A MOLECULAR REAPPRAISAL OF THE SYSTEMATICS OF THE LEAF-EARED MICE *PHYLLOTIS* AND THEIR RELATIVES

UNA RE-EVALUACIÓN MOLECULAR DE LA SISTEMÁTICA DEL GÉNERO *PHYLLOTIS* Y SUS GRUPOS HERMANOS

Steppan, S. J., O. Ramirez, J. Banbury, D. Huchon, V. Pacheco, L. I. Walker, and A. E. Spotorno

ABSTRACT

The tribe Phyllotini includes the ecologically abundant and well studied leaf-eared mice *Phyllotis*. We report on phylogenetic analyses of both mitochondrial (1,185 bp of the complete cytochrome *b* gene (cyt *b*) and flanking tRNA) and nuclear (1,300 bp of the nuclear recombination activating gene 1, RAG1) DNA for 13 species of *Phyllotis*, including 2 undescribed species. We also include published and new sequences for 19 other phyllotine species. Sampling within species is relatively high, with 5 of the species represented by 7 to 17 individuals each. The results clarify relationships among phyllotine genera, test monophyly of *Phyllotis*, resolve some relationships within *Phyllotis*, and confidently resolve the position of the newly discovered species. Notably, we confirm previous cyt *b* results, but in contrast to morphological hypotheses, that *Graomys* and *Andalgalomys* are not closely related. Relationships near the base of Phyllotini are poorly supported by cyt *b* but receive somewhat better support from the more slowly evolving RAG1. *Graomys* and *Eligmodontia* do not appear to be members of a clade that includes *Phyllotis* and *Auliscomys*. *Phyllotis wolffsohni* should be removed from *Phyllotis* and placed in *Tapecomys*. The data do not support recognition of *Paralomys* because the type species (*Phyllotis gerbillus*) is closely related to 3 other northwestern species (*andium, amicus*, sp. nov. 1) that together fall within *Phyllotis*. A second undescribed species, from Argentina, appears to be the sister species to *P. osilae*, and together these are the sister-group to all other *Phyllotis* s. s. The *darwini* species-group is supported by cyt *b* data, and within that group, the widespread species *P. xanthopygus* is characterized by deep divergences, high genetic diversity, and paraphyly with respect to at least 2 morphological species, *limatus* and *bonariensis*. Two or 3 possible cryptic species are revealed by the phylogenies. Problematic sequences from GenBank are identified. Finally, with respect to molecular clock estimates of divergence dates, our application of different calibrations and methods suggests that phyllotine divergence dates estimated by Salazar-Bravo et al. (2001) may be too old.

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Key words: *Phyllotis*, cytochrome b, RAG1, phylogenetics, biogeography, cryptic species

RESUMEN

La tribu Phyllotini incluye a los ecológicamente abundantes y bien estudiados ratones orejones del género *Phyllotis*. En este trabajo, presentamos un análisis filogenético basado en secuencias del genoma mitocondrial (1,185 pb del gen completo del citocromo b (cyt *b*) y el adyacente RNAt) y nuclear (1,300 pb del gen nuclear activante de recombinación 1, RAG 1), incluyendo a 13 especies de *Phyllotis*, 2 de ellas aún no descritas. Además, hemos incluído nuevas secuencias y otras previamente publicadas para otras 19 especies de filotinos. La muestra dentro del nivel de especie fue relativamente alta, 5 de las especies estuvieron representadas con un número que varió entre 7 y 17 individuos. Los resultados resuelven las relaciones filogenéticas entre los géneros de filotinos, prueban la monofília de *Phyllotis*, resuelven algunas relaciones dentro de *Phyllotis*, y resuelve la posición de las nuevas especies descubiertas. Notablemente, confirmamos los resultados previos basados en secuencias del cyt *b* pero es incongruente con la hipótesis, basada en datos morfológicos, que *Graomys* y *Andalgalomys* no están cercanamente relacionados. Las relaciones cercana a la base del clado Phyllotini son pobremente soportadas por el cyt *b* pero reciben un mejor soporte del RAG1, cuya tasa de mutación es más lenta. *Graomys* y *Eligmodontia* no parecen ser miembros del clado que incluye a *Phyllotis* y *Auliscomys*. *Phyllotis wolffsohni* debería ser removido de *Phyllotis* y ubicado en *Tapecomys*. Los datos no apoyan el reconocimiento de *Paralomys* debido a que la especie tipo (*Phyllotis gerbillus*) está cercanamente relacionada a otras 3 especies del noroeste (*andium, amicus,* sp. nov. 1) que juntas forman parte del clado de *Phyllotis*. Una segunda aún no descrita especie de Argentina, parece ser la especie hermana de *P. osilae*, y juntas son el grupo hermano de todas las otras especies de *Phyllotis* s. s. El grupo de especies *darwini* está soportado por los datos provenientes del cyt *b*, y dentro de este grupo, *P. xanthopygus*, la especie de más amplia distribución geográfica, se caracterizó por profundas divergencias, alta diversidad genética, y parafília con respecto a 2 especies morfológicas, *limatus* y *bonaerensis*. Dos o 3 especies crípticas se observan en las filogénias. Se han identificado secuencias problemáticas de GenBank. Finalmente, con respecto a la antigüedad de las divergencias estimadas por reloj molecular, nuestra aplicación de diferentes calibraciones y métodos sugiere que Salazar-Bravo et al. (2001) las ha sobrestimado.

Palabras claves: *Phyllotis*, citocromo b, RAG1, filogenética, biogeografía, especies crípticas

INTRODUCTION

The genus *Phyllotis* (Rodentia: Sigmodontinae) has had a long systematic history and captured the attention of some of the leading mammalogists, starting with the original collection of 2 species by Charles Darwin on the voyage of the *Beagle*, followed by their subsequent description by Waterhouse (1837) and later revisionary studies by Pearson (1958) and Hershkovitz (1962). Members of the genus are widespread throughout the

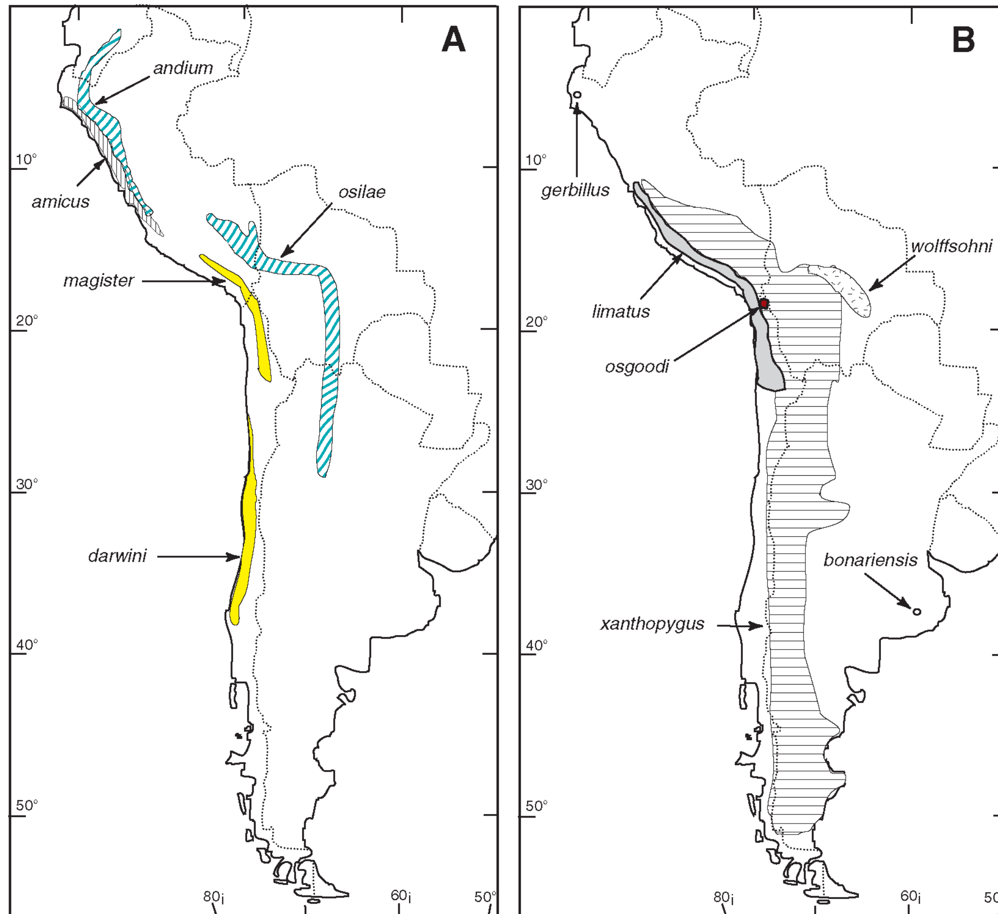

Figure 1. Distribution of *Phyllotis* species included in this study. Panels A and B separate species with overlapping distributions for visual clarity.

central and southern Andes (Fig. 1) and often are the most abundant mammal at a location. Their appearance, ecology, and taxonomic complexity led Pearson to describe them as the South American equivalent of *Peromyscus* (1958:391). Group membership has varied considerably in different treatments, including the more recent studies by Pearson and Patton (1976), Olds and Anderson (1989), Braun (1993), and Steppan (1993, 1995, 1998).

The primary objective of this study is to assess the monophyly of *Phyllotis*, its placement within Phyllotini, and to provide a phylogenetic hypothesis for the genus. These objectives are best achieved by thorough taxonomic sampling within putative members as well as among phyllotines in general, so that monophyly can be tested exhaustively. We have therefore examined all available phyllotine species for which sequences of cytochrome *b* (cyt *b*) have been reported and combined those with 61 new cyt *b* and 85 new recombination activating gene 1 (RAG1) sequences we generated. Relationships within the *P. darwini* species group are very complex, and a detailed

phylogeographic study is being conducted separately (Albright et al., in prep). We include here selected exemplars of the major clades in the *darwini* species group.

The secondary objective is to provide phylogenetic hypotheses for 2 newly discovered species from Peru and Argentina. Remarkably, after the 2 revisions by Pearson (1958) and Hershkovitz (1962), only 1 other new species has been described (*P. bonariensis*; Crespo, 1964), until now. The complete descriptions of these species will be published elsewhere (Pacheco et al., unpubl. ms.; Jayat et al., this volume).

Morphological studies have largely agreed on the composition of Phyllotini; *Andalgalomys, Andinomys, Auliscomys, Calomys, Chinchillula, Eligmodontia, Euneomys, Galenomys, Graomys, Irenomys, Loxodontomys, Neotomys,* and *Phyllotis*. Two more recently described genera, *Salinomys* (Braun and Mares, 1995) and *Tapecomys* (Anderson and Yates, 2000), both appear to be well supported as members of this tribe. The inclusion of the Puna mouse (*Punomys*) in Phyllotini has been much more unsettled, with Olds and Anderson (1989) including it and Steppan (1995) excluding it. Nearly all have also included *Reithrodon*, although Vorontsov (1959) created the Reithrodontini for this genus. Inclusion of *Reithrodon* was strengthened when Olds and Anderson (1989) proposed the *Reithrodon* group for *Reithrodon, Euneomys*, and *Neotomys*, all with sigmoidal or nearly sigmoidal molars. Steppan (1995) concurred and added some supporting characters, such that the *Reithrodon* group was possibly the best supported clade within the phyllotines. New fossil finds appeared to provide additional support to the *Reithrodon* group, presenting possible transitional forms between an *Auliscomys*/*Loxodontomys*-like ancestor and the extant members of the group (Steppan and Pardiñas, 1998; Ortiz et al., 2000). However, molecular data have strongly refuted several of these hypotheses. *Reithrodon* was found to be unrelated to phyllotines, arising from an unresolved basal radiation of sigmodontines, based on mitochondrial (cyt *b* -- Smith and Patton 1999; D'Elia, 2003) and nuclear genes (IRBP -- D'Elía, 2003; Weksler, 2003; RAG1, GHR, c-myc -- Steppan et al., 2004a). *Euneomys* has been found to be neither a phyllotine nor member of a *Reithrodon* group (IRBP -- D'Elia 2003; RAG1, GHR, c-myc -- Steppan et al., unpubl. data). No molecular data are yet available for *Neotomys*, but given the results regarding the other 2 genera, it likely is not a phyllotine either. Furthermore, *Andinomys* (Smith and Patton, 1999; Steppan et al., 2004a) and *Irenomys* (Smith and Patton, 1999; D'Elía, 2003; Steppan et al., 2004a) appear to be additional "unique lineages" of sigmodontines, not closely related to other tribes. Therefore, all of these genera are removed from Phyllotini. There is molecular support for the inclusion of most of the remaining genera, and among these 11 (*Auliscomys, Andalgalomys, Calomys, Chinchillula, Eligmodontia, Galenomys, Graomys, Loxodontomys, Phyllotis, Salinomys, Tapecomys*) those with published sequences or available tissues are included here. For this study, we lack data only for *Galenomys* and *Chinchillula*.

Morphological data (predominantly dental and cranial) have not resulted in any strongly supported hypotheses among genera within Phyllotini (Steppan and Sullivan, 2000), and few even within genera. Steppan (1995) analyzed 98 morphological characters for all recognized genera and found only 2 intergeneric clades with greater than 80% bootstrap support; the *Reithrodon* group and *Graomys* plus *Andalgalomys*. Both of these clades have subsequently been refuted by molecular data (e.g., Smith and Patton, 1999; Anderson and Yates, 2000), although not thoroughly tested. A subsequent reanalysis of Steppan's (1995) morphological data with faster computers, more thorough search strategies, and some additional taxa resulted in lower bootstrap scores for these clades (Steppan and Sullivan, 2000) and in fact, most intergeneric clades had less than 10%

bootstrap support and only the *Reithrodon* group displayed greater than 30% bootstrap support. The morphological study by Braun (1993) also failed to demonstrate any strongly supported clades. To date, morphological data have not been very informative about phyllotine phylogeny, other than to suggest that internal branches might be short.

Similarly, morphological data have been equivocal regarding monophyly of *Phyllotis*. Braun (1993) found that *Phyllotis* was not monophyletic and she resurrected *Paralomys* for the grouping of *amicus* and *gerbillus,* although her *Paralomys* was also paraphyletic. Steppan (1995) found these species to form a clade in the bootstrap consensus tree, although notably not in any of the most parsimonious trees. *P. wolffsohni* appeared to be well outside *Phyllotis*, but again, intervening nodes were not well supported. Therefore, morphological data suggested that *Phyllotis* as then construed likely was paraphyletic and that *wolffsohni, amicus, gerbillus*, and *andium* might all need to be removed (Braun 1993; Steppan 1995). Within *Phyllotis*, only one moderately well-supported clade appeared, that of the *darwini* species group: *P. darwini*, *P. xanthopygus*, *P. caprinus*, and *P. limatus* (Steppan and Sullivan, 2000). *P. magister* and *P. definitus* might be sister to that group but support was again weak (*P. bonariensis* was not examined).

Molecular Studies

No published molecular studies have expressly targeted phyllotines although several have included a variety of phyllotines (e.g., Salazar-Bravo et al., 2001). DNA hybridization indicated that *Phyllotis* was more closely related to *Loxodontomys* than either were to *Calomys, Graomys*, or *Eligmodontia* (Dickerman, 1992). Several studies agree that *Calomys* is the sister group to all other phyllotines, including mitochondrial (Engel et al., 1998; Smith and Patton, 1999) and nuclear data (Steppan et al., 2004a). Anderson and Yates (2000) showed that *Andalgalomys* was not closely related to *Graomys*, contra Steppan (1993; 1995) and Braun (1993).

Within *Phyllotis*, Steppan (1998) found that cyt *b* was equivocal regarding the inclusion of *P. wolffsohni*. *Phyllotis andium* and *P. amicus* appeared to be sister taxa and within the genus, although outgroup sampling was too limited for a definitive test of the validity of *Paralomys* sensu Braun (1993). A *darwini* species group was supported, including *P. darwini* and *P. magister* as sister species and a genetically diverse *P. xanthopygus* that was paraphyletic with respect to *P. limatus*. More detailed sampling confirmed that *P. limatus* was a very recent offshoot from *P. xanthopygus* (Kuch et al., 2002).

Several hypotheses can be drawn from these morphological and the limited cyt *b* data: 1) *P. amicus* and *P. gerbillus* are sister species and are not members of a strict *Phyllotis* clade, 2) *Phyllotis* includes *amicus, andium,* and *gerbillus*, but not *wolffsohni*, 3) *Phyllotis* does not include *wolffsohni*, *amicus*, or *gerbillus*, 4) *Phyllotis* does not include those 3 plus *andium*, 5) the *darwini* species group comprises *P. darwini, P. caprinus, P. xanthopygus*, and *P. limatus*, to the exclusion of *P. magister* and *P. definitus* (except in a more inclusive clade), 6) *Graomys* and *Andalgalomys* form a clade.

In 1958, Pearson remarked that he preferred to focus on the taxonomic questions of species, subspecies, and their delimitation rather than more inclusive genus-level questions because it is there that "one deals with more tangible, less subjective evidence" (Pearson, 1958, p. 385). With the advent of cladistic methods and DNA sequence data on the one hand and the proliferation of species concepts (and a growing recognition

of the amorphous nature of species) it seems that the situation is now reversed. Thus the subject of this paper is the more tangible delimitation of phylogenetic relationships among species and a reassessment of generic-level systematics of the genus *Phyllotis* and its phyllotine relatives.

Objectives

We assess the systematics of *Phyllotis* and its place within the phyllotines by analyzing new sequences for mitochondrial cyt *b* and nuclear RAG1. Using genes from both genomes, we achieve a less biased estimate of the phylogeny than might be achieved using mitochondrial data alone — mitochondria reflect maternal lines of inheritance only. We combine our new data with previously published cyt *b* sequences available from GenBank.

METHODS

Specimens Sequenced

We sequenced 101 specimens for cytochrome *b*, including 65 specimens representing 13 species (one of them undescribed) in the genus *Phyllotis*. In addition, 2 sequences of another undescribed species were contributed by Jayat et al. (this volume). Eighteen phyllotine species were added to clarify relationships within the tribe Phyllotini, including 5 species of the designated outgroup, *Calomys*. Locality information and GenBank accession numbers are listed in Appendix 1. We downloaded all available cyt *b* sequences for phyllotines (Steppan, 1998; Smith and Patton, 1999; Anderson and Yates, 2000; Salazar-Bravo et al., 2001; Catanesi et al., 2002; Kuch et al., 2002), but excluded some from this study in an effort to reduce computational time: only a partial survey of *Calomys* sequences were included because it would replicate analyses of Salazar-Bravo et al. (2001); there was little variation among *Graomys centralis* and *G. griseoflavus* from Catanesi et al. (2002); samples of *Auliscomys boliviensis* (AF387810) and *Andalgalomys olrogi* (AY070231) were identical and not closely related to congeners in either genus and thus their identity is questionable.

Sixty-nine specimens were sequenced for nuclear RAG1, representing 10 recognized species of *Phyllotis* and 2 undescribed species. Nine other phyllotine species, including 3 species of *Calomys*, were added in hopes of clarifying the relationships within the tribe Phyllotini.

DNA Extraction, Amplification, and Sequencing

Complete genomic DNA was extracted from frozen or EtOH-preserved tissue according to standard phenol/chloroform extraction techniques (Sambrook et al., 1989). We amplified the entire cytochrome *b* (cyt *b*) gene using polymerase chain reaction (PCR) with primers P484 and P485 (Steppan et al., 1999) or MVZ05 and MVZ14 (Smith and Patton 1999). This resulted in 1185 nucleotides, including 1144 in cyt *b* and 41 in the 3′ flanking tRNA. *Phyllotis osgoodi* was amplified in a small fragment from an extraction derived from a dried skin using primers L14925 and H15052 (Kuch et al., 2002), yielding a 127 bp fragment. DNA amplification was performed at 50µl

reaction volumes containing 1 ng/µl whole genomic extract, 5 µl of 10X buffer, 3 µM $MgCl_2$, 2.5 µM of DMSO, 0.3 mM of dNTP, 2.5 µM of each primer and 0.03 U/µl of Amplitaq Gold (Perkin-Elmer) in buffer supplied by the manufacturer. Typical cycling conditions were initial denaturation at 94°C for 15 minutes followed by 40-45 cycles of denaturation (45s at 94°C), primer annealing (45s at 51°C), DNA extension (1 min 15s at 72°C), and a final extension for 6 min at 72°C. Five µl of amplification product for each sample were electrophoresed on a 1% TBE agarose gel, stained by ethidium bromide, and visualized with UV light. The products were purified by precipitation with polyethylene glycol (PEG) in saline (NaCl) and re-suspended in 25µL deionized water. DNA concentration was measured by UV absorption.

Sequencing was performed on double-stranded templates with ABI Big-dye terminator sequencing chemistry on ABI (Perkin Elmer) 373A and 3100 automated sequencers. All individuals were sequenced completely in both directions for the entire cyt *b* gene with the amplification primers and the following sequencing primers: B12p (Steppan, 1998), and P1185 (Steppan et al., 1999). Sequences were aligned with Sequencher 4.1 (Genecodes). There were no insertions or deletions (indels) allowing unambiguous alignment by eye.

We amplified a 1,000 or 1,300 base pair (bp) segment in the 5′ region of RAG1 using PCR techniques using 2 primer pairs S70/S105 (Steppan et al., 2004b) and S116/142 (AGATGTTTAGGGTGMGATCC and GAGGAAGGTRTTGACACGGATG, respectively). The smaller fragment falls entirely within and the larger fragment falls primarily within the more variable divergent domain (Fugmann et al., 2000; Steppan et al., 2004b). This divergent domain constitutes approximately the first 1,000 bp of these fragments, comprising the 5′ end. DNA amplification was performed at 50µl reaction volumes containing 2ng/µl whole genomic extract, 4mM $MgCl_2$, 2.5µM of DMSO, 0.3mM of dNTP, 2.5µM of each primer and 0.03 U/µl of Amplitaq Gold (Perkin-Elmer) in 1X Gold buffer supplied by the manufacturer. Typical cycling conditions were initial denaturation at 94°C for 12 min followed by 40 cycles of denaturation (45s at 94°C), primer annealing (45s at 52°C), DNA extension (1 min 30s at 72°C), and a final extension for 6 min at 72°C. Purification and sequencing for RAG1 were as for cyt *b* with one exception. When multiple polymorphic sites were detected in a sample (as evidenced on chromatograms by superimposed pairs of peaks, typically half-height, and confirmed by sequencing in both directions), PCR products were cloned using the pCR 2.1-TOPO vector. Three to 8 clones for each sample were sequenced with clone primer M13F. Alternative haplotypes were then determined. For sequences with only 1 heterozygous site, haplotypes were designated as the 2 alternative resolutions. Direct sequencing of PCR products used the amplification primers and internal primers S102 and S106 (Steppan et al., 2004b). Alignment of RAG1 was done by eye with reference to the protein sequence because RAG1 has no introns. One indel, consisting of a single amino acid, was detected.

Phylogenetic Analyses

Phylogenetic analyses were conducted on nucleotide sequences with equally weighted maximum-parsimony (MP), maximum likelihood (ML), and Bayesian methods. *Calomys* was designated as the outgroup based on prior mitochondrial (Engel et al., 1998; Smith and Patton, 1999), nuclear DNA (Steppan et al., 2004a), and morphological studies (Braun, 1993; Steppan, 1995). Individuals with identical haplotypes were

consolidated into single OTUs for phylogenetic analyses. Maximum-parsimony and ML methods were conducted using the program PAUP* (Swofford, 2002). All MP analyses used heuristic searches with tree bisection-reconnection (TBR) branch swapping and 20 random-addition replicates. A sequential optimization approach (Swofford et al., 1996; Fratti et al., 1997) was used to estimate the cyt *b* ML phylogeny. Initial trees were generated by MP. The ML parameter values were estimated under a nested array of substitution models for the MP tree (Swofford et al., 1996; Fratti et al., 1997) as implemented in ModelTest 3.04 (Posada and Crandall, 1998). When reconstructing a phylogenetic tree, adding new parameters is not always justified and can lead to erroneous results (Nei and Kumar, 2000). For this reason it is important to choose the model for which each parameter contributes significantly to explain the data. Fourteen different nucleotide substitution models were evaluated for among-site variation under equal rates, a portion of the sites assumed to be invariable (I), rates among all sites assumed to vary according to a gamma distribution (Γ; Yang, 1994), and a combination of invariable sites and gamma-distributed rates. Because each of the models can be considered a special case of the most general, GTR + I + Γ, likelihood-ratio tests (LRT) can be used to test for significant differences in the contributions of each model parameter to explaining the data (Yang et al., 1995). Both the LRT and the Akaike Information Criterion (an alternative model selection method) selected the same model. The GTR + I + Γ was a significantly better fit for cyt *b* than simpler models. We then conducted a ML search using the preferred model with parameters fixed at the values estimated on the MP tree. Heuristic searches were conducted with 20 random-addition replicates and TBR branch swapping. Model parameters were re-estimated from the initial ML tree, and the process repeated until the topology of the re-estimated tree matched that of the tree from the previous search, never requiring more than one iteration.

Robustness of the results for both MP and ML was estimated by means of 200 bootstrap replicate searches (Felsenstein, 1985). MP bootstrap was limited to 400 maxtrees. Due to computational demands, the ML bootstrap analysis was done on a 40-processor cluster of computers using Condor job management, stepwise addition with 5 random addition sequence for each replicate, TBR branch-swapping, and each addition sequence replicates restricted to a 3,000 rearrangement limit.

Bayesian analyses were conducted with MrBayes 3.01 (Huelsenbeck and Ronquist, 2003). Bayesian analysis used the GTR + I + Γ model as in the ML analyses. We conducted a Metropolis-Coupled Markov chain Monte Carlo (MCMC) analysis with 4 chains, with a heating parameter of 0.2, for 7,000,000 generations, sampling trees every 100 generations. The likelihood converged on a stable value by 50,000 generations and clade frequencies, examined in successive 200,000 generation bins, stabilized by 1 million generations. We excluded the first 2 million generations from the sampling (as the "burn-in" period).

For RAG1, we followed the procedures for cyt *b* with the following differences. The HKY85 + I model (unequal base-frequencies, different transition and transversion rates) was a significantly better fit for RAG1 than simpler models, and more complicated models would result in over-parameterization of the data. Bootstrapping with maximum likelihood used 3 random addition sequence replicates and a 2,000 rearrangement limit. Bayesian analysis used the HKY85 + I model as in the ML analyses. We ran 4 MCMC chains, with a heating parameter of 0.2, for 10,000,000 generations, partitioning the data by codon position (i.e., each codon position had parameters

estimated separately), sampling trees every 300 generations. The likelihood converged on a stable value by 50,000 generations, and we excluded the first 2 million generations from the sampling (as the "burn-in" period).

The mitochondrial and nuclear data sets were analyzed separately and not combined because many individuals were heterozygous for RAG1. Given some evidence of possible introgression or incomplete lineage sorting (see Results), cyt *b* and RAG1 must be viewed as independently assorting loci that would be expected to have different evolutionary histories. The assumption of phylogenetic methods that operational taxonomic units (OTUs, in this case individuals) are monophyletic would be violated for RAG1 as would be the assumption that characters coded for OTUs share identical genealogies (i.e., OTUs not hybrids), making it invalid to concatenate the data from the 2 genes.

RESULTS

Phyllotine Phylogenetics

As expected, the mitochondrial data were much more variable than the nuclear. For cyt *b*, 478 of 1185 characters were parsimony informative whereas only 68 out of 1334 were so for RAG1. After the divergence of *Calomys*, the basal split among the phyllotines sampled here, based on cyt *b* sequences, are the clade of *Graomys* and *Eligmodontia* on one branch and remaining phyllotines on the other. Support for these 2 clades was weak, with only 31/35% ML and 42/41% MP bootstrap values on the respective branches (Figs. 2 and 3). Bayesian analysis gave a slightly different picture, providing little support for the *Graomys*/*Eligmodontia* clade (the group was most likely paraphyletic because *Eligmodontia* is the sister-group to the remaining phyllotines with 0.55 posterior probability (pp)), but somewhat surprisingly, monophyly of the second clade was strongly supported (pp = 1.00). In either case, cyt *b* data did not support a close relationship between *Graomys* and *Andalgalomys*. The RAG1 data were equivocal regarding the latter point, yielding a polytomy for this region of the tree (Fig. 4) but supporting a *Graomys*/*Andalgalomys* clade in 55% of MP bootstrap replicates. We lacked RAG1 data for several key taxa, including *Eligmodontia* and *Salinomys*, and their inclusion could easily break up the *Graomys*/*Andalgalomys* clade. RAG1 data provided support for a *Phyllotis* group that included *Auliscomys, Loxodontomys*, and *"P." wolffsohni* (see comment below) with 75% ML bootstrap. Bootstrap values for RAG1 were higher than for cyt *b* in the deeper regions of the tree, while much lower near the tips.

The cyt *b* data weakly supported a monophyletic *Auliscomys*, with ML and Bayesian trees showing *A. pictus* and *A. sublimis* as sister taxa (Fig. 2; 61%, 0.95 pp) whereas MP separated them (Fig. 3). Although the optimal cyt *b* trees place *Loxodontomys* close to *Auliscomys* (or just to *A. pictus*), in agreement with traditional arrangements, that support was very weak and the clade was not present in the RAG1 tree (Fig. 4). The same lack of support for intergeneric relationships was seen throughout the basal portions of the phyllotine tree. The one well-supported clade was that consisting of *Tapecomys* and *"P." wolffsohni*. Based on cyt *b* (*Tapecomys primus* was not sequenced for RAG1) this clade received 100% bootstrap and posterior probabilities in all analyses. The placement of the *Tapecomys*/*P. wolffsohni* clade was relatively unstable as well, being associated either with *Auliscomys*/*Loxodontomys* (cyt *b* ML, Fig. 2), *Andalgalomys*/

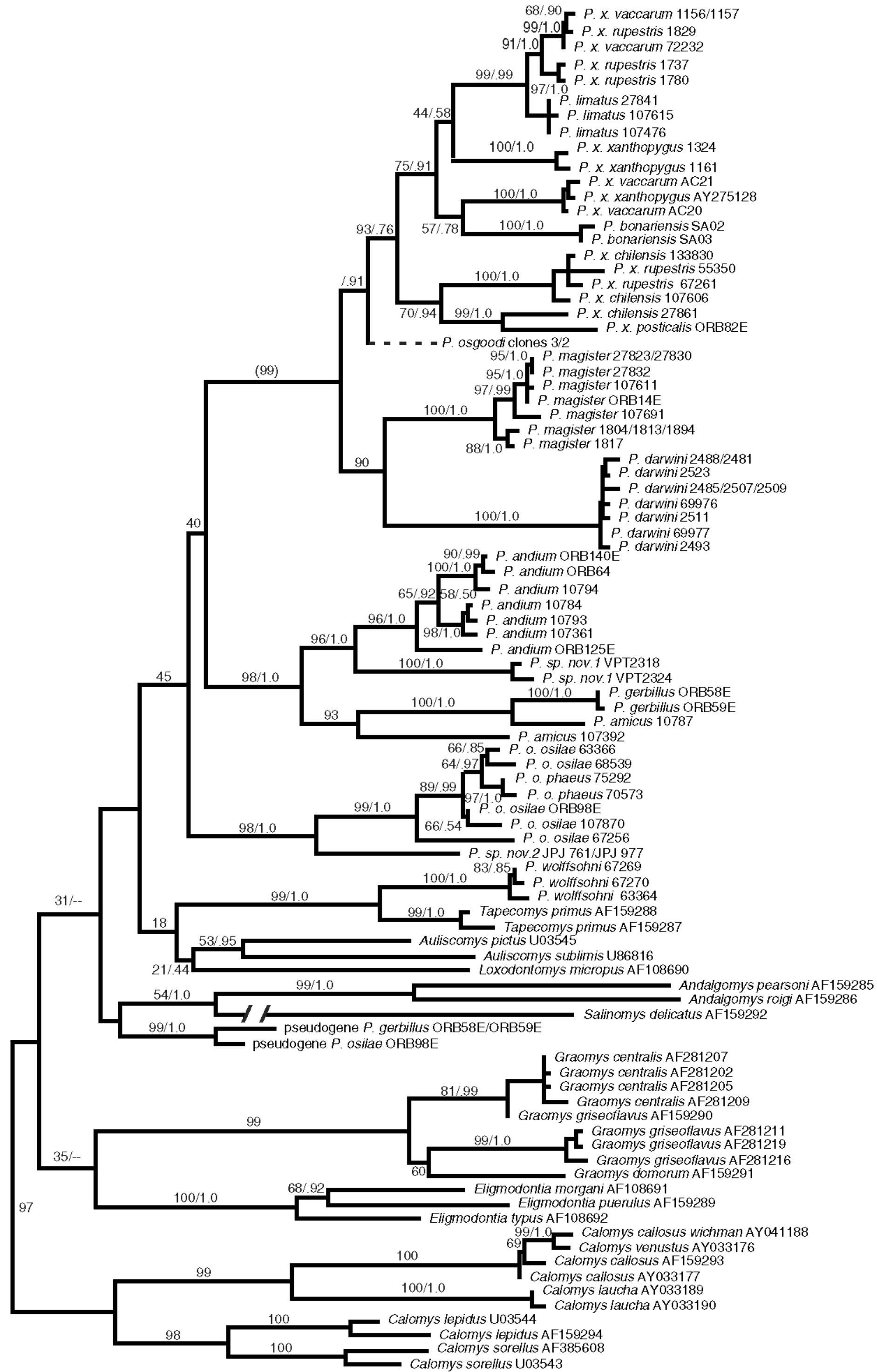
68/.90
P. x. vaccarum 1156/1157
99/1.0
P. x. rupestris 1829
91/1.0
P. x. vaccarum 72232
P. x. rupestris 1737
99/.99
P. x. rupestris 1780
97/1.0
P. limatus 27841
44/.58
P. limatus 107615
P. limatus 107476
100/1.0
P. x. xanthopygus 1324
75/.91
P. x. xanthopygus 1161
P. x. vaccarum AC21
100/1.0
P. x. xanthopygus AY275128
P. x. vaccarum AC20
93/.76
57/.78
100/1.0
P. bonariensis SA02
P. bonariensis SA03
P. x. chilensis 133830
100/1.0
P. x. rupestris 55350
P. x. rupestris 67261
/.91
P. x. chilensis 107606
70/.94
99/1.0
P. x. chilensis 27861
P. x. posticalis ORB82E
P. osgoodi clones 3/2
95/1.0
P. magister 27823/27830
95/1.0
P. magister 27832
(99)
97/.99
P. magister 107611
P. magister ORB14E
100/1.0
P. magister 107691
P. magister 1804/1813/1894
88/1.0
P. magister 1817
P. darwini 2488/2481
90
P. darwini 2523
P. darwini 2485/2507/2509
P. darwini 69976
P. darwini 2511
100/1.0
P. darwini 69977
40
P. darwini 2493
90/.99
P. andium ORB140E
100/1.0
P. andium ORB64
P. andium 10794
65/.92
58/.50
P. andium 10784
96/1.0
P. andium 10793
98/1.0
P. andium 107361
96/1.0
P. andium ORB125E
100/1.0
P. sp. nov.1 VPT2318
45
98/1.0
P. sp. nov.1 VPT2324
100/1.0
P. gerbilius ORB58E
100/1.0
P. gerbilius ORB59E
93
P. amicus 10787
P. amicus 107392
66/.85
P. o. osilae 63366
64/.97
P. o. osilae 68539
P. o. phaeus 75292
89/.99
P. o. phaeus 70573
99/1.0
97/1.0
P. o. osilae ORB98E
98/1.0
66/.54
P. o. osilae 107870
P. o. osilae 67256
P. sp. nov.2 JPJ 761/JPJ 977
83/.85
P. wolffsohni 67269
100/1.0
P. wolffsohni 67270
31/--
99/1.0
P. wolffsohni 63364
99/1.0
Tapecomys primus AF159288
18
Tapecomys primus AF159287
53/.95
Auliscomys pictus U03545
Auliscomys sublimis U86816
21/.44
Loxodontomys micropus AF108690
99/1.0
Andalgomys pearsoni AF159285
54/1.0
Andalgomys roigi AF159286
Salinomys delicatus AF159292
99/1.0
pseudogene P. gerbilius ORB58E/ORB59E
pseudogene P. osilae ORB98E
Graomys centralis AF281207
Graomys centralis AF281202
Graomys centralis AF281205
81/.99
Graomys centralis AF281209
99
Graomys griseoflavus AF159290
Graomys griseoflavus AF281211
99/1.0
Graomys griseoflavus AF281219
35/--
Graomys griseoflavus AF281216
60
Graomys domorum AF159291
68/.92
Eligmodontia morgani AF108691
100/1.0
Eligmodontia puerulus AF159289
97
Eligmodontia typus AF108692
99/1.0
Calomys callosus wichman AY041188
69
Calomys venustus AY033176
100
Calomys callosus AF159293
99
Calomys callosus AY033177
100/1.0
Calomys laucha AY033189
Calomys laucha AY033190
100
Calomys lepidus U03544
98
Calomys lepidus AF159294
100
Calomys sorellus AF385608
Calomys sorellus U03543
0.01 substitutions/site

Figure 2 (facing page). Maximum likelihood phylogram of cyt b sequences using the GTR+Γ+I model of evolution. *Calomys* is designated the outgroup. The branch leading to *Salinomys* has been shortened to precisely 1/2 the actual length for visual clarity. Numbers above branches are bootstrap percentages and Bayesian posterior probabilities. The bootstrap percentage in parentheses was estimated from analyses excluding the short *P. osgoodi* sequence; the provisional nature of the placement of that sequence is indicated by the dashed branch leading to *P. osgoodi*. Taxon names are followed by specimen or accession number. Numbers beginning in "AF," "AY," or "U" are GenBank accessions. Some bootstrap values less than 50% or posterior probabilities less than 0.50 are deleted.

Salinomys (cyt *b* MP, Fig. 3), or near the base of Phyllotini (as represented by *wolffsohni*, RAG1, Fig. 4). After exclusion of *wolffsohni*, the remaining species of *Phyllotis* formed a moderately supported clade (42% ML and 36% MP bootstrap but 1.00 pp).

We detected a presumptive nuclear pseudogene copy (numt) of a portion of cyt *b* in *P. osilae* and both individuals of *P. gerbillus* as evidenced by the presence of internal stop codons, a frame shift deletion, high proportion of non-synonymous substitutions, and very short branches on the ML tree. The placement of this pseudogene clade at the base of the "*Phyllotis* group" and the ratio of terminal to internal branch lengths indicate that transfer of a copy to the nucleus occurred early in phyllotine history. Therefore, other species in that generic group likely contain the pseudogene as well. The pseudogene was preferentially amplified in these 2 taxa when using the primer pair P484/P1185 but was never amplified using the cyt *b* flanking pair P484/P485.

Relationships within *Phyllotis*

Relationships within genera generally were better supported than those among genera. Within *Phyllotis* sensu stricto 3 major cyt *b* clades were evident: an *osilae* group that includes the undescribed species from Argentina (*Phyllotis* sp. nov. 2), an *andium/amicus* group that includes *P. gerbillus* and the undescribed species from Peru (*Phyllotis* sp. nov. 1), and the *darwini* species group. Monophyly of each of these 3 groups was well supported (67-93% MP bootstrap, 76-98% ML bootstrap, 0.91-1.00 pp). Support values for the *darwini* group were moderate when the short (127 bp) *P. osgoodi* sequence was included but high when it was excluded (e.g., MP bootstrap rises from 67% to 100%).

Within the *andium* group there appear to be 2 species pairs: *P. gerbillus* with *P. amicus* and *P. andium* with *Phyllotis* sp. nov. 1. Monophyly of both species pairs was moderately to well supported. *Phyllotis amicus* appears to be paraphyletic based on both cyt *b* and RAG1. Specimens of *P. amicus* from Lima, Peru (MUSN 10787, MUSN 10789) are much more closely related to *P. gerbillus* than to *P. amicus* (FMNH 107392). After re-examination, all voucher specimens appear to have been properly identified (*P. amicus (*FMNH 107392) from Arequipa, Peru was not re-examined for this study) and the 2 Lima *P. amicus* specimens clearly are not *P. gerbillus*, although specimen MUSN 10789 has relatively small incisors more similar to *P. gerbillus* than typical *amicus*. RAG1 optimal trees did not include a monophyletic *andium* group but all members of that group shared a single amino acid insertion that is the only insertion or deletion among phyllotines and was not seen in any of the other 140 muroid rodent species we have sequenced (Steppan et al., 2004a, unpublished data).

P. x. vaccarum 1156/1157
P. x. rupestris 1829
P. x. vaccarum 72232
P. x. rupestris 1737
P. x. rupestris 1780
P. limatus 107615
P. limatus 107476
P. limatus 27841
P. x. xanthopygus 1324
P. x. xanthopygus 1161
P. x. vaccarum AC20
P. x. xanthopygus AY275128
P. x. vaccarum AC21
P. bonariensis SA02
P. bonariensis SA03
P. x. rupestris 55350
P. x. rupestris 67261
P. x. chilensis 133830
P. x. chilensis 107606
P. x. chilensis 27861
P. x. posticalis ORB82E
P. magister 27823/27830
P. magister 27832
P. magister 107611
P. magister ORB14E
P. magister 107691
P. magister 1804/1813/1894
P. magister 1817
P. osgoodi clones 3/2
P. darwini 69977
P. darwini 2493
P. darwini 2511
P. darwini 2485/2507/2509
P. darwini 2523
P. darwini 69976
P. darwini 2488/2481
P. andium ORB140E
P. andium ORB64E
P. andium 10794
P. andium 10784
P. andium 10793
P. andium 107361
P. andium ORB125E
P. sp. nov. 1 VPT2318
P. sp. nov. 1 VPT2324
P. gerbillus ORB58E
P. gerbillus ORB59E
P. amicus 10787
P. amicus 107392
P. osilae 63366
P. osilae 68539
P. o. osilae ORB98E
P. o. osilae 107870
P. o. phaeus 75292
P. o. phaeus 70573
P. o. osilae 67256
P. sp. nov. 2 JPJ 761/JPJ 977
P. wolffsohni 67269
P. wolffsohni 67270
P. wolffsohni 63364
Tapecomys primus AF159288
Tapecomys primus AF159287
Andalgomys pearsoni AF159285
Andalgomys roigi AF159286
Salinomys delicatus AF159292
Loxodontomys micropus AF108690
Auliscomys pictus U03545
pseudogene P. gerbillus ORB58E/ORB59E
pseudogene P. o. osilae ORB98E
Auliscomys sublimis U86816
Graomys centralis AF281207
Graomys centralis AF281202
Graomys centralis AF281205
Graomys centralis AF281209
Graomys griseoflavus AF159290
Graomys griseoflavus AF281211
Graomys griseoflavus AF281219
Graomys griseoflavus AF281216
Graomys domorum AF159291
Eligmodontia morgani AF108691
Eligmodontia typus AF108692
Eligmodontia puerulus AF159289
Calomys callosus AF159293
Calomys callosus AY033177
Calomys callosus wichman AY041188
Calomys venustus AY033176
Calomys laucha AY033189
Calomys laucha AY033190
Calomys lepidus U03544
Calomys lepidus AF159294
Calomys sorellus AF385608
Calomys sorellus U03543
10 changes

Figure 3 (facing page). Maximum parsimony phylogram of cyt b sequences, one random tree of 970 equally parsimonious trees, each 3330 steps long. The only nodes to collapse in the strict consensus are within tip local-population level clades within species. Numbers above branches are bootstrap proportions. Numbers in parentheses were estimated from analyses excluding the short *P. osgoodi* sequence.

The *darwini* species group was well supported by cyt *b* (99% MP bootstrap, 99% ML bootstrap) but not directly by RAG1 (Fig. 4). Based on cyt *b*, it includes *P. darwini, P. magister, P. osgoodi,* the geographically widespread *P. xanthopygus*, and 2 peripheral species that are embedded in a *xanthopygus* complex, *P. limatus* and *P. bonariensis*. Parsimony and likelihood disagreed regarding the relationships among some of the species. Parsimony placed *P. magister* (plus *P. osgoodi*) as the sister group to the *xanthopygus* complex (Fig. 3), rather than as the sister species to *P. darwini*, as did ML (Fig. 2) and RAG1 (Fig. 4). The ML RAG1 tree indicated that 2 specimens of *P. darwini* (LCM 2493, 27558) possessed an allele that was otherwise characteristic of *P. magister*, suggesting introgression, but this result was not stable. MP did not resolve the relationship of this allele to either species (forming a trichotomy) and the allele in question was placed in a *P. darwini* clade in 52% of ML bootstrap replicates. *Phyllotis osgoodi*, for which we have only 127 bp of cyt *b* sequence, is closely related to either the *xanthopygus* complex (ML) or to *P. magister* (MP).

The *xanthopygus* complex is very diverse genetically, with up to 19% sequence divergence for cyt *b* among populations (GTR+Γ+I ML distances). *Phyllotis darwini* and *P. magister* exhibited very little diversity in contrast (1.4% and 3.35% maximum ML sequence divergence respectively), and *P. andium* and *P. osilae* exhibited intermediate genetic diversity (6.3% and 9.1% maximum ML sequence divergence respectively). Two groups were evident within the *xanthopygus* complex based on both genes -- a northern/altiplano group comprised of *P. xanthopygus posticalis, P. x. chilensis,* and some *P. x. rupestris*, and a widespread, lower elevation clade comprised of *P. limatus, P. bonariensis, P. x. xanthopygus, P. x. vacarrum,* and some *P. x. rupestris*.

DISCUSSION

Phyllotine Systematics

Data from cytochrome *b* appears to be insufficient to resolve many of the branching events within Phyllotini. The data from the nuclear gene RAG1 corroborate some of the cyt *b* findings but weakly conflict in other areas, suggesting that some of the internal branches are short enough that differential lineage sorting may have occurred, resulting in incongruence between gene trees and species trees. We thus interpret the results in these regions with greater caution than the support values would themselves suggest. The balance of evidence suggests that there is a *Phyllotis* group that contains *Auliscomys, Loxodontomys*, probably *Tapecomys*, and possibly the *Andalgalomys/Salinomys* clade. Previous molecular studies have clearly demonstrated that, contrary to morphological studies by Steppan and coworkers (Steppan, 1995; Steppan and Pardiñas, 1998; Ortiz et al., 2000), *Reithrodon* and *Euneomys* (and probably *Neotomys* as well) do not belong in this group (Engel et al., 1998; Smith and Patton, 1999; D'Elía, 2003; Weksler, 2003;

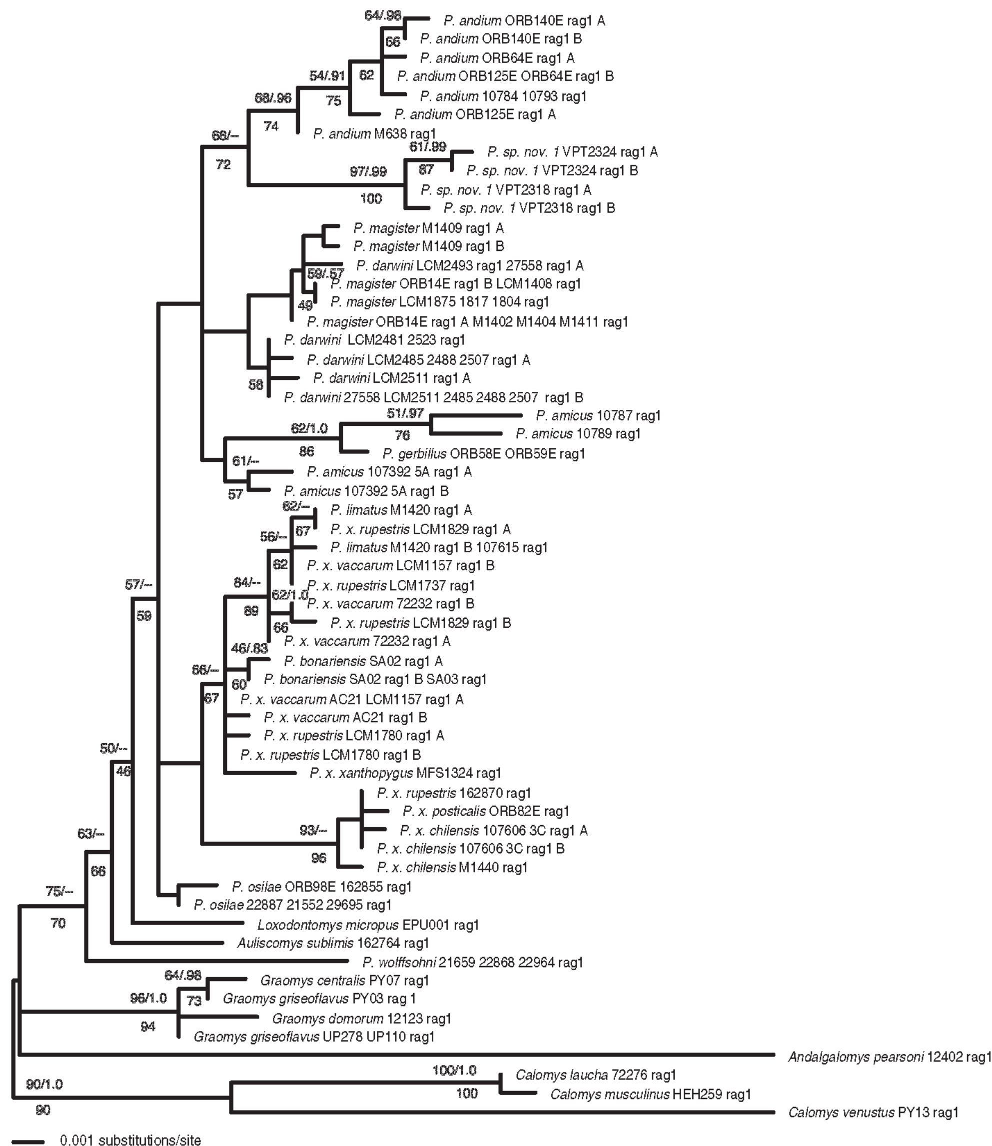

Figure 4. Maximum likelihood phylogram of RAG1 sequences, one of 4 trees with -L = 3055.77. Differences among the optimal trees are limited to rearrangements within *P. magister*. "A" and "B" refer to the 2 alleles when individuals are heterozygous. Numbers above branches are bootstrap proportions (>50%) and Bayesian posterior probabilities (>0.50). Numbers below branches are MP bootstrap proportions.

Steppan et al., 2004a). *Graomys* does not appear to be a member of the *Phyllotis* group and may be most closely related to *Eligmodontia*. It also does not appear to be most closely related to *Andalgalomys*, contrary to the morphological results of Braun (1993) and Steppan (1993; 1995; but cf. Steppan and Sullivan, 2000).

Perhaps the strongest conclusion from these results is that *wolffsohni* should be removed from *Phyllotis* and placed in *Tapecomys*. The monophyly of this clade is strongly supported in all analyses. Although we lack RAG1 data for *Tapecomys primus*, *wolffsohni* still falls well outside a *Phyllotis* clade. The association between these 2 species is not that surprising. They share several derived morphological features (e.g., ridged and posteriorly divergent interorbital region) but more significantly, they have nearly identical karyotypes that are distinct from all other phyllotines. Anderson and Yates (2000) noted this but attributed the similarity to convergence. Evidence that the 2 taxa are distinct species comes from their reciprocal monophyly, genetic distances similar to other species pairs, and the fact that they were clearly distinguishable in a morphological phylogeny (*Tapecomys primus* was listed as *species nova* in Steppan, 1993).

After removal of *wolffsohni*, *Phyllotis* appears monophyletic. There is no support for *Paralomys*, either consisting of *amicus* and *gerbillus* (Braun, 1993; Nowak, 1999) or *gerbillus* alone (Cabrera, 1961). Recognition of *Paralomys* would require removal of *andium* from *Phyllotis* as well as elevation of the *osilae* clade to generic status.

Systematics of *Phyllotis*

We included 2 undescribed species in this study, one of which is closely related to *P. andium* and the other closely related to *P. osilae*. Notably, *Phyllotis* sp. nov. 1 from Peru has a long branch for RAG1, and the 2 individuals are distinguished from *P. andium* by 6 apomorphic changes (4 unique synapomorphies for *Phyllotis.* sp. nov. 1 and 2 unique synapomorphies shared by all *P. andium*), providing evidence that this is not just a local population of *P. andium*. Even after the removal of *wolffsohni*, the number of species in *Phyllotis* increases to at least 15. There may be additional "cryptic" species in *Phyllotis*, that is, taxa recognized first by DNA and for which we have not yet identified phenotypic differences. The possible paraphyly of *P. amicus* suggests one case and the deep divergences within the paraphyletic *xanthopygus* complex suggest another. It is notable that the genetic differences between *P. gerbillus* and 2 of the *P. amicus* (which come from Lima within previous estimates of the known range) is less than that seen within most species, and the location of the other *P. amicus* (FMNH 107392) is in Arequipa Province, nearly 400 km SE of the previously known southern limits of *P. amicus* (Hershkovitz, 1962). This raises the possibility that FMNH 107392 is a new species and that the marked pelage and dental characters that make *P. gerbillus* the most derived species in the genus (and formed the basis for removal to its own genus *Paralomys*) have evolved only recently, perhaps within the last 1 MY. The issue involving the *xanthopygus* complex will be analyzed in more detail elsewhere (Albright et al., unpubl. ms.).

The systematics of *P. gerbillus* have been particularly uncertain. Our results suggest that this specialized coastal desert species is derived from the nearby and similar sized *P. amicus*. Together these 2 species are the sister group to another northern species pair, *P. andium* and *Phyllotis* sp. nov. 1.

Our data are unable to resolve the basal relationships within the *darwini* species group. Maximum likelihood for cyt *b* and RAG1 favor a close relationship between *P. darwini* and *P. magister*, both Pacific slope species from central Chile and northern Chile to central Peru, respectively (Fig. 1). Both of these species exhibit little genetic diversity, even though the geographic sampling is fairly extensive. For *P. magister*, we have

samples spanning nearly the entire latitudinal range, covering 800 km. The genetic diversity within *P. magister* or *P. darwini* is much less than that seen in *P. xanthopygus*.

Problematic Sequences

We identified several sequences on GenBank that seemed out of place phylogenetically or had unusual characteristics. The cyt *b* sequence for *Salinomys* was divergent and was estimated by likelihood to have a very long branch leading to it (Fig. 2). The branch leading to *Salinomys* appears to have 3-5 times the average amino acid substitution rate for phyllotines and is the only sequence included in this study to have an unusually long branch (unpubl. analysis). Given that this mouse lives around salt flats (Braun and Mares, 1995), a very unusual and physiologically demanding environment for rodents, it would be interesting to determine whether the unusual sequence characteristics were caused by adaptation or sequencing errors. We cannot exclude the alternative possibility that this sequence might be a numt (nuclear copy of a mitochondrial gene), although in that case we would expect a short terminal branch because of a reduced rate of third-position substitutions.

Our analyses also detected a probable taxonomic problem with *Graomys* that likely is due to changing taxonomy and the separation of *G. centralis* from *G. griseoflavus*. The Bolivian *G. griseoflavus* sequence from Anderson and Yates (2000) groups closely with Argentine specimens that Catanesi et al. (2002) elevated to *G. centralis* based on karyotype differences. Interestingly, the *G. domorum* of Anderson and Yates (2000) is more closely related to the *G. griseoflavus* of Catanesi et al. (2002) than to their own "*griseoflavus*," but diverges near the base of the genus. This suggests that the old concept of *G. griseoflavus* may have been composite. Unfortunately, Catanesi et al. (2002) did not make morphological comparisons of their 2 karyotype groups to museum specimens or the type specimens, and Anderson and Yates (2000) did not report their karyotypes, so the resolution of this question awaits further study. These data do support the presence of at least 3 species in *Graomys*.

Finally, re-sequencing the specimens from Steppan (1998) detected errors in several published sequences. Most of these were minor random errors that did not affect the topology, and updates have been submitted to GenBank. However, the published sequence for *P. osgoodi*, amplified from a dried skin fragment, was found to be a composite of 2 contaminating sequences; a *P. osilae* and a *P. limatus*. The hybrid nature of the sequence resulted in a most parsimonious placement of "*osgoodi*" in an intermediate basal position on the tree, not far from where the new cloned sequences suggest it might actually belong. However, our sequence is still very short and should be confirmed with fresh tissue samples. Although the unstable resolution of *A. sublimis* (which was not re-sequenced) suggests that it might contain sequencing errors, data from several nuclear genes and another individual indicate that the uncertainty from cyt *b* may correctly reflect the absence of many synapomorphies because of very short internodes (Steppan and Adkins, unpubl. data).

Biogeography and Dating

Phylogenetic relationships among genera are too uncertain to confidently propose biogeographic patterns. Within *Phyllotis*, the 3 primary clades are distributed on the eastern slopes of the Andes (*P. osilae* and *Phyllotis* sp. nov. 2), northwestern Peru

(*andium/gerbillus* clade), and the more widespread *darwini* group, absent only from the northern areas. It is unclear whether *P. darwini* and *P. magister* represent sequential Pacific-slope sister-groups to the *xanthopygus* complex. If they were sequential sister-groups, that would support a "bathtub" model of peripheral-isolate speciation in the clade. In that model, successive range expansions of the ancestral *xanthopygus* complex would result in sequential colonization of the Pacific-slope region followed by speciation and eventual reciprocal monophyly between the Pacific-slope isolate and the widespread ancestor. The bathtub metaphor suggests *P. xanthopygus* "spilling over" the confining Andes.

We have not employed a tree-wide molecular clock despite failing to reject a clock model for cyt *b* (after excluding *Salinomys*; $-L_{clock} = 14{,}261.2$, $-L_{unconstrained} = 14{,}185$; $p_{(d.f.\ 176)} \approx 0.1$) because we are not confident that fossil calibrations using sigmodontines can be placed accurately enough on the phylogeny to avoid overconfidence in and over-interpretation of the resulting dates. Salazar-Bravo et al. (2001) used an *Auliscomys/Loxodontomys* divergence of 4 Mya (based on the first fossil occurrence of *Auliscomys formosus*) and a global clock to derive a time scale of *Calomys* evolution. They estimated that *Calomys* radiated around 8 Mya, and diverged from other phyllotines at 10-12 Mya (i.e., *A. formosus* is 33-40% of the age of the phyllotines). These estimates are somewhat at odds with a nuclear gene-based estimate that would place the *Calomys*/phyllotine split between 4.0-5.5 (the range of molecular clock and relaxed-clock estimates ± 1 sd, based on a revised *Mus-Rattus* divergence at 9.5 Mya; Steppan et al., 2004a) and the earlier South American radiation of sigmodontines at 7.6 Mya. We caution that there do not appear to be definitive apomorphies that place *A. formosus* in a clade with extant *Auliscomys*, nor that would unequivocally place it in a more-inclusive clade along with *Loxodontomys*. Thus, the common ancestor of *Auliscomys* and *Loxodontomys* could have lived several My before or after *A. formosus*. We also note that the *Auliscomys/Loxodontomys* clade has relatively short branches, suggesting that lower evolutionary rates there may result in overestimating divergence dates when extrapolated elsewhere on the tree. The *Mus-Rattus* calibration used by Steppan et al. (2004a) is much better constrained, although possibly too distant phylogenetically to apply to cyt *b* data with confidence. Using a *Mus-Rattus*-based ML rate for cyt *b* of 7.3% divergence per My (Steppan et al., 2003), we approximate the following divergences: basal split in Phyllotini at 4.8-7.4 Mya (minimum-maximum point estimates), basal split in *Calomys* at 4.5 Mya (mean), *A. pictus/Loxodontomys* at 3.0 Mya, *Tapecomys primus* vs. *wolffsohni* at 1.5 Mya (mean), basal split in *Phyllotis* at 3.0-5.1 Mya (range), basal split in *P. xanthopygus* at 1.6-2.3 Mya (range). Thus a revised *Mus-Rattus* calibration yields dates in Phyllotini that are younger than estimated by Salazar-Bravo et al. (2001), and with a slightly different distribution (e.g., the *Auliscomys-Loxodontomys* split is 41-62% as old as the ancestral phyllotine rather than 33-40%). Each of these dates should be viewed as having considerable errors associated with them and a tree-based bayesian approach yielded older dates (unpubl. data). Clearly, better fossil calibrations are needed.

CONCLUSIONS

Our analyses based on both mitochondrial and nuclear genes have clarified some relationships among phyllotine genera and refined our concept of *Phyllotis*. Notably,

Graomys and *Eligmodontia* do not appear to be members of a clade that includes *Phyllotis* and *Auliscomys*, and *wolffsohni* should be removed from *Phyllotis* and placed in *Tapecomys*. The data do not support recognition of *Paralomys* because the type (*P. gerbillus*) is closely related to 3 other northwestern species (*andium, amicus*, sp. nov. 1) that together fall within *Phyllotis*. The *darwini* species-group is supported by cyt *b* data, and within that group, the widespread species *xanthopygus* is characterized by deep divergences, large genetic diversity, and paraphyly with respect to at least 2 morphological species, *limatus* and *bonariensis*. The data allow us to place 2 undescribed species fairly precisely. Finally, our application of different calibrations and methods suggests that phyllotine divergence dates estimated by Salazar-Bravo et al. (2001) may be too old.

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