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ORIGINAL PAPER

Chromosomal variability and evolution in the tribe Phyllotini (Rodentia, Cricetidae, Sigmodontinae)

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Abstract Sigmodontinae is a group of rodents with a rich and complex diversification in South America. Among them, the tribe Phyllotini comprises at least ten genera and exhibits high chromosome variability. It was proposed that chromosome evolution in mammals is influenced by centromeric meiotic drive (CMD). This mechanism of chromosome transmission generates a bimodal distribution of karyotypes, which are either mostly acrocentric or mostly biarmed. Here, we reviewed and analyzed chromosome data from Phyllotini to contrast them with that predicted by the CMD model. Additionally, we analyzed the chromosome data in a phylogenetic framework. When only one karyotype was considered per polymorphic species, the distribution resembles the CMD model, although it is not completely bimodal. The position of most polymorphic species in the center of the distribution and the presence of XY_1Y_2 chromosomes in a species with exclusively biarmed autosomes suggested that the CMD model is applicable to some particular species. Within a phylogenetic framework, some genera are characterized by high fundamental numbers (FNs), such as *Calomys*, *Phyllotis*, and *Andalgalomys*, and others by low FN (*Loxodontomys*, *Auliscomys*). This suggests that FN is a good marker for inferring some intra- and intergeneric relationships. However, the chromosome data are not coincident with the close molecular relationship obtained between *Andalgalomys* and *Salinomys*, because these species have respectively the maximum and minimum diploid number (2n) found in the tribe. There are 87 described karyotypes, but only one species has 2n = 52, considered ancestral for sigmodontines, or 2n = 70, proposed as ancestral for phyllotines. This suggests a major chromosomal restructuring at the base of the phyllotine radiation.

Keywords South American rodents · Evolution · Chromosomes · Centromeric meiotic drive · Phylogeny

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Introduction

The subfamily Sigmodontinae comprises a lineage of cricetid rodents that radiated and differentiated in South America. Multiple studies, based on different lines of evidence such as morphology, parasitology, and molecular genetics, corroborate their common origin. However, the taxa that arrived to South America, their time of arrival, and the evolutionary processes that brought the current diversity are still controversial (Hershkovitz 1962; Reig 1981, 1986; Smith and Patton 1999; D'Elía 2003; D'Elía et al. 2003; Parada et al. 2013; Pardiñas et al. 2014). Within Sigmodontinae, the approximately 380 recognized species are distributed in 84 extant genera (Patton et al. 2015). Most of these genera are grouped into various tribes: Abrotrichini, Akodontini, Ichthyomyini, Oryzomyini, Phyllotini, Reithrodontini, Sigmodontini,

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Thomasomyini, and Wiedomyini. The number and content of these suprageneric clusters vary according to different authors (Hershkovitz 1962; Reig 1981, 1986; Steppan 1995; Smith and Patton 1999; D'Elía et al. 2007; Patton et al. 2015).

The tribe Phyllotini has been studied under various approaches and is recognized as a monophyletic group by all authors. However, its composition and phylogenetic relationships have changed considerably over the years (Hershkovitz 1962; Olds and Anderson 1989; Braun 1993; Steppan 1995; Steppan et al. 2007; Pardiñas et al. 2014). Early studies, largely based on morphology, indicated that the tribe was the most diverse among Sigmodontinae (see rev. in Reig 1986). Subsequent molecular studies also recover the tribe as a natural group, but composed only by 11 genera: *Andalgalomys*, *Auliscomys*, *Calomys*, *Eligmodontia*, *Galenomys*, *Graomys*, *Loxodontomys*, *Phyllotis*, *Salinomys*, and *Tapecomys*, with *Calassomys*, newly described genus, included in this tribe (Smith and Patton 1999; D'Elía 2003; D'Elía et al. 2007; Steppan et al. 2007; Salazar-Bravo et al. 2013; Pardiñas et al. 2014).

Most phylogenetic hypotheses for phyllotines were proposed after analyzing only a fraction of genera and/or species that compose the tribe (Smith and Patton 1999; Spotorno et al. 2001; D'Elía 2003; Parada et al. 2013; Salazar-Bravo et al. 2013; Carrizo and Catalano 2015). The most inclusive phylogenetic approach, based on molecular characters, and including almost all currently recognized genera (except *Galenomys* and *Calassomys*), and more than one species per genus, was performed by Steppan et al. (2007). This work recovered almost all relationships previously obtained by morphological evidence, although some other morphological relationships are strongly contradicting (Steppan et al. 2007).

Chromosome studies have contributed to delimit taxa and to understanding genetic diversity in the tribe (Pearson and Patton 1976; Spotorno et al. 2001). Phyllotines have extensive chromosome variability, with diploid numbers (2n) ranging from 18 in *Salinomys* to 78 in *Andalgalomys* (Olds et al. 1987; Lanzone et al. 2005) and fundamental numbers (FNs) from 30 in *Auliscomys* to 130 in *Andalgalomys* (Olds et al. 1987; Walker and Spotorno 1992). Furthermore, a species with multiple sex chromosomes XY_1Y_2, which is infrequent in mammals, was described in this group (Lanzone et al. 2011a). Early studies in Phyllotini suggested an ancestral karyotype in the tribe with 2n = 70, FN = 68, (Pearson and Patton 1976), and independent fusions as the main rearrangements leading to reductions in chromosome numbers (Spotorno et al. 2001). Additionally, new evidence based on chromosomal painting technique, combined with molecular phylogeny, supported 2n = 52 (FN = 52) as the plesiomorphic condition for the subfamily Sigmodontinae (Swier et al. 2009).

Chromosome evolution has been the subject of much study and controversy, both theoretically and practically (Patton and Sherwood 1983; King 1993; Faria and Navarro 2010). The major factors related to these evolutionary processes are the selective value of each rearrangement (whether the heterozygous is neutral, underdominant, or hetorotic, and whether the homozygous has some selective advantages) and the population parameters where the rearrangements arise (e.g., effective population size and metapopulational structure). Additionally, it was proposed that chromosome evolution in mammals is strongly influenced by centromeric meiotic drive (CMD). This mechanism operates in the asymmetric meiosis of females and favors the segregation to the oocyte (and transmission to the next generation) of metacentric or acrocentric chromosomes, alternatively. This process repeatedly results in karyotypes with predominance of biarmed or acrocentric chromosomes, generating a bimodal distribution when the percentages of both chromosome types in each karyotype are calculated in a lineage (Pardo-Manuel de Villena and Sapienza 2001; Yoshida and Kitano 2012).

The purpose of our work was to review the chromosome data of the tribe Phyllotini and perform a meta-analysis to investigate the chromosome evolution in the tribe, within the framework of current chromosome and phylogenetic hypotheses.

Materials and methods

We performed an extensive review of the literature and compiled chromosome information for 55 species, subspecies, and some innominate taxa of phyllotine rodents (Online Resource 1). First, we described the frequencies and distribution of all diploid (2n) and fundamental numbers (FNs) of autosome arms to investigate variability in both parameters.

To test the CMD model, we used only autosomes as described by Pardo-Manuel de Villena and Sapienza (2001) to avoid bias due to differences in sex chromosomes and to the existence of XY_1Y_2 in *Salinomys delicatus* (Lanzone et al. 2011a). Chromosomes of each karyotype were classified as acrocentric (acrocentric or telocentric chromosomes) or biarmed (metacentric and submetacentric chromosomes). To calculate the percentage of acrocentrics in the karyotypes, we first considered all cytotypes for polymorphic species (species in which were described several cytotypes and having odd 2n and/or FN). Since a single number of acrocentric chromosomes cannot be computed for polymorphic species, the maximum and minimum numbers were calculated. With the maximum of acrocentrics, we obtained the p (relative frequency of acrocentric autosomes) and q (relative frequency of biarmed autosomes) values necessary to estimate the expected binomial distribution under a random model of chromosome segregation with nine intervals. After that, we divided the observed autosomal complements according to the percentage of acrocentric chromosomes into these categories as in Pardo-Manuel de Villena and Sapienza (2001). Finally, we performed a chi-square test with Yates's correction to compare the expected and observed distribution of chromosome types.

Phylogenetic analysis

For the phylogenetic analysis, we included all species of Phyllotini for which there are available sequences in GenBank. The DNA data used comprise 54 terminals, including all Phyllotini recognized genera, and *Abrothrix jelskii* (Abrotrichini), *Irenomys tarsalis* (*Incertae sedis*), *Nectomys squamipes* (Oryzomyini), *Reithrodon auritus* (Reithrodontini), *Sigmodon hispidus* (Sigmodontini), and *Thaptomys nigrita* (Akodontini), being *S. hispidus* used to root the tree (Online Resource 1: Table 1). We included mitochondrial cytochrome b (Cyt-b), and nuclear fragments of recombination activating gene 1 (RAG-1), and interphotoreceptor retinoid binding protein (IRBP). These sequences are the most-sampled fragments for Phyllotini and related groups (Steppan et al. 2007; Parada et al. 2013; Salazar-Bravo et al. 2013; Pardiñas et al. 2014; Carrizo and Catalano 2015).

Sequences alignment was performed using MAFFT v6.240 (Katoh and Toh 2008) under the strategy E-INS-i and default parameters for gaps opening and extension, being the resulting alignments visually inspected and manually edited. The length of each gene fragment after the alignment was 1140 bp for Cyt-b, 758 bp for IRBP, and 1310 bp for RAG1. The final molecular dataset included 54 species for Cyt-b, 27 species for IRBP, and 30 species for RAG1.

For the parsimony phylogenetic analysis, we treated DNA sequences under static homology hypotheses. The analysis was performed using TNT (Goloboff et al. 2008). The shortest trees were found by submitting 1000 different random addition sequences (RAS) to the tree bisection-reconnection branch-swapping method (TBR), retaining 100 trees per replication. Internal branches were considered unsupported and collapsed during searches if any possible states were shared between ancestor and descendent nodes. Additionally, the support indices Parsimony Jackknife and Bootstrap CG frequencies were estimated over 1000 RAS plus TBR, keeping 100 trees per replicate. Trees were edited with Tree Graph 2 (Stöver and Müller 2010). In addition to parsimony, we also analyzed the data under a maximun likelihood (ML) criterion with PhyML (Guindon et al. 2010). This analysis was run under a general time reversible model (GTR), and the branch support was calculated by 500 replicates of bootstrapping (Felsenstein 1985).

Results

Description and basic statistics

Eighty-seven different karyotypes were described for the group, including monomorphic, polytypic, and polymorphic species (excluding the newly proposed genus *Calassomys*). In the tribe Phyllotini, the vast majority of species had unique karyotypes (Online Resource 1). The exceptions were several species of *Phyllotis* and two of *Eligmodontia* that shared the 2n and FN, and some species of *Calomys*, *Eligmodontia*, *Graomys*, and *Phyllotis* with chromosomal polymorphisms and polytypisms (Online Resource 1). In two species of *Eligmodontia*, several Robertsonian (Rb) variants were described, and *Graomys* presented a widely distributed Rb polymorphism. In this last genus, two species shared polymorphic inversions. This type of polymorphic rearrangements was also described in *Phyllotis* and *Calomys* (Online Resource 1).

When we plotted the 2n and FN of all chromosome variants, a wide variation was observed in both parameters. However, FNs showed a greater dispersion (SD 2n = 12.78, FN = 19.36; quartile range 2n = 18.00, FN = 23.50) and were distributed more discontinuously than 2n (Fig. 1a, b). In both, 2n and FN, there were very few odd numbers. The odds 2n were concentrated exclusively within the range of 31 to 37 chromosomes, and odds FNs were found in 45, 47, 57, and 71 chromosome arms.

Sex chromosomes were quite variable in the tribe. X chromosomes presented different morphologies (metacentric, submetacentric, or acro-telocentric), even within the same genus, but none of these variants has been described as polymorphic. The X chromosome always was median to big in size. Y chromosomes can be metacentric, submetacentric, or acrotelocentric and in general were small in size. In only one species, *E. morgani*, the Y chromosome variants formed a polymorphism.

The CMD model

Considering all chromosomes together, and including all karyotypes described for polymorphic species, the maximum number of acrocentric chromosomes was 1531, with 982 being biarmed, and the minimum number of acrocentric chromosomes was 1500, with 1000 being biarmed. Thus, between 60.92 and 60.00 % of all chromosomes described in the tribe were acrocentric (Online Resource 1). This results in $p = 0.61$ (frequency of acrocentric autosomes) and $q = 0.39$ (frequency of biarmed autosomes), for the maximum of acrocentrics, a criterion chosen because for polymorphic species of *Graomys* and *Eligmodontia*, acrocentrics seem to be the ancestral condition, and biarmed chromosomes the derived one (see discussion for further justification).

Taking into account all chromosome complements of each species, karyotypes with both types of chromosomes predominated in the tribe (Online Resource 1, Fig. 2b). This distribution departed not only from the binomial distribution expected under a random model ($\chi^2 = 4075.70$, df = 8, $p < 0.0001$; Fig. 2a) but also from the bimodal one expected under the CMD model. However, when only one karyotype was considered per polymorphic species, namely the one with the highest number of acrocentrics (see Online Resource 1), again the distribution departed from the expected binomial distribution

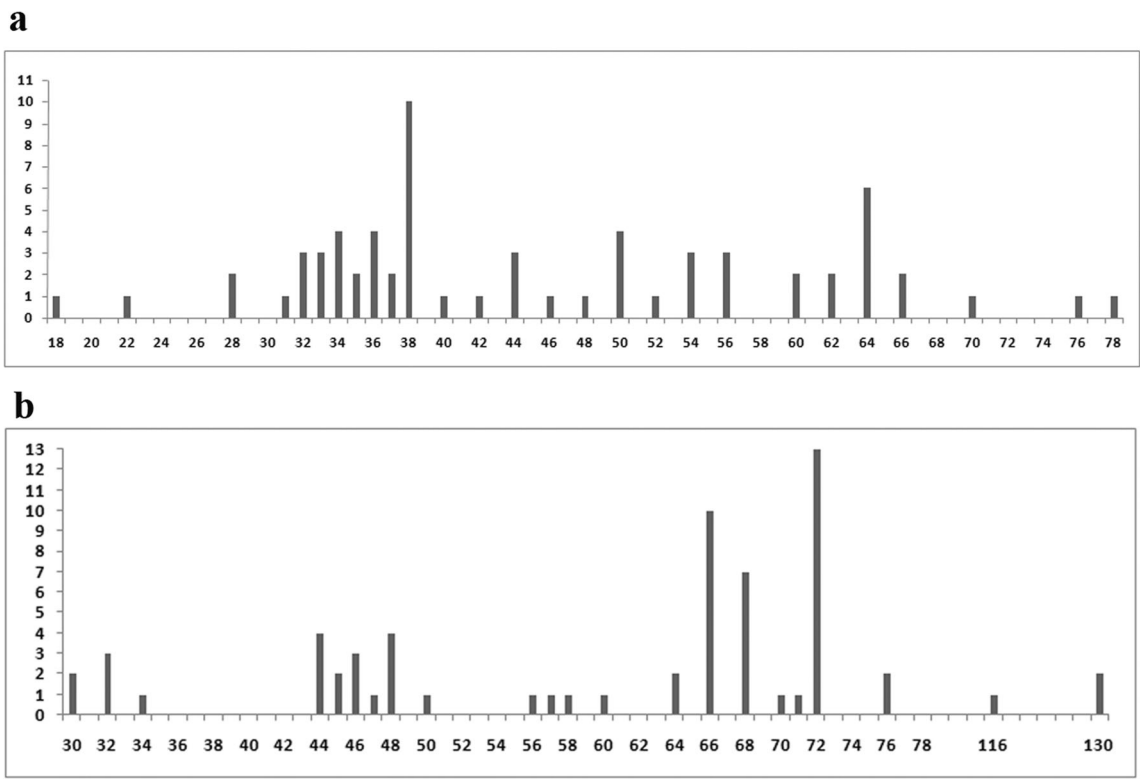

Fig. 1 Bar graphs showing the distribution and frequency of **a** diploid numbers (2n) and **b** fundamental numbers (FN) found in the tribe Phyllotini

($\chi^2 = 5607.42$, df = 8, $p < 0.0001$) and was closer to the CMD model, although not completely bimodal (Fig. 2c). The correlation between diploid number and percentage of acrocentric chromosomes was low but statistically significant (df = 1.85, $F = 7.63$, $R = 0.29$, $p = 0.007$).

Phylogenetic analysis

The parsimony phylogenetic analysis recovered six most parsimonious trees of 4910 steps, which differ in *Calomys callosus*, *Calomys laucha*, and *Phyllotis darwini* position (see Fig. 3 for strict consensus tree). The Phyllotini tribe was recovered as monophyletic, with *Calassomys apicalis* as the basal taxa. Despite this, their phylogenetic position is not supported statistically, while the clustering for all remaining phyllotines has a moderate statistical support (Fig. 3). In this sense, all Phyllotini-recognized genera were recovered as monophyletic groups (Fig. 3). In *Phyllotis*, two main clades were recovered, one of them is composed by *P. osilae* with 2n = 70, NF = 68, plus *P. anitae* and *P. alisosiensis* (both without chromosomes information), while the other involves all *Phyllotis* species with 2n = 38, FN = 72, except *P. andium* which has the same FN but 2n = 64, and *P. bonariensis* with no data available. Others polytypic genera as *Eligmodontia*, *Graomys*, and *Calomys* have very diverse chromosome complements, having *Calomys* elevate FNs and *Graomys* intermediate FNs. The species of *Andalgalomys* share high 2n and FNs, and those of *Auliscomys* share low 2n and FNs. Both species of *Tapecomys* have similar 2n and FNs.

At suprageneric level, all relationships have very low statistical support. One suprageneric clade group *Eligmodontia* with *Graomys*, two polytypic genera, with great chromosomal variability, and that include polymorphic species. Other clades recovered with high chromosomal divergence, and poor statistical support, involve the cluster of the species with the highest (*Andalgalomys pearsoni*) and lowest (*S. delicatus*) 2n and FNs reported for the tribe (Fig. 3 and Online Resource 2).

The maximum likelihood analyses recover similar relationships between phyllotine groups (Online Resource 2), with *C. apicalis* as the basal taxa of the group. However, the clade including *S. delicatus* plus *Andalgalomys* species is not recovered as sister group of *Tapecomys wolffsonhi* + *T. primus* (as in parsimony).

Despite the great chromosomal diversity in Phyllotini, the ancestral diploid and fundamental number 52 proposed for sigmodontines was not recorded in any of the phyllotines compiled here. Just one species, *Eligmodontia moreni*, presented similar diploid and fundamental number (2n = 52, FN = 50). In this last genus, *E. hirtipes* and *E. dunaris* (not included in the phylogenetic analysis) had the diploid and fundamental number (2n = 50/FN = 48) closest

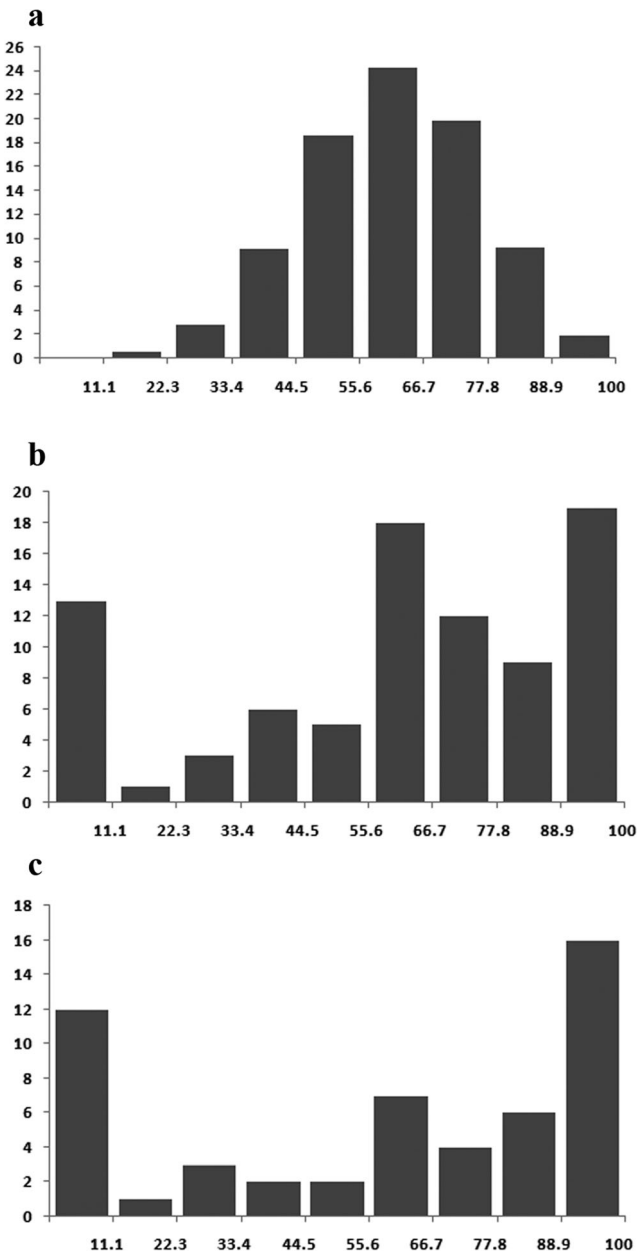

Fig. 2 Bar graphs showing **a** the expected distribution of karyotypes displaying different percentages of acrocentric chromosomes under a random model with nine categories. **b** Observed distribution of karyotypes with different percentages of acrocentric chromosomes considering all chromosome complements found in phyllotines. **c** Observed distribution of karyotypes with different percentages of acrocentric chromosomes considering only one karyotype per species

to the ancestral karyotype proposed for sigmodontines and this FN was shared with *E. puerulus*. Some species of *Calomys*, *Tapecomys*, and *Phyllotis* had diploid numbers close to 52, but all had higher FNs. On the other hand, several species of *Calomys* shared the FN considered ancestral for phyllotines (FN = 68), but none had 2n = 70. The only species with the proposed ancestral karyotype for the tribe (2n = 70, NF = 68) was *P. osilae*.

Discussion

Chromosome variability in Phyllotini and the CMD model

The tribe Phyllotini has high chromosome variability, and its karyotypes are good simple markers for taxonomic identification. Some exceptions are found in highly variable species such as *Graomys griseoflavus* and *Eligmodontia puerulus* or in genera comprising several species with the same karyotype, as observed in the *Phyllotis* clade with 2n = 38. However, despite the apparent chromosome constancy observed in *Phyllotis*, some species with identical conventional karyotypes differ in C and G-banding patterns (Walker et al. 1984). On the other hand, there are many different chromosome complements described for *Calomys*, which were found in populations with uncertain taxonomic status. Interestingly, despite the many different published karyotypes, few polymorphisms have been described for this last genus (Forcone et al. 1980; Lisanti et al. 1996).

In sigmodontine rodents, some species show constant and other highly variable chromosome complements (Fagundes et al. 1998; Swier et al. 2009). This contrast between slowly and rapidly evolving taxa is common among rodents (Romanenko et al. 2012). In some phyllotines, chromosome polymorphisms were reported in more than one species of the same genus, as for *Eligmodontia* and *Graomys*. Moreover, these genera have species with very divergent karyotypes, and spontaneous mutants were detected in both (Ortells et al. 1989; Zambelli et al. 1994), suggesting that more chromosome variants are generated and maintained in these clades than in others. Among all possible rearrangements, in the tribe, only Rb translocations and inversions were detected in polymorphic form (Forcone et al. 1980; Lisanti et al. 1996; Lanzone et al. 2011b; 2014; Labaroni et al. 2014), as is common in rodents (Patton and Sherwood 1983; Piálek et al. 2001; Lanzone et al. 2002).

In mammals, biarmed and uniarmed chromosomes are nearly equally represented, whereas acrocentrics represent 50.6 % of all chromosomes compiled by Pardo-Manuel de Villena and Sapienza (2001). However, more than 60 % of all chromosomes described in phyllotines are acrocentrics. The numerical superiority of acrocentrics in Phyllotini supports the proposed hypothesis that the ancestral chromosome complement in sigmodontines in general and in phyllotines in particular was predominantly acrocentric (Pearson and Patton 1976; Spotorno et al. 2001; Swier et al. 2009).

Firstly in mammals and then extended to other taxa (see Bidau and Martí 2004), it was proposed that chromosome evolution is influenced by centromeric meiotic drive (CMD). Our review and assessment of chromosome constitutions in the tribe Phyllotini is not compatible with the expected distribution under this model, but neither supports a random model of chromosome segregation. In contrast to that predicted under the CMD model, there are several karyotypes in the center of the

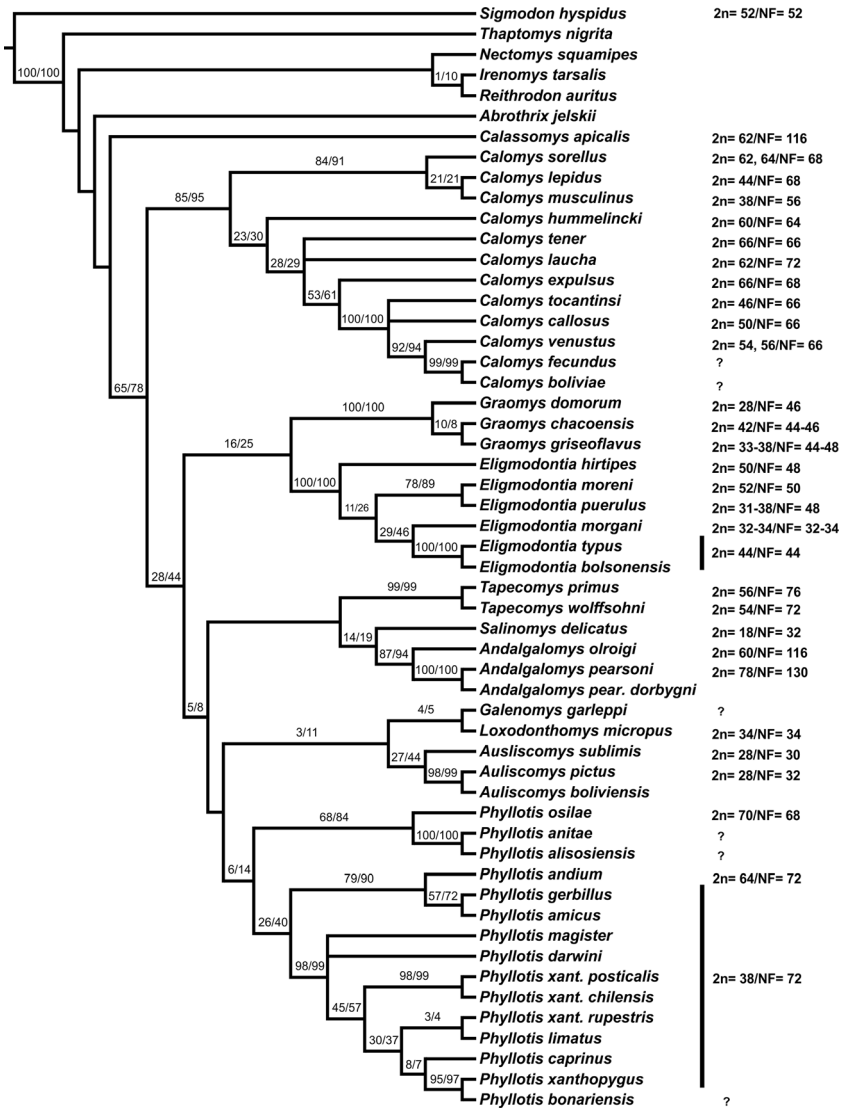

Fig. 3 Phylogenetic hypothesis obtained by the analysis of the molecular dataset (Cyt-b, RAG-1, and IRBP). Strict consensus of the six trees obtained by parsimony analysis. *Numbers* above branches indicate jackknife and bootstrap support. Chromosomal characteristics (2n and FN) of each species are indicated to the right of the tree

distribution that possess both chromosome types nearly equally represented. Many of these karyotypes belong to polymorphic species. When only one karyotype per species is considered, the distribution is closer to the expected one, although it is not completely bimodal. While the general model is rejected, the observations that polymorphic species are in the center of the distribution appear to favor the CMD model, at least for these species. Chromosomal polymorphisms can represent transient stages before fixation of a chromosome type. In polymorphic Rb species, the process appears to occur from acrocentric chromosomes to the fixation of metacentric ones. In both, *E. puerulus* and *G. griseoflavus*, the sister species related to the Rb species has more acrocentric chromosomes. While this can reflect the ancestry of acrocentrics in Phyllotini (Pearson and Patton 1976; Spotorno et al. 2001), this tendency to pass from acrocentric to biarmed chromosomes has been suggested previously for other phyllotines, like *Calomys*, and for other rodents too (Espinosa et al. 1997; Salazar-Bravo et al. 2001; Piálek et al. 2001). Within these three genera (*Eligmodontia*, *Graomys*, and *Calomys*), independent reduction and/or increase in the 2n appear to have occurred (Salazar-Bravo et al. 2001; this study), depending on which karyotype is considered ancestral for the tribe and for each genus.

Another observation that favors the CMD, at least for some species, is the presence of XY_1Y_2 chromosome systems in *S. delicatus* (Lanzone et al. 2011a). Under the CMD model, species with biarmed chromosomes tend to fix this type of sex chromosomes (Yoshida and Kitano 2012), and *S. delicatus* has a karyotype with exclusively biarmed autosomes. However, it is necessary to consider that the CMD model is applicable mainly to Rb translocations, although tandem fusions should also be subject to the same mechanisms of non-random segregation (Pardo-Manuel de Villena and Sapienza 2001). If Rb rearrangements predominated in the karyotypic evolution of

phyllotines, the diploid numbers should be correlated with the percentage of acrocentric chromosomes. But the low correlation between both parameters, together with the observation of a greater dispersion and discontinuity of FN than 2n, strongly suggest that several other rearrangements were also very important in the evolution of phyllotine rodents. The high frequency of other rearrangements and the direct observation of polymorphisms for inversions indicate that the CMD can operate in some species. Notwithstanding, a more general mechanism that can be applied to the other chromosome changes could account for the karyotype evolution in the tribe.

Interspecific relationships and chromosomal variations

In phyllotines, most genera were recovered as monophyletic by successive studies based in molecular or/and morphological characters (Braun 1993; Steppan 1995; Steppan et al. 2007; Salazar-Bravo et al. 2013; Carrizo and Catalano 2015; this study). Within these genera, most species differ from each other by several chromosome changes, suggesting a strong cytogenetic component in the intra-generic diversification (Spotorno et al. 2001). Several studies have indicated the potential pathways by which these chromosomal changes, necessary to generate diversity within polytypic genera, can occur (Pearson and Patton 1976; Walker and Spotorno 1992; Espinosa et al. 1997; Salazar-Bravo et al. 2001; Almeida et al. 2007; Lanzone et al. 2011b). They indicated that in some lineages, some rearrangements are more frequent than others, but in most cases, several different types of chromosome changes are necessary to include all the karyotypic diversity detected within each genus, suggesting that in most lineages, karyotype evolution is not restricted to only one type of chromosome rearrangement.

In our phylogenetic analysis, *Tapecomys wolffsohni* is recovered with high support as sister of *T. primus*. The taxonomic removal of *Phyllotis wolffsohni* to *Tapecomys* was proposed by Steppan et al. (2007) based on molecular data and is sustained also by morphologic and chromosome data (Patton et al. 2015; Online Resource 1). Excluding *wolffsohni* from the genus, *Phyllotis* appears monophyletic and composed by two main clades. One of them includes *P. osilae*, *P. anitae*, and *P. alisosiensis*, with karyotypic data only available for *P. osilae* (2n = 70/FN = 68), while the other clade grouped all species with FN = 72. Thus, in this genus, the FN appears to be more phylogenetically informative than 2n, but more cytogenetic studies are needed to understand the chromosome evolution of *Phyllotis*.

Intergeneric relationships and chromosomal variations

At higher taxonomic level, relationships among genera are generally unresolved, and most suprageneric clades have low statistical support or only are recovered in some analyses (Braun 1993; Steppan 1995; Steppan et al. 2007; Parada et al. 2013; Salazar-Bravo et al. 2013, this study). A close relationship between *Ausliscomys* and *Loxodontomys* was proposed primarily by Steppan et al. (2007), and both taxa share low 2n and FN. However, this relationship was not obtained in some studies (Parada et al. 2013; Salazar-Bravo et al. 2013). On the other hand, the close relationship obtained in molecular studies between *Andalgalomys* and *Salinomys* (Steppan et al. 2007; Parada et al. 2013; Salazar-Bravo et al. 2013; Carrizo and Catalano 2015; this study) is intriguing given chromosome data. Both genera exhibit the highest (*Andalgalomys*) and lowest (*Salinomys*) diploid number observed in the tribe, and the transformation of one karyotype into another requires extensive chromosome restructuring of the whole complement. However, both taxa have been recovered in different positions by different studies (Braun 1993; Steppan 1995; Salazar-Bravo et al. 2013; Carrizo and Catalano 2015), so this phylogenetic relationship should be taken with caution. Also, *Tapecomys* and *Loxodontomys* are recovered as closely related in some analyses (Salazar-Bravo et al. 2013; Parada et al. 2013; Carrizo and Catalano 2015), but these taxa have highly divergent chromosome complements. The use of different species of phyllotines as terminals could be the cause of differences among the proposed phylogenetic hypothesis for the tribe (Steppan et al. 2007; Parada et al. 2013; Salazar-Bravo et al. 2013; this study). In general, the relations among most genera are still controversial. A denser sampling of terminals and the analysis of more characters are needed in order to obtain a holistic view of the Phyllotini intergeneric relationship, which is fundamental to better understand its chromosome evolution.

The ancestral karyotype

The phylogenetic hypothesis for the Sigmodontinae has varied over time and with authors (Reig 1981; Smith and Patton 1999; D'Elía 2003; Steppan et al. 2004; Parada et al. 2013; Salazar-Bravo et al. 2013). One of the most current interpretations of molecular and biogeographic data indicates that *Sigmodon* could represent one of the living lineages of the ancestral stock of murid rodents that gave rise to the sigmodontine radiation. After arriving in South America, this initial lineage rapidly radiated into all Sigmodontinae tribes (Oryzomyalia) to finally give rise to all current species (Steppan et al. 2004). The ancestral karyotype considered for *Sigmodon* is 2n = 52, FN = 52, proposed as ancestral for all sigmodontines (Swier et al. 2009). The absence of this chromosome complement in Phyllotini can be related to its high chromosome variability and/or to early chromosome differentiation events in the ancestral stock.

In earliest studies for Phyllotini, the proposed ancestral karyotype for the tribe was 2n = 70, FN = 68 (Pearson and Patton 1976), but only one species (*P. osilae*) has this karyotype. The genus *Calomys*, considered basal in the tribe by Steppan et al. (2007), presents a high 2n variation, but none of its species has

2n = 52 or 2n = 70. All species in this genus have a higher FN than that proposed as ancestral for Sigmodontinae. The possible exception of *Calomys musculinus* with FN = 48, reported by Massoia et al. (1968), should be confirmed with new chromosome data, because this is the only study to report this FN and the spermatogonial metaphase presented by the authors shows poor chromosome morphology definition. In *Calomys*, the proposed ancestral FN is the same as for phyllotines FN = 68 (Pearson and Patton 1976; Bonvicino et al. 2010), and this high FN is present in several species of the genus (Online Resource 1).

A new genus assigned to tribe Phyllotini, *Calassomys*, with a karyotype composed by 2n = 62, FN = 116, was recently discovered (Pardiñas et al. 2014). This chromosome description resembles *Andalgalomys* karyotypes, although both genera were not related in the phylogenetic analyses, and these high 2n and FN could have been acquired by independent events. The taxonomic position of *Calassomys* apicalis is contradictory, being recovered as the most basal genus in the tribe (Pardiñas et al. 2014; this study), or like sister taxa of *Calomys*, or nested inside *Calomys* (Salazar-Bravo et al. 2013). Our results do not contradict the inclusion of *Calassomys* in the tribe Phyllotini, and the lack of support could be consequence of the lack of nuclear DNA sequences (IRBP and RAG1). The different phylogenetic relationships obtained in different studies could be consequence of several causes: the outgroup design (our phylogenetic analysis was not performed to test the relations among Sigmodontinae tribes), the inclusion of different numbers of phyllotine species (excluding *Calassomys* 47 terminals, versus 20 in Salazar-Bravo et al. 2013, 10 used by Parada et al. 2013, and 25 in Carrizo and Catalano 2015), the different information sources (Cyt-b, versus IRBP gene in Salazar-Bravo et al. 2013, both genes combined in Parada et al. 2013, and the addition of RAG in Carrizo and Catalano 2015 and this study) and methods of analysis (MP, Bayesian and ML).

In the most inclusive phylogenetic hypothesis for Sigmodontinae, *Delomys* was recovered as sister to all Phyllotini (Parada et al. 2013; Pardiñas et al. 2014). This genus has tree species with chromosome complements that range between 2n = 72–82 and FN = 80–90, being 2n higher than those of *Calassomys* and *Calomys* (Gonçalves and De Oliveira 2014). Thus, all three ancestral lineages related to the Phyllotini clade have high 2n and FN, suggesting the ancestry of these chromosome characteristics.

In recent years, modern cytogenetic methods such as chromosome painting have contributed significantly to understanding the karyotypic evolution in several taxa. The data from Sigmodontinae indicates conservation of whole linkage groups of several chromosomes, as inferred by the relatively low frequency of chromosome probes that hybridized in more than one site in other karyotypes (Swier et al. 2009; Romanenko et al. 2012; Suárez et al. 2015; Leão Pereira et al. 2016). Despite that, only the smallest autosome pair and the X (with the exception of the neo-X of *S. delicatus*) appear conserved as independent linkage groups since the origin of the subfamily. Several syntenic blocks that probably were part of the ancestral Sigmodontinae karyotype were identified (Leão Pereira et al. 2016). However, most painting data for Sigmodontinae are incomplete, and studies on Phyllotini are absent (Romanenko et al. 2012). In sigmodontines, the ancestral karyotype is suspected to be 2n = 52 FN = 52 (Swier et al. 2009), a chromosome number close to the ancestral one proposed for several rodent clades (Romanenko et al. 2012). Yet, in phyllotines, none of the karyotypes reported has these exact chromosome characteristics. Our results indicate that the presence of 2n = 52 in *E. moreni* can be the product of homoplasy, but additional data with banding techniques are needed to support this hypothesis. Additionally, the mapping of karyotypes in the phylogeny suggests an early increase of the linkage groups and chromosome arms at the base of Phyllotini radiation. Thus, the clear non-conservation of chromosomes number and morphology throughout the tribe show significant contribution of chromosomes in the evolution of the phyllotine rodents as previously proposes (Reig 1986; Spotorno et al. 2001), reinforcing the role of chromosome changes in the sigmodontine radiation.

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