ZOOLOGIA 34: e19864
ISSN 1984-4689 (online)
zoologia.pensoft.net

ZOOLOGIA An International Journal for Zoology

RESEARCH ARTICLE

Taxonomic identification using geometric morphometric approach and limited data: an example using the upper molars of two sympatric species of *Calomys* (Cricetidae: Rodentia)

Natália Lima Boroni[1,3], Leonardo Souza Lobo[2,3], Pedro Seyferth R. Romano[3], Gisele Lessa[3]

[1]Departamento de Zoologia, Universidade Federal de Minas Gerais. 31270-901 Belo Horizonte, MG, Brazil.
[2]Laboratório de Processamento de Imagem Digital, Museu Nacional, Universidade Federal do Rio de Janeiro. 20940-040 Rio de Janeiro, RJ, Brazil.
[3]Departamento de Biologia Animal, Universidade Federal de Viçosa. 36570-900 Viçosa, MG, Brazil.
Corresponding author: Natália Lima Boroni (natalia_boroni@hotmail.com)

http://zoobank.org/A73FD3AB-66CC-47D2-BFCA-B4FF8569D0BC

ABSTRACT. The taxonomic identification of micromammals might be complicated when the study material is fragmented, as it is the case with pellets and fossil material. On the other hand, tooth morphology generally provides accurate information for species identification. Teeth preserve notably well, retaining their original morphology, unlike skulls and mandibles, which can get crushed or have missing parts. Here, we explored a geometric morphometrics approach (GM) to identify fragmented specimens of two sympatric *Calomys* Waterhouse, 1837 species – *Calomys tener* (Winge, 1888) and *Calomys expulsus* (Lund, 1841) – using the morphology of intact molars as the basis for identification. Furthermore, we included some specimens of uncertain taxonomic identification to test their affinities and the utility of the shape of the molar to identify incomplete specimens. We evaluated the variations in the shape of the first upper molar (M1) among 46 owl pellets specimens of *Calomys*, including *C. expulsus* (n = 15), *C. tener* (n = 15), and unidentified specimens treated as *Calomys* sp. (n = 16) through GM analysis using 17 landmarks. The data was explored using PCA, PERMANOVA, and Discriminant analyses over the Procrustes residuals matrix were applied to evaluate inter- and intraspecific shape differences. Also, we evaluated whether allometric shape differences could impact the data, but found no evidence of a correlation between size and shape. Our results support that shape differences in the M1 are effective for discriminating between *C. tener* and *C. expulsus*. Moreover, the unidentified specimens do not represent a third shape but could be identified with confidence either as *C. tener* or *C. expulsus*. Our results show that even with fragmentary materials, GM is a feasible and useful tool for exploring inter-specific shape differences and assisting in taxonomic identification as a complement to traditional qualitative description of diagnostic features in poorly preserved specimens.

KEY WORDS. Landmarks, morphology, owl pellets, Sigmodontinae, taxonomy.

INTRODUCTION

The complex morphology of the molars (with the cones, flexes, and lophs) is a source of information for the study of cricetid rodent taxonomy, as these structures provide diagnostic characteristics for subfamilies (Reig 1977). Over the years, it has been demonstrated that geometric morphometrics is a useful tool for systematics, for example taxonomic identification by analysis of molar shape differences among rodents, especially of fossil material (Polly and Head 2004, Kryštufek and Janžekoviè 2005, Macholán 2006, Marcolini et al. 2009). Enamel hardness protects the molars so they often are the only intact structure of a fragmented skull and form the only known elements of several extinct taxa in the fossil record (Reig 1977).

Among the genera with complicated taxonomy and great morphological similarity between some species is *Calomys* Waterhouse, 1837 (Almeida et al. 2007). They are small cricetid rodents distributed mainly in areas of dry vegetation, with wide distribution in South America (Bonvicino et al. 2010). The Brazilian species can be separated into two major groups, based on skull and body size measurements: a group of larger-bodied *Calomys* that includes *Calomys callosus* (Rengger, 1830), *Calomys expulsus* (Lund, 1840), *Calomys tocantinsi* Bonvicino, Lima & Almeida, 2003, *Calomys callidus* (Thomas, 1916), and *Calomys cerqueirai* Bonvicino, Oliveira & Gentile, 2010; and a group of smaller-bodied individuals with two species, *Calomys tener* (Winge, 1888) and *Calomys laucha* (G. Fisher, 1814). Despite this morphometric clustering, the smaller body size group is not monophyletic and *Calomys laucha* shares a more recent common ancestor with *C. expulsus* than *C. tener* does (Almeida et al. 2007). In contrast to their general morphological and morphometric similarity, the karyotype differs greatly between species of *Calomys*, making cytogenetic studies jointly with molecular data useful for species discrimination within this genus (Bonvicino and Almeida 2000, Salazar-Bravo et al. 2013, Almeida et al. 2007, Bonvicino et al. 2010).

In Brazil, *C. tener* and *C. expulsus* are both widely distributed (Salazar-Bravo 2015). The first occurs mainly in the Cerrado and Atlantic Forest borders; and the second occurs in the Caatinga and Cerrado (Bonvicino et al. 2008). Sympatry between these species is common, especially in the central region of Brazil (Bonvicino et al. 2010, Salazar-Bravo 2015). The morphometry, karyotype, distribution, and ecological differences between these two species had been described by Bonvicino and Almeida (2000), but there is little data on the dental morphology of either species. These species have the same diploid number, but differ in the fundamental number (Bonvicino and Almeida 2000). Morphologically, these two species can be distinguished by their size, and some cranial measurements such as the lengths of the skull and molar series (Bonvicino et al. 2010), but owing to the size variation and ontogenetic development, species identification might be inaccurate (Hingst-Zaher et al. 2000).

Although karyotypes and gene sequences may be useful for discriminating among *Calomys* species (Bonvicino and Almeida 2000, Almeida et al. 2007, Bonvicino et al. 2010), these data are rarely available from fossil and subfossil material. Even considering that *C. tener* is a little smaller than *C. expulsus* in some cranial characters, is difficult to separate both species when the material available for study is incomplete. Fragmented material is often found in owl pellets, fossils and sub fossils, and *Calomys* remains are very common in these samples throughout South America (e.g., Pardiñas et al. 2000, 2002, Salles et al. 2006, Scheibler and Christoff 2007).

Calomys and other members of the tribe Phyllotini generally share simplified molars and complete loss of the mesoloph and mesolophid (Hershkovitz 1962), but discrete dental characters useful for identification of *C. tener* and *C. expulsus* are lacking (see Hershkovitz 1962). In the absence of such features and in face of the difficulties to identify either species with fragmentary remains, the variation between them need to be studied and verified using alternative quantitative tools. Many studies, not only involving rodents, have explored the use of morphometric methods with superposition of forms for identifying different taxa, including molar analysis (e.g., Rohlf and Slice 1990, Bookstein 1991, Rohlf 1999, Becerra and Valdecasas 2004, Macholán 2006, Marcolini et al. 2009, Matthews and Stynder 2011). With this methodology, a complete identification is not always possible, but at least one can reduce the number of steps and time necessary for a correct identification (Becerra and Valdecasas 2004). In the Neotropics, however, studies investigating the usefulness of geometric morphometrics of the molar for taxonomic identification are unusual, and only skull and post-cranial elements are generally employed (e.g., Corti et al. 2001, Cordeiro-Estrela et al. 2006, 2008, Morgan 2009, Astúa et al. 2015).

The aim of present study is to explore geometric morphometric analysis to identify fragmented materials (modern and fossil) of small vertebrates based on molars; and to assess whether this method allows for accurate identifications. For this we applied this technique in one area: species-level identification of *Calomys* (*C. expulsus* and *C. tener*) based on their upper molars.

MATERIAL AND METHODS

The *Calomys* specimens analyzed were from owl pellets collected in the Natural Monument Peter Lund, Cordisburgo, in the central karst region of Minas Gerais, the Bambuí group, Brazil (Fig. 1). At the Natural Monument Peter Lund the pellets were collected inside the Salitre cave (19°07’17”S, 44°28’24”W) during the Park Management Plan.

In the Köppen climate classification system, the regional climate is Aw tropical humid, characterized by hot, rainy summers and dry winters (Travassos 2010). The average annual temperature in Cordisburgo is 22°C and the average annual precipitation ranges from 1250 to 1500 mm (Travassos 2010). In the karst area of Cordisburgo, much of the Cerrado has been replaced by agriculture and silviculture of *Eucalyptus* spp. (Travassos 2010). In the surrounding area and limestone outcrops, there is a semi-deciduous forest conditioned by the type of rock and climate, and it is possible to identify riparian and gallery forests along major drainages (Travassos 2010).

For species identification, we analyzed complete and fragmented skulls, maxillae and mandibles, examining the cranial sutures and morphology of the teeth. However, some fragments did not allow a consistent identification to the species level, thus restricting classification to the level of genus. The nomenclature of the species was based on that described by Patton et al. (2015). The molar teeth nomenclature was based on Reig (1977). All collected materials were deposited in the Mastozoology collection of the Museu de Zoologia João Moojen, Universidade Federal de Viçosa, Brazil (MZUFV 3861, MZUFV 3862, MZUFV 3863).

With the fragmented skull, the best characteristics used to differentiate *C. tener* from *C. expulsus* was the presence of alisphenoid strut, which is observed only in *C. tener* (Salazar-Bravo 2015) (Suppl. material 1), and the length of the molar series, which is on average 3.4 mm (range 3.1–3.9 mm) in *C.*

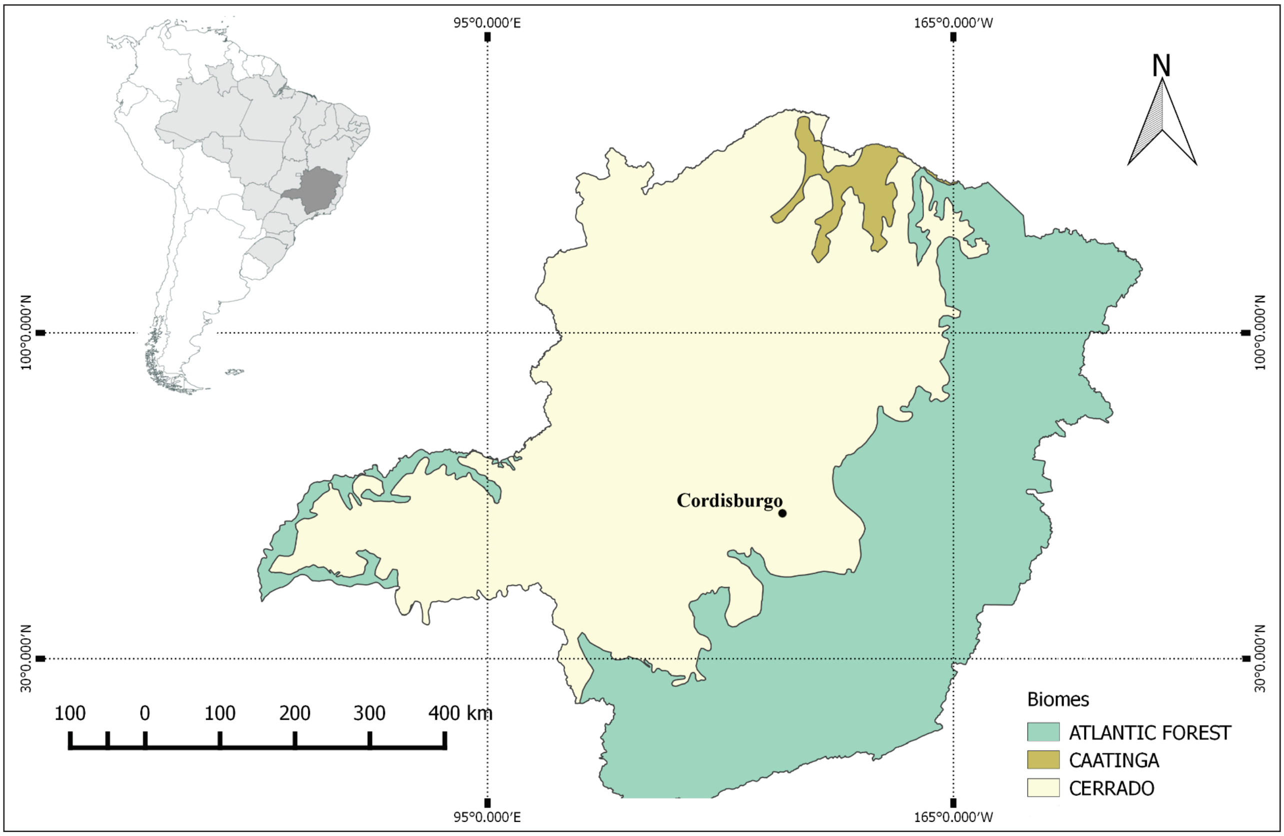


Figure 1. Map of Minas Gerais (Brazil), with the study location, municipality of Cordisburgo.

tener and on average 4.0 mm (range 3.8–4.2 mm) in *C. expulsus* (Bonvicino and Almeida 2000, Bonvicino et al. 2010). Due to an overlap in the measurements of the length of the molar series and difficulties to observe the presence of alisphenoid strut in all skulls due to fragmentation of the posterior part of the skull, some specimens were only identified as *Calomys* sp.

A total of 124 skulls were analyzed and separated into classes of dental wear, including 32 of *C. tener*, 57 of *C. expulsus,* and 35 of *Calomys* sp. (Suppl. material 2). For all analyses only the first right molar of *Calomys* skulls was analyzed, all of which had been previously identified as being of the same age based on wear class (see Suppl. materials 2).

The wear category class 2 showed the most numerically balanced sample between *C. tener*, *C. expulsus,* and *Calomys* sp. (Table 1) and, for that reason, was chosen for the morphometric analysis (see Suppl. material 3 for further information about the specimens analyzed). This subset included 46 specimens (15 of *C. tener*, 15 of *C. expulsus* and 16 of. *Calomys* sp.) Comparison of specimens from the same age category eliminated this potential source of age-related variation in enamel morphology due to tooth wear caused by chewing. Moreover, it minimized the allometric effect in the data, since the size of the specimens showed little variation.

Table 1. Specimens examined and the respective sample sizes by dental wear category.

	Calomys tener	*Calomys expulsus*	*Calomys* sp.
Class 1	7	10	8
Class 2	15	15	16
Class 3	9	20	8
Class 4	–	11	2
Total	32	57	35

The landmarks were digitalized using TpsDig v.2.17 (Rohlf 2015). We selected 17 landmarks corresponding to type II (points of maximum curvature, *sensu* Bookstein 1991), spanning the enamel folds connecting the main cusps of the first molar, which are described as follows (Fig. 2). Landmark 1, anterior extremity of anterolabial conule; Landmark 2, posterior extremity of anteroflexus; Landmark 3, anterior extremity of anterolabial conule; Landmark 4, lateral extremity of anterolabial conule;

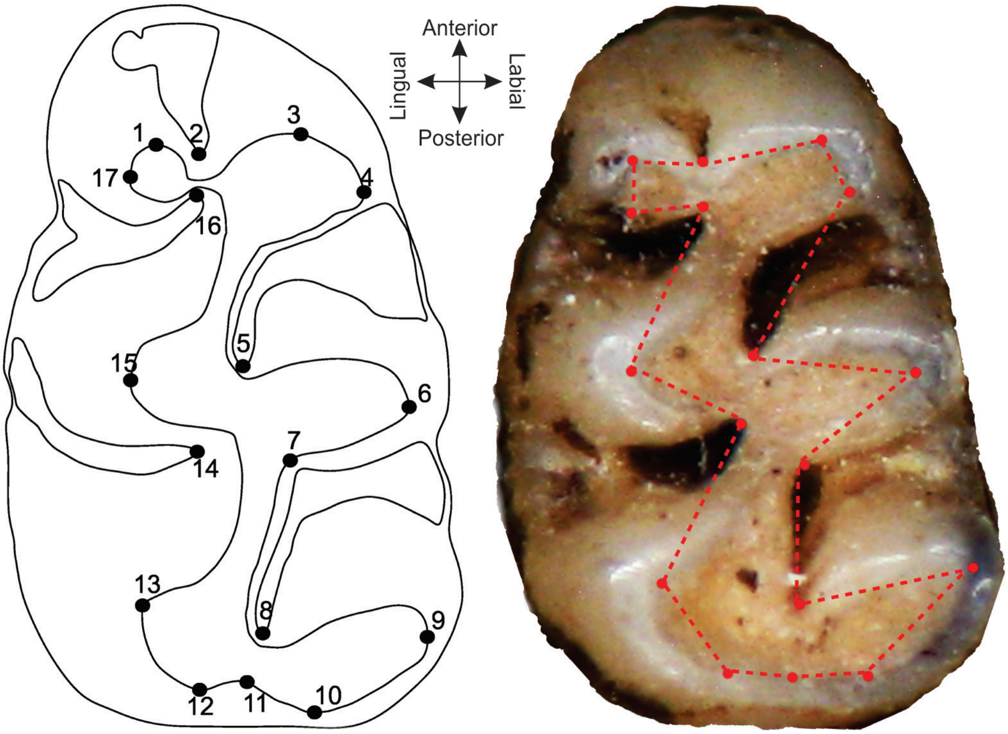


Figure 2. Landmarks of the molar used in this study. For landmarks description, see text.

Landmark 5, posterior extremity of protoflexus; Landmark 6, lateral extremity of protocone; Landmark 7, anterior extremity of hypoflexus; Landmark 8, posterior extremity of hypoflexus; Landmark 9, lateral extremity of hypocone; Landmark 10, posterior extremity of hypocone; Landmark 11, hypocone in contact with the metacone; Landmark 12, posterior extremity of metacone; Landmark 13, lateral extremity of metacone; Landmark 14, medial extremity of metaflexus; Landmark 15, lateral extremity of paracone; Landmark 16, medial extremity of paraflexus; and Landmark 17, lateral extremity of anterolingual conule.

The multivariate analyses were performed using the MorphoJ v. 1.05f (Klingenberg 2011). After digitizing all landmarks using TpsDig, the centroid sizes of all specimens were calculated from the original coordinate matrix. The centroid size is the square root of the summed squared distances of each landmark to the centroid and can be used as a measure of general size of specimens (Zelditch et al. 2004). The matrices of coordinates were then superimposed using Procrustes standardization (the generalized least square method, GLS) to remove differences in size, orientation, and position between specimens (Zelditch et al. 2004). All further statistics were performed using the Procrustes residuals to analyze differences in shape and the centroid size values to evaluate differences in size.

For increased reliability of data, an analysis of error in digitization of anatomical landmarks was performed following the protocol of Adriaens (2007) using the TpsSmall v. 1.26 (Rohlf 2015). For this analysis, 12 specimens that were duplicated to evaluate the accuracy in digitization of anatomical landmarks were used. The results of the analysis of error are presented in Suppl. material 4.

From this experimental design, a preliminary principal component analysis (PCA) was performed using only species anatomically distinguishable, namely, 15 specimens of *C. tener* and 15 specimens of *C. expulsus*. This PCA was conducted to visualize the projection of individuals from the two axes of greatest variation, thereby detecting the distribution pattern in the graph. Then, we included the 16 specimens that could not be definitively identified as *C. tener* or may be *C. expulsus*. This addition aimed to increase the sample, totaling to 46 specimens, and explore the projection of *Calomys* sp. in the two axes of the PCA in the presence of distinguishable specimens. The second goal of adding specimens of *Calomys* sp. was to classify the unidentified specimens to one or another expected species (*C. tener* or *C. expulsus*). The wireframe, which helps in comparing the shapes of the specimens plotted in the PCA, was used whenever necessary to describe and discuss the shape of the specimens.

After each *Calomys* spp. was assigned to *C. tener* or *C. expulsus* through the pattern observed in the PCA, a Discriminant analysis (DA) was performed using MorphoJ v. 1.05f (Klingenberg 2011) and with 10.000 replications in the permutation test. As the Procrustes distances did not meet assumptions of normality, homoscedasticity and homogeneity of covariance (Suppl. material 5), the differences between the molar morphology of *C. tener* and *C. expulsus* were tested by PERMANOVA with adjustment of Euclidean distance (Anderson 2005). This analysis is analogous to ANOVA, however is a non-parametric test used to perform a comparison between two or more groups. In this test the significance is computed by permutation of group membership and $p < 0.05$ as the criterion of significance. "*Calomys* sp." potentially identified as *C. tener* or *C. expulsus* into the PC1 vs. PC2 individual projections were also tested by PERMANOVA. Both analyzes were performed using 10.000 replications in permutation test using PAST v.3.11 (Hammer et al. 2001).

Lastly, a pooled regression within each species (multivariate regression) was performed to evaluate whether the differences in the size of the tooth were correlated to the pattern of differentiation of the PCA projection. For this analysis, we used the centroid size of specimens as the independent variable and Procrustes coordinates as the dependent variable. Also, we performed a permutation test with 10,000 rounds to evaluate whether the dependence of shape (Procrustes distances) on the size (centroid size) of the tooth is significant.

RESULTS

The first PCA was performed only with *C. expulsus* and *C. tener* together. The PC1 and PC2 totaled 29.5% and 13.8% of the total variance, respectively, explaining 43.3% of the shape variation within the sample. It was possible to visualize either species separated in PC1 vs PC2 individual projections (Suppl. material 6). PERMANOVA test showed significant difference between *C. expulsus* and *C. tener* (p-value = 0.001 F = 6.079). The second PCA, which includes also the *Calomys* sp. specimens, showed a similar pattern as that of the previous PCA, with PC1 and PC2 holding respectively 28.7% and 12.3%, amounting 41% of the variation (Fig. 3). Discriminant analysis of first upper molar Procrustes coordinates classified 100% of the specimens into the correct groups; when the cross-validation technique was applied, 82.6% of specimens were recovered into the correct groups (Table 2). The permutation test using the Procrustes distance values (0.0484, p-value = < 0.0001) and Mahalanobis distances (6.4366, T-square = 0.0008, p-value = 0.0006) corroborated a distinction in the shape of the molar between species. The result of the PERMANOVA test for *Calomys* sp. (identified as *C. tener* or *C. expulsus* by PCA) together with the other specimens of *C. expulsus* and *C. tener* was significant (p-value = 0.0001 F = 8.283).

The variation expressed by PC1 showed differences in shape of the oclusal between both species. These differences are illustrated based on the wireframe view of mean shape (Fig. 4) and disparate shapes (Fig. 5) of *C. tener* and *C. expulsus*. In general, *C. tener* showed a more retracted shape in the lingual-labial axis, due to an expansion of the cusps in *C. expulsus* compared to *C. tener*; flexus retraction and smaller aperture angles of the lingual flexus in *C. expulsus* compared to *C. tener*. The anterolingual conule of *C. tener* was retracted in the anterior-posterior axis compared to *C. expulsus*, was located more anteriorly, and was more oblique in relation to the anteromedian flexus. When compared with *C. expulsus*, *C. tener* had a retraction of the anterolabial conule in the labial-lingual axis and suffered another retraction in relation to the anterior and labial extremities (between the anatomical landmarks 3 and 4). The paraflexus of *C. tener* compared to *C. expulsus* was expanded in the anterior-posterior axis, and the paracone was retracted in the labial-lingual axis. The protoflexus of *C. tener*, when compared with that of *C. expulsus*, was expanded and the protocone was retracted on both the orientation axes. The metacone of *C. tener*, when compared with that of *C. expulsus*, was retracted in both the axes. The hypoflexus of *C. tener*, in relation to *C. expulsus*, was expanded and the hypocone was retracted in its anterior part. The posterior region of the molar of *C. tener*, in relation to *C. expulsus*, was retracted in the lingual region (landmarks 10, 11, and 12) and anterior-posterior axis.

CT 02 (*C. tener*) can be distinguished from the other individuals by observing the shape differences explained by PC2 and illustrated in Fig. 6. CT 02 differed from the mean value for PC2 scores owing to the following morphological disparities: expansion of the anterior-posterior axis and slight retraction of the labial-medial axis of the anterolabial conule (landmarks 3 and 4); expansion of anterior-posterior axis in the protoflexus (landmark 5) (the anterior extremity of hypoflexus (landmark 7) did not expand proportionally), which caused a narrowed protocone in CT 02; slight retraction of posterior extremity of hypocone; and lingual expansion of metacone (landmark 13) and posterior displacement of anterior extremity of metaflexus (landmark 14).

To evaluate whether tooth size influences the patterns of variation in oclusal surface, we performed a multivariate regression with the Procrustes coordinates residual (shape) on the centroid size (size measurement). This regression showed a weak relationship that accounted for just 5.3% of the shape variation predicted by the size (Fig. 7), pursuant the permutation test accepting the null hypothesis of independence between the variables (p-value = 0.2118).

Table 2. Discriminant analysis results (classification/misclassification table) for *Calomys expulsus* and *Calomys tener*.

	Discriminant function – Allocated to		Total	Percentage
	Calomys expulsus	*Calomys tener*		
Calomys expulsus	22	0	22	100
Calomys tener	0	24	24	100
	Cross-validation – Allocated to			
	Calomys expulsus	*Calomys tener*		
Calomys expulsus	19	3	22	86.4
Calomys tener	5	19	24	79.2

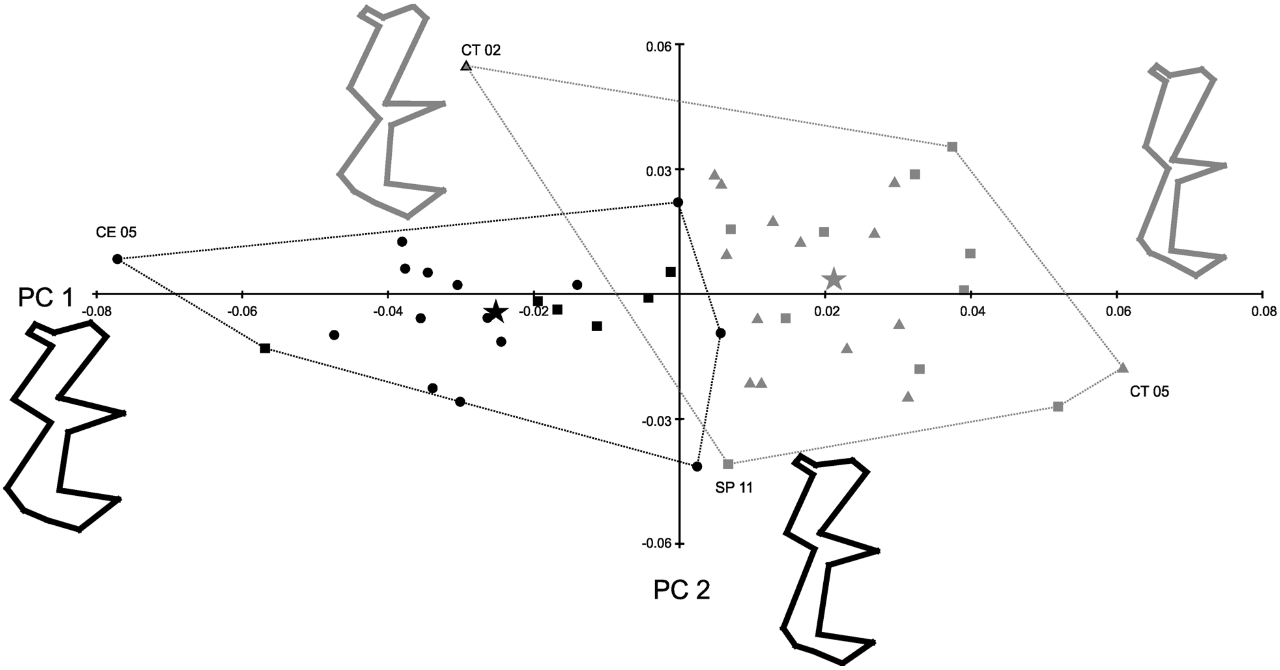
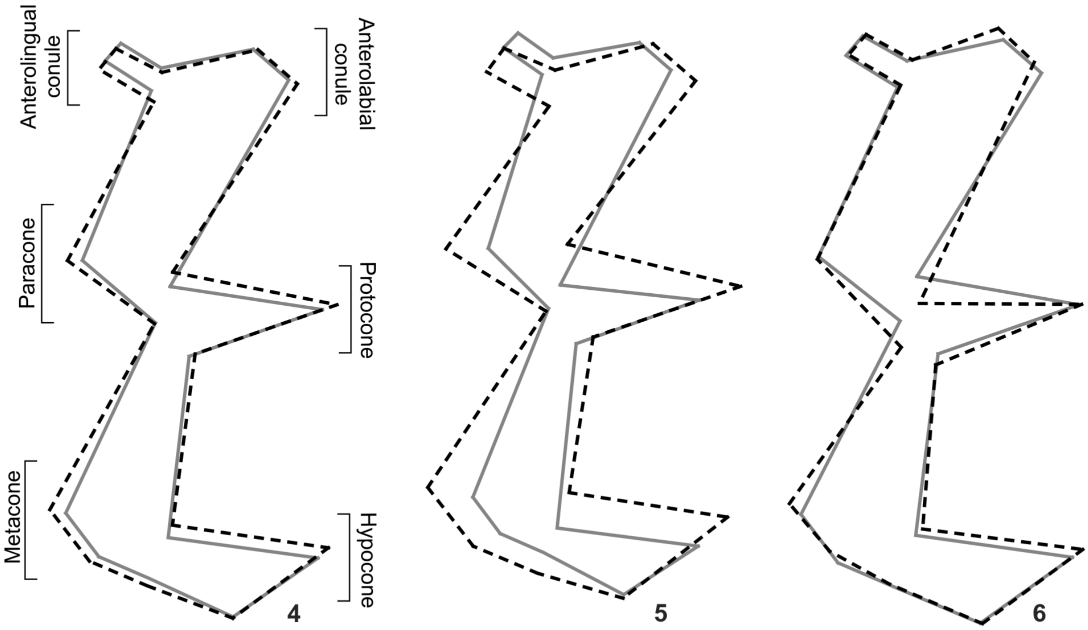


Figure 3. PCA showing the individual projections of *Calomys* sp., *C. expulsus* and *C. tener* in the two major axis (PC1 28.7%, PC2 12.3% of the variance). The wireframes illustrate shape differences between most different specimens: CE 09, CT 05, CT 02, and SP 11. Black circles: *C. expulsus*; Gray triangle: *C. tener*; Black square: *Calomys* sp. identified as *C. expulsus*; Gray square: *Calomys* sp. identified as *C. tener*; Gray triangle with black edge: specimen CT 02; Stars: mean shape for each species.



Figures 4–6. Shape differences wireframes in PC1. Gray solid lines indicate *C. tener* (positive PC1 values) and black dotted lines indicate *C. expulsus* (negative PC1 values), see Fig. 3 for individual projections. (**4**) Differences between the mean shapes of *C. tener* and *C. expulsus*; (**5**) differences between the disparate shape of *C. tener* represented by CT 05 and *C. expulsus* represented by CE 09; (**6**) shape differences wireframes in PC2. Gray solid lines indicate the mean of shape of PC2 value and black dotted lines indicate specimen CT 02 (*C. tener* outlier).

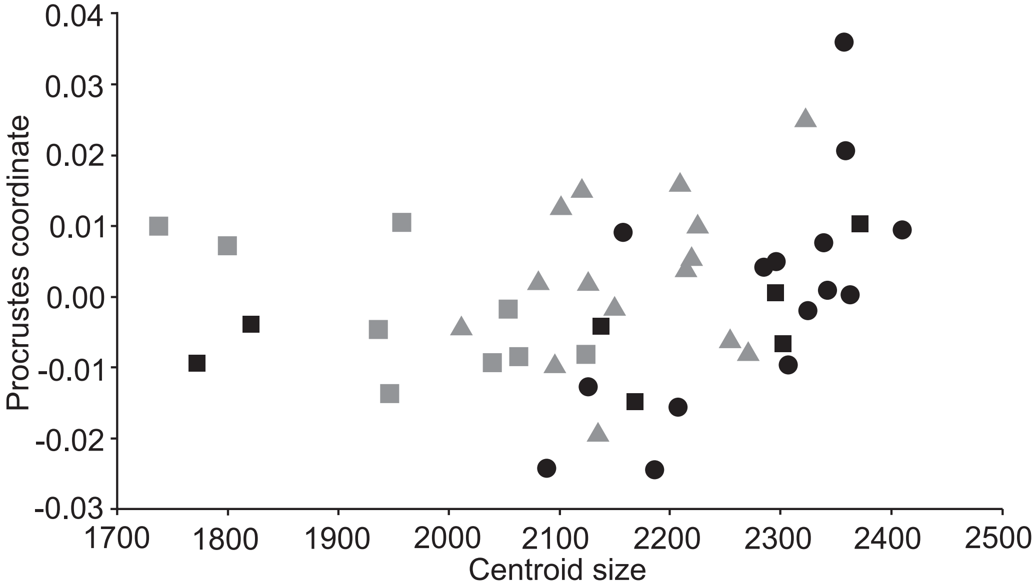


Figure 7. Pooled regression within the two species, between shape (dependent variable) and centroid size (independent variable). Black circles: *C. expulsus*; Gray triangle: *C. tener*; Black square: *Calomys* sp. identified as *C. expulsus*; Gray square: *Calomys* sp. identified as *C. tener*.

DISCUSSION

Calomys tener and *C. expulsus* are grouped into different clades (Almeida et al. 2007), however, neither species has discrete craniodental characters for species diagnosis (Hershkovitz 1962, Cordeiro-Estrela et al. 2006), and commonly used morphometric measures also overlapped (see Bonvicino et al. 2010). Nevertheless, the analyses performed in the present study indicate that the presence of the alisphenoid strut can be used to identify either species.

The overlap areas in morphometric cranial analyses using orthogonal matrix transformation techniques as PCA, irrespective of whether traditional or geometric, have also been identified in other species of other genera and in *Calomys* (Cordeiro-Estrela et al. 2006, 2008, Astúa 2009, Bonvicino et al. 2010, Martínez and Di Cola 2011). Furthermore, the results obtained in the present study for M1 shape are in agreement with those reported in other studies that showed a divergence in the shape of *Calomys* skull, but with small areas of intersection (Cordeiro-Estrela et al. 2006, 2008).

The main criterion for distinguishing *C. tener* from *C. expulsus* by traditional morphometrics is the size of the body (Bonvicino and Almeida 2000). *Calomys expulsus* can be differentiated from *C. tener* by having a larger body, smaller ear, proportionally smaller tail (generally), more robust skull, smaller braincase, length of molar row close to 4.0 mm in *C. expulsus*, 3.0 mm in *C. tener* (Winge 1888, Bonvicino and Almeida 2000). However, Cordeiro-Estrela et al. (2006) analyzed the skull of *C. expulsus* and *C. tener* using geometric morphometrics and noted that allometry is not the main source of variation between the two species, despite its contribution to their differences. The main morphological differences between these two species are related to the patterns of shape variation (Cordeiro-Estrela et al. 2006), as observed in the present study through molar analysis.

As mentioned earlier, *C. expulsus* and *C. tener* occur in sympatry, and were differentiated in the present study through the presence of alisphenoid strut and length of maxillary molars. We observed a high similarity between our analysis and previous identification based on both characteristics, with more than 80% accuracy in DA. However, our results indicate that tooth size characters are not useful to diagnose either species. Thus, we suggest that the use of size characters (e.g., Bonvicino and Almeida 2000, Bonvicino et al. 2010) to distinguish *C. tener* and *C. expulsus* should be avoided. Cordeiro-Estrela et al. (2008) arrived at a similar conclusion considering the skull centroid size of *C. laucha* and *Calomys musculinus* (Thomas, 1913).

In our results, CT02 deserves mention. This specimen was identified as *C. tener* because it had alisphenoid strut and a 3.7 mm long molar. However, the shape of this specimen, at least observing the differences explained by PC1, reveals more similarity with *C. expulsus*. Since CT 02 is a fragmentary specimen, our conclusion regarding its identification is limited. This specimen could be *C. tener* with larger molars or perhaps *C. expulsus* with alisphenoid strut and small molars. On the other hand, another possibility is that CT02 is a *C. cerqueirai*, a species described from near the investigation area of the present study (see Bonvicino et al. 2010). However, we are not able to confirm this hypothesis, since *C. cerqueirai* shows no discrete cranial or dental characters or difference in size, when compared with *C. expulsus* (Bonvicino et al. 2010). As described by Bonvicino et al. (2010), the main differences between *C. cerqueirai* to *C. expulsus* are a Cinnamon-Brown overall dorsal coloration, head paler than dorsum, small ears with short brownish hairs, and sharply bicolored tail. Also, some descriptive cranial measurements, as palatal bridge, breath of incisive foramen, breadth of first maxillary molar, are overlapped. Unfortunately, we could not add *C. cerqueirai* specimens in our analysis and there are only twelve specimens deposited in other institutions (Bonvicino et al. 2010, Mesquita and Passamani 2012, Colombi and Fagundes 2015.) But since neither species (*C. cerqueirai* and *C. expulsus*) has alisphenoid strut (Salazar-Bravo 2015), the most likely identification of specimen CT02 is outliner *C. tener*.

We observed a pattern of differentiation in the oclusal shape of class 2, among sympatric specimens of *C. expulsus* and *C. tener*. In this class, the cusps of *C. tener* (as protocone and hipocone) were more retracted, whereas the cusps of *C. expulsus* are expanded in the lingual-labial axis (when compared with *C. tener*); the flexus (as paraflexus and protoflexus) are expanded and the aperture angles are larger in *C. tener* than in *C. expulsus*. Different molar morphologies in rodents can represent adaptations to different diets and can be relevant to avoid intraspecific competition (Parra et al. 1999, Renaud et al. 1999). This could mean that the differences in the shape of the molar, observed between *C. tener* and *C. expulsus*, observed in this study, could reflect different diets. This hypothesis needs testing, since the diet of *C. expulsus* and *C. tener* has not been published in the literature. A study on the outline of the first molar of extinct murine rodents demonstrated that different diet groups could be distinguished through geometric morphometric analysis (Cano et al. 2013). However, in marmots and some Didelphidae it has been demonstrated that diet is weakly correlated with molar shape (Caumul and Polly 2005, Chemisquy et al. 2015).

The possibility to perform taxonomic identifications based on molar shape, principally in rodents, is a very useful, especially when working with fossil materials (Macholán 2006, Barrón-Ortiz et al. 2008, Marcolini et al. 2009, Matthews and Stynder 2011). Matthews and Stynder (2011) distinguished *Aethomys* fossil specimens from extant specimens based on molar shape using geometric morphometric analysis; however, they emphasized that although the shape of the molar is important, a small sample size might hamper a complete analysis. Limitations in analyses owing to insufficient sample size are common, particularly in paleontological samples (Matthews and Stynder 2011), as it was in ours. Is important to point out that geometric morphometrics and features from the molars can be used in other types of studies beyond taxonomic identifications, as for example: correlating changes in molar shape with paleoclimate aspects (McGuire 2010), phylogenetic (Caumul and Polly 2005), morphological evolution and environmental variations (Renaud and Van Dam 2002).

Many studies using small mammal fossils or owl pellets for taxonomic identification are restricted to the level of genus (e.g. Salles et al. 1999, 2006, Bonvicino and Bezerra 2003, Scheibler and Christoff 2007, Rocha et al. 2011). With a good taxonomic identification it is possible to go beyond listing the species that occur at an area. The possibility to identify unknown specimens with an accurate morphology-based taxonomic assignments permits us to gain a better understanding of geographic distribution, migration and environmental response (Polly and Head 2004). Approaches using different quantitative, statistics and morphological analyzes can provide more scientifically accurate and reliable results (Polly and Head 2004).

This is the first study using molars found in owl pellets for taxonomic identification through geometric morphometrics in South America. Our results were effective for the identification of dubious *Calomys* sp. specimens. All of them were identified either as *C. tener* or *C. expulsus* based on the individual projections of the PC1. The use of size as a diagnostic character is not effective for molar differentiation of *C. tener* and *C. expulsus*, as the regression test showed no evidence of correlation between size and shape. These results demonstrated the huge potential of geometric morphometric analysis as a tool in the rodent taxonomic identification using molars, especially in works with fragmented material (modern or fossil). This technique can be extensively useful principally by paleontologists, who have to identify isolated morphological elements, as molars of small vertebrates. This method can be also applied to other genera (e.g. *Oligoryzomys* Bangs, 1900 and *Akodon* Meyen, 1833) and to other groups such as bats and marsupials, wherever accurate taxonomic identifications of morphologically similarity and sympatric species are needed.

ACKNOWLEDGMENTS

We are grateful to Ulyses Pardiñas for the initial support in the identification of the owl pellets. Rodolfo Stumpp and the reviewers for all relevant comments. We thank the staff of Peter Lund National Monument for allowing us to use their facilities for the collection of samples. We are also grateful to Prof. Weyder Santana for permitting us to use the stereoscopic microscope and camera. The Federal Forest Institute (IEF) granted permits for the collection of specimens. We also thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the student scholarship. This study was supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG APQ-02262-12), grants to Gisele Lessa and was developed during 2013 at the Graduation Program in Animal Biology of the Federal University of Viçosa.

LITERATURE CITED

Adriaens D (2007) Protocol for error testing in landmark based geometric morphometrics. http://www.fun-morphometrics.

morph.ugent.be/Miscel/Methodology/Morphometrics.pdf [Accessed: 18/03/2016]

Almeida FC, Bonvicino CR, Cordeiro-Estrela P (2007) Phylogeny and temporal diversification of *Calomys* (Rodentia, Sigmodontinae): Implications for the biogeography of an endemic genus of the open/dry biomes of South America. Molecular Phylogenetics and Evolution 42: 449–466. https://doi.org/10.1016/j.ympev.2006.07.005

Anderson MJ (2005) Permutational multivariate analysis of variance. Auckland, Department of Statistics, University of Auckland.

Astúa D (2009) Evolution of scapula size and shape in didelphid marsupials (Didelphimorphia: Didelphidae). Evolution 63: 2438–2456. https://doi.org/10.1111/j.1558-5646.2009.00720.x

Astúa D, Bandeira I, Geise L (2015) Cranial morphometric analyses of the cryptic rodent species *Akodon cursor* and *Akodon montensis* (Rodentia, Sigmodontinae). Oecologia Australis 19: 143–157. https://doi.org/10.4257/oeco.2015.1901.09

Barrón-Ortiz CR, Riva-Hernández G, Barrón-Corvera R (2008) Morphometric analysis of equid cheek teeth using a digital image processor: A case study of the Pleistocene Cedazo local fauna equids, Mexico. Revista Mexicana de Ciencias Ggeológicas 25: 334–345

Becerra JM, Valdecasas AG (2004) Landmark superimposition for taxonomic identification. Biological Journal of the Linnean Society 81: 267–274. https://doi.org/10.1111/j.1095-8312.2003.00286.x

Bonvicino CR, Almeida FC (2000) Karyotype, morphology and taxonomic status of *Calomys expulsus* (Rodentia: Sigmodontinae). Mammalia 64: 339–351. https://doi.org/10.1515/mamm.2000.64.3.339

Bonvicino CR, Bezerra AMR (2003) Use of regurgitated pellets of Barn Owl (*Tyto alba*) for inventory small mammals in the Cerrado of central Brazil. Studies on Neotropical Fauna and Environment 38: 1–5. https://doi.org/10.1076/snfe.38.1.1.14030

Bonvicino CR, Oliveira JA, D'andrea OS (2008) Guia dos roedores do Brasil, com chaves para gêneros baseadas em caracteres externos. Rio de Janeiro, Centro Pan-Americano de Febre Aftosa, OPAS/OMS.

Bonvicino CR, Oliveira JA, Gentile R (2010) A new species of *Calomys* (Rodentia: Sigmodontinae) from Eastern Brazil. Zootaxa 25: 19–25.

Bookstein FL (1991) Morphometric tools for landmark data: geometry and biology. Cambridge University Press, New York.

Caumul R, Polly PD (2005) Phylogenetic and environmental components of morphological variation: Skull, mandible, and molar shape in Marmots (*Marmota*, Rodentia). Evolution 59: 2460–2472. https://doi.org/10.1111/j.0014-3820.2005.tb00955.x

Cano GA, Hernández FM, Álvarez-Sierra M (2013) Dietary ecology of Murinae (Muridae, Rodentia): A geometric morphometric approach. PLOS ONE 8: 1–7. https://doi.org/10.1371/journal.pone.0079080

Chemisquy MA, Prevosti FJ, Martin G, Flores DA (2015) Evolution of molar shape in didelphid marsupials (Marsupialia: Didelphidae): analysis of the influence of ecological factors and phylogenetic legacy. Zoological Journal of the Linnean Society 173: 217–235. https://doi.org/10.1111/zoj.12205

Colombi VH, Fagundes V (2015) First record of *Calomys cerqueirai* (Rodentia: Phyllotini) in Espirito Santo (Brazil) with description of the 2n = 36, FNA = 66 karyotype. Mammalia 79: 479–486. https://doi.org/10.1515/mammalia-2014-0076

Cordeiro-Estrela P, Baylac M, Denys C, Marinho-Filho J (2006) Interspecific patterns of skull variation between sympatric Brazilian vesper mice: Geometric morphometrics assessment. Journal of Mammalogy 87: 1270–1279. https://doi.org/10.1644/05-MAMM-A-293R3.1

Cordeiro-Estrela P, Baylac M, Denys C, Polop J (2008). Combining geometric morphometrics and pattern recognition to identify interspecific patterns of skull variation: Case study in sympatric Argentinian species of the genus Calomys (Rodentia: Cricetidae: Sigmodontinae). Biological Journal of the Linnean Society 94: 365–378. https://doi.org/10.1111/j.1095-8312.2008.00982.x

Corti M, Aguilera M, Capanna E (2001) Size and shape changes in the skull accompanying speciation of South American spiny rats (Rodentia: Proechimys spp.). Journal of Zoology 253: 537–547. https://doi.org/10.1017/S0952836901000498

Hershkovitz P (1962) Evolution of neotropical Cricetine rodents (Muridae) with special reference to the phyllotine group. Fieldiana Zoology 46: 1–524. https://doi.org/10.5962/bhl.title.2781

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological Statistics Software Package for education and data analysis. Palaeontologia Electronica 4: 1–9.

Hingst-Zaher E, Marcus L, Cerqueira R (2000) Application of geometric morphometrics to the study of post-natal size and shape changes in the skull of *Calomys expulsus*. Hystrix 11: 99–113. https://doi.org/10.4404/hystrix-11.1-4139

Klingenberg CP (2011) MorphoJ: An integrated software package for geometric morphometrics. Molecular Ecology Resources 11: 353-357. https://doi.org/10.1111/j.1755-0998.2010.02924.x

Kryštufek B, Janžekovič F (2005) Relative warp analysis of cranial and upper molar shape in rock mice *Apodemus mystacinus* sensu lato. Acta theriologica 50: 493–504. https://doi.org/10.1007/BF03192642

Macholán M (2006) A geometric morphometric analysis of the shape of the first upper molar in mice of the genus Mus (Muridae, Rodentia). Journal of Zoology 270: 672–681. https://doi.org/10.1111/j.1469-7998.2006.00156.x

Marcolini F, Piras P, Martin RA (2009) Testing evolutionary dynamics on first lower molars of Pliocene *Ogmodontomys* (Arvicolidae, Rodentia) from the Meade Basin of Southwestern Kansas (USA): A landmark-based approach. PALAIOS 24: 535–543. https://doi.org/10.2110/palo.2008.p08-114r

Matthews T, Stynder DD (2011) An analysis of the Aethomys (Murinae) community from Langebaanweg (Early Pliocene, South Africa) using geometric morphometrics. Palaeogeography, Palaeoclimatology, Palaeoecology 302: 230–242. https://doi.org/10.1016/j.palaeo.2011.02.003

Martínez JJ, Di Cola V (2011) Geographic distribution and phenetic skull variation in two close species of *Graomys* (Rodentia, Cricetidae, Sigmodontinae). Zoologischer Anzeiger 250: 175–194. https://doi.org/10.1016/j.jcz.2011.03.001

McGuire J (2010) Geometric morphometrics of vole (*Microtus californicus*) dentition as a new paleoclimate proxy: Shape change along geographic and climatic clines. Quaternary International 212: 1–8. https://doi.org/10.1016/j.quaint.2009.09.004

Mesquita AO, Passamani M (2012) Composition and abundance of small mammal communities in forest fragments and vegetation corridors in Southern Minas Gerais, Brazil. Revista de Biologia Tropical 60: 1335–1343. https://doi.org/10.15517/rbt.v60i3.1811

Morgan CC (2009) Geometric morphometrics of the scapula of South American caviomorph rodents (Rodentia: Hystricognathi): form, function and phylogeny. Mammalian Biology-Zeitschrift für Säugetierkunde 74: 497–506. https://doi.org/10.1016/j.mambio.2008.09.006

Pardiñas UF, D'Elía G, Ortiz PE (2002) Sigmodontinos fósiles (Rodentia, Muroidea, Sigmodontinae) de América del Sur: estado actual de su conocimiento y prospectiva. Mastozoología Neotropical 9: 209–252.

Pardiñas UF, Moreira GJ, Garcia-Esponda CM, Santis LJ (2000) Deterioro ambiental y micromamiferos durante el holoceno en el nordeste de la estepe patagônica. Revista Chilena de Historia Natural l73: 9–21. https://doi.org/10.4067/S0716-078X2000000100002

Parra V, Loreau M, Jaeger JJ (1999) Incisor size and community structure in rodents: two tests of the role of competition. Acta Oecologica 20: 93–101. https://doi.org/10.1016/S1146-609X(99)80021-6

Patton JL, Pardiñas UFG, D'elía G (2015) Mammals of South America, vol. 2. The University of Chicago Press, Chicago. https://doi.org/10.7208/chicago/9780226169606.001.0001

Polly PD, Head JJ (2004) Maximum-likelihood identification of fossils: taxonomic identification of Quaternary marmots (Rodentia, Mammalia) and identification of vertebral position in the pipesnake *Cylindrophis* (Serpentes, Reptilia). In: Elewa AMT (Ed.) Morphometrics applications in biology and paleontology. Springer, New York, 197–221. https://doi.org/10.1007/978-3-662-08865-4_14

Reig OA (1977) A proposed unified nomenclature for the enamelled components of the molar teeth of the Cricetidae (Rodentia). Journal of Zoology 181: 227–241. https://doi.org/10.1111/j.1469-7998.1977.tb03238.x

Renaud S, Michaux J, Mein P, Aguilar JP, Auffray JC (1999) Patterns of size and shape diferentiation during the evolutionary radiation of the European Miocene murine rodents. Lethaia 32: 61–71. https://doi.org/10.1111/j.1502-3931.1999.tb00581.x

Renaud S, Van Dam J (2002) Influence of biotic and abiotic environment on dental size and shape evolution in a Late Miocene lineage of murine rodents (Teruel Basin, Spain). Palaeogeography, Palaeoclimatology, Palaeoecology 184: 163–175. https://doi.org/10.1016/S0031-0182(02)00255-9

Rocha RG, Ferreira E, Leite YLR, Fonseca C, Costa LP (2011) Small mammals in the diet of barn owls, *Tyto alba* (Aves: Strigiformes) along the mid-Araguaia River in central Brazil. Zoologia 28: 709–716. https://doi.org/10.1590/S1984-46702011000600003

Rohlf FJ (1999) Shape statistics: Procrustes superimposition and tangent spaces. Journal of Classification 16: 197–223. https://doi.org/10.1007/s003579900054

Rohlf FJ (2015) The tps series of software. Hystrix, the Italian Journal of Mammology 26: 9–12. https://doi.org/10.4404/hystrix-26.1-11264.

Rohlf FJ, Slice D (1990) Extensions of the Procrustes method for the optimal superimposition of landmarks. Systematic Zoology 39: 40–59. https://doi.org/10.2307/2992207

Salazar-Bravo J, Pardiñas UF, D'Elía G (2013) A phylogenetic appraisal of Sigmodontinae (Rodentia, Cricetidae) with emphasis on phyllotine genera: systematics and biogeography. Zoologica Scripta 42: 250–261. https://doi.org/10.1111/zsc.12008

Salazar-Bravo J (2015) Genus *Calomys*. In: Patton J, Pardiñas UFJ, D'elía G (Eds) Mammals of South America, vol. 2. The University of Chicago Press, Chicago, 481.

Salles LO, Cartelle C, Guedes PG, Boggiani P, Janoo A, Russo CAM (2006) First report on Quaternary mammals from the Serra da Bodoquena, Mato Grosso do Sul, Brazil. Boletim do Museu Nacional 521: 1–12

Salles LO, Carvalho GS, Weksler M, Sicuro FL, Abreu F, Camardella AR, Guedes PG, Avilla LS, Abrantes EAP, Sahate V, Costa ISA (1999) Fauna de mamíferos do Quaternário de Serra da Mesa (Goiás, Brasil). Publicações Avulsas do Museu Nacional 78: 1–15

Scheibler DR, Christoff AU (2007) Habitat associations of small mammals in southern Brazil and use of regurgitated pellets of birds of prey for inventorying a local fauna. Revista Brasileira de Biologia 67: 619–625. https://doi.org/10.1590/S1519-69842007000400005

Travassos LE (2010) Considerações sobre o carste da região de Cordisburgo, Minas Gerais, Brasil. Belo Horizonte, Tradição Plana.

Winge H (1888) Jordfundne og nulevende Gnavere (Rodentia) fra Lagoa Santa, Minas Geraes, Brasilien. E Museo Lundii 1: 1–200.

Zelditch LM, Swiderski DL, Sheets HD, Fink WL (2004) Geometric Morphometrics for Biologists: A Primer. Elsevier Academic Press, San Diego, 436 pp.

Submitted: 13 April 2016
Received in revised form: 3 March 2017
Accepted: 10 March 2017
Editorial responsibility: Diego Astúa de Moraes

Author Contributions: NLB and LL conducted the experiments, analyzed the data and wrote the paper. PSRR analyzed the data and wrote the paper. GL reviewed the paper.

Competing Interests: The authors have declared that no competing interests exist.

Supplementary material 1

Alisphenoid strut
Authors: Natália Lima Boroni, Leonardo Souza Lobo, Pedro Seyferth R. Romano, Gisele Lessa
Data type: multimedia

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/subtbiol.34.e19864.suppl1

Supplementary material 2

Wear categories
Authors: Natália Lima Boroni, Leonardo Souza Lobo, Pedro Seyferth R. Romano, Gisele Lessa
Data type: measurement

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/subtbiol.34.e19864.suppl2

Supplementary material 3

Specimens of the wear category class 2
Authors: Natália Lima Boroni, Leonardo Souza Lobo, Pedro Seyferth R. Romano, Gisele Lessa
Data type: species data

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/subtbiol.34.e19864.suppl3

Supplementary material 4

Protocol for testing error in the digitalization
Authors: Natália Lima Boroni, Leonardo Souza Lobo, Pedro Seyferth R. Romano, Gisele Lessa
Data type: statistical data

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/subtbiol.34.e19864.suppl4

Supplementary material 5

Tests for normality, hornoscedasticity and homogeneity
Authors: Natália Lima Boroni, Leonardo Souza Lobo, Pedro Seyferth R. Romano, Gisele Lessa
Data type: statistical data

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/subtbiol.34.e19864.suppl5

Supplementary material 6

PCA with Calomys terrer and *C. expulsus*
Authors: Natália Lima Boroni, Leonardo Souza Lobo, Pedro Seyferth R. Romano, Gisele Lessa
Data type: statistical data

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/subtbiol.34.e19864.suppl6