Acta Theriol (2012) 57:277–287
DOI 10.1007/s13364-011-0070-9

SHORT COMMUNICATION

The phylogenetic relationships of the Andean swamp rat genus *Neotomys* (Rodentia, Cricetidae, Sigmodontinae) based on mitochondrial and nuclear markers

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Received: 15 June 2011 / Accepted: 18 December 2011 / Published online: 11 January 2012

Abstract The aim of this study was to assess the phylogenetic position of the South American cricetid genus *Neotomys* using two molecular markers: one nuclear (*Irbp*) and one mitochondrial (*mt-cyb*). This genus is currently considered as *incertae sedis* in the Sigmodontinae radiation. The phylogenetic relationships were estimated using three approaches: Bayesian inference, maximum likelihood and parsimony. We found the genus *Neotomys* closely related to the genera *Euneomys* and *Irenomys*, which are also considered *incertae sedis*. Our results suggest a common origin for this group of genera; this fact should be reflected in the taxonomy as a supra generic group with a tribal level. However, further and deeper analysis of both molecular and morphological data are needed to diagnose and formalize the proposed tribe. The relationships of this clade to the other members of Sigmodontinae were not clear as assessed by these data sets. The three genera are distributed around the Central and Southern Andes in South America evidencing that the Andes have played an important role in the diversification of several tribes of sigmodontine rodents.

Keywords *Neotomys* · Sigmodontinae · Phylogenetics · *Irbp* · Cytochrome *b*

Communicated by: Mabel D. Giménez

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The Andean swamp rat *Neotomys*, is a monotypic genus of cricetid rodent distributed along the Central Andean highlands (Barquez 1983; Musser and Carleton 2005) and currently considered as *incertae sedis* in the Sigmodontinae radiation (D'Elía 2006a). The Sigmodontinae genera have been typically grouped into supra generic entities early in their zoological classifications (Vorontsov 1959; Reig 1980; Steadman and Ray 1982; Voss 1988; Olds and Anderson 1989). These pioneer works were not based on quantitative phylogenetic approaches, but these authors developed one of the first tribal-level classifications among muroid rodent subfamily. Later, several authors applied phylogenetic approaches using both, morphological and molecular characters, to assess phylogenetic relationships either of the whole subfamily or within tribes (Braun 1993; Steppan 1993; 1995; Engel et al. 1998; Smith and Patton 1999; D'Elía 2003; D'Elía et al. 2003; Weksler 2003; D'Elía et al. 2006b; Weksler 2006). The results of these studies have confirmed the existence of several supra generic natural groups but also changed the limits and genera composition of many tribes. Additionally, some genera (i.e., *Juliomys*, *Punomys*, *Andinomys*, *Irenomys*, *Euneomys*) could not be placed into any tribe or monophyletic group less inclusive than Sigmodontinae and are provisionally considered as

incertae sedis (Smith and Patton 1999; D'Elía 2003; D'Elía et al. 2006a).

Neotomys ebriosus was first described by Thomas (1894) from specimens collected in Vitoc valley, Junín department, Peru. Later Thomas (1921) described *Neotomys vulturnus* from Sierra de Zenta, Jujuy province, Argentina. Afterwards, Sanborn (1947) reviewed the genus and considered *N. vulturnus* as a subspecies of *N. ebriosus*. The genus *Neotomys* was traditionally considered either as a Phyllotini in *sensu lato* or as a member of the *Reithrodon* group in *sensu stricto* (Olds and Anderson 1989). To date, phylogenetic analysis including the genus *Neotomys* have been conducted only based on morphological evidence. Steppan (1995) based on 98 characters, recovered a clade consisting of *Neotomys*, *Reithrodon*, and *Euneomys*, supporting the *Reithrodon* group. On the other hand, Braun (1993) recovered *Reithrodon* and *Neotomys* as sister genera based on 46 morphological characters, but *Euneomys* was not included in this clade. Later, the *Reithrodon* group was not recovered as monophyletic by D'Elía (2003) in his molecular analysis (using two genes: *Irbp* and *mt-cyb*) of the phylogenetic relationships of Sigmodontinae. He supports the view of Vorontsov (1959 cited in Reig 1980) in the sense that *Reithrodon* together with other fossil species conforms the tribe Reithrodontini (D'Elía 2003, see also Ortiz et al. 2000). Despite the absence of molecular data to assess the phylogenetic position of *Neotomys*, D'Elía et al. (2006a) considered *Neotomys* as *incertae sedis* based on the polyphyletic nature of the *Reithrodon* group and the fact that neither *Reithrodon* nor *Euneomys* are members of tribe the Phyllotini (D'Elía 2003). In this context, molecular data of *Neotomys* may be useful to resolve the phylogenetic relationships of these genera.

Here, we use evidence from two molecular markers to assess the phylogenetic position of the genus *Neotomys* in the Sigmodontinae radiation. We used one nuclear marker, the first exon of the gene encoding interphotoreceptor retinoid binding protein (*Irbp*) and one mitochondrial marker, the cytochrome *b* (*mt-cyb*) gene. Both markers have been widely employed in phylogenetic studies of sigmodontine rodents (Smith and Patton 1999; D'Elía 2003; D'Elía et al. 2003; Weksler 2003; D'Elía et al. 2006b; Weksler 2006).

The type locality of *N. ebriosus vulturnus*, was originally indicated by Thomas in Sierras de Zenta and later relocated by Díaz and Barquez (2007) in Sierras de Tilcara, Jujuy province. During a field trip to the type locality of *N. ebriosus vulturnus*, we collected two specimens in Sierras de Tilcara, 12 km ESE of Maimará, 14 km ESE of Tilcara (23°39.926 S 65°17.917 W), 4, 092 m. The specimens are now deposited at the Colección Mamíferos Lillo (CML), Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán, collection numbers CML 7679 and CML 7680 and original field numbers LT-RMB 77 and LT-RMB 56, respectively.

Total deoxyribonucleic acid (DNA) was extracted from the specimen CML 7680 following the protocol of salt extraction (Bruford et al. 1992), precipitated in absolute ethanol, and dried and stored in TE buffer (Tris-EDTA) pH 8. The *mt-cyb* gene was amplified using the primers Mus14095 and Mus15398 (Anderson and Yates 2000) and the cycling protocol described in Ferro and Martínez (2009). The first exon of *Irbp* gene was amplified using PCR beads (Qiagen, UK) following Weksler (2003). Double-stranded PCR products were purified and sequenced by Macrogen USA (http://www.macrogenusa.com) using BigDye Terminator in an ABI3730×l DNA automatic analyzer. A sequence of 1,138 bp of *mt-cyb* gene was obtained using the same amplification primers. For *Irbp* sequencing, we used two additional internal primers (F and E2; Weksler 2003) and obtained a sequence of 1,278 bp. Both sequences were deposited in GenBank under the following accession numbers: HM061604 and HM061605 for *mt-cyb* and for *Irbp*, respectively.

In order to assess the phylogenetic position of *Neotomys* in the Sigmodontinae radiation, we included in our taxonomic sampling sequences obtained from GenBank of all the available genera of sigmodontine tribes and the *incertae sedis* considered by D'Elía et al. (2007) (Table 1). The two matrices (1,143 bp for *mt-cyb* and 1,278 bp for *Irbp*) were aligned independently using the default parameters of Muscle program version 3.6 (Edgard 2004). The two data sets were then analyzed independently and together by means of three approaches: Bayesian inference, maximum likelihood, and maximum parsimony analysis. We performed a Bayesian inference of phylogenetic relationships using MrBayes 3.1 (Ronquist and Huelsenbeck 2003). The models that best fitted our data sets were selected using AIC-corrected (AICc) criterion implemented in ModelTest Server 1.0 (http://darwin.uvigo.es/software/modeltest_server.html) which uses Modeltest 3.8 (Posada and Crandall 1998). Instead, the TVG+Γ+I model, which was selected by Modeltest 3.8, we used the GTR+Γ+I model for both genes because the former model cannot be implemented in MrBayes 3.1, and we thereby proceeded to realize the next complex model. The Bayesian analyses were initiated with two random starting trees with four chains each one (one cold and three heated chains) and run for 15 million generations for *Irbp* and 20 million generations for *mt-cyb* and the combined analysis of both genes. The Markov chains were sampled every 1,000 generations. Of the resulting trees, the first 25% were discarded as burn-in, while the remaining trees were summarized in 50% majority rule consensus tree. Branch lengths were estimated using mean values of branch lengths of sampled trees after discarded the burn-in samples. In order to determine the number of

Table 1 Taxonomic sample employed for the phylogenetic analyses indicating the species included; the tribe according to D'Elía et al. (2007) and the GenBank accession numbers for each gene

Species	Sigmodontinae tribe	*mt-cyb* GenBank accession numbers	*Irbp* GenBank accession numbers
Cricetus cricetus	–	AJ973392	AY277410
Scotinomys teguina	–	EF990029	AY163639
Neotoma micropus	–	EF989953	EF989853
Peromyscus melanosis	–	EU574701	EF989891
Arvicola terrestres	–	AY332709	AY277407
Tylomys nudicaudus	–	DQ179812	AY163643
Euneomys chinchilloides	*Incertae sedis*	AY275115	AY277446
Andinomys edax	*Incertae sedis*	AF159284	–
Irenomys tarsalis	*Incertae sedis*	U03534	AY277450
Juliomys pictipes	*Incertae sedis*	EU157764	AY163588
Delomys sublineatus	*Incertae sedis*	AF108687	AY163582
Neotomys ebriosus	*Incertae sedis*	HM061604	HM061605
Calomys lepidus	Phyllotini	EU579473	AY163580
Graomys chacoensis	Phyllotini	EU579472	EU649037
Eligmodontia typus	Phyllotini	EU377643	AY277445
Phyllotis xanthopygus	Phyllotini	AY956739	AY277471
Andalgalomys pearsoni	Phyllotini	AF159285	EU649038
Auliscomys pictus	Phyllotini	U03545	AY277434
Salinomys delicatus	Phyllotini	EU377608	–
Tapecomys primus	Phyllotini	AF159288	–
Apeomys lugens	Thomasomyini	–	DQ003722
Rhipidomys nitela	Thomasomyini	EU579475	AY163636
Thomasomys baeops	Thomasomyini	DQ914654	AY163642
Chilomy instans	Thomasomyini	AF108679	–
Rhagomys rufescens	Thomasomyini	AY206770	DQ003723
Bibimys labiosus	Akodontini	DQ444329	AY277436
Oxymycterus nasutus	Akodontini	EF661854	AY277468
Blarinomys breviceps	Akodontini	AF108668	AY277437
Kunsia tomentosus	Akodontini	AF108670	AY277455
Akodon azarae	Akodontini	DQ444328	AY163578
Deltamys kempi	Akodontini	AY195862	AY277444
Juscelinomys huanchacae	Akodontini	AY275119	AY277453
Lenoxus apicale	Akodontini	U03541	AY277456
Necromys urichi	Akodontini	AY273919	AY277463
Scapteromys tumidus	Akodontini	AY445570	AY277477
Thalpomys cerradensis	Akodontini	AY273916	AY277481
Thaptomys nigrita	Akodontini	AF108666	AY277482
Reithrodon auritus	Reithrodontini	EU579474	AY163634
Abrothrix andinus	Abrotrichini	AF108671	AY277418
Geoxus valdivianus	Abrotrichini	U03531	AY277448
Chelemys macronyx	Abrotrichini	U03533	AY277441
Notiomys edwarsi	Abrotrichini	EU416275	AY163602
Pearsonomys annectens	Abrotrichini	AF108672	AY851749
Wiedomys pyrrhorhinos	Wiedomyini	EU579477	AY163644
Sigmodon leucotis	Sigmodontini	EU652909	EU635712
Rheomys raptor	Ichthyomyini	–	AY163635
Neusticomys monticolus	Ichthyomyini	–	EU649036

Table 1 (continued)

Species	Sigmodontinae tribe	*mt-cyb* GenBank accession numbers	*Irbp* GenBank accession numbers
Oligoryzomys nigripes	Oryzomyini	DQ826004	AY163612
Euryoryzomys russatus	Oryzomyini	EU579486	AY163625
Nectomys squamipes	Oryzomyini	AF181283	EU273419
Scolomys ucayalensis	Oryzomyini	EU579518	AY163638
Aegialomys xanthaeolus	Oryzomyini	EU074632	EU273420
Amphinectomys savamis	Oryzomyini	EU579480	AY163579
Cerradomys scotti	Oryzomyini	EU579482	EU649040
Ereoryzomys polius	Oryzomyini	EU579483	AY163624
Handleyomys rostratus	Oryzomyini	EU579491	EU649045
Holochilus sciureus	Oryzomyini	EU579497	EU649049
Hylaeamys laticeps	Oryzomyini	EU579498	EU649050
Lundomys molitor	Oryzomyini	EU579501	AY163589
Melanomys chrysomelas	Oryzomyini	EU340018	EU649053
Microryzomys minutus	Oryzomyini	EU258535	AY163592
Neacomys spinosus	Oryzomyini	EU579504	AY163597
Nephelomys albigularis	Oryzomyini	DQ224407	AY163614
Nesoryzomys fernandinae	Oryzomyini	EU579506	EU649058
Oecomys concolor	Oryzomyini	EU579508	AY163606
Oreoryzomys balneator	Oryzomyini	EU258534	EU649068
Oryzomys couesi	Oryzomyini	EU074662	EU273425
Pseudoryzomys simplex	Oryzomyini	EU579516	EU649070
Sigmodontomys alfari	Oryzomyini	EU340016	EU649071
Sooretamys angouya	Oryzomyini	EU579512	EU649072
Transandinomys talamancae	Oryzomyini	EU579514	EU649074
Zygodontomys brevicauda	Oryzomyini	EU579519	EU649075

discarded trees as burn-in, we plotted the log likelihood values of cold chains over the sampled generations. Standard deviation of split frequencies was sufficiently low (<0.005) and all the values of potential scale reduction factor (PSRF) in the evolution model were close to 1.00. These parameters were used as convergence diagnostic. Maximum likelihood analyses were performed at RAxML BlackBox on CIPRES portal (http://www.phylo.org/sub_sections/portal/; Stamatakis et al. 2008). We used the same model as the indicated for Bayesian inference (GTR+Γ+I). Node support values were evaluated by means of 1,000 bootstrap replicates. We performed maximum parsimony analyses using the program TNT (Goloboff et al. 2003, 2008a). Gaps were treated as missing data, and characters were considered under equal weights and implied weights (Goloboff et al. 2008b). This latter approach weights characters according to a concave function of homoplasy (Goloboff 1993). The concavity constant (k) is set by the user and negatively correlates with how strongly homoplasious characters are down-weighting. DNA sequence data are usually more homoplasious than morphological data. Therefore, large k values (>10) for DNA sequence data are preferable to avoid extreme down-weighting (Arnedo et al. 2009). We chose k values of 6, 15, 50, and 100. The optimal trees were estimated by means of the driven search option of New Technology Search implemented in TNT (Goloboff 1999). The approach used here consisted in finding 100 times the minimum length. Robustness of the nodes was evaluated by 1,000 bootstrap (Felsenstein 1985) and jackknife (Farris et al. 1996) replicates. Jackknife was performed with a removal probability of 0.36. Finally, we assessed the incongruence of both genes using the incongruence length difference (ILD) test (Farris et al. 1994, 1995). We made 1,000 replications in order to estimate statistical significance. All the trees were rooted using the following taxa as outgroups: *Cricetus cricetus* (Cricetinae), *Scotinomys teguina*, *Neotoma micropus*, *Peromyscus melanotis* (Neotomyinae), *Arvicola terrestris* (Arvicolinae), and *Tylomys nudicaudus* (Tylomyinae).

The genetic distances between members of the same tribe versus different tribes were calculated with the program MEGA 5.01 (Tamura et al. 2011) using maximum composite likelihood method and uncorrected p distances. Tamura

et al. (2004) showed that pairwise distances and the related substitution parameters are accurately estimated by maximizing the composite likelihood, which is defined as a sum of related log-likelihoods. Estimates of variance were performed by means of 1,000 bootstrap replicates. According to phylogenetic results (see below), we included *Neotomys*, *Euneomys*, and *Irenomys* in a group that was compared with others previous recognized tribes (e.g., Phyllotini, Akodontini, etc.). The composition of each tribe is listed in Table 1.

The multiple-sequence alignment yielded no internal gaps; all of them were external resulting from the use of different length sequences deposited in GenBank. There were 319 of 1,278 and 553 of 1,143 phylogenetic informative sites for *Irbp* and *mt-cyb*, respectively. The three criteria used here to estimate phylogenetic relationships (Bayesian inference, maximum likelihood, and maximum parsimony) yielded similar results regarding to the phylogenetic position of the genus *Neotomys* either for the two data sets (*Irbp* and *mt-cyb*) analyzed separately or combined. The genus *Neotomys* was established to be closely allied to *Irenomys* and *Euneomys*, constituting a monophyletic group (Figs. 1, 2, 3).

The equal weight parsimony analysis yielded 435 minimum length trees (length=1,262, consistency index=0.567, retention index=0.609) for the *Irbp* data set (Fig. 1). However, the implied weighting approach drastically reduced the number of parsimony trees to 9, 6, 2, and 1 tree for k=6, 15, 50, and 100, respectively. Main changes in the results using this approach were between tribe's relationships. However, all the implied weighting analysis recovered every Sigmodontinae tribe as monophyletic just as depicted in the strict consensus tree of equal weight analysis (Fig. 1). The clade formed by *Neotomys*, *Euneomys*, and *Irenomys* was always highly supported by parsimony analysis with bootstrap and jackknife values of 96% and 98%, respectively. Maximum likelihood and Bayesian inference analysis for the *Irbp* data set yielded similar results to parsimony analysis regarding to the phylogenetic relationships of genus *Neotomys*, which also grouped together with *Euneomys* and *Irenomys* with high support values (bootstrap and posterior probability; Fig. 1).

The *mt-cyb* data set analyzed under the maximum likelihood approach recovered all sigmodontine tribes as monophyletic with two exceptions. *Rhagomys* was not recovered within the Thomasomyini, and *Juliomys* was recovered within the tribe Oryzomyini (Fig. 2). However, support values for the recognized tribes were low to moderately high (e.g., 77% for Abrotrichini and 74% for Phyllotini). As for the *Irbp* data set, the analysis of *mt-cyb* data alone recovered a very well supported clade formed by *Neotomys*, *Irenomys*, and *Euneomys* (Fig. 2).

Bayesian inference recovered almost all the recognized tribes as monophylectic with exception of Thomasomyini. *Rhagomys* was grouped as sister genus of the node composed by *Reithrodon*, *Euneomys*, *Irenomys*, and *Neotomys*. These last two genera were related (Bayesian posterior probability=1.00; Fig. 2). Unlike the maximum likelihood analysis, the tribe Oryzomyini was recovered as monophyletic by Bayesian inference with moderate support (Bayesian posterior probability=0.86).

The equal weighted parsimony analysis of the *mt-cyb* data set, recovered three minimum length trees (length=7,459, consistency index=0.154, retention index=0.313). The strict consensus tree was highly unresolved and with low support values of bootstrap and jackknife for the Sigmodontinae tribes. The genus *Euneomys* was recovered as sister genus of *Neotomys*, although with low support (52 and 56 of bootstrap and jackknife, respectively). When the implied weighting method was conducted for *mt-cyb* data set, one completely resolved tree was obtained for k=6, 15, 50, and 100. Although weakly supported, almost all the tribes were recovered. The exceptions were Thomasomyini and Oryzomyini for k=6 and 15 and Thomasomyini for k=50 and 100. Under implied weights, the genus *Neotomys* was always recovered together with *Euneomys* and *Irenomys* in a monophyletic group.

Finally, the analysis conducted on the combined matrix of *Irbp* and *mt-cyb* sequences yielded similar results to the independent analysis concerning the phylogenetic position of *Neotomys*. The Bayesian phylogram (50% majority rule consensus) of partitioned analysis of the two genes is presented in Fig. 3. All previously recognized tribes were recovered as monophyletic, with only two exceptions. First, *Rhagomys* was recovered as sister genus of *Juliomys* but not within the tribe Thomasomyini (Fig. 3). Second, the two members of the tribe Ichthyomyini included in this study, *Rheomys* and *Neusticomys*, were not closely related to each other. Nevertheless, the genus *Neotomys* still grouped together with *Irenomys* and *Euneomys* in very well supported clade (Bayesian posterior probability=1.00). The maximum likelihood analysis, for the combined data set, confirms the close relationship among these three genera (Fig. 3). Although subtle topological differences are apparent in comparison with Bayesian inference, the tree obtained by maximum likelihood analysis recovered *Neotomys*, *Irenomys*, and *Euneomys* as a high supported monophyletic group (99% bootstrap). Again, *Rhagomys* was recovered outside of the Thomasomyini and closely related to *Juliomys*. However, the remaining sigmodontine tribes were recovered as monophyletic in this analysis (Fig. 3). The equal weight maximum parsimony analysis recoverd two trees of 8,814 steps (consistency index=0.211, retention index=0.343; Fig. 3). The topology of the strict consensus is different to those obtained by the Bayesian phylogenetic inference and maximum likelihood analyses. Even when not all recognized tribes were recovered as monophyletic (e. g., Abrotrichini, Thomasomyini, and Ichthyomyini), the parsimony analysis still support the monophyletic nature of *Neotomys*, *Euneomys*, and *Irenomys*, with

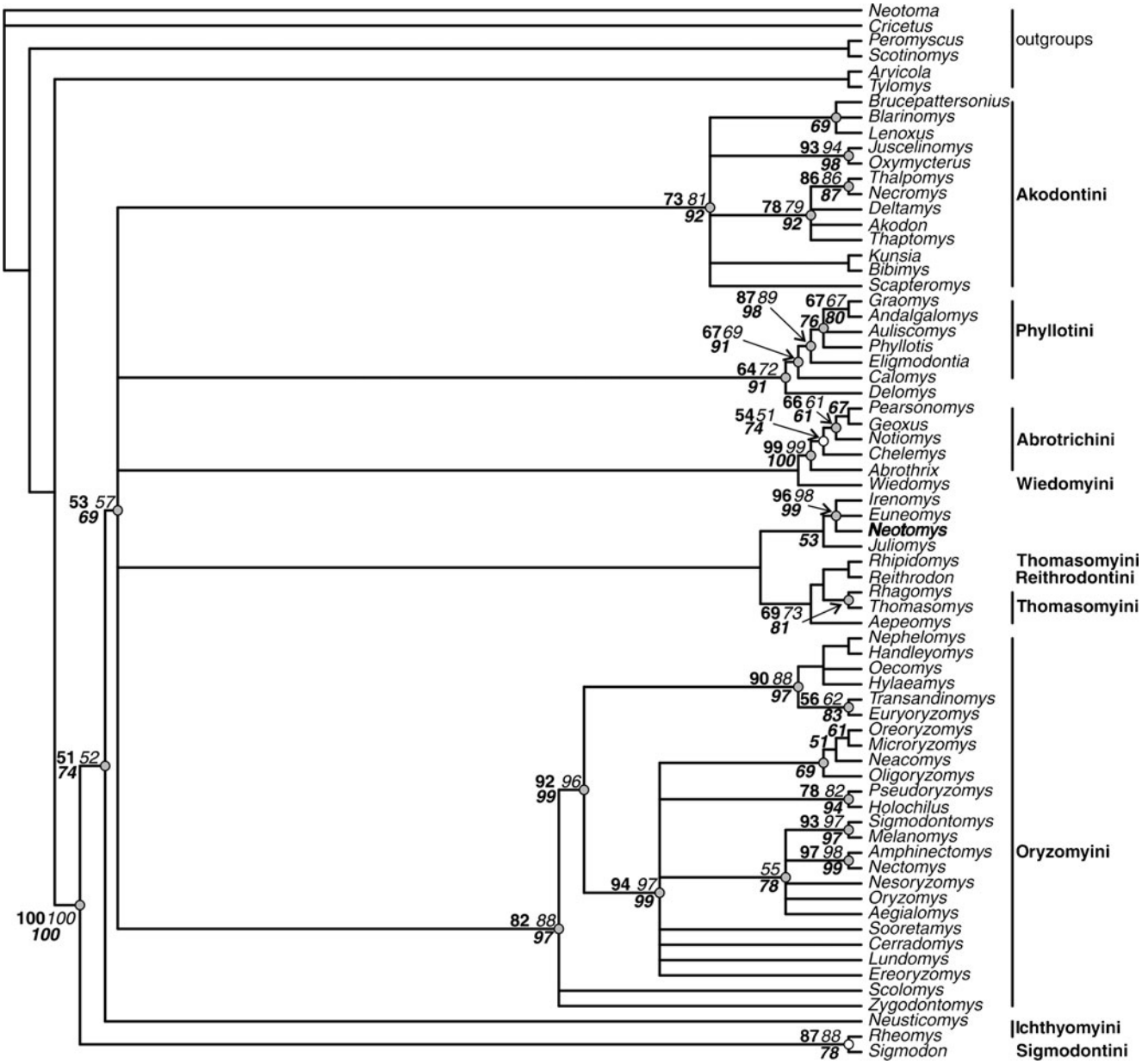

Fig. 1 Strict consensus tree of 435 minimum length trees obtained by parsimony (equal weight analysis) using *Irbp* sequences. Values of 1,000 bootstrap and jackknife replicates are indicated in bold and italics, respectively (only values above 50% are shown). Support values of bootstrap obtained by maximum likelihood analysis, for shared nodes with parsimony, are showed in bold italics. Also, the posterior probability support of Bayesian inference for each shared node are indicated by means of symbols: *white symbols* values between 0.75 and 0.89, *grey symbols* values of 0.90 or higher

relatively high support values (70% bootstrap and 84% jackknife). The different values of k in implied weighting approach produced one resolved tree. The results of ILD test indicate that the matrices of the *Irbp* and *mt-cyb* genes were significantly incongruent ($p<0.01$).

Within and between tribes, genetic divergences, expressed as maximum composite likelihood, were compared with the group formed by *Neotomys*, *Euneomys*, and *Irenomys* for both genes *Irbp* and *mt-cyb* (Tables 2 and 3). The within-group divergence values of the *Neotomys* group (1.6%) was similar to the within-group divergence values of recognized tribes (1.2–2.5%) for the *Irbp* sequence (Table 2). Accordingly, mean values of between-group divergence for the *Neotomys* group (2.6–6.1%) fell within the range of variation for comparisons among recognized tribes (2.6–8.0%; see Table 2). On the other hand, within-group divergence values of the *Neotomys* group for *mt-cyb* sequences were slightly higher (24.3%) than the observed values for the other recognized tribes (13.5–23.8%). However, between-group divergence values for the *Neotomys* group range from 26.3% to 31.2%,

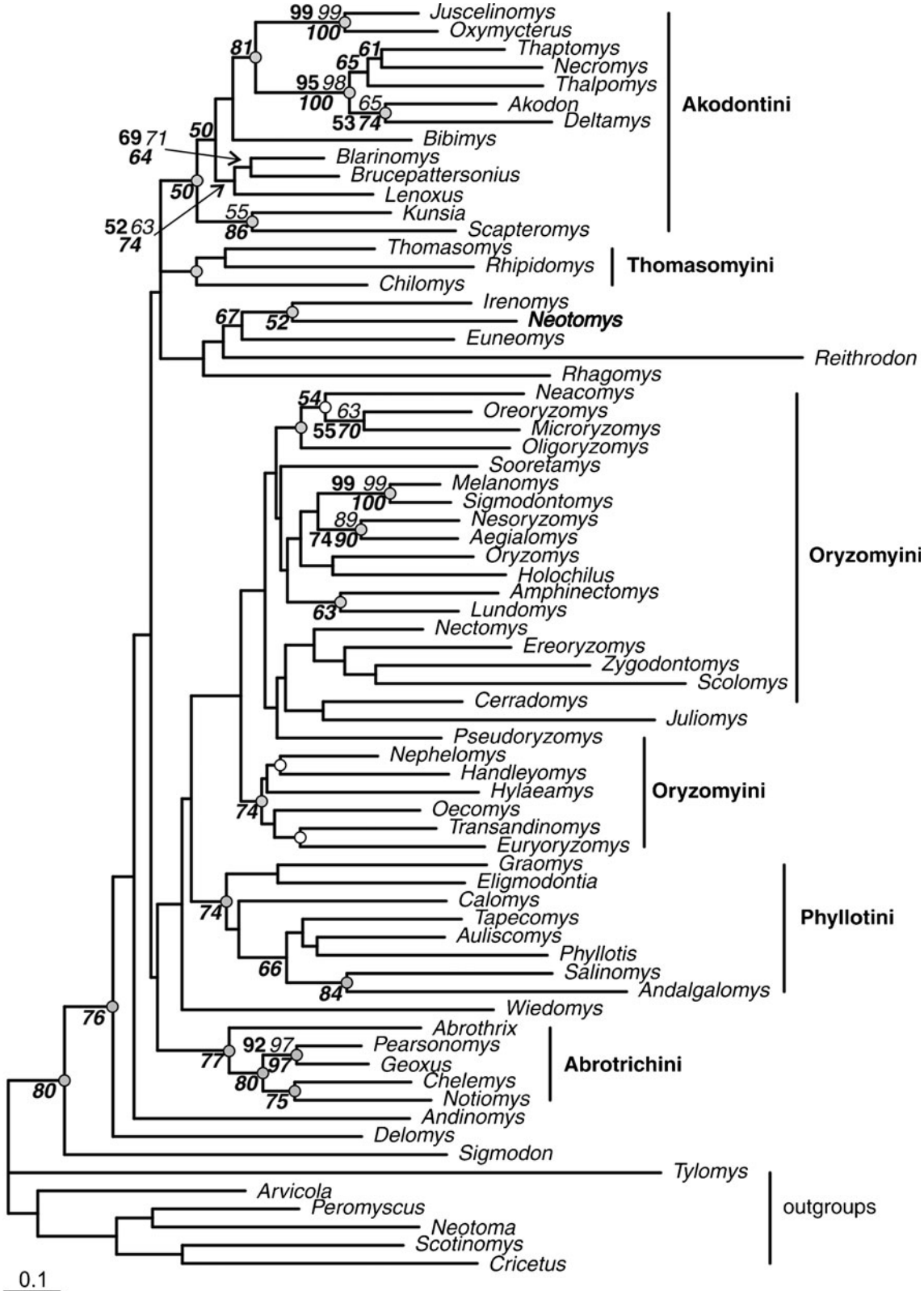

Fig. 2 Maximum likelihood tree [-37,890.165, the estimated parameters were: *A*=0.259, *C*=0.281, *G*=0.207, *T*=0.254, Gamma parameter α=0.579, and *I*=0.437; rate substitution matrix (*AC*)=6.073, (*AG*)=7.902, (*AT*)=5.385, (*CG*)=0.699, (*CT*)=45.182, and (*GT*)=1] obtained using *mt-cyb* data set. Bootstrap nodal supports (only values above 50% are shown) are indicated in bold italics. Values of 1,000 bootstrap and jackknife replicates of equal weight maximum parsimony analysis, for shared nodes, are indicated bold and italics, respectively. The posterior probability support of Bayesian inference for the shared nodes are indicated by means of symbols on nodes: *white symbols* values between 0.75 and 0.89, *grey symbols* values of 0.90 or higher

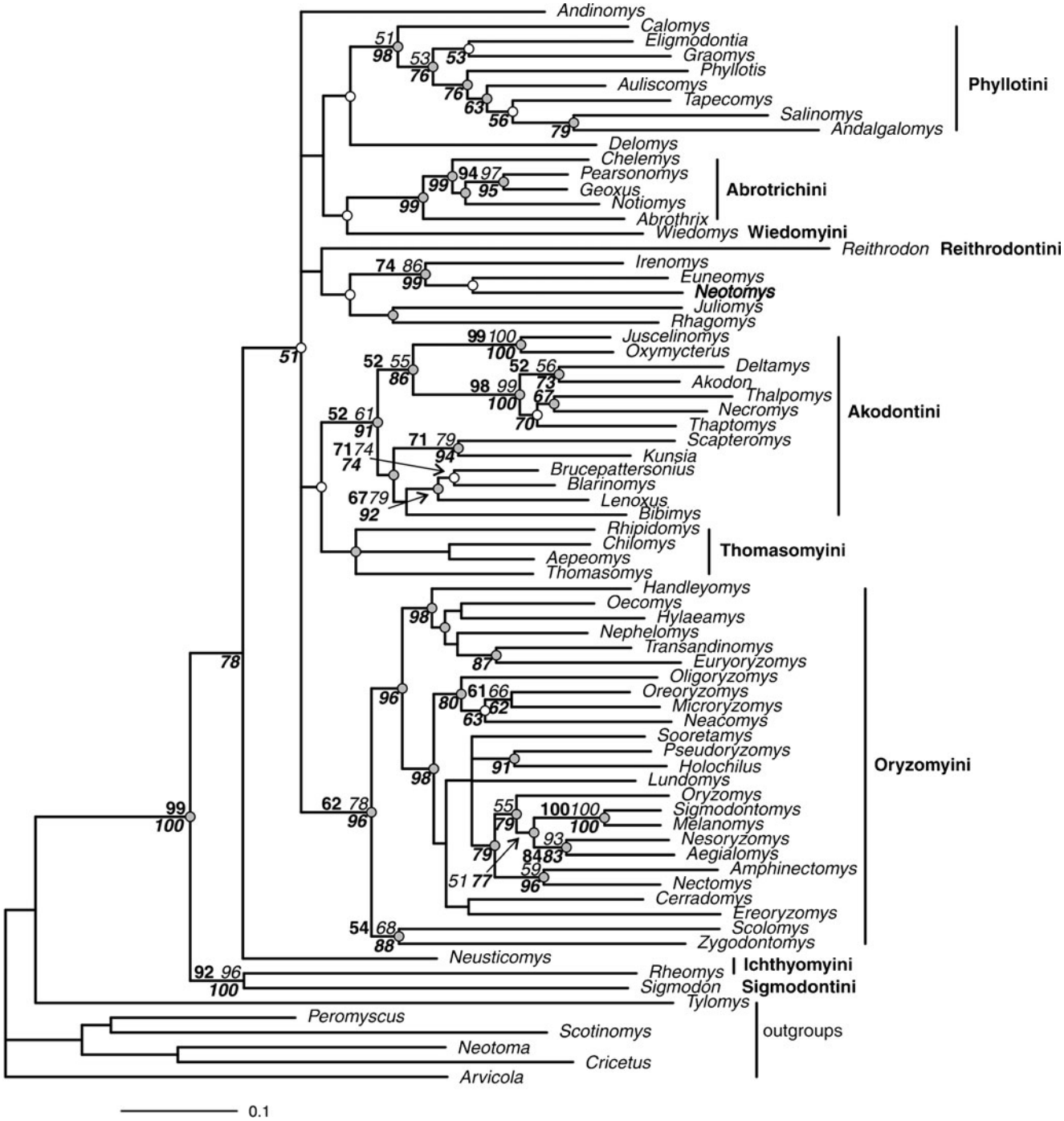

Fig. 3 Bayesian inference 50% majority rule consensus tree obtained by means of partitioned analysis of *Irbp* and *mt-cyb* data sets. The posterior probability support of Bayesian inference for each node is indicated by means of symbols on nodes: *white symbols* values between 0.75 and 0.89, *grey symbols* values of 0.90 or higher. The values in bold and italics represent support values for common nodes obtained with 1000 bootstrap and jackknife replicates of parsimony (equal weight analysis; only values above 50% are shown), respectively. Additionally, support values of bootstrap obtained of maximum likelihood analysis is showed in bold italics

whereas the range of variation of between-group divergence among recognized tribes was 15.2–38.6% (Table 3). The uncorrected *p* distances for *Irbp* were very similar to those obtained by maximum composite likelihood. Contrary, and probably as a consequence of multiple hits, the uncorrected *p* distances obtained for *mt-cyb* data set were lower than those of maximum composite likelihood method. However, proportions of divergences between and within groups are similar to those obtained by the maximum composite likelihood method for *mt-cyb* (results not shown).

Table 2 Mean and standard error (between parentheses) of maximum composite likelihood distances, expressed as percentage of divergence, of *Irbp* sequences within and between the recognized tribes of Sigmodontinae and the clade obtained in phylogenetic analyses integrated by *Neotomys*, *Euneomys*, and *Irenomys*, here called the *Neotomys* group

Irbp

Tribes	1	2	3	4	5	6	7	8	9	10
1. Abrotrichini (*N*=5)	*1.2 (0.3)*									
2. Akodontini (*N*=13)	3.6 (0.6)	*2.1 (0.3)*								
3. Ichthyomyini (*N*=2)	4.3 (0.7)	4.0 (0.7)	*1.7 (0.5)*							
4. *Neotomys* group (*N*=3)	3.2 (0.5)	3.0 (0.5)	3.6 (0.6)	*1.6 (0.4)*						
5. Oryzomyini (*N*=25)	4.0 (0.6)	4.1 (0.6)	5.0 (0.7)	3.5 (0.5)	*2.5 (0.3)*					
6. Phyllotini (*N*=6)	3.9 (0.6)	3.7 (0.5)	4.5 (0.7)	3.2 (0.6)	4.4 (0.6)	*2.1 (0.3)*				
7. Reithrodontini (*N*=1)	3.6 (0.7)	2.9 (0.5)	3.8 (0.7)	2.9 (0.6)	3.9 (0.6)	3.2 (0.6)	*n/a*			
8. Sigmodontini (*N*=1)	7.3 (1.1)	7.2 (1.0)	5.2 (0.9)	6.1 (0.9)	8.0 (1.0)	7.4 (1.1)	7.0 (1.1)	*n/a*		
9. Thomasomyini (*N*=4)	3.0 (0.5)	2.8 (0.4)	3.5 (0.6)	2.3 (0.4)	3.4 (0.5)	3.0 (0.5)	2.6 (0.5)	6.6 (1.0)	*2.1 (0.4)*	
10. Wiedomyini (*N*=1)	3.0 (0.6)	3.1 (0.6)	4.0 (0.7)	2.6 (0.6)	3.9 (0.7)	3.4 (0.6)	3.2 (0.7)	7.0 (1.1)	2.6 (0.5)	*n/a*

n/a Not available values

Our phylogenetic reconstructions assess the phylogenetic position of *Neotomys* in the Sigmodontinae radiation from a molecular perspective. The results obtained in this work suggest that *Neotomys*, together with *Euneomys* and *Irenomys*, belongs to a monophyletic group of genera distinct to the Phyllotini and not closely related to *Reithrodon*. Conversely, the phylogenetic position of *Neotomys*, inferred from morphological data, suggested a close relationship to *Reithrodon* (Braun 1993) or to *Reithrodon* and *Euneomys* (Steppan 1993, 1995). Our molecular phylogenetic analysis revealed that *Neotomys* is allied to *Irenomys* and *Euneomys*. The phylogenetic position of *Irenomys* had been also elusive. Steppan (1995) identified an *Andinomys* group including *Andinomys* and *Irenomys*, while D'Elía et al. (2006b) found *Irenomys* closely related to *Euneomys*. Beside this, *Irenomys* had no clear affinities with other genera, either by means of morphological or molecular phylogenies (Braun 1993; Smith and Patton 1999; D'Elía 2003). The well-supported *Neotomys*, *Irenomys*, and *Euneomys* clade we have recurrently recovered would probably deserve a tribal status in order to taxonomically identify this monophyletic group of genera. The within and between group genetic differences for *Irbp* support this suggestion. The values of consistency index and retention index of *Irbp* data set are higher than *mt-cyb* one. This evidence indicates that the *Irbp* data set has lower levels of homoplasy than the *mt-cyb* data set. Recently, Feijoo et al. (2010) in a comprehensive analysis of the systematics of *Abrothrix lanosus* showed the congruence between *Irbp*-based phylogeny and morphological variation within *Abrothrix*, contrary to the *mt-cyb*-based

Table 3 Mean and standard errors (between parentheses) of maximum composite likelihood distances, expressed as percentage of divergence, of *mt-cyb* sequences within and between the recognized tribes of Sigmodontinae and the clade obtained in phylogenetic analyses integrated by *Neotomys*, *Euneomys*, and *Irenomys*, here called the *Neotomys* group

Mt-cyb

Tribes	1	2	3	4	5	6	7	8	9
1. Abrotrichini (*N*=5)	*13.5 (2.4)*								
2. Akodontini (*N*=13)	22.2 (3.1)	*19.6 (2.5)*							
3. Neotomys group (*N*=3)	29.4 (4.5)	30.7 (4.4)	*24.3 (4.1)*						
4. Oryzomyini (*N*=25)	16.6 (2.7)	22.1 (3.1)	28.5 (4.2)	*15.2 (2.3)*					
5. Phyllotini (*N*=8)	17.5 (2.8)	24.8 (3.5)	28.9 (4.3)	17.4 (2.6)	*17.2 (2.8)*				
6. Reithrodontini (*N*=1)	28.1 (4.7)	30.3 (4.5)	28.2 (4.8)	29.6 (4.9)	34.4 (5.7)	*n/a*			
7. Sigmodontini (*N*=1)	16.2 (3.3)	23.3 (3.8)	31.2 (5.0)	15.2 (2.8)	19.3 (3.4)	28.5 (5.8)	*n/a*		
8. Thomasomyini (*N*=4)	20.1 (3.0)	24.5 (3.2)	27.2 (3.8)	21.4 (3.0)	22.0 (3.1)	32.0 (5.1)	22.4 (3.7)	*23.8 (3.7)*	
9. Wiedomyini (*N*=1)	18.8 (3.6)	23.1 (3.7)	26.3 (4.7)	21.0 (3.7)	18.8 (3.3)	38.6 (7.1)	20.9 (4.6)	19.8 (3.4)	*n/a*

n/a Not available values

phylogeny which was largely incongruent. We believe that more evidence should be achieved, both molecular and morphological, to refute or confirm our results. Furthermore, additional morphological data will provide more robustness to our results and the hypothesis concerning the tribal status of this clade. From a molecular perspective, an essential point in further analysis should be the inclusion of other *incertae sedis* genera such as *Punomys* and *Andinomys* (including *Irbp* sequence) as well as additional genes to elucidate the phylogenetic relationship of this group. A formal definition and diagnosis of the proposed tribe will be desirable such as the recently formalized Abrotrichini tribe (D'Elía et al. 2007) that was previously identified by means of molecular phylogenetic analysis (Smith and Patton 1999; D'Elía 2003).

The three genera that comprise this lineage are mainly distributed along the Andes. *Neotomys* is in the highlands of the Central Andes (Pardiñas and Ortiz 2001; Anderson 1997; Barquez 1983; Sanborn 1947). *Irenomys* inhabits the Patagonic forest of the southern Andes of Chile and Argentina, whereas the genus *Euneomys* is distributed from the highlands of the southern Andes to Patagonian region (Musser and Carleton 2005; D'Elía et al. 2006a; Martin 2010; Pardiñas et al. 2010). The geographic distribution of this monophyletic group of genera suggests that the Andes may have played an important role in the diversification of these genera as well as for several tribes of Sigmodontinae such as Akodontini and Phyllotini (Reig 1984; Braun 1993).

In our analysis, the genus *Neotomys* was found to be part of a quite stable, well-supported, and previously unrecognized lineage in Sigmodontinae radiation. These results provide enlightenment for the understanding of the sigmodontine radiation and clues for further investigations about the systematic relationships of these rodents.

Acknowledgments We are grateful to CONICET for the fellowship given to JJM and LIF and for the financial support through PIP CONICET 6197 to Ulyses F. J. Pardiñas and Rubén M. Barquez. We are also grateful to Noemí Gardenal for her kindness in providing lab infrastructure support. We thank two anonymous reviewers whose valuable comments improved considerably this work.

References

Anderson SA (1997) Mammals of Bolivia, taxonomy and distribution. Bull Am Mus Nat His 231:1–652

Anderson S, Yates TL (2000) A new genus and species of phyllotine rodent from Bolivia. J Mammal 8:18–36

Arnedo AA, Hormiga G, Scharff N (2009) Higher-level phyloegentics of linyphiid spiders (Araneae, Linyphiidae) based on morphological and molecular evidence. Cladistics 25:231–262

Barquez RM (1983) La distribución de *Neotomys ebriosus* Thomas en la Argentina y su presencia en la provincia de San Juan (Mammalia, Rodentia, Cricetidae). Hist Nat 22:189–191

Braun JK (1993) Systematic relationships of the tribe Phyllotini (Muridae: Sigmodontinae) of South America. Oklahoma Mus Nat Hist, Spec Publ 50 pp

Bruford ME, Hanotte O, Brookfield JFY, Burke T (1992) Single-locus and multilocus DNA fingerprinting. In: Hoelzel AR (ed) Molecular genetic analysis of populations, a practical approach. Oxford University Press, Oxford, pp 225–269

D'Elía G (2003) Phylogenetics of Sigmodontinae (Rodentia, Muroidea, Cricetidae), with special reference to the akodont group, and with additional comments on historical biogeography. Cladistics 19:307–323

D'Elía G, González EM, Pardiñas UFJ (2003) Phylogenetic analysis of sigmodontine rodents (Muroidea), with special reference to the akodont genus *Deltamys*. Mamm Biol 68:351–364

D'Elía G, Teta P, Pardiñas UFJ (2006a) *Incertae sedis*, Subfamilia Sigmodontinae, Familia Cricetidae. In: Barquez RM, Díaz MM, Ojeda RA (eds) Mamíferos de Argentina. Sistemática y distribución, SAREM, Tucumán, Argentina, pp 197–202

D'Elía G, Luna L, González EM, Patterson B (2006b) On the Sigmodontinae radiation (Rodentia, Cricetidae): an appraisal of the phylogenetic position of *Rhagomys*. Mol Phylogenet Evol 38:558–564

D'Elía G, Pardiñas UFJ, Teta P, Patton JL (2007) Definition and diagnosis of a new tribe of sigmodontine rodents (Cricetidae: Sigmodontinae), and a revised classification of the subfamily. Gayana 71:187–194

Díaz MM, Barquez RM (2007) The wild mammals of Jujuy province, Argentina: systematics and distribution. In: Kelt DA, Lessa EP, Salazar-Bravo JA, Patton JL (eds) The quintessential naturalist: honoring the life and legacy of Oliver Pearson. University of California Publications in Zoology, pp. 417–578

Edgard RC (2004) Muscle: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797

Engel SR, Hogan KM, Taylor JF, Davis SK (1998) Molecular systematics and paleobiogeography of the South American sigmodontine rodents. Mol Biol Evol 15:35–49

Farris JS, Källersjö M, Kluge AG, Bult C (1994) Testing significance of incongruence. Cladistics 10:315–319

Farris JS, Källersjö M, Kluge AG, Bult C (1995) Constructing a significance test of incongruence. Syst Biol 44:570–572

Farris JS, Albert VA, Källersjö M, Lipscomb D, Kluge AG (1996) Parsimony jackknifing outperforms neighbor-joining. Cladistics 12:99–124

Feijoo M, D'Elía G, Pardiñas UFJ, Lessa EP (2010) Systematics of the southern Patagonian–Fueguian endemic *Abrothrix lanosus* (Rodentia: Sigmodontinae): phylogenetic position, karyotypic and morphological data. Mamm Biol 75:122–137

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Ferro LI, Martínez JJ (2009) Molecular and morphometric evidence validates a Chacoan species of the grey leaf-eared mice genus *Graomys* (Rodentia: Cricetidae: Sigmodontinae). Mammalia 73:265–271

Goloboff PA (1993) Estimating character weights during tree search. Cladistics 9:83–91

Goloboff P (1999) Analyzing large data sets in reasonable times: solutions for composite optima. Cladistics 15:415–428

Goloboff P, Farris J, Nixon K (2003) TNT: Tree analysis using new technology. Program and documentation. Available at: http://www.zmuc.dk/public/phylogeny/tnt

Goloboff P, Farris J, Nixon K (2008a) TNT, a free program for phylogenetic analysis. Cladistics 24:774–786

Goloboff PA, Carpenter JM, Arias JS, Miranda Esquivel DR (2008b) Weighting against homoplasy improves phylogenetic analysis of morphological data sets. Cladistics 24:1–16

Martin GM (2010) Mammalia, Rodentia, Cricetidae, *Irenomys tarsalis* (Philippi, 1900): new records for Argentina and filling gaps. Check List 6:561–563

Musser GG, Carleton MD (2005) Superfamily Muroidea. In: Wilson DE, Reeder DM (eds) Mammal species of the world. A taxonomic and geographic reference, 3rd edn. The Johns Hopkins University Press, Baltimore, pp 894–1531

Olds N, Anderson S (1989) A diagnosis of the tribe Phyllotini (Rodentia, Muridae). In: Redford KH, Eisenberg JF (eds) Advances in neotropical mammalogy. Sandhill Crane Press, Gainesville, pp 55–74

Ortiz PE, Pardiñas UFJ, Steppan SJ (2000) A new fossil phyllotine (Rodentia: Muridae) from Northwestern Argentina and relationships of the *Reithrodon* group. J Mammal 81:37–51

Pardiñas UFJ, Ortiz PE (2001) *Neotomys ebriosus*, an enigmatic South American rodent (Muridae, Sigmodontinae): its fossil record and present distribution in Argentina. Mammalia 65:244–250

Pardiñas UFJ, Teta P, Chebez JC, Martínez FD, Ocampo S, Navas DO (2010) Mammalia, Rodentia, Sigmodontinae, *Euneomys chinchilloides* (Waterhouse, 1839): range extension. Check List 6:167–169

Posada D, Crandall KA (1998) ModelTest: testing the model of DNA substitution. Bioinformatics 14:817–818

Reig OA (1980) A new fossil genus of South American cricetid rodents allied to *Wiedomys*, with and assessment of the Sigmodontinae. J Zool 192:257–281

Reig OA (1984) Distribuiçao geografica e historia evolutiva dos roedores muroideos sudamericanos (Cricetidae: Sigmodontinae). Rev Brasil Genet 7:333–365

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Sanborn CC (1947) The South American Rodents of the genus *Neotomys*. Field Zool 31:51–57

Smith MF, Patton JL (1999) Phylogenetic relationships and the radiation of sigmodontine rodents in South America: evidence from cytochrome *b*. J Mammal Evol 6:89–128

Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML Web-Servers. Syst Biol 75:758–771

Steadman DW, Ray CE (1982) The relationships of *Megaoryzomys curioi* an extinct cricetine rodent (Muroidea: Muridae) from the Galapagos Islands, Ecuador. Smiths Contrib Paleobiol 51:1–23

Steppan SJ (1993) Phylogenetic relationships among the Phyllotini (Rodentia: Sigmodontinae) using morphological characters. J Mamm Evol 1:187–214

Steppan SJ (1995) Revision of the Tribe Phyllotini (Rodentia: Sigmodontinae), with a phylogenetic hypothesis for the sigmodontinae. Field Zool 80:1–112

Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Science USA 101:11030–11035

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Thomas O (1894) Descriptions of some new neotropical Muridae. Field Zool 14:346–366

Thomas O (1921) On a further collection of mammals from Jujuy obtained by Mr. E. Budin. Ann Mag Nat Hist Ser 9, 8:608–617

Vorontsov NN (1959) The system of the hamster (Cricetinae) in the sphere of the world fauna and their phylogenetic relations. Biuletin Moskovskogo Obshtschestva Ispitately Prirody, Otdel Biologia 64:134–137

Voss RS (1988) Systematics and ecology of ichthyomyine rodents (Muroidea): patterns of morphological evolution in a small adaptive radiation. Bull Amer Mus Nat Hist 188:259–493

Weksler M (2003) Phylogeny of neotropical oryzomyine rodents (Muridae: Sigmodontinae) based on the nuclear IRBP exon. Mol Phylogenet Evol 29:331–349

Weksler M (2006) Phylogenetic relationships of oryzomyne rodents (Muroidea: Sigmodontinae): separate and combined analyses of morphological and molecular data. Bull Amer Mus Nat Hist 296:146