Molecular phylogenetics of *Oligoryzomys fulvescens* based on cytochrome *b* gene sequences, with comments on the evolution of the genus *Oligoryzomys*

Filogenia molecular de *Oligoryzomys fulvescens* basada en secuencias del gen citocromo *b*, con comentarios sobre la evolución del género *Oligoryzomys*

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Abstract. Using sequences from the mitochondrial cytochrome *b* gene, we analyze the phylogeographic structure of *Oligoryzomys fulvescens* and discuss phylogenetic relationships among 13 species of *Oligoryzomys* including an evaluation of the taxonomic status of *O. vegetus*. We also examine host-hantaviral associations in light of the resulting phylogenetic reconstructions. Gene phylogenies constructed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) optimality criteria were largely congruent. All analyses recovered the genus *Oligoryzomys* as monophyletic, with *O. microtis* as sister group of the remaining taxa. *O. fulvescens* was polyphyletic and represented by two geographic groups: *O. fulvescens* sensu stricto from Mexico and Middle America, recovered with high nodal support in all analyses, and two samples of *O. fulvescens* from Venezuela. Our phylogenetic reconstructions lend support to the hypothesis that ancestors of the genus *Oligoryzomys* entered South America, diversified in lowland habitats prior to diversification to the south. Whether the clade representing *O. fulvescens* sensu stricto and *O. vegetus* evolved in situ in Central America or is derived from a South American lineage that reinvaded North America is unknown. However, given the relatively low levels of genetic differentiation among samples of *O. fulvescens* sensu stricto, we hypothesize that this taxon expanded its range northward in Mexico relatively recently.

Key words: *Oligoryzomys fulvescens*, polyphyly, North and Central America, DNA phylogenies, hantavirus

Resumen. Usando secuencias del gen citocromo *b*, analizamos la estructura filogeográfica de *Oligoryzomys fulvescens* y discutimos las relaciones filogenéticas entre 13 especies de *Oligoryzomys* incluyendo la evaluación del estatus taxonómico de *O. vegetus*. También examinamos las asociaciones hopedero-hantavirus en función de los resultados filogenéticos. Las filogenias obtenidas bajo máxima parsimonia, máxima versimilitud e inferencia Bayesiana fueron congruentes. El genero *Oligoryzomys* resultó ser un grupo monofilético, con *O. microtis* como grupo hermano de los taxa restantes. *O. fulvescens* fue polifilético y estuvo representado por dos grupos geográficos: *O. fulvescens* sensu stricto de México y Centro América. Nuestros resultados apoyan la propuesta de que los antecesores del género *Oligoryzomys* entraron a Suramérica y se diversificaron en hábitats de zonas bajas antes de extender su diversificación hacia el sur. Desconocemos si el clado de *O. fulvescens* sensu stricto y *O. vegetus* evolucionó en

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Centro América o es derivado de un linaje Suramericano que reinvadió el norte del continente. Sin embargo, considerando la poca diferenciación genética dentro de *O. fulvescens* sensu stricto, proponemos que recientemente este linaje extendió su rango hacia el norte de México.

Palabras clave: *Oligoryzomys fulvescens*, polifilia, Norte y Centro América, filogenias de ADN, hantavirus.

INTRODUCTION

Members of the genus *Oligoryzomys* represent a relatively diverse radiation of small, Neotropical sigmodontine mice. *Oligoryzomys* occur throughout South America from Tierra del Fuego northward into Central America and Mexico (Hall, 1981; Emmons, 1997) and have been identified as vectors for hantaviruses (see Khaiboullina et al., 2005 and Rivera et al., 2007 for recent summaries). Musser and Carleton (2005) recognized 18 *Oligoryzomys* species, provisionally divided among five species groups (Carleton and Musser, 1989), but did not include *O. mesorrius* (Andrades-Miranda et al., 2001) in their summary. Bonvicino and Weksler (1998) described *O. stramineus* and later Weksler and Bonvicino (2005) recognized two new forms (*O. moojeni* and *O. rupestris*), but also considered *O. elirurus* and *O. delticola* as junior synonyms of *O. nigripes*. Gallardo and Palma (1990) recognized *O. megellanicus* as a species-level taxon with close affinities to *O. longicaudatus*. Silva and Yonenaga-Yasuda (1991) documented the karyotypes of two undescribed Brazilian *Oligoryzomys*. In addition, S. Lima et al. (2003) referred to a third undescribed colilargo from Brazil. Therefore, it is certain that the number of described species underestimates the actual biodiversity in this group (Musser and Carleton, 2005), especially given the amount of inter- and intraspecific karyotypic diversity documented in the genus as summarized by Weksler and Bonvicino (2005).

Of the 18 or so *Oligoryzomys* species currently recognized, most are found exclusively in South America (Musser and Carleton, 2005); only *O. fulvescens* and *O. vegetus* occur in Central and North America (Hall, 1981). *Oligoryzomys vegetus* is restricted to eastern Panama and Costa Rica (Carleton and Musser, 1995), whereas *O. fulvescens* occurs in the Sierra Madre Oriental and Occidental of Mexico south and east into Central America and northern South America (Carleton and Musser, 1989; Musser and Carleton, 2005). Mexican and Middle American populations of *O. fulvescens* are divided into 8 subspecies (Hall, 1981; Carleton and Musser, 1995). Relatively little is known regarding genetic variation within or among northern taxa relative to their South American counterparts. Dickerman and Yates (1995) used allozymic data to compare a single sample of *O. fulvescens* from Hidalgo, Mexico, with four South American species of *Oligoryzomys*. Later, Myers et al. (1995) examined a 401 base pair segment of the cytochrome *b* (Cyt*b*) gene and developed a phylogenetic hypothesis of relationships among seven *Oligoryzomys* species, including *O. vegetus* from Central America. However, recent estimates of phylogenetic relationships among species of *Oligoryzomys* using molecular data have not included North American taxa (Rivera et. al., 2007; Trott et al., 2007). The purpose of this paper is to examine the phylogeographic structure of *O. fulvescens*, emphasizing North and Central American samples. In addition, we evaluate the taxonomic status of *O. vegetus*, which co-occurs with *O. fulvescens* in Costa Rica and eastern Panama as well as samples referable to *O. fulvescens* in northern South America. We also examine host-hantaviral associations in light of our phylogenetic reconstructions. Finally, we comment on the phylogenetic structure present among the other species of *Oligoryzomys* included in our data set and corresponding biogeographic implications.

METHODS AND MATERIALS

Specimens and DNA sequencing

We included 45 specimens representing 13 species of *Oligoryzomys* and several outgroup taxa (sensu Watrous and Wheeler 1981—*Oreoryzmys balneator*, *Micororyzomys minutus* and *Neacomys minutus*) following the hypothesized relationships described by Weksler et al. (2006). Of these, 36 *Oligoryzomys* and three outgroup sequences were generated in our laboratories and the others were obtained from GenBank (Appendix I). Specimen identifications from GenBank were followed with the exception of AY452199 from Paraguay, which was identified as *O. fornesi*, but according to G. D'Elía (pers. comm.) should be regarded as *O. flavescens*. In addition, sample 29 from Nicaragua was identified *O. fulvescens nicaraguae*, but is instead regarded as *O. vegetus* (see discussion).

Total genomic DNA was extracted from liver, muscle, or tail clips using the Qiagen DNeasy™ Tissue Kit (Cat. No. 69504). Four microliters of DNA extraction product were electophoresed on 1.75-2.0% agarose gels stained with ethidium bromide to qualitatively estimate amount of genomic DNA. If DNA bands were relatively bright, then samples were diluted prior to polymerase chain

reaction (PCR) amplification. Two polymerase chain reactions were performed to amplify nearly the entire (Cyt*b*) gene (Saiki et al., 1988). Primer pairs used were as follows: L14724 (Irwin et al., 1991) / CB3H (Palumbi, 1996) or H627 (this study) and F1 (Whiting et al., 2003) / H15767 (Edwards et al., 1991). For the alternate overlapping set (approximately 750 and 600 bp, respectively) we used MVZ05 (Smith and Patton, 1993) / 752R (Bradley et al, 2000) or F1 (Whiting et al., 2003) / CB40 (Hanson and Bradley, in prep). The primer sequences are as follows: L14724, 52 -CGA AGC TTG ATA TGA AAA ACC ATC GTT G-32 ; CBH3, 52 -GGC AAA TAG GAA RTA TCA TTC-32 ; H627, 52 -GTC GGA GTT WGA GTT TAG WCC TGA-32 ; F1, 52 -TGA GGA CAR ATA TCH TTY TGR GG, H15767, 52 -ATG AAG GGA TGT TCT ACT GGT TG-32 ; MVZ05, 52 -CGA AGC TTG ATA TGA AAA ACC ATC GTT G-32 ; 752R, 52 -GAG ACC CCG ATA ATT ACA CTC CTG C-32 ; and CB40, 52 -CCA CTA YCA GCA CCC AAA GC-32 . The total length of the combined sequences, exclusive of the outer primers, typically was approximately 1,050 bp. Amplification reactions were performed in 25 µl volumes containing 1.0 µl template DNA (approximate concentration estimated on a 2% agarose gel), 4 µl dNTPs (1.25 mM), 2 µl 10X *Taq* buffer, 0.5 µl of each primer (100 µM), 3 µl $MgCl_2$ (25 mM), 14 µl distilled water, and 0.25 µl *Taq* polymerase (5 u/µl; Promega Corp., Madison, WI). Thermal profiles for the PCR reactions were either: 2-4 min at 94ÚC, 39 cycles (1min at 94ÚC, 1 min at 50ÚC, and 1 min at 72ÚC), plus 3 min at 72ÚC, or 2 min at 95°C, 30—40 cycles (30 sec at 95°C, 45 sec at 49°C, then 1 min 20 sec at 72°C, and a final extension cycle of 8 min at 72°C. Four microliters of double-stranded PCR amplified product were assayed by electrophoresis on a 2% agarose gel. The remaining product (ca. 21 µl) was purified using a Millipore Multiscreen™ PCR 96-Well Filtration System (Cat. No. MANU03050). Sequencing was performed using the Applied Biosystems Big Dye v.3.1 Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, CA).

Excess dye terminator was removed using Millipore Multiscreen™ Filter Plates for High Throughput Separations (Cat. No. MAHVN4510). Cytochrome *b* sequences were determined using Perkin-Elmer ABI Prism 377 or ABI 3100-*Avant* automated sequencer housed in the DNA Sequencing Center at Brigham Young University and Texas Tech University, respectively. To verify the accuracy of our data, we included negative controls in every reaction, complementary strands of each DNA fragment were sequenced and sequences were edited manually using the original chromatograph data in the program Sequencher version 4.1.1 (Gene Codes Co., 2000). All sequences have been deposited on the GenBank database (see Appendix I for accession numbers).

Phylogenetic analyses

Gene phylogenies were estimated using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) optimality criteria. Genetic distance values, MP and ML analyses as well as calculations of pair-wise genetic distances were performed using PAUP* 4.0 (Swofford, 2003). MP analyses were conducted with equal character weighting using a heuristic search with tree-bisection reconnection (TBR) branch swapping. For MP trees, branch support for nodes was assessed using nonparametric bootstrapping (Felsenstein, 1985) with 10000 bootstrap replicates of 100 random sequence additions. Bootstrap values $\geq$ 70% were considered well supported (Hillis and Bull 1993).

Under the ML criterion, the model of evolution most appropriate for our data was selected using Akaike Information Criterion (AIC—Akaike 1974) as implemented in MODELTEST v3.6 (Posada and Crandall, 1998). The general time reversible model with invariable sites and rate heterogeneity (GTR + I+ Ã) was selected as the best-fit model of nucleotide substitution. The base frequencies were A= 0.3181, C=0.3056, G=0.1118, and T=0.2645; transversion rates were (A-C) 3.0119, (A-G) 13.8081, (A-T) 2.5571, (C-G) 1.7213, (C-T) 25.8284; the proportion of invariable sites (I) was 0.4586, and the gamma distribution shape parameter (Ã) was 0.6681.

Bayesian inference was conducted using MrBayes 3.0b4 software. In this method, a posterior probability of a phylogeny is estimated by sampling trees from the overall distribution of posterior probabilities using Metropolis-coupled Markov Chain Monte Carlo to sample phylogenies according to their posterior probabilities (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Each of the two simultaneous analyses was started from a different, randomly chosen tree and 4 simultaneous incrementally heated chains were run for 10 x 10^6 generations, with sampling every 1000^{th} interval. To ensure the Markov Chain had become stable, ln-likelihood values for sampling points were plotted against generation time. All sample points prior to reaching stationarity (conservatively, the first 10% of the total trees) were discarded as «burn in». Posterior probabilities for individual clades obtained from independent analyses were compared for congruence (Huelsenbeck and Imennov, 2002; Huelsenbeck et al., 2002; Nylander et al., 2004).

Hypothesis testing

Alternative phylogenetic hypotheses were tested with ML-based approaches. Tree searches were conducted with constraints configured to match tree topologies for each a priori hypothesis. Alternative tree topologies were evaluated for statistical significance using the Shimodiara and Hasegawa test (S-H; Shimodiara and Hasegawa, 1999) as implemented in PAUP* 4.0 (Swofford, 2003). Ten thousand bootstrap replicates were performed using the S-H test by resampling the partial likelihoods for each site (RELL model).

RESULTS

Sequence variation

A 751-801 bp portion of the Cyt*b* gene was compared across all samples (see Appendix I). Mitochondrial DNA nucleotide composition was similar to those reported for the majority of mammals (Irwin et al. 1991) and no internal stop codons were detected – indicating the sequences we generated represented functional mitochondrial sequences rather than pseudogenes. There were 288 variable characters in the data matrix, of which 208 were parsimony informative. In this study, guanines occurred less frequently (11.19%) relative to adenine, cytosine and thymine (31.79%, 30.56% and 26.46%, respectively). Mean uncorrected pairwise distances among species of *Oligoryzomys* ranged from 4.6% between samples of *O. flavescens* and *O. andinus* to 13.1% between *O. microtis* and *O. flavescens*.

Phylogenetic analyses

Parsimony analysis with equal character weighting resulted in a single most parsimonious tree (Fig. 1) of 1010 steps (CI = 0.49, RI = 0.62). This analysis supported monophyly of the genus *Oligoryzomys* (clade I) with

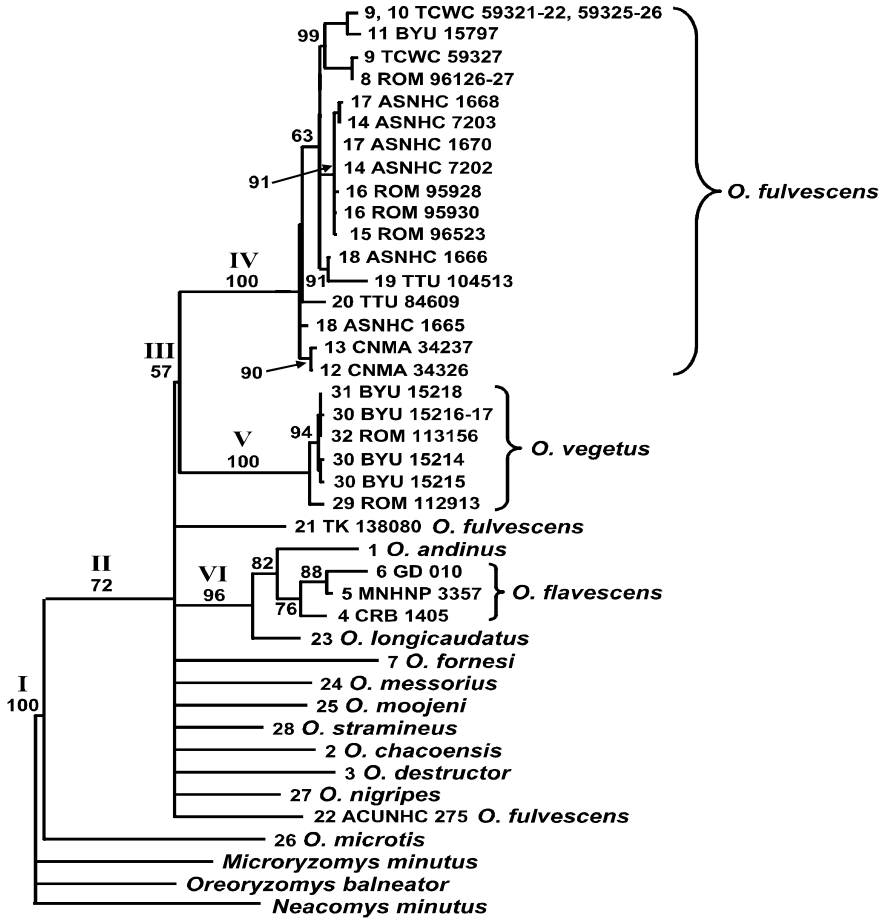

Figure 1. Single most parsimonious tree (1010 steps; CI = 0.49; RI = 0.62) derived from analysis of cytochrome *b* sequence data for 42 samples of *Oligoryzomys* and three outgroup taxa (*Oreoryzomys balneator, Microryzomys minutus* and *Neacomys minutus*). Nodal support is represented by bootstrap values derived from 10000 pseudoreplicates and only strongly support nodes (BP > 70%) are shown. The six clades referenced in text and in Table 1 are labeled with Roman Numerals. Terminals are numbered and correspond to collecting localities provided in Appendix I. For each terminal, sample numbers are followed by species designations or by the museum acronym and voucher number.

strong bootstrap support (BP=100). Within this clade, *O. microtis* was recovered as the sister group of the remaining *Oligoryzomys* samples. Percent genetic distances between samples of *Oligoryzomys* and *O. microtis* ranged from 10.1 to 13.1%. *Oligoryzomys fulvescens* (clade IV, samples 21 and 22) was not recovered as a monophyletic entity. Instead, clade IV, represented by samples of *O. fulvescens* from Mexico and Honduras (and hereafter referred to as *O. fulvescens* sensu stricto), formed the sister group to *O. vegetus* (hereafter including *O. f. nicaraguae*) with low bootstrap support (BP=57), all together these samples formed clade III. Percent genetic distances among samples of *O. fulvescens* sensu stricto ranged from 0 to 3.6%. Samples 21 and 22 differed from each other by 6.6% and from samples of *O. fulvescens* (sensu stricto) by 7.5 to 9.6%.

This analysis also recovered three South American taxa as closely related and comprising a well supported clade VI (BP=96): *O. andinus*, *O. flavescens*, and *O. longicaudatus* (percent sequence divergence ranged from 2.1% within *O. flavescens* to 6.8% between *O. flavescens* and *O. andinus*). Relationships among the remaining *Oligoryzomys* samples were unresolved.

Tree topologies generated from ML and BI optimality criteria using the GTR+I+Ã model of evolution generated identical tree topologies, both of which were more resolved than that produced using MP. The ML tree (lnL=-5713.51) with Bayesian posterior probability values for nodal support (Fig. 2) recovered the same relationships as in Fig. 1 in addition to recovering two new relationships not previously depicted. The first clade consisted of

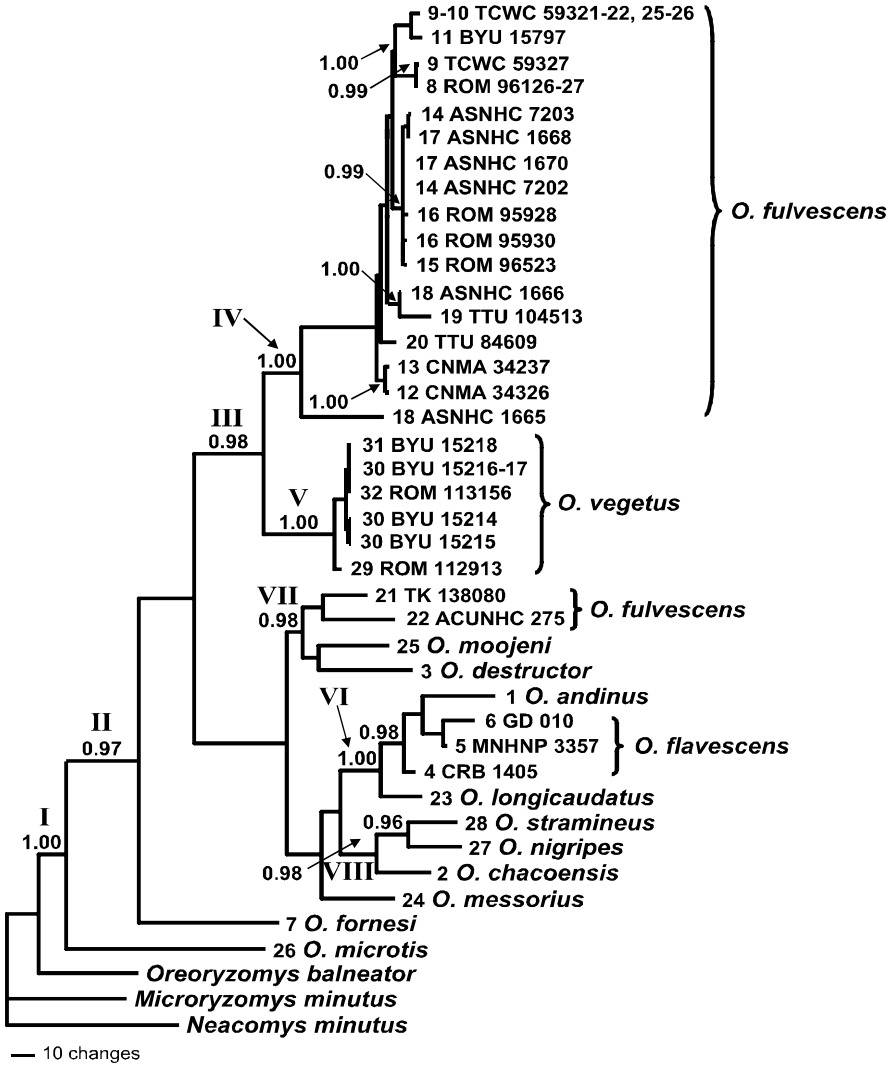

Figure 2. Phylogram of a single tree (lnL=-5713.51) generated from maximum likelihood analysis of the cytochrome -*b* gene for 42 samples of *Oligoryzomys* and three outgroup taxa (*Oreoryzomys balneator, Microryzomys minutus* and *Neacomys minutus*). Numbers are posterior probabilities derived from a Bayesian analysis that yielded an identical tree topology (not shown). The eight clades referenced in text and in Table 1 are labeled with Roman Numerals. Terminals are numbered as in Figure 1.

South American *O. fulvescens* (samples 21 and 22) together with *O. moonjeni* and *O. destructor* (p*P* = 0.98; clade VII) and there was significant support (p*P* = 0.98) for the clade comprised of *O. stramineus, O. nigripes,* and *O. chacoensis* (clade VIII).

Constraint tests

Using ML (S-H test) optimality criteria, we tested 11 a priori hypotheses derived from the literature as summarized in Table 1. The majority of hypotheses evaluated resulted in significantly less likely tree topologies with the exceptions of tests 3, 4, and 8. These three tests involved constraining monophyly of South American samples of *Oligoryzomys fulvescens* (21 and 22) with *O. fulvescens* from Mexico and Central America, monophyly of clade III, and the sister group relationship between *O. microtis* and *O. fornesi*.

DISCUSSION

Monophyly of the genus Oligoryzomys and deep level phylogenetics

Previous studies on the phylogenetic relationships among members of the genus *Oligoryzomys* using morphological (Carleton and Musser, 1989; Steppan, 1995; Weksler, 2006), allozymes (Dickerman and Yates, 1995) and molecular data (Myers et al., 1995; Rivera et al., 2007; Weksler, 2003; 2006) have concluded that this taxon is monophyletic. This study includes greater taxon sampling than previous analyses and our results strongly support the hypothesis that the genus *Oligoryzomys* is a monophyletic taxon. Hypothesis test 1, forcing non-monophyly of *Oligoryzomys*, resulted in a significantly less likely tree (Table 1). Our analyses also document that *O. microtis* forms a clade relative to other *Oligoryzomys* taxa. In turn, ML and BI tree topologies (Fig. 2) indicate that *O. fornesi* constitutes the second, internal clade within the genus, although its phylogenetic position based on MP was unresolved.

Additionally, the view that *O. microtis* and *O. fornesi* are conspecific (Carleton and Musser, 1989; Olds and Anderson, 1987) could not be rejected (Table 1). However, Myers et al. (1995) also recovered these two taxa in separate clades and they differ karyotypically (summarized in Weksler and Bonvinco, 2005). Therefore, we concur with Weksler and Bonvinco (2005) who maintain the two as distinct taxa.

Recovery of *O. microtis* as a basal clade within *Oligoryzomys* together with the magnitude of genetic divergence we documented agrees with the results of Myers et al. (1995) and those of Rivera et al. (2007) and is consistent with the proposal by Carleton and Musser (1989) that *O. microtis* belongs in its own species group. However, these results contrast with the discussion by Voss et al (2001). They compared Mexican (*O. fulvescens* sensu stricto) and Central American (*O. f. costaricensis*) specimens with Amazonian specimens referable to *O. microtis* including material from the type locality of *O. microtis* (redefined therein) and found no obvious quantitative or qualitative morphological characters that separated *O. fulvescens* from *O. microtis*. As a result, Voss et al. (2001) suggested that *O. fulvescens* sensu lato and *O. microtus* may be conspecific, but allowed that increased sample sizes or other data sets might shed light on this issue. We tested this hypothesis by constraining *O. microtis* and *O. fulvescens* sensu stricto as sister taxa, resulting in significantly less likely and longer tree topologies (Table 1). Given the phylogenetic position of *O. microtis* based on genetic data and chromosomal differences between these two taxa (summarized in Weksler and Bonvicino, 2005), we hypothesize that this taxon represents a distinct, basal lineage within the genus *Oligoryzomys* and predict that inclusion of sequence data from nuclear markers will support recognition of *O. microtis* as a monotypic subgenus within *Oligoryzomys*.

Systematics of Oligoryzomys fulvescens

Oligoryzomys fulvescens, as currently recognized, occurs from Tamaulipas, Mexico, into northern South America (Musser and Carleton, 2005). However, we consistently recovered *O. fulvescens* sensu lato as polyphyletic in all analyses and consisting of two moieties: clade IV (*O. fulvescens* sensu stricto) and South American samples of *O. fulvescens* (samples 21 and 22). Constraint tests that forced monophyly of these groups resulted in trees that approached significance at $p < 0.05$ (test 3, Table 1). Alternatively, *O. fulvescens* sensu stricto formed the sister group to *O. vegetus* (clade III) and altering this relationship also did not result in a significantly longer tree. However, recognition of two clades within *O. fulvescens* is supported by karyotypic data. *O. fulvescens* from Veracruz, Mexico, possesses a diploid (2n) = 60 and fundamental number (FN) = 74 karyotype (Haiduk et al., 1979), whereas *O. fulvescens* from Venezuela has a 2n = 60 and FN = 72 (Kiblisky, 1969). Gardner and Patton (1976) report that *O. fulvescens* from Costa Rica possesses a 2n = 54, FN = 68 chromosomal complement, but based on the collecting locality; this individual likely represents *O. vegetus*. Our results suggest that *O. fulvescens* sensu stricto should be regarded as a separate taxonomic entity from Venezuelan *O. fulvescens*.

Table 1. Topological tests of selected a priori hypotheses evaluated using Shimodiara-Hasegawa test (S-H). The log likelihood (ML) score was -5713.51. Clade designations and sample numbers are as presented in Figures 1 and 2.

	S-H test	
Hypothesis	Log Score Difference	p-value
1. Monophyly of clade I (genus *Oligoryzomys*–Carleton and Musser, 1989; Myers et al., 1995)	66.66	0.0010
2. Monophyly of clade II (all *Oligoryzomys* taxa except *O. microtis*–Carleton and Musser, 1989)	18.45	0.0140
3. Monophyly of clade IV, samples 21 and 22 (*O. fulvescens* sensu lato–Carleton and Musser, 1989).	23.78	0.0760
4. Monophyly of clade III (*O. fulvescens* sensu stricto, *O. vegetus* and *O. f. nicaraguae*– Carleton and Musser, 1995)	7.98	0.1430
5. Monophyly of clade IV (*O. fulvescens* sensu stricto–Carleton and Musser, 1995)	57.07	<0.0001
6. Monophyly of the *nigripes* group, samples 3, 23 and 27 (Carleton and Musser, 1989)	105.96	<0.0001
7. Monophyly of the *andinus* group, samples 1 and 2 (Carleton and Musser, 1989)	68.31	<0.0001
8. Monophyly of *O. microtis* and *O. fornesi* (Carleton and Musser, 1989; Olds and Anderson, 1987)	14.33	0.0680
9. Monophyly of *O. microtis* and *O. fulvescens* sensu stricto (Voss et al. 2001)	57.06	<0.0001
10. Monophyly of *O. fornesi* and *O. longicaudatus* (Myers et al., 1995)	53.33	0.0030
11. Monophyly of *O. andinus* and *O. microtis* (Myers et al., 1995)	101.61	<0.0001

According to Jones and Engstrom (1986:13) «two fairly well-defined subspecies [of *O. fulvescens*] occur in Nicaragua». *Oligoryzomys fulvescens nicaraguae* is larger cranially and more darkly colored than is *O. f. costaricensis*. The former subspecies occurs in higher elevations in the eastern portion of the country, whereas the latter occurs in lower elevations to the west. We sequenced a single individual from Rivas Province in Nicaragua (sample 29) and tentatively assigned it to *O. f. nicaraguae* based on its geographic location. We rejected monophyly of this specimen with *O. fulvescens* sensu stricto at high significance (test 5, Table 1). Therefore, regardless of the subspecific name applied to sample 29, our results demonstrate that this specimen is closely related to samples of *O. vegetus*.

Status of Oligoryzomys vegetus

Compared to *Oligoryzomys fulvescens*, *O. vegetus* is typically larger in both external (total length and hind foot length) and cranial measurements (Carleton and Musser, 1995). In addition, the capsular process on the lower mandibles in specimens of *O. vegetus* usually extends above the ventral rim of the sigmoid notch when viewed laterally or medially, whereas this process is not typically visible in specimens of *O. fulvescens* (see Fig. 5 in Carleton and Musser, 1995). However, there is no qualitative or quantitative character that separates these two taxa absolutely. For example, about 16% of specimens identified as *O. vegetus* from Costa Rica possessed a «*fulvescens*-like» capsular process morphology, whereas 7% of *O. fulvescens* examined had the «*vegetus*-like» morphology (Carleton and Musser, 1995). We scored the relative positions of the capsular process for specimens of *Oligoryzomys* from Costa Rica and Nicaragua included in this analysis and all specimens except BYU 15216 were identified as *O. vegetus* based on this character. BYU 15216 possessed the «*fulvescens*-like» capsular morphology. However, all specimens from Costa Rica and Nicaragua, including BYU 15216, formed a clade (referable to *O. vegetus*) relative to examples of *O. fulvescens* from Mexico and Honduras which we regard as *O. fulvescens* sensu stricto (Figs. 1 and 2). Further, a 401 bp Cyt*b* sequence of *O. vegetus* from the Panamanian Chiriqui (Myers et al. 1995) groups with the Costa Rican and Nicaraguan samples included in this study (analyses not shown). Therefore, our data support Carleton and Musser's (1995) recognition of *O. vegetus* as a species-level taxon, herein defined to be the sister group of *O. fulvescens* sensu stricto rather than *O. fulvescens* sensu lato (Carleton and Musser, 1989). Our findings also extend the known range of *O. vegetus* from Panama and the Cordillera de Tilarán in Costa Rica (Carleton and Musser, 1995) north and west to the Rivas Province in southern Nicaragua.

Phylogenetic relationships among South American Oligoryzomys

Although our primary objective was to elucidate relationships among North and Central American *Oligoryzomys*, our Cyt*b* sequence data provide insight into genealogical relationship among taxa restricted to South America and together with data for North and

Central American taxa and allow us to comment on the evolution of the genus. The only South American group of taxa that we recovered consistently in all our analyses and with high bootstrap or posterior probabilities was clade VI, consisting of *O. andinus, O. flavescens* and *O. longicaudatus* (Figs. 1 and 2). Our ML and Bayesian phylogenetic estimates recovered two additional clades with significant posterior probabilities. Clade VII consisted of Venezuelan *O. fulvescens* together with *O. moojeni* and *O. destructor*. Likewise, nodal support for Clade VIII, consisting of *O. chacoensis, O. nigripes* and *O. stramineus* also was high (p*P* = 0.98).

In addition to recognizing the *O. fulvescens* and *O. microtis* species groups, Carleton and Musser (1989) allocated *O. andinus* and *O chacoensis* to the *O. andinus* group, recognized the *O. nigripes* group as consisting of *O. delticola, O. destructor, O. elirurus, O. longicaudatus,* and *O. nigripes*, and proposed the *O. flavescens* group comprised of *O. flavescens* and several undescribed taxa from Bolivia, Brazil and Peru (*O. elirurus, O. delticola* and the undescribed taxa allocated to the *O. flavescens* group were not included in our analyses). We tested the monophyly of the *O. andinus* group and found no support for a close relationship between *O. andinus* and *O. chacoensis* based on our sequence data. Likewise, forcing monophyly of *O. destructor, O. longicaudatus* and *O. nigripes* resulted in a significantly less likely tree topology than our optimal tree (Table 1). In fairness, a morphological cladistic data set is not available to test the alternative: whether the morphological data can reject our hypothesis based on sequence data, and our results might differ with additional taxon or gene sampling. However, we infer that existing morphological data are largely uninformative at this taxonomic level.

Myers et al. (1995) estimated genealogical relationships among eight *Oligoryzomys* taxa using a 401 base pair segment of the Cyt*b* gene. They consistently found a well supported sister group relationship between *O. fornesi* and *O. longicaudatus*. Myers et al (1995) also recovered *O. andinus* and *O. microti*s as sister taxa, but with less nodal support. However, constraining our ML tree topology to match these two hypotheses (tests10 and 11) resulted in significantly less likely trees. We suspect that this discrepancy in the case of the *fornesi-longicaudatus* clade involves specimen identification. We are referring to samples of *Oligoryzomys* from Paraguay as *O. flavescens* rather than *O. fornesi* as did Myers et al (2005). The second incongruence identified might be explained increased taxon sampling, outgroup choice, and/or a larger number of nucleotides sequenced between our study and that of Myers et al (1995).

Recently, Rivera et al. (2007) examined phylogenetic relationships among six species of *Oligoryzomys* from Argentina (*O. chacoensis, O. nigripes, O. destructor, O. flavescens, O. longicaudatus* and *O. microtis* and) using restriction sites and sequences from the mitochondrial D-loop region. The results by Rivera et al. (2007) regarding the placement of these taxa were similar to ours in that they recovered a monophyletic *Oligoryzomys* with *O. microtis* as the basal taxon relative to other species and arranged *O. flavescens* and *O. longicaudatus* as sister taxa. We tested the genealogical relationships developed by Rivera et al. (2007) against ours by pruning our data set to match theirs and rerunning our ML analysis. Constraining the Rivera topology to ours resulted in trees that were not significantly less likely (log score difference 7.36—p = 0.365).

Oligoryzomys and hantaviruses

Hantaviruses are negative-stranded RNA viruses in the family Bunyaviridae that infect murid rodent hosts, including the New World subfamily Sigmodintinae (Johnson et al., 1997). A total of 29 hantaviruses has been identified in North and South American sigmodontine rodents to date (Schmaljohn and Hjelle, 1997; J. Mills, pers. comm.). Investigators typically document a one-to-one correspondence between a particular hantavirus and its rodent host (Mills et al., 2007) with only one clear case of host switching among New World hantaviruses (Morzunov et al., 1998) involving *Peromyscus leucopus* in the eastern United States. Moreover, a subset of studies document multiple rodent hosts for a single hantavirus, which is believed to be the result of spillover from the primary host to other, short-term hosts (Rawlings et al., 1996; Rowe et al., 1995; Schmaljohn and Hjelle, 1997). Interestingly, there are now 10 hantaviruses associated with seven species of *Oligoryzomys* as follows: *O. chacoensis*, Bermejo virus (Levis et al., 1998; Padula et al., 2002); *O. flavescens*, Lechiguanas virus (Levis et al., 1998) and Central Plata virus (Delfraro et al., 2003); *O. fornesi*, Anajatuba virus (Plyusnin, 2002); *O. fulvescens*, Choclo virus (Vincent et al., 2000) and Maporal virus (Fulhorst et al., 2004); *O. longicaudatus*, Andes virus (Levis et al., 1998) and Orán virus (Levis et al., 1998); *O. microtis*, Río Mamoré virus (Hjelle et al., 1996); and *O. nigripes*, Juquitiba virus (Suzuki et al., 2002). Therefore, three taxa (*O. flavescens*, *O. fulvescens*, and *O. longicaudatus*) each are implicated as hosts for two types of hantaviruses. Sample 21 (*O. fulvescens* from Venezuela) in our data set represents the rodent host for Maporal virus (Fulhorst et al., 2004). The fact that *O. fulvescens* (sensu lato) serves as host to different viruses is consistent with our hypothesis that *O. fulvescens* (sensu stricto) and

O. fulvescens from Venezuela likely represent two species-level lineages. Unfortunately, we cannot address the multiple hantaviral associations for *O. flavescens* and *O. longicaudatus*, but suggest that those species also represent composite biological entities.

Historical biogeography

Sigmodontine rodents, including ancestors of *Oligoryzomys*, are thought to have entered South America from Central America prior to the formation of a permanent land bridge connecting the two continents (Marshall et. al., 1982, Webb and Rancy, 1996). Under this scenario, proto-oryzomyines dispersed to South America along island arcs prior to formation of the Panamanian land bridge (Simpson, 1980). Recent estimates for the time closure of the Panamanian portal are between 2.5 and 3.5 million years ago (mya—Coates et al., 1992; Graham, 1992; Hooghiemstra, 1994), followed by orogeny in the Isthmian region which resulted in formation of upland habitats approximately 2.5 mya (Graham, 1989). Therefore, proto-oryzomyine forms likely colonized South America no later 2.5 mya (late to mid-Pliocene) but the timing could have been considerably earlier, even in the late Miocene (>5.2 mya) if waif dispersal or island hopping occurred (Marshall, 1979). According to Miller et al. (1987), global temperatures declined approximately 4ºC from the mid-Pliocene to the early Pleistocene, resulting in an increase of cool to cold temperature plant pollens found in Central America and northern South American palynofloras (Graham, 1999) subsequent to closure of the Panamanian portal. Detailed analysis of middle Pliocene (about 3 mya) pollen and spore flora from central Panama document an appearance of grass pollen indicative of a more developed tropical dry forest component (Graham, 1991). Given the timing of climatic and resulting vegetation changes in the isthmian region, it is likely that lowland tropical forests rather than tropical dry forest conditions predominated during the time that proto-oryzomyines entered South America (Marshall, 1979). Subsequent diversification of oryzomyines and the early evolution of the genus *Oligoryzomys* likely occurred in northern South America during the late Miocene and through the mid-Pliocene. Given that the northern Andean highlands had developed approximately one-half the modern elevation 10 mya (Gregory-Wodzicki, 2000), considerable habitat diversity must have existed in northern South America (lowland tropical rainforests, tropical savannahs and montane forests) during the time early *Oligoryzomys* lineages diversified. This scenario is not inconsistent with modern habitat preferences for basal lineages of *Oligoryzomys* (*O. microtis* and *O. fornesi*). *Oligoryzomys microtis* is found in Amazonian forests, whereas *O. fornesi* inhabits more open lowland habitats. The other, more derived South American clade we recovered (clade VII) consisted of *O. andinus, O. flavescens* and *O. longicaudatus*. All taxa in the group are found in the central or southern portions of the continent and two (*O. andinus* and *O. longicaudatus* prefer montane habitats. The existence of this clade is consistent with the notion that evolution in the genus involved diversification from north to south and from low to relatively high elevation habitats, a hypothesis first articulated by Marshall (1979).

CONCLUSIONS

The phylogenetic signal in our data is not sufficient to hypothesize the evolutionary events giving rise to northern taxa. However, *O. fulvescens* sensu stricto and *O. vegetus* appear to be sister taxa. Whether these forms evolved in situ in Central America or represent a northward reinvasion from South America cannot be determined. Given the low amount of sequence divergence present within Mexican *O. fulvescens*, it is likely that populations found along the eastern versant of Mexico represent a relatively recent northward expansion.

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Appendix I. List of *Oligoryzomys* and outgroup taxa included in this study with population number, collection location, museum voucher numbers and GenBank accession numbers (* = sequences reported for the first time). Geographic abbreviations are: AR = Argentina; BO = Bolivia; BR = Brazil; CR = Costa Rica; EC = Ecuador; HN = Honduras; MX = México; NI = Nicaragua; PG = Paraguay; PR = Perú; VZ = Venezuela. Museum abbreviations are as follows: ACUNHC = Abilene Christian University Natural History Collections; AMNH = American Museum of Natural History; ASNHC = Angelo State Natural History Collections; BYU = Monte L. Bean Museum, Brigham Young University; CNMA = Colección Nacional de Mamíferos, Universidad Nacional Autonóma de México; CRB = Cibele R. Bonvicino, voucher at Museu Nacional, Rio de Janeiro, Brazil; MNHNP = Museo Nacional de Historia Natural de Paraguay; MN = Museu Nacional do Rio de Janeiro; MVZ = Museum of Vertebrate Zoology, University of California, Berkeley; ROM = Royal Ontario Museum; TCWC = Texas Cooperative Wildlife Collection, Texas A&M University; TTU = The Museum; Texas Tech University; UFPB = Universidade Federal da Paraíba. In addition, TK and GD = TTU, and Guillermo D'Elía field numbers, respectively.

Sample No.	Scientific Name	Country: State/Province	Locality	Musuem Voucher No.	GenBank Accession No.	No. of base pairs
1	*O. andinus*	BO: Oruro	2 km E. of Huancaroma	AMNH 260406	AY452200	801
2	*O. chacoensis*	PG: Boqueron	La Lomita	TTU 104514	EU258543*	801
3	*O. destructor*	EC: Pichincha	60 km N Quito, Tandayapa Valley	ACUNHC 898	EU258544*	801
4	*O. flavescens*	BR: Sao Paulo	Pedreira	CRB 1405	EU258545*	801
5	*O. flavescens*	PG: Neembucu	Estancia Yacare	MNHNP3357	EU258542*	751
6	*O. flavescens*	PG: Misiones	620 m S Hotel Centu Cue, 26Ú15'06"S, 57Ú1'35"W	GD010	AY452199	801
7	*O. fornesi*	BR: Goiás	Serra da Mesa	MN 36746	DQ826022	801
8	*O. fulvescens*	MX: Tamaulipas	4 km W La Carbonera, 46.5 km ESE San Fernando	ROM 96126 ROM 96127	EU294238* EU294239*	801

Sample No.	Scientific Name	Country: State/Province	Locality	Musuem Voucher No.	GenBank Accession No.	No. of base pairs
9	*O. fulvescens*	MX: Tamaulipas	Los Cedros, Reserva de la Biosfera El Cielo, 23 03' 00.00000'' N, 99 09' 09.06000'' W, 329 m;	TCWC 59321 TCWC 59325 TCWC 59326 TCWC 59327	EU294242* EU294244* EU294245* EU294246*	801
10	*O. fulvescens*	MX: Tamaulipas	San José, Reserva de la Biosfera El Cielo	TCWC 59322	EU294243*	801
11	*O. fulvescens*	MX: Puebla	La Gloria, Apulco, 10 km N Zacapoaxtla, 1,500 m	BYU 15797	EU294235*	801
12	*O. fulvescens*	MX: Veracruz	18 km NE Teocelo	CNMA 34236	EU294248*	801
13	*O. fulvescens*	MX: Veracruz	Cascadas de Texolo, 1.5 km SE Xico	CNMA 34237	EU294247*	801
14	*O. fulvescens*	MX: Tabasco	14 km E and 27 km S El Triunfo	ASNHC 7203 ASNHC 7202	EU294236* EU294237*	801
15	*O. fulvescens*	MX: Yucatán	Laguna Becanchen	ROM 96523	EU294240*	801
16	*O. fulvescens*	MX: Campeche	52 km SW Champotón	ROM 95928 ROM 95930	EU294229* EU294230*	801
17	*O. fulvescens*	MX: Chiapas	6.6 km S (by road) Palenque	ASNHC 1668 ASNHC 1670	EU294231* EU294232*	801
18	*O. fulvescens*	MX: Chiapas	12 km N Berriozabal	ASNHC 1665 ASNHC 1666	EU294233* EU294234*	801
19	*O. fulvescens*	MX: Chiapas	Mapastepec, Tutuan, El Rancho Trébol	TTU 104513	EU258548*	801
20	*O. fulvescens*	HN: Olancho	4 km E Catacamas (Esculea de Sembrador)	TTU 84609	EU258547*	801
21	*O. fulvescens*	VZ: Portuguesa	Hato Maporal, near Caño Delgadito	TK 138080	DQ227457*	801
22	*O. fulvescens*	VZ: Amazonas	Pozon, 50 km NE Puerto Ayacucho	ACUNHC 275	EU258537*	751
23	*O. longicaudatus*	AR: Rio Negro	12 km W Bariloche	MVZ 155842	U03535	801
24	*O. messorius*	BR: Roraima	Surumu	MN 37751	DQ826024	801
25	*O. moojeni*	BR: Goiás	Serra da Mesa	MN 37441	DQ826021	794
26	*O. microtis*	BO: Santa Cruz	Dinamarca, UTM 20K 0457277 8113921	BYU 19014	AY439000	
27	*O. nigripes*	PG: Itapua	3.2 km N, 0.4 km E Ape Aime	TTU 104515	EU258550*	
28	*O. stramineus*	BR: Goiás	Legalito Farm	UFPB 1827	DQ826026	794
29	*O. vegetus* (formerly *O. f. nicaraguae*)	NI: Rivas	unknown	ROM 112913	EU258538*	801
30	*O. vegetus*	CR: Alajuela	Finca Rosalia, 3 km N Laguna Fraijanes, San Isidro, 1,650 m	BYU 15214 BYU 15215 BYU 15217	EU294250* EU294252* EU294251*	801
31	*O. vegetus*	CR: Cartago	Rio Macho, Instituto Costarricense de Electricidad	BYU 15218	EU294249*	801