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Phylogenetic relationships of the pygmy rice rats of the genus *Oligoryzomys* Bangs, 1900 (Rodentia: Sigmodontinae)

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Sequences from two mitochondrial genes (cytochrome *b* and NADH1) were used to produce a molecular phylogeny for 12 named and two undescribed species of the genus *Oligoryzomys*. All analyses placed *Oligoryzomys microtis* as the most basal taxon, a finding consistent with previous studies that suggested the west-central Amazon as a centre of origin for the tribe Oryzomyini to which *Oligoryzomys* belongs. Biogeographically, this suggests that *Oligoryzomys* had a South American origin, and later advanced northwards, entering Central America and Mexico more recently. Different analyses have provided consistent support for several additional clades that did not necessarily agree with the species groups hypothesized by previous studies. A molecular clock derived for these data suggests an origin for the genus of 6.67 Mya, with most speciation within the genus occurring between 3.7 and 1.5 Mya.

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ADDITIONAL KEYWORDS: cytochrome *b* – NADH1 – Neotropics – phylogeny.

INTRODUCTION

New World mice are conventionally recognized within the subfamily Sigmodontinae, which includes about 450 species (Steppan, Adkins & Anderson, 2004; Musser & Carleton, 2005). However, some authors divide this taxon into the Sigmodontinae *s.s.*, which is predominantly South American in distribution, with approximately 300 species, and the almost exclusively North American Neotominae (Steppan *et al.*, 2004). There are four major hypotheses concerning the arrival and radiation of sigmodontine rodents in

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South America: (1) sigmodontines evolved in North America prior to 7 Mya (Upper Miocene), and reached South America by waif dispersal from Central America about 6 Mya in the Upper Miocene (Marshall, 1979; Marshall *et al.*, 1982); (2) sigmodontines differentiated in tropical North America before spreading to South America by overland dispersal after the establishment of the Panamanian land bridge by Plio–Pleistocene times, about 3.5 Mya (Patterson & Pascual, 1972; Baskin, 1978; Simpson, 1980; Webb, 1991); (3) South American sigmodontines differentiated '*in situ*' from an invading northern ancestor arriving by over-water dispersal during Miocene times before the Panamanian land bridge, about 8 Mya (Hershkovitz, 1966, 1972; Savage, 1974; Reig, 1980, 1981, 1986); and (4) sigmodontines differentiated rapidly in South America from an ancestral form arriving by overland dispersal through the already formed Panamanian land bridge, approximately 3 Mya (Simpson, 1940, 1950). The occurrence of some fossil sigmodontine mice already differentiated in current genera date back to the Pliocene Montehermosan of Argentina, before the establishment of the Panamanian land bridge. This suggests an earlier origin for the entrance of these taxa into South America (Reig, 1981). In fact, calibrations based on molecular phylogeny suggest an origin of about 5–9 Mya (Engel *et al.*, 1998) and 10 Mya (Spotorno, 1986; Smith & Patton, 1999; Steppan *et al.*, 2004). A recent classification proposed by Steppan *et al.* (2004) based on nuclear genes recognized a new taxon within sigmodontines, Oryzomyalia, which includes important components of the South American mouse radiation, such as the tribe Oryzomyini and all of their related taxa. This tribe includes 28 genera (Weksler *et al.*, 2006), among which are the rice rats of the genus *Oligoryzomys* Bangs, 1900. This taxon was originally included as a subgenus within *Oryzomys*, but Carleton & Musser (1989) recognized *Oligoryzomys* as a valid genus based on several morphological features, including cranial, tooth, and stomach morphology, as well as its reduced body size, tail longer than head and body, short and broad hindfoot, small skull, and a convex interorbital bone. In the last decade the genus *Oligoryzomys* has been the focus of epidemiologic studies, as several species of the genus constitute the major reservoir of *Hantavirus*, which is an emerging infectious disease agent that causes a cardiopulmonary syndrome in humans, with lethal consequences in some cases (Lee & van der Groen, 1989). Different species of *Oligoryzomys* are the reservoirs of different *Hantavirus* strains, and they seem to constitute a coevolutionary association (Yates *et al.*, 2002; Rivera *et al.*, 2007). Regarding their habitats, *Oligoryzomys* species may be found in a variety of environments, from high-elevation Puna habitat in the central Andes Mountains (*Oligoryzomys andinus* Osgood, 1914) to lowland tropical and subtropical areas (*Oligoryzomys eliurus* Wagner, 1845 and *Oligoryzomys chacoensis* Myers and Carleton, 1981). The genus has a wide distribution in the Neotropics extending from Mexico (*Oligoryzomys fulvescens* Saussure, 1860), through Central America (*Oligoryzomys vegetus* Bangs, 1902), and southwards to Patagonia (*Oligoryzomys longicaudatus* Bennett, 1832; Fig. 1A and B).

Tate (1932) recognized about 30 species of *Oligoryzomys*, whereas Hershkovitz (1966) believed that *Oligoryzomys* represented a single species, with different forms described as subspecies. Musser & Carleton (1993) recognized 15 taxa of *Oligoryzomys*, but recent revisions by Musser & Carleton (2005) added three more, giving a total of 18 species. Weksler & Bonvicino (2005) described two new endemic species from Brazil (*Oligoryzomys moojeni* and *Oligoryzomys rupestris*) but, at the same time placed *O. eliurus* and *Oligoryzomys delticola* (Thomas, 1917) as junior synonyms of *Oligoryzomys nigripes* (Olfers, 1818), thus the total number of species remains the same as considered by Musser & Carleton (2005). Most species of the genus have been described based on morphology and chromosome number. In fact, most species have different karyotypes with 2*n* between 46 and 70 (Andrades-Miranda *et al.*, 2001; Weksler & Bonvicino, 2005).

Little is known about the evolutionary relationships within *Oligoryzomys*. Based on morphology, Carleton & Musser (1989) concluded that the genus is a monophyletic group, and recognized 12 species and three undescribed forms that were placed into five groups: the *fulvescens* group, including *O. fulvescens*, *Oligoryzomys arenalis* (Thomas, 1913), and *O. vegetus*; the *microtis* group, including *Oligoryzomys microtis* (Allen, 1916); the *andinus* group, including *O. andinus* and *O. chacoensis*; the *flavescens* group, including *Oligoryzomys flavescens* (Waterhouse, 1837) and three undescribed species; and the *nigripes* group, including *O. nigripes*, *O. eliurus*, *Oligoryzomys destructor* (Tschudi, 1844), *O. delticola*, and *O. longicaudatus*. Dickerman & Yates (1995) analysed the allozyme variation among selected oryzomyine taxa, including five species of *Oligoryzomys*, and concluded that the genus is monophyletic. Similarly, based on 401-bp sequences of the mitochondrial cytochrome *b* (cyt B) gene among eight species of the genus, Myers, Lundrigan & Tucker (1995) concluded that the genus was a natural group. Later, Weksler (2003) arrived at the same conclusion based on a phylogeny of the oryzomyine rodents, using sequences of the first exon of the IRBP nuclear gene that included five species of *Oligoryzomys*. More recently, from the results presented by Rivera *et al.*

Figure 1A. Approximate geographic distribution of species of the genus *Oligoryzomys*, including undescribed taxa *Oligoryzomys sp.* 1 and *Oligoryzomys sp.* B (the distribution of *Oligoryzomys sp.* B is according to trapping records reported in Carleton and Musser, 1989).

(2007) based on *d-loop* sequences, it is also possible to conclude the monophyly of the genus, although they evaluated only the systematic relationships of the species that occur in Argentina (seven taxa). Finally, a recently published study by Miranda *et al.* (2008) based on cyt B sequences including 12 species of *Oligoryzomys* also concluded the monophyly of the genus. All the latter studies agreed on the monophyly of *Oligoryzomys*, but none of them recognized the species groups as proposed by Carleton & Musser (1989). Since then, no other study designed to evaluate the phylogenetic relationships of most of the currently known species of *Oligoryzomys* has been published.

The above information suggests that *Oligoryzomys* is a monophyletic group. We tested this hypothesis and evaluated whether the species groups proposed by Carleton & Musser (1989) constitute natural groups (clades). We evaluated the phylogeny in 12 species of *Oligoryzomys* [seven species overlapped with those considered in Miranda's (2008) study]. In addition, we included two undescribed forms: *Oligoryzomys* sp. 1 (field catalogue of one of us, MIC) and *Oligoryzomys* sp. B (Carleton & Musser, 1989). Another major objective was to calibrate a molecular clock to hypothesize the time of origin and radiation of *Oligoryzomys*, and contrast those results with hypotheses about the origin and radiation of sigmo-

Figure 1B. *Continued*.

dontines in the Neotropics. To accomplish these goals, we sequenced the cyt B and nicotinamide dinucleotide dehydrogenase subunit 1 (NADH1) mitochondrial genes in 16 specimens of the 14 taxa plus two out-groups. Aligned sequences were analysed phylogenetically using different optimality criteria for each molecular marker and for the total evidence matrix.

MATERIAL AND METHODS

DNA SEQUENCING AND ALIGNMENT

DNA was extracted from frozen tissue (mainly liver) from 16 specimens: 14 *Oligoryzomys* species and two out-groups (see below). Capture and handling procedures followed guidelines approved by the American Society of Mammalogists (Gannon *et al.*, 2007). According to Musser & Carleton's (2005) species account, we lack six species of *Oligoryzomys*, for which samples were unavailable: a new form described from Brazil, *Oligoryzomys stramineus* (Bonvicino and Weksler, 1998); *Oligoryzomys griseolus* (Osgood, 1912) from Venezuela, of which there have been no recent captures (M. Aguilera to R.E. Palma, pers. comm.); *Oligoryzomys victus* (Thomas, 1898) from Lesser Antilles, which is presumably extinct (Musser & Carleton, 2005); *Oligoryzomys magellanicus* (Bennet, 1835) (Isla Harrison, Magallanes, Chile); *O. arenalis* (Lambayeque, Perú), and *Oligoryzomys brendae* (Massoia, 1998) (although see below). In addition, we could not get samples from two other Brazilian forms: *O. moojeni* and *O. rupestris*, described by Weksler & Bonvicino (2005). DNA was extracted according to the techniques outlined in Laird *et al.* (1991). In most cases we sequenced a single specimen of each species, except for *O. longicaudatus* and *Oligoryzomys sp.* 1 (Table 1). We

Table 1. List of species, collection/museum numbers, localities, and GenBank accessession numbers of *Oligoryzomys* sequenced for the cytochrome *b* and NADH1 mitochondrial genes

Museum/ catalog	Species	Locality	Cytochrome *b* access	NADH1 access	Coordinates
NK101588	*O. fulvescens*	Panamá, Prov. Los Santos, Península de Azuero	EU 192164	EU 192190	07°46′04″S, 80°17′04″W
KU142065	*O. vegetus*	Costa Rica, Prov. Punta Arenas, Monteverde, Cerro Amigos, 1760 m a.s.l.	EU 192165	EU 192189	not available
NK13425	*O. microtis*	Bolivia, Depto Beni, 3 km S Rurrenabaque, 365 m a.s.l.	EU 192172	EU 192191	14°30′S, 67°34′W
NK21532	*O. flavescens*	Bolivia, Depto Chuquisaca, 9 km by road N of Padilla, 2000 m a.s.l.	EU 192170	EU 192177	19°18′S 64°22′W
NK42266	*O. eliurus*	Brasil, Sao Paulo, Depto Guariba	EU 192163	EU 192182	21°25′30.9″S, 48°15′24.9″W
NK22846	*O. destructor*	Bolivia, Depto Cochabamba, Tinkursiri, 17 km E of Totora, 2950 m a.s.l.	EU 192171	EU 192176	17°45′S, 65°02′W
NK72388	*O. chacoensis*	Paraguay, Depto Boquerón, Fortin Toledo, 600 m a.s.l.	EU 192173	EU 192183	22°01′20.3″S, 60°36′2.5″W
NK11547	*O. andinus*	Bolivia, Depto Oruro, 2 km W of Huancaroma, 3730 m a.s.l.	AY 452200	EU 192186	17°40′S, 67°30′W
GD259	*O. fornesi*	Paraguay, Depto Paraguarí, Costa Río Tebicuary, 1.2 km aguas abajo	EU 192158	EU 192184	26°24.050S, 57°02.340W
GD569	*O. delticola*	Uruguay, Depto Rivera, Lunarejo (propiedad Sr. Abelenda)	EU 192162	EU 192181	31°06′S, 55°58′W
MIC210	*Oligoryzomys sp.* 1	Argentina, Prov. Catamarca, Dept Ambato, Las Juntas	EU 192167	EU 192178	28°06′34″S, 65°55′0″W
MIC211	*Oligoryzomys sp.* 1	Argentina, Prov. Catamarca, Dept Ambato, Las Juntas	EU 192168	EU 192180	28°06′34″S, 65°55′0″W
MIC203	*Oligoryzomys sp.* 1	Argentina, Prov. Catamarca, Dept Ambato, Las Juntas	EU 192169	EU 192179	28°06′34″S, 65°55′0″W
GD547	*O. nigripes*	Paraguay, Depto Paraguarí, Costa del Río Tebuicary	EU 192161	EU 192175	26°30.816S, 57°14.444W
MUSA2625	*Oligoryzomys sp. B 3203*	Perú, Depto Puno, Prov. Sandia, Distrito Limbani, Pueblo de Limbani	EU 192159	EU 192185	not available
JCT1960	*O. longicaudatus*	Chile, Magallanes, Prov. Antarctica Chilena, Isla Navarino, Bahía Inútil	EU 192160	EU 192187	54°59′S, 68°13′W
NK95245	*O. longicaudatus*	Chile, Aysén, Prov. Coyhaique, Forestal Mininco	AY 346567	EU 192188	45°31′03″S, 71°51′49″W
NK37843	*Transandinomys talamancae*	Ecuador, Depto El Oro, Río Puyango	EU 192166	EU 192192	03°53′00″S, 80°07′00″W
NK27671	*Holochilus brasiliensis*	Bolivia, Depto Beni, San Ramón, Río Mamoré	EU 192174	EU 192193	13°16′19″S, 64°37′33″W

GD, field catalogue of Guillermo D'Elía, Universidad de Concepción, Chile; JCT, field catalogue number of Juan Carlos Torres-Mura, Museo Nacional de Historia Natural, Santiago, Chile; KU, Kansas University Natural History Museum, The University of Kansas, USA; MIC, field catalogue of María Inés Carma; MUSA, Museo de la Universidad de San Agustín, Arequipa, Perú; NK, voucher reference number used for the Museum of Southwestern Biology, University of New Mexico, New Mexico, USA.

amplified the cyt B and the NADH1 mitochondrial genes via the polymerase chain reaction (PCR; Saiki *et al.*, 1988) using Taq DNA Polymerase (Invitrogen) and primers L (MSB) 5′-GACATGAAAAATCATCGT TGTAATTC-3′ and MVZ-14 (Smith & Patton, 1993) for the cyt B gene, and 16S.f2 5′-TACGACCTCGATG TTGGATCAGG-3′ and Met.r1 5′-GGGGTATGGGCC CRARAGC-3′ for the NADH1 gene. The PCRs were performed using the following thermal profiles. For cyt B: 35 cycles of 94 °C denaturation for 40 s; 42 °C annealing for 40 s; and a 72 °C extension for 1 min 20 s. For NADH1: 35 cycles of 94 °C denaturation for 40 s; 50 °C annealing for 35 s; and a 72 °C extension for 1 min 20 s. Double-stranded PCR products were purified using QIAquick (Qiagen). Sequencing was conducted through cycle sequencing (Murray, 1989) using the PCR primers labelled with the Big Dye Terminator kit (Perkin Elmer), and the sequencing reactions were analysed in an ABI Prism 310 automated sequencer. The PCR products were sequenced at least two times to ensure sequence fidelity. Sequences were aligned by eye and using ClustalX to maintain amino acid sequences (Thompson *et al.*, 1997). We also used MacClade 3.08 (Maddison & Maddison, 1992) to translate nucleotide codons into amino acids. Alignment was conducted for each data set, as well as for the complete data matrix. All sequences were entered into GenBank, and accession numbers are given in Table 1. The substitution rate was evaluated for both genes using the best-fitting nucleotide substitution model obtained with Modeltest (Posada & Crandall, 1998). Through this approximation we demonstrated that although both molecular markers belong to the same genome, they showed different evolutionary trends (Fig. 2).

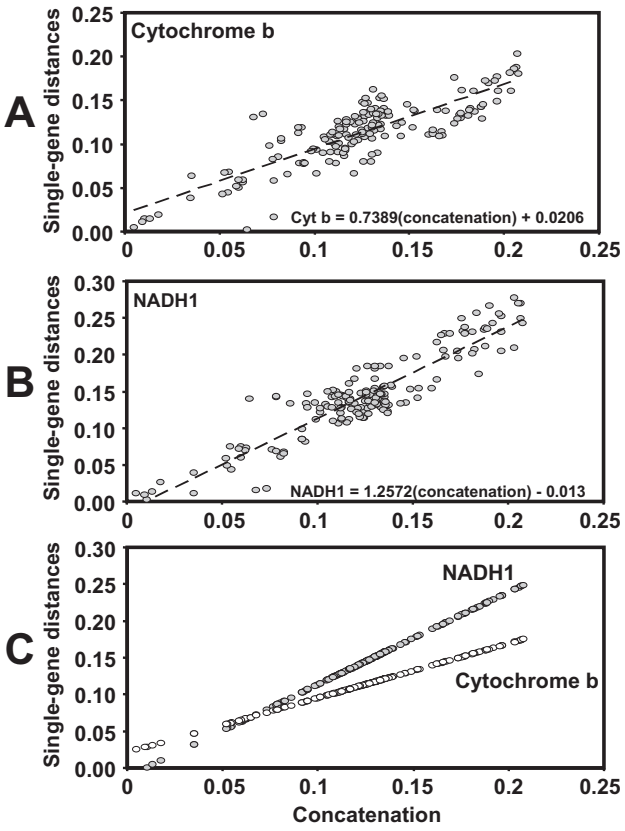

Figure 2. Nucleotide-based pairwise distances calculated independently for each gene (A, cytochrome *b*; B, NADH1), vs. pairwise distances calculated from the concatenation of two genes (see Material and methods for the calculation of distances).

Phylogenetic analyses and clock calibration

Phylogenetic reconstruction was performed through maximum parsimony (MP) using PAUP* 4.0b10 (Swofford, 2002). Both mitochondrial data sets (cyt B and NADH1) were analysed separately and as a combined data matrix. Congruence between cyt B and NADH1 data sets was tested using the partition homogeneity test (Farris *et al.*, 1994) implemented in PAUP* 4.0b10 with 1000 replicates, excluding invariant characters (Cunningham, 1997). For parsimony analysis we treated all characters as unordered with four possible states (A, C, G, and T), and we used the characters that were phylogenetically informative. As the transition/transversion (ts/tv) rate was 4 : 1 for each mitochondrial marker, we performed weighted parsimony (WP). For WP, a heuristic search was performed with ten random additions, and branch swapping was performed via tree bisection reconnection (TBR; Nei & Kumar, 2000). The reliability of nodes was estimated by non-parametric bootstrap (Felsenstein, 1985) after 1000 pseudoreplications. Phylogenetic trees were rooted with the out-group criterion using two oryzomyine taxa, *Holochilus brasiliensis* and *Transandinomys talamancae* (formerly known as *Oryzomys talamancae*; Weksler, Percequillo & Voss, 2006). *Holochilus brasiliensis* is part of the sister clade to which *Oligoryzomys* belongs, whereas *T. talamancae* corresponds to a more distant related lineage within Oryzomyini (Weksler, 2006). In addition, to allow comparison with published data from other sigmodontines, and particularly from other oryzomyine taxa, we calculated the distance values between pairwise taxa using Kimura's two parameter (K2P) model (Kimura, 1980) for the cyt B gene.

The Markov Chain Monte Carlo (MCMC) method within a Bayesian framework (hereafter BMCMC) was used to estimate the posterior probability of phylogenetic trees. The MCMC procedure ensures that trees are sampled in proportion to their probabilities of occurrence under the model of gene-

sequence evolution. Approximately 22 000 000 phylogenetic trees were generated using the BMCMC procedure, sampling every 1000 trees to ensure that successive samples were independent. The first 50 trees of the sample were removed to avoid including trees sampled before convergence of the Markov Chain. A general likelihood-based mixture model (MM), based on the general time-reversible (GTR) model (see Rodríguez *et al.*, 1990) of gene-sequence evolution, was used to estimate the likelihood of each tree, as described by Pagel & Meade (2004, 2005). This model accommodates cases in which different sites in the alignment evolved in qualitatively distinct ways, but does not require prior knowledge of these patterns or partitioning of the data. These analyses were conducted using the software BayesPhylogenies, available from http://www.evolution.reading.ac.uk/BayesPhy.html. In order to find the best MM of gene-sequence evolution, we obtained the likelihood of the trees by first using a simple GTR matrix, then using a GTR matrix plus the gamma-distributed rate heterogeneity model (1GTR + G), and then continuing to add up to six GTR + G matrices. For the posterior analyses, only the combination of matrices with the fewest number of parameters that significantly increased the likelihood was used, which was evaluated using a one-way ANOVA for balanced data sets in Statistica 6.0 (StatSoft Inc.), and then by a posterior Newman–Keuls test (Zar, 1996). Assumptions of normality of data and homogeneity of variance were previously evaluated. Posterior probabilities for topologies were then assessed as the proportion of trees sampled after burn-in, in which that particular topology was observed.

As our results did not support the generalized molecular clock model (Likelihood Ratio = 74.34, $d.f. = 17$, $P < 0.0001$), we used a relaxed molecular clock by running BEAST v.1.4.8 (Drummond *et al.*, 2006), which employs a BMCMC to co-estimate topology, substitution rates, and node ages. Posterior probability distributions of node ages were obtained for the concatenated two-gene alignment. The GTR + G + I model with rate variation (six gamma categories) was implemented for the concatenated genes. The analysis implemented a Yule branching rate prior, with rate variation across branches asumed to be uncorrelated and lognormally distributed (Drumond *et al.*, 2006). The MCMC chain was run for 10 000 000 generations (burn-in 10 000 generations), with parameters sampled every 1000 steps. Examination of MCMC samples using TRACER 1.4 (Rambaut & Drummond, 2003) suggested that the independent chains were each adequately sampling the same probability distribution: effective sample sizes for all parameters of interest were greater than 500.

We used two points of fossil calibration based on Pardiñas, D'Elía & Ortiz (2002): C1, an *O. flavescens* fossil specimen from the Ensenadense level dated to a mean time of 1.5 Mya; and C2, an *O. eliurus* fossil specimen from the Lujanense level dated to a mean time of 0.24 Mya. As these datings do not have an associated error, we used additional data reported by Schultz *et al.* (2004) for the Argentinean Pampa. By using radiometric $^{40}Ar/^{39}Ar$, they dated a Pleistocene site as 0.23 ± 0.03 Mya, which is equivalent to the stratus of the *O. eliurus* fossil. This error was incorporated into the molecular clock analysis as part of the prior probability through a uniform distribution where the mean corresponded to the fossil age and the error corresponded to the radiometric error. We did not find an error associated with C1; however, we used a standard deviation equivalent to half the Ensenadense level (1.5 ± 0.64 Mya). Thus, we used the fossil calibration as an uncertain age, and the node age estimation was set to normal distribution. With the former parameters, we first estimated divergence at the root of the tree, then the in-group divergence, and finally the divergence at several clades, as shown in Figure 3.

Dispersal–vicariance analysis

To infer the history of biogeographic distributions in *Oligoryzomys*, we used the known distributions of each species, as coded in the Appendix, optimized on the combined-data tree using dispersal–vicariance analysis (DIVA 1.1; Ronquist, 1996, 1997). This program infers ancestral distributions based on a three-dimensional cost matrix derived from a simple biogeographical model. The advantage of this approach is that it does not require a general a priori hypothesis of area relationships.

RESULTS

Nucleotide variation analyses

The final alignments were 977 bp for the cyt B gene and 959 bp for the NADH1 gene, which provided a combined data matrix of 1936 characters. The overall base composition for each gene was as follows: cyt B, A, 31%; C, 29%; T, 28%; and G, 12%; and NADH1, A, 35%; C, 30%; T, 27%; and G, 8%.

The K2P distance for the cyt B gene (Table 2) exhibited values that ranged between 0.722% for specimens of the same locality, and 1.345% for specimens of the same species from different localities, such as island and continent representatives (e.g. *O. longicaudatus*). However, the K2P distance values between different species varied between 5.7% (*O. longicaudatus–Oligoryzomys fornesi* [Massoia, 1973]) to 15% (e.g. *O. microtis–O. andinus*). Other values between recognized *Oligoryzomys* species were about

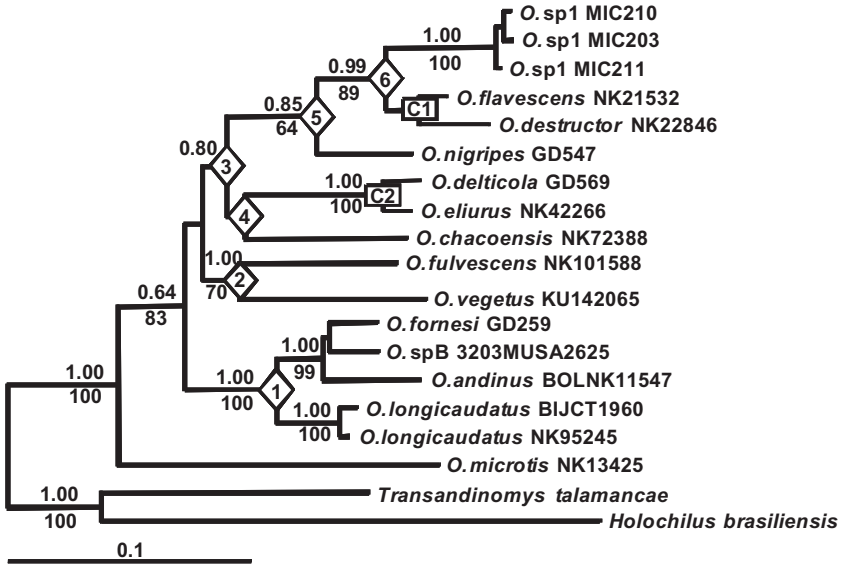

Figure 3. *Oligoryzomys* phylogeny based on weighted parsimony analysis (WP) and the Bayesian Markov Chain Monte Carlo method (BMCMC). The phylogeny was obtained for the combined mitochondrial cytochrome *b* and NADH1 sequence data, whereas BMCMC represents a consensus tree of the $N = 21\,950$ trees from the converged Markov chain. A posterior probability above 0.5 and bootstrap values over 50% are represented on each node. C1 represents calibration time 1 = 1.5 Mya; C2 represents calibration time 2 = 0.24 Mya (according to Pardiñas *et al.*, 2002; see Material and methods). Diamonds on nodes represent that clade number for the clock calibration using BEAST.

10% between the sister taxa *O. fulvescens* and *O. vegetus*, or about 6% between *O. flavescens* and *O. destructor*. The K2P distance values among representatives of the same taxon (the unnamed *Oligoryzomys sp.* 1) were less than 1%, and the K2P distance between the other unnamed species *Oligoryzomys sp.* B and its closest relative *O. fornesi* was 3.6% (Table 2).

Phylogenetic analyses

The two genes used in the present study are functionally independent, and exhibit unique evolutionary patterns (Fig. 2). The rate of nucleotide substitution for each gene was relatively homogeneous across the length of their sequences, but NADH1 has a much higher rate of substitution than cyt B. The predicted distance values (Fig. 2C) show different slopes (*t-Student* = 12.48; *d.f.* = 169; $P < 0.001$), evidencing two evolutionary rates in the molecular markers analysed. On the other hand, the partition homogeneity test suggested that our data sets were not significantly incongruent ($P = 0.01$), following the criteria of Cunningham (1997). Therefore, these data were combined for further phylogenetic analysis.

A similar topology was obtained with both the MP via WP and BMCMC analyses. The WP resulted in a single most parsimonious tree that was 2517 steps long: consistency index, *CI* = 0.5217; retention index, *RI* = 0.6033. Of the total 1936 characters combining both genes, 485 were parsimony informative. Both, WP and BMCMC (Fig. 3) showed the same topology and hypothesized *O. microtis* as the most basal taxon within *Oligoryzomys*, with 100% bootstrap and 1.00 posterior probability support, respectively (Fig. 3). The WP and BMCMC trees exhibit a split that recovered a well-supported clade (1.00 and 100) that included (((*O. fornesi*, *Oligoryzomys* sp. B), *O. andinus*), *O. longicaudatus*) on one clade, and all of the other species of *Oligoryzomys* on the other clade. In the latter clade we recovered a split between two major groupings: (*O. fulvescens*, *O. vegetus*) on one side, and a major clade that included ((*O. delticola*, *O. eliurus*), *O. chacoensis*) in a sister relationship with a clade that included (((*O. flavescens*, *O. destructor*), *Oligoryzomys sp.* 1), *O. nigripes*).

Age estimations from the relaxed clock analysis estimated 6.67 ± 0.02 Mya for the divergence between *Oligoryzomys* with respect to the out-groups, and 5.27 ± 0.052 Mya for the split between *O. microtis* and the rest of the species (Fig. 3). Other age estimations were as follows: 2.2 ± 0.043 Mya for clade 1 (((*O. fornesi*, *Oligoryzomys sp.* B), *O. andinus*), *O. longicaudatus*); 3.35 ± 0.035 Mya for clade 2 (*O. fulvescens*, *O. vegetus*); 3.71 ± 0.035 Mya for clade 3, composed of clade 4 ((*O. eliurus*, *O. delticola*), *O. chacoensis*) and clade 5 *O. nigripes*; and 1.54 ± 0.031 Mya for clade 6, composed of (((*O. destructor*, *O. flavescens*), *Oligoryzomys sp.* 1).

Table 2. Kimura's two parameter (K2P) model distance values among pair-wise *Oligoryzomys* spp. for cytochrome *b* sequences

Taxon name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1 *O. fornesi* GD259	–																		
2 *Oligoryzomys* sp B. 3203MUSA2625	0.037	–																	
3 *O. andinus* NK11547	0.067	0.070	–																
4 *O. longicaudatus* BIJCT1960	0.079	0.055	0.083	–															
5 *O. longicaudatus* NK95245	0.057	0.058	0.082	0.013	–														
6 *O. nigripes* GD547	0.098	0.092	0.124	0.083	0.089	–													
7 *O. delticola* GD569	0.094	0.091	0.120	0.079	0.085	0.009	–												
8 *O. eliurus* NK42266	0.096	0.094	0.112	0.079	0.087	0.030	0.032	–											
9 *O. chacoensis* NK72388	0.098	0.099	0.116	0.085	0.091	0.113	0.113	0.104	–										
10 *O. fulvescens* NK101588	0.096	0.095	0.119	0.096	0.097	0.107	0.108	0.101	0.108	–									
11 *O. vegetus* KU142065	0.118	0.116	0.133	0.110	0.111	0.108	0.111	0.114	0.115	0.101	–								
12 *Oligoryzomys sp.* 1 MIC210	0.111	0.109	0.119	0.112	0.109	0.110	0.109	0.105	0.106	0.110	0.099	–							
13 *Oligoryzomys sp.* 1 MIC203	0.105	0.103	0.116	0.113	0.111	0.111	0.110	0.107	0.108	0.108	0.103	0.010	–						
14 *Oligoryzomys sp.* 1 MIC211	0.111	0.109	0.117	0.112	0.109	0.110	0.109	0.104	0.100	0.109	0.103	0.007	0.009	–					
15 *O. flavescens* NK21532	0.130	0.128	0.132	0.128	0.129	0.133	0.132	0.123	0.106	0.122	0.121	0.057	0.060	0.050	–				
16 *O. destructor* NK22846	0.146	0.142	0.150	0.145	0.143	0.140	0.138	0.132	0.135	0.134	0.145	0.098	0.101	0.094	0.063	–			
17 *O. microtis* NK13425	0.146	0.138	0.151	0.138	0.14	0.136	0.137	0.134	0.136	0.144	0.138	0.102	0.107	0.098	0.090	0.108	–		
18 *Transandinomys*	0.123	0.113	0.138	0.106	0.104	0.138	0.135	0.129	0.118	0.114	0.127	0.124	0.127	0.127	0.145	0.153	0.144	–	
19 *Holochilus*	0.159	0.153	0.155	0.152	0.152	0.174	0.172	0.164	0.172	0.173	0.191	0.187	0.184	0.184	0.188	0.214	0.207	0.189	–

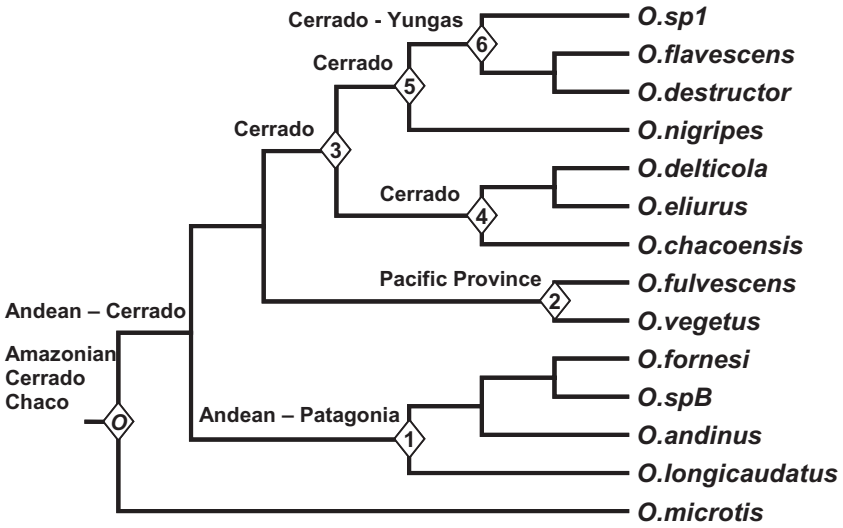

Figure 4. Dispersal–vicariance analysis with geographic regions optimized onto the topology of the Bayesian consensus tree. The hypothetical ancestral distributions obtained through this method are listed above the branches.

DIVA ANALYSIS

Optimization of geographic distributions of taxa using dispersal/vicariance analysis (Fig. 4) revealed the basal clade (constituted by *O. microtis*) to be from the Amazonian, Cerrado, and Chaco ecoregions of South America. The next succesive basal clade, clade 1, is from the highlands of the Andes and Patagonia; clade 2 is from the Pacific Province; clades 3, 4, and 5 are from the Cerrado; whereas clade 6 is from the Cerrado and Yungas (see Fig. 4).

DISCUSSION

PHYLOGENETIC ANALYSES AND NUCLEOTIDE VARIATION

Both WP and Bayesian analyses recovered *O. microtis* as the most basal taxon in the evolution of the genus *Oligoryzomys*. A similar conclusion was reached by Rivera *et al.* (2007), as well as by Miranda *et al.* (2008): Miranda *et al.* (2008) based their study on cyt B sequences that included some of the species considered in this study. Except for *O. microtis* forming its own group and the sister relationship of *O. fulvescens* and *O. vegetus*, we did not recover any other species groups proposed by Carleton & Musser (1989). One of the well-supported clades obtained in our study (((*O. fornesi*, *Oligoryzomys* sp. B), *O. andinus*), *O. longicaudatus*) was supported by the phylogeny of Myers *et al.* (1995), based on partial cyt B sequences: they found that *O. fornesi* was more closely related to *O. longicaudatus*, and not to *O. microtis* as had been hypothesized by Carleton & Musser (1989) based on morphology. The inclusion of *O. andinus* in this clade does not agree with Myers *et al.* (1995), who recovered this species as sister to *O. microtis*. However, high bootstrap support and posterior probability values makes the hypothesized (((*O. fornesi*, *Oligoryzomys* sp. B), *O. andinus*), *O. longicaudatus*) clade obtained in our study more likely. *Oligoryzomys* sp. B (*sensu* Carleton & Musser, 1989) should be treated carefully, as this taxon was included in the *flavescens* group by Carleton & Musser (1989). The latter authors proposed that *Oligoryzomys sp.* B could be the Andean counterpart of *O. flavescens*, as the former is distributed above 2000 m a.s.l. in the central Andes. Carleton & Musser (1989) reported *Oligoryzomys sp.* B from the eastern Puna and Amazon slopes of the Andes, and from the Pacific side of the southern Peruvian Andes between 3000 and 4000 m a.s.l. The specimen of *Oligoryzomys sp.* B analysed by us was trapped in Limbani, Puno department, Peru, which was one of the collecting localities reported for this taxon by Carleton & Musser (1989). However, the *Oligoryzomys sp.* B analysed by us was recovered as sister to *O. fornesi* from the Chaco region, and is highly divergent with respect to *O. flavescens*. Indeed, the K2P distance value between sister taxa *Oligoryzomys sp.* B and *O. fornesi* was 3.7% for the cyt B gene, whereas the K2P cyt B distance value between *Oligoryzomys* sp. B and *O. flavescens* was 12.7%. The K2P value (3.7%) obtained for *Oligoryzomys* sp. B with respect to *O. fornesi* represents nearly a half or one-third of the nucleotide distance value obtained between well-recognized species (Steppan, 1998; Smith & Patton, 1999; Palma, Marquet & Boric-Bargetto, 2005a; this study, e.g. *O. andinus–O. longicaudatus*, *O. vegetus–O. fulvescens*), suggesting that *Oligoryzomys sp.* B might be speciating. This inter-

pretation agrees with previous work in other sigmodontine taxa (e.g. Oryzomyini and Phyllotini) that demonstrated a genetic distance between subspecies of rodents in a range that varied by less than 4% (Myers *et al.*, 1995; Steppan, 1998). *Oligoryzomys andinus*, on the other hand, appeared as sister to the (*O. fornesi*, *Oligoryzomys sp.* B) union, contrary to the relationship with *O. chacoensis* proposed by Carleton & Musser (1989). Finally, the basal part of the former clade recovered both *O. longicaudatus* specimens – one from Navarino Island (54°S) and the other from Coyhaique (45°S) on the continent – as having a 1% K2P distance value. This slight nucleotide difference between the insular and continental representatives of *O. longicaudatus* confirms previous results about the strong genetic/molecular homogeneity of this species along its wide distributional range in the southern Andes (Gallardo & Palma, 1990; Palma *et al.*, 2005b).

Another well-supported relationship was that between *Oligoryzomys* sp. 1 and its closest relatives *O. flavescens* and *O. destructor*. These samples were collected in Catamarca Province, north-west Argentina. Cranial and dental morphology supported differences with related species, although a name has yet to be assigned (M.I. Carma unpubl. data). Our molecular analyses supported the validity of this new taxon, and the individuals studied seem to constitute a valid species when contrasted with their sister taxa *O. flavescens* and *O. destructor*. The K2P distance value for the cyt B gene between *Oligoryzomys* sp. 1 and *O. flavescens* varied around 6.0%, whereas the distance between *Oligoryzomys* sp. 1 and *O. destructor* was about 10% for the same gene. This new taxon (*Oligoryzomys* sp. 1) is probably an offshoot of one of these two latter taxa, most likely a peripheral isolate of *O. flavescens*, as the southern distributional limit of *O. destructor* seems to be Chuquisaca, in Bolivia (Carleton & Musser 1989). In the last species account, Musser & Carleton (2005) included a new species of *Oligoryzomys*, *O. brendae*, as distributed from Tucuman and Catamarca. The *Oligoryzomys* sp. 1 representatives in this study are from Catamarca in the north-west of Argentina. We are not sure if the *Oligoryzomys* sp. 1 reported by us in this study is the same *O. brendae* presented in Musser & Carleton (2005), as no formal description for *O. brendae* is yet available (the citation in Musser and Carleton refers to a meeting presentation), and hence this name as published constitutes a *nomen nudum*.

The phylogenetic relatedness of *O. vegetus* and *O. fulvescens* with respect to other *Oligoryzomys* spp. is well supported in both phylogenetic analyses. Both optimality criteria recovered these two species as sister taxa. The close relationship between these two species seems plausible, given their biogeographic relatedness in Central America and the morphological characteristics that relate both taxa together with *O. arenalis* in the *fulvescens* group (Carleton & Musser 1989).

The next clade in the WP and Bayesian tree recovered *O. delticola* and *O. eliurus* in a sister relationship, and as being closely related to *O. chacoensis* as a first out-group. The first two taxa together with *O. nigripes* and *O. longicaudatus* are part of the *nigripes* species group *sensu* Carleton & Musser (1989). Our molecular mitochondrial results give no support for the *nigripes* species group, particularly with regards to the inclusion of *O. nigripes*, *O. longicaudatus*, and *O. destructor*. According to our results, the union between *O. eliurus* and *O. delticola* exhibits short branch lengths in the WP and BMCMC total evidence tree, and the K2P distance value for the cyt B gene between these two species is low (~3%), a value close to that obtained for subspecies in other related sigmodontine taxa (Steppan, 1998; Smith & Patton, 1999; Palma *et al.*, 2005a; this study). We obtained strong support for the relationship between *O. delticola* and *O. eliurus*, and other studies have proposed *O. delticola* to be a junior synonym of *O. nigripes* (Bonvicino & Weksler, 1998; Francés & D'Elía, 2006), based on karyotypes and GTG-banding similarities. Further work based on morphology and chromosomes have stated that it is difficult to separate *O. delticola* and *O. eliurus* from *O. nigripes* (Weksler & Bonvicino, 2005), and more recently Paresque *et al.* (2007) proposed leaving *O. delticola* and *O. eliurus* as junior synonyms of *O. nigripes*. Our results, however, showed *O. nigripes* as part of a different clade, compared with *O. delticola* and *O. eliurus*, with a moderate support in both the WP and BMCMC analyses (Fig. 3). Based on our results, we believe that *O. nigripes* is a different species with respect to *O. eliurus* and *O. delticola*, and that these two taxa could constitute the same species.

BIOGEOGRAPHY

The fossil record of oryzomyines is poor. The earliest records from South America are from the Pleistocene (Steppan, 1998; Pardiñas *et al.*, 2002). According to molecular evidence, the hypothesized time of arrival of sigmodontines in South America was prior to the formation of the Panamanian land bridge, and was achieved by waif dispersal via island hoping and/or rafting (Steppan *et al.*, 2004; Smith & Patton, 2007). The occurrence of oryzomyine forms in Central and North America must be a back dispersal from the south, probably as part of, or as a by-product of, the Great American Interchange (Simpson, 1980) once North and South America were connected via Central America.

Oligoryzomys (together with *Zygodontomys*, another component of the oryzomyine radiation) is one of the oryzomyines that does not have a known pattern of geographic distribution (Weksler, 2006). In trying to explain the patterns of geographic distribution for oryzomyines in the Neotropics, Weksler (2006) found that most species of oryzomyines followed a *trans*-Andean (west to the Andes) or *cis*-Andean (east to the Andes) distribution, or occurred in the Andes as a whole. However, this pattern does not fit *Oligoryzomys*, as this genus ranges from Mexico to Patagonia.

The position of *O. microtis* at the base of *Oligoryzomys* radiation supports earlier claims that the origin of the genus (and the tribe for that matter) should be localized in the premontane forests of the northern Andes Mountains or the western Amazon lowland forests (Reig, 1986; Weksler, 2006). This hypothesis was independently proposed by Reig (1986) based on the number of taxa found in the northern Andes Mountains (Ecuador, Colombia, and Venezuela). Reig (1986) recognized 14 species of oryzomyines in that area, five of which were endemic. Twenty years later, Weksler (2006) proposed the premontane forests of the northern Andes Mountains and the western Amazon lowland forests as two candidate places for the probable centre of origin of oryzomyines. Our results suggest that the Amazon lowlands, the Cerrado, and the Chaco are the most parsimonious areas of origin, which partially agrees with Weksler (2006) and mostly agrees with Miranda *et al.* (2008). Our molecular clock estimates have hypothesized a time of 6.67 Mya for the differentiation of *Oligoryzomys* spp., which falls within the time range given for the initial diversification of oryzomyines (between 5 and 9 Mya). This date has been proposed by Smith & Patton (1999), based on cyt B sequences, and by Steppan *et al.* (2004), based on four nuclear genes. The hypothesized time of origin for the genus corresponds to the end of Miocene (and even earlier than that, see Steppan *et al.*, 2004), and is about the time suggested for the probable arrival of early sigmodontines in South America (Spotorno, 1986; Smith & Patton, 1999; Steppan *et al.*, 2004). From a biogeographic perspective, that period was characterized by the formation of a vast array of habitats, not only for oryzomyines, but also for the radiation of sigmodontines in general. This was a time when forests and woodlands (subtropical and temperate) covered most of the continent (Hinojosa & Villagrán, 2005). Increasing orogenic events associated with the rising of the Andes Mountains resulted in a gradual cooling and drying, which increased the spread of woodlands and savannas, and the contraction of forests (Potts & Behrensmeyer, 1992; Garzione *et al.*, 2008). In addition, our molecular clock calibration hypothesized two other major pulses for the radiation of *Oligoryzomys*: the first being about 3.7–3.0 Mya, which allowed the diversification of the species in clade 3 (Fig. 3). The second major pulse was the diversification of forms included in clades 1 and 6, between 2.2 and 1.5 Mya. The latter diversification rate corresponded to a period of alternating interglacial and glacial events in the Pleistocene (Holling & Schilling, 1981). Thus, the timing obtained through molecular clock calibration for *Oligoryzomys* spp. placed this taxon in a scenario of strong habitat change on the continent that may have promoted the differentiation of several taxa.

The recent work by Miranda *et al.* (2008) proposed a north-to-south gradient of dispersal for the different species of *Oligoryzomys* in South America, first occupying the Amazon and the Cerrado ecogeographic zones. Our results do not allow us to verify this north-to-south gradient, although we agree that the Amazon and the Cerrado must be the ancestral area for the radiation of *Oligoryzomys*. We thus suggest that the radiation of *Oligoryzomys* occurred in four areas stemming from a widely distributed ancestral form such as *O. microtis*. These included the following groups.

1. An Andean–Chacoean group including *O. fornesi*, *Oligoryzomys sp.* B, *O. andinus*, and *O. longicaudatus*, with an estimated diversification time of about 2.2 Mya from an Andean Patagonian ancestral distribution (Fig. 4). This diversification left *Oligoryzomys sp.* B and *O. andinus* in the highlands of the central Andes, *O. fornesi* in the foothills of the Andes and part of the Chaco region, and *O. longicaudatus* in the southern lowlands that ranges from the Andes Mountains of Argentina and Chile southwards to Patagonia.
2. A group that relates *O. flavescens* from part of the Chaco, the Monte Desert, and the east–central portion of Argentina with *O. destructor* from the west–central Amazonia, and south to subtropical areas of Paraguay and Argentina. This clade gave rise to a new species, *Oligoryzomys sp.* 1, in the north-western portion of Argentina that could be an offshoot of *O. flavescens*. At the base of this radiation is *O. nigripes*, which occurs in northern Argentina (Formosa and Misiones provinces) and east of the Paraguay River. This radiation originated from an ancestor-form inhabitant of the Cerrado.
3. A group closely associated with group 4, a clade containing *O. eliurus* from the Brazilian Caatinga and Cerrado, and *O. delticola* from Uruguay and the delta of Paraná River. Basal to this relationship is *O. chacoensis* from the Chaco, Cerrado, and Caatinga of Brazil. All these forms originated from a Cerrado ancestor.

4. Finally, *O. fulvescens* was recovered as a sister taxon to *O. vegetus*, which occurs in Central and southern North America, and may be a southern invader to the north, after the re-establishment of the Panamanian bridge by Plio–Pleistocene times, once the bridge between Central and South America was re-established (Simpson, 1980). In fact, time calibration for the split between *O. fulvescens* and its sister taxon *O. vegetus* gave a diversification of about 3.35 Mya, a time where the bridge between both continents was already set. *Oligoryzomys vegetus*, on the other hand, could be a peripheral isolate of *O. fulvescens*.

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APPENDIX

Ecogeographic zones of distribution for the *Oligoryzomys* spp. used in the DIVA analysis

Species	Ecogeographic zones of distribution
Oligoryzomys andinus	Sechura desert; Central Andean wet puna; Bolivian Yungas[1]
Oligoryzomys chacoensis	Chaco; Chiquitano dry forests; Cerrado[1]
Oligoryzomys delticola	Humid pampas; Uruguayan savanna[1,2,3,4,5]
Oligoryzomys destructor	Napo moist forests; Ucayali moist forests; Bolivian Yungas[1]
Oligoryzomys eliurus	Cerrado; Caatinga; Atlantic rainforests[1,5]
Oligoryzomys flavescens	Humid chaco; Uruguayan savanna; humid pampas; Argentine espinal; Argentine monte; Cerrado; Caatinga; Atlantic rainforests[1]
Oligoryzomys fornesi	Humid chaco; Cerrado; Caatinga; Atlantic rainforests[1,2,6]
Oligoryzomys fulvescens	Moist forests of west and east versants of south Mexico; Llanos; Moist forests of Guiana and northernmost Brazil[1,7,8]
Oligoryzomys longicaudatus	Chilean matorral; Valdivian rainforests; Magellanic subpolar forests; Patagonian steppe[9,10]
Oligoryzomys microtis	Amazon forests of Brazil, Perú, and Bolivia; Humid Chaco[1,5]
Oligoryzomys nigripes	Humid Chaco; Cerrado; Caatinga; Atlantic rainforests[1,4,5]
Oligoryzomys vegetus	Lower montane and montane forests of Costa Rica and Panamá[1]
Oligoryzomys sp. 1	Southern Andean Yungas[1]
Oligoryzomys sp. B	Altoandina[11]

[1]Musser & Carleton (2005); [2]Myers & Carleton (1981); [3]Espinosa & Reig (1991); [4]Bonvicino & Weksler (1998); [5]Andrades-Miranda *et al.* (2001); [6]Lacher & Alho (2001); [7]Gardner & Patton (1976); [8]Haiduk, Bickham & Schmidly (1979); [9]Palma *et al.* (2005a); [10]Belmar-Lucero *et al.* (2009); [11]Carleton & Musser (1989).