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Notes on Geographic Distribution

Calomys tener (Winge, 1887) (Rodentia: Cricetidae: Sigmodontinae): Filling gaps

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Abstract: *Calomys tener* is a widely distributed species in Brazil. Herein we report the second record of *C. tener* in state of Rio Grande do Sul about 80 km northwestwards from the previous southernmost known limit in Brazil.

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The genus *Calomys* comprises ca. 13 recognized species of small sigmodontine rodents distributed mainly in dry vegetation areas of South America (Bonvicino *et al*. 2010). Seven of these species occur in Brazilian territory (Mattevi *et al*. 2005; Paglia *et al*. 2012), ranging throughout the Cerrado, Caatinga, Pantanal, Pampa and Atlantic Forest morphoclimatic domains (Bonvicino *et al.* 2003; 2010; Paglia *et al*. 2012). *Calomys tener* is a wide distributed species, known from localities throughout the Brazilian states of Tocantins and Mato Grosso in the north, to Rio Grande do Sul, southern Brazil (Haag *et al.* 2007) and the Argentine province of Misiones (Massoia 1988), whereas the known western and eastern limits comprehend eastern Bolivia (Anderson 1997) and the Brazilian state of Espírito Santo, respectively (Bonvicino *et al.* 2010).

On 01 August 2011 we captured an adult female of *Calomys tener* (Figures 1 and 2), with the third molar erupted and molar occlusal surface indicating an advanced level of wear, according to Kunz *et al.* (1996) and Cordeiro-Estrela *et al.*(2008), in an altered grassland area during a small mammal survey conducted in *Banhado Grande* locality, municipality of Viamão, northern Rio Grande do Sul Coastal Plain (30°00′42″ S, 50°57′54″ W; 33 m a.s.l.) (Figure 3). The specimen was deposited in the mammalian collection of the *Museu de Ciências Naturais La Salle*, *Centro Universitário La Salle*, Canoas, Rio Grande do Sul (MCN-MAM 42).

The field expeditions were carried out monthly from September 2010 to August 2011 during three consecutive nights. For sampling small mammals we used 90 pitfall traps and 90 wire traps distributed in three transect lines. In each transect there were 30 trapping stations spaced 20 m from the next which consisted, each one, by a pitfall (20 L bucket) and a wire trap (14x14x30 cm or 20x20x40 cm). The wire traps were baited with slices of fresh corn covered with a mixture of peanut butter, sardine and vanilla essence. The total trapping effort amounted to 5,584 trap-nights. Throughout the study we captured 51 individuals of *Calomys tener* in pitfalls and wire traps. Other small mammals captured in sympatry were: *Calomys laucha* (Fischer, 1814) (n=9), *Deltamys kempi* Thomas, 1917 (n=13), *Holochilus brasiliensis* (Desmarest, 1819) (n=1), *Mus musculus* Linnaeus, 1758 (n=9), *Necromys lasiurus* (Lund, 1840) (n=1) and *Oligoryzomys flavescens* (Waterhouse, 1837) (n=47). The identification of these specimens was based on morphological characters. Collection was authorized by *Instituto Chico Mendes de Conservação da Biodiversidade* (process number 24673-2) and these are also deposited in the mammalian collection of the *Museu de Ciências Naturais La Salle*, *Centro Universitário La Salle*, Canoas, Rio Grande do Sul (see Appendix 2).

A fragment of 890 bp of Cytochrome b gene (*cyt b*) from MCN – MAM 42 was sequenced and the sequence identity was first investigated by using BLAST (Basic Local Alignment Tool) (Altschul *et al*. 1990) in order to observe its similarity levels with other sequences deposited in the GenBank. We also performed a Neighbor-Joining (NJ) analysis in MEGA5 (Tamura *et al*. 2011), which included the *cyt b* sequence from one specimen here surveyed and

Figure 1. *Calomys tener* (adult female, MCN-MAM 42) from Banhado Grande, municipality of Viamão, Rio Grande do Sul, Brazil. Picture by Diana Gonçalves Dellagnese.

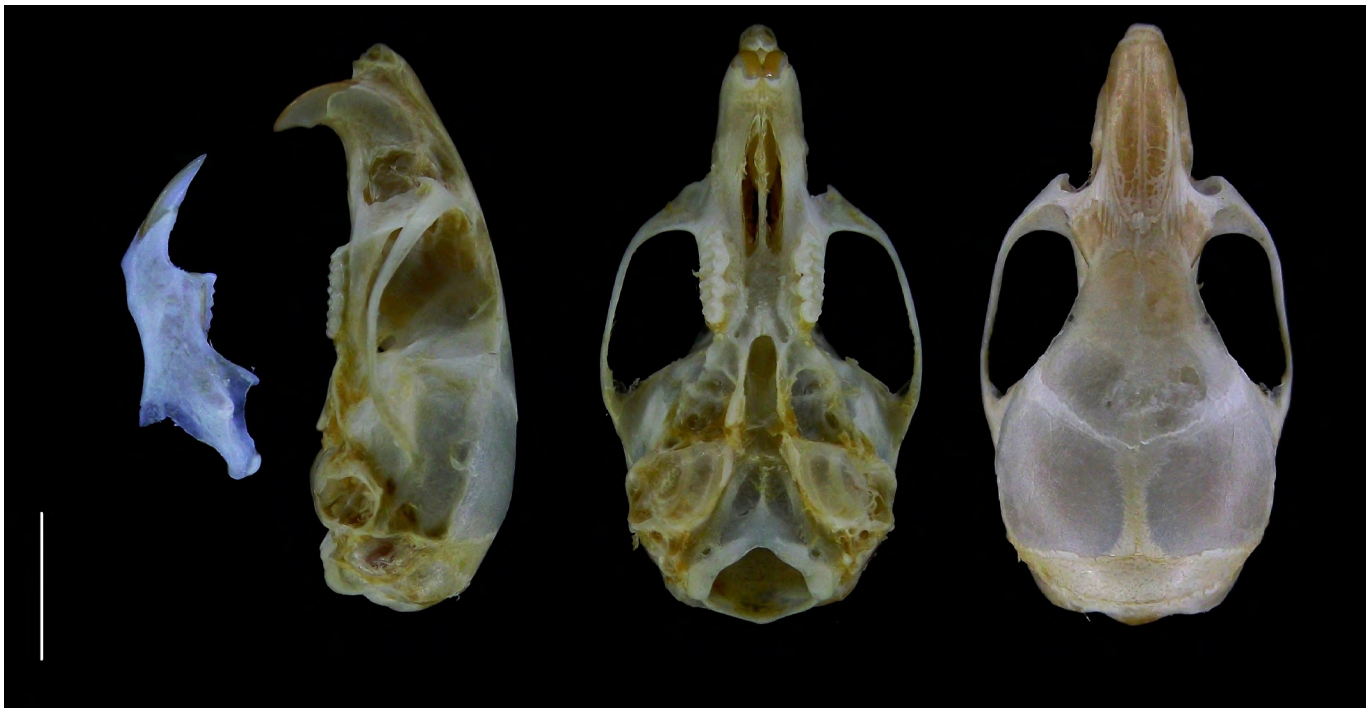

Figure 2. Dorsal, ventral and lateral views of the skull and mandible of *Calomys tener* (adult female, MCN-MAM 42) from Banhado Grande, municipality of Viamão, Rio Grande do Sul, Brazil. Pictures by Eduardo Coelho. Scale bar = 10 mm.

specimens from other 10 recognized species of *Calomys* generated in the studies of Salazar-Bravo *et al*. (2001; 2002) and Almeida *et al.* (2007), obtained from GenBank (Figure 4; Appendix 1). The specimen here surveyed grouped with the specimen of *C. tener* from Goiás, Central Brazil (Almeida *et al*. 2007) with a high level of bootstrap support (100%). The new *cyt b* sequence was deposited in GenBank (access number: JX975467). Kimura 2-parameter (K2P) evolutionary distances between *Banhado Grande* specimen and sequences from the specimens utilized in NJ analysis are shown in Table 1. Cranial measurements (Table 2) were within the range presented by Bonvicino *et al.* (2003) for C. *tener*, except by the length of diastema (LD) and breadth of rostrum (BRO). The specimen presented tawny dorsal pelage, lighter on the face; the venter was lighter, but hairs gray-based and with color limits with dorsum less conspicuous when compared to sympatric *C. laucha*. White hairs behind ears also distinguished these two species in the region, being a conspicuous characteristic in *C. laucha* and less conspicuous in *C. tener*.

This is the second record of *Calomys tener* for the state of Rio Grande do Sul. Although it not extends its distribution, we confirm the presence of this species in a region approximately 80 km northwestwards from the locality of Quintão, the previous southernmost known limit in Rio Grande do Sul (Haag *et al*. 2007). Quintão (30°24′ S and 50°16′ W – the coordinates provided in Haag *et al*. 2007 are erroneous) is located in the coastal zone of municipality of Palmares do Sul, which is completely inserted in Pampa biome (MMA/IBAMA, 2010). The physiognomy in this area is characterized mainly by coastal grasslands and dunes. On the other hand, the municipality of Viamão is located in the southern border of Atlantic Forest biome, which is also in agreement with the habitat of occurrence cited for the species (Musser and Carleton 2005; Leite and Patterson 2008).

Calomys species are morphologically very similar, being the cytogenetic analysis and DNA sequences the most appropriate methods for species identification (Almeida *et al*. 2007; Haag *et al*. 2007). *Calomys laucha* presents 2n=62-64 and FN=68-72 while the karyotype of *C. tener* is characterized by 2n=66 and FN=66-70 (Salazar-Bravo *et al*. 2001; Almeida *et al*. 2007; Haag *et al*. 2007; Bonvicino *et al*. 2010). The identification of *C. tener* specimens from Quintão was based on karyotype and cyt *b* sequences (Haag *et al*. 2007) as well as the occurrence of *C. laucha* in Rio Grande do Sul, known from Taim Ecological Station [32°32′ S and 52°32′ W – the coordinates cited in Haag *et al*. (2007) are erroneous, correctly cited in Mattevi *et al*.(2005)] in southern Coastal Plain, Pampa biome, and from a transitional area between Pampa and Atlantic Forest in Viamão (this study). Another record of *C. laucha* is based only on karyotype data from Faxinalzinho (27°20′ S and 52°40′ W), northern Meridional Plateau, in Atlantic Forest biome (Badzinski *et al*. 2012).

Although the high local abundance of *Calomys tener* obtained in the present study, it may represent an uncommon species in Rio Grande do Sul considering the capture effort accumulated on a period of ten

Table 1. Evolutionary distances (K2P) between *Calomys tener* MCN-MAM 42 from Banhado Grande, municipality of Viamão, Rio Grande do Sul, and *Calomys* specimens utilized in Neighbour-Joining (NJ) analysis (see Figure 4), based on 890 bp of Cytochrome b gene.

	MCN-MAM 42
C. callosus	16.01
C. expulsus	19.16
C. hummelincki	19.51
C. laucha	16.36
C. lepidus	18.17
C. musculinus	17.04
C. sorellus	16.38
C. tener	**1.27**
C. tocantinsi	16.64
C. venustus	16.25

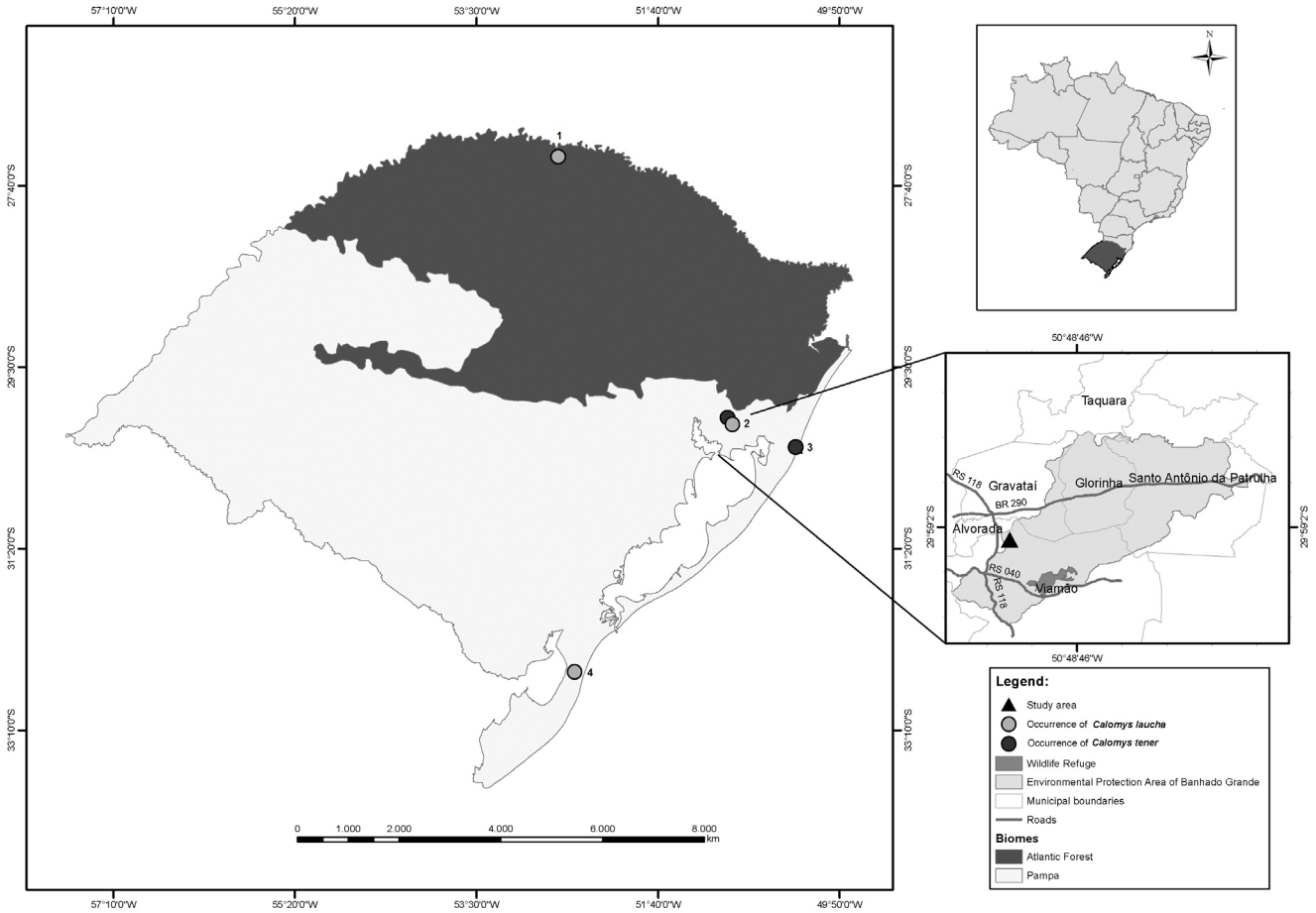

Figure 3. New records of *Calomys tener* and selected records of *Calomys laucha* from Rio Grande do Sul, Brazil. In the larger map the biome limits follows MMA/IBAMA (2010), in the inset map, the boundaries of wildlife refuge, environmental protection area and municipalities are shown (based on SEMA 2013). Localities: 1. Faxinalzinho RS (Badzinski *et al.* 2012), 2. Banhado Grande RS (this study), 3. Quintão RS (Haag *et al.* 2007), 4. Taim RS (Haag *et al.* 2007).

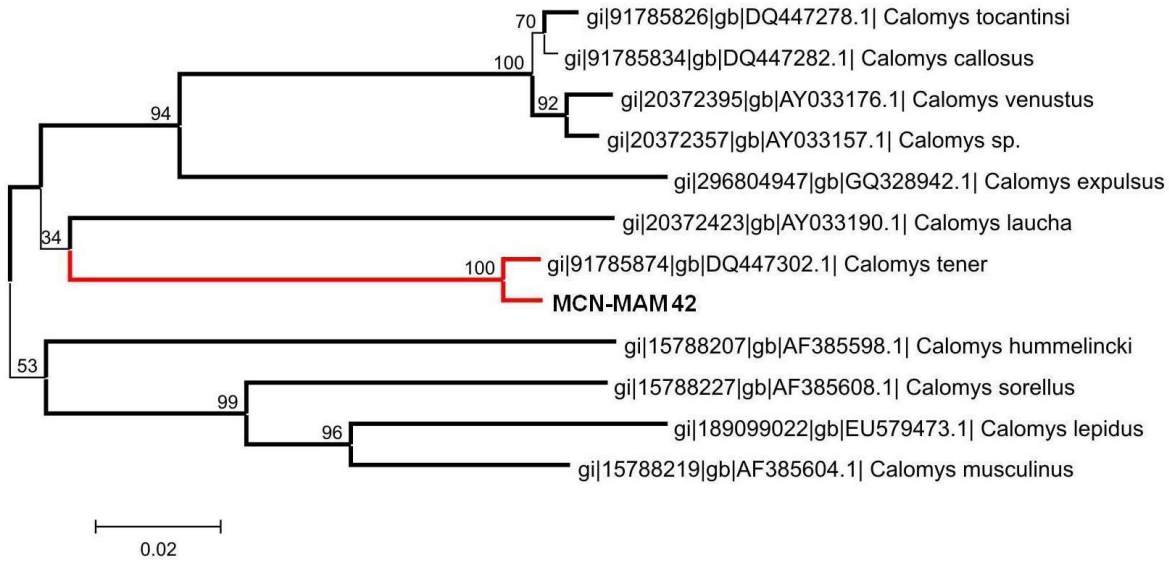

Figure 4. Neighbor-Joining tree based on 890 bp of cytochrome b sequences of *Calomys* specimens. MCN-MAM 42 is the specimen of *C. tener* from Banhado Grande, municipality of Viamão, Rio Grande do Sul, and the other specimens were downloaded from GenBank (See Appendix 1). Bar indicates substitutions per site.

years of small mammal surveys conducted in different physiognomies of the state (*e.g.* Cademartori *et al.* 2002; 2004; Dalmagro and Vieira 2005; Iob and Vieira 2008; Quintela *et al.* 2012; 2013; Sponchiado *et al.* 2012) and the record in only two localities until now (Haag *et al.* 2007 and this study). *Calomys laucha*, on the other hand, seems to be a common species in southern coastal dune systems (*e.g.* Camargo *et al.* 2006; Haag *et al.* 2007; Quintela *et al.* 2013), with one record that may represents its northernmost limit of distribution in a shrubby area in the north region of the state (Badzinski *et al.* 2012). Since this is the first record of sympatry between these two morphologically similar species in the genus, the real distribution limits

in the state of both species should be investigated and also its representativeness in the distinct vegetation physiognomies in order to better understand the biogeography of these still poorly known species in southern Brazil.

Table 2. Cranial measurements (in mm) of *Calomys tener* adult female, MCN-MAM 42, from Banhado Grande, municipality of Viamão, Rio Grande do Sul. For measurements definitions see Bonvicino *et al*. (2003).

GSL	22.0	BM1	0.9
CIL	19.5	RL	8.1
BOC	5.1	BRO	3.7
LD	4.7	ORL	7.6
PB	3.7	LIB	3.4
M1M	4.5	ZB	11.4
LIF	4.5	BB	9.6
BIF	1.4	CH	7.3
LM	3.4	BZP	2.3

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Appendix 1. Data of *Calomys* specimens utilized in Neighbor-Joining (NJ) analysis of cytochrome b sequences.

SPECIES	VOUCHER / TISSUE	GENBANK ACESSION NUMBER	LOCALITY/COUNTRY	NUMBER OF BP	REFERENCE
Calomys callosus	LBCE4474	DQ447282	Aquidauana, Mato Grosso do Sul, Brazil	1143	Almeida *et al.* (2007)
Calomys expulsus	LBCE8748	GQ328942	Luziânia, Goiás, Brazil	1143	Nascimento *et al.* (2012)
Calomys hummelincki	not informed	AF385598	Falcón, Isiro, Venezuela	1143	Salazar-Bravo *et al.* (2001)
Calomys laucha	not informed	AY33190	Tarija, Estancia Bolivar, Bolivia	1143	Salazar-Bravo *et al.* (2002)
Calomys lepidus	MVZ171562	EU579473	Puno, Peru	1143	Hanson and Bradley (unpublished)
Calomys musculinus	NK23706	AF385604	Tarija, Bolivia	1143	Salazar-Bravo *et al.* (2001)
Calomys sorellus	not informed	AF385608	Arequipa, Caylloma, Peru	1143	Salazar-Bravo *et al.* (2001)
Calomys sp.	not informed	AY033157	Beni, Bolivia	1143	Salazar-Bravo *et al.* (2002)
Calomys tener	CRB2382	DQ447302	Mimoso de Goiás, Goiás, Brazil	1143	Almeida *et al.* (2007)
Calomys tocantinsi	ARB55	DQ447278	PARNA Araguaia, Tocantins, Brazil	1143	Almeida *et al.* (2007)
Calomys venustus	not informed	AY033176	Santiago del Estero, Argentina	1143	Salazar-Bravo *et al.* (2002)

Appendix 2. List of voucher specimens from Banhado Grande, RS deposited in the mammalian collection of the Museu de Ciências Naturais La Salle, Centro Universitário La Salle, Canoas RS.

Calomys laucha (G. Fischer, 1814) - MCN-MAM 43; *Deltamys kempi* Thomas, 1917 - MCN-MAM 45; *Holochilus brasiliensis* (Desmarest, 1819) - MCN-MAM 48; *Mus musculus* Linnaeus, 1758 - MCN-MAM 47; *Necromys lasiurus* (Lund, 1840) - MCN-MAM 51; *Oligoryzomys flavescens* (Waterhouse, 1837) - MCN-MAM 46.