



Taxonomic status of large- and middle-sized *Calomys* (Cricetidae: Sigmodontinae) from the southern central Andes inferred through geometric morphometrics of the skull

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Calomys is one of the most polytypic and widely distributed genera of the Neotropical rodent fauna. The taxonomic hierarchy for the large- and middle-sized morphotypes from the southern central Andes (SCA; *C. boliviae*, *C. callosus*, *C. fecundus*, and *C. venustus*) has fluctuated repeatedly from synonyms or subspecies to valid species. As a first approach to the taxonomic resolution of the taxa complex of this genus inhabiting the SCA, we applied an integrative quantitative assessment of currently recognized species' cranial discrimination through the analysis of geometric morphometrics. The morphometric analyses revealed the presence of 3 distinct species of *Calomys* in northwestern and central Argentina. We corroborated the presence of a large amount of intraspecific variation with substantial overlap for the 3 species in the morphospace. The assessment indicates that 24% of skull size variation is due to differences among species. *Calomys boliviae* (including *C. fecundus*) was the largest among the studied species, whereas the differences were subtle between *C. venustus* and *C. callosus*. The relative contribution of interspecific differences to the total skull shape variation was lower than that of size and different among the cranial views—dorsal, ventral, and lateral—analyzed (between 8% and 16%). Moreover, static allometric size changes had a major effect on skull shape differences between species. So, including ecoregions and size-free shape, variables improved significantly the amount of interspecific differentiation. We highlight the usefulness of morphometric assessments to clarify and contribute to the taxonomy of Neotropical mice.

Calomys es uno de los géneros más politípicos y ampliamente distribuidos de la fauna de roedores Neotropicales. La taxonomía de las especies grandes y medianas del sur de los Andes centrales (SAC; *C. boliviae*, *C. callosus*, *C. fecundus* y *C. venustus*) ha fluctuado repetidamente desde sinónimos o subespecies a especies válidas. Como una primera aproximación a la resolución taxonómica del complejo de taxones de este género que habitan el SAC, se aplicó una evaluación cuantitativa integradora a la discriminación craneal de las especies reconocidas actualmente mediante análisis de morfometría geométrica. El análisis morfométrico reveló la presencia de tres especies distintas de *Calomys* en el noroeste y el centro de Argentina. Corroboramos la presencia de una gran cantidad de variación intraespecífica, con un solapamiento sustancial de las tres especies en el morfoespacio. La evaluación indica que el 24% de la variación total en el tamaño del cráneo se debe a las diferencias entre especies. *Calomys boliviae* (incluyendo *C. fecundus*) fue la más grande entre las especies estudiadas, mientras que entre *C. callosus* y *C. venustus* las diferencias fueron sutiles. La contribución relativa de las diferencias interespecíficas a la variación total de la forma del cráneo fue menor que la del tamaño y diferente entre las vistas craneales analizadas -dorsal, ventral y lateral (entre 8 y 16%). Por otra parte, los cambios de tamaño alométrico estático

tuvieron un efecto sustancial en la diferenciación de la forma craneal entre las especies. Por lo tanto, la inclusión de las ecoregiones y de variables de forma libres del efecto alométrico mejoró significativamente la cantidad de diferenciación interespecífica. Destacamos la utilidad de las evaluaciones morfométricas para aclarar y contribuir a la taxonomía de los ratones neotropicales.

Key words: allometric trajectories, ecogeographic variation, Phyllotini, skull, species differentiation, taxonomy

Calomys Waterhouse, 1837 is one of the most polytypic genera of the tribe Phyllotini (Hershkovitz 1962; Haag et al. 2007). It comprises small- to middle-sized sigmodontines and is one of the most widely distributed genera of the Neotropical rodent fauna (Salazar-Bravo et al. 2001). This genus, whose species are colloquially known as “vesper mice” or “lauchas,” is one of the few sigmodontine genera restricted to arid and semiarid habitats, particularly in southern South America. It occurs in Argentina, Bolivia, southeastern Brazil, Chile, Paraguay, Peru, and Uruguay, and it also inhabits isolated sites in Venezuela and Colombia (Eisenberg and Redford 1999; Musser and Carleton 2005). The most recent taxonomic reports (Bonvicino et al. 2010; Quintela et al. 2014) included 13 species in the genus, 6 of which have been recorded in the southern central Andes (SCA—Musser and Carleton 2005; Díaz et al. 2006): *Calomys boliviae* (Thomas 1901) with *fecundus* Thomas 1926 as a synonym, *C. callosus* (Rengger 1830), *C. laucha* (Fischer 1814), *C. lepidus* (Thomas 1884), *C. musculus* (Thomas 1913), and *C. venustus* (Thomas 1894). In SCA, *C. boliviae* (= *C. fecundus*) is relatively large while *C. callosus* and *C. venustus* are medium sized. *Calomys boliviae* (= *C. fecundus*) occurs mainly in the Yungas and transitional areas of the Chaco ecoregions. *Calomys callosus* has a distribution similar to that of *C. boliviae* (Fig. 1), and also inhabits the Yungas and Chaco ecoregions. Meanwhile, *C. venustus* has a southern distribution, and occurs in the Chaco and Espinal ecoregions (Salazar-Bravo et al. 2002; Díaz and Barquez 2007; Salazar-Bravo 2015).

Species of the genus *Calomys* appear to be morphologically uniform (Salazar-Bravo et al. 2001; Almeida et al. 2007). Consequently, when Hershkovitz (1962) reviewed the genus based on body size and cranial morphology, he reduced the 10–15 previously identified species (Ellerman 1941; Cabrera 1961) to only 4 species: 2 highland species (*C. lepidus*, *C. sorellus*), a large lowland species (*C. callosus*), and a small lowland species (*C. laucha*), while he considered all other previously described morphotypes as junior synonyms or subspecies (Hershkovitz 1962). Later, morphological studies and karyological analysis provided evidence for revalidation of some names, most of them listed under *C. callosus* and *C. laucha* by Hershkovitz (1962), and for descriptions of new species (Massoia et al. 1968; Lisanti et al. 1976; Pearson and Patton 1976; Williams and Mares 1978; Vitullo et al. 1984; Reig 1986; Corti et al. 1987; Pérez-Zapata et al. 1987; Olds 1988; Bonvicino and Almeida 2000; Bonvicino et al. 2003).

In contrast to their morphological uniformity, *Calomys* species show great interspecific variation in chromosome numbers and shapes (Salazar-Bravo et al. 2001; Almeida et al. 2007). In their latest taxonomic compilation, Musser and Carleton (2005) listed 12 species, but noted that the constant listing of specimens

as indeterminate species (e.g., Anderson 1997; Salazar-Bravo et al. 2001) and the report of new karyotypic variants (Espinosa et al. 1997; Lima and Kasahara 2001) make clear that a thorough review of alpha taxonomy of the genus is necessary.

The taxonomy and nomenclature of the SCA small-sized species (body length less than 150 mm), *C. laucha*, *C. lepidus*, and *C. musculus*, is relatively well resolved and its validity is not a matter of frequent discussion (although see González-Ittig et al. 2014, for a recent discussion of several populations of *C. laucha*). The case for the nominal forms of medium- and large-sized species (body length larger than 150 mm; *C. boliviae*, *C. callosus*, *C. fecundus*, and *C. venustus*) is different. The taxonomic status of these species has changed repeatedly from synonyms or subspecies to valid species (Musser and Carleton 1993; Salazar-Bravo et al. 2002; Dragoo et al. 2003). *Calomys boliviae* was considered by Musser and Carleton (1993) to be a valid species, including *fecundus* as a synonym, while both species were considered to be synonyms of *venustus* by Olds (1988) and Anderson (1997). Based on molecular characters and chromosomal data, Salazar-Bravo et al. (2002) and Dragoo et al. (2003) considered *C. fecundus* to be a valid species, even Salazar-Bravo et al. (2001) included this species in Argentina, which had been previously reported only in Bolivia. Later, *boliviae* was suggested as a possible senior synonym of *fecundus* (Salazar-Bravo et al. 2003; Díaz et al. 2006; Díaz and Barquez 2007). Otherwise, *C. venustus* and *C. callosus* were considered as distinct species by Olds (1988) and Anderson (1997). In particular, Olds (1988) discriminated both species on body size and considered *boliviae* to be a synonym of *venustus*. However, *venustus* was considered a synonym of *callosus* by Musser and Carleton (1993) and Díaz and Barquez (2002). Finally, molecular studies support the differentiation of these last 2 species, and suggest that *callosus* is not present in Argentina (Salazar-Bravo et al. 2001; Dragoo et al. 2003). Data on molecular differentiation are consistent with chromosomal evidence and indicate shallow differentiation between the SCA *Calomys* species: *C. boliviae* and *C. venustus* have $2n = 54$ and $2n = 56$, respectively (Salazar-Bravo et al. 2002), while *C. callosus* has $2n = 50$ (Pearson and Patton 1976; Bonvicino et al. 2010). All species have autosomal fundamental numbers of 66 (Bonvicino et al. 2010).

Regardless of the specific epithets used, in SCA 2 general forms can be distinguished: 1 large sized and another medium sized. Nevertheless, even the boundaries between these morphotypes are diffuse and difficult to resolve: the specific determination may be hampered by individual variations in size, mainly due to ontogenetic growth (Araújo 2000; Hingst-Zaher et al. 2000; Cordeiro-Estrela et al. 2008) that can lead to erroneous identification in juveniles or young adult specimens. Both

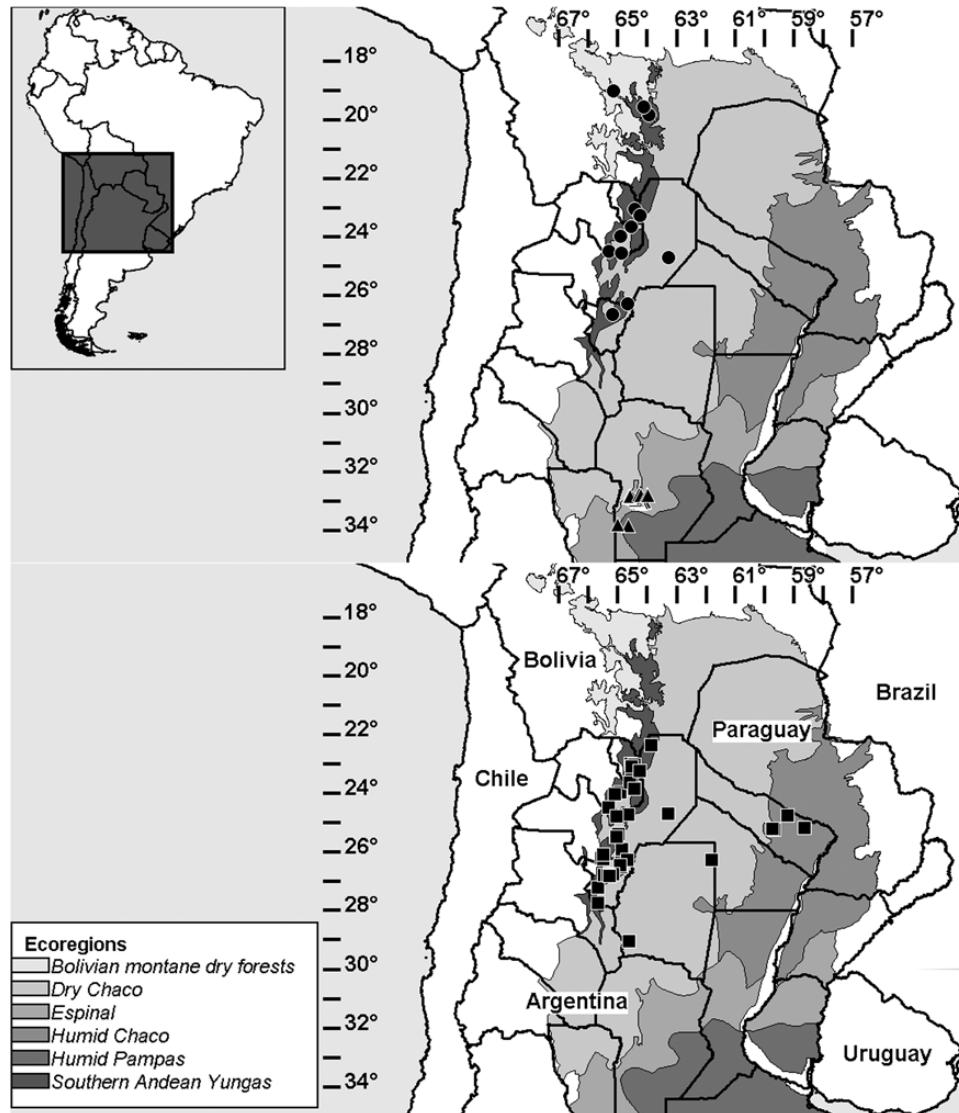


Fig. 1.—Study area and geographic provenance of large- and middle-sized *Calomys* specimens studied from northern and central Argentina and Bolivia. Black circles = *Calomys boliviae*, black triangles = *C. venustus*, and black squares = *C. callosus*. Ecoregions as defined by Burkart et al. (1999) and Olson et al. (2001).

size, which is certainly an important variable, and qualitative morphological differences are not enough to achieve good rates of species discrimination.

Taxonomic assessments are important in order to quantify biodiversity, and morphological, genetic, and cytogenetic evidence is essential to better identify species. Taxonomic clarification is especially important when the species of concern are implicated in zoonotic and epidemiologic problems, as is the case with species of *Calomys*. For example, recent reports indicate that *C. boliviae* (= *C. fecundus*) may be a reservoir of Laguna Negra virus, an etiologic agent of Hantavirus Pulmonary Syndrome in northwestern Argentina (Pini et al. 2012).

Because the mammalian skull contains important diagnostic characters, in this work we use geometric morphometrics to provide a quantitative assessment of cranial shape in *Calomys*. We evaluate intraspecific and interspecific variation in large- and middle-sized *Calomys* as a 1st step in the taxonomic

revision of the *Calomys* species present in SCA. Previous studies (Cordeiro-Estrela et al. 2006, 2008; Martínez and di Cola 2011) have demonstrated the usefulness of geometric morphometric methods as tools to identify species with similar morphologies. These methods enable size to be partitioned from shape (Bookstein 1991), which makes it possible to assess patterns of skull size and shape differentiation between large- and medium-sized species of *Calomys* inhabiting the SCA.

In this study, we use a geometric morphometric approach to test if there are 2 or 3 different forms of *Calomys* in SCA: 1 large-sized form assignable to *C. boliviae* (= *C. fecundus*) and 1 or 2 medium-sized forms, attributable to *C. callosus* or *C. venustus*. Also, we analyze patterns of intraspecific variation in the sympatrically distributed species (*C. callosus* and *C. boliviae*). Because species identification can be difficult as a result of variation in size, static allometric trajectories between species are also analyzed.

MATERIALS AND METHODS

We assessed morphometric variation in 187 adult and sub-adult specimens (with the molars fully erupted and variable tooth wear) belonging to the 3 analyzed species. Skulls were obtained from the systematic collections of the Universidad Nacional de Tucumán (Colección Mamíferos Lillo, CML, San Miguel de Tucumán, Tucumán, Argentina), the Universidad Nacional de Río Cuarto (Colección UNRC, CUNRC, Río Cuarto, Córdoba, Argentina), and the Museo Nacional de Historia Natural (Colección Boliviana de Fauna, CBF, La Paz, Bolivia). Examined specimens are listed in the Appendix I. The collection localities of the specimens whose skulls we studied cover a large part of the natural distribution of these species in northwestern Argentina (Fig. 1).

We photographed dorsal, lateral, and ventral views in order to analyze size and shape variation using a geometric approach. Photographs were made using a Sony DSC-H5 digital camera mounted on a copy stand at a fixed distance from the specimens and with a fixed focal length using ambient light. Millimeter graph paper was used as a background in each picture, to provide a scale for size. Specimen sequence was randomized during photography and during landmark digitization. Landmarks were used only on the left side of each structure to minimize the influence of asymmetry on landmark configurations.

The landmarks were chosen based on positional homology and ease of identification (Fig. 2). In total, 12, 15, and 18 landmarks were used on the dorsal, lateral, and ventral views of the skull, respectively. Of the 187 specimens, 158 were sufficiently intact so that all 3 views could be used (*C. boliviae* $N = 10$, *C. callosus* $N = 91$, and *C. venustus* $N = 57$; see [Supporting Information S1](#) for details). Landmark digitization was performed in program TPSDig2 (Rohlf 2005). Landmark definitions are provided in [Table 1](#).

Landmark configurations were scaled to unit centroid size and superimposed using the least-square generalized Procrustes method (Rohlf and Slice 1990). Skull size was estimated using centroid size: the square root of the sum of squared distances of each landmark to the center of the configuration (Bookstein 1991). Sexual dimorphism in size and shape was evaluated using Analyses of Variance (ANOVAs) and Discriminant Function Analysis, respectively. Statistical shape differences between sexes were estimated using the T^2 statistic in conjunction with 10,000 permutations.

Skull size differences across species were assessed using box plots of centroid size for each skull view independently, and from a single proxy of skull size obtained by summing the centroid sizes of separate views for each individual. We used ANOVA and an a posteriori Tukey honest significant difference test to assess differences in species means. Intraspecific differences were analyzed relative to ecoregions that are depicted in Fig. 1. The variance associated with species and ecoregions was estimated using linear models. Significance of regression parameters was assessed using a permutation test with 10,000 replicates.

In order to reduce the dimensionality of the shape variable data set, we used Principal Components Analyses (PCA) for each cranial view. The number of principal components to be

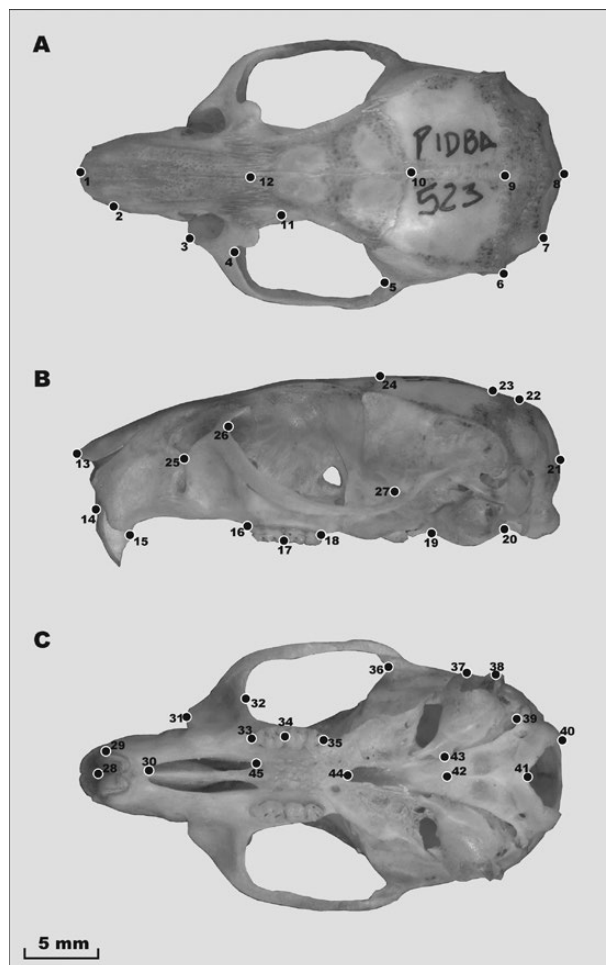


Fig. 2.—Landmarks positioning for geometric morphometric analysis for the A) dorsal, B) lateral, and C) ventral views of the skull of large- and middle-sized *Calomys*. Landmark definition is shown in [Table 1](#).

analyzed was selected by measuring the correlation between the matrix of Procrustes shape distances in the full shape space and pairwise Euclidean distances in the reduced shape space (Cardini et al. 2007). The correlations were carried out using the first 3 principal components, then the first 5, and increasing so on by 5. As we obtained highly significant correlations using the first 10 principal components (that accounted for 82–89% of variance), we performed subsequent analysis using the first 10 principal components. The variance accounted for by species and ecogeographic differences was estimated by linear models.

The PCs of the covariance matrix of superimposition residuals were used as new shape variables, thus reducing the dimensionality of the data set and providing orthogonal variables. A pooled PCA using previous PC scores (i.e., the first 10 PCs for each view) from the 3 separate views (dorsal, ventral, and lateral) (Fernandes et al. 2009) was carried out to explore interspecific shape differences. A Canonical Variate Analysis (CVA) was performed using the first 5 principal components of the combined PCA (60% of variation) in order to consider the main axis of variation explained by the 3 cranial views. Rates of correct classification were estimated by comparing

Table 1.—Definition of landmarks used in 3 cranial views. See Fig. 2 for more details.

Landmark	Definition
Dorsal view	1. Rostralmost point of the nasal bones
	2. Intersection of the rostral curvature of the nasal process of the incisive and the nasal bones in a dorsal projection
	3. Rostralmost point of the zygomatic plate
	4. Rostralmost point of the zygomatic arch
	5. Caudalmost point of the orbit
	6. Intersection of the parietal-interparietal and interparietal-occipital sutures
	7. Lattermost point of the occipital
	8. Caudal end of the curvature of the occipital bone
	9. Intersection of the sagittal and parietal-interparietal sutures
	10. Intersection of the coronal and sagittal sutures
	11. Narrowest point of the interorbital region
	12. Intersection of the nasofrontal suture in the midline
Lateral view	13. Rostralmost point of nasal
	14. Inner extreme point of incisor at body of premaxillary bone
	15. Point at intersection between premaxillary and posterior end of incisive alveolus
	16. Rostralmost point of molar row on alveolar process of maxilla
	17. Intersection between first molar and second molar
	18. Caudalmost point of molar row on alveolar process of maxilla
	19. Rostralmost point of the tympanic bulla at the intersection with the squamosal
	20. Caudalmost point of the tympanic bulla at the intersection with the occipital
	21. Caudalmost point of the occipital
	22. Intersection between occipital and interparietal
	23. Intersection between interparietal and parietal
	24. Intersection between parietal and frontal
	25. Rostralmost point of zygomatic plate
	26. Rostralmost point of the orbit
	27. Caudalmost point of the orbit
Ventral view	28. Rostralmost point of the upper incisors next to the midline
	29. Lateralmost point of the incisive alveolus
	30. Rostralmost point of the rostral palatine fissure
	31. Rostralmost point of the zygomatic plate
	32. Rostralmost point of the orbit
	33. Rostralmost point of the molar row
	34. Intersection between the first molar and second molar at the midline
	35. Caudalmost point of the molar row
	36. Caudalmost point of the orbit
	37. Rostral end of the external opening of the bony auditory canal
	38. Caudal end of the external opening of the bony auditory canal
	39. Caudalmost point of the intersection between the tympanic bulla and jugular foramen
	40. Lateralmost point of the foramen magnum
	41. Rostralmost point of the foramen magnum
	42. Intersection at the midline between basioccipital and basisphenoid
	43. Suture between basisphenoid and basioccipital where it contacts the tympanic bulla
	44. Caudalmost point of the suture between palatine bones and the rostral border of the mesopterygoid fossa
	45. Caudalmost point of the palatine fissure

the a priori assignments with those based on the CVA. Each specimen was assigned to a given species based on the minimum Mahalanobis distance to the group mean. Group assignments were cross-validated by a jackknife resampling routine. Procrustes distances between species and associated *P* values based on 10,000 permutations were estimated using program MorphoJ (Klingenberg 2011). The same analyses were carried out for each cranial view independently.

Allometric effects on shape variation were examined in each species by multivariate regression of Procrustes shape variables on log centroid size using 10,000 permutations against the null hypothesis of independence between size and shape. The amount of allometric shape variation was quantified as a percentage of the total shape variation. Because vectors such as

PCs, regression vectors, etc., correspond to directions in shape tangent space, a straightforward method to compare such vectors is to compute the angle between them (Cheverud 1982; Klingenberg and Marugán-Lobón 2013). This was inferred using the arccosine of the inner product of the 2 vector elements under comparison. Within MorphoJ (Klingenberg and Marugán-Lobón 2013), vector angles were tested against the null hypothesis that within the shape tangent space, the vectors would have random directions.

RESULTS

Results of the ANOVA indicated significant sexual size dimorphism in 2 of the 3 species: *C. callosus* and *C. venustus*

Table 2.—Statistical tests for sexual dimorphism. Sexual dimorphism in size (SSD) and shape (SShD) were evaluated by analysis of variance and T^2 statistics, respectively. Cb = *C. boliviae*; Cc = *C. callosus*; Cv = *C. venustus*.

Cranial view	Species	SSD F	P (N)	SShD Procrustes distance	$T^2 P$ (N)
Dorsal	Cb	0.398	0.563 (6)	0.026	0.82 (6)
	Cc	8.323	< 0.01 (52)	0.012	0.653 (52)
	Cv	29.17	< 0.01 (57)	0.014	0.051 (57)
Lateral	Cb	0.591	0.485 (6)	0.028	0.538 (6)
	Cc	5.673	0.021 (48)	0.009	0.531 (48)
	Cv	22.93	< 0.01 (57)	0.015	0.035 (57)
Ventral	Cb	0.318	0.629 (4)	0.025	1 (4)
	Cc	8.62	0.005 (48)	0.009	0.275 (48)
	Cv	23.41	< 0.01 (57)	0.014	< 0.001 (57)

(Table 2). In both cases, males tended to be larger than females. Sexual differences in skull shape were significant only in *C. venustus*, in which the lateral and ventral views of skull indicated sexual dimorphism (Table 2). Results of the ANOVA revealed significant differences ($F_{2,155} = 25.82$, $P < 0.01$) in skull size across the species of *Calomys* studied. Species differences accounted for 24% ($P < 0.01$) of the total skull size variation. We found significant differences between the larger *C. venustus* and the smaller *C. callosus* ($P < 0.01$). *Calomys boliviae* presented the largest skull among the 3 species ($P < 0.01$ for the comparison with *C. callosus* and $P < 0.01$ for the comparison with *C. venustus*) (Fig. 3). Differences among ecoregions accounted for less than 10% of the variation in the total centroid size. On the other hand, species and ecoregions, considered jointly, did not improve the percentage of variance explained (Table 3). We did not find evidence for ecogeographic differences at the intraspecific level in *C. boliviae* and *C. callosus*. In the latter species, individuals from the Dry Chaco tended to have a larger skull (Fig. 3) (Supporting Information S2). When each cranial view was considered independently, species differences accounted for almost 30% ($P < 0.01$) of the total skull size variation in the 3 species. However, this percentage was lower for the centroid size of the ventral cranial view (17.90%, $P < 0.01$) (Table 3). Ecoregions accounted for almost 16% of total size variation in dorsal and lateral views, but ventral centroid size was not significant. Although significant, the percentages of variation explained did not improve when species differentiation and ecoregions were considered jointly (Table 3).

The PCA of the combined matrix of shape variables from the 3 cranial views is shown in Fig. 4. The first principal component (28.24% of total variation) separated *C. venustus* from *C. boliviae*. The second principal component (13.90% of total variation) showed differentiation among the specimens of *C. callosus* and *C. venustus*.

Overall, the CVA (Fig. 5) correctly classified species 81% of the time (Table 4). Interspecific differences accounted for 7.93% of total shape variation in dorsal view, while this percentage reached 16.4% for the lateral view. Species differences for the ventral view accounted for 12.5% (Table 3). We obtained better discrimination between species using the lateral and ventral views (Supporting Information S3). Procrustes mean shape distances suggested significant differences between species. *Calomys boliviae* and *C. callosus* were most similar

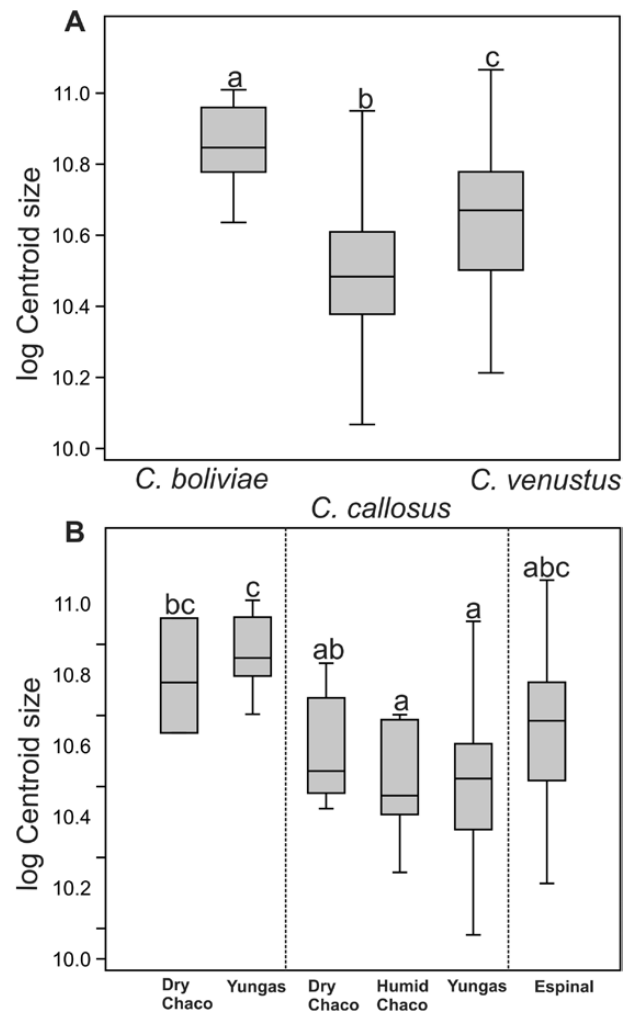


Fig. 3.—Box-and-whisker plot showing the A) interspecific and B) intraspecific differences and ranges of variation in the sum of centroid sizes of the 3 cranial views. The boxes delimit the 25th and 75th quartiles, the horizontal bar indicates the median, and the whiskers extend to the most extreme data points. Letters indicate statistical differences at $P < 0.05$.

with respect to dorsal and lateral views of the skull (0.0145, $P = 0.019$ and 0.0251, $P < 0.01$, respectively); while *C. callosus* and *C. venustus* were the most similar based on the ventral view (0.0174, $P < 0.01$). Ecoregions accounted for percentages of shape variation similar to those of the interspecific differences (Table 3). Moreover, when both sources of variation

Table 3.—Percentage of variance (R^2) accounted by species and ecoregion differences for size and shape variables derived from geometric morphometrics.

Variable	N	Species		Ecoregions		Species × ecoregions	
		% R^2	P	% R^2	P	% R^2	P
Dorsal centroid size	177	31.02	< 0.01	15.95	< 0.01	31.46	< 0.01
Lateral centroid size	175	31.45	< 0.01	16.20	< 0.01	33.47	< 0.01
Ventral centroid size	171	17.90	< 0.01	2.69	0.21	19.34	< 0.01
Centroid size sum	158	24.03	< 0.01	9.33	< 0.01	23.56	< 0.01
Dorsal shape	177	7.93	< 0.01	7.77	< 0.01	10.88	< 0.01
Lateral shape	175	16.42	< 0.01	16.96	< 0.01	20.82	< 0.01
Ventral shape	171	12.52	< 0.01	12.15	< 0.01	16.01	< 0.01
Dorsal size-free shape ^a	177	14.09	< 0.01	13.68	< 0.01	16.92	< 0.01
Lateral size-free shape ^a	175	23.85	< 0.01	26.46	< 0.01	28.71	< 0.01
Ventral size-free shape ^a	171	11.86	< 0.01	14.60	< 0.01	15.71	< 0.01

^a Size-free shape refers to shape variables whose allometric effect was removed.

(interspecific and ecogeographical differences) were taken into account, the percentages of variance explained improved significantly (Table 3).

Multivariate regression of shape on log centroid size revealed that shape changes were significantly related with changes in size for *C. callosus* and *C. venustus* (Fig. 6). Allometry in *C. callosus* explained 7%, 15%, and 14% of the total shape variation in dorsal, lateral, and ventral landmark configurations, respectively. The percentage of allometry in *C. venustus* was always greater than 20% in the 3 cranial views (Table 5). In general, with increasing skull size, the braincase became more slender and the rostrum (e.g., nasals) became more elongated (Fig. 6). The variance accounted by interspecific and ecogeographical differences improved when size-free shape variables were considered, especially for the dorsal and lateral cranial views (Table 3). Correlations between regression vectors among species were highly significant for the lateral and ventral cranial views; *C. callosus* and *C. venustus* had similar allometric trajectories (Table 5), whereas *C. boliviae* had an allometric trajectory different than those of the other 2 species.

DISCUSSION

In an effort to clarify the taxonomic richness of medium- and large-sized *Calomys* species in the SCA, we performed an integrated morphometric analysis. Our results suggest the presence of 3 morphologically and morphometrically distinct species of *Calomys*: 2 medium-sized and 1 large species. The morphological similarity of medium- and large-bodied *Calomys* species is remarkable (Almeida et al. 2007). The identification and taxonomy of *Calomys* species inhabiting northern and central Argentina, specially the forms called *boliviae*, *callosus*, *callidus*, *fecundus*, and *venustus*, is difficult. In our study, we assess *C. boliviae*, *C. callosus*, and *C. venustus*, and we consider *C. fecundus* as a synonym of *C. boliviae* (Salazar-Bravo et al. 2003; Díaz et al. 2006; Díaz and Barquez 2007). We did not study *C. callidus* because the distribution area of this species does not reach the SCA territory.

We have been able to corroborate the presence of *C. boliviae* and *C. callosus* as widely distributed species in the SCA

and also confirm the presence of a third species, *C. venustus*, in central Argentina. Salazar-Bravo et al. (2001) showed that, according to molecular evidence, these are 3 different and well-defined species.

Body size is important in discriminating between species (e.g., Cardini and Elton 2008; Cordeiro-Estrela et al. 2008; Elton et al. 2010; Martínez and di Cola 2011). Our geometric morphometric assessment indicates that 24% of total variation in skull centroid size is due to species differences. *C. boliviae* is the largest among the species studied, followed by *C. venustus*. Size differences were subtle but statistically significant between *C. venustus* and *C. callosus*. Our PCA revealed that cranial depth and orbital length may be useful characters to discriminate between species. However, it is important to note that use of size to discriminate among species is dependent on whether the differences are a result of isometry or allometry (Cordeiro-Estrela et al. 2006, 2008).

Skull shape is also important in species discrimination for *Calomys* (Cordeiro-Estrela et al. 2006, 2008). The relative contribution of interspecific differences to the total shape variation was lower than that of size and different among the cranial views analyzed. In lateral and ventral views, they accounted for more than 10%, while for the dorsal view the percentage was lower. Values improved when we controlled for allometry, indicating that size has an effect on shape differences for these species. Cordeiro-Estrela et al. (2006) reported negligible effects of size on allometric shape differentiation in 2 Brazilian species of *Calomys* (*C. expulsus* and *C. tener*). However, they attributed their small allometric effects on the absence of landmarks in the rostrum of the studied specimens. The authors based their conclusion on a study of laboratory-bred specimens that found divergent allometric patterns in the rostrum between the 2 species during ontogeny (Araripe 2000). We included landmarks on the rostrum and found significant allometric effects, and were able to discriminate among species as did Cordeiro-Estrela et al. (2008) for 3 species of *Calomys* from central Argentina (2 small- and 1 middle-sized species). Shape changes in the skull, attributable to increased body size, involve a reduction in the breadth of the skull, with a reduction in cranial depth and an elongation of the rostrum. Similar allometric

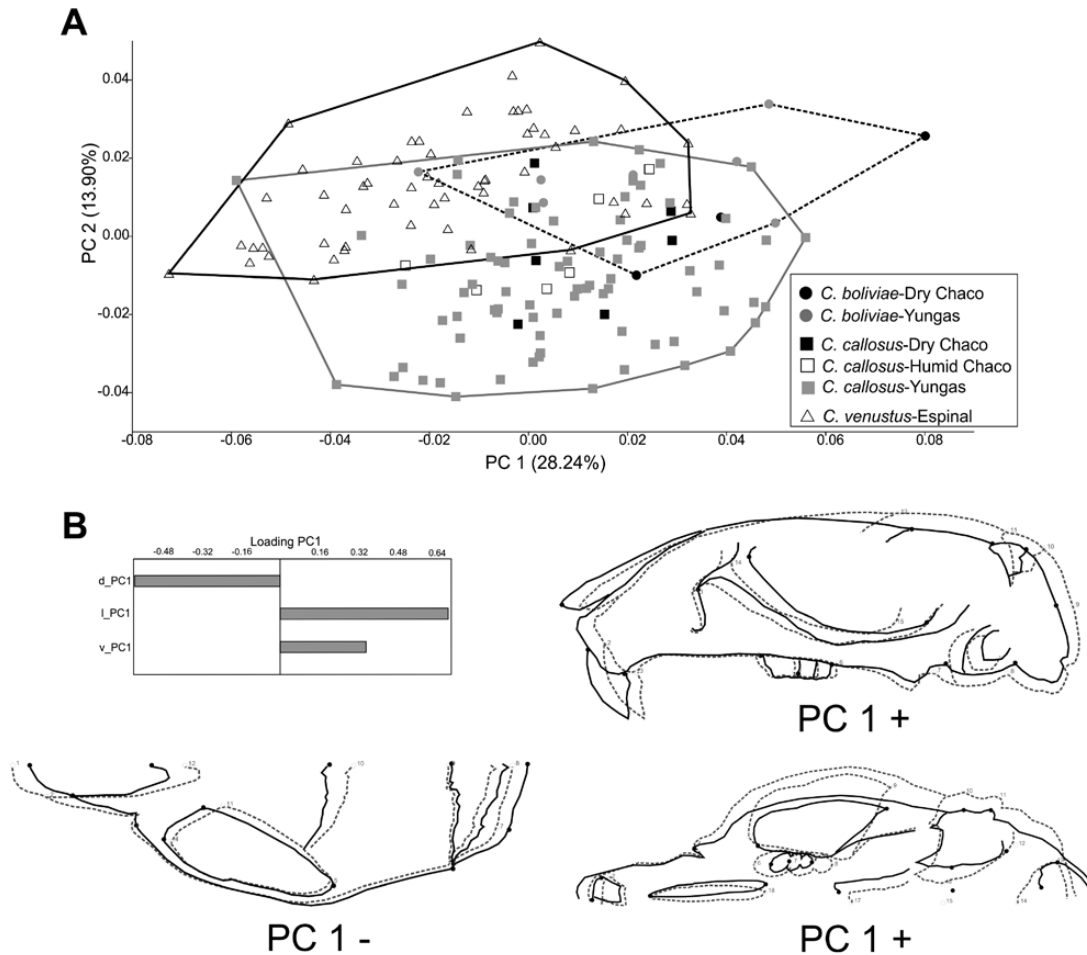


Fig. 4.—A) Principal Component Analysis for the combined data set of shape variables for 3 cranial views of large- and middle-sized *Calomys* skulls. The smallest convex polygon that envelops the range variation of each species is depicted. B) Loadings of the main axis of variation for the 3 cranial views and shape changes associated with the main axis of variation. The mean shapes are illustrated with gray dotted lines while shape changes corresponding to the main axis of variation are illustrated with solid black lines.

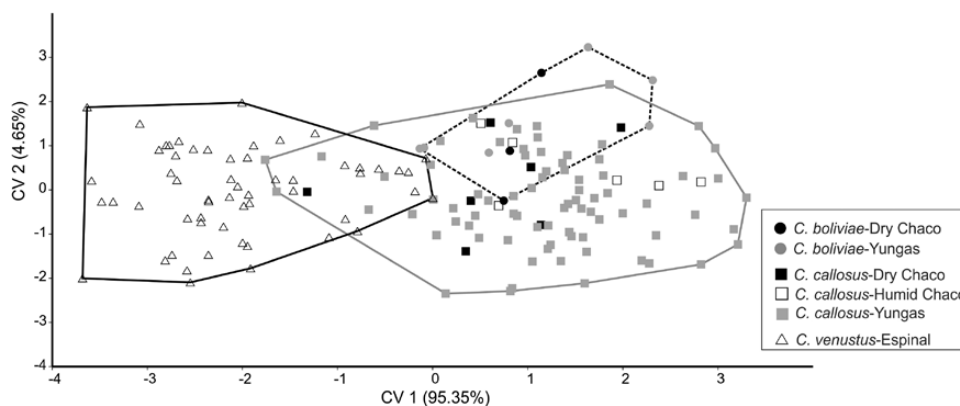


Fig. 5.—Canonical Variates Analysis for the combined data set of shape variables of the 3 cranial views of large- and middle-sized *Calomys* skulls. The minimum convex polygon of each species is depicted.

patterns were found in 2 small-sized species, *C. musculinus* and *C. laucha* (Cordeiro-Estrela et al. 2008).

Similar developmental mechanisms may lead to convergences in both morphology and modularity. For example, *C. callosus* and *C. venustus* share a similar static allometric trajectory that suggests similar patterns of skull integration. As noted by

Cheverud (1996) and Wagner and Altenberg (1996), different external selective pressures might be another source of dissimilarity in integration patterns across species. In other words, selection may mold gene networks underlying groups of functionally related traits to yield genetically integrated modules that respond to selection as a single unit. Integration patterns

do not constrain morphological evolution over long time scales. Moreover, integration patterns influence a species' response to shifting selection pressures over ecological time scales (Sanger et al. 2012).

Our distributional data indicate that *C. boliviae* and *C. callosus* are sympatric species. Actually, both species have been recorded in 7 localities in the provinces of Jujuy, Salta, and Tucumán (see Appendix I). Moreover, phylogenetically close species with similar ecological requirements tend to differ morphologically as a result of character displacement, presumably to minimize effects of interspecific competition (Brown and Wilson 1956). In this sense, these 2 species differ in skull size variation, but the skull shape differentiation was subtle. Astonishingly, differences in the skull size between these 2 species were more pronounced in the Yungas ecoregion, whereas the interspecific differences were subtle and not significant in the Dry Chaco ecoregion. Skull shape differences between

species when considering ecoregions tend to be subtle and there is not a clear pattern of character displacement. According to some authors (Klingenberg and Leamy 2001; Workman et al. 2002), size is a more labile variable than shape to ecological and environmental factors, whereas shape is more resilient to changes in the environment (Breuker et al. 2008; Debat et al. 2009; Martínez et al. 2014). It is possible then, that *C. boliviae* and *C. callosus* may be avoiding competition through character displacement via size, a hypothesis that deserves further research.

Our results support the hypothesis that *C. venustus* in central Argentina is a distinct lineage from *C. callosus*. Musser and Carleton (1993) considered *C. venustus* to be a synonym of *C. callosus*, but Olds (1988) and Anderson (1997) treated *C. callosus* and *C. venustus* as distinct species. Olds (1988) considered *C. boliviae* to be synonym of *C. venustus*, implying that *C. venustus* is larger than *C. callosus*. The validity of *C. venustus* is supported by morphological, cytogenetic, and molecular evidence (Gardenal et al. 1977; Olds 1988; Salazar-Bravo et al. 2001). Salazar-Bravo et al. (2002) suggested that this species is limited to the Espinal ecoregion.

However, *C. venustus* occurs in the Espinal and southern Chacoan habitats in central Argentina (Córdoba, San Luis, and Santiago del Estero provinces—Salazar-Bravo 2015) and is documented in the provinces of Jujuy, Salta, and Tucumán by some authors (e.g., Díaz and Barquez 2007). Our Argentinean records correspond to the Espinal ecoregion and are located in the province of Córdoba.

Table 4.—Geometric morphometric classification rates obtained from Canonical Variate Analysis using jackknife cross-validation method. Percentages of correct classification are shown between parentheses.

Species	N	Predicted membership		
		<i>C. boliviae</i>	<i>C. callosus</i>	<i>C. venustus</i>
<i>C. boliviae</i>	10	8 (80%)	2	0
<i>C. callosus</i>	91	21	66 (72.53%)	4
<i>C. venustus</i>	57	1	2	54 (94.74%)

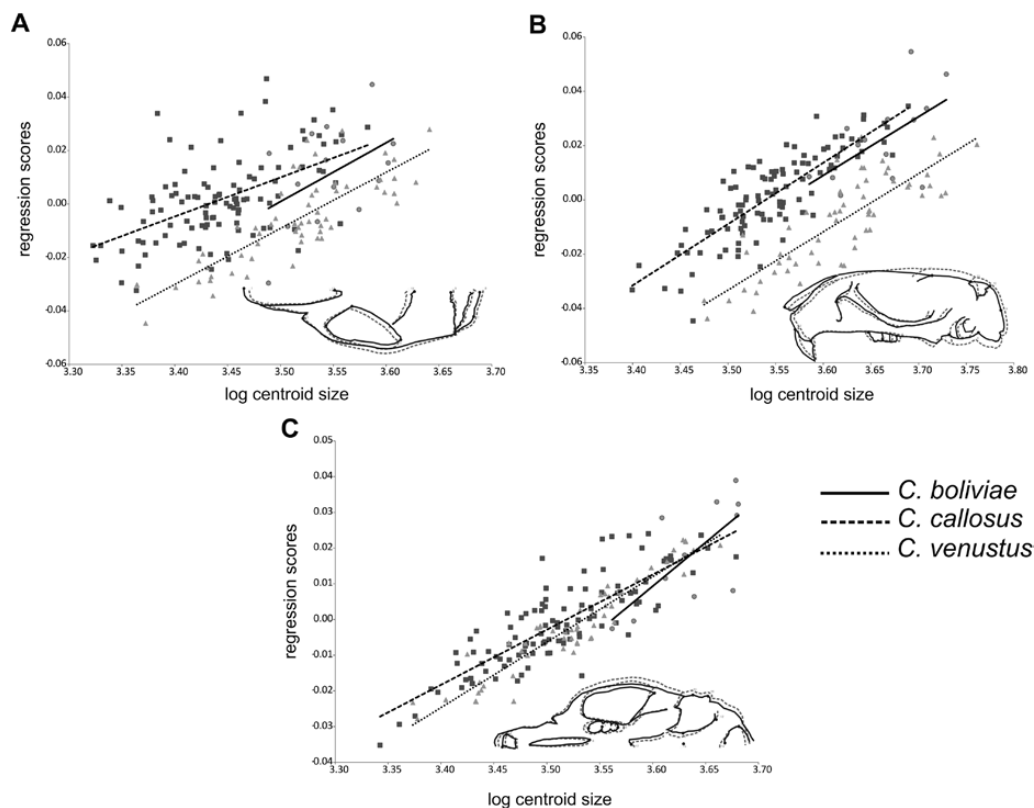


Fig. 6.—Allometric shape variation in the 3 species of *Calomys* for each cranial view. Shape changes corresponding to the regression vector are displayed with continuous black lines. Mean shape is displayed with gray dotted lines: A) dorsal, B) lateral, and C) ventral cranial views.

Table 5.—Statistical tests for allometry in *Calomys*. The percentage of allometry, the amount of shape variation explained by size variation, in the 3 species of *Calomys* for the 3 cranial views is shown. *P* values for the test of allometry against the hypothesis of no relationship between shape and size. The angles (below the diagonal) and their statistical significance (above the diagonal) against the null hypothesis that the regression vectors have random directions in the shape tangent space are shown. Cb = *C. boliviae*; Cc = *C. callosus*; Cv = *C. venustus*.

Cranial view	Species	Allometry, %	<i>P</i>	Cb	Cc	Cv
Dorsal	Cb	10.70	0.147		< 0.01	< 0.01
	Cc	7.31	< 0.01	39.91°		< 0.01
	Cv	22.22	< 0.01	45.02°	26.76°	
Lateral	Cb	12.35	0.093		< 0.01	< 0.01
	Cc	14.74	< 0.01	39.49°		< 0.01
	Cv	22.52	< 0.01	37.38°	22.73°	
Ventral	Cb	18.83	0.062		< 0.01	< 0.01
	Cc	14.34	< 0.01	28.58°		< 0.01
	Cv	33.38	< 0.01	32.31°	24.22°	

Until now, morphological characters in these *Calomys* species were insufficient to distinguish between them (Salazar-Bravo 2015). In fact, it is not always possible to ascertain in the published literature which species was actually studied, as the medium- and large-sized species of *Calomys* from southern Bolivia and northern Argentina share many morphological characters, and because they are often sympatric.

Distributional analyses based only on published occurrence localities may be problematic. For example, Salazar-Bravo (2015) noted that in his distributional assessment for *C. venustus*, he only included individuals identified on the basis of chromosomal and molecular and/or biochemical data. This suggests not only that the species’ taxonomic limits of medium- and large-sized *Calomys* from southern Bolivia and northern Argentina are not well known but also the geographic limits of these species are poorly defined (Salazar-Bravo 2015).

Our work provides some taxonomic resolution for 3 species of *Calomys* using cranial morphometrics. We corroborate the presence of a large amount of intraspecific variation and a substantial overlap for species in morphospace. Although statistically, discrimination between close species can be achieved using multivariate shape and size data, diagnostic features useful for field biologists are elusive (Tabatabaei Yazdi and Adriaens 2013). Multiple lines of evidence (i.e., molecular, cytogenetic, and morphological) are needed to achieve supported species boundaries and provide a better resolution of species relationships and to clarify taxonomic issues.

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SUPPORTING INFORMATION

The Supporting Information document is linked to this manuscript and is available at Journal of Mammalogy online (jmmal.oxfordjournals.org). The material consists of data provided by the author that is published to benefit the reader. The posted material is not copyedited. The contents of all supporting data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Supporting Information S1.—Sample size for morphometric variation at interspecific level in *Calomys* from the southern central Andes.

Supporting Information S2.—Box-and-whisker plot showing the interspecific differences and ranges of variation in centroid size for the 3 cranial views -dorsal, ventral, and lateral- independently.

Supporting Information S3.—Principal Component Analysis for the shape variables of the cranial views of large- and middle-sized *Calomys*’ skulls. Convex hulls for the species are depicted in each case. Shape changes associated with the first principal component are depicted along the axis.

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APPENDIX I

List of used specimens of *Calomys* species included in this study. Each species specimens are alphabetically arranged according to the province, the department, and the locality from which they come. After the name of the species, country, province, and department, the number of specimens is indicated in parentheses. Following each locality, the number of specimens is shown, and the acronym of the museum or collector and catalogue number assigned to each specimen are provided in parentheses.

Order Rodentia: Suborder Sciurognathi: Family Cricetidae: Subfamily Sigmodontinae: Tribe Phyllotini:

Calomys boliviae (15):

ARGENTINA (11).

JUJUY (5): EL CARMEN (3): Along Hwy 9 at border with Salta province, 4,600 ft, 2 (CML 3600, 3601); Arroyo El Cano, 7 km N Río El Saladillo, 1 (MD 28); LEDESMA (2): 12 km W Fraile Pintado sobre Río Ledesma, 1 (CML 8307); 13 km SW Yuto, sobre Arroyo Yuto, 1 (CML 9246). SALTA (4): ANTA (1): Los Colorados, 17 km E Santo Domingo, 3 (CML 3014); ORÁN (3): Abra Grande, 1 (CML 5860); Ingenio San Martín del Tabacal, 1 (CML 5951); Ingenio San Martín del Tabacal, Lote “Milagros,” 1 (CML 5853).

TUCUMÁN (2): BURRUYACÚ (2): Finca El Jagüel, a 4.5 km ruta provincial 304, camino a Requielme, 1 (CML 9249); Río Loro, 1 (CML 608).

BOLIVIA (4).

CHUQUISACA (4): 2 km SW Monteagudo, 1,130 m, 1 (CBF 1074); Laguna Seca, 14 km E El Salvador, 1 (CML 3073); Río Limón, 1,300 m, 2 (CBF 1082, 1091).

Calomys callosus (115)

ARGENTINA (115)

SIN LOCALIDAD ESPECÍFICA, 1 (CML 2243).

FORMOSA (9): PATIÑO (7): Cruce entre ruta 95 y riacho Pilagá, 7 km N cruce rutas 81 y 95, 4 (LIF 76, 77, 84, 90); Estancia Paguazú, 8 km N cruce rutas 81 y 95 y 6 km E, 2 (LIF 100, 104); Estancia Santa Catalina, 5 km Cogoy, 1 (CML 2066); PILCOMAYO (2): Ruta provincial 3 entre Tres Lagunas y Laguna Gallo, app. 12 km W Tres Lagunas, 2 (CML 8376, 8377).

JUJUY (17): DR. MANUEL BELGRANO (1): Río Las Capillas, 15 km N Las Capillas por ruta 20, 957 m, 1 (CML 4325); EL CARMEN (1): Límite entre Salta y Jujuy, Abra de Santa Laura, sobre ruta nacional 9, 1,397 m, 1 (CML 9272); LEDESMA (11): 12 km W Fraile Pintado sobre Río Ledesma, 2 (CML 9265, 9266); 13 km SW Yuto, sobre Arroyo Yuto, 5 (CML 9245, 9247, 9261, 9263, 9264); 2.5 km W El Bananal, 437 m, 3 (CML 9258, 9259, 9260); Parque Nacional Calilegua, Arroyo Sauzalito, 1 (CML 2937); SANTA BÁRBARA (4): Laguna La Brea, 1 (CML 9271); Laguna La Brea, sobre ruta 1, 3 km SW camino a Las Siete Aguas, 3 (CML 9268, 9269, 9270).

SALTA (36): ANTA (6): Los Colorados, 17 km E Santo Domingo, 4 (CML 3013, 3015, 3062, 3069); Parque Nacional El Rey, 2 (CML 604, 664); CANDELARIA (1): Río Los Sauces, 12 km NW del Jardín, ruta 6, 1 (LIF 191); GENERAL GÜEMES (3): Cabeza de Buey, Campo La Peña, 3 (CML 672, 874, 897); GENERAL JOSÉ DE SAN MARTÍN (3): Tartagal, Laguna de las Catas, 4 km E Itaú, 2 (CML 2339, 2340, 2341); METÁN (3): 15 km N Metán por ruta 9 y 5 km W, 986 m, 2 (CML 8338, 8381); Río Las Conchas, 5.7 km W Metán, sobre el río, 996 m, 1 (CML 9276); ORÁN (19): 43.7 km NW cruce rutas 50 y 18, camino a Isla de Cañas, 789 m, 2 (CML 9307, 9339); Abra Grande, 5 (CML 5892, 5952, 5954, 5955, 5956); El Oculito, ruta provincial 18, km 25, sobre Río Blanco (camino a San Andrés), 1 (LIF 784); Ingenio San Martín del Tabacal, 6 (CML 5569, 5570, 5571, 5953, 5957,

7347); Ingenio San Martín del Tabacal, Lote "Milagros," 5 (CML 5845, 5850, 5855, 5857, 5858); ROSARIO DE LA FRONTERA: Bonete Punco, 13 km S Los Baños por ruta provincial 13 S, 817 m, 1 (CML 8382).

SANTIAGO DEL ESTERO (4): ALBERDI (1): 1 km S, 2 km E Pampa de los Guanacos, 1 (CML 3599); CHOYA (3): Salinas de Ambargasta, app. 8 km SE Cerro Rico, 141 m, 2 (CML 9277, 9278, 9279).

TUCUMÁN (48): BURRUYACÚ (6): Alrededores Dique El Cajón, 2 km N, 712 m, 1 (CML 9281); Finca El Jagüel, a 4.5 km ruta provincial 304, camino a Requielme, 2 (CML 9250, 9282); Piedra Tendida, 8 km W Dique El Cajón, 3 (CML 5978, 9251, 9286); LA COCHA (11): Dique San Ignacio, 5 (CML 2887, 2888, 2889, 2890, 2900); Dique San Ignacio, 580 m, 3 (CML 7174, 7175, 7176); Dique San Ignacio, 589 m, 3 (CML 9290, 9291, 9292); MONTEROS (1): Reserva Provincial La Florida, 7 km W Ibatín, sobre Río Pueblo Viejo, 1 (CML 9293); TAFÍ VIEJO (15): 5 km SW Siambón, 3,100 ft, 9 (CML 7103, 7104, 7105, 7106, 8840, 9252, 9298, 9299, 9302); La Agüadita, 2 (CML 9336, 9337); Río Grande, 5 km S El Siambón, 920 m, 4 (CML 8378, 9253, 9254, 9297); TRANCAS (6): 12 km San Pedro de Colalao, Chulca, sobre Río Chulca, 1 (CML 6108); A 12 km San Pedro de Colalao, Chulca, sobre Río Chulca, 1 (CML 9303); Quebrada Río Chilca, 1 (CML 423); San Pedro de Colalao, 3 (CML 946, 947, 1588); YERBA BUENA (9): Horco Molle, 1 km residencias Universidad Nacional de Tucumán, predios Facultad de Agronomía y Zootecnia, 4 (CML 2283, 2287, 2305, 2324); Horco Molle, 650 m, 1 (CML 2809); Horco Molle, Reserva Flora y Fauna, ladera S y SE Cerro Abel Peirano, pastizal y arbustal, 1 (CML 2758); Horco Molle, Río Muerto, cultivo de maíz de López, 2 (CML 2752, 2753); Marcos Paz, 1 (CML 1378).

Calomys venustus (57)

ARGENTINA (57).

CÓRDOBA (57): Zona rural de la ciudad de Río Cuarto, 55 (CUNRC 27678, 27688, 27689, 27721, 27726, 27741, 27750, 27788, 27794, 27816, 27833, 27845, 27873, 27878, 27884, 27921, 27937, 27939, 27940, 27949, 27952, 27963, 27985, 27991, 27994, 28006, 28007, 28044, 28049, 28052, 28053, 28056, 28061, 28065, 28072, 28073, 28080, 28088, 28092, 28102, 28104, 28143, 28146, 28148, 28156, 28182, 28183, 28193, 28196, 28213, 28228, 28250, 28251, 28252, 28261); Coronel Moldes, 2 (28989, 28990).