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A new species of *Calomys* (Rodentia, Sigmodontinae) from Central Brazil identified by its karyotype

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Ten species of small rodents of genus *Calomys* are found in South America. Three of these ten species are known to occur in Brazil: *C. tener*, *C. laucha* and *C. expulsus* (= *C. callosus expulsus*). Almost all *Calomys* karyotypes are made up of acrocentric pairs. In this paper we describe a new karyotype with 2n = 46 (FN = 66), including 11 meta/submetacentric and 11 acrocentric autosomal pairs. This is not related to any described *Calomys* karyotype. The X chromosome is a medium submetacentric and the Y is a small acrocentric. This new karyotype is briefly compared to karyotype of the sympatric species *C. tener* (2n = 66, FN = 66). The reduced diploid number and small amount of pericentromeric heterochromatin observed in the biarmed chromosomes that contrasts to large blocks seen in acrocentrics seem to indicate that centric fusion and loss of constitutive heterochromatin have led to the new karyotype. Cytogenetic evidence suggests strongly that a new species with 2n = 46 from Central Brazil should be described in the genus *Calomys*.

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The vesper mice *Calomys* (Phyllotine, Sigmodontinae) are found in South America: Peru, Bolivia, Chile, Argentina, Paraguay, Central and Eastern Brazil, and in isolated pockets of Colombia and Venezuela (EISENBERG and REDFORD 1999; MUSSER and CARLETON 1993).

EISENBERG and REDFORD (1999) list seven *Calomys* species that differ in size when in areas of sympatry: *C. boliviae*, *C. callosus*, *C. laucha*, *C. lepidus*, *C. musculinus*, *C. sorellus* and *C. tener*. On the other hand, MUSSER and CARLETON (1993) assign nine species for the genus. They include two species in addition to the ones listed above: *C. callidus* and *C. hummelincki*. Recently, Bonvicino (pers. com.) revised Brazilian specimens of *Calomys* (*C. callosus expulsus*). She recognized distinct characteristics to assign *expulsus* a legitimate species status. Following Bonvicino, we find that three species should occur in Brazil: *C. tener*, *C. laucha* and *C. expulsus*.

Calomys show, in addition to a wide geographic distribution, karyotypic differences that concern both diploid number (2n = 36 to 2n = 66) and the number of autosome arms (FN = 48 to FN = 82). They represent a complex group in which more than one karyotype have been reported for a single species (Table 1). In Brazil, species with 2n = 66 presented FN = 68 and 70 (*C. expulsus*, referred as *C. callosus*) and FN = 66 (*C. tener*), and the karyotypes displayed almost exclusive acrocentric chromosomes.

Fusion is thought to be the most frequent mechanism in chromosomal evolution of the Phyllotine rodents. From an ancestral karyotype with solely telocentric (acrocentric) chromosomes, multiple tandem and centric fusions are thought to reduce diploid number and FN, resulting in different derived karyotypes such as are observed by the living members in the group (PEARSON and PATTON 1976; WALKER et al. 1979; WALKER and SPOTORNO 1992).

In this paper we describe a new karyotype for *Calomys*, from a sample from Central Brazil, with 2n = 46 and FN = 66. This is believed to belong to a new species for the genus.

MATERIAL AND METHODS

Our sample is composed by 22 specimens: 18 animals of *Calomys* sp. trapped in Parque Nacional do Araguaia, state of Tocantins (eight females, two males and two embryos) and in Vila Rica (two females) and Cocalinho (three females and one male), state of Mato Grosso; and 5 animals of *Calomys tener* from Itapetininga (two males) and Rio Claro (one male and one female), state of São Paulo, and from Gaúcha do Norte (one female), state of Mato

Table 1. *Recognized species of the genus Calomys and their diploid number and number of autosome arms (FN). Classification follows* MUSSER *and* CARLETON *(1993)*

Species	2n	FN[1]	Karyotype			Locality	Reference
			Autosomes	X	Y		
C. boliviae[2]	50	66	15A, 9M	M	A	Bolivia	PEARSON and PATTON 1976
C. callidus	48	?	?			Eastern Paraguay, Central and Eastern Argentina	VITULO et al. 1984; CORTI et al. 1987
C. callosus	36	48	10A, 7M	M	A	Peru, Paraguay	REIG 1986; PEARSON and PATTON 1976
C. expulsus[3]	66	68	30A, 2M	A	A	Central Brazil	SVARTMAN and ALMEIDA 1992
	66	70[4]	29A, 3M	A	A	Northeastern Brazil	SOUZA 1981
C. hummelincki	–	–	–	–	–	Northern Venezuela, Northeastern Colombia, Isla Curaçao and Aruba	MUSSER and CARLETON 1993
C. laucha	62	72	24A, 6M	A	D	Argentina	MASSOIA et al. 1968; PEARSON and PATTON 1976; REIG 1986
	64	76	24A, 7M	A	D	Argentina	PANZETTA 1969
	64	70	27A, 4M	M	A	Argentina	GARDENAL et al. 1977
	64	68	28A, 3M	S	A	Argentina and Uruguay	BRUM-ZORRILLA et al. 1990
	64[5]	?	?	?	?	Southernmost Brazil	MATTEVI (pers. com.) in KASAHARA and YONENAGA-YASSUDA 1984
C. lepidus	36	68	17M	A	A	Peru, West Bolivia, Northeastern Chile and Northwestern Argentina	REIG 1986; PEARSON and PATTON 1976
C. musculinus	38	48	8A, 10M	S	A	Argentina	FORCONE et al. 1980; LISANTI et al. 1996; MASSOIA et al. 1968
C. sorellus	62, 64[6]	68	28A, 3M	A	A	Peru	PEARSON and PATTON 1976
C. tener[7]	66	66	31A, 1M	A	A	Southeastern and Central Brazil	YONENAGA 1975; SVARTMAN and ALMEIDA 1992
Calomys sp.	46	66	11A, 11M	S	A	Central Brazil	Present report

A = acro (or subtelocentric); M = metacentric; S = submetacentric, D = dot-like chromosome.
[1] Number of autosome arms.
[2] Synonym of *C. fecundus*.
[3] Formerly named as *C. callosus expulsus*, but elevated to species status after revision of Bonvicino (pers. com.).
[4] Formerly refered as *C. callosus*. Karyotype identical to that described by SVARTMAN and ALMEIDA (1992), but FN is different due to erroneous classification of the morphology of pair 2.
[5] Formerly named *C. callosus* by Mattevi but we are inclined to suggest that it belongs to *C. laucha* based on its 2n value. The karyotype was not detailed.
[6] Diploid number variation due to a single autosomal fusion.
[7] Formerly considered as a subspecies of *C. laucha*, but regarded as a full species in MUSSER and CARLETON (1993). *Calomys* sp. (YONENAGA 1975) was considered as *C. tener* by REIG (1986) and by SVARTMAN and ALMEIDA (1992).

Grosso (Fig. 1, Appendix 1). The vegetation of Parque Nacional do Araguaia, in Tocantins, and Cocalinho, in Mato Grosso (Central Brazil), is characterized by "cerrado", a dry, deciduous and tropical forest. Vila Rica and Gaúcha do Norte, in Mato Grosso, are transitional areas between Amazonian rain forest and "cerrado" in Central Brazil. Itapetininga and Rio Claro, in São Paulo, are regions of "cerrado" in Southeastern Brazil. Voucher specimens from Mato Grosso and São Paulo were deposited in Museu de Zoologia da Universidade de São Paulo (MZUSP) and specimens from Tocantins were deposited in Museu Nacional do Rio de Janeiro (MNRJ).

Metaphase preparations were obtained from bone marrow, after in vivo injection of colchicine, and from fibroblasts, derived from tail or ear biopsies, which were cultured in Dulbecco's Modified Eagle Medium supplemented with 20 % fetal bovine serum, according to conventional procedures. Cells were spread onto clean slides, air dried, and stored at − 20°C until use. GTG- and CBG-banding and Ag-NOR (nucleolar organizer regions) staining followed standard procedures.

Fig. 1. Sampling localities of *Calomys* sp. (1,3,4) and *C. tener* (2,5,6).

RESULTS

The new karyotype for the genus *Calomys* with 2n = 46 and FN = 66, includes 11 pairs of meta/submetacentrics (pairs 1 to 11) and 11 acrocentric pairs (pairs 12 to 22) with gradual decrease in size. The X is a well-distinguished medium submetacentric chromosome and the Y is a small acrocentric, indistinguishable from the two pairs 21 and 22 of the same size (Fig. 2a). Constitutive heterochromatin revealed by C-banding, is confined to large blocks in the centromeric region of acrocentric autosomes, except for pairs 14, 19, 21 and 22. On the other hand, C-bands are almost absent in the biarmed chromosomes. The Y is an easily characterized entirely heterochromatic acrocentric chromosome. No interstitial C-bands were observed in any of the chromosomes (Fig. 2b). All chromosome pairs can be identified after G-banding pattern (Fig. 3).

The karyotype of *C. tener* presented 2n = 66 and FN = 66, with almost exclusive acrocentric chromosomes (pairs 1 to 31) with gradual variation in size, and a small metacentric pair (pair 32). The X is an easily distinguished medium submetacentric chromosome and the Y is a small acrocentric, indistinguishable from the others (Fig. 4a). C-banding pattern shows conspicuous pericentromeric blocks of heterochromatin in all acrocentrics (mainly pairs 2, 4, 9 and 11), in the X and the small metacentric chromosomes. The Y is easy to distinguish and entirely heterochromatic, even though it is less intensely stained than the autosome pericentric regions. The pair 1 presented an interstitial band slightly stained above the pericentromeric block (Fig. 4b). G-banding allows the recognition of all homologous chromosomes (Fig. 5).

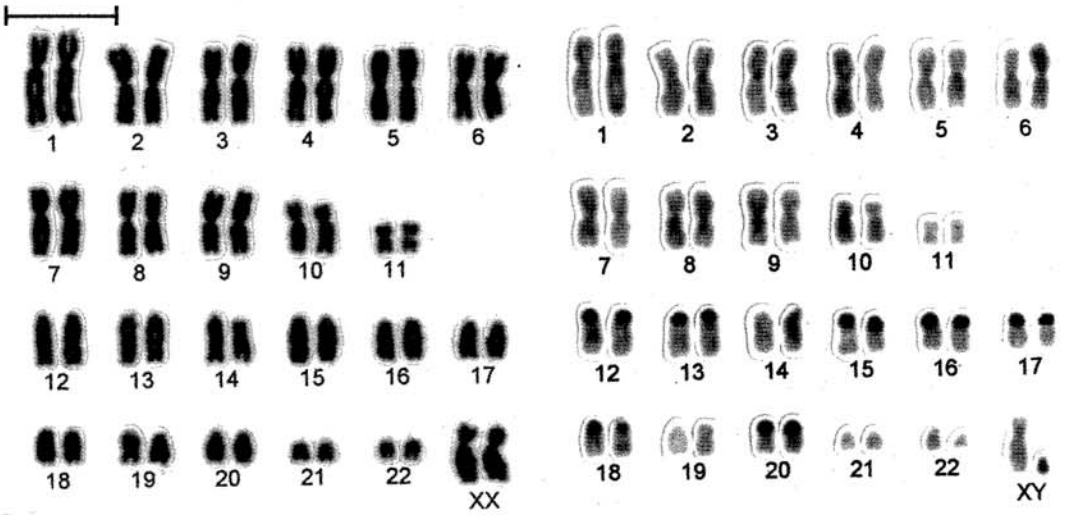

Fig. 2. Karyotypes of *Calomys* sp. (2n = 46, FN = 66) after conventional staining (female) and CBG-banding (male). Bar = 10 μm.

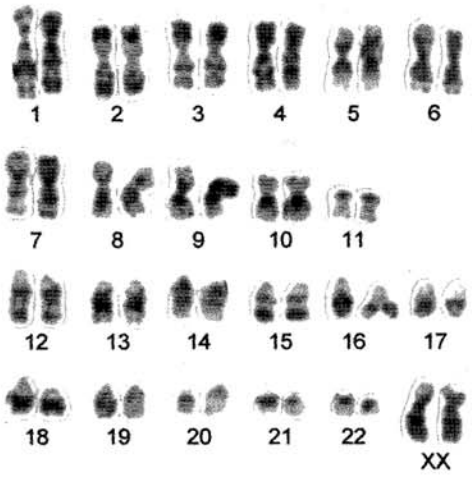

Fig. 3. GTG-banded karyotype of a female of *Calomys* sp. (2n = 46, FN = 66).

Multiple telomeric NORs were detected in both karyotypes, with inter and intra-individual variation: from 5 to 12 in 40 metaphases of *Calomys* sp. and from 2 to 18 in 80 metaphases *C. tener*. Ag-NORs were almost exclusively observed in the short arm of acrocentrics. In *C. tener*, NORs were detected in the telomere of long arm and in both telomeres of some acrocentrics in low frequency (Fig. 6a–f).

DISCUSSION

The cytogenetic comparative analysis of the sympatric *Calomys* sp. (2n = 46) and *C. tener* (2n = 66), both with FN = 66, lead us to suggest that at least 11 centric fusions and a concomitant loss of heterochromatin are the most probable rearrangements in the evolution of the 2n = 46 karyotype. This assumption is supported by the observation of pericentromeric heterochromatin in the majority of acrocentric chromosomes from 2n = 66 *C. tener* karyotype that contrasts to the almost complete absence of heterochromatin in biarmed chromosomes of 2n = 46 karyotype. *C. tener* has been reported to have conspicuous pericentromeric heterochromatin in all acrocentric autosomes (SVARTMAN and ALMEIDA 1992). Since Robertsonian fusions has been reported to be common in the karyotype evolution in rodents (GARDNER and PATTON 1976), we believe that most of the heterochromatin in the ancestor karyotype is lost during the events of fusion of chromosomes that led to the reduced karyotype of *Calomys* sp.

PEARSON and PATTON (1976) summarized the relationship among the phyllotines based on karyotypic analysis and suggested a hypothetical ancestral karyotype for the group with 2n = 70 (FN = 68), with successive events of fusions and inversions of chromosomes generating the reduced diploid number and the variation of number of autosome arms (FN). Accordingly, *Calomys* sp. (2n = 46) could be considered to represent the most derived karyotype of the genus in Brazil.

Multiple NORs are common in *Calomys*: they varied from 6 to 16 in *C. callosus expulsus*; from 9 to 13 in *C. tener* (SVARTMAN and ALMEIDA 1992); and from 5 to 9 in *C. callosus* (SOUZA 1981). Ours ranged from 2 to 18 in *C. tener* and 5 to 12 in *Calomys* sp. This suggests that probably Ag-NORs bearing chromosomes would not be involved in the events of centric fusion of chromosomes that have led into the establishment of the 2n = 46 karyotype.

The taxonomy of *Calomys* is confused and chromosomal data have been helpful in the characterization and discrimination of taxa. The most recent revisions of South American mammals (NOWAK 1991; MUSSER and CARLETON 1993; EISENBERG and REDFORD 1999) list nine species. Nevertheless, our chromosomal revision (Table 1) would suggest that there are 11 *Calomys* species.

Some *Calomys* species are karyologically invariable. *C. boliviae* (2n = 50), *C. lepidus* (2n = 36), *C. musculinus* (2n = 38), *C. callosus* (2n = 36) and *C. callidus* (2n = 48) are well characterized by morphological and chromosomal data. *C. sorellus* from Peru presented 2n = 64, but a derived karyotype was found with 2n = 62. This is due to a single autosomal fusion (PEARSON and PATTON 1976). *C. hummenlincki* has unknown karyotype but is considered to be a separate species (REIG 1986, MUSSER and CARLETON 1993).

An unnamed species of *Calomys* described by Yonenaga (2n = 66, FN = 66) was provisionally assigned as *C. tener* by REIG (1986). Later, SVARTMAN and ALMEIDA (1992) affirmed that the karyotype from the sample from Southeastern Brazil (YONENAGA 1975) is identical to *C. tener* karyotype (2n = 66, FN = 66) from Central Brazil.

Conversely, *C. laucha* presented two diploid numbers (2n = 62 and 64) and four different fundamental numbers (FN = 68, 72, 74 and 82). This species deserves a revision. Table 1 shows that different karyotypes were assigned to *C. callosus*: 2n = 36, 2n = 64 and 2n = 66. The 2n = 36 form was restricted to Peru and Paraguay and seems to belong to *C. callosus* (REIG 1986; PEARSON and PATTON 1976). The 2n = 64 karyotype assigned to *C. callosus* from southernmost Brazil by Mattevi (personal communication in KASAHARA and YONENAGA-YASSUDA 1984) has not been described in detail but we suggest that these

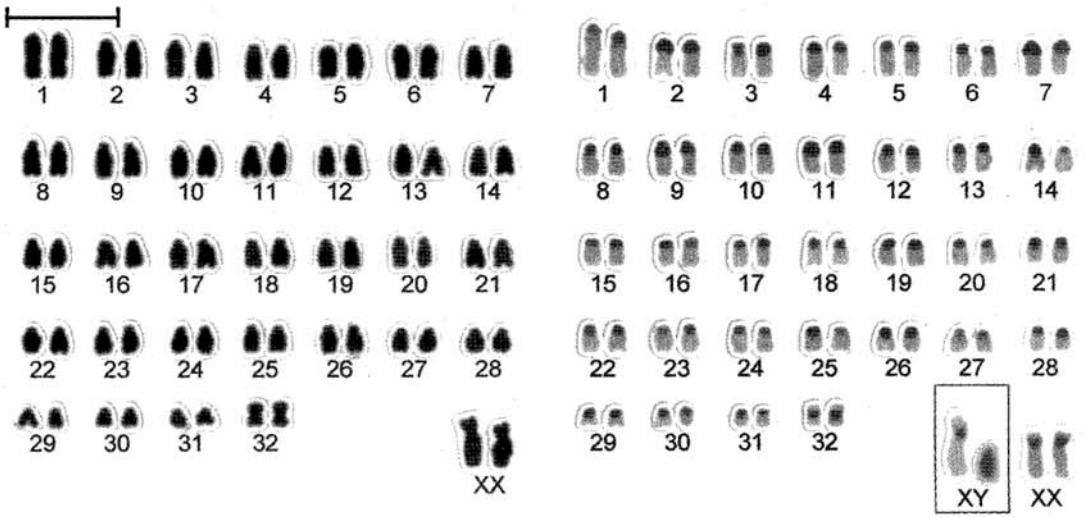

Fig. 4. Karyotypes of *Calomys tener* (2n = 66, FN = 66) after conventional staining (female) and CBG-banding technique (female). Sexual pair of male is in the square. Bar = 10 μm.

animals could be related to *C. laucha* (2n = 64) like the ones from Argentina and Uruguay (BRUM-ZORRILLA et al. 1990). As for the 2n = 66 form, SVARTMAN and ALMEIDA (1992) assigned the 2n = 66, FN = 68 karyotype to *C. callosus expulsus*. Firstly, *expulsus* had been considered to be a synonym of *callosus* (MUSSER and CARLETON 1993) but has been recently distinguished as a species by Bonvicino (pers. com.). On the basis of the discrepancies of diploid numbers we believe that these three forms deserve species status.

Karyotype has been most helpful in the assessment of *Calomys* species. In some cases it contributes to fully identify a species. On the basis of the synopsis of chromosomal data of genus *Calomys* and the geographical distribution of *Calomys* sp. (2n = 46) we argue that a new species should be recognized.

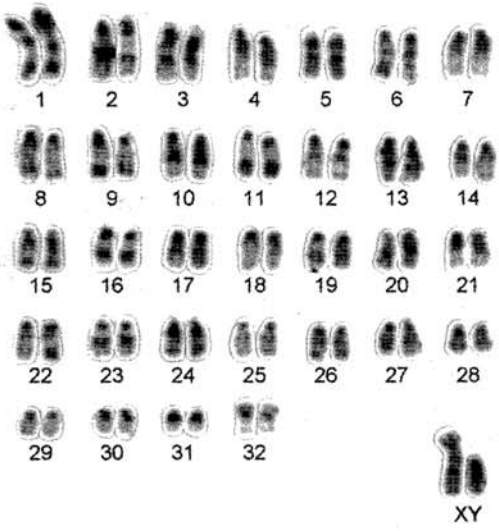

Fig. 5. GTG-banded karyotype of a male of *Calomys tener* (2n = 66, FN = 66).

Fig. 6a–f. Ag-NORs of three specimens of *Calomys* sp. **(a–c)** and three specimens of *C. tener* **(d–f). a** 14 Ag-NORs with 2 associations. **b** 9 Ag-NORs. **c** 9 Ag-NORs with 2 associations. **d** 10 Ag-NORs. **e** 7 Ag-NORs with 1 association. **f** 7 Ag-NORs. Note one Ag-NOR at telomere of long arm of small acrocentric observed in low frequency.

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APPENDIX 1

Mato Grosso state: 2n = 66: Gaúcha do Norte (13°11′S, 53°15′W): CIT 596. 2n = 46: Vila Rica (09°54′S, 51°12′W): CIT 745; Cocalinho (14°23′S, 50°59′W): CIT 781–784.

Tocantins state: 2n = 46: Parque Nacional do Araguaia (11°47′S, 49°31′W): CIT 1144, CIT 1149–1151, CIT 1197, CIT 1206, CIT 1210, CIT 1213, CIT 1217, CIT 1220, CIT 1222.

São Paulo state: 2n = 66: Itapetininga (23°35′S, 48°03′W): CIT 1322, CIT 1332; Rio Claro (22°24′S, 47°33′W): BIO 286–287.

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