ZOOLOGIA 30 (2): 242–247, April, 2013
http://dx.doi.org/10.1590/S1984-46702013000200016

SHORT COMMUNICATION

Scapteromys aquaticus (Rodentia: Sigmodontinae) in Brazil with comments on karyotype and phylogenetics relationships

Cibele R. Bonvicino[1,2], Fabiano A. Fernandes[3,4], Maria C. Viana[1], Bernardo R. Teixeira[2] & Paulo S. D'Andrea[2]

[1] *Divisão de Genética, INCA. Rua André Cavalcanti 37, 20230-130 Rio de Janeiro, RJ, Brazil.*
[2] *Laboratório de Biologia e Parasitologia de Mamíferos Reservatórios Silvestres, Instituto Oswaldo Cruz. Avenida Brasil 4365, 21045-900, Rio de Janeiro, RJ, Brazil.*
[3] *Laboratório de Ecoepidemiologia da Doença de Chagas, Instituto Oswaldo Cruz. Avenida Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brazil.*
[4] *Corresponding author. E-mail: fabianoaf66@yahoo.com*

ABSTRACT. The swamp rats are distributed in Argentina, southern Paraguay, Uruguay and southern Brazil, with two species currently accepted: *Scapteromys aquaticus* Thomas, 1920 and *Scapteromys tumidus* Waterhouse, 1837. While *S. aquaticus* occurs in Argentina, Paraguay and western Uruguay, *S. tumidus* occurs in Brazil and Uruguay. Here we report for the first time the occurrence of *S. aquaticus* in gallery forest remnants in Southern Brazil. Karyologic analysis showed 2n = 32 and FNa = 40. Phylogenetic analyses, based on DNA sequences from the mitochondrial cytochrome-b gene indicate that the Brazilian and the Argentinian specimens of *S. aquaticus* shared one haplotype, while median joining analysis showed lack of population structure. This register, plus the karyotype data available for Brazilian population, recovered four karyomorphotypes in Brazil, corresponding to the two known species of *Scapteromys* and two unnamed species. This scenario indicates that more multidisciplinary studies are necessary to understand the actual diversity of *Scapteromys*.

KEY WORDS. New record; southern Brazil; species limits; swamp rat.

Species of swamp rats, *Scapteromys* Waterhouse, 1837 (Akodontini), are distributed in Argentina, Southern Paraguay, Uruguay and Southern Brazil (D'Elía & Pardiñas 2004) with two species: *Scapteromys aquaticus* Thomas, 1920 and *Scapteromys tumidus* Waterhouse, 1837 (Musser & Carleton 2005).

Scapteromys spp. occur in semi-aquatic habitats and areas near watercourses and swamps. There are no specific data about *S. aquaticus* habits but *S. tumidus* feeds on invertebrates (Suarez & Bonaventura 2001) and is associated with flooded microhabitats with low plant coverage (Bonaventura *et al*. 2003). Karyologic studies of this genus showed a wide variation, with four different karyotypes (Freitas *et al*. 1984, D'Elía & Pardiñas 2004). *Scapteromys aquaticus* populations are characterized by a diploid number (2n) of 32, occurring in Argentina (Brum-Zorrilla *et al*. 1986, Fronza *et al*. 1976) and southern Paraguay (D'Elía & Pardiñas 2004), while the *S. tumidus* population in Uruguay (Brum-Zorrilla *et al*. 1972, 1986) and southern Brazil (Freitas *et al*. 1984) showed 2n = 24. Two other karyotypes have been reported in the Brazilian population, 2n = 34 in the state of Rio Grande do Sul and 2n = 36 in the state of Paraná (Freitas *et al*. 1984, Pedó *et al*. 2010).

D'Elía & Pardiñas (2004) presented a systematic study of populations of *Scapteromys*, based on molecular and morphological evidence, and suggested two main clades: one named *S. aquaticus*, formed by Argentinean and Paraguayan populations together with one population from western Uruguay, and the other, *S. tumidus*, constituted by the remaining Uruguayan populations. Unfortunately, populations from Brazil were not analyzed.

Here, we report for the first time the occurrence of *S. aquaticus* with 2n = 32, extending the geographic distribution to Southern Brazil.

A zoologic and epidemiologic survey was conducted in the municipality of São Borja, Rio Grande do Sul in May 2009 (Fig. 1). Its climate is subtropical humid, with a mean annual temperature of 20°C, with minimum and maximum temperatures in December-January and July-August, respectively. São Borja is limited by the Uruguay river on the west. Altitude of study areas varied from 59-86 m above sea level. Small mammals were captured with live traps (Tomahawk® and Sherman® models) with baits containing a mixture of peanut butter, banana, oat and bacon. During this study, 14 *S. aquaticus* speci-

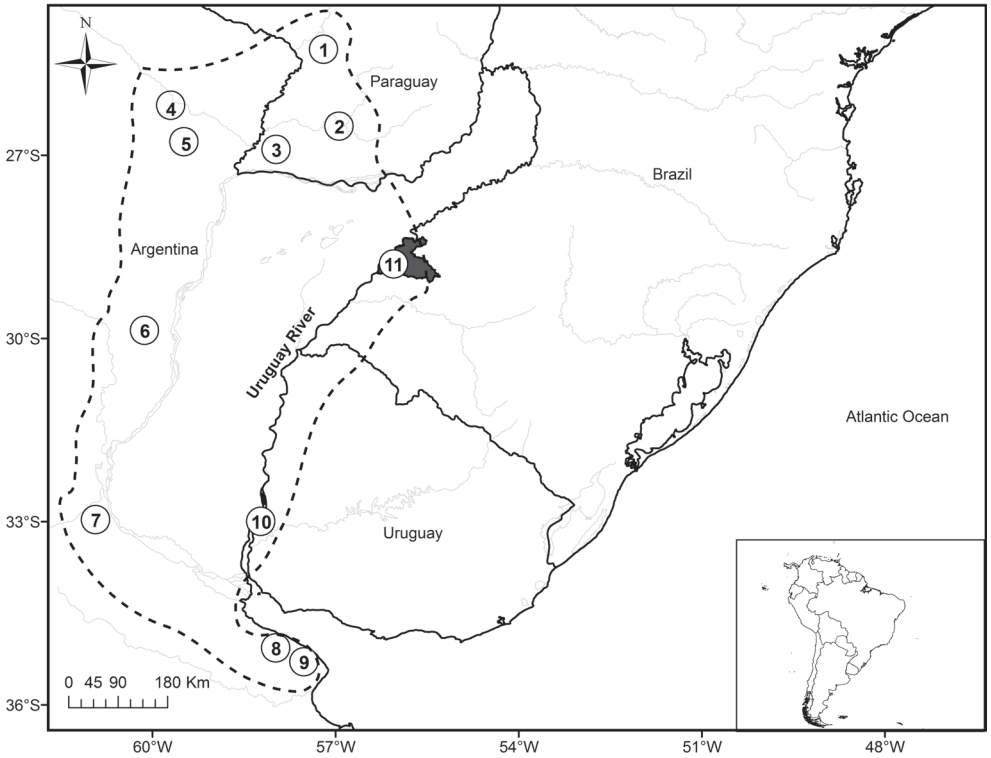

Figure 1. Collecting sites of *Scapteromys aquaticus* samples used in the analysis. Paraguay: 1) Estancia Ype kua, 2) Costa del Río Tebicuary, 3) Estancia Yacaré 0.87 km WNW of Puesto San Fernando. Argentina: 4) 17km W Colonia Villafañe, 5) Selvas del Río de Oro, 6) Rio Paraná 0.5km W Esquina, 7) Ramallo, 8) La Balandra, 9) Punta de Índio. Uruguay: 10) Las Cañas. Brazil: 11) São Borja.

mens were collected (7 females LBCE 12337, 12339, 12536, 12541, 12544-45, 12349, and 7 males LBCE 12314-15, 12338, 12341, 12350, 12542-43) and karyotyped for confirming identification. Specimens occurred in gallery forest remnants near Uruguay river (28°37'08"S, 56°01'12"W) and in shrubby vegetation around swamps (28°38'39"S, 55°59'54"W and 28°38'34"S, 55°59'57"W).

Chromosome preparations were obtained from short-term bone marrow cultures with 80% RPMI 1640, 20% fetal calf serum, ethidium bromide (5 mg/ml) and colchicine (10^{-6}) for two hours at 37°C, following by hypotonic shock with KCl (0.075M) for 30 minutes, pre fixation and fixation with Carnoy (3 methyl alcohol:1 acetic acid). Estimates of fundamental number were restricted to autosome pairs.

Voucher specimens were deposited in the mammal collection of Laboratório de Biologia e Parasitologia de Mamíferos Reservatórios Silvestres (LBCE), Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil (Table I). Fieldwork was carried out with authorization (License number 13373, to P.S. D'Andrea) from Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio).

DNA was isolated from livers of twelve specimens preserved in 100% ethanol with the phenol-cloroformio protocol (Sambrook *et al.* 1989). Cytochrome *b* DNA (cyt*b*; ca. 801 bp) was PCR amplified with primers L14724 (Irwin *et al*. 1991) and MVZ16 (Silva & Patton 1993), with a pre-denaturation step at 94°C for 3 min; 33 cycles of denaturation at 94°C for 20 sec, annealing at 48°C for 15 sec, extension at 72°C for 60 sec, and final extension of 72°C for 2 min. Amplicons were purified with GFXä PCR DNA and Gel Band Purification kit (GE Healthcare, Brazil) and sequenced with the same PCR primers and labeled with XL and BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing was carried out with an ABI Prism™ 3130 platform.

Phylogenetic analyses included sequence data from 28 *Scapteromys* deposited in GenBank: 25 *S. aquaticus* (AY445527 – AY445551), three *S. tumidus* (AY445561, AY445563, AY445565), and two other used as outgroups, one *Kunsia tomentosus* (Lichtenstein, 1830) (AY445526) and one *Blarinomys breviceps* (Winge, 1887) (AY275112).

Genetic distance estimates were carried out with complete deletion using Kimura's two parameters, with MEGA (version 5; Tamura *et al*. 2011). Estimates of maximum-likelihood (ML) analysis were carried out with PAUP 4.0b10 (Swofford 2003), and bootstrap analysis were based on 1,000 replicates. Bayesian analyses were carried out with Mr.Bayes (Ronquist &

Table I. List of *Scapteromys* haplotypes, number of specimens, field-museum number #GenBank accession number, and locality of collection.

Haplotypes	Number of specimens	Specimen #GenBank	Locality
1	3	LBCE12314, 12339, 12541	Brazil: Rio Grande do Sul, São Borja
2	1	LBCE12338	Brazil: Rio Grande do Sul, São Borja
3	1	LBCE12341	Brazil: Rio Grande do Sul, São Borja
4	4	LBCE1239, 12350, 12542, 12544	Brazil: Rio Grande do Sul, São Borja
4	1	AY445536	Argentina: Chaco, Selvas del Río de Oro
5	1	AY445527	Argentina: Cordillera, 0.5 km W Esquina
6	5	AY445528 – AY445532	Argentina: Buenos Aires, La Balandra
7	3	AY445533 – AY445535	Argentina: Buenos Aires, Ramallo
8	1	AY445537	Argentina: Chaco, Selvas del Río de Oro
9	1	AY445538	Argentina: Chaco, Selvas del Río de Oro
10	1	AY445539	Argentina: Formosa, 17 km W Colonia Villafañe
11	1	AY445540	Argentina: Formosa, 17 km W Colonia Villafañe
12	1	AY445541	Paraguay: Neembucú, Estancia Yacaré
13	3	AY445542 – AY445544	Paraguay: Paraguarí, río Tebicuary
14	1	AY445545	Paraguay: Corrientes, Estancia Ypekua
15	6	AY445546 – AY445551	Uruguay: Río Negro, Las Cañas

Huelsenbeck 2003). NETWORK (version 4.5.1.6; available at http://www.fluxus-engineering.com) was used for reconstructing a median-joining (MJ) network (Bandelt *et al.* 1999) to evaluate sub-population structure and geographic distribution patterns. MJ was calculated using variable sites only.

Intraspecific analyses were carried out with DNAsp 5 (Librado & Rozas 2009) and Arlequin 3.11 (Excoffier & Schneider 2005) for computing global and pairwise estimates of gene flow and for estimating haplotype and nucleotide diversity. Inferences of past events like population expansion or decline were based on estimates of neutrality tests: Tajima's D (Tajima 1989), Fu and Li's *F* and *D* (Fu 1997). Mismatch distribution analyses was based on an assumed stepwise growth model (Rogers & Harpending 1992) with DNAsp 5 (Librado & Rozas 2009).

Karyologic analysis of *S. aquaticus* collected in São Borja showed 2n = 32, FNa = 40 (Table II). The autosome complement is composed by five biarmed pairs, three large sized and two small sized and ten acrocentric pairs decreasing in size from median to small (Fig. 2). The X chromosome is a median sized acrocentric and the Y chromosome is a small sized biarmed chromosome.

Cytochrome *b* DNA, comprising 801 bp from nine specimens, showed four haplotypes, two of them shared by more than one specimen (H1 by three specimens, H4 by four specimens; Table I). These data, together with 25 *S. aquaticus* retrieved from GenBank, accounted for 15 haplotypes (Table I). Only one haplotype (H4), in 4 Brazilian specimens (LBCE12339, 12350, 12542, 12544), was shared by one specimen from Argentina (AY445536).

Analysis of all *S. aquaticus* sequences showed 21 variable sites (18 transitions and three transversions), with genetic distance estimates ranging from 0.0 to 2.7%. Genetic distance estimates between *S. aquaticus* haplotypes from São Borja varied from 0.0 to 0.5%, while genetic distance estimates between *S. aquaticus* from São Borja and other localities varied from 0.0 to 1.1%. Genetic distances estimates between Paraguayan, Uruguayan and Argentinean specimens were $\leqslant$0.6%.

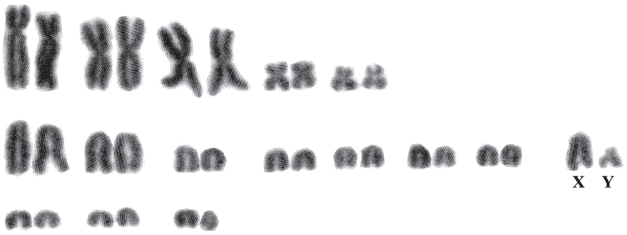

Figure 2. Conventional Giemsa coloration of *Scapteromys aquaticus* (male) from São Borja, Rio Grande do Sul.

Phylogenetic analyses confirmed the monophyly of *Scapteromys* (Fig. 3), grouping haplotypes from Paraguay, Argentina, Uruguay and Brazil lacking a geographic structure.

MJ network (Fig. 4) showed a star-like topology, indicating lack of geographic structure and showing the most frequent haplotype from Brazil in a central position. The majority of Argentinean and Paraguayan haplotypes (H4) were directly connected with this haplotype, while the haplotype from Uruguay was connected with one haplotype from Brazil (H3) connected to the central haplotype.

Pairwise genetic distance estimates (Fst) ranged from 0.17 (between São Borja and Argentinean haplotypes) to 0.68 (be-

Table II. Karyologic data on *Scapteromys* specimens with field or museum number, diploid number (2n), autosome fundamental number (FN), locality of collection.

Taxon	Specimen	2n/FN	Locality	References
S. tumidus	2 M, 1 F	24/40	Brazil: Rio Grande do Sul, Porto Alegre	1
S. tumidus	2 M	24/40	Brazil: Rio Grande do Sul, Pelotas	1
S. tumidus	6 M, 3 F	24/40	Brazil: Rio Grande do Sul, Rio Grande	1
S. tumidus	7 M, 2 F	24/40	Brazil: Rio Grande do Sul, Bagé	1
S. tumidus	5 M, 2 F	24/40	Uruguay, Fray Bentos	2
S. aquaticus	7M: LBCE 12314-15, 12338, 12341, 12350, 12542-43; 5F: LBCE 12337, 12339, 12349, 12536, 12541, 12544	32/40	Brazil: Rio Grande do Sul, São Borja	TS
S. aquaticus	several	32/40	Argentina: Punta Lara	3
Scapteromys 36	1 M	36/40	Brazil: Paraná, São José dos Pinhais	1
Scapteromys 34	5 M, 1 F	34/40	Brazil: Rio Grande do Sul, Cambará do Sul	1

M) male, F) female, TS) this study, 1) Freitas *et al.* (1984), 2) Brum-Zorrilla *et al.* (1972), 3) de Fronza *et al.* (1976).

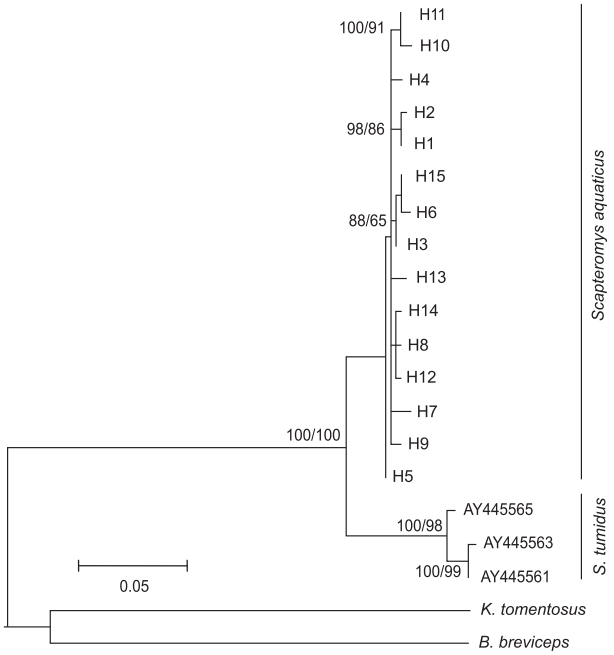

Figure 3. Maximum likelihood phylogeny (GTR+I model) and Bayesian analyses for cytochrome b of *Scapteromys aquaticus*. Numbers close to branches are boostrap values. For clarity, only boostrap values for the main groups for *Scapteromys* are shown. Branches represent each haplotype indicated by numbers listed as indicated on Table I.

tween Uruguayan and Paraguayan haplotypes). Other estimates accounted for 0.27 between São Borja and Paraguayan haplotypes, 0.57 between São Borja and Uruguayan haplotypes, 0.20 between Argentinean and Paraguayan, and 0.46 between Uruguayan and Argentinean haplotypes.

Tajima's D neutrality test and the more sensitive Fu and Li's tests resulted in negative and significant values (Tajima's $D = -1.22$, $p < 0.05$; Fu and Li's $D = -0.85$, $p < 0.05$; Fu and Li's $F = -1.14$, $p < 0.05$). Additional evidence of a recent history of population expansion from São Borja in Southern Brazil was provided by analysis of mismatch distribution showing a typical unimodal distribution under the exponential population expansion model, when considering only São Borja haplotypes or all haplotypes (data not show).

In the latest taxonomic review of the genus *Scapteromys*, the distribution of *S. aquaticus* included Argentina, Paraguay and the Uruguayan area of Las Cañas in Department of Río Negro (D'Elia & Pardiñas 2004). The *Scapteromys* specimens from Brazil herein karyotyped showed 2n = 32, FNa = 40, similarly to *S. aquaticus* from Argentina and Paraguay (Brum-Zorrilla *et al.* 1986, Fronza *et al.* 1976) and confirmed that the distribution of this species extends to the south of Rio Grande do Sul in Brazil. The only detail is the X chromosome of Brazilian specimens that was a medium size acrocentric while, in Punta Lara (Argentina), the X chromosome was found to be acrocentric and submetacentric in one female specimen, and acrocentric in other females (Fronza *et al.* 1976).

Three other *Scapteromys* karyotypes were reported in Brazil: 2n = 24 for *S. tumidus* and two other karyomorphotypes, 2n = 34 and 36 in specimens without species identification (Freitas *et al.* 1984). The *S. aquaticus* herein karyotyped were collected in the most southern region of Brazil, while the *S. tumidus* and *Scapteromys* sp. karyotyped by Freitas *et al.* (1984) were collected in northern and eastern Rio Grande do Sul, Santa Catarina and Paraná states. These findings were in agreement with the proposition of Freitas *et al.* (1984) that more than one species of *Scapteromys* occurs in Brazil, with *S. aquaticus* being restricted to the most southern region.

A previous review of the tribe Akodontini showed the monophyly of *S. aquaticus* (D'Elia & Pardiñas 2004). Our find-

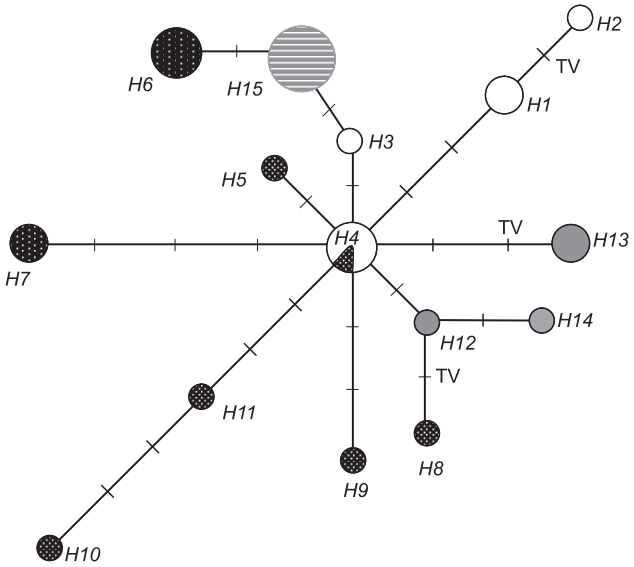

Figure 4. Median-joining network of *Scapteromys aquaticus*. Numbers following the haplotypes sequence listed in Table I. White circles represent Brazilian specimens, grey circles represent Paraguayan specimens, lined circle Uruguayan specimens and dotted circles Argentinean specimens. Circle sizes correspond to number of individuals carrying a given haplotype. Small lines to connecting branches denote the number of nucleotide substitutions. (TV) Transversion.

ings showed that Brazilian *S. aquaticus* grouped with specimens from Argentina, Paraguay and Uruguay but without a geographic structure. The Brazilian (n = 4) and the Argentinean (n = 1) specimens of *S. aquaticus* shared a single haplotype, while haplotypes from Uruguay and Paraguay were exclusive of these populations (Fig. 4).

The star-like topology of the MJ network (Fig. 4), and the Brazilian haplotype (H4) most frequent and probably the most ancestral one (Crandall & Templeton 1993), suggested low differentiation between localities, with the most frequent haplotypes in at least two populations (Brazilian and Argentinean) and unique haplotypes connected by one or few nucleotide changes, presumably derived from H4.

Geographic barriers, like the Uruguay river, between populations from Argentina and Brazil, usually could be associated with strong levels of genetic differentiation, but the effect of this river as main promoter of genetic divergence was not observed for *S. aquaticus*. The same pattern was reported between populations from Argentina and Uruguay (Fig. 1; Las Cañas – D'Elia & Pardiñas 2004). This scenario demonstrated a low level of diversity in these populations suggesting a recent history of population expansion without influence of the river.

This new record plus the karyotype data available for Brazilian *Scapteromys* population showed four karyomorphotypes in Brazil, corresponding to the two known *Scapteromys* species and two unnamed species. This data indicate that the actual diversity of *Scapteromys* genus is underestimated and more multidisciplinary studies are necessary for understand this scenario.

ACKNOWLEDGMENTS

We are grateful to H.N. Seuánez (INCA, Brazil) for reviewing the final version of the manuscript. Clerical and technical help came from L. Monnerat and K.R. Lobo. The collaboration in fieldwork by the field team of the Laboratório de Biologia e Parasitologia de Mamíferos Silvestres Reservatórios and Laboratório de Biologia de Tripanossomatídeos (A.M. Jansen, A.L.R. Roque, and F.L. Rocha), IOC/FIOCRUZ, was most useful. Work supported by CNPq grant to CRB (302951/2007-5).

LITERATURE CITED

Bandelt, H.J.; P. Forster & A. Rohl. 1999. Median-joining networks for inferring intraspecific phylogenies. **Molecular Biology and Evolution 16**: 37-48.

Bonaventura, S.M.; V. Pancotto; N. Madanes & V. Vicari. 2003. Microhabitat use and density of sigmodontine rodents in *Spartina densiflora* freshwater marshes, Argentina. **Mammalia 67** (3): 367-378.

Brum-Zorrilla, N.; N. Lafuente & P. Kiblisky. 1972. Cytogenetic studies in the cricetid rodent *Scapteromys tumidus* (Rodentia-Cricetidae). **Specialia 28**: 1373.

Brum-Zorrilla, N.; G. Oliver; T.G. Fronza & R. Wainberg. 1986. Karyological studies of South American rodents (Rodentia: Cricetidae). I. Comparative chromosomic analysis in *Scapteromys* taxa. **Caryologia 39**: 131-142.

Crandall, K.A. & A.R. Templeton. 1993. Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. **Genetics 134** (3): 959-969.

D' Elía, G. & U.F.J. Pardiñas. 2004. Systematics of Argentinean, Paraguayan, and Uruguayan Swamp Rats of the genus *Scapteromys* (Rodentia, Cricetidae, Sigmodontinae). **Journal of Mammalagy 85**: 897-910.

Excoffier, L.L.G. & S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. **Evolutionary Bioinformatics Online 1**: 47-50.

Freitas, T.R.O.; M.S. Mattevi & L.F. Oliveira. 1984. Unusual C-band patterns in three karyotypically rearranged forms of *Scapteromys* (Rodentia, Cricetidae) from Brazil. **Cytogenetics and Cell Genetics 38**: 39-44.

Fronza, T.G.; R.L. Wainberg & B.E. Llorente. 1976. Polimorfismo del cromosoma X y significacion filogenética del cariotipo de la "Rata aquatica" *Scapteromys aquaticus* (Rodentia, Cricetidae) de la ribera de Punta Lara (Argentina). **Mendeliana 1**: 41-48.

Fu, Y. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. **Genetics 147**: 915-925.

Hall, T.A. 2007. **Bioedit**. Ibis Biosciences, Carlsbad, CA.

Irwin, D.M.; T.D. Kocher & A.C. Wilson. 1991. Evolution of the cytochrome *b* gene of mammals. **Journal of Molecular Evolution 32**: 128-144.

Librado, P. & J. Rozas. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. **Bioinformatics 25**: 1451-1452.

Musser, G.M. & M.D. Carleton. 2005. Superfamily Muroidea, p. 894-1531. *In*: D.E. Wilson & D.M. Reeder (Eds). **Mammal Species of the World. A taxonomic and geographic reference.** Baltimore, The Johns Hopkins University Press, 3rd ed.

Pedó, E.; T.R.O. Freitas & S.M. Hartz. 2010. The influence of fire and livestock grazing on the assemblage of non-flying small mammals in grassland-Araucaria Forest ecotones, southern Brazil. **Zoologia 27** (4): 533-540.

Rogers, A.R. & H. Harpending. 1992. Population Growth Makes Waves in the Distribution of Pairwise Genetic Differences. **Molecular Biology and Evolution 9** (3): 552-569.

Ronquist, F. & J.P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. **Bioinformatics 19**: 1572-1574.

Sambrook, J.; E.F. Fritsch & T. Maniatis. 1989. **Molecular cloning: a laboratory manual.** Cold Spring Harbor, Cold Spring Harbor Laboratory Press.

Silva, M.N.F. & J.L. Patton. 1993. Amazonian phylogeography: mtDNA sequences variation in arboreal echimyid rodents (Caviomorpha). **Molecular and Phylogenetics Evolution 2**: 243-255.

Suárez, O.V. & S.M. Bonaventura. 2001. Habitat use and diet in sympatric species of rodents of the low Paraná delta, Argentina. **Mammalia 65** (2): 167-176.

Swofford, D.L. 2003. **PAUP*. Phylogenetic Analysis Using Parsimony (*and Others Methods).** Sunderland, Sinauer Associates, version 4.0b.

Tajima, F. 1989. Statistical methods to test for nucleotide mutation hypothesis by DNA polymorphism. **Genetics 123**: 585-595.

Tamura, K.; D. Peterson; N. Peterson; G. Stecher; M. Nei & S. Kumar. 2011. **MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28**: 2731-2739.

Submitted: 27.I.2012; Accepted: 15.X.2012.
Editorial responsibility: Walter A.P. Boeger