The Linnean Society of London
ZOOLOGICAL Journal of the Linnean Society

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

Journal:	*Zoological Journal of the Linnean Society*
Manuscript ID:	ZOJ-02-2009-0545.R2
Manuscript Type:	Original Article
Keywords:	phylogenetic systematics < Phylogenetics, Rodentia < Taxa, Neotropical < Geography, South America < Geography

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 center of origin for the tribe Oryzomyini to which *Oligoryzomys* belongs. Biogeographically, this suggests that *Oligoryzomys* had a South American origin and later advanced northward entering Central America and Mexico more recently. Different analyses provided consistent support for several addition clades, which did not necessarily agree with 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.

ADDITIONAL KEYWORDS: cytochrome *b* - NADH1 – Neotropics – *Oligoryzomys* - phylogeny - Sigmodontinae.

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 *sensu stricto*, 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 South America: 1) sigmodontines evolved in North America prior to seven Million years ago (Mya; Upper Miocene) and reached South America by waif dispersal from Central America about six 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 originally was 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 hind foot, small skull, and a convex interorbital bone. In the last decade the genus *Oligoryzomys* has been the focus of epidemiologic studies since 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*., 2000; 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 (*O. andinus*) to lowland tropical and subtropical areas (*O. eliurus*, *O. chacoensis*). The genus has a wide distribution in the Neotropics extending from Mexico (*O. fulvescens*), through Central America (*O. vegetus*), and southward to Patagonia (*O. longicaudatus*; Figs. 1a and 1b).

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 for a total of 18 species. Weksler & Bonvicino (2005) described two new endemic species from Brazil (*O. moojeni* and *O. rupestris*) but, at the same time placed *O. eliurus* and *O. delticola* as junior synonyms of *O. nigripes*, 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 2n between 46-70 (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*, *O. arenalis* and *O. vegetus*; the *microtis* group including *O. microtis*; the *andinus* group including *O. andinus* and *O. chacoensis*; the *flavescens* group including *O. flavescens* and three undescribed species, and the *nigripes* group including *O. nigripes, O. eliurus, O. destructor, O. delticola* and *O. longicaudatus*. Dickerman & Yates (1995) analyzed 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* gene among eight species of the genus, Myers (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. (2007) based on *d-loop* sequences, it is also possible to conclude about the monophyly of the genus, although they evaluated only the systematic relationships of the species that occur in Argentina (7 taxa). Finally, a recent published study by Miranda *et al*. (2008) based on cytochrome *b* sequences including 12 species of *Oligoryzomys* also concluded about 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* (we overlapped in 7 species if compared with those considered in Miranda's [2008] study). In addition, we included two undescribed forms, *Oligoryzomys* sp1 (field catalog of one of us, MIC) and *O*. spB (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 sigmodontines in the Neotropics. To accomplish these goals, we sequenced the cytochrome *b* and the nicotinamide dinucleotide dehydrogenase subunit 1 (NADH1) mitochondrial genes in 16 specimens of the 14 taxa plus two

outgroups. Aligned sequences were analyzed 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 outgroups (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 6 species of *Oligoryzomys* for which samples were unavailable: a new form described from Brazil, *O. stramineus*; *O. griseolus* from Venezuela for which there are no recent captures (Marisol Aguilera to REP, personal communication); *O. victus* from Lesser Antilles which is presumably extinct (Musser & Carleton, 2005); *O. magellanicus* (Isla Harrison, Magallanes, Chile); *O. arenalis* (Lambayeque, Perú), and *O. brendae* (although see below). In addition, we could not get samples from other two 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 *O.* sp1 (Table 1). We amplified the cytochrome *b* (cyt B) and the NADH dehydrogenase subunit 1 mitochondrial genes via the polymerase chain reaction (PCR; Saiki *et al*., 1988) using Taq DNA Polymerase (Invitrogen) and primers L (MSB) 5'-GAC ATG AAA AAT CAT CGT TGT AAT TC-3' and MVZ-14 (Smith & Patton, 1993) for the cyt B gene, and 16S.f2 5'-TAC GAC CTC GAT GTT GGA TCA GG-3' and Met.r1 5'-GGG GTA TGG GCC CRA RAG C-3' for the NADH1 gene. The PCRs were performed using the

following thermal profiles: for cyt B (35 cycles) 94˚ C denaturation for 40 sec; 42˚ C annealing for 40 sec and 72˚ C extension for 1 min 20 sec. For NADH1 (35 cycles) 94˚ C denaturation for 40 sec, 50˚ C annealing for 35 sec and 72˚ C extension for 1 min 20 sec. Double-stranded PCR products were purified using the methods of QIAquick (Qiagen). Sequencing was conducted through cycle sequencing (Murray1989) using the PCR primers labeled with the Big Dye Terminator kit (Perkin Elmer, Norwalk, Connecticut) and the sequencing reactions were analyzed in an ABI Prism 310 automated sequencer (Foster City, California). The PCR products were sequenced at least two times to ensure sequence fidelity. Sequences were aligned by eye to maintain amino acid sequence and using Clustal X (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 software (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).

Phylogenetic analyses and clock calibration

Phylogenetic reconstruction was performed through maximum parsimony (MP) using PAUP* 4.0 b10 (Swofford, 2002). Both mitochondrial data sets (cyt B and NADH1) were analyzed 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* 4.0 b10 with 1000 replicates excluding invariant characters (Cunningham, 1997). For parsimony analysis we treated all characters as unordered with four possible states (A, C, G, T) and we used those characters that were phylogenetically informative. Since transition-transversion (ts/tv) rate was 4:1 for each mitochondrial marker, we performed weighted parsimony (WP). For weighted parsimony, a heuristic search was performed with 10 random addition 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 outgroup criterion using two oryzomyine taxa, *Holochilus brasiliensis* and *Transandinomys talamancae* (formerly known as *Oryzomys talamancae*; Weksler, Percequillo & Voss, 2006). *H. 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 the Kimura Two-Parameter (K2P) model (Kimura, 1980) for the cytochrome *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 1,000 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 Rodriguez *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 at the website 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 were determined. 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 the program Statistica 6.0 (StatSoft, Inc. 2001, Tulsa, OK, USA), and then a posterior Newman-Keuls test (Zar, 1996). Assumptions of normality of data and homogeneity of variance were previously evaluated. Posterior probabilities for topologies then were assessed as the proportion of trees sampled after burn-in, in which that particular topology was observed.

Since our results did not support the generalized molecular clock model (LR = 74.34, df = 17, $p < 0.0001$) we used a relaxed molecular clock by performing BEAST v.1.4.8 (Drumond *et al*., 2006), which employs a Bayesian Markov Chain Monte Carlo (MCMC) to co-estimate topology, substitution rates and node ages. Posterior probability distributions of node ages were obtained for the concatenated 2-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 1,000 steps. Examination of MCMC samples using TRACER v. 1.4 (Rambaut & Drumond, 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 *et al*. (2002): (C1) a *O. flavescens* fossil specimen from the Ensenadense level, which was dated at mean time of 1.5 Mya; and (C2) a *O. eliurus* fossil specimen from the Lujanense level, which was dated at mean time of 0.24 Mya. Since these datations do not have an error associated 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 *O. eliurus* fossil. That error was incorporated in the molecular clock analysis as part of the prior probability through an uniform distribution where the mean corresponded to the fossil age and the error to the radiometric error. We did not find and error associated to 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. Having the former parameters, we first estimated divergence at the root of the tree, then the ingroup divergence, and finally the divergence at several clades as shown in Fig. 3.

Dispersal-vicariance analysis

To infer the history of biogeographic distributions in *Oligoryzomys*, we used the known distributions of each species, as coded in Appendix 1, 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. Overall base composition for each gene was: A = 31%, C = 29%, T = 28% and G = 12% for the cyt B gene, and A = 35%, C = 30%, T = 27% and G = 8% for the NADH1 gene.

K2P distance for the cyt B gene (Table 2) exhibited values that ranged between 0.722% for specimens of the same locality, 1.345% for specimens of the same species from different localities such as island and continent representatives (e.g., *O. longicaudatus*). However, K2P distance values between different species varied between 5.7 (*O. longicaudatus*-*O.fornesi*) to 15% (e.g., *O. microtis*-*O. andinus*). Other values between recognized *Oligoryzomys* species were about 10% between sister taxa *O. fulvescens* and *O. vegetus*, or about 6% between *O. flavescens* and *O. destructor*. K2P distance values among representatives of the same taxon (the unnamed *O.* sp1) were less

than 1%, and the other unnamed species *O*. spB 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 that of the cyt B. gene. The predicted-distance values (Fig. 2C) show different slopes ($ts = 12.48$; $df = 169$; $p < 0.001$) evidencing two evolutionary rates in the molecular markers analyzed. 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 both, with maximum-parsimony via weighted parsimony and BMCMC analyses. The WP resulted in a single most parsimonious tree 2517 steps long, CI = 0.5217 and 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 a 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*, O. spB), *O. andinus*), *O. longicaudatus*) on one clade, and all 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 sister relationship to a clade that included (((*O. flavescens*, *O. destructor*), *O*. sp1), *O. nigripes*).

Age estimations from the relaxed clock analysis estimated 6.67 ± 0.02 Mya for the divergence between *Oligoryzomys* with respect to the outgroups, and 5.27 ± 0.052 Mya for the split between *O. microtis* and the rest of the species (Fig. 3). Other age estimations were 2.2 ± 0.043 Mya for clade 1 (((*O. fornesi, O*. spB), *O. andinus*), *O. longicaudatus*); 3.35 ± 0.035 for clade 2 (*O. fulvescens*, *O. vegetus*); 3.71 ± 0.035 Mya for clade 3 composed by clades 4 ((*O. eliurus, O. delticola*), *O. chacoensis*) and clade 5 *O. nigripes*; clade 6 composed by (((*O. destructor, O. flavescens*), *O*. sp1) recovered 1.54 ± 0.031 Mya.

DIVA analysis

Optimization of geographic distributions of taxa according to Appendix 1 onto the tree topology 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 1 is from the highlands of the Andes and Patagonia; clade 2 is from the Pacific Province; 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 weighted parsimony 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 Miranda *et al*. (2008), the latter based 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 (((*fornesi,* spB*), andinus*), *longicaudatus*) was supported by the phylogeny of Myers, Lundrigan & Tucker (1995) based on partial cytochrome *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 (((*fornesi,* spB*), andinus*), *longicaudatus*) clade, obtained in our study, as more likely. *Oligoryzomys* spB (sensu Carleton & Musser, 1989) should be treated carefully since this taxon was included in the *flavescens* group by Carleton & Musser (1989). The latter authors proposed that *O*. spB could be the Andean counterpart of *O. flavescens* since the former is distributed above 2000 m in the central Andes. Carleton & Musser (1989) reported *O*. spB from the eastern Puna and Amazon slopes of the Andes and from the Pacific side of the southern Peruvian Andes between 3000-4000 m. The specimen of *O*. spB analyzed 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, *O*. spB analyzed by us was recovered as sister to *O. fornesi* from the Chaco region and highly divergent with respect to *O. flavescens*. Indeed, K2P distance value between sister taxa *O*. spB and *O. fornesi* was 3.7% for the cyt B gene, whereas K2P cyt B. distance value

between spB and *O. flavescens* was 12.7%. The K2P value (3.7%) obtained for spB 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, 2005a; this study, e.g. *O. andinus*-*O. longicaudatus*, *O. vegetus*-*O. fulvescens*), suggesting that *O.* spB might be speciating. This interpretation agrees with previous work in other sigmodontine taxa (e.g., Oryzomyini, Phyllotini) that demonstrated a genetic distance between subspecies of rodents in a range that varied < 4% (Myers et al., 1995; Steppan, 1998). *Oligoryzomys andinus*, on the other hand, appeared as sister to the (*O. fornesi, O.*spB) 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 one from Coyhaique (45˚ S) in 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* sp1 and its closest relatives *O. flavescens* and *O. destructor*. These samples were collected in Catamarca province, northwest Argentina. Cranial and dental morphology supported differences with related species although a name has yet to be assigned (M. I. Carma *et al*., unpublished 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*. K2P distance value for the cyt B. gene

between sp1 and *O. flavescens* varied around 6.0%, whereas between sp1 and *O. destructor* the distance value was about 10% for the same gene. This new taxon (sp1) probably is an offshoot of one of these two latter taxa, most likely a peripheral isolate of *O. flavescens*, since the southern distributional limit of *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 sp1 representatives in this study are from Catamarca in the northwest of Argentina. We are not sure if the sp1 reported by us in this study, is the same *O. brendae* presented in Musser & Carleton (2005) since no formal description for *O. brendae* is yet available (citation in Musser and Carleton's 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 weighted parsimony and Bayesian tree recovered *O. delticola* and *O. eliurus* in sister relationship and closely related to *O. chacoensis* as first outgroup. 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 inclusion of *nigripes*, *longicaudatus* and *destructor*. According to our results, the

union *eliurus-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 (ca. 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 a strong support for the relationship *delticola-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 *delticola* and *eliurus* from *O. nigripes* (Weksler & Bonvicino, 2005), and more recently Paresque *et al*., (2007) proposed to leave *O. delticola* and *O. eliurus* as junior synonyms of *O. nigripes*. Our results however, showed *O. nigripes* as part of a different clade if compared with *O. delticola* and *O. eliurus*, with a moderate support in both the WP and BMCMC analysis (Fig. 3). Based on our results, we believe that *O. nigripes* is a different species with respect to *eliurus* and *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, D' Elía & Ortiz, 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 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), or occurred in the Andes. However, this pattern does not fit for *Oligoryzomys* since this genus ranges from Mexico to Patagonia.

The position of *Oligoryzomys 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, 5 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 a probably center 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 agreed with Weksler (2006) and mostly with Miranda *et al*. (2008). Our molecular clock estimates 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 given 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 that increased woodlands and savannas and the contraction of forests (Potts & Behrensmeyer, 1992; Garzione *et al*., 2008). In addition, our molecular clock calibration hypothesized other two major pulses for the radiation of *Oligoryzomys*, being the first about 3.7-3.0 Mya that allowed diversification of species in clade 3 (Fig. 3). The second major pulse was 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 northern-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 to verify this

northern-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 steming from a widely distributed ancestral form such as *O. microtis*. These included: 1) an Andean-Chacoean group including *O. fornesi*, *O.* spB*, 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 *O.* spB 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 southward 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, south to subtropical areas of Paraguay and Argentina. This clade gave rise to a new species, *O.* sp1, in the northwestern 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) closely associated to (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* recovered as sister taxon to *O. vegetus,* which occurs in Central and southern North America, may be a southern invader to the north, after the reestablishment 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. *O. vegetus* on the other hand, could be a peripheral isolate of *O. fulvescens*.

ACKNOWLEDGEMENTS

This work is dedicated to the memory of Terry L. Yates who died while performing this work. We appreciate the comments of Jennifer K. Frey who helped to improve this manuscript, and the laboratory support from Dusan Boric-Bargetto. We thank Juan Carlos Torres-Mura for the loan of specimens from Navarino Island. We thank the following for loans of tissue samples and specimens: Guillermo D'Elía, Blas Armien, Enrique Lessa, the Museum of Southwestern Biology, University of New Mexico, and the University of Kansas Museum of Natural History. This work was supported by grants NIH-Hantavirus Chile and Panamá, FONDAP-CASEB 1501-0001 Programa 2, DIUC 205.113.070-1.0, FONDECYT 1990156, FONDECYT 3050092 and FONDECYT 1070331. The collecting permits of Servicio Agricola Ganadero (SAG) and Corporación Nacional Forestal from Chile are also acknowledged.

REFERENCES

Andrades-Miranda J, Oliveira LFB, Lima-Rosa CAV, Nunes AP, Zanchin NIT, Mattevi MS. 2001. Chromosome studies of seven species of *Oligoryzomys* (Rodentia: Sigmodontinae) from Brazil. *Journal of Mammalogy* **82**: 1080-1091.

Baskin JA. 1978. *Bensonomys*, *Calomys*, and the origin of the phyllotine group of Neotropical cricetines (Rodentia: Cricetidae). *Journal of Mammalogy* **59**: 125-135.

Belmar-Lucero S, Godoy P, Ferrés M, Vial P, Palma RE. 2009. Range expansion of *Oligoryzomys longicaudatus* (Rodentia, Sigmodontinae) in Patagonian Chile, and first record of Hantavirus in the region. *Revista Chilena de Historia Natural* in press.

Bonvicino CR, Weksler M. 1998. A new species of *Oligoryzomys* (Rodentia, Sigmodontinae) from Northeastern and Central Brazil. *Zeitschrift fur Saugetierkunde* **63**: 90-103.

Carleton MD, Musser GG. 1989. Systematic studies of oryzomyine rodents (Muridae, Sigmodontinae): a synopsis of *Microryzomys*. *Bulletin of the American Museum of Natural History* **191**: 1-83.

Cunningham CW. 1997. Is incongruence between data partitions a reliable predictor of phylogenetic accuracy? Empirically testing a iterative procedure for choosing among phylogenetic methods. *Systematic Biology* **46:** 464-478.

Dickerman AW, Yates TL. 1995. Systematics of *Oligoryzomys*: protein electrophoresis analyses. *Journal of Mammalogy* **76**: 172-188.

Drumond AJ, Ho SYW, Phillips MJ, Rambaut A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biology* **4**: 699-710.

Engel SR, Hogan KM, Taylor JF, Davis SK. 1998. Molecular systematics and paleobiogeography of the South American sigmodontine rodents. *Molecular Biology and Evolution* **15**: 35-49.

Espinosa MB, Reig OA 1991. Cytogenetics and karyosystematics of South American oryzomyine rodents (Cricetidae, Sigmodontinae). III. Banding karyotypes of Argentinean *Oligoryzomys*. *Z. Saugetierkunde* **56**: 306-317.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using bootstrap. *Evolution* **39**: 783-791.

Francés J, D´Elía G. 2006. *Oligoryzomys delticola* es sinónimo de *O. nigripes*. *Journal of Neotropical Mammalogy* **13**: 123-131.

Gallardo MH, Palma RE. 1990. Systematics of *Oryzomys longicaudatus* (Rodentia: Muridae) in Chile. *Journal of Mammalogy 71*: 333-343.

Gannon WL, Sikes RS and the Animal Care and Use Committee of the American Society of Mammalogists. 2007. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *Journal of Mammalogy* **88**: 809-823.

Gardner AL, Patton JL. 1976. Karyotypic variation in oryzomyine rodents (Cricetinae) with comments on chromosomal evolution in the Neotropical cricetine complex. *Ocassional Papers, Museum of Zoology Louisiana State University* **49**: 1-47.

Garzione CN, Hoke GD, Libarkin JC, Withers S, MacFadden B, Eiler J, Ghosh P, Mulch A. 2008. Rise of the Andes. *Science* **320**: 1304-1307.

Haiduk MW, Bickham JW, Schmidly DJ. 1979. Karyotypes of six species of *Oryzomys* from Mexico and Central America. *Journal of Mammalogy* **60**: 610-615.

Hershkovitz P. 1966. South Americam swamp and fossorial rats of the scapteromyine group (Cricetinae, Muridae) with comments on the glans penis in murid taxonomy. *Zeistchrift für Säugetierkunde* **31**: 81-149.

Hershkovitz P. 1972. The recent mammals of the Neotropical region: A zoogeographic and ecological review. In: Keast A, Erk FC, and Glass B, eds. *Evolution, Mammals, and Southern Continents*. Albany, New York: State University Press, 311-431.

Hinojosa LF, Villagrán C. 2005. Did South American Mixed Paleofloras evolve under thermal equability or in the absence of an effective Andean barrier during the Cenozoic?. *Palaeogeography, Palaeoclimatology, Palaeoecology* **217** :1-23.

Holling JT, Schilling DH. 1981. Late Wisconsin-Weichselian mountains glaciers and small ice caps. In: Denton GH, Hughes TJ, eds. *The last great ice sheets*. New York: John Wiley and Sons, 179-206.

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16:** 111-120.

Lacher TEJ, Alho CJR. 2001. Terrestrial small mammal richness and habitat associations in an Amazon Forest-Cerrado contact zone. *Biotropica* **33:** 171-181.

Laird PW, Zuderveld A, Linders K, Rudnicki MA, Jaenisch R, Berns A, 1991. Simplified mammalian DNA procedure. *Nucleic Acids Research* **19**: 4293.

Lee H, van der Groen G. 1989. Hemorragic fever with renal syndrome. *Progress in Medical Virology* **36**: 62-102.

Maddison WP, Maddison DR. 1992. MacClade: Analysis of phylogeny and character evolution. Version 4.04. Sinauer Associates, Inc., Sunderland, Massachusetts, USA.

Marshall LG. 1979. A model for paleobiogeography of South American cricetine rodents. *Paleobiology* **5:** 126-132.

Marshall LG, Webb SD, Sepkoski JJ, Raup DM. 1982. Mammalian evolution and the Great American Interchange. *Science* **215**: 1351-1357.

Miranda GB, Oliveira LFB, Andrades-Miranda J, Langguth A, Callegari-Jacques S, Mattevi MS. 2008. Phylogenetic and phylogeographic paterns in Sigmodontine rodents of the genus Oligoryzomys. *Journal of Heredity* in press.

Murray V. 1989. Improved double-stranded DNA sequencing using the linear polymerase chain reaction. *Nucleic Acids Research* **17**: 8889.

Musser GG, Carleton MD. 1993. Family Muridae. In: Wilson DE, Reeder DM, eds. *Mammal species of the world: a taxonomic and geographic reference*. Washington, DC: Smithsonian Institution Press, 501-755.

Musser GG, Carleton MD. 2005. Superfamily Muroidea. In: Wilson DE, Reeder DM, eds. *Mammal species of the world: a taxonomic and geographic reference*. Third Edition. Baltimore, Maryland: John Hopkins University Press, 894-1531.

Myers P, Carleton MD. 1981. The species of *Oryzomys* (*Oligoryzomys*) in Paraguay and the identity of Azara's 'rat sixieme ou rat a tarse noir'. *Miscelaneous Publications Museum of Zoology*, *University of Michigan* **161**: 1-41.

Myers P, Lundrigan B, Tucker PK. 1995. Molecular phylogenetics of oryzomyine rodents: the genus *Oligoryzomys*. *Molecular Phylogenetics and Evolution* **4:** 372-382.

Nei M, Kumar S. 2000. *Molecular Evolution and Phylogenetics*. Oxford University Press, USA.

Pagel M, Meade A. 2004. A phylogenetic mixture model for detecting pattern-heterogeneity in gene sequence or character state data. *Systematic Biology* **53**: 571-581.

Pagel M, Meade A. 2005. Mixture models in phylogenetic inference. In: Gascuel O, ed. *Mathematics of evolution and phylogeny*. Oxford University Press, USA.

Palma RE, Marquet PA, Boric-Bargetto D. 2005a. Inter- and intraspecific phylogeography of small mammals in the Atacama Desert and adjacent areas of northern Chile. *Journal of Biogeography* **32**: 1931-1941.

Palma RE, Rivera-Milla E, Salazar-Bravo J, Torres-Pérez F, Pardiñas UFJ, Marquet PA, Spotorno AE, Meynard AP, Yates TL. 2005b. Phylogeography of *Oligoryzomys longicaudatus* (Rodentia: Sigmodontinae) in temperate South America. *Journal of Mammalogy* **86**: 191-200.

Pardiñas UFJ, D´Elía G, Ortiz PE. 2002. Sigmodontinos fósiles (Rodentia, Muroidea, Sigmodontinae) de América del Sur: Estado actual de su conocimiento y prospectiva. *Journal of Neotropical Mammalogy* **9**: 209-252.

Paresque R, de Jesús Silva MJ, Yonenaga-Yasuda Y, Fagundes V. 2007. Karyological geographic variation of *Oligoryzomys nigripes* Olfers, 1818 (Rodentia, Cricetidae) from Brazil. *Genetics and Molecular Biology* **30**: 43-53.

Patterson B, Pascual R. 1972. The fossil mammal fauna of South America. In: Keast A, Erk, FC, Glass B, eds. *Evolution, mammals, and southern continents*. Albany, New York: State University Press, 247-309.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817-818.

Potts R, Behrensmeyer AK. 1992. Late Cenozoic Terrestrial Ecosystems. In: Behrensmeyer AK, Damuth JD, DiMichele WA, Potts R, Hans-Dieter S, Wing SL, eds. *Terrestrial ecosystems through time: evolutionary paleoecology of terrestrial plants and animals*. Chicago: The University of Chicago Press, 419-541.

Rambaut A, Drummond AJ. 2003. Tracer version 1.2 (computer program). Available: http://evolve.zoo.ox.ac.uk.

Reig OA. 1980. A new fossil genus of South American cricetid rodents allied to *Wiedomys*, with and assessment of the Sigmodontinae. *Journal of Zoology London* **192**: 257-281.

Reig OA. 1981. Teoría del origen y desarrollo de la fauna de mamíferos de América del Sur. Museo Municipal de Ciencias Naturales, Mar del Plata, Argentina.

Reig OA. 1986. Diversity patterns and differentiation of high Andean rodents. In: Vuilleumier F, Monasterio M, eds. *High altitude tropical biogeography*. London: Oxford University Press, 404-439.

Rivera PC, Gonzalez-Ittig RE, Rossi-Fraire HJ, Levis S, Gardenal CN. 2007. Molecular identification and phylogenetic relationships among the species of the genus *Oligoryzomys* (Rodentia, Cricetidae) present in Argentina, putative reservoirs of hantaviruses. *Zoologica Scripta* **36**: 231-239.

Rodríguez F, Oliver JF, Marín A, Medina JR. 1990. The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology* **142**: 485-501.

Ronquist F. 1996. DIVA version 1.1. Computer program and manual. Available from Uppsala University at: <http://www.eb-c.uu.se/systzoo/research/diva.html>.

Ronquist F. 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Systematic Biology* **46**: 195-203.

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**: 487-491.

Savage JM. 1974. The isthmian link and the evolution of Neotropical mammals. *Contributions in Science, Natural History Museum Los Angeles County* **260**: 1-51.

Schultz PH, Zárate M, Hames B, Koeberl C, Bunch T, Storzer D, Renne P, Wittke J. 2004. The Quaternary impact record from the Pampas, Argentina. *Earth and Planetary Sciences Lettes* **219**: 221-238.

Smith MF, Patton JL. 1993. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. *Biological Journal of the Linnean Society* **50**: 149-177.

Smith MF, Patton JL. 1999. Phylogenetic relationships and the radiation of Sigmodontine rodents in South America: Evidence from cytochrome *b*. *Journal of Mammalian Evolution* **6**: 89-128.

Smith MF, Patton JL. 2007. Molecular phylogenetics and diversification of South American Grass Mice, genus *Akodon*. In: Kelt DA, Lessa EP, Salazar-Bravo J, Patton JL, eds. The Quintessential Naturalist: honoring the life and legacy of Oliver P. Pearson. Berkeley, California: University of California Publications, Zoology, 827-858.

Simpson GG. 1940. Mammals and Landbridges. *Journal of the Washington Academy of Sciences* **30**: 137-163.

Simpson GG. 1950. History of the fauna of Latin America. *American Scientist* **38**: 361-389.

Simpson GG. 1980. *Splendid isolation: the curious history of South American mammals*. Yale Univ. Press, New Haven, Connecticut, USA.

Spotorno AE. 1986. Systematics and evolutionary relationships of Andean phyllotine and akodontine rodents. Unpublished Ph. D. Thesis. University of California Berkeley, California, USA.

Steppan SJ. 1998. Phylogenetic relationships and species limits within *Phyllotis* (Rodentia: Sigmodontinae): concordance between mtDNA sequence and morphology. *Journal of Mammalogy* **79**: 573-593.

Steppan SJ, Adkins RM, Anderson J. 2004. Phylogeny and divergence-date estimates of rapid radiations in muroid rodents based on multiple nuclear genes. *Systematic Biology 53*: 533-553.

Swofford DL. 2002. PAUP*: Phylogenetic Analyses Using Parsimony (* and other methods). Version 4.0b10. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

Tate GHH. 1932. The taxonomy history of the South and Central American cricetid rodents of the genus *Oryzomys*. Part 2: Subgenera *Oligoryzomys*, *Thallomyscus* and *Melanomys*. *American Museum Novitates* **580**: 1-17.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL_X Windows interface: flexible strategies for multiple sequence

alignment aided by quality analysis tools. *Nucleic Acids Research* **25**: 4876-4882.

Webb SD. 1991. Ecogeography and the Great American Interchange. *Paleobiology* **17**: 266-280.

Weksler M. 2003. Phylogeny of Neotropical oryzomyine rodents (Muridae: Sigmodontinae) based on the nuclear IRBP exon. *Molecular Phylogenetics and Evolution* **29**: 331-349.

Weksler M. 2006. Phylogenetic relationships of oryzomyine rodents (Muroidea: Sigmodontinae): separate and combined analyses of morphological and molecular data. *Bulletin of the American Museum of Natural History* **296**: 1-149.

Weksler M, Bonvicino CR. 2005. Taxonomy of pigmy rice rats genus *Oligoryzyomys* Bangs, 1900 (Rodentia, Sigmodontinae) of the Brazilian Cerrado, with the description of two new species. *Arquivos Museo Nacional Rio de Janeiro* **63**: 113-130.

Weksler M, Percequillo AR, Voss RS. 2006. Ten new genera of Oryzomyine Rodents (Cricetidae: Sigmodontinae). *American Museum Novitates* **3537**: 1-29.

Yates TL, Mills JN, Parmenter RR, Vande Castle JR, Calisher CH, Nichol ST, Abbott KD, Young JC, Morrison ML, Beaty BJ, Dunnum JL, Baker RJ, Salazar-Bravo J, Peters CJ. 2002. The ecology and evolutionary history of an emerging disease: Hantavirus pulmonary syndrome. *Bioscience* **52**: 989-998.

Zar J. 1996. *Biostatistical analysis*. Prentice Hall, Upper Saddle River, New Jersey, USA.

Figure Legends

Figures 1a and 1b. Approximate geographic distribution of species of the genus *Oligoryzomys*, including undescribed taxa *O*. sp1 and *O*. spB (distribution of *O*. spB is according to trapping records reported in Carleton and Musser, 1989).

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

Figure 3. *Oligoryzomys* phylogeny based on weighted parsimony analysis (WP) and Bayes Markov Chain Monte Carlo method (BMCMC). The phylogeny was obtained for the combined mitochondrial cytochrome *b* and NADH1 sequence data, whereas for BMCMC represents a consensus tree of the n = 21950 trees from the converged Markov chain. Posterior probability above 0.5 and bootstrap values over 50% are represented on each node. C1 represents calibration time 1 = 1.5 Mya and 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 clock calibration using BEAST.

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

Table 1. List of species, collection/museum numbers, localities, and GenBank accesses 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.	EU 192165	EU 192189	not available
NK13425	*O. microtis*	Bolivia, Depto. Beni, 3 KM S RURRENABAQUE, 365 M	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.	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, 17km E of Totora, 2950 m	EU 192171	EU 192176	17° 45' S 65° 02' W
NK72388	*O. chacoensis*	Paraguay, Depto. Boquerón, FORTIN TOLEDO, 600M	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.	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° 24.050 S, 57° 02.340 W
GD569	*O. delticola*	Uruguay, Depto. Rivera, Lunarejo (propiedad Sr. Abelenda)	EU 192162	EU 192181	31° 06' S, 55° 58' W
MIC210	*O. sp1*	Argentina, Prov. Catamarca, Dept. Ambato, Las Juntas	EU 192167	EU 192178	28°06´34" S, 65°55´0" W
MIC211	*O. sp1*	Argentina, Prov. Catamarca, Dept. Ambato, Las Juntas	EU 192168	EU 192180	28°06´34" S, 65°55´0" W
MIC203	*O. sp1*	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°30.816 S, 57° 14.444 W
MUSA2625	*O. spb3203*	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"
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"

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

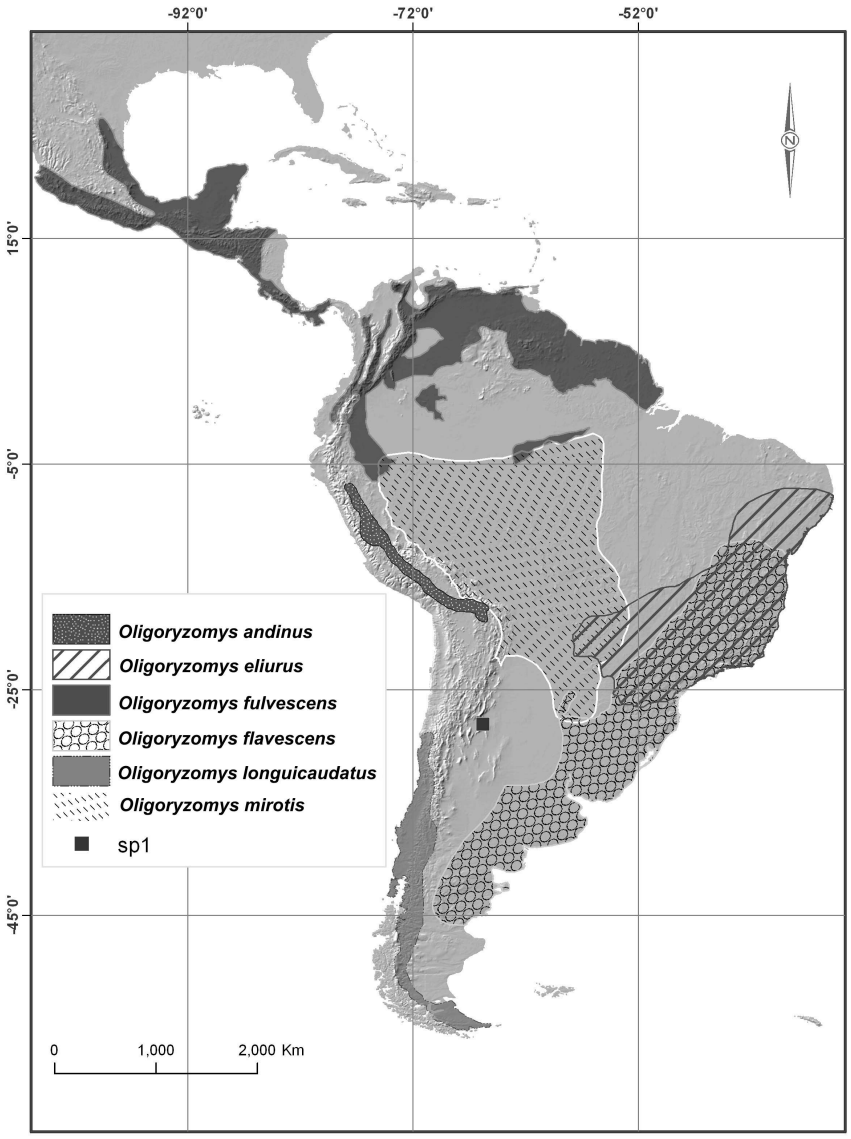
Table 2. K2P distance values among pair-wise *Oligoryzomys* spp. for cytochrome *b* sequences.

	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	*O.*spB 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	*O.*sp1 MIC210	0.111	0.109	0.119	0.112	0.109	0.110	0.109	0.105	0.106	0.110	0.099	-							
13	*O.*sp1 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	*O.*sp1 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	-

Appendix 1. Ecogeographic zones of distribution for *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[2, 6, 1]
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 and Carleton 2005; [2]Myers and Carleton 1981; [3]Espinosa and Reig 1991; [4]Bonvicino and Weksler 1998; [5]Andrades-Miranda et al. 2001; [6]Lacher and Alho 2001 [7]Gardner and Patton 1976; [8]Haiduk et al. 1979; [9]Palma et al. 2005; [10]Belmar-Lucero et al. in press; [11]Carleton and Musser 1989



215x279mm (300 x 300 DPI)

215x279mm (300 x 300 DPI)

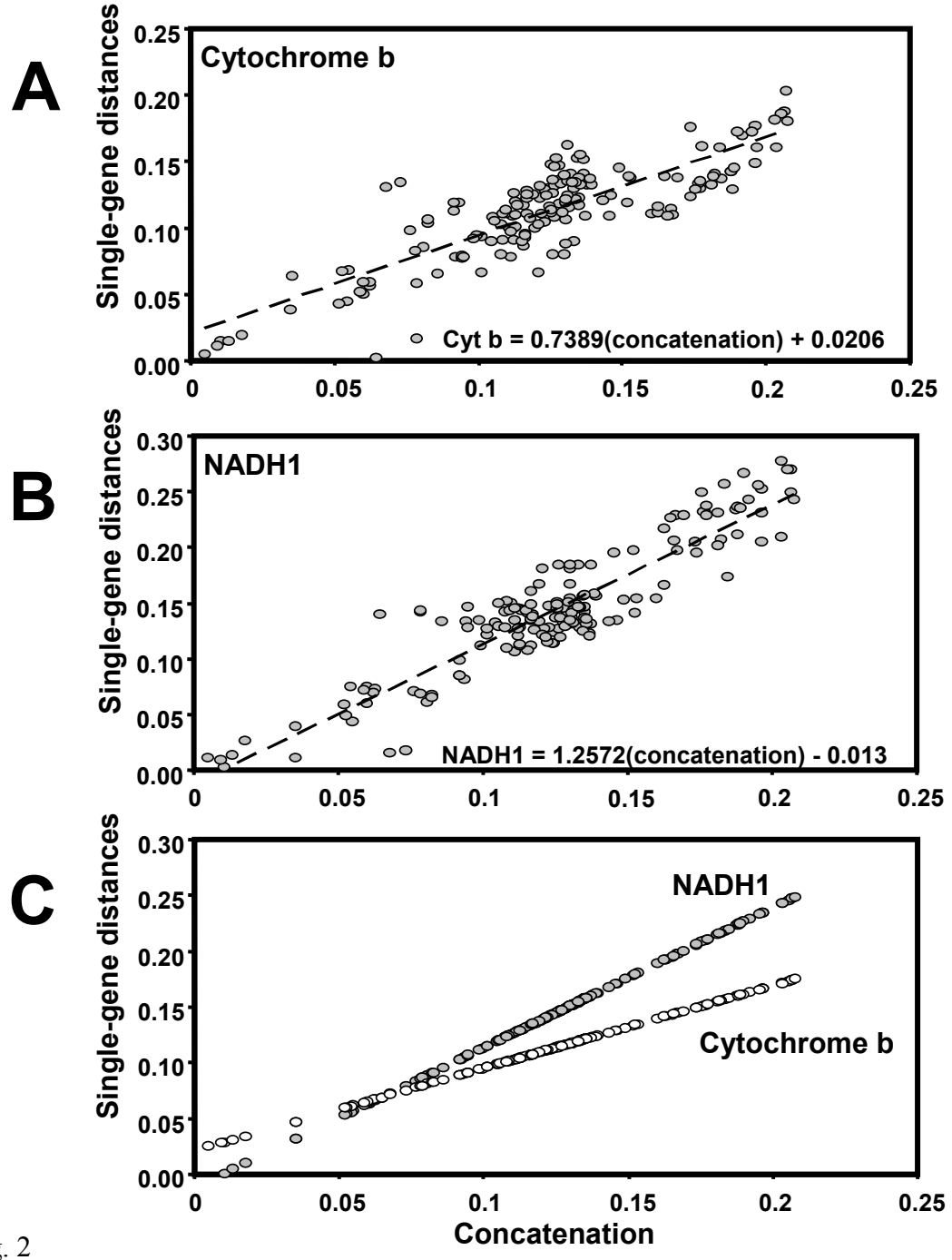
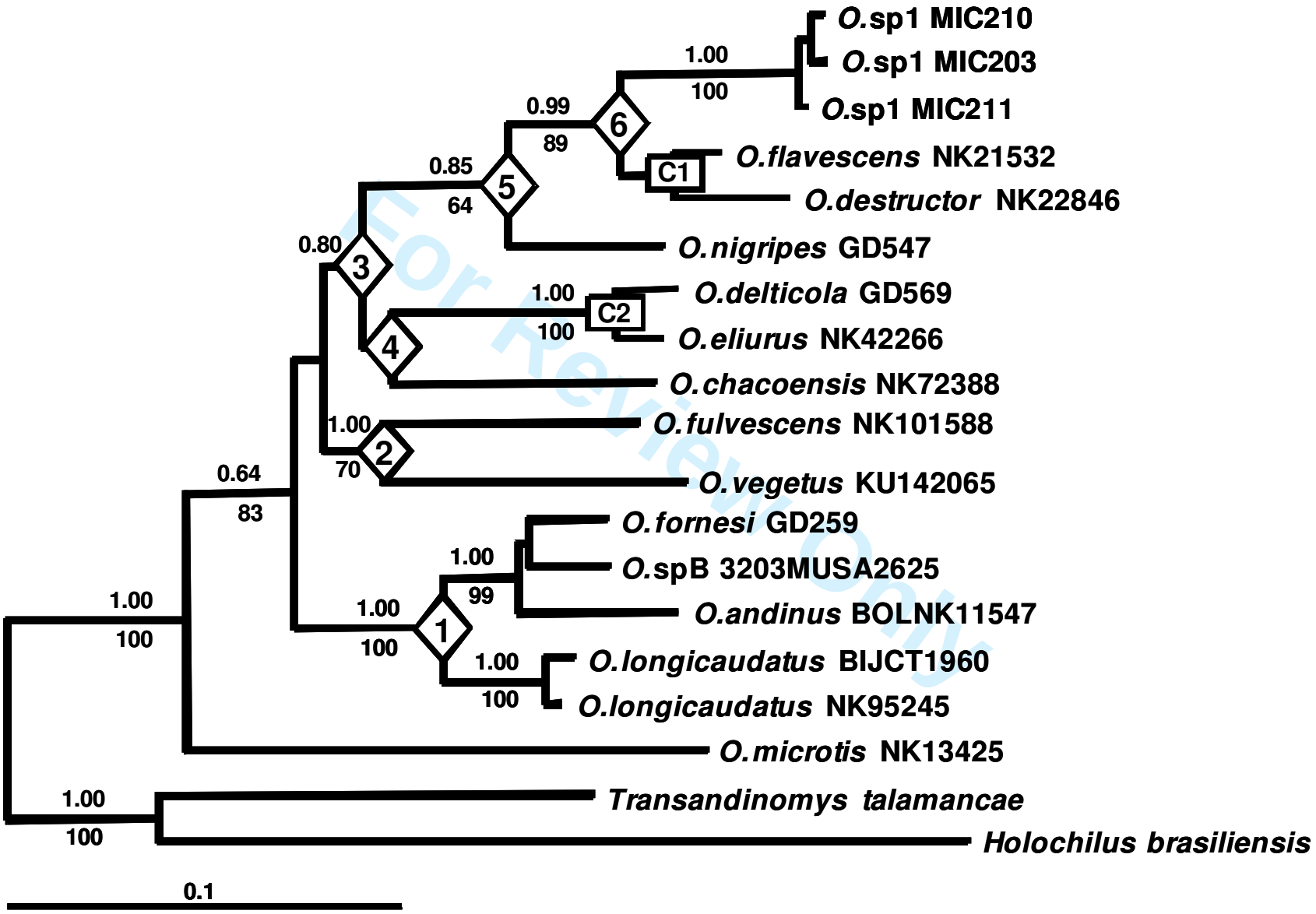

A
Cytochrome b
Single-gene distances
Cyt b = 0.7389(concatenation) + 0.0206
B
NADH1
Single-gene distances
NADH1 = 1.2572(concatenation) - 0.013
C
NADH1
Cytochrome b
Single-gene distances
Concatenation
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0
0.05
0.1
0.15
0.2
0.25

Fig. 2



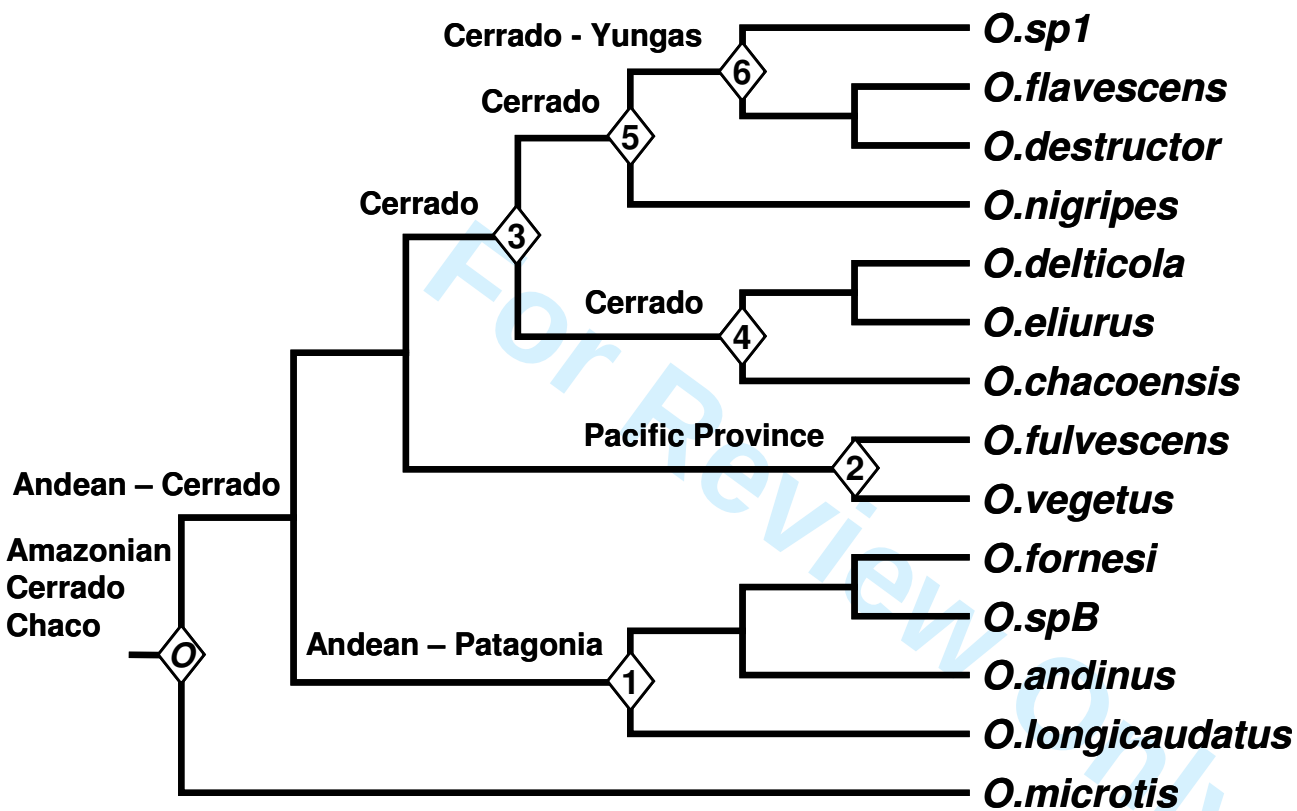


Fig. 4