



Mastozoología Neotropical

ISSN: 0327-9383

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Sociedad Argentina para el Estudio de los
Mamíferos
Argentina

Garcia, João P.; Oliveira, João A.; Corrêa, Margaret M. O.; Pessôa, Leila M.
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Mastozoología Neotropical, vol. 17, núm. 1, enero-junio, 2010, pp. 53-60

Sociedad Argentina para el Estudio de los Mamíferos
Tucumán, Argentina

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MORPHOMETRICS AND CYTOGENETICS OF *Gracilinanus agilis* AND *Cryptonanus* spp. (DIDELPHIMORPHIA: DIDELPHIDAE) FROM CENTRAL AND NORTHEASTERN BRAZIL

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ABSTRACT: Species of the didelphid genera *Gracilinanus* and *Cryptonanus* are morphologically and cytogenetically very similar. Several qualitative characters, some of which exhibit intraspecific polymorphisms, have been used to distinguish these genera, but more data are needed to characterize them better. Samples of *G. agilis* and *Cryptonanus* spp. from nine localities in central and northeastern Brazil were analyzed. Multivariate analyses of craniodontal measurements and descriptive statistics of external body measurements indicate that *G. agilis* is conspicuously larger than *Cryptonanus* spp., and that general size is the main factor distinguishing these forms. Size differences can be combined with qualitative characters making the differentiation between *G. agilis* and *Cryptonanus* spp. easier. Cytogenetic analyses, including the first description of C-bands and Ag-NORs of *G. agilis*, revealed that the karyotypes of *G. agilis* and *Cryptonanus* sp. from Barão de Melgaço, Mato Grosso, are very similar, except for the fourth autosomal pair and the X chromosome.

RESUMEN: Morfometría y citogenética de *Gracilinanus agilis* y *Cryptonanus* spp. (Didelphimorphia: Didelphidae) del centro y nordeste del Brasil. Los didélfidos *Gracilinanus* y *Cryptonanus* poseen una morfología y citogenética muy semejantes. Estos géneros han sido diferenciados por caracteres polimórficos cualitativos, pero más datos son necesarios para caracterizarlos mejor. Fueron analizadas muestras de *G. agilis* y *Cryptonanus* spp. de nueve localidades de centro y nordeste de Brasil. Los análisis multivariados de las medidas craneodontarias y las estadísticas descriptivas de las medidas corporales externas indican que *G. agilis* es claramente mayor que *Cryptonanus* spp. y que el tamaño general es el principal factor para distinguir esas formas. La variación en tamaño puede ser asociada a los caracteres cualitativos para facilitar la diferenciación entre *G. agilis* y *Cryptonanus* spp. Los análisis citogenéticos, incluyendo la primera descripción del bandeado C y las Ag-NORs de *G. agilis*, revelaron que los cariotipos de *G. agilis* y *Cryptonanus* sp. de Barão de Melgaço, Mato Grosso, son muy semejantes, excepto por el cuarto par de autosomas y el cromosoma X.

Key words. Differentiation. General size. Karyotypes. Mouse opossums.

Palabras clave. Cariotipos. Diferenciación. Marmosinos. Tamaño general.

INTRODUCTION

The genus *Gracilinanus* Gardner and Creighton, 1989, as recently restricted by Voss et al. (2005) comprises six species: *G. aceramarcae* (Tate, 1931), *G. agilis* (Burmeister, 1854), *G. dryas* (Thomas, 1898), *G. emiliae* (Thomas, 1909), *G. marica* (Thomas, 1898) and *G. microtarsus* (Wagner, 1842). These are long-tailed, small-sized (head and body, 85-130 mm; tail, 90-150mm; weight < 50g), pouchless opossums, with a dark circumocular mask (Gardner and Creighton, 1989; Voss et al., 2005). The dorsal pelage ranges from bright reddish brown to dull brownish gray and the ventral pelage ranges from white to pale orange with gray-based hairs present to a greater or lesser extent. The tail ranges from moderately long to very long and can be unicolored fuscous or weakly bicolored through its length. The presence of maxillary fenestrae and of a secondary foramen ovale formed by the anteromedial bullar process are considered two of the most important diagnostic cranial characters for the genus (Costa et al., 2003).

Before the genus *Cryptonanus* was described by Voss et al. (2005), the forms now recognized as *C. agricolai* (Moojen, 1943), *C. chacoensis* (Tate, 1931), *C. guahybae* (Tate, 1931), *C. ignitus* (Díaz, Flores and Barquez, 2002), and *C. unduaviensis* (Tate, 1931) were included within *Gracilinanus*. Although species of *Gracilinanus* and *Cryptonanus* are considered very similar, Voss et al. (2005) described some discrete morphological characters that could be used to distinguish these genera. In spite of some polymorphisms, most species of *Cryptonanus* lack a rostral process in the premaxillae, a secondary foramen ovale, and maxillary fenestrae; the second upper premolar is shorter than the third one; and the upper canine has accessory cusps. Besides these characters, these authors also suggested that *Cryptonanus* specimens have shorter rostrums and smaller orbits, but they remarked that these proportions are ontogenetically variable and that available samples were too small

for statistically compelling morphometric analyses.

Cytogenetic data on *Gracilinanus* and *Cryptonanus* are scarce. The karyotypes of *G. microtarsus* and *C. chacoensis* from Argentina (the latter identified as *Marmosa agilis chacoensis*) were described by Wainberg et al. (1979). These authors recorded the diploid number (2n) and described the chromosomal morphology for both species. Carvalho et al. (2002) described the 2n and the number of autosomal arms (FN) of *G. agilis* and *C. agricolai* (the latter identified as *G. emiliae*), and the distribution of both the constitutive heterochromatin (C-bands) and the silver stained nucleolar organizer regions (Ag-NORs) in *G. microtarsus* and *C. agricolai*. All of these species showed 2n = 14 and FN = 24. The karyotypes of the species listed above, except *C. chacoensis*, were described on the basis of Brazilian specimens.

Three species of *Gracilinanus* are known to occur in Brazil: *G. emiliae*, known from two localities in Pará state (Voss et al., 2001); *G. microtarsus*, known from mesic habitats of the Atlantic Forest; and *G. agilis*, known from dry forests and gallery forests of central Brazil (Costa et al., 2003). Three species of *Cryptonanus* have also been recorded for Brazil: *C. chacoensis*, known from northern Pantanal (Rossi et al., 2006); *C. agricolai*, known from the Caatinga and Cerrado of east-central Brazil; and *C. guahybae*, known from a few localities in the state of Rio Grande do Sul (Voss et al., 2005). Two areas of sympatry are known between species of *Gracilinanus* and *Cryptonanus* in Brazil. *Gracilinanus microtarsus* is sympatric with *C. guahybae* in Rio Grande do Sul and *G. agilis* is sympatric with *C. agricolai* and *C. chacoensis* in central and northeastern Brazil.

Herein we evaluate the morphometric and cytogenetic variation in *Gracilinanus agilis*, the smallest species of this genus (Costa et al., 2003), which is known to occur sympatrically with species of *Cryptonanus*. Considering that most of the qualitative morphological characters previously used to differentiate these gen-

era are polymorphic, we intend to evaluate if morphometric data can be used to distinguish *G. agilis* from *Cryptonanus* spp. We complement the characterization of these taxa with cytogenetic data, including the first description of C-bands and Ag-NORs of *G. agilis* and the karyotype with Ag-NORs of *Cryptonanus* sp. from Barão de Melgaço, Mato Grosso.

MATERIAL AND METHODS

We analyzed 59 specimens of *G. agilis* and 8 specimens of *Cryptonanus* spp. from 9 localities in central and northeastern Brazil (Fig. 1). Based on provisional diagnoses given in Voss et al. (2005), specimens of *Cryptonanus* present in our restricted samples could be assigned to the nominal forms *agricolai* (specimens from Goiás, Bahia and Ceará) and *chacoensis* (specimens from Mato Grosso). However, due to the generic level of the comparisons made here, we treated these forms as *Cryptonanus* spp., to avoid misidentifications caused by the relatively unclear status of these forms diagnoses (Voss et al., 2005). Thirteen specimens of *G. agilis* and two of *Cryptonanus* sp. from Barão de Melgaço, Mato Grosso, were karyotyped. More data on localities and specimens are presented in Appendix.

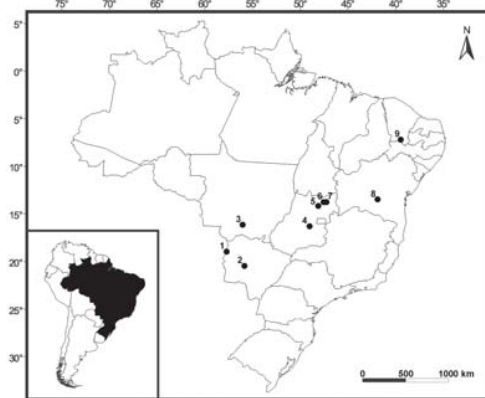


Fig. 1. Collection sites of specimens of *Gracilinanus agilis* and *Cryptonanus* spp. in central and northeastern Brazil. State of Mato Grosso do Sul: Corumbá (1) and Aquidauana (2); State of Mato Grosso: Barão de Melgaço (3); State of Goiás: Anápolis (4), Serra da Mesa (5), Teresina de Goiás (6), and Cavalcante (7); State of Bahia: Chapada Diamantina (8); State of Ceará: Crato (9).

We recorded 20 cranial and dental measurements with digital calipers accurate to 0.01 mm. Definitions of the following characters can be found in Hershkovitz (1992), Costa et al. (2003), and Voss et al. (2005): greatest skull length (GSL), condylobasal length (CBL), basal skull length (BSL), rostral length (ROL), nasal length (NAL), braincase breadth (BCB), zygomatic breadth (ZYB), postorbital breadth (POB), interorbital breadth (IOB), rostral breadth (ROB), cranial depth (CRD), length between first incisor and last molar (IM4), length from canine to last molar (CM4), length from first to last molar (MM4), length from first to third molar (MM3), palatal length (PAL), palatal breadth (PAB), least pterygoid breadth (LPB), petrosal bulla breadth (PBB), and alisphenoid bulla breadth (ABB). In addition to these measurements, we also recorded mandibular length (MDL, measured from the anterior extremity of the dentarium to the condyloid process). We recorded the following external measurements from museum skin tags: head and body length (HB), length of tail (LT), length of hind foot (including the claw, HF), and length of ear (Ear).

All measurements, craniodental and external, were taken from adult specimens (age classes 6 and 7 of Tribe, 1990). Significant sexual dimorphism was detected within samples of *G. agilis*, but unfortunately we could not test this dimorphism in *Cryptonanus* spp. because our restricted sample contains only one female. Males and females of both taxa were grouped in all analyses because we presumed that intergeneric differences should be greater than intraspecific differences.

Descriptive statistics for external measurements were calculated. All craniodental measurements were log-transformed for multivariate statistical analyses. Missing values (2.4%) were estimated by the expectation-maximization method (Strauss et al., 2003). A principal component analysis was employed in the variance-covariance matrix to search of the main patterns of craniometric variation in the total dataset including available samples of adult specimens of *G. agilis* and *Cryptonanus* spp. Character correlations with principal components were portrayed as vector plots. Multivariate statistical analyses were performed on MatLab 4.3 (The MathWorks) using routines written by R. Strauss (available at <http://www.biol.ttu.edu/Strauss/Matlab/matlab.htm>).

We employed the technique of Ford and Hamerton (1956) for mitotic preparations. C-banding and Ag-NOR sites were detected employing the techniques of Sumner (1972) and Howell and

Black (1980), respectively. The chromosomal nomenclature follows Patton (1967).

RESULTS

Principal Component Analysis revealed that a single axis (PC1) accounts for most of the variance (approximately 82%) of craniodental measurement data. Two major clusters were detected along the first axis: one cluster comprises *G. agilis* samples and the other comprises samples referred to *Cryptonanus* spp. (Fig. 2). Most characters are positively associated with the PC1, indicating that it can be interpreted as a general size vector (Fig. 3). The second axis (PC2) accounts for approximately 6% of the variance, while the other 12% are distributed over the third to the fifth axes. Along the second axis, the local samples that compose the cluster of *G. agilis* are notably overlapped, whereas the local samples of *Cryptonanus* cluster show no superposition. The sample from Crato, composed by the type and paratype of *C. agricolai*, does not overlap with the remaining samples referred to *Cryptonanus*, which are distributed along PC2 (Fig. 2). Least pterygoid breadth and postorbital breadth are the characters that best separate the subgroups of *Cryptonanus* (Fig. 3).

Analysis of external measurements reveals overlap between the observed range of variation in *G. agilis* and *Cryptonanus* spp. for HB, LT, Ear, and HF. Nevertheless, for all external measurements mean values of *G. agilis* are higher than those of *Cryptonanus* spp. (Table 1).

Cytogenetic analyses of specimens of *G. agilis* from Barão de Melgaço, Mato Grosso, showed $2n = 14$ and $FN = 24$, with six pairs of biarmed autosomes. Pairs 1, 2 and 3 are submetacentric; pair 4 is metacentric; and pairs 5 and 6 are subtelocentric. Sex chromosomes are the smallest pair in the karyotype. The X chromosome is submetacentric and the Y is acrocentric (Fig. 4A). Analyses of C-banding patterns showed small blocks of constitutive heterochromatin located at the pericentromeric regions of all autosomes and the X chromosome. The Y chromosome is entirely hetero-

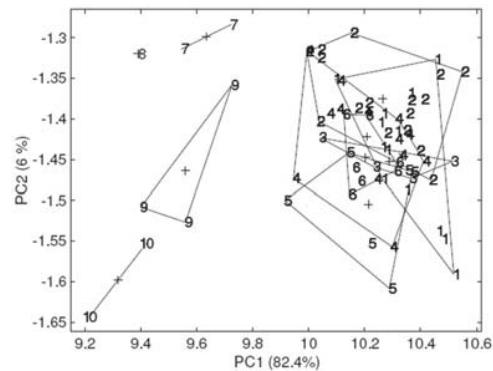


Fig. 2. First and second components of principal component analysis of 21 craniodental measurements of *Gracilinanus agilis* (1-6) and *Cryptonanus* spp. (7-10) from central and northeastern Brazil. Localities are Barão de Melgaço (1,7), Cavalcante (2), Teresina de Goiás (3), Aquidauana + Corumbá (4), Chapada Diamantina (5, 8), Serra da Mesa (6), Serra da Mesa + Anápolis (9), and Crato (10, type series of *C. agricolai*).

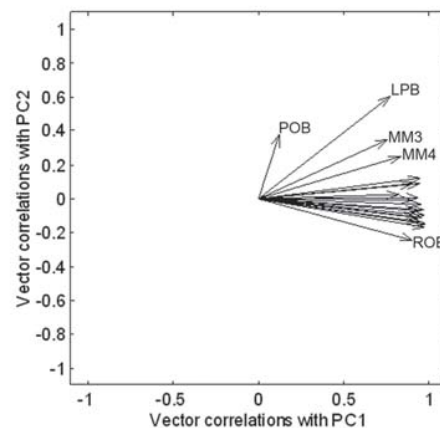


Fig. 3. Vector plot of loadings of first and second components of the principal component analysis of 21 craniodental measurements of *Gracilinanus agilis* and *Cryptonanus* spp. from several localities from central and northeastern Brazil. POB – postorbital breadth; LPB – least pterygoid breadth; MM3 – length from first to third molar; MM4 – length from first to fourth molar; ROB – rostral breadth. Some measurement acronyms are omitted.

Table 1

Descriptive statistics of external measurements of *Gracilinanus agilis* and *Cryptonanus* spp. from central and northeastern Brazil.

| | <i>G. agilis</i> (n=38) | | | <i>Cryptonanus</i> spp. (n=6) | | |
|-----|-------------------------|-----|-----|-------------------------------|-----|-----|
| | \bar{X} | Min | Max | \bar{X} | Min | Max |
| HB | 106 | 79 | 129 | 83 | 75 | 88 |
| LT | 146 | 121 | 164 | 112 | 100 | 125 |
| HF | 18 ^a | 12 | 23 | 15 | 11 | 20 |
| Ear | 21 ^b | 17 | 24 | 16 ^c | 15 | 17 |

^a n = 37; ^b n = 35; ^c n = 3.

chromatic (**Fig. 4B**). The analysis of Ag-NORs revealed that these regions are only present on the short arm of autosome pair 6 (**Fig. 4C**).

Cryptonanus sp. from Barão de Melgaço, Mato Grosso, showed 2n = 14 and NF = 24, with six pairs of biarmed autosomes. Pairs 1, 2, 3, and 4 are submetacentric; pairs 5 and 6 are subtelocentric. Sex chromosomes are the smallest pair in the karyotype. The X chromosome is subtelocentric (**Fig. 5A**). The analysis of Ag-NORs revealed that these regions are only present on the short arm of autosome pair 6 (**Fig. 5B**).

DISCUSSION

Multivariate analyses of craniodental traits revealed that adult samples of *G. agilis* and *Cryptonanus* spp. are clearly distinguishable based on general size. The interspecific differences are so conspicuous that intraspecific differences such as sexual dimorphism, which is known for *G. agilis* (see Costa et al., 2003), do not prevent species cluster formation by the analyses.

Our results indicate that the differences between *Cryptonanus* spp. and *G. agilis* can be attributed to general cranial size and size-correlated (allometric) shape variation. Although there are conspicuous size differences between *G. agilis* and *Cryptonanus* spp., the identification of the latter becomes easier if qualitative characters pointed out by Voss et al. (2005) are combined with craniodental and body measurements.

In spite of our restricted sample, it is possible to observe a difference between external measurement means of adults of *G. agilis* and *Cryptonanus* spp. However, juvenile, subadults, and even unusual small adults of *G. agilis* can be hard to distinguish from specimens of *Cryptonanus* spp. if only external measurements are considered. External quali-

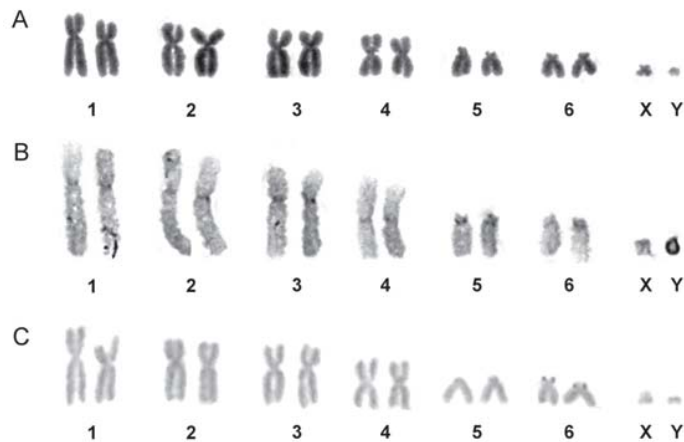


Fig. 4. Karyotype of *Gracilinanus agilis* from Barão de Melgaço, Mato Grosso, Brazil. A) Giemsa staining (MN64376); B) C-banding (MN64403); C) Ag-NORs (MN64403).

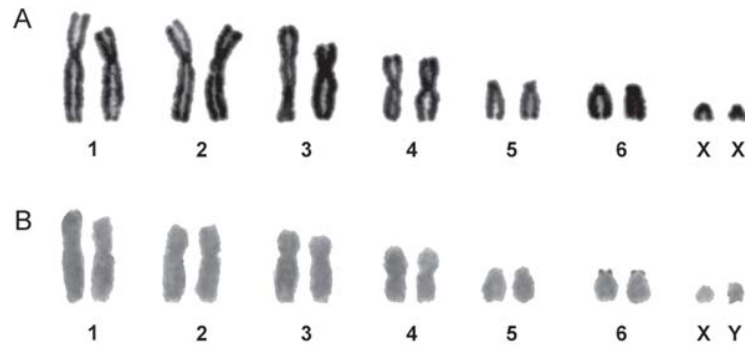


Fig. 5. Karyotype of *Cryptonanus* sp. from Barão de Melgaço, Mato Grosso, Brazil. A) Giemsa staining (MN64347); B) Ag-NORs (MN64260).

tative traits as well as craniodental quantitative and qualitative traits must be used to guarantee the correct diagnoses of these taxa. Therefore, field identifications may not be attempted on the exclusive basis of external measurements.

Although the aim of this paper was to evaluate the distinctiveness of *G. agilis* and *Cryptonanus* spp. based on morphometrics and cytogenetics, we believe that one qualitative character must be emphasized: one specimen of *Cryptonanus* from Anápolis, State of Goiás, presented gray-based ventral fur. According to distributional patterns presented by Voss et al. (2005) this specimen should be assigned to *C. agricolai*. In the provisional diagnosis of this species, all but one specimens presented self-whitish ventral fur (Voss et al., 2005); the specimen from Anápolis constitutes the second exception to this pattern. This corroborates the lack of knowledge about variation in *Cryptonanus* (see Voss et al., 2005), which is a consequence of the reduced number of specimens in museum collections.

Regarding cytogenetic traits, *G. agilis* and *Cryptonanus* sp. from Barão de Melgaço, Mato Grosso, have very similar karyotypes, which is not unexpected because other didelphid species that have the same diploid number usually also have the same fundamental number and distribution pattern of Ag-NORs and C-bands (Souza et al., 1990; Carvalho et al., 2002; Svartman and Vianna-Morgante, 2003).

The two karyotypic differences between *G. agilis* and *Cryptonanus* sp. from Barão de Melgaço are in the fourth autosome pair, which is metacentric in the former and submetacentric in the latter, and in the X chromosome, which is submetacentric in the former and subtelocentric in the latter. The other autosomes and the position of Ag-NORs are identical in both species. We were not able to compare the Y chromosomes of both species.

As this is the first description of the Ag-NORs and C-bands in *G. agilis* and of the karyotype of *Cryptonanus* sp. from Mato Grosso, we present a brief comparison of our data with literature records for other congeneric species. The pattern of Ag-NORs and C-bands distribution reported by us for *G. agilis* is the same as that described for *G. microtarsus* from the states of Santa Catarina and Rio Grande do Sul, Brazil (Carvalho et al., 2002). The pattern of Ag-NORs distribution we reported for *Cryptonanus* sp. from Barão de Melgaço is also identical to that described for specimens attributed by Voss et al. (2005) to *C. agricolai* from the State of Goiás, Brazil (Carvalho et al., 2002). These findings are in accord with the high degree of karyotypical conservatism previously attributed to didelphids (Reig et al., 1977; Souza et al., 1990; Carvalho et al., 2002; Svartman and Vianna-Morgante, 2003).

A major concern in taxonomy of mouse opossums is the lack of knowledge on their

intraspecific variation, which is a consequence of the reduced number of specimens in museum collections. Increasing these samples is a key point to improve the understanding about the differences between *G. agilis* and *Cryptonanus* spp. Furthermore, this would enable us to shed more light on the provisional diagnoses of species of *Cryptonanus* given by Voss et al. (2005).

ACKNOWLEDGEMENTS

To Stella Maris Franco for technical support in Museu Nacional mammal collection and Paulo Sérgio D'Andrea for the permission to study the specimens under his care. To A.L. Gomes for helping with the map. To Guillermo D'Elía and an anonymous referee for the valuable suggestions to improve this manuscript. J.P. Garcia receives financial support from FAPERJ scholarship and L.M. Pessôa and J.A. Oliveira receive research grants from CNPq.

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APPENDIX

All voucher specimens analyzed in this study are deposited in the Museu Nacional (MN), Rio de Janeiro, Brazil. The cell suspension samples (*) used in the chromosomic analysis are deposited in the Laboratório de Mastozoologia, Departamento de Zoologia – IB/UFRJ.

Cryptonanus spp. BAHIA: Chapada Diamantina (13°32'S, 41°50'W) – MN67674. CEARÁ: Crato (7°14'S, 39°24'W) – MN1494, MN1495 (paratype and holotype of *C. agricolai*, respectively). GOIÁS: Anápolis (16°19'S, 48°57'W) – MN50165; Serra da Mesa (14°12'S, 48°03'W) – MN36216, MN36305. MATO GROSSO: Barão de Melgaço (16°11'S, 55°58'W) – MN64260*, MN64347*.

Gracilmanus agilis: BAHIA: Chapada Diamantina – MN67582, MN67599, MN67606, MN67607, MN67611, MN67697. GOIÁS: Cavalcante (13°47'S, 47°27'W) – MN46538, MN46539, MN46540, MN46541, MN46542, MN46543, MN46544, MN46545, MN46546, MN46547, MN46548, MN46549, MN46550, MN46551, MN46552, MN46567; Serra da Mesa – MN36023, MN36047, MN36085, MN36201, MN36279, MN36292, MN36735; Teresina de Goiás (13°46'S, 47°15'W) – MN42979, MN42981, MN42982, MN42987. MATO GROSSO: Barão de Melgaço – MN64286*, MN64376*, MN64403*, MN64412*, MN64488*, MN64497*, MN64498*, MN64499*, MN64539*, MN64628*, MN64663*, MN64695*, MN64696*, MN64712. MATO GROSSO DO SUL: Aquidauana (20°28'S, 55°47'W) – LBCE4823, LBCE4824, LBCE4835, LBCE4840, LBCE4846, LBCE4854, LBCE4876, LBCE4888, LBCE6145, LBCE6149; Corumbá (19°00'S, 57°39'W) – LBCE5691, LBCE5700, LBCE6147.