Zootaxa 2336: 19–25 (2010)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

A new species of *Calomys* (Rodentia: Sigmodontinae) from Eastern Brazil

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Abstract

On the basis of combined analyses of karyotypic, molecular and morphologic data, we herein describe a new *Calomys* species collected in a transitional area between the Atlantic Forest and the Cerrado morphoclimatic domains of eastern Brazil. This new taxon differs from all other Brazilian *Calomys* species by its diploid number (2n=38), the lowest among Brazilian *Calomys* species, and by its yellowish snout. Phylogenetic analyses based on cytochrome *b* DNA suggest that this species belongs to the larger-bodied species group within *Calomys*, together with *C. expulsus*, *C. callidus*, *C. callosus*, and *C. tocantinsi*.

Key words: molecular phylogeny, karyotype, cytochrome *b*, morphometrics, description

Introduction

Calomys Waterhouse, 1837 is a genus of small sigmodontine rodents, mainly distributed in dry vegetation areas across a wide geographic range in South America. The latest taxonomic compilation (Musser & Carleton 2005) included 12 species in the genus, five of which occur in Brazil: *Calomys callosus* (Rengger, 1830), *Calomys expulsus* (Lund, 1841), *Calomys tener* (Winge, 1837), *Calomys laucha* (Fischer, 1914), and *Calomys tocantinsi* Bonvicino, Lima and Almeida, 2003. Another species, *Calomys callidus* (Thomas, 1916), was recently reported from the Brazilian state of Rondônia (Mattevi *et al.* 2005). The description of a new karyomorph (2n=36, FNa=66; Geise *et al.* 1996) and associated molecular analysis indicating the presence of an additional evolutionary lineage within the genus (Almeida *et al.* 2007), raises to seven the number of *Calomys* species occurring in Brazil. Previous works considered *C. fecundus* (Thomas, 1926) a valid species (Salazar-Bravo *et al.* 2001; Almeida *et al.* 2007), but we follow Musser and Carleton (2005) in considering *C. fecundus* a junior synonym of *C. boliviae* (Thomas, 1901). Many of these species inhabit the open vegetation formations of the Caatinga and Cerrado morphoclimatic domains, which cover a very large part of South America (ca. 2,650,000 km^2). Together with the Chaco, this area is referred to as the open diagonal belt, due to its predominant xeric formations and grasslands dissected by semi-deciduous forests (Eiten 1972; Reis 1976).

Recent taxonomic studies of Cerrado species based on karyological and molecular analyses have contributed to a better understanding of *Calomys* in Brazil, with the description of new species (Bonvicino & Almeida 2000; Bonvicino *et al.* 2003) and the recognition of species previously known from elsewhere in South America (Mattevi *et al.* 2005). Intrageneric phylogenetic relationships have been proposed on the basis of morphology (Hershkovitz 1962; Steppan 1995), karyology (Pearson & Patton 1976; Espinosa *et al.* 1997), and molecular data (Salazar-Bravo *et al.* 2001; Almeida *et al.* 2007). This last study focused on Brazilian species and confirmed the occurrence of an undescribed form from the Brazilian Cerrado included in the larger-bodied species group, a monophyletic assemblage including *C. expulsus*, *C. callosus*, *C. venustus*, *C. tocantinsi*, and *C. fecundus* (= *C. boliviae*) (Almeida *et al.* 2007).

Accepted by P. Cordeiro-Estrela: 2 Nov. 2009; published: 15 Jan. 2010

Calomys species are natural reservoirs for several infectious agents associated with human diseases. *Calomys musculinus* (Thomas, 1913), for example, is the natural reservoir of Junín virus, the agent of Argentinian hemorrhagic fever (Porcasi *et al.* 2005), and three species are known to be the main natural carriers of Laguna Negra (LN) virus, which accounts for hantavirus pulmonary syndrome: *C. callosus* in central Bolivia (Carroll *et al.* 2005) and northwestern Argentina (Levis *et al.* 2004), *C. laucha* in Paraguay (Johnson *et al.* 1997), and *Calomys* sp. in Mato Grosso, Brazil (Travassos *et al.* 2008).

Here we describe a new species of *Calomys* from a transitional area between the Cerrado and Atlantic Forest morphoclimatic domains (Ab'Saber 1970), based on karyotypic, molecular (mitochondrial cytochrome *b* gene) and morphologic analyses.

Material and methods

Examination of external, cranial, and dental morphology in addition to karyotypic analysis was performed on specimens collected in several Brazilian localities (Fig. 1). Skins and skulls are or will be housed in the mammal collection of Museu Nacional, Universidade Federal do Rio de Janeiro (MN), Rio de Janeiro. The following abbreviations refer to field numbers: CRB = C.R. Bonvicino, LBCE = Laboratório de Biologia e Parasitologia de Mamíferos Reservatórios Silvestres, and SVS = Secretaria de Vigilância em Saúde, Ministério da Saúde. We karyotyped (individuals marked with # in Table 1) and/or used DNA sequence data of *Calomys* specimens from the Brazilian localities listed in Tables 1 and 2.

Chromosome preparations were obtained from 2-hour bone marrow cultures at 37°C in RPMI 1640 medium supplemented with 20% fetal calf serum, ethidium bromide (5µg/mL) and colchicine (10^{-6}M). FNa refers to the autosomal fundamental number. Karyotypic data were compared with previously published data (Table 1). We analyzed cytochrome *b* DNA sequence data from 12 species of *Calomys*, all reported in previous studies and currently available in GenBank (Table 2), and from *Auliscomys micropus* (GenBank AF108690), *Phyllotis limatus* (GenBank AY956740) and *Eligmodontia puerulus* (GenBank EU377651), which were used as outgroups based on previous phylogenetic studies (Almeida et al. 2007). The p-distance was used for calculating genetic distances between haplotypes and the Kimura 2-parameter model (K2p) for constructing neighbor-joining dendrograms with MEGA 4.0 (Tamura *et al.* 2007). Maximum parsimony trees were obtained through heuristic searches using the tree-bisection-reconnection branch-swapping algorithm in PAUP 4.0b10 (Swofford 1999), with all sites equally weighted and a random addition sequence with 10 replications. Confidence intervals for neighbor-joining and maximum parsimony trees were obtained by bootstrap analyses based on 1,000 replicates. Maximum likelihood analyses were carried out with heuristic searches with 10 addition sequence replicates in PAUP 4.0b10, and bootstrap values were obtained based on 1,000 replicates using GARLI (Zwickl 2006).

For morphologic comparisons we used the specimens listed in Appendix 1. Morphological descriptions and comparisons were based on qualitative external and cranial characters, using the terminology of Voss (1988, 1993). Capitalized color nomenclature follows Ridgway (1912). For morphometric characterizations, external measurements comprised head-body length (HB), tail length (T), hind foot length with claw (HF), ear length (E), weight (W) as recorded on specimen tags, and 16 cranial dimensions defined by Bonvicino and Weksler (1998), taken with the aid of a digital caliper: greatest skull length (SL), condylo-incisive length (CI), breadth of the occipital condyles (BO), length of diastema (LD), palatal bridge (PB), length of incisive foramen (LI), breadth of incisive foramen (BI), length of maxillary molar row (LM), breadth of first maxillary molar (M1), orbital length (OL), least interorbital breadth (IB), zygomatic breadth (ZB), breadth of braincase (BB), rostrum length (RL), and rostrum breadth (RO). The two last variables were not available for all specimens and were not included in the multivariate analyses. Only adult animals (with all molar teeth erupted and functional) were included in morphometric comparisons.

To analyze craniometric variation we carried out a Principal Component Analysis on the covariance matrix of log-transformed measurements, and to identify characters that most contributed to the differentiation among samples we performed a Canonical Variate Analysis. Missing data (1.99%) were estimated by the Expectation-Maximization method (Dempster *et al.* 1977; Strauss *et al.* 2003). This algorithm, as well all multivariate analyses, was implemented in MatLab version 4.2 (The Mathworks 1992), using functions written by R. E. Strauss available at http://www.faculty.biol.ttu.edu/strauss/Matlab/Matlab.htm (accessed in March, 2009).

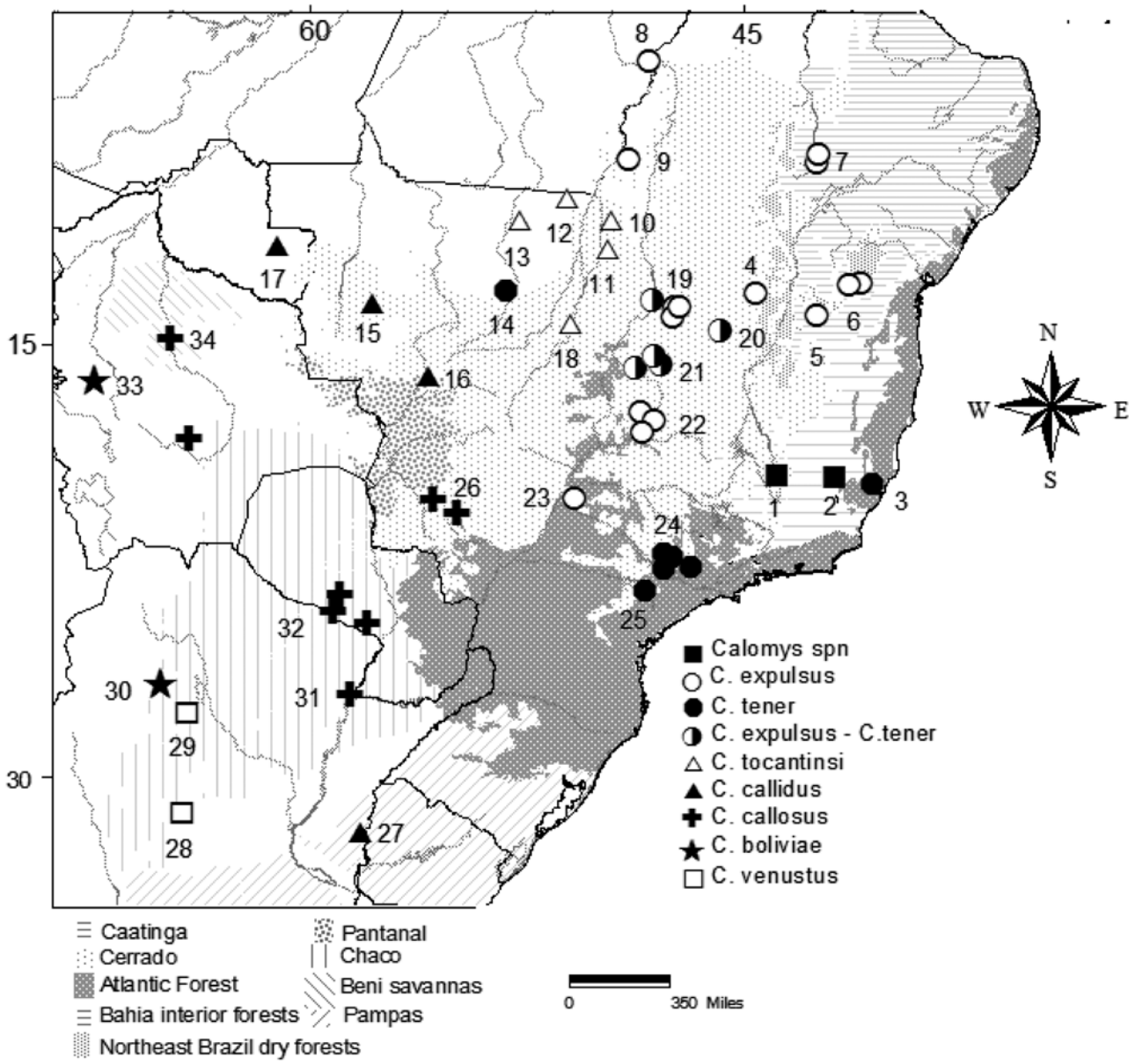

FIGURE 1. Localities of specimens used in the analysis, selected localities of karyotyped specimens and type localities of *Calomys* taxa of larger-body size. Numbers refer to localities specified in Table 1 and Appendix 1.

TABLE 1. Diploid (2n) and autosomal fundamental numbers, (FNa), localities of specimens, and other published data on *Calomys* species. Species names in parentheses refer to the names used in the original publications. States of Brazil (BRA) and Venezuela (VEN), departments of Bolivia (BOL), Peru (PER) and Uruguay (URU), and provinces of Argentina (ARG) are underlined. Other country abbreviations are Aruba (ARU) and Netherlands Antilles (NEA). Numbers in parentheses refer to the localities plotted in Figure 1. # refers to karyotyped specimens.

Taxa	2n	FNa	Locality and specimen studied	Source
Calomys sp.n.	38	66	BRA: Minas Gerais, Capitão Andrade (2) MN 71971#-72-73	This study
Calomys sp.n. (*C.expulsus*)	36	66	BRA: Minas Gerais, Lagoa Santa (1)	Geise *et al*. 1996
C. bolivae (*C. fecundus*)	54	66	ARG: Chuquisaca, Monteagudo, Chuhuayacu, Tarija, Tucumilla, Carapari, Camatindy, Porvenir.	Salazar-Bravo *et al*. 2002
C. callidus	48	66	ARG: Entre Rios, Parque Nacional El Palmar (27)	Vitullo *et al.* 1984; 1990
C. callidus	48	66	BRA: Rondônia, Pimenta Bueno (17)	Mattevi et al. 2005
C. callidus	48	66	BRA: Mato Grosso, Campo Novo do Parecis (15) SVS MT 310#, SVS MT 326#; Barão de Melgaço (16) MN 64031	This study
C. callosus	50	66	BRA: Mato Grosso do Sul, Aquidauana (26) LBCE 4474#, MN 71981, Sidrolândia (26) MN 71974-76#	This study
C. callosus (*C. fecundus*)	50	66	BOL: Beni, 160km N Trinidad (34)	Pearson and Patton 1976
C. expulsus	66	68	BRA: Bahia, Caetité (5) MN 63372#, Correntina (4) CRB 2733; Goiás, Mimoso de Goiás (21) MN 71951-53#, 71958#, MN 67076-77#, 71961#; São Paulo, Aparecida D'Oeste (23) CRB 2745#; Piauí, Coronel José Dias (7) MN 63309#	This study
C. expulsus	66	68	BRA: Distrito Federal, Brasília (21); Goiás, Minaçu (19), Ipameri (22), Caldas Novas (22), Corumbaíba (22)	Svartman 1989; Mattevi *et al*. 2005
C. expulsus	66	68	BRA: Tocantins, Piquizeiro (8), São Sebastião (9)	Lima 2001
C. expulsus	66	68	BRA: Bahia, Cocos (20), Jaborandi (20); Goiás, Alto Paraíso (19), Cavalcante (19), Teresina de Goiás (19); Piauí, São João do Piauí (7)	Bonvicino and Almeida 2000; Bonvicino *et al*. 2003
C. expulsus	66	68	BRA: Bahia, Itaetê (6), Mucugê (6)	Pereira and Geise 2007
C. hummelincki	60	64	VEN: Falcón, Represa El Isiro; Lara, Curarigua; Apure, Puerto Páez; Monagas, El Merey; Bolívar, Sipao. ARU: Aruba. NEA: Curaçao	Martino et al. 2002
C. laucha	64	72	ARG: Córdoba, Laguna Larga	Ciccioli and Poggio 1993**
C. laucha	64	68	BRA, Rio Grande do Sul, Taim	Mattevi *et al*. 2005
C. laucha	64	70	URU: Rocha, Laguna Negra	Brum-Zorrilla *et al*. 1990
C. laucha *	64	68	URU: Maldonado, Bella Vista; Artigas, Colonia Palma; Rocha, Laguna Negra; San José, Concordia; Colonia, Rosario	Brum-Zorrilla *et al*. 1990
C. lepidus	36	68	PER: Junín and Puno	Pearson and Patton 1976
C. af. lepidus (*C. lepidus*)	44	68	ARG: Jujuy, Laguna de Pozuelos	Espinosa *et al*. 1997
C. musculinus	38	62	ARG: Córdoba, Laguna Larga	Ciccioli and Poggio 1993**
C. musculinus	38	56-57	ARG: Córdoba, Chucul	Lisanti *et al*. 1996
C. musculinus	38	56-58	ARG: Córdoba, Las Higueras	Forcone *et al*. 1980
C. musculinus murillus	38	48	ARG: Buenos Aires	Massoia *et al*. 1968
C. sorellus *	64	68	PER: Departments from Ancash to Puno	Pearson and Patton 1976
C. tener	66	66	BRA: Espírito Santo, Santa Tereza (3) SVS ES254#; Bahia, Jaborandi (20) MN 62645; Goiás, Mimoso de Goiás (21) MN 67075#	This study
C. tener	66	66	BRA: Goiás, 40km SW Minaçu (19)	Mattevi *et al*. 2005

continued next page

TABLE 1. (continued)

Taxa	2n	FNa	Locality and specimen studied	Source
C. tener	66	66	BRA: Distrito Federal (21); Bahia, Cocos (20); São Paulo, Campinas (24), Itirapina (24), Pedreira (24), Rio Claro (24)	Svartman 1989; Bonvicino and Almeida 2000
C. tener	66	66	BRA: Mato Grosso, Gaúcha do Norte (14)	Fagundes *et al*. 2000
C. tener (*Calomys* sp.)	66	66	BRA: São Paulo, Itapetininga (25)	Yonenaga 1975
C. tocantinsi	46	66	BRA: Tocantins, Lagoa da Confusão (10), Formoso do Araguaia (11), P.N. do Araguaia	Lima and Kasahara 2001; Bonvicino *et al*. 2003
C. tocantinsi (*Calomys* sp.)	46	66	BRA: Mato Grosso, Vila Rica (12), Cocalinho (18); Tocantins, P.N. do Araguaia	Fagundes *et al*. 2000
C. tocantinsi	46	66	BRA: Mato Grosso, São José do Xingu (13) MN 71970#	This study
C. venustus	56	66	ARG: Córdoba	Salazar-Bravo et al. 2002
Calomys sp. (*C. l. laucha*)	62	72	ARG: Buenos Aires, Parque Pereyra	Massoia *et al*. 1968
Calomys sp. (*C. laucha*)	56	60	URU: Artigas	Brum-Zorrilla *et al*. 1990
Calomys sp. (*C. laucha*)	64	70	ARG: Córdoba, Laboulaye. URU: Rocha, Laguna Negra	Brum-Zorrilla *et al*. 1990
Calomys sp. (*C. laucha*)	64	72	ARG: Córdoba, Laguna Larga; Corrientes, Esquina	Brum-Zorrilla *et al*. 1990

* Despite the apparent similarity between the *C. sorellus* and *C. laucha* karyotypes (both 2n=64, FNa=68), the morphology of the chromosomes is different. In C. *laucha*, two of the three metacentric autosomes are the largest in the complement, whereas in *C. sorellus* the metacentric chromosomes are medium-sized and small.

** These authors included subtelocentric chromosomes as biarmed chromosomes; this may be the cause of the apparently different chromosome complements.

Results

Karyotypic analysis of one *Calomys* sp.n. specimen showed 2n=38, FNa=66 (Fig. 2). The autosomal complement was composed of 15 pairs of biarmed chromosomes varying in size from large to small, and three pairs of small-sized acrocentric chromosomes. The X chromosome was a medium-sized submetacentric, and the Y chromosome a small acrocentric.

The analyses of 10 *C. expulsus* specimens resulted in 2n=66, FNa=68 (Fig. 2). The autosome complement comprised 2 pairs of biarmed chromosomes (one large pair of submetacentrics and one medium pair of metacentrics), and 30 acrocentric pairs gradually varying in size. The X chromosome was a large subtelocentric and the Y chromosome a small acrocentric. Two *C. tener* specimens had 2n=66, FNa=66 (Fig. 2), the autosome complement comprising 1 pair of small biarmed chromosomes and 31 acrocentric pairs gradually varying in size. The X chromosome was a large subtelocentric and the Y chromosome a small acrocentric. The karyotypes of 12 *C. callosus* specimens were all 2n=50, FNa=66 (Fig. 2); the autosome complement comprised 9 pairs of biarmed chromosomes (eight large pairs and one small pair) and 15 acrocentric pairs varying in size from large to small. The X chromosome was a large submetacentric chromosome and the Y chromosome a small acrocentric. Two *C. callidus* specimens had a karyotype of 2n=48, FNa=66 (Fig. 2); the autosome complement comprised 10 pairs of biarmed chromosomes (8 large pairs and one small pair), and 13 acrocentric pairs varying in size from large to small. The X chromosome was a large biarmed and the Y chromosome a small acrocentric. *C. tocantinsi* (one specimen) had 2n=46, FNa=66 (Fig. 2). The autosome complement comprised 11 pairs of biarmed chromosomes (8 large pairs and one small pair), and 11 acrocentric pairs varying in size from large to small. The X chromosome was a large submetacentric and the Y was a small acrocentric chromosome.

The chromosome complement of larger-bodied species showed a trend of reduction in diploid number, from the most basal species with 2n=66, through other species with intermediate diploid numbers, to a minimum of 2n=36 and 38 in *Calomys* sp.n. Genetic distance estimates between *Calomys* sp.n. haplotypes varied from 0.1 to 0.2%, with three haplotypes, one of which was shared by one specimen from Capitão

TABLE 2. P- distance estimates (x100) between cytochrome b sequences used in molecular analyses, with GenBank accession number, species, and museum or field number ("number"). Shaded cells are distances within the same species. 2n= diploid number, FNa= autosomal fundamental number, *specimen without karyologic data. States in Brasil (BRA) are Bahia (BA), Minas Gerais (MG), Goiás (G), Mato Grosso (MT), Mato Grosso do Sul (MS), Tocantins (TO); Argentina (ARG); Bolivia (BOL), Venezuela (VEN). DQ447276 (field number CEG40) from Lagoa Santa shares the same haplotype with DQ447274 from Capitão Andrade.

	GenBank	Species and number	Locality	2n/FNa	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	DQ447275	*Calomys* sp.n.	BRA: MG, Lagoa Santa	36/66														
2	DQ447274	*Calomys* sp.n. MN71971, MN71972	BRA: MG, Capitão Andrade	38/66	0.1													
3	DQ447273	*Calomys* sp.n.MN71793	BRA: MG, Capitão Andrade	38/66	0.2	0.1												
4	DQ447279	*C. callidus* MN64031	BRA: MT, Barão de Melgaço	48/66	6.5	6.4	6.3											
5	DQ447282	*C.callosus* MN71981	BRA: MS, Aquidauana	50/66	6.2	6.1	6.0	0.3										
6	DQ447278	*C. tocantinsi*	BRA: TO, PN Araguaia	46/66	7.0	6.9	6.8	0.8	1.1									
7	AY033173	*C. boliviae*	ARG: Tucumán	*	6.3	6.2	6.3	2.3	2.2	2.6								
8	AY033176	*C. venustus*	ARG: Santiago del Estero	*	6.2	6.1	6.0	2.4	2.3	2.7	1.3							
9	DQ447283	*C.expulsus* CRB2733	BRA: BA, Correntina	66/68	13.1	13.0	12.9	13.0	13.0	12.4	13.0	13.2						
10	AY033189	*C. laucha*	BOL: Tarija, Est. Bolivar	*	13.8	13.7	13.8	14.9	14.9	14.9	15.5	15.6	14.0					
11	DQ447302	*C. tener* MN67075	BRA: GO, Mimoso de Goiás	66/66	15.9	15.7	15.6	15.5	15.3	15.3	15.9	15.5	15.8	14.6				
12	AF385598	*C. hummelincki*	VEN: Falcón, Isiro	*	16.9	16.8	16.9	16.1	16.0	16.2	16.2	16.5	16.9	17.3	16.9			
13	AF385608	*C. sorellus*	Peru: Arequipa, Caylloma	*	18.3	18.2	18.3	19.9	19.5	20.0	19.5	19.9	19.0	19.9	16.9	18.1		
14	AF385606	*C. lepidus*	BOL: Tarija, Iscayachi	*	18.7	18.6	18.5	18.8	18.9	19.1	19.8	19.7	19.0	19.5	17.3	19.3	14.2	
15	AF385604	*C. musculinus*	BOL: Tarija, Tucumilla	*	16.6	16.5	16.4	17.3	17.2	17.6	18.8	18.4	17.3	16.3	14.6	17.0	11.5	10.8

Andrade and one specimen from Lagoa Santa (Table 2). Distance estimates between *Calomys* sp.n. and all other *Calomys* species ranged from 6.0 to 18.7%, and were higher than for any other species pairs in the larger-bodied monophyletic assemblage, except those including *C. expulsus*. The p-distance estimates were 1.3% between *C. venustus* and *C. boliviae*, 2.3% between *C. venustus* and *C. callosus*, and 2.7% between *C. venustus* and *C. tocantinsi*.

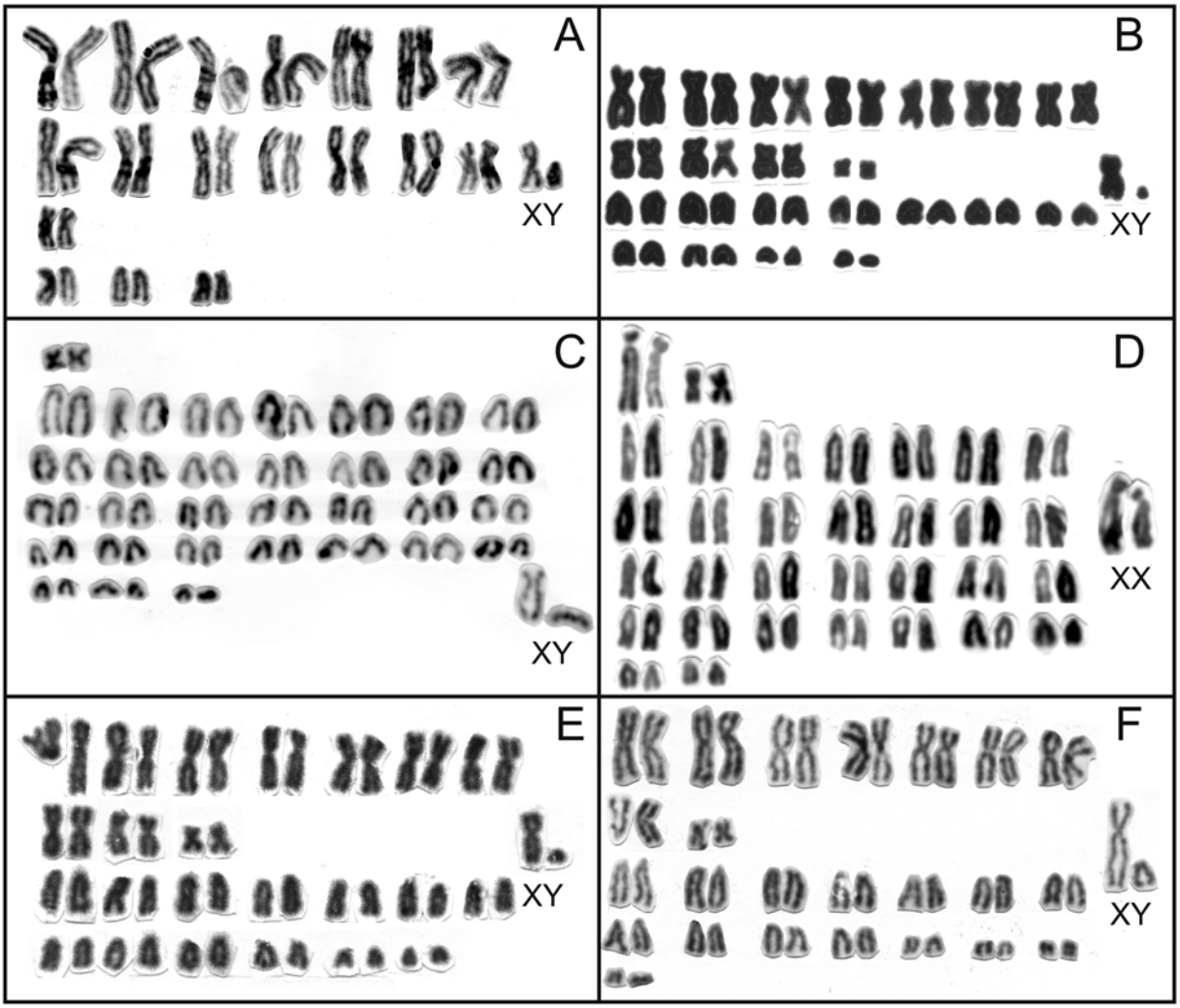

FIGURE 2. Karyotypes (conventional Giemsa staining) of (A) *Calomys* sp.n., male MN 71971, (B) *C. tocantinsi*, male MN 62731 (C) *C. tener*, male MN 62645, (D) *C. expulsus*, female MN 63372, (E) *C. callidus*, male SVS 400, (F) *C. callosus*, male LBCE 4474.

Neighbor-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) topologies grouped *Calomys* species in a monophyletic arrangement; in ML and NJ, the *Calomys* clade divided into two subclades, one with *C. musculinus* and *C. lepidus*, with *C. sorellus* as its sister branch, and the other with the remaining *Calomys* species (Fig. 3). This latter clade included *C. hummelincki* as the most basal offshoot, followed by *C. tener* and *C. laucha* (in NJ and ML); these were subsequently followed by *C. expulsus*, leaving an internal clade comprising two groups, one with *C. boliviae*, *C. venustus*, *C. callosus*, *C. tocantinsi* and *C. callidus*, and the other with *Calomys* sp.n. In MP *Calomys* split into three lineages, one with *C. hummelincki*, one with *musculinus-lepidus-sorellus*, and the third containing the remaining *Calomys* species in the same topological arrangement as in the NJ and ML analyses. These topologies were coincident with the karyotypic data, and placed *C. expulsus* (2n=66, FNa=68) as the sister group to remainder of the larger-bodied species group (Fig. 3). To derive the karyotype of *C. venustus* from that of *C. expulsus* requires one inversion in a

small autosome pair and centric fusions in five or six autosome pairs; to derive the *C. callosus* karyotype, centric fusions in two more autosome pairs are necessary; to derive the *C. callidus* karyotype requires a centric fusion in one further autosome pair; to derive the *C. tocantinsi* karyotype, a centric fusion involving yet another autosome pair is required; finally, to derive the *Calomys* sp.n. karyotype from that of *C. expulsus*, centric fusions in two more autosome pairs are necessary.

The first three principal components (PCs) explained 83.5% of the total craniometric variation, with PC1 accounting for 68.9%. All character loadings on PC1 had positive signs, indicating that PC1 can be interpreted as a general size vector. Convex hulls depicting groups of scores belonging to different species revealed a complete separation between the sample of *C. tener* and the remaining ones, which showed considerable overlap with respect to PC1 but were slightly separated along PC2 (Fig. 4).

Since size differences between *C. tener* and the remaining species obviate further comparisons, this species was excluded from the Canonical Variant Analysis. The first three canonical axes summarized 94.7% of the variation among groups, and interpolation of group scores with respect to CV1 showed complete separation between the distribution of *C. tocantinsi* and the completely overlapping distributions of *C. callosus*, *C. expulsus*, and *Calomys* sp.n., with *C. callidus* showing intermediate values. Considerable superimposition was revealed along CV2, which accounted for 27.7% of the variation among groups. *Calomys* sp.n. scores were separated from those of the remaining samples along CV3, which accounted for 13.8% of the discrimination among samples (Fig. 4). The variables most associated with this function that may contribute to the differentiation between *Calomys* sp.n. and the remaining samples are breadth of first maxillary molar (M1) and length of maxillary molar row (LM) (Fig. 4).

Karyotypic, molecular and morphologic data showed that *Calomys* sp.n. specimens (2n=38, FNa=66) belong to a hitherto undescribed species, which is described below.

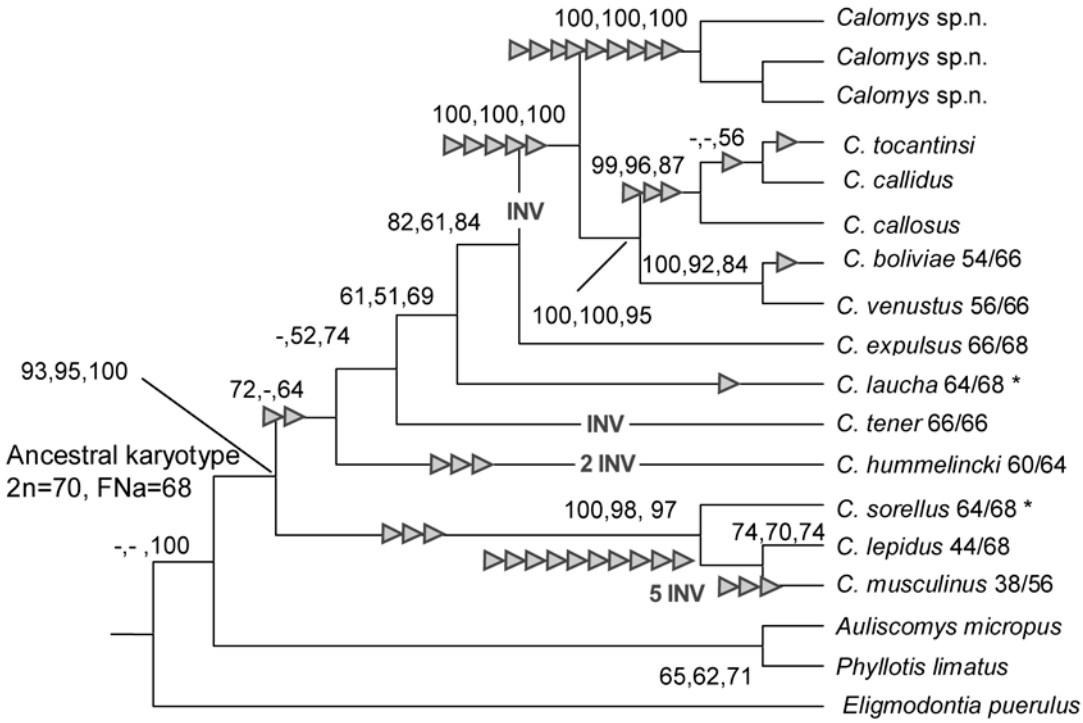

FIGURE 3. Maximum likelihood (ML) cladogram showing phylogenetic relationships among *Calomys* and selected muroid species based on 1140 bp of the cytochrome b gene. Numbers near nodes are bootstrap support values for neighbor-joining, maximum parsimony and ML analysis. INV indicates a pericentric inversion, and a gray triangle a Robertsonian fusion. See footnote of table 1 for the meaning of the asterisk.

***Calomys cerqueirai* new species**

Holotype. An adult male (MN 71971) consisting of skin, skull, mandible, partial postcranial skeleton and ethanol-preserved liver tissue sample, field number LBCE 5556 (Fig. 5). It was collected by R. Gentile on July 30, 2003, in grassy vegetation along a small stream at the type locality. The liver sample is stored at the Laboratório de Biologia e Parasitologia de Mamíferos Reservatórios Silvestres, Instituto Osvaldo Cruz-FIOCRUZ, Rio de Janeiro, under original field number LBCE 5556.

Paratypes. Two young males (MN 71972, 71973) consisting of skin, skull, mandible, partial skeleton and ethanol-preserved liver tissues, field numbers LBCE 5579 and 5583 collected along "Córrego Café" beyond the city center (19º02'33"S, 41º49'33"W).

Type locality. Margins of "Buraco do Cachorro" stream, situated near the head of "Café" stream, close to Capitão Andrade city center (19º04'35"S, 41º52'30"W), Capitão Andrade Municipality, state of Minas Gerais, Southeastern Brazil.

Capitão Andrade is a small city situated in a hilly region approximately 230 meters above sea level, in a characteristic rural landscape. It is located in a transitional zone between the Atlantic Forest and Cerrado morphoclimatic domains (Ab'Saber 1970), with predominant semi-deciduous tropical forest vegetation, in the Rio Doce Basin. The "Morro do Café" hill separates two main streams, "Café" and "Perdida", which run across the municipality and flow into the Rio Doce, 20 km beyond the municipality's center. Specimens were captured in rural areas along streams. Transects were characterized by open vegetation, predominantly grass, shrubs and scattered trees, since the original forest vegetation has been removed even on hills, which are now used for farming. This study was conducted during winter (July), when the vegetation was very dry, except alongside the streams. The three animals were captured in grassy vegetation, less than 2 meters from the stream margin.

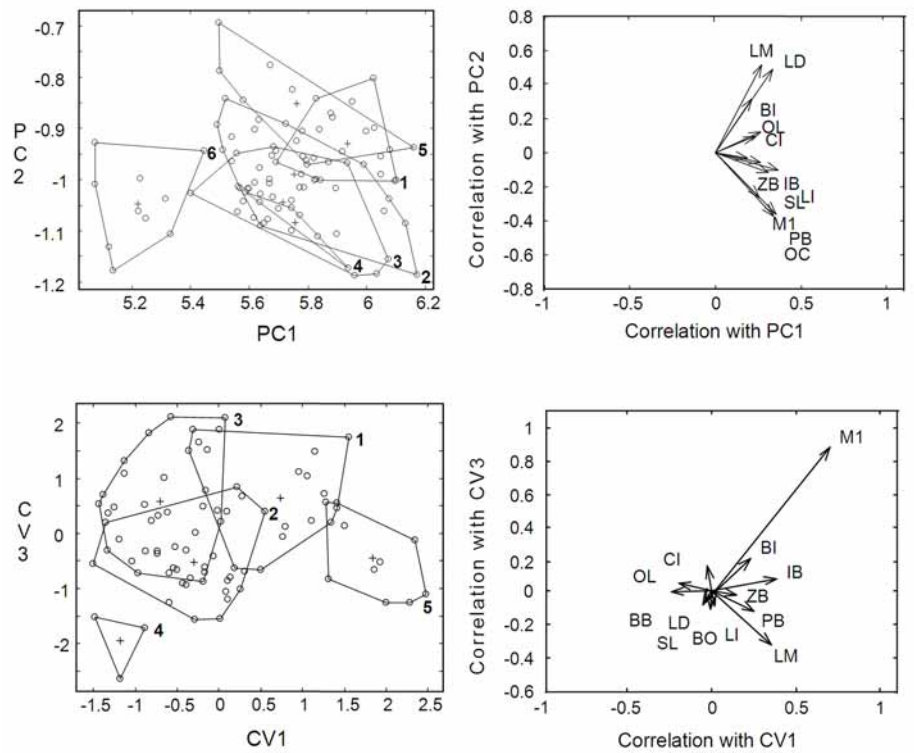

FIGURE 4. Left: Convex hulls for scores of specimens belonging to different species on the first and second principal components (upper) and on the first and third canonical functions (lower): 1) *Calomys callidus*; 2) *C. callosus*; 3) *C. expulsus*; 4) *Calomys* sp.n. 5) *C. tocantinsi*; 6) *C. tener.* Right: Vector plots of character correlations associated with the principal components (upper) and canonical functions (lower).

Other specimens. Two specimens (GenBank accession numbers DQ447275 and DQ447276) from Lagoa Santa, Minas Gerais state, were not examined, but form a monophyletic clade with Capitão Andrade specimens, and one of them (DQ447276) shares the same cytochrome *b* haplotype with the holotype (MN 71971) and the paratype MN 71972 of *C. cerqueirai*. To derive the karyotype of Lagoa Santa specimens (2n=36, FNa=66) and Capitão Andrade specimens (2n=38, FNa=66), one centric fusion involving two acrocentric pairs, or a centric fission involving one biarmed pair, is necessary. These specimens from Lagoa Santa were karyotyped by Geise *et al.* (1996), and sequenced by Almeida *et al*. (2007).

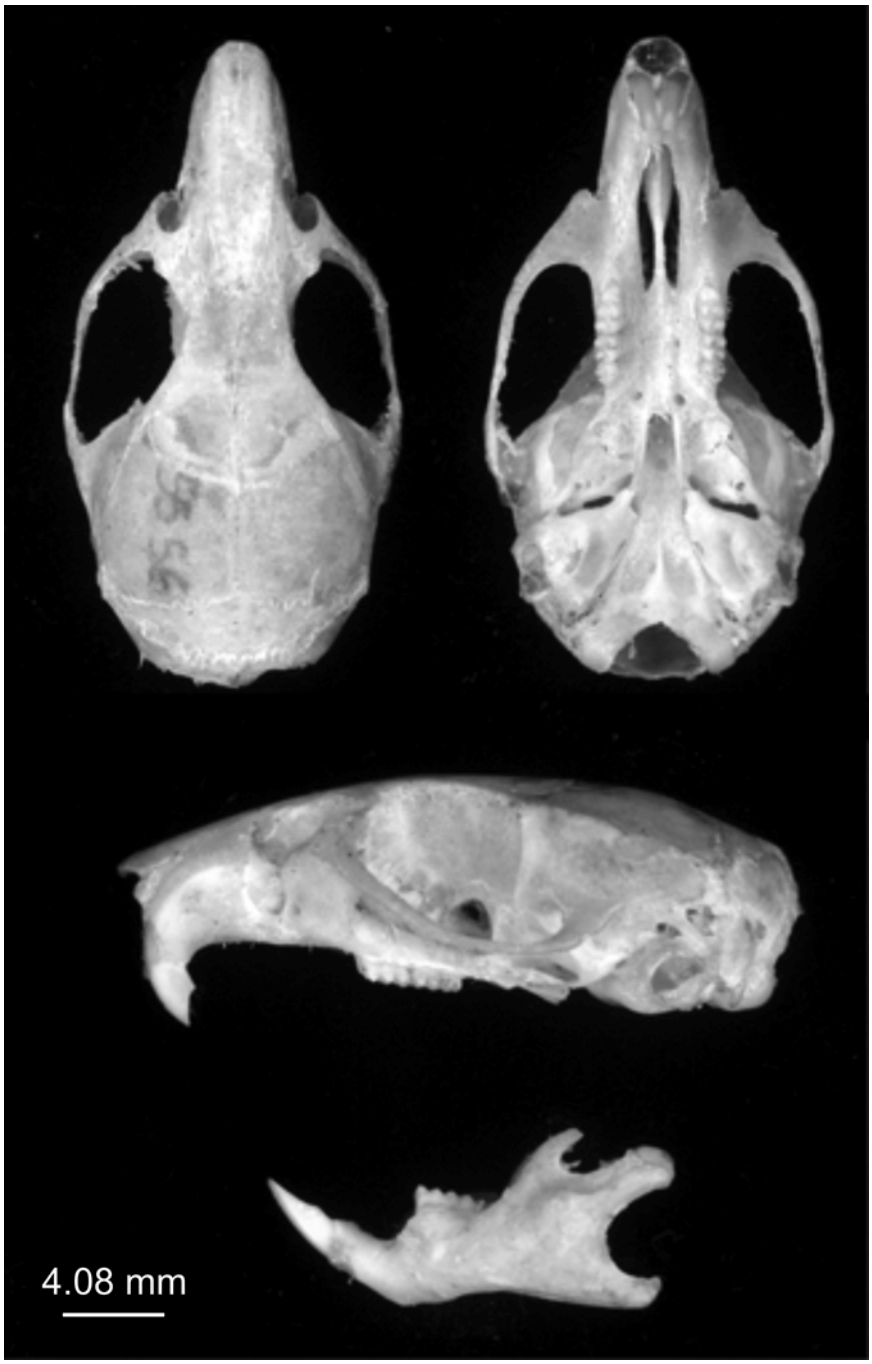

FIGURE 5. Dorsal, ventral and lateral views of skull, and lateral view of mandible of *Calomys* sp.n. holotype (male MN 71971).

Etymology. This species is named after Dr. Rui Cerqueira for his long-standing dedication to the development of Brazilian Mammalogy.

Diagnosis. A large sized *Calomys* species with tail shorter than head and body, overall dorsal coloration light brown-yellowish, orange snout, and tail sharply bicolored. This species has five autapomorphic sites in relation to the sequences of the other *Calomys* species analyzed, in bases 189, 297, 423, 879, 1122 of the cytochrome b gene.

Measurements. See table 3.

Distribution. Known from the type locality and from Lagoa Santa, Minas Gerais state, Brazil.

Morphological description. A small-sized sigmodontine rodent (Table 3) with tail shorter than head and body (75-87% of head and body length), moderately long and narrow hind feet, and small, rounded pinnae (16-18% of head and body length). Dorsal pelage is soft and dense, Cinnamon-Brown overall. Head color as on dorsum, but hairs surrounding nose Mars Yellow to Ochraceous-Tawny (Fig. 6). Two types of hairs of similar length (8-10mm) present on dorsum: a rather homogeneously melanic type and a banded type. On head and dorsum, banded hairs have a long plumbeous base (7mm) and an Ochraceous-Tawny distal band. Melanic hairs are entirely black distally and mixed with banded hairs, producing the Cinnamon-Brown tone of the dorsum. Body laterals slightly lighter than dorsum due to the reduction of black guard hairs. Ventral pelage well delineated from laterals, covered with whitish hairs with gray bases. Pinnae internally covered by entirely Orange hairs, or hairs with long Orange bands, and externally covered by sparse brownish hairs. Patch of completely white hairs behind the ear. Fore- and hindfeet dorsally furred with entirely white hairs; silvery ungual tufts present on distal phalanxes of digits 2-5 in manus and digits 1-5 in pes. Tail sharply bicolor, with white hairs ventrally and dark hairs dorsally.

FIGURE 6. Enlarged dorsal view of heads of C. *expulsus* (MN 71962, left) and C. *cerqueirai* (holotype, MN 71971, right).

Cranial characters: skull with relatively short rostrum and laterally expanded braincase. Frontal bones with anteriorly convergent lateral outlines and supraorbital edges, but without distinct supraorbital crests. Nasals short. Zygomatic plate slightly projected anteriorly. Zygomatic notch moderately deep and broad. Incisive foramina long, reaching line of M1 protoflexus. The bony palate extends behind the end of upper molar row by a distance about equal to the length of M3, and bears one pair of posterolateral palatal pits.

TABLE 3. Descriptive statistics of measurements –mean (mm), sample size (in parentheses) and range of variation (mm) – of *Calomys* sp.n. and selected samples of *C. expulsus*, *C. callosus*, *C. callidus*, *C. tocantinsi*, and *C. tener* from Brazil. VAR = variables, see material and methods for abbreviations. * = CT (body and tail length). F = females, M = males.

VAR	*Calomys* sp.n.	*C. expulsus*	*C. callosus*	(F) *C. callidus*	(M) *C. callidus*	(F) *C. tocantinsi*	(M) *C. tocantinsi*	*C. tener*
HB	99 (2) 86–112	99.7 (211) 64–122	98 (27) 83–123	173.8*(12) 160–194	182.7*(25) 155–210	90.1 (10) 77–105	91.8 (8) 79–116	77.5 (10) 74–88
T	80 (2) 75–85	72.1 (21) 45–88	72.3 (27) 59–90	72.8 (12) 64–86	75.8 (25) 50–87	66.6 (10) 60–75	68.4 (8) 61.6–75	60.6 (10) 38–77
HF	21.5 (2) 21–22	20.2 (21) 18–22	20.9 (27) 17–23	17.9 (12) 16–20	19.2 (25) 17–23	21.2 (10) 20–22.5	21.2 (8) 20–22	16.6 (10) 15–17
E	17 (2) 16–18	17 (21) 14–19	16.8 (27) 15–19	15.7 (12) 14–18	15.4 (25) 14–17	17 (10) 13.5–21	16.5 (8) 14–18	14.2 (10) 13–16
W	30 (2) 25–35	28.2 (21) 19–40	29.9 (27) 20–46.9	30.5 (12) 21–42	35.8 (25) 25–51	25.3 (8) 14.6–31	25.3 (8) 14.6–31	14.5 (10) 10–17
SL	26 (3) 24–28	25.5 (21) 23.7–28.2	25 (22) 22.5–27.8	25.0 (5) 24.3–26.3	26.7 (11) 23.7–28.4	24.8 (8) 22.9–26	25.0 (7) 24.2–28.6	22.1 (10) 21–23.5
CI	24.3 (3) 22.8–26.2	23.7 (21) 22.2–26.2	22.9 (24) 20.2–26	23.3 (5) 22.8–24.5	24.8 (11) 22.4–26.9	22.5 (10) 19.7–24.3	23.5 (8) 20.9–26.5	19.9 (10) 18.8–21
BO	6.3 (3) 6.1–6.4	5.8 (21) 5.5–6.2	5.8 (23) 5.3–6.4	6.1 (5) 5.9–6.7	6.1 (11) 5.7–6.3	5.9 (11) 5.6–6.3	6.0 (8) 5.7–6.6	5.1 (10) 4.7–5.5
LD	6.4 (3) 5.9–6.7	6.4 (21) 5.8–7.8	6.2 (26) 5.3–7.2	6.5 (5) 6.4–6.6	6.6 (11) 4.7–7.1	6 (11) 5.2–6.8	6.2 (8) 5.1–7.3	5.3 (10) 5.0–5.8
PB	4.7 (3) 4.5–4.8	4.2 (21) 3.7–4.8	4.5 (24) 3.8–5.2	4.7 (5) 4.4–5.1	5.0 (11) 4.3–5.5	4.7 (11) 4.2–5.3	4.9 (8) 4.2–5.9	3.7 (10) 3.5–4.2
LI	5.9 (3) 5.6–6.4	5.6 (21) 4.9–6.5	5.6 (27) 4.8–6.5	5.6 (5) 5.3–6	5.9 (11) 5.3–6.4	4.8 (11) 4.2–5.4	5.3 (8) 4.6–5.9	4.6 (10) 3.8–5.1
BI	1.8 (3) 1.7–2.0	1.7 (21) 1.5–2.2	1.7 (27) 1.5–2.1	1.8 (5) .7–1.9	1.9 (11) 1.6–2.1	1.6 911) 1.4–1.9	1.8 (8) 1.5–2.1	1.5 (10) 1.3–1.7
LM	4.1 (3) 3.9–4.4	4.0 (21) 3.8–4.2	4.1 (27) 3.8–4.4	4.1 (5) 4.0–4.3	4.1 (11) 3.8–4.4	4.2 (11) 3.8–4.5	4.3 (8) 4.1–4.6	3.4 (10) 3.1–3.9
M1	1.3 (3) 1.2–1.5	1.2 (21) 1.1–1.4	1.2 (27) 1.1–1.3	1.3 (5) 1.3–1.4	1.3 (11) 1.2–1.4	1.3 (11) 1.2–1.4	1.3(8) 1.2–1.4	1.0 (10) 0.9–1.1
OL	9.1 (3) 8.8–9.5	9.4 (21) 8.8–10.3	9.1 (27) 7.8–10.4	8.8 (5) 8.6–8.9	9.5 (11) 8.8–10.0	8.1 (11) 7.5–9.5	9.1 (8) 8.4–10.3	8 (10) 7.1–8.3
IB	4.3 (3) 4.1–4.6	4.2 (21) 3.83–4.7	4.2 (27) 3.8–4.0	4.4 (5) 4.3–4.5	4.6 (11) 4.3–5.0	4.2 (11) 3.8–4.5	4.3 (8) 3.8–4.6	3.7 (10) 4.1–3.4
ZB	14 (3) 13.5–14.7	13.3 (21) 12.5–14.0	13.3 (27) 12.3–14.4	13.2 (5) 12.7–14.3	14.2 (11) 12.6–14.7	13.4 (11) 11.8–14.2	13.8 (8) 12.6–15.3	11.5 (10) 10.6–12.2
BB	11.3 (3) 11.2–11.5	10.8 (21) 10.2–11.4	11.1 (27) 10.4–11.8	11.1 (5) 10.6–11.6	11.5 (10) 11.1–12.1	10.7 (10) 9.9–11.1	10.9 (8) 10.5–11.2	9.8 (10) 9.5–10.4
LR	9.4 (3) 8.8–10.3	9.1 (21) 8.2–10.4	-	9.3 (5) 8.8–10.3	9.8 (11) 8.2–10.7	8.8 (9) 8.2–9.6	9.1(7) 8.1–10.4	7.6 (10) 7.0–8.5
BR	4.9 (3) 4.6–5.5	4.8 (21) 4.4–5.4	-	5 (5) 4.6–5.4	5.2 (11) 4.8–5.6	4.6 (11) 4–4.9	4.6(8) 4.3–5.0	4.2 (10) 3.9–4.5

Narrow mesopterygoid fossa with U-shaped anterior notch. Sphenopalatine vacuities well developed. Parapterygoid fossae shallow and very large. Posterior openings of oval foramina large, without an alisphenoid strut. Hamular process delimiting a small postglenoid foramen and a large subsquamosal fenestra. Squamosal alisphenoid groove leading to small sphenofrontal foramen, and large stapedial foramen indicating a primitive carotid circulation pattern (Voss 1988). The lateral wall of the mastoid is perforated by a medium sized fenestra.

Dental characters: Incisor opisthodont, upper series (M1-M3) parallel sided. A shallow anteromedian flexus in M1 divides the anterocone into subequal anterolingual and anterolabial conules. M2 squared and M3 small and rounded.

Karyotype. 2n=38, FNa=66 in the holotype (see results), and 2n=36, FNa=66 in specimens from Lagoa Santa (Geise et al. 1996).

Comparisons. see discussion.

Natural history. All specimens were captured in rural areas along streams, in grassy vegetation, less than 2 meters from the stream margins. The holotype was infested with four individuals of *Taenia* sp., and 113 of *Hepsilon* sp.; MN 71973 (LBCE 5583) was infested with unidentified nematodes, and MN 71971 (LBCE 5556) with 62 individuals of *Hepsilon* sp. and four specimens of an unidentified nematode.

Discussion

Our results, based on combined karyotypic, mitochondrial DNA and morphologic data, are indicative of the high diversity and complexity of the genus *Calomys*. The karyotype herein reported for *Calomys cerqueirai* (2n=38, FNa=66, and 2n=36, FNa=66) differed from all others previously found in *Calomys* species (Table 1). It differed from *Calomys expulsus* (2n=66, FNa=68) in both diploid number and autosomal fundamental number. Although sharing the same autosomal fundamental number, its chromosome complement was very different from *C. tocantinsi* (2n=46, FNa=66), *C. callidus* (2n=48, FNa=66) and *C. tener* (2n=66, FNa=66), as well as from *C. laucha* (2n=64, FNa=68), another species occurring in Brazil. *C. lepidus* (2n=36, FNa=68) showed karyotypic similarity with *Calomys cerqueirai* (2n=38 or 2n=36, FNa=66) but these species differed by more than 14% at cytochrome *b* level (see fig. 4). Although *C. musculinus* shared the same 2n=38, it differed markedly in its autosomal fundamental number, which varied from 48 to 62 (Table 1). The phylogeny of *Calomys* species herein obtained corroborated Pearson and Patton's (1976) hypothesis of the 2n=70 karyotype as the ancestral condition in *Calomys* (Fig. 3).

Despite the striking karyotypic differences between *Calomys cerqueirai* and *C. callosus, C. expulsus, C. tocantinsi* and *C. callidus*, these species showed similar cranial measurements (Table 1), while *C. tener* differed from them in both karyotypic and morphometric attributes. Morphologic data on *C. laucha*, the other species known to occur in Brazil, were not available; however, this is a small species, more similar in size to *C. tener*. Body size and cranial measurements allowed the separation of two groups of Brazilian *Calomys*, a larger-bodied species group, including *C. callosus, C. expulsus, C. tocantinsi, C. callidus* and *C. cerqueirai*, and a smaller-bodied species group, including *C. tener* and *C. laucha*.

As with other species in the genus, the five larger-bodied species have a head-body length longer than the tail and a patch of white hairs behind the ears. They share several morphologic characteristics, such as a delicate skull with a medium-sized, narrow rostrum; a long incisive foramen; a large zygomatic plate relative to cranium size, with deep zygomatic notches; an interorbital region without developed postorbital ridges; an interparietal bone as broad as the parietals; and the width of the parapterygoid plate greater than the width of the mesopterygoid fossa. With respect to dental characters, these species show parallel upper molar rows and an anteromedian flexus in M1. Despite such similarities, *Calomys cerqueirai* is distinguishable from other species of larger-bodied *Calomys* by the following morphologic characteristics: a Cinnamon-Brown overall dorsal coloration, as opposed to Olive-Brown in *C. expulsus* and Dark Olive in *C. callosus, C. callidus* and *C.*

tocantinsi. In *Calomys cerqueirai*, the head is paler than the dorsum due to a reduction in the number of completely black hairs, resulting in a Mars Yellow to Ochraceous Tawny snout region, while in *C. callidus*, *C. callosus*, *C. expulsus* and *C. tocantinsi* the head and dorsum have the same coloration. *Calomys cerqueirai* has small ears (16-18mm) externally covered by short brownish hairs, unlike the other species, in which the external hairs of the ear are black. *Calomys cerqueirai* has a sharply bicolor tail, in which it is similar to *C. callosus* and *C. callidus*, but different from *C. expulsus*, in which the limit between the upper dark and lower light parts of the tail is less well defined.

Because *C. cerqueirai* and *C. expulsus* are potentially sympatric in Lagoa Santa, we compared our specimens of *C. cerqueirai* and the type material of *C. expulsus* from Lund's collection in the Zoological Museum, University of Copenhagen (ZMK) based on photographs available at http://www.zmuc.dk/VerWeb/lund/lund_mammals.html. Skins ZMK 238 and ZMK 239 show the same pelage coloration as other *C. expulsus* examined by us, and differ from the skins of *C. cerqueirai* in the characters cited above.

It has been estimated that *Calomys* was split by two sequential events of evolutionary divergence; the first separating Andean from lowland species, and the second isolating species north of the Amazon from those farther south, which took place in the Pliocene between 3 and 4 million years before the present (MYBP). A speciose larger-bodied clade of lowland species, distributed to the south of the Amazon forest in association with the dry forests and ecotones of the Cerrado and adjacent biomes, diversified during the Pleistocene (Almeida *et al*., 2007). These larger-bodied species included *C. expulsus*, C. *fecundus* (= *C. boliviae*), *C. venustus*, *C. tocantinsi*, *C. callosus*, *Calomys* sp. from Beni (Almeida *et al*. 2007), *C. cerqueirai* and *C. callidus*. At least five *Calomys* species of this group occur in the Cerrado of Brazil, namely *C. expulsus*, *C. callosus*, *C. tocantinsi*, *C. callidus* and *C. cerqueirai*. Sympatry in *Calomys* is common between species of larger body size and smaller body size: *C. expulsus* and *C. tener* are sympatric in Minas Gerais state (Lagoa Santa), several localities in Goiás state and the Federal District (Brasília). However, true sympatry of two larger-bodied species, or of two smaller-bodied species is rare: *C. expulsus* and *C. tener* are sympatric, albeit not syntopic, with *C. cerqueirai* in Lagoa Santa, Minas Gerais state. The sympatry and morphologic similarities among larger-bodied *Calomys* species can lead to species misidentification, as reflected in the complex taxonomic history of this genus. Here we used only karyotyped samples and samples from type localities in order to minimize this problem.

In Brazil, all *Calomys* species are mainly distributed in the Cerrado, although they also occur in the Caatinga, in the western borders of the Atlantic Forest, in the transitional region with the Amazonian forest, and in the Pampas of southern Brazil. *Calomys cerqueirai* inhabits the transitional region between the Cerrado and the Atlantic Forest of Minas Gerais state (in Lagoa Santa and Capitão Andrade). *Calomys expulsus* occurs in the Caatinga (states of Pernambuco, southern Piauí, northern Minas Gerais, central-western Bahia), and Cerrado (states of Tocantins, Goiás, Mato Grosso do Sul, Minas Gerais, northern São Paulo, and the Federal District). *Calomys tener* occurs in the borderlands of the Atlantic Forest (states of São Paulo and Espírito Santo) and in the Cerrado (states of Minas Gerais, Mato Grosso, Mato Grosso do Sul, Goiás, Bahia, and the Federal District). *Calomys tocantinsi* is apparently endemic to the Cerrado of Central Brazil (states of Tocantins and eastern Mato Grosso). *Calomys callosus* occurs in the southwestern Brazilian Cerrado (state of Mato Grosso do Sul); *Calomys callidus* is found in the northwestern Cerrado (western Mato Grosso and southern Rondônia) and in the transition with the Amazonian forest (Rondônia state); and *C. laucha* is known from the Pampas (state of Rio Grande do Sul).

As is the case with many other sigmodontine rodents, the genus *Calomys* contains several morphologically very similar species. The combination of DNA sequencing and karyologic and morphologic analyses has proved to be an indispensable tool for disclosing this underestimated diversity. Due to the important role of these rodents as wild reservoirs of hantaviruses and arboviruses, which are generally host specific, it is very important to reveal and describe this cryptic variability in order to better understand the dynamics of such endemic disease organisms.

Acknowledgements

We are grateful to J.S. Garcia, P. Borodin, S.F.C. Neto, V.C. Vaz, J.W.F. Costa, and to the field team of the Laboratório de Biologia e Parasitologia de Mamíferos Silvestres Reservatórios, IOC/FIOCRUZ, for their collaboration in fieldwork. To P.S. D'Andrea for specimens from Aquidauana, Corumbá, Campo Novo do Parecis and Santa Teresa; to F. Almeida for measurements of specimens. We are also grateful to J. Salazar-Bravo, C. J. Tribe and an anonymous referee for revising previous versions of this manuscript. License to collect the specimens was granted by Brazilian Institute of Environment and Natural Resources (IBAMA). This work was supported by fellowships and grants from CNPq to CRB (302951/2007-5) and JAO (306801/

2007-8), from FAPERJ to CRB and FAPERJ – APQ1 (E-26/170.971/2004) to RG, and from PAPES III – FIOCRUZ to RG (0250.250.214).

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APPENDIX 1. Specimens used in morphometric analyses (numbers in parentheses refer to localities in figure 1; N.L. = not located)

Calomys sp.n.—BRAZIL: Minas Gerais state, (2) Capitão Andrade, MN 71971-73; *C. callidus*—BRAZIL: Mato Grosso state, (15) Campo Novo do Parecis, SVS 333-34, 338, 340, 343, 346, 350, 353, 376-77, 401, 409, 411, 416, 420; *C. callosus*—BOLIVIA: Dept. Santa Cruz, (N.L.) La Hoyada, 30 km S Valle Grande, MVZ 134660-61, 134661; PARAGUAY: Dept. Boquerón, (NL) Rio Verde, 30 km by road NW Villa Hayes (MVZ 145218-20); Dept. Presidente Hayes, (47) 69 km NW Villa Hayes by road, MVZ 145221, 145223; (47) 295 km NW Villa Hayes by road, Chaco Experimental Station, MVZ 145224-44; (47) 213 km NW Villa Hayes by road, MVZ 145222; BRAZIL: Mato Grosso do Sul state, (26) Corumbá, Fazenda Alegria, MN 71977-78, 71980; *C. expulsus*—BRAZIL: Goiás state, (21) Corumbá de Goiás, Morro dos Cabeludos, MN 61577-78, 61581, CRB 500; (19) Teresina de Goiás, Fazenda Vão dos Bois, MN 43027-30, 43032-34, 43036; (19) 60 km SSW Cavalcanti, Fazenda Fiandeira, MN 61583-87; (19) 5 km N Alto Paraíso, MN 61588-89; (21) Mimoso de Goiás, Fazenda Cadoz, MN 71964; Piauí state, (7) Coronel José Dias, Zabelê, MN 63301; (7) João Costa, MN 63270; Bahia state, (20) Jaborandi, Fazenda Sertão do Formoso, MN 61606, 61658; *C. tocantinsi*—BRAZIL: Tocantins state, (11) Formoso do Araguaia, holotype MN 62731-33, 62736-40, 62742-43; *Calomys tener*—BRAZIL: Goiás state, (21) Corumbá de Goiás, Morro dos Cabeludos, MN 61575-76, 61580; (19) Teresina de Goiás, Fazenda Vão dos Bois, MN 43026, 43035; São Paulo state, (24) Pedreira, Fazenda Fortaleza, MN 61590-91; (24) Itirapina, MN 61592; (24) Campinas, MN 61605; (24) Rio Claro, Fazenda São José, MN 61593.