Zoologica Scripta

Phylogenetic relationships among species of the genus *Oligoryzomys* (Rodentia, Cricetidae) from Central and South America

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Submitted: 17 May 2010
Accepted: 28 July 2010
doi:10.1111/j.1463-6409.2010.00446.x

González-Ittig, R. E., Salazar-Bravo, J., Barquez, R. M. & Gardenal, C. N. (2010). Phylogenetic relationships among species of the genus *Oligoryzomys* (Rodentia, Cricetidae) from Central and South America. — *Zoologica Scripta*, *39*, 511–526.

The genus *Oligoryzomys* includes several species very similar in external morphology, which has resulted in a confusing specific taxonomy. Accurate species identification is particularly important because several species of *Oligoryzomys* act as natural hosts of hantaviruses affecting humans. Here, we assign specific status to individuals from a wide geographical area of Argentina and Chile using sequences of the mtDNA control region. We also compare cytochrome *b* sequences of 14 species recognized from Central and South America to infer the phylogenetic relationships within the genus. In addition, the results were analysed using available data on chromosome numbers, and the host–parasite relationships reported for the genus *Hantavirus*. We confirm the geographical distribution of *Oligoryzomys longicaudatus* (Argentina, Chile), *Oligoryzomys nigripes* (Argentina, Paraguay, Brazil), *Oligoryzomys chacoensis* (Argentina, Bolivia, Paraguay), *Oligoryzomys fornesi* (Argentina, Paraguay), *Oligoryzomys destructor* (Argentina, Bolivia) and *Oligoryzomys microtis* (Bolivia, Brazil). *Oligoryzomys longicaudatus* is strongly related to the *Oligoryzomys flavescens* complex, which comprises four clades; *O. nigripes* is closely related to *Oligoryzomys stramineus*, and *Oligoryzomys vegetus*, to *Oligoryzomys fulvescens* from Central America. *Oligoryzomys chacoensis*, *O. destructor*, *O. fornesi*, *O. longicaudatus*, *O. microtis*, *O. nigripes*, *O. stramineus*, *Oligoryzomys moojeni*, *Oligoryzomys rupestris*, *O. fulvescens* and *O. vegetus* are confirmed as valid species, whereas *O. flavescens*, *Oligoryzomys magellanicus*, *Oligoryzomys griseolus*, *Oligoryzomys victus*, *Oligoryzomys andinus* and *Oligoryzomys arenalis* need exhaustive revision. The sister species to all the remaining entities of the genus was *O. microtis*, suggesting an Amazonian origin for the genus.

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Introduction

Oligoryzomys Bangs (1900) (Rodentia, Cricetidae, Sigmodontinae) is the most speciose genus of the tribe Oryzomyini, distributed from Mexico through Central America to all countries in South America (Musser & Carleton 2005). Members are small-bodied, long-tailed, nocturnal, terrestrial and frequently arboreal; they feed on fruit, seeds and insects. The number of species cited for

the genus ranges from 12 to 20 (Honacki *et al.* 1982; Reig 1986; Carleton & Musser 1989); in their synthesis on the taxonomy and geographical distribution of Mammals species of the world, Musser & Carleton (2005) recognize 18 species of *Oligoryzomys*. The genus presents high chromosomal variation, with diploid numbers ranging from $2n = 46$ to 70 (Silva & Yonenaga-Yassuda 1997; Andrades-Miranda *et al.* 2001; Lima *et al.* 2003; Weksler & Bonvicino 2005).

Several species of *Oligoryzomys* have been recognized as natural hosts of hantaviruses acting as aetiological agents of the Hantavirus Pulmonary Syndrome (HPS), a severe respiratory illness that causes high mortality in humans (Levis *et al.* 1998; Padula *et al.* 2000). Argentina has the highest number of HPS cases reported in South America (Padula *et al.* 2000) and a high diversity of hantaviral genotypes. According to Rivera *et al.* (2007) at least five putative species of *Oligoryzomys* are associated with hantaviruses in Argentina: *Oligoryzomys chacoensis* (natural host of the Oran hantavirus), *Oligoryzomys nigripes* (associated with the Juquitiba virus; Padula *et al.* 2007), *Oligoryzomys flavescens* populations from the eastern region of the country (host of the Lechiguanas/Central Plata hantaviruses; Levis *et al.* 1998; Delfraro *et al.* 2003), western populations of *O. flavescens* (associated with the Bermejo hantavirus; Rivera *et al.* 2007) and *Oligoryzomys longicaudatus* (reservoir of Andes hantavirus; Levis *et al.* 1998; Padula *et al.* 2004).

Despite the important role of *Oligoryzomys* in the transmission of human diseases, the specific taxonomy is quite unclear, mainly because of the high similarity in morphological characters among species and the lack of revisions involving regional differences and comparative analyses of holotypes.

Different molecular markers have been employed to infer the phylogeny of *Oligoryzomys*. On the basis of cytochrome *b* (Cyt *b*) gene sequences, Myers *et al.* (1995) analysed the phylogenetic relationships among species of five genera of the tribe Oryzomyini, including eight species of *Oligoryzomys*. Dickerman & Yates (1995) used protein electrophoresis data to infer the systematics of five species from Central and South America. In those two reports and in Weksler (2003), the monophyly of the genus was established using sequences of the nuclear IRBP gene, but relationships among species were not properly elucidated; the phylogenetic reconstructions showed weak statistical support. Using sequences of the IRBP and Cyt *b* genes of 11 species (most of them from Brazil), Miranda *et al.* (2009) observed two main species groups: the 'Amazon-Cerrado' group and the 'Pampa-Andean' group; the authors suggest that the genus originated in northern South America and later inhabited the southern regions. Using PCR-RFLP and sequences of the mtDNA control region to identify *Oligoryzomys* species from Argentina, Gonzalez-Ittig *et al.* (2002) and Rivera *et al.* (2007) found seven well-defined groups supported by high bootstrap values; nonetheless, the relationships among *O. nigripes*, *O. chacoensis* and *Oligoryzomys destructor* remained unresolved. Rivera *et al.* (2007) also considers that *O. flavescens* would be a species complex given that the genetic variants of the specimens assigned to this entity grouped in three related clades.

The mtDNA control region is characterized by a high evolutionary rate compared with other mitochondrial segments (Saccone *et al.* 1991) and is particularly suitable for phylogenetic studies among very closely related taxa (Sbisà *et al.* 1997). Because of historical bias, most reports on *Oligoryzomys* including molecular data have used the Cyt *b* region as marker of choice; for this reason, at present it is not possible to compare molecular data on the mtDNA control region of the species present in Argentina with those from other South American regions.

To contribute to the knowledge of the taxonomy, distribution and evolutionary relationships of *Oligoryzomys*, here we analysed sequences of the mtDNA control region in individuals from a vast geographical area of Argentina and some specimens from Chile. Once the specific status of these specimens was accurately assigned according to the control region, we included specimens of each clade in an estimation of the phylogenetic relationships among species using Cyt *b* sequences of individuals from Paraguay, Bolivia, Chile, Peru, Brazil, Venezuela, Panama, Costa Rica and Mexico. We also integrated molecular results with chromosomal data, and with host-parasite relationships reported for the genus *Hantavirus*.

Materials and methods

Samples

Tissues were collected from live-trapped animals belonging to 18 localities from Argentina, two from Chile and one from Uruguay (Fig. 1, Table 1). The following localities were sampled: *Argentina*: Salta Province: Orán ($n = 7$); Metán ($n = 2$). Jujuy Province: in the vicinity of San Salvador de Jujuy ($n = 3$). Chaco Province: Selvas del Río de Oro ($n = 4$); Camino a Isla Cerrito ($n = 2$); Parque Nacional Chaco ($n = 4$). Misiones Province: Cuñapirú ($n = 2$); Reserva Provincial Urugua-í ($n = 1$). Formosa Province: Ibarreta ($n = 1$). Tucumán Province: El Siambón ($n = 1$). Santa Fe Province: Berna ($n = 3$). Buenos Aires Province: near La Plata ($n = 1$); La Balandra ($n = 3$). Córdoba Province: Pampa de San Luis ($n = 1$); Capilla de los Remedios ($n = 1$). La Pampa Province: Reserva Provincial Parque Luro ($n = 1$). Río Negro Province: Bariloche ($n = 3$); El

Fig. 1 Circles: Sampling localities of specimens of *Oligoryzomys* from Argentina (the political division is shown), Chile and Uruguay listed in Table 1. Squares: geographical locations listed in Table S1, Supporting information for individuals with available Cyt *b* sequences in GenBank.

Bolsón (*n* = 1). *Chile*: Lake District: Neltume (*n* = 1); Bio Bio region: El Prado (*n* = 1). *Uruguay*: San José Department: Punta de Valdez (*n* = 2). Specimens were assigned to the different species of *Oligoryzomys* on the basis of external morphology according to Carleton & Musser (1989), Díaz *et al.* (1997) and Díaz (2000).

Table 1 Field identification code of the specimens, location of sampling sites, GenBank accession number and Museums or Collections where the voucher specimens are deposited.

Identification number of specimens	Sample location (numbers in parentheses correspond to circles in Fig. 1)	Latitude	Longitude	Control region Accession numbers	Cyt *b* Accession numbers	Source
Or 22496	(1) Orán, Salta Province, Argentina	23°08′S	64°19′W	AY863417	GU185903	INEVH
Or 22498	(1) Orán, Salta Province, Argentina	23°08′S	64°19′W	GU185872	GU185904	INEVH
Or 22522	(1) Orán, Salta Province, Argentina	23°08′S	64°19′W	GU185890		INEVH
Or 22523	(1) Orán, Salta Province, Argentina	23°08′S	64°19′W	GU185891	GU185914	INEVH
Or 22526	(1) Orán, Salta Province, Argentina	23°08′S	64°19′W	GU185889		INEVH
Or 22531	(1) Orán, Salta Province, Argentina	23°08′S	64°19′W	AY863415	GU185913	INEVH
PIDBA 973	(2) Metán, Salta Province, Argentina	25°08′S	65°01′W	GU185878		CML
PIDBA 986	(2) Metán, Salta Province, Argentina	25°08′S	65°01′W	GU185877	GU185900	CML
Jy 1245	(3) San Salvador, Jujuy Province, Argentina	24°12′S	65°19′W	AY863418	GU185901	INEVH
Jy 1303	(3) San Salvador, Jujuy Province, Argentina	24°12′S	65°19′W	GU185874		INEVH
Jy 1332	(3) San Salvador, Jujuy Province, Argentina	24°12′S	65°19′W	GU185875	GU185902	INEVH
LIF 093	(4) Ibarreta, Formosa Province, Argentina	25°13′S	59°43′W	GU185873		CML
Roro 006	(5) Selvas de Río Oro, Chaco Province, Argentina	26°29′S	58°58′W	GU185886		CENPAT
Roro 007	(5) Selvas de Río Oro, Chaco Province, Argentina	26°29′S	58°58′W	AY863421	GU185898	CENPAT
Roro 014	(5) Selvas de Río Oro, Chaco Province, Argentina	26°29′S	58°58′W	GU185885		CENPAT
Roro 040	(5) Selvas de Río Oro, Chaco Province, Argentina	26°29′S	58°58′W	GU185867	GU185905	CENPAT
Ol 9-MACN 22830	(6) Parque Nacional Chaco, Chaco Province, Argentina	26°49′S	59°40′W	GU185883	GU185920	MACN
Ol 14-MACN 22834	(6) Parque Nacional Chaco, Chaco Province, Argentina	26°49′S	59°40′W	GU185887	GU185917	MACN
Ol 15-MACN 22835	(6) Parque Nacional Chaco, Chaco Province, Argentina	26°49′S	59°40′W	GU185884	GU185919	MACN
Ol 40-MACN 22837	(6) Parque Nacional Chaco, Chaco Province, Argentina	26°49′S	59°40′W	GU185888	GU185918	MACN
LIF 121	(7) Camino Isla Cerrito, Chaco Province, Argentina	27°19′S	58°46′W	GU185870		CML
LIF 122	(7) Camino Isla Cerrito, Chaco Province, Argentina	27°19′S	58°46′W	GU185869	GU185910	CML
CP 007	(8) Cuñapirú, Misiones Province, Argentina	27°04′S	55°03′W	DQ926658	GU185906	CENPAT
CP 008	(8) Cuñapirú, Misiones Province, Argentina	27°04′S	55°03′W	GU185866		CENPAT
Ol 105-MACN 22262	(9) Reserva Provincial Urugua-í, Misiones Province, Argentina	25°59′S	54°05′W	GU185871	GU185907	MACN
B3	(10) Berna, Santa Fe Province, Argentina	29°16′S	59°52′W	GU185881		CENPAT
B2	(10) Berna, Santa Fe Province, Argentina	29°16′S	59°52′W	GU185882		CENPAT
B6	(10) Berna, Santa Fe Province, Argentina	29°16′S	59°52′W	AY863422	GU185899	CENPAT
PIDBA 1201	(11) El Siambón, Tucumán Province, Argentina	26°42′S	65°27′W	GU185876		CML
SLBCH	(12) Pampa de San Luis, Córdoba Province, Argentina	31°20′S	64°47′W	AY863413	GU185916	MZ-UNC
CE 11	(13) Capilla de los Remedios, Córdoba Province, Argentina	31°30′S	63°54′W	GU185892	GU185915	MZ-UNC
UP 45	(14) La Balandra, Buenos Aires Province, Argentina	34°49′S	58°02′W	DQ926659	GU185908	CENPAT
UP 46	(14) La Balandra, Buenos Aires Province, Argentina	34°49′S	58°02′W	GU185868	GU185909	CENPAT
UP 51	(14) La Balandra, Buenos Aires Province, Argentina	34°49′S	58°02′W	GU185879	GU185924	CENPAT
BA 850	(15) La Plata, Buenos Aires Province, Argentina	34°58′S	57°56′W	AY863414	GU185925	IMBICE
PL 32022	(16) Reserva Provincial Parque Luro, La Pampa Province, Argentina	36°55′S	64°17′W	GU185880	GU185923	INEVH
Bar 23403	(17) Bariloche, Río Negro Province, Argentina	41°09′S	71°27′W	AY863416	GU185912	INEVH
Bar 23404	(17) Bariloche, Río Negro Province, Argentina	41°09′S	71°27′W	GU185893	GU185911	INEVH
Bar 23394	(17) Bariloche, Río Negro Province, Argentina	41°09′S	71°27′W	GU185897		INEVH
Bol 504	(18) El Bolsón, Río Negro Province, Argentina	41°58′S	71°31′W	GU185896		URES
N4	(19) Neltume, Los Lagos region, Chile	39°48′S	71°57′W	GU185894		CMIEE
P1	(20) El Prado, Bio Bio region, Chile	36°39′S	71°49′W	GU185895		CMIEE
PV27	(21) Puntas de Valdez, San José Department, Uruguay	34°35′S	56°42′W		GU185921	INEVH
PV32	(21) Puntas de Valdez, San José Department, Uruguay	34°35′S	56°42′W		GU185922	INEVH

INEVH, Instituto Nacional de Enfermedades Virales Humanas, Pergamino, Buenos Aires, Argentina; URES, Unidad Regional de Epidemiología y Salud Ambiental Zona Andina, Bariloche, Río Negro, Argentina; CENPAT, Centro Nacional Patagónico, Puerto Madryn, Chubut, Argentina; CMIEE, Colección de Mamíferos del Instituto de Ecología y Evolución, Universidad Austral de Chile, Valdivia, Chile; MZ-UNC, Museo de Zoología, Universidad Nacional de Córdoba, Córdoba, Argentina; CML, Colección Mamíferos Lillo, Instituto Miguel Lillo, Tucumán, Argentina (PIDBA: Programa de Investigaciones de Biodiversidad Argentina); IMBICE, Instituto Multidisciplinario de Biología Celular, La Plata, Buenos Aires, Argentina; MACN, Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia', Buenos Aires, Argentina.

All animals were killed by ether inhalation. Liver, kidneys or tail tips were removed and preserved in 85% alcohol (J.T. Baker Inc., Phillipsburg, NJ, USA). Field identification codes of the specimens, location of capture sites and institutions where the vouchers are deposited are indicated in Table 1.

DNA extraction and PCR amplifications

A biosafety level 1 vertical laminar flow cabinet was used to manipulate tissues potentially infected with Hantavirus. DNA extraction from liver, kidney or tail tip was performed on each individual according to the CTAB (cetyl-trimethyl-ammonium-bromide) protocol (Del Sal *et al.* 1989). Proteinase K and RNAse were used in the extraction procedure to inactivate potential virus particles present in the samples.

The mtDNA control region was amplified using primers 464 and 282 with the sequences: 5′-TGAATTGGAGGACAACCAGT-3′ and 5′-AAGGCTAGGACCAAACCT-3′, respectively, and a segment of approximately 1250 bp was obtained. This fragment included the complete control region, and partial sequences of the Cyt *b* gene (~110 bp), 12s rRNA gene, and the complete flanking proline and phenylalanine tRNAs. Amplification conditions were those described by Gonzalez-Ittig *et al.* (2002); in a 50-μL volume, we used: 240 μM each of dATP, dGTP, dCTP, dTTP; 200 nM of each primer, 1× reaction buffer, 2.5 mM $MgCl_2$, 1.0 U of Taq polymerase (Amersham Biosciences, Little Chalfont, UK) and 10 ng of total DNA. The reaction started with denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 90 s and extension at 72 °C for 90 s. Finally, there was a hold period of 5 min at 72 °C.

The complete Cyt *b* gene was amplified with primers Mus 14095 (5′-GACATGAAAAATCATCGTTGTAATTC-3′) and Mus 15398 (5′-GAATATCAGCTTTGGGTGTTGRTG-3′) (Anderson & Yates 2000). The reaction started with denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 45 s and extension at 72 °C for 45 s. Finally, there was a hold period of 5 min at 72 °C.

Sequencing

All sequencing reactions were performed by Macrogen USA Inc (http://www.macrogenusa.com) in an ABI 3730x1 DNA automatic analyser (PE Applied Biosystems, Forster City, CA, USA) using the same primers mentioned above. Sequences obtained in this study have been deposited in GenBank; accession numbers are listed in Table 1.

To avoid erroneous inclusion of Numts (nuclear sequences of mitochondrial origin) in the data sets, the codons of the 110 bp of the Cyt *b* gene of the PCR product obtained with primers 464/282 and the complete PCR product obtained with primers Mus14095/Mus15398 were translated into aminoacids to check for non-functional mutations (González-Ittig & Gardenal 2008).

Phylogenetic analyses

We first sequenced the mtDNA control region of the specimens listed in Table 1 and performed all the phylogenetic analyses as detailed below. We included the sequences published in Rivera *et al.* (2007) with the following GenBank accession numbers: AY863413, AY863414, AY863415, AY863416, AY863417, AY863418, AY863421, AY863422, DQ926658 and DQ926659, which were used as references to confirm the morphological classification of the specimens included in this study or to assign the specific name to a few individuals identified as *Oligoryzomys* sp.

To incorporate in the present phylogenetic analysis sequences of all the species of the genus *Oligoryzomys* available in GenBank (Table S1, Supporting information), we sequenced the Cyt *b* gene of 2–4 specimens included in each of the clades obtained using the control region. We rooted the trees using sequences of the following outgroups: *Pseudoryzomys simplex*, *Holochilus chacarius* and *Euryoryzomys russatus*.

Nucleotide alignments were produced using Multiple Alignment using Fast Fourier Transform (Katoh *et al.* 2002; Katoh & Toh 2008; v6.717; http://mafft.cbrc.jp/alignment). This program implements progressive alignments and adds extra steps to improve the alignment guide tree. We used the strategies L-INS-i and G-INS-I, with gap opening penalty: 1.53, gap extension penalty: 0.1.

As there are few sequences encompassing the complete Cyt *b* gene in GenBank, the aligned matrix used in this study had several missing characters in the 5′ and/or the 3′ end. We performed a preliminary analysis using the complete matrix, and also cutting the ends to decide which matrix was the most appropriate to maximize node supports; the complete matrix produced nodes with higher statistical support.

Both the mtDNA control region and Cyt *b* sequence data sets were analysed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian methods. MP was performed with TNT ver 1.1. (Goloboff *et al.* 2008) and PAUP 4.0.b10 (Swofford 1998). Characters were unordered and equally weighted; in the control region dataset, gaps were treated as a fifth character state. No gaps were present it the Cyt *b* dataset. In TNT and PAUP, we performed a heuristic search of 1000 iterations of random taxon addition using the tree bisection-reconnection (TBR) branch swapping algorithm. Trees of MP analyses resulting in more than one most parsimonious tree were summarized in strict consensus trees. Nonparametric bootstrap support values were calculated based on 1000 replicate searches. We used the pruning option of the TNT program to explore if one or few taxa were moving among different distant positions, which could result in poorly resolved consensus trees.

To determine the most appropriate model of nucleotide substitution for the data set, sequences were analysed with MODELTEST 3.7 (Posada & Crandall 1998). Likelihood

scores for 56 different models were computed through ML using the program PAUP. For the control region data set, the HKY+G model was selected using the Akaike criterion, and the following corrections were employed for subsequent Bayesian inferences: a base frequency of A = 0.3591, C = 0.2531, G = 0.0960, T = 0.2918; a transition/transversion ratio of 1.6350; a gamma distribution with alpha = 0.4140; the total likelihood was −lnL = 7956.8907. For the Cyt *b* data set, the TrN+I+G model was selected using the Akaike criterion, and the following corrections were used for subsequent Bayesian inferences: a base frequency of A = 0.3239, C = 0.3067, G = 0.1052, T = 0.2642; a ratio matrix of AC = 1.0000, AG = 6.1276, AT = 1.0000, CG = 1.0000, CT = 8.5201, GT = 1.0000; a proportion of invariable sites of 0.4617, and a gamma distribution with alpha = 0.8924; the total likelihood was −lnL = 5988.6284. Bayesian inferences were performed using MRBAYES 3.1.2 (Ronquist & Huelsenbeck 2003). Two independent runs were performed simultaneously on the data, each one using one cold and three heated chains. We discarded the first 25% of the samples as 'burn in' to ensure stationarity. After 1 million generations for the control region data set and 4 million generations for the Cyt *b* data set, the average standard deviation of split frequencies between the two independent runs at completion was 0.006 and 0.004, respectively. This value suggested convergence of the two runs on a stationary distribution. ML trees were constructed using the on line version of program PhyML ver 3.0 (http://www.atgc-montpellier.fr/phyml/) (Guindon *et al.* 2005). Even though MODELTEST selected the HKY+G and TrN+I+G models, which are not included in the on line version of PhyML, we used the HKY85 and TN93 models for the control region and Cyt *b* datasets, respectively, which are the most similar to those previously selected. The model parameters described above were incorporated for the analyses and 1000 bootstrap replicates were performed.

As the Kimura 2 parameters (K2P) genetic distance (Kimura 1980) has been previously used in Sigmodontinae rodent characterizations (Smith & Patton 1999), we calculated this distance for within- and between-clade comparisons.

Results

The general topology of the consensus trees obtained with MP, ML and Bayesian procedures based on the control region data were almost identical (Fig. 2), with minor differences in the relationships among sequences within clades. Using the pruning procedure implemented in TNT, no taxa were identified as moving among different distant positions in the MP procedure. The supporting values of the nodes were usually higher using the Bayesian approach than in the other two procedures. Under MP criteria, of a total of 1244 characters, 383 were informative; 60 most parsimonious trees of 1518 steps were found and Consistency and Retention indexes were 0.69 and 0.83, respectively. In the tree obtained with data of the control region, the clade of *O. destructor* appears as the sister taxon of the rest of the species of *Oligoryzomys* (Fig. 2). The clade formed by sequences of specimens assigned to *O. longicaudatus* from the south of Chile and Argentina is closely related to those of the *O. flavescens* complex (Fig. 2). In this complex, three clades were recognized: one composed of individuals from a wide geographical range in the eastern and central regions of Argentina, identified as *O. flavescens*; another clade comprising sequences of specimens from the Chaco province, classified as *Oligoryzomys fornesi*; and another clade containing sequences of individuals from Córdoba and Orán (referred to as *O. flavescens* West in the present work).

To compare these results with those using a different gene as genetic marker, we carried out a phylogenetic analysis based on sequences of Cyt *b*, which included the sequences from GenBank as listed in the Table S1, Supporting information. The Bayesian consensus tree obtained is shown in Fig. 3. The ML consensus tree and the MP strict consensus tree (based on 11880 most parsimonious trees) presented several polytomies, mainly in the nodes close to the root of the tree; all of them were best resolved using the Bayesian approach. Nonetheless, the three methods provided identical species assignment for all individuals and congruent phylogenetic relationships in all the trees. Under MP criteria, the Cyt *b* matrix presented 802 characters, with 233 parsimony-informative sites. The tree length was 997, and the Consistency and Retention indexes were 0.42 and 0.71, respectively. Using the pruning procedure implemented in TNT, we identified the sequence L37400 (belonging to *Oligoryzomys andinus*) as moving among different distant positions (indicated in Fig. 3).

The sister species to all the other species of the genus was *Oligoryzomys microtis* from Brazil and Bolivia. The next node corresponds to the clade formed by *O. destructor* and *O. andinus*. It should be noted that the sequence L37402 from Bolivia, designated as *Oligoryzomys* sp. by Myers *et al.* (1995), clustered with the sequences of individuals classified as *O. destructor* from northern Argentina. The association between *O. andinus* and *O. destructor* was weakly supported in the ML trees; in the MP tree, no relationship between these taxa was found, which is consistent with the moving position of *O. andinus*. By excluding this sequence, the node recovering *O. microtis* as the sister species of the *Oligoryzomys* was gained; the general topology became almost identical to that obtained in the Bayesian and ML consensus trees.

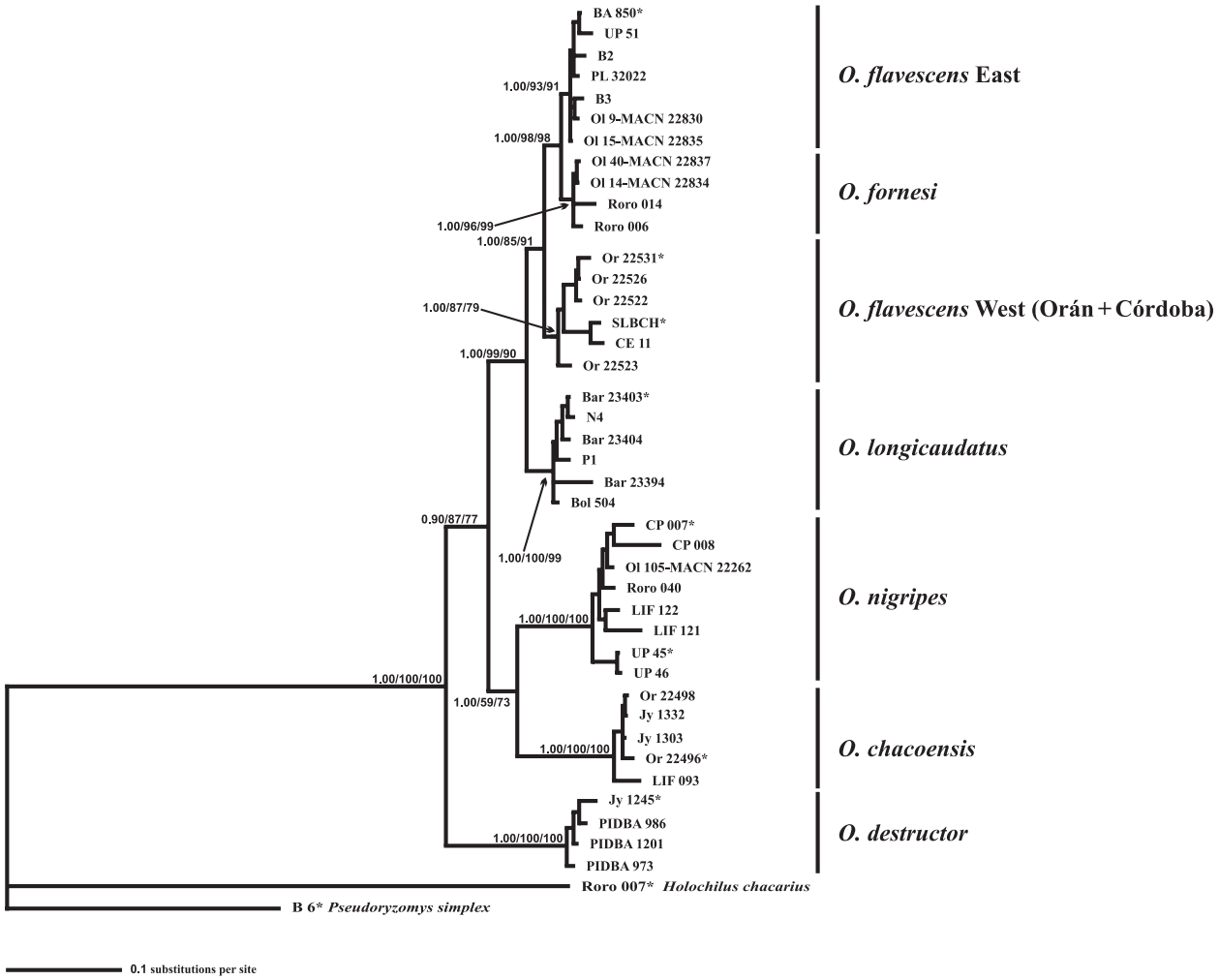

Fig. 2 Phylogram of the Bayesian consensus tree obtained from the control region data set (1244 bp) after 1 million generations. The topology is identical to that of maximum parsimony (MP) and maximum likelihood (ML) after 1000 replicates. The order of the bootstrap values in the nodes is: Bayesian posterior probabilities/ML/MP. Sequences of *Pseudoryzomys simplex* and *Holochilus chacarius* were used as outgroups. *: are sequences of specimens used in Rivera *et al.* (2007).

Interestingly, the four sequences from Mexico assigned in GenBank to *Oligoryzomys fulvescens* form a clade that is related to one containing a sequence assigned to *Oligoryzomys vegetus* from Panama (Myers *et al.* 1995) and three sequences from Costa Rica assigned to *O. fulvescens*. In addition, the sequence DQ227457, also assigned in GenBank to *O. fulvescens* from Venezuela, clustered with *Oligoryzomys* sp. from northern Brazil but not with *O. fulvescens* from Mexico or to those of Costa Rica; in Fig. 3 they are referred to as *Oligoryzomys* sp. 2 and sp. 1, respectively, as it is evident that the species assignments in GenBank were incorrect. The clade comprising the sequences from Venezuela and northern Brazil is closely related to the one including *Oligoryzomys moojeni* from central Brazil.

The *O. flavescens* complex was represented by several groups of sequences (Fig. 3): (i) *O. flavescens* West, (ii) *O. flavescens* East (with sequences from Argentina and Uruguay), (iii) a group of sequences from Brazil also classified in GenBank as *O. flavescens* and (iv) a group of sequences recognized as *O. fornesi*. This topology is in agreement with that observed using control region sequences (Fig. 2).

Furthermore, this study confirms the geographical distribution of several species: *O. longicaudatus* in Argentina and Chile, *O. nigripes* in Argentina, Paraguay and Brazil (strongly related to *Oligoryzomys stramineus* from Brazil), *O. chacoensis* in Argentina, Bolivia and Paraguay, *O. fornesi* in Argentina and Paraguay, and *O. microtis* in Bolivia and Brazil.

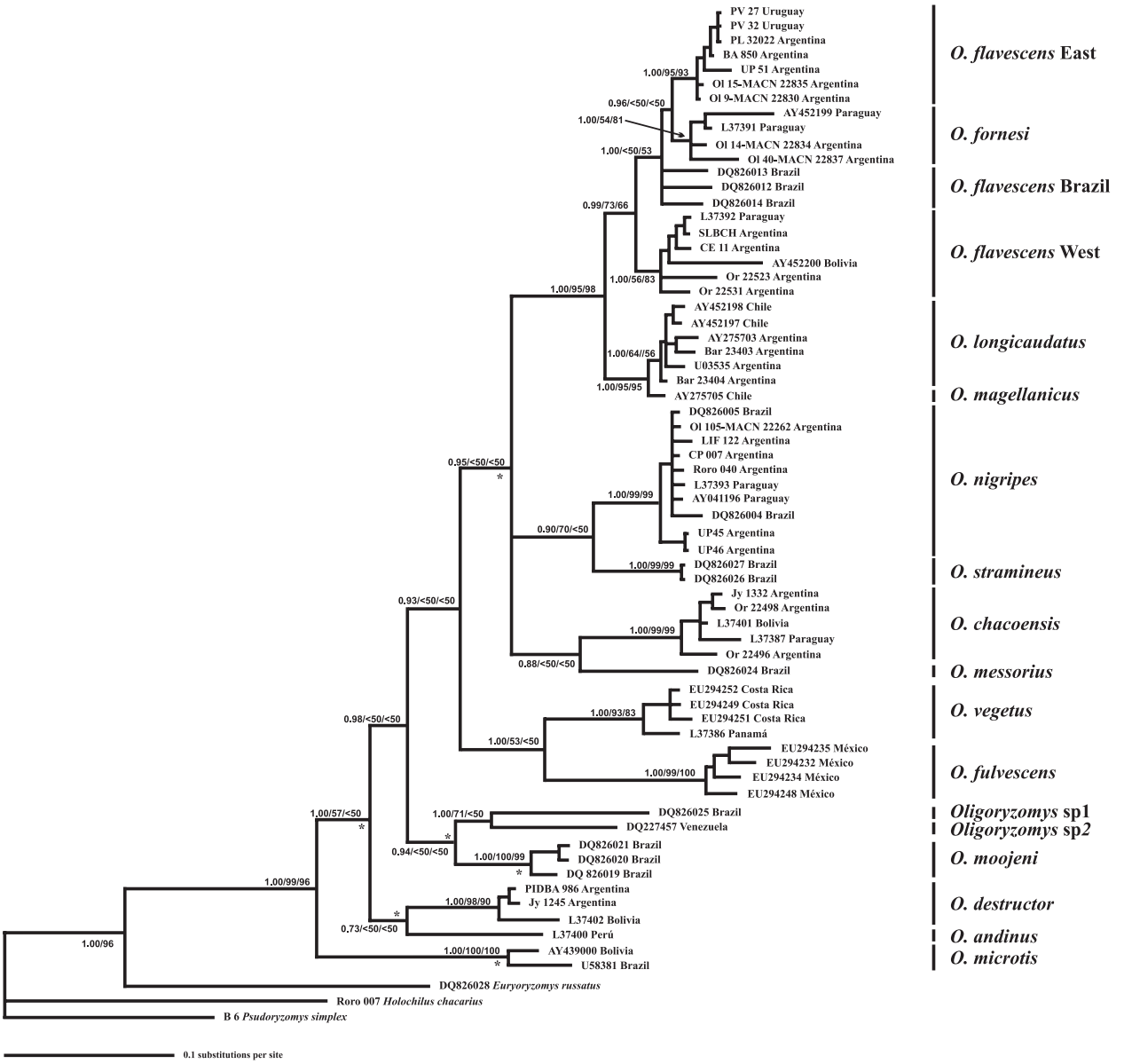

Fig. 3 Phylogram of the Bayesian consensus tree obtained from the cytochrome *b* data set (802 bp) after 4 million generations. The order of the bootstrap values in the nodes is as follows: Bayesian posterior probabilities/ML/MP. Sequences from GenBank are indicated with their Accession number. Sequences of the specimens analysed in this study are designated as in Table 1. Sequences of *Pseudoryzomys simplex*, *Holochilus chacarius* and *Oryzomys russatus* were used as outgroups. *Indicates the alternative positions of the sequence L37400 (*Oligoryzomys andinus*) using the pruning procedure implemented in TNT.

The K2P genetic distances among groups within the *O. flavescens* complex are relatively small, ranging from 1.89 to 4.59 for the Cyt *b* gene and from 2.58 to 4.99 for the control region. The entity classified as *O. flavescens* West presents the largest genetic distance in the complex and one of the largest within-clade distance. *Oligoryzomys longicaudatus* is separated from the *O. flavescens* complex by a relatively small genetic distance, compared with the distances among the remaining species present in Argentina (Table 2).

Discussion

In this article, we analysed a larger number of individuals from a much wider geographical range compared with our

Table 2 Matrix of the Kimura 2 parameter genetic distances among eight of the *Oligoryzomys* species analysed in this study. (a) Matrix obtained from the Cyt *b* gene data set (802 nucleotides). (b) Matrix obtained from the control region data set (1244 nucleotides). In bold, distances within clade

(a) Species	1	2	3	4	5	6	7	8
1 *Oligoryzomys flavescens* East	**0.65 ± 0.44**							
2 *Oligoryzomys fornesi*	2.91 ± 0.85	**2.38 ± 1.53**						
3 *O. flavescens* Brazil	1.89 ± 0.38	2.77 ± 1.05	**1.09 ± 0.53**					
4 *O. flavescens* West	3.79 ± 0.92	4.59 ± 1.41	3.48 ± 1.03	**2.42 ± 1.55**				
5 *Oligoryzomys longicaudatus*	4.55 ± 0.42	5.71 ± 0.97	4.03 ± 0.41	5.02 ± 0.99	**1.09 ± 0.32**			
6 *Oligoryzomys nigripes*	10.05 ± 0.65	10.79 ± 0.91	9.13 ± 0.62	10.73 ± 0.64	8.16 ± 0.74	**1.24 ± 0.63**		
7 *Oligoryzomys chacoensis*	10.53 ± 1.24	11.25 ± 1.27	9.8 ± 1.1	11.09 ± 1.14	10.2 ± 1.04	8.77 ± 0.63	**1.53 ± 0.74**	
8 *Oligoryzomys destructor*	11.39 ± 1.14	12.39 ± 1.37	11.33 ± 1.4	11.62 ± 1.51	11.84 ± 0.96	11.64 ± 0.52	10.17 ± 0.95	**2.8 ± 2.1**
(b) Species	1	2	3	4	5	6	7	
1 *O. flavescens* East	**1.2 ± 0.48**							
2 *O. fornesi*	2.58 ± 0.52	**1.33 ± 0.8**						
3 *O. flavescens* West	4.7 ± 0.67	4.99 ± 0.73	**3.18 ± 1.32**					
4 *O. longicaudatus*	5.58 ± 0.63	5.98 ± 0.78	6.39 ± 0.87	**2.05 ± 1.08**				
5 *O. nigripes*	11.65 ± 0.79	12.13 ± 1.01	12.96 ± 1.12	11.54 ± 1.03	**3.62 ± 1.27**			
6 *O. chacoensis*	12.94 ± 0.36	13.26 ± 0.5	13.35 ± 0.78	12.09 ± 0.78	12.61 ± 0.9	**1.42 ± 1.19**		
7 *O. destructor*	13.58 ± 0.47	13.92 ± 0.53	14.49 ± 0.98	14.32 ± 1.02	15.58 ± 0.74	16.58 ± 0.4	**1.54 ± 0.44**	

previous study (Rivera *et al.* 2007), confirming the usefulness of sequences of the mtDNA control region to identify individuals of the following nominal species of *Oligoryzomys* cited for Argentina: *O. chacoensis*, *O. nigripes*, Eastern *O. flavescens*, Western *O. flavescens*, *O. fornesi* and *O. longicaudatus*. In species acting as reservoirs, this information is essential to determine the geographical area in which a human disease can become endemic.

Phylogenetic relationships among the species were estimated using two segments of the mtDNA, the control region and the Cyt *b* gene. In the former, the topology of the trees obtained on the basis of 42 sequences using MP, ML and Bayesian methods was almost identical and all the nodes were strongly supported. In a previous work (Rivera *et al.* 2007) using both RFLP and sequence data (nine sequences) of the control region, the clade containing *O. chacoensis* was closer to the root of the tree compared with *O. nigripes* and *O. destructor*, but phylogenetic relationship between the latter two species was not resolved. Results from the present study, which are statistically well supported, revealed that *O. destructor* is the sister taxon to a clade including *O. chacoensis* and *O. nigripes* (Fig. 2).

Because most previous estimations of phylogenetic relationships among species of *Oligoryzomys* from South American countries other than Argentina were performed using the Cyt *b* gene as molecular marker, we also analysed sequences of this gene in all the samples from Argentina and compared them with those present in GenBank for specimens from Uruguay, Bolivia, Brazil, Chile, Paraguay, Peru, Venezuela, Panama, Costa Rica and Mexico. All the 'Argentinean' clades recovered using the control region as well as the relationships among the different lineages were corroborated by the Cyt *b* gene trees (Fig. 3), providing solid evidence for the phylogenetic inference performed.

The Oligoryzomys flavescens *complex (Eastern and Western* Oligoryzomys flavescens*, Brazilian* Oligoryzomys flavescens *and* Oligoryzomys fornesi*)*

When analysing individuals from Argentina classified as *O. flavescens* based on morphological characters, Rivera *et al.* (2007) reported three clades in the control region MP tree: 'Córdoba', 'Oran' and 'East'. Our current results, based on a higher number of sequences and localities sampled, reveal a unique, well supported 'West' group including Córdoba and Oran, an *O. flavescens* East group consistent with the previous study, and another group including the sequences corresponding to individuals classified as *O. fornesi*. A similar picture was obtained using the Cyt *b* gene for the samples from Argentina, but another group formed by the haplotypes from Brazil is now retrieved within the clade of *O. flavescens*. According to Andrades-Miranda *et al.* (2001) and Weksler & Bonvicino (2005), the Brazilian *O. flavescens* (chromosome number of $2n$ = 64–67/FN = 66–72) is distributed in the south Atlantic Forest and in the Cerrado biome of central Brazil and has not been associated with any Hantavirus.

The form 'East' is distributed in eastern Argentina and Uruguay (the type locality is Maldonado, Uruguay); its chromosome number is similar to the Brazilian form ($2n$ = 64–66/FN = 66–68; Brum-Zorrilla *et al.* 1988) and has been recognized as the natural host of the hantavirus genotype Lechiguanas (or Central Plata) in Argentina and

Uruguay (Levis *et al.* 1998; Delfraro *et al.* 2003). Espinosa & Reig (1991) and Aniskin & Volobouev (1999) reported that *O. flavescens* from north-western Argentina (Tucumán and Jujuy provinces) and southern Bolivia (Tarija) presented $2n$ = 64–68/FN = 66–70, with very similar chromosomal morphology and banding patterns than those of *O. flavescens* from Uruguay and Eastern Argentina. The variation in chromosome number among these three entities of *O. flavescens* is related to the presence of B chromosomes (Sbalqueiro *et al.* 1991).

Previous studies with a reduced number of samples have provided confusing species assignments. For example, Myers *et al.* (1995) recognized specimens pertaining to two groups within *O. fornesi*, one East and one West (individual UMMZ 133833, L37392, Table S1, Supporting information) from West Paraguay. In this study, the sequence L37392 clusters within *O. flavescens* West, suggesting that this specimen was wrongly assigned to *O. fornesi*. A similar case was observed for individuals AMNH 230986 (L37400) from Peru and NK 11547 (AY452200) from Bolivia. They were classified as *O. andinus*, but in this work the specimen from Bolivia also clusters within the *O. flavescens* West group (Fig. 3, Table S1, Supporting information). Carleton & Musser (1989) reported a species referred to as *Oligoryzomys* sp. B, which would be the Andean counterpart of *O. flavescens*. Because the distribution of *Oligoryomzys* sp. B described in that work overlaps, at least in part, with that of the form we refer to as *O. flavescens* West, they probably represent the same taxonomic entity. Elucidating the systematics and geographical distribution of *O. flavescens* West is necessary because it is the reservoir of the Bermejo hantavirus genotype, which causes HPS in northern Argentina and southern Bolivia (Padula *et al.* 2002; Rivera *et al.* 2007). The K2P genetic distance values observed in the four groups recovered with the Cyt *b* dataset range between 1.89 and 4.59 (Table 2a), which is in agreement with the assumption that *O. flavescens* is a species complex (Rivera *et al.* 2007).

The high nodal support for the inclusion of *O. fornesi* ($2n$ = 62/FN = 64; Bonvicino & Weksler 1998) within the *O. flavescens* complex deserves special attention, as this relationship was previously observed by other authors. In a study of the phylogenetic relationships among Oryzomyine rodents using sequences of the nuclear IRBP gene in combination with morphological characters, Weksler (2003, 2006) and Miranda *et al.* (2009) also found a close relationship between the clades of *O. fornesi* and *O. flavescens*. Taking into account the available chromosomal and molecular data and that *O. flavescens* appears as parafiletic, we consider that *O. fornesi* should be a valid species. Nevertheless, additional molecular, cytogenetical and alpha taxonomical analyses are needed to properly characterize the different groups of *O. flavescens*, which most likely should be reclassified into different specific names.

Massoia (1973), in the original description of *O. fornesi*, and then Myers & Carleton (1981) using a phenetic clustering method, stated that this species was related to *O. flavescens*, not to *O. microtis*, as suggested by Olds & Anderson (1987) and Carleton & Musser (1989). In the present phylogenetic trees (Figs 2 and 3), all statistical approaches cluster *O. fornesi* within the *O. flavescens* complex, whereas *O. microtis* from the Amazonian basin, was recovered in a very different branch. This result strongly supports that obtained by Myers *et al.* (1995), in which *O. microtis* and *O. fornesi* appear as unrelated.

As already mentioned, the karyotype $2n$ = 62/FN = 64 would correspond to *O. fornesi*, whereas individuals $2n$ = 64/FN = 66 should be assigned to *O. microtis* (Weksler & Bonvicino 2005). The habitat of *O. fornesi* is characterized by open vegetation and forests of the Humid Chaco (Argentina and Paraguay) and Cerrado (Brazil) morphoclimatic domains (probably also the Caatinga in Brazil). *Oligoryzomys fornesi* is sympatric with the form *O. flavescens* East in the Chaco domain in Argentina (Contreras & Berry 1983; in this study, in the Parque Nacional Chaco) and in Paraguay (Bonvicino & Weksler 1998). In contrast, *O. microtis* occurs in the Amazon biome. In this study, the latter taxon (represented by sequences from Brazil and Bolivia) is recovered as the basal species to all other members of the genus. A similar result was obtained by Miranda *et al.* (2009) using also the Cyt *b* gene. Regarding *O. fornesi*, these authors obtained a very different result according to the gene they considered: in the Cyt *b* tree, the species appears close to the root, but in the ML tree using the nuclear gene IRBP, the node grouping individuals of *O. fornesi* occurs in a distal position, closely related to that of *O. flavescens*, which is in agreement with our results based on Cyt *b* and control region data (Figs 2 and 3). It is highly probable that Miranda *et al.* (2009) used two pseudogene sequences (Numts) of the Cyt *b* gene since, as stated by Bensasson *et al.* (2001), they can resemble ancestral sequences or molecular 'fossils'. The confusion in the phylogenetic relationships between *O. microtis* and *O. fornesi* is also evident in Myers & Carleton (1981), who wrongly identified specimens from Mamore, Department of Beni in the Bolivian Amazon, as *O. fornesi*. In fact, specimens from that region of Bolivia correspond to *O. microtis*, which is the natural reservoir of the hantavirus genotype Río Mamore (Bharadwaj *et al.* 1997; Powers *et al.* 1999).

The possible presence of Oligoryzomys destructor *in Argentina*

The type locality of *O. destructor* is Río Chinchao, in the Yungas of the Huanuco Department, Peru (Musser &

Carleton 2005). The Yungas domain is unevenly distributed on the eastern slopes of the Andean and subandean mountains in Colombia, Ecuador, Peru, Bolivia and Argentina. Capllonch *et al.* (1997) reported its presence in the Yungas of Argentina. Later, Pardiñas & Galliari (1998) stated that this assumption was not properly supported because of the lack of morphological and morphometric studies comparing those samples with the holotype. However, the specimens included in our present study (Table 1) correspond to the taxonomic entity described by Capllonch *et al.* (1997) and also cited in Díaz & Barquez (2007). Although we included sequences of only four individuals, our laboratory database have similar haplotypes from more than 20 specimens of this species (data not shown), captured at different localities of the Yungas in Catamarca, Tucumán, Salta and Jujuy provinces, northwestern Argentina, which confirms the cohesion of this taxonomic entity. We agree with Pardiñas & Galliari (1998) that additional evidence is needed to confirm the presence of *O. destructor* in this country. However, for simplicity, in this article we will continue using this name.

In the Cyt *b* phylogenetic tree, the sequence of specimen AMNH 263838 (L37402 in Fig. 3) from Río Limón in the Bolivian Yungas (*Oligoryzomys* sp. in Myers *et al.* 1995) clusters within the *O. destructor* lineage. However, the K2P genetic distance between this specimen and individuals PIDBA 986 and Jy 1245 from Argentina is 3.9% and 4.1%, respectively, which are higher than the genetic distances among specimens from Argentina (1.54 ± 0.44% for control region and 0.39% for Cyt *b* data). New data on specimens from Bolivia and Peru are needed to clarify the taxonomic status of specimens from this geographical region.

Relationships between Oligoryzomys nigripes *and* Oligoryzomys stramineus

In this study, the *O. nigripes* clade is represented by samples from Argentina, Paraguay and Brazil. This species inhabits the Atlantic Rain Forest in Brazil (Andrades-Miranda *et al.* 2001; Weksler & Bonvicino 2005; Miranda *et al.* 2009), and the Paranaense Rain Forest which extends along the rivers Paraná, Paraguay and Uruguay (Myers & Carleton 1981; Francés & D'Elía 2006; Rivera *et al.* 2007). According to Massoia (1973), Myers & Carleton (1981), Weksler & Bonvicino (2005) and Paresque *et al.* (2007), *Oligoryzomys eliurus* is a synonym of *O. nigripes*. Although sequences of specimens from Bolivia and Uruguay are not available in GenBank, the present results support the assumption that *O. nigripes* is a single taxonomic unit with broad geographical distribution. Its karyotype $2n = 62/FN = 78–82$ was described by Andrades-Miranda *et al.* (2001) and Weksler & Bonvicino (2005). Accordingly, the specimen NK 22527 (AY041196) identified as *O. fornesi* by Rinehart *et al.* (2005) is in fact *O. nigripes* (Fig. 3, Table S1, Supporting information). This species is the natural host of the pathogenic Juquitiba hantavirus in south-eastern Brazil (Suzuki *et al.* 2004), north-eastern Argentina (Misiones province), (Padula *et al.* 2007) and Uruguay (Delfraro *et al.* 2008).

In our study, the Cyt *b* tree reveals that the clade containing *O. nigripes* is strongly related to that of *O. stramineus* ($2n = 52/FN = 68–70$; Bonvicino & Weksler 1998; Andrades-Miranda *et al.* 2001; Weksler & Bonvicino 2005), a species endemic to the Cerrado and Caatinga domains in Brazil (Bonvicino & Weksler 1998). This close relationship was also observed by Miranda *et al.* (2009) using both the Cyt *b* and IRBP genes and Weksler (2003, 2006) based on the IRBP gene and morphological characters.

The distribution of Oligoryzomys chacoensis

The geographic origin of individuals grouped in the *O. chacoensis* clade ($2n = 58/FN = 74$; Myers & Carleton 1981; Espinosa & Reig 1991) confirms that this species inhabits the Chaco of Argentina, Bolivia and Paraguay (Figs 1 and 3); it has also been captured in several localities of the Yungas and in an ecotone between Yungas and Prepuna in Jujuy, Argentina (Díaz & Barquez 2007). Its citation for Brazil would correspond to an erroneous classification of specimens belonging to other species (Bonvicino & Weksler 1998). *Oligoryzomys chacoensis* has been recognized as the reservoir of the Oran hantavirus genotype (Gonzalez Della Valle *et al.* 2002; Rivera *et al.* 2007).

The Oligoryzomys fulvescens *group*

Oligoryzomys fulvescens, distributed from southern Mexico throughout Central America to Venezuela, Colombia, Ecuador, and northern Brazil, was proposed as a species complex (Carleton & Musser 1989). In this study, sequences from Mexico clearly group in a clade different from those from Costa Rica, which includes the individual from Panama (UMMZ 116911; L37386) classified as *O. vegetus* by Carleton & Musser (1995) and Myers *et al.* (1995). *Oligoryzomys fulvescens fulvescens* from Mexico has a chromosome number $2n = 60/FN = 74$. Gardner & Patton (1976) reported a $2n = 54/FN = 68$ for one specimen from Costa Rica identified as *O. fulvescens*, which must clearly belong to *O. vegetus*. The type locality of *O. fulvescens* is in Mexico (Orizaba, Veracruz State) and that of *O. vegetus* is in Panama (Volcán de Chiriqui, Chiriqui Province; Musser & Carleton 2005). Carleton & Musser (1995) observed that these two species can occur in sympatry in Costa Rica and western Panama, and reported important morphological and morphometric differentiation between them. These

observations, data from cytogenetics and the molecular results presented here, confirm that *O. vegetus* and *O. fulvescens* are valid species.

The use of the name *O. fulvescens*, however, is controversial. For example, specimen TK 138080 from Venezuela is identified in GenBank as *O. fulvescens* (DQ227457, Fig. 3), but is related to *Oligoryzomys* sp. 1 (DQ826025) from north-eastern Brazil (locality 'v' in Fig. 1), and not to *O. fulvescens* from Mexico (therefore we refer to this sequence as *Oligoryzomys* sp. 2). The accurate specific assignment of individuals of *Oligoryzomys* from Central and northern South America has epidemiological importance since specimens assigned to *O. fulvescens* from western Venezuela have been associated with Maporal hantavirus (Fulhorst *et al.* 2004), whereas *O. fulvescens* from Panama has been associated with Choclo hantavirus (Vincent *et al.* 2000). If each hantavirus were associated with a specific rodent host (Schmaljohn *et al.* 1985), individuals from Panama and Venezuela could belong to different species. Besides, considering that *O. fulvescens* and *O. vegetus* occur in sympatry in western Panama, it is necessary to reexamine the specific classification of individuals positive for Choclo hantavirus.

Regarding those specimens from Venezuela, Osgood (1912) described the species *Oligoryzomys griseolus*, which is present at high altitudes; Musser & Carleton (2005) stated that this form resembles *O. vegetus* in morphological traits. Considering our present results and those from the literature, the comparison of *Oligoryzomys* sp. 2 with *O. griseolus* and with the form $2n = 62/FN = 74$ referred to as *O. longicaudatus* variant 3 in Gardner & Patton (1976), all from Venezuela, will contribute to clarify the taxonomy of these forms.

Other species presenting taxonomic and phylogenetic uncertainties

Two species of *Oligoryzomys* from Brazil have been recently described (Weksler & Bonvicino 2005): *O. moojeni* ($2n = 70/FN = 74$) and *Oligoryzomys rupestris* ($2n = 44$–$46/FN = 52$–53; Silva & Yonenaga-Yassuda 1997). Unfortunately, only sequences of *O. moojeni* are published in GenBank. In this study and in that of Miranda *et al.* (2009) using Cyt *b* sequences, the species *O. moojeni* is closely related to *Oligoryzomys* sp. 1 from northern Brazil and to *Oligoryzomys* sp. 2 from Venezuela. It is interesting to highlight that the phylogenetic relationships among these forms present a biogeographic correlate since the northern South America species (*Oligoryzomys* sp. 1, *Oligoryzomys* sp. 2 and *O. moojeni*) are closely related. A similar figure is observed for the Central American species *O. fulvescens* and *O. vegetus*. Geographical distribution data should not be ignored in a taxonomic revision of the genus.

In Peru, several species have been described revealing phylogenic conflicts. According to our Bayesian Cyt *b* and ML trees, *O. andinus* (accession number L37400) is related to *O. destructor*. However, because *O. andinus* ($2n = 60/FN = 70$; Gardner & Patton 1976) appears to be shifting from place to place, relationships among basal lineages in *Oligoryzomys* are unsatisfactorily resolved in our analysis (Fig. 3). Moreover, *Oligoryzomys arenalis* from the arid coastal areas (described by Osgood 1914; and cited in Pacheco 2002), not included in our present study, should be taken into account in future studies. There is also a controversy with the classification of individual MVZ 139219 from Puquio, Department of Ayacucho, initially assigned by Gardner & Patton (1976) to *O. longicaudatus* var 1 ($2n = 68/FN = 74$–76) and later classified as *Oligoryzomys* sp. B in Carleton & Musser (1989). In this paper, we propose that *O. flavescens* West would be a synonymous of *Oligoryzomys* sp. B, but the chromosome number described in Espinosa & Reig (1991) and Aniskin & Volobouev (1999) does not match with that of the sample from Peru. Most of the specimens assigned to *Oligoryzomys* sp B. in Carleton & Musser (1989) were obtained in the eastern side of the Andes mountains (like all individuals of *O. flavescens* West), but individual MVZ 139219 was captured in the western side, and it probably represents another species. Weksler & Bonvicino (2005) include the karyotype of this specimen as part of a 'nigripes' group.

Little information is available for some species, such as *Oligoryzomys victus* from Saint Vincent in the Western Antilles islands; it was described by Thomas (1898), later revised by Ray (1962) and is now apparently extinct (MacPhee & Fleming 1999).

In conclusion, in the context of previous available information, our analyses consider the following valid species of *Oligoryzomys*: *O. chacoensis*, *O. destructor*, *O. fornesi*, *O. longicaudatus*, *O. microtis*, *O. nigripes* (*Oligoryzomys delticola* and *O. eliurus* being synonymous), *O. stramineus*, *O. moojeni*, *O. fulvescens* (from Mexico), *O. vegetus* (from Costa Rica and Panama) and *O. rupestris*. However, the specific status of the several groups of *O. flavescens*, *Oligoryzomys magellanicus*, *O. griseolus*, *O. victus*, *O. andinus* and *O. arenalis* are still dubious and should be revised carefully in further studies combining molecular and morphological analyses from samples covering a wider geographical range.

The geographic origin of the genus Oligoryzomys

Reig (1984) and Engel *et al.* (1998) proposed that species of *Oligoryzomys* originated by a rapid diversification process. The position of *O. microtis* at the base of the *Oligoryzomys* radiation revealed by our data and by those of Miranda *et al.* (2009) support the idea of an Amazonian origin for the genus. Reig (1986) considered that the

diversification could have begun in premontane forest of the northern Andes or the western lowland Amazon. This agrees with the proposal of Steppan *et al.* (2004), who conclude that the Oryzomyalia inhabiting Central and North America (selected species of *Oryzomys*, *Oligoryzomys*, *Melanomys* and *Sigmodontomys*) represents re-colonizations of that continent from South America. In the present phylogenetic analysis of *Oligoryzomys*, the fact that basal nodes are represented by South American taxa reinforces previous hypotheses. Miranda *et al.* (2009) suggested that the genus had spread from northern South America to the southern regions of the continent. According to our results, the clades grouping *O. flavescens*, *O. longicaudatus* and *O. magellanicus* are recovered in a distal position; these species inhabit the southern regions of Argentina and Chile, supporting that proposal.

Large extensions in South America have not been sampled yet (see Fig. 1) and new species could be described from those regions. Studies using an integrative approach involving a larger sampling area, multilocus molecular data, detailed morphological and chromosomal analyses and niche-based distribution modelling should be performed to delimit the species boundaries and to get a better approach to the evolutionary history of the genus *Oligoryzomys*.

Acknowledgements

We are very grateful to Ulyses F.J. Pardiñas (CENPAT, Puerto Madryn, Argentina), Pablo Teta (MACN-UBA, Buenos Aires, Argentina) and Ignacio Ferro (PIDBA, Tucumán, Argentina) for providing samples and especially for the morphological classification of many of the specimens used in this study. We also thank Silvana Levis (INEVH, Pergamino, Argentina), Milton Gallardo (Universidad Austral de Chile, Valdivia, Chile), Gustavo E. Cantoni (URES, Bariloche, Argentina), Eduardo R. Herrero (URES, Bariloche, Argentina) and Javier Pereira (ACEN, Buenos Aires, Argentina), for providing some of the specimens. We thank Camilo Mattoni (FCEFyN, UNC, Córdoba, Argentina) for his advice on phylogenetic analyses and Jacqueline Miralles-Salazar for lab work assistance. Michael Carleton provided valuable comments that helped to improve the manuscript. This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET PIP 11420090100289), Argentina, by the Secretaría de Ciencia y Tecnología (SECYT), Universidad Nacional de Córdoba, Argentina. JSB thanks the financial support from the National Science Foundation (INT 94-17252 and DEB 06-16305) and the Department of Biological Sciences of Texas Tech University. REGI, RMB and CNG are Researchers of CONICET.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Cytochrome *b* sequences extracted from GenBank used in the present study for comparison purposes. In bold, new species assignment according to our present data, with previous classification in parentheses.

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