ELSEVIER

Mammalian Biology
Zeitschrift für Säugetierkunde

www.elsevier.de/mambio

SHORT COMMUNICATION

Morphometrics of *Graomys* (Rodentia, Cricetidae) from Central-Western Argentina

Juan José Martínez[a,*], Jerónimo Matías Krapovickas[b], Gerardo Raúl Theiler[c]

[a]*Cátedra de Genética de Poblaciones y Evolución, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Av. Vélez Sarsfield 299 Córdoba, Argentina*
[b]*Cátedra de Paleontología, Sección Paleovertebrados, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Av. Vélez Sarsfield 1611 Córdoba, Argentina*
[c]*Cátedra de Introducción a la Física y Química Biológicas, Facultad de Odontología, Universidad Nacional de Córdoba, Pabellón Argentina, Ciudad Universitaria, Córdoba, Argentina*

Received 18 July 2008; accepted 3 November 2008

Keywords: *Graomys*; Cytogenetics; Morphometrics; Multivariate analysis; Argentina

The taxonomy and systematics of the sigmodontine rodents of the tribe Phyllotini are a matter of continuous revision (Braun 1993; Steppan 1993, 1995; Steppan et al. 2007). In this sense, the species of the genus *Graomys* (Thomas, 1916), with a wide geographical distribution range in South America, lack a comprehensive systematic revision.

Graomys centralis was originally described as a subspecies of *Eligmodontia* (*E. griseoflavus centralis*) by Thomas (1902), on the basis of five specimens from Cruz del Eje (Córdoba, Argentina). In this description, Thomas pointed out the lower size of tympanic bullas of *centralis* specimens in relation to the other subspecies of the genus, especially when compared to *cachinus* and *chacoensis*. However, Hershkovitz (1962) presented the species *cachinus*, *centralis*, *chacoensis*, *lockwoodi*, and *medius*, as synonyms of *griseoflavus*. Musser and Carleton (1993), however, indicated the species homogeneity as "highly suspicious".

Several works have shown that *G. griseoflavus* includes numerous cytotypes ($2n = 34$, 35, 36, 37, 38, 41, and 42) that are morphologically indistinguishable (Wainberg and Fronza 1974; Zambelli et al. 1994). The cytotypes $2n = 36$, 37, and 38 are karyotypically mixed populations and occur mainly in the Monte Desert and Patagonic steppe, whereas cytotype $2n = 42$ occurs in the Espinal and in Western Chaco ecoregions (Theiler et al. 1999).

Laboratory crossing tests demonstrated post-zygotic isolation between animals with $2n = 42$ and $2n = 36$–38. The reproductive isolation was clearly asymmetric; there was no copula between males $2n = 36$–38 and females $2n = 42$, whereas the reciprocal crossings (females $2n = 36$–38 $\times$ males $2n = 42$) produced viable sterile hybrid offspring with 39 or 40 chromosomes (Theiler and Blanco 1996a). Theiler and Blanco (1996b) also found reinforcement of this post-zygotic isolation by mechanisms involving olfactory discrimination during female estrus. Females discriminated males of compatible chromosome complement from those that would produce non-viable offspring or sterile hybrids. On the basis of these evidences, Theiler and Blanco (1996b) concluded that $2n = 42$ cytotype and $2n = 36$–38 complex are two sibling species.

Phylogenetic studies based on *Cyt b* and *D-loop* fragments of the mtDNA showed that animals with $2n = 41$ and $2n = 42$ form a separate clade from individuals with $2n = 34$–38, the latter being included in another well-supported group (Catanesi et al. 2002,

*Corresponding author.
E-mail address: juan_jmart@yahoo.com.ar (J.J. Martínez).

1616-5047/$ - see front matter
doi:10.1016/j.mambio.2008.11.003

2006). This result confirms the existence of at least two sibling species.

Several authors (Theiler 1997; Tiranti 1998; Theiler et al. 1999) described $2n = 42$ cytotypes in individuals found in localities near Cruz del Eje, the site where the type specimens of *Graomys centralis* Thomas (1902) was obtained.

Graomys centralis was validated on the basis of concrete evidence (Theiler 1997; Musser and Carleton 2005; Díaz et al. 2006). Rodents from the *Graomys* complex showing a $2n = 42$ cytotypes were included in this taxon. However, the morphometric variation of this species from *G. griseoflavus*, has never been analyzed. In this study, we present the first comparative morphometric study of specimens of the species *G. centralis* and *G. griseoflavus*. We also examine the diploid chromosome number of specimens from Cruz del Eje, type locality for *G. centralis*.

Five individuals (two males and three females) were live-trapped in a woody area located about 8 km from Cruz del Eje (30°43′S, 64°49′W; Córdoba province). The karyotypes of these specimens were obtained according to Theiler and Blanco (1996a).

Forty-three adult skulls (M3 erupted) belonging to previously karyotyped *G. centralis* and *G. griseoflavus* specimens (Theiler 1997) were examined; 23 additional skulls of non-karyotyped specimens were included for subsequent identification. All the examined specimens are listed below, indicating locality and catalogue number. The localities are shown in Fig. 1. The karyotyped specimens have not been accessioned yet and are currently identified with the collector's initials (GRT, Gerardo R Theiler). The non-karyotyped specimens identified as *G. griseoflavus* are deposited in the Universidad Nacional de Río Cuarto collection (CUNRC), Grupo de Investigaciones en Ecología de Poblaciones (GIEP), Universidad Nacional de Río Cuarto, Río Cuarto, Argentina.

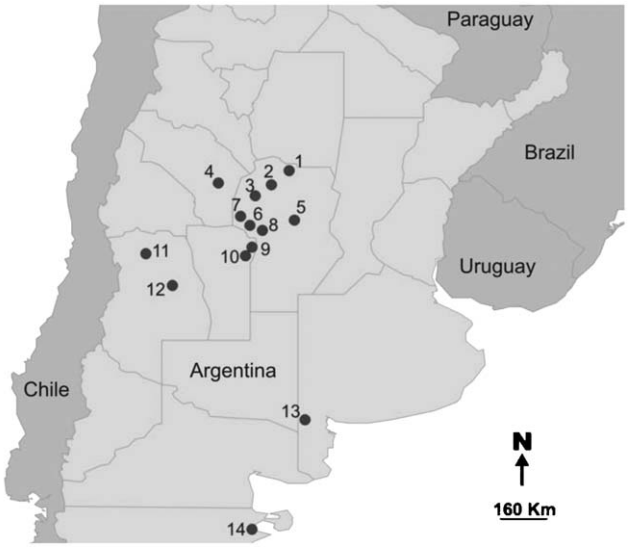

Fig. 1. Location of *Graomys* specimens examined. The localities were (1) Villa de María de Río Seco, (2) Déan Funes, (3) Cruz del Eje, (4) Chamical, (5) Santiago Temple, (6) Villa Dolores, (7) San Vicente, (8) Yacanto, (9) Papagayos, (10) Villa del Carmen, (11) Mendoza, (12) Ñacuñán, (13) Médanos, and (14) Puerto Madryn.

Specimens examined: *Graomys centralis* $2n = 42$, (20). Argentina: Chamical, La Rioja (GRT 024, 025, 026, 027, 028, 041, CUNRC h10, CUNRC m20); Villa de María de Río Seco, Córdoba (GRT 029. 030); Deán Funes, Córdoba (GRT 037, 038, 039); Santiago Temple, Córdoba (GRT 035, 036); Cruz del Eje, Córdoba (GRT 031, 032, 033, 034, 040).

Graomys griseoflavus $2n = 36$–38, (23). Argentina: Papagayos, San Luis (GRT 001, 002, 003); Mendoza, Mendoza (GRT 010, 011, 012, 013, 014, 015); Ñacuñán, Mendoza (GRT 005, 006, 007, 008, 009); Médanos, Buenos Aires (GRT 016, 017, 018, 019, 020, 021, 022, 023); Puerto Madryn, Chubut (GRT 004).

Non-karyotyped specimens (24). Argentina: Villa de María de Río Seco, Córdoba (CUNRC 1522, 1523, 1524, 1526, 4004, 4005, 4006, 4047, 4052, 4066, 4137); San Vicente, Córdoba (CUNRC, 2354), Yacanto, Córdoba (CUNRC 2387, 2816); Villa Dolores, Córdoba (CUNRC 50103); Cruz del Eje, Córdoba (CUNRC 2178, 2180, 2195, 4264, 4283, 4952, 4953, 4954, 44781);Villa del Carmen, San Luis (CUNRC 43462).

We also made univariate and multivariate analyses using 19 cranial measurements that were taken with a caliper to the nearest 0.02 mm. The measurements are as follows: Greatest length of skull (GLS), basal length (BL), palatal length (PL), diastema length (DL), zygomatic breadth (ZB), least interorbital breadth (LIB), breadth of braincase (BB), rostral breadth (RB), nasal length (NL), nasal width (NW), length of incisive foramen (LIF), length of maxillary tooth row (LM), length of mandibulary tooth row (Lm), mastoidal breadth (MB), postpalatal length (PPL), length between molars (LBM), condylo-first molar length (C1ML), length of tympanic bulla (LTB), and width of tympanic bulla (WTB).

A *t*-test was performed to evaluate univariate mean differences between the species across the variables. A principal components analysis (PCA) was performed on the correlation matrix to characterize the morphological variation among the karyotyped and non-karyotyped specimens. The first eight principal components (92% of total variation) were used for a discriminant analysis to obtain the rates of correct classification of previously karyotyped animals; the values of discriminant function were used to examine the classification of non-karyotyped animals. Statistical analyses were performed with Infostat (2008) and PAST (Hammer et al. 2001).

The cytogenetic study showed that all individuals examined from Cruz del Eje had similar karyotypes with

42 chromosomes (Fig. 2). Pairs 1–17 are acrocentric decreasing in size. Pair 18 is medium-sized submetacentric chromosomes, and pairs 19 and 20 are composed of small submetacentric chromosomes. The X chromosome is a large submetacentric and Y is a small acrocentric. Starting from the cytotype $2n = 42$ in *G. centralis*, two centric fusions (15–17 and 16–18) originated cytotype $2n = 38$. A third fusion involving chromosomes 1 and 6 produced cytotype $2n = 36$. The $2n = 37$ cytotype is the heterozygote for 1–6 Robertsonian fusion. Finally, a fourth centric fusion has been described involving chromosomes 2 and 5, yielding cytotypes $2n = 34$ and $2n = 35$ in heterozygotes (Zambelli et al. 1994; Theiler 1997).

Fourteen of 19 measurements differed significantly and were greater in *G. griseoflavus* than in *G. centralis*. On the other hand, measurements from the type *G. centralis* Thomas (1902) were more similar to those of specimens with $2n = 42$ (classified by us as *G. centralis*) than to measurements of *G. griseoflavus* specimens ($2n = 36$–38) (Table 1).

The first three principal components (PC) on 19 measurements (Fig. 3) explained 77.5% of the total variation, 65% of the variation being explained by the first PC. All the measurements are positively correlated with the first PC, which is interpreted as a measure of size. Almost all the non-karyotyped individuals are more related to *G. centralis* specimens than to *G. griseoflavus* ones.

The discriminant analysis correctly classified 95.35% of the specimens previously classified on the basis of their karyotypes. Two specimens (one of each species) were misclassified: one from Chamical in La Rioja province and the other from Papagayos in San Luis province. Considering the high rate of correct classification, the status of each non-karyotyped

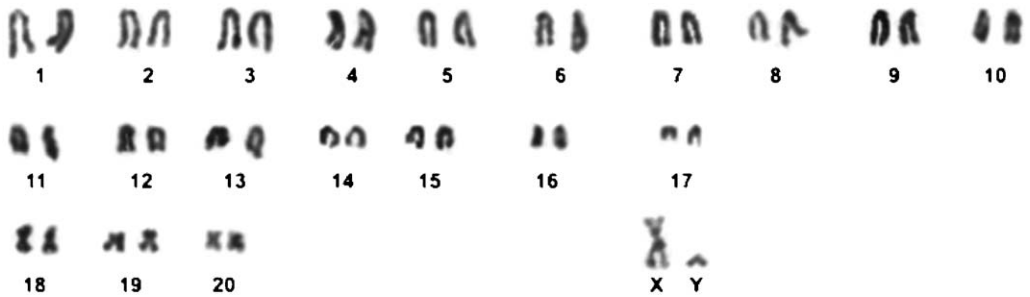

Fig. 2. Standard Giemsa-stained bone marrow karyotype of one male of *Graomys* from Cruz del Eje, Cordoba with $2n = 42$; type locality of *Graomys centralis*.

Table 1. Descriptive statistics (mean ± S.D.) of cranial measurements (mm) of *G. centralis*, *G. griseoflavus*, and the type of *G. centralis* (Thomas, 1902). *P* of *t* test of mean differences was estimated.

Measurement	*G. centralis* ($N = 20$)	*G. griseoflavus* ($N = 23$)	*G. centralis* (holotype)	*P*
GLS	33.27 ± 1.71	35.15 ± 1.71	33.50	0.0009
BL	28.42 ± 1.76	32.82 ± 1.41	26.00	<0.0001
PL	6.14 ± 0.52	6.33 ± 0.48	–	0.2204
DL	8.61 ± 0.70	9.51 ± 0.69	8.50	0.0001
ZB	17.24 ± 1.21	17.81 ± 0.71	17.00	0.0750
LIB	5.51 ± 0.62	5.49 ± 0.38	5.40	0.8846
BB	14.07 ± 0.58	14.75 ± 0.64	13.50	0.0007
RB	5.57 ± 0.41	5.59 ± 0.41	–	0.9117
NL	13.75 ± 0.88	14.52 ± 1.11	14.00	0.0177
NW	3.92 ± 0.32	3.99 ± 0.33	–	0.4727
LIF	7.21 ± 0.43	7.99 ± 0.53	–	<0.0001
LM	5.35 ± 0.25	5.51 ± 0.26	4.50	0.0409
Lm	5.25 ± 0.22	5.43 ± 0.30	–	0.0417
MB	12.44 ± 0.44	12.88 ± 0.43	–	0.0018
PPL	11.40 ± 0.81	12.72 ± 0.75	–	<0.0001
LBM	6.58 ± 0.43	6.83 ± 0.27	–	0.0320
C1ML	19.75 ± 1.01	21.18 ± 0.74	–	<0.0001
LTB	6.35 ± 0.33	7.22 ± 0.34	6.00	<0.0001
WTB	4.48 ± 0.44	5.28 ± 0.40	–	<0.0001

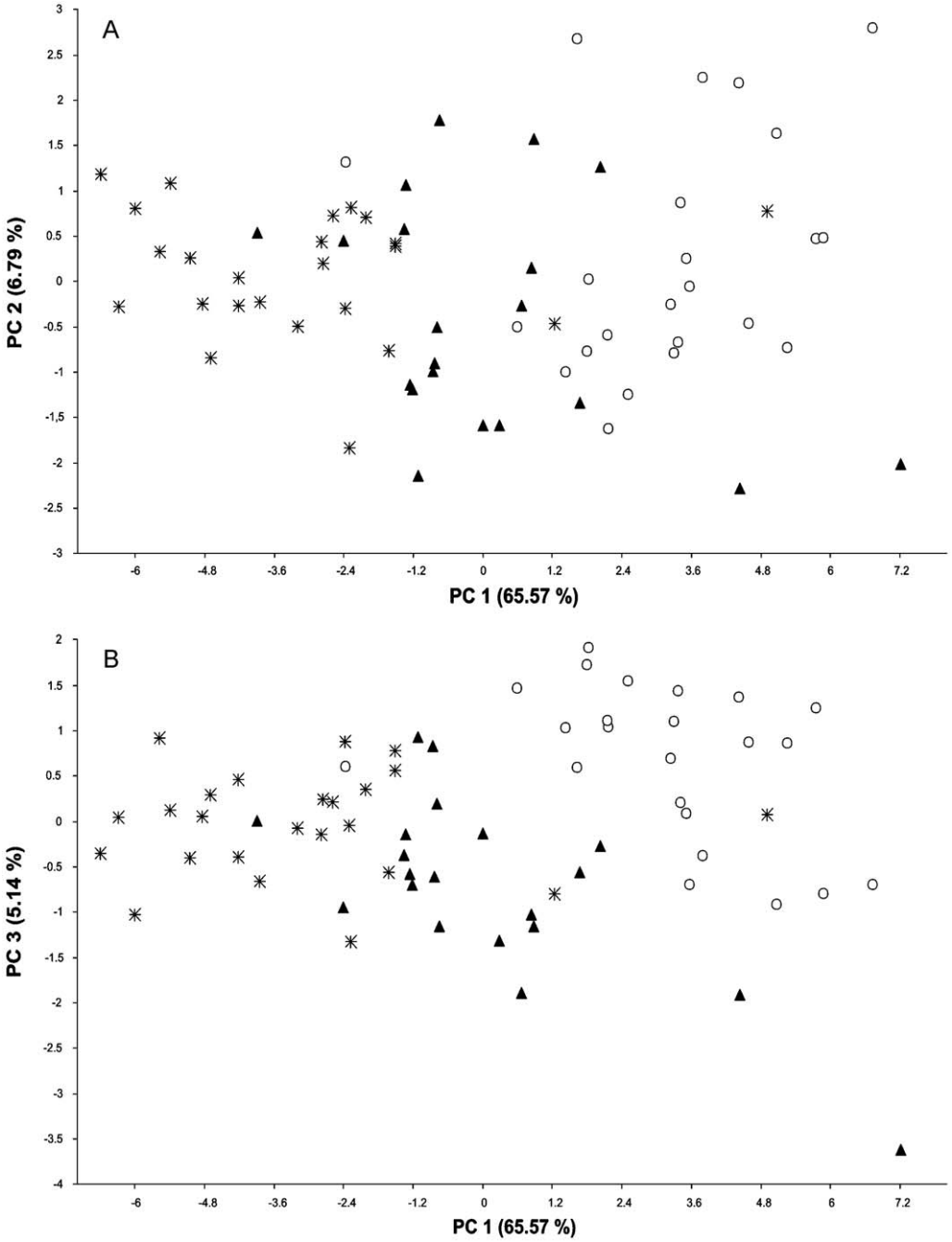

Fig. 3. Principal component analysis (PCA) using all standardized cranial characters in *Graomys* specimens ($N = 67$): (A) Plot of principal component 1 versus principal component 2. (B) Plot of principal component 1 versus principal component 3. ▲: *G. centralis* specimens; ○: *G. griseoflavus* specimens; ∗: non-kayotyped specimens.

individual was assigned based on a discrimination function analysis.

The centroids in the discriminant space are −2.17 (−3.45–−0.14) and 1.87 (−0.45–3.95) for *G. centralis* and *G. griseoflavus*, respectively. Almost all the non-karyotyped specimens were classified as *G. centralis*, except two individuals, CUNRC 43462 and CUNRC 50103, from Villa del Carmen and Villa Dolores, respectively. The first specimen (CUNRC 43462) is 30 km away from Papagayos, San Luis Province, where individuals of *G. griseoflavus* occur. The other specimen, CUNRC 50103, was collected 80 km away from Papagayos in San Luis Province, a site very close to the localities of Yacanto (21 km) and San Vicente (18 km), where the specimens were classified as *G. centralis*. The coexistence of these two species in the

same area might be due to a transitional physiognomy between Chaco and Monte ecoregions. Further field studies in the area are needed to elucidate this situation.

In a recent study, Lanzone et al. (2007) proposed a new taxonomic status for individuals from Chumbicha, Catamarca province, type locality of *Graomys medius*, on the basis of cytogenetic and morphometric evidence. They suggested that *G. medius* should be synonymized with *G. centralis*. These authors also pointed out the smaller size of individuals from Chumbicha than that of *G. griseoflavus* specimens from Ñacuñán. In addition, they remarked that specimens from Villa Hayes, in Paraguay, have $2n = 42$ and stated that "If only one *Graomys* species inhabits the Chaco biome, the name *chacoensis* Allen (1901) should be applied to those specimens".

In this study, we analyzed the morphometric variation of *G. centralis* from central-western Argentina, where the main studies of the genus were conducted, and distinguished that species from *G. griseoflavus* specimens. This discrimination, however, was possible mainly because of size differences; therefore, other methods, such geometric morphometrics, should be implemented to detect slight differences due to shape changes. In addition, the karyotypes of animals from Cruz del Eje, type locality of *G. centralis*, are provided for the first time. A comprehensive morphometric study of individuals representing the complete genus and the range of distribution in South America may clarify the species taxonomy.

Acknowledgements

We are grateful to José Priotto and Jaime Polop from Universidad Nacional de Río Cuarto who kindly permitted the access to animals in their care. We also thank Teresa Sanchez and Veronica Bertero from CIPAL, Universidad Nacional de Córdoba. Guillermo Albrieu assisted with the English version. Financial support was provided by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) to JJM. JJM is a postgraduate student of the Doctorado en Ciencias Biológicas, Universidad Nacional de Córdoba.

References

Braun, J.K., 1993. Systematic relationships of the tribe Phyllotini (Muridae: Sigmodontinae) of South America. Special Publications, Oklahoma Museum Natural History, pp. 1–50.

Catanesi, C.I., Vidal-Rioja, L., Crisci, J.V., Zambelli, A., 2002. Phylogenetics relationships among Robertsonian karyomorphs of *Graomys griseoflavus* (Rodentia, Muridae) by mitochondrial cytochrome b DNA sequencing. Hereditas 136, 130–136.

Catanesi, C.I., Vidal-Rioja, L., Zambelli, A., 2006. Molecular and phylogenetic analysis of mitochondrial control region in Robertsonian karyomorphs of *Graomys griseoflavus* (Rodentia, Sigmodontinae). Mastozool. Neotrop. 13, 21–30.

Díaz, M.M., Teta, P., Pardiñas, U.F.J., Barquez, R.M., 2006. Tribu Phyllotini Vorontsov, 1959. In: Barquez, R.M., Díaz, M.M., Ojeda, R.A. (Eds.), Mamíferos de Argentina. Sistemática y distribución. Sarem, Tucumán, Argentina.

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4, 1.

Hershkovitz, P., 1962. Evolution of neotropical cricetine rodents (Muridae) with special reference to the Phyllotine group. Fieldiana: Zoology 46, 1–524.

InfoStat, 2008. InfoStat Versión 2008. Grupo InfoStat, FCA. Universidad Nacional de Córdoba, Argentina.

Lanzone, C., Novillo, A., Súarez, N.S., Ojeda, R.A., 2007. Cytogenetics and redescription of Graomys (Sigmodontinae) from Chumbicha, Argentina. Mastozool. Neotrop. 14 (2), 249–255.

Musser, G.G., Carleton, M.D., 1993. Family Muridae. In: Wilson, D.E., Reeder, D.M. (Eds.), Mammal Species of the World. A Taxonomic and Geographic Reference, second edition. Smithsonian Institution's Press, Washington, pp. 501–756.

Musser, G.G., Carleton, M.D., 2005. Superfamily Muroidea. In: Wilson, D.E., Reeder, D.M. (Eds.), Mammal Species of the World. A Taxonomic and Geographic Reference, vol. 2, third edition. Johns Hopkins University Press, Baltimore, MD, pp. 894–1531.

Steppan, S.J., 1993. Phylogenetic relationships among the Phyllotini (Rodentia: Sigmodontinae) using morphological characters. J. Mamm. Evol. 1, 187–213.

Steppan, S.J., 1995. Revision of the tribe Phyllotini (Rodentia: Sigmodontinae), with a phylogenetic hypothesis for the Sigmodontinae. Fieldiana: Zoology 80, 1–112.

Steppan, S.J., Ramirez, O., Banbury, J., Huchon, D., Pacheco, V., Walker, L.I., Spotorno, A.E., 2007. A molecular reappraisal of the systematics of the leaf-eared mice Phyllotis and their relatives. In: Kelt, D.A., Lessa, E.P., Salazar-Bravo, J., Patton, J.L. (Eds.), The Quintessential Naturalist: Honoring the Life and Legacy of Oliver P. Pearson, vol. 134. University of California Publications in Zoology, pp. 1–981.

Theiler, G.R., 1997. Patrones de evolución en *Graomys griseoflavus* (Rodentia, Muridae). Un caso de especiación rápida. Ph.D. Dissert. Facultad de Ciencias Exactas, Físicas y Naturales. Universidad Nacional de Córdoba, Argentina.

Theiler, G.R., Blanco, A., 1996a. Patterns of evolution in *Graomys griseoflavus* (Rodentia, Muridae). II. Reproductive isolation between cytotypes. J. Mammal. 77, 776–784.

Theiler, G.R., Blanco, A., 1996b. Patterns of evolution in *Graomys griseoflavus* (Rodentia, Muridae). III. Olfactory discrimination as a premating isolation mechanism between cytotypes. J. Exp. Zool. 274, 346–350.

Theiler, G.R., Gardenal, C.N., Blanco, A., 1999. Patterns of evolution in *Graomys griseoflavus* (Rodentia, Muridae). IV. A case of rapid speciation. J. Evol. Biol. 12, 970–979.

Thomas, O., 1902. On mammals collected at Cruz del Eje, Central Cordoba, by Mr. P. O. Simmons. Ann. Mag. Nat. Hist. 7 (9), 237–245.

Tiranti, S.I., 1998. Cytogenetics of *Graomys griseoflavus* (Rodentia: Sigmodontinae) in central Argentina. Z. Saugetierkd. 63, 32–36.

Wainberg, R.L., Fronza, T.G., 1974. Autosomic polymorphism in *Phyllotis griseoflavus griseoflavus* Waterhouse, 1837 (Rodentia, Cricetidae). Boll. Zool. 41, 19–24.

Zambelli, A., Vidal-Rioja, L., Wainberg, R., 1994. Cytogenetic analysis of autosomal polymorphism in *Graomys griseoflavus* (Rodentia, Cricetidae). Z. Saugetierkd. 59, 14–20.