *Akodon serrensis* collected in the Pró-Mata area, southern Brazil. Scale: 1 cm.

Table 1. Species, accession number, voucher specimen number, base pair (bp), country and reference of the sequences extracted from GenBank used in the phylogenetic reconstruction.

SPECIES	ACCESSION	VOUCHER	BP	COUNTRY	REF.
Akodon mystax	AY273907	MN 48070	1140	Brazil	D'Elia (2003)
Akodon mystax	AF184054	MN48041	1140	-	Geise *et al.* (2001)
Akodon paranaensis	EF621302	CIT 1342	801	Brazil	D'Elia *et al.* (2008)
Akodon paranaensis	EF101885	LMT405	1140	-	Goncalves *et al.* (2007)
Akodon reigi	EF621305	GD 624	801	Uruguay	D'Elia *et al.* (2008)
Akodon reigi	AY195865	MNHN 3682	1140	Uruguay	D'Elia *et al.* (2003)
Akodon montensis	AY273906	MN 48066	1140	Brazil	D'Elia (2003)
Akodon montensis	AF184055	FMNH141602	1140	-	Geise *et al.* (2001)
Akodon montensis	AY195864	GD 513	1140	Paraguay	D'Elia *et al.* (2003)
Akodon cursor	AF184051	MZUSP 29257	1140	-	Geise *et al.* (2001)
Akodon cursor	EF206814	haplotype 12	1140	-	Montes *et al.* (2008)
Akodon boliviensis	M35691	-	1140	-	Smith and Patton (1991)
Akodon azarae	AY702964	GD 327	1140	Uruguay	Pardiñas *et al.* (2005)
Akodon azarae	AY702963	CNP 751	801	Argentina	Pardiñas *et al.* (2005)
Akodon albiventer	AY494838	FMNH 129978	1140	-	Smith and Patton (1999)
Akodon albiventer	AY341042	NK 96060	1000	-	Palma *et al.* (2005)
Deltamys kempi	AY195860	UP 42 (cytb) gene	1140	-	D'Elia *et al.* (2003)
Deltamys kempi	AY195862	MNHN 4151	1140	Uruguay	D'Elia *et al.* (2003)
Akodon serrensis	AY273908	LG VA1	1140	Brazil	D'Elia (2003)
Akodon serrensis	AF184058	MN35927	1140	-	Geise *et al.* (2001)
Akodon serrensis	EF622508	-	1140	-	Montes *et al.* (unpublished)
Akodon serrensis	EF101889	LMT436	1140	-	Goncalves *et al.* (2007)
Necromys lactens	EU260470	OMNH 34512	1140	-	Braun *et al.* (2008)
Thalpomys cerradensis	AY310356	-	751	-	Andrade *et al.* (2004)
Thalpomys cerradensis	AY273916	MZUSP 30397	1140	Brazil	D'Elia (2003)
Thaptomys nigrita	AF108666	-	1140	-	Smith and Patton (1999)

distribution to the south of Brazil. These reports suggest the existence of basic biological gaps on the knowledge, like the distribution, of small mammal species in southern Brazil. Moreover, it highlights the necessity of integrated methods for the improvement of species taxonomic identification, mainly in community ecology studies about richness, composition and abundance estimates (*e.g.* Lima *et al.* 2010).

In terms of altitude, the records of *A. serrensis* presented in this note were in much lower level than those reported by Geise *et al.* (2004) (above 2,000 m) and Testoni *et al.* (2012) (between 920 and 1,200 m). This fact corroborates the suggestion made by Christoff (pers. comm.) that on the southern region of its distribution this rodent occurs in lower altitudes. The Rio Grande do Sul State has no high-altitude areas (above 1,000 m), like other areas were the species was registered previously. Moreover, the highest areas of southernmost Brazil are not sampled as well as areas of the southeastern of Brazil, so gaps still occurs on the knowledge of rodents distribution.

The phylogenetic relationships of *A. serrensis* are still unclear. The results found by Gonçalves *et al.* (2007) suggested that the genus *Akodon*, as currently understood, was not a monophyletic assemblage due to the placement of *A. serrensis* outside *Akodon*. Other genetic studies have suggested a major phylogenetic proximity of *A. serrensis* with the monophyletic genus *Thaptomys* (D'Elía 2003; Hass *et al.* 2011). The present study does not intend to elucidate this question, only to points out that this species probably is not part of the genus *Akodon*. Indeed, Figure 2 shows that *A. serrensis* formed a separate clade from the remaining species of the genus, as was found in previous phylogenetic studies (D'Elía 2003; Gonçalves *et al*. 2007; Hass *et al*. 2011).

Finally, we would like to stress the importance of sampling effort of Neotropical rodents, and its deposit in scientific collections. The knowledge of this taxonomic group is very limited, with little information about its ecology, natural history and phylogeny. Neotropical rodents are very important biodiversity maintainers, because they are food resource for larger vertebrates (Juarez and Marinho-Filho 2002; Bisceglia *et al.* 2008; Sousa and Bager 2008; Specht *et al.* 2008; Abreu *et al.* 2010) and act like seed predators and dispersers (Jansen and Forget 2001; Horn *et al.* 2008; Iob and Vieira 2008), thus performing a relevant ecological role in Neotropical forests. The adequate knowledge about rodent diversity in this region is essential for conservation and management strategies to be drawn properly.

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