



The population of *Ctenomys* from the Ñacuñán Biosphere Reserve (Mendoza, Argentina) belongs to *Ctenomys mendocinus* Philippi, 1869 (Rodentia: Ctenomyidae): molecular and karyotypic evidence

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Abstract

Subterranean tuco-tucos of the genus *Ctenomys* are caviomorph rodents comprising a complex of over 50 nominal species found in the southern half of South America. The validity of several nominal forms awaits a proper assessment. The population of *Ctenomys* from Ñacuñán Biosphere Reserve (Mendoza, Argentina) has been classically considered to represent a distinct species and has been commonly referred as *Ctenomys* “*eremophilus*”. Based on molecular and cytogenetic analysis we assessed the taxonomic status of the *Ctenomys* population of Ñacuñán. Specimens analyzed showed two very similar chromosome complements (2n=48 and 2n=50), the latter being widely distributed in populations of *C. mendocinus*. Similarly, haplotypes recovered from Ñacuñán specimens are very similar and sister to those recovered from specimens of *C. mendocinus*. Considering this evidence we conclude that the individuals of *Ctenomys* from Ñacuñán population should be assignable to *C. mendocinus*.

Key words: Caviomorpha, Karyotype, Monte desert, Ñacuñán Reserve, species limits, taxonomy, Tuco-tucos

Introduction

Subterranean tuco-tucos of the genus *Ctenomys* Blainville, 1826 are caviomorph rodents with an impressive chromosomal diversity, with diploid numbers ranging from 10 to 70 and fundamental numbers from 16 to 84 (Reig *et al.* 1990). The extensive chromosomal diversity is not only at interspecific level, but also intraspecific variability occurs in many species of the genus (e.g., Freitas, 2007). Tuco-tucos are a complex of over 50 nominal species found in the southern half of South America. This is one of the most explosively speciating genus of mammals and chromosomal rearrangements may have played a key role in its diversification (Reig 1989; but see D’Anatro & D’Elía 2011). In spite of several molecular phylogenies which shed light on the main pattern of tuco-tuco diversification (see Parada *et al.* 2011 and references therein) much taxonomic work is needed in order to assess the status of several nominal forms, including several currently considered synonyms, with the final goal of clarify the number of living species.

A good example of this confuse taxonomic scenario is that of the *Ctenomys* population from Ñacuñán Biosphere Reserve (Mendoza, Argentina; hereafter Ñacuñán), nominated in a meeting abstract as *C. “eremophilus”* by Contreras and Roig (1975). Later the same population has been referred as to *C. “eremicus”* (Contreras 1979) and *C. “eremophilus”* (Contreras 1981); both names were again proposed without a formal description. Technically, *C. “eremophilus”* is not a *nomen nudum* (i.e., naked name) given that, although very poorly, Contreras and Roig (1975) intended to describe the putative new form. But certainly, *C. “eremophilus”* is not available, because its proposition constitutes an invalid nomenclatural act given that, as made, it contravenes some of the

provision of The Code, in particular the criteria of availability (ICZN, 1999; see article 13 regulating the publication requirements of names proposed after 1930). The latter two names, *C. "eremicus"* and *C. "eremophilus"*, are, as indicated by [Galliari et al. \(1996\)](#) for *C. "eremophilus"*, *nomina nuda*. However, several authors have kept using the name *C. "eremophilus"* to refer to tuco-tucos from the Ñacuñán (e.g. [Borruel et al. 1998](#); [Rosi et al. 2009](#)).

Ñacuñán (34° 02'S, 67° 58'W) is located 200 km southwest of the city of Mendoza, in the lowland Monte desert region (Roig & Rossi 2001). Ecological studies show that at Ñacuñán *Ctenomys* occurs over the whole types of habitat, although its highest activity was recorded in sand dune habitats with soft soils ([Albanese et al. 2010](#)). Populations of *Ctenomys* in other Monte localities around Ñacuñán are referred to *C. mendocinus* Philippi, 1869 a widespread species in west-central Argentina ([Rosi et al. 2002](#)). In the abstract where the name *C. "eremophilus"* was proposed no diagnostic character is mentioned. However, a morphometric analysis conducted on skull measurements indicated that the population of Ñacuñán is to some extent smaller than a sample assigned to *C. mendocinus* (Rosi et al. 1992). A previous cytogenetic study conducted by [Massarini et al. \(1991a\)](#) reported, on the basis of one female from Ñacuñán a karyotype of 2n=50 (FN=80), which is similar to the karyotype of 2n=48 reported for *C. mendocinus*. These authors stated that the "small karyotypic differences" between the 2n=48 and 2n=50 karyomorphs are "not conclusive enough to indicate species-level differentiation."

Besides the nomenclatorial issue previously referred, the degree of distinction of *C. "eremophilus"* respect to other species of *Ctenomys*, in particular *C. mendocinus*, remains unclear; therefore, the need of erect a new name for the population of *Ctenomys* from Ñacuñán has to be further assessed. Given these antecedents, the aim of this study is to evaluate the taxonomic status of the population of *Ctenomys* from Ñacuñán by means of cytogenetic and genetic analyses.

Material and methods

Voucher specimens, tissue samples, and cell suspensions are housed in the Mammal Collection of the Instituto Argentino de las Zonas Áridas (CMI, IADIZA), CCT CONICET, Mendoza, Argentina. Seven specimens of *Ctenomys* (6 males: CMI 07219; CMI 07220; CMI 07225; CMI 07226; CMI 07227; CMI 07229 and 1 female: CMI 07222) from Ñacuñán were karyotyped using standard chromosome techniques. Mitotic metaphases from direct bone marrow were obtained following a colchicine-hypotonic technique (Verma & Babu 1995). Non-differential chromosome staining was performed in phosphate-buffered Giemsa (pH=6.8). C-bands were induced by the barium hydroxide technique of Sumner (1972). Ten metaphase spreads were counted for each specimen. Fundamental Numbers (FN) refer only to autosomes ([Patton 1967](#)).

Phylogenetic analysis was based on 41 complete sequences (1140 base pairs) and 5 partial sequences of the mitochondrial gene that codes for the cytochrome *b* (*cyt-b*) protein. As previous to this study the placement of variants from *Ctenomys* from Ñacuñán within the radiation of *Ctenomys* is uncertain, taxonomic coverage was broad as a way to provide a robust test for their placement. Taxonomic coverage encompasses haplotypes representing 39 species of tuco-tucos, including all available sequences of the *C. mendocinus* species group (sensu [Massarini et al. 1991b](#)). GenBank accession numbers of the analyzed haplotypes of *Ctenomys* are presented in Figure 3. Sampling includes sequences of 2 specimens from Ñacuñán (CMI 07219: GenBank accession number JN542717; CMI 07222: JN542718) acquired in the present study following the protocol outlined in [Parada et al. \(2011\)](#). The outgroup consisted of 4 sequences gathered from representatives of the closely related family Octodontidae (GenBank accession numbers: AF007058 and AF007060), Echimyidae (L23341) and Capromyidae (AF422915). Sequence alignment was done as in [Parada et al. \(2011\)](#).

Base substitutions per site were computed using the observed p-distance model and pairwise deletion using MEGA 5.03 ([Tamura et al. 2011](#)). Maximum parsimony (MP) analysis was done with TNT 1.1, May 2011 ([Goloboff et al. 2008](#)). The shortest trees were found using the default settings for "New Technology Search." The relative support for each clade was obtained with 400 Poisson bootstrap (Pb) replicates and with Bremer support (Bs). Model based analysis was done using the model of sequence evolution HKY85, which was selected by ModelGenerator with BIC criteria ([Keane et al. 2006](#)). Bayesian inference was done with BEAST v1.6.1 ([Drummond & Rambaut 2007](#)) using 4 substitution categories, an estimated gamma distribution and an estimated proportion of invariable sites. An uncorrelated lognormal clock, a mean substitution rate of 1.0 substitution per site

along with a Yule prior on branching rates were used. Two independent 10 million-length runs were used with the first 1 million of generations of each run discarded as burn in. Posterior probabilities (P) were used as an estimate of branch support.

Results

Our cytogenetic analysis shows that the population of Ñacuñán is polymorphic; two different karyomorphs are displayed (Fig. 1 and 2). We found specimens bearing the already known karyomorph of $2n = 50$, FN= 80 (Massarini *et al.* 1991a, Massarini *et al.* 1991b) and specimens displaying a $2n=48$, FN= 76 (Fig. 1). The $2n= 50$, FN=80 karyomorph is indistinguishable from that reported for a female from Ñacuñán by Massarini *et al.* (1991a) and consists of 16 pairs of biarmed chromosomes (metacentric to subtelocentric) and eight pairs of acrocentric chromosomes; the X is a metacentric chromosome and the Y is a small subtelocentric chromosome (Fig. 2). Individuals with a $2n=48$, FN= 76 had an autosome complement of 15 pairs of biarmed chromosomes (metacentric to subtelocentric) and 8 pairs of acrocentric chromosomes. The pair 1 of the autosome complement is a subtelocentric and heteromorphic pair. The X is a metacentric chromosome and the Y is a subtelocentric chromosome (Fig. 1). Importantly, the $2n=48$, FN= 76 cytotype from Ñacuñán, reported for the first time here, is alike to the karyotype reported for specimens of *C. mendocinus* from different localities from Mendoza Province (Massarini *et al.* 1991b).

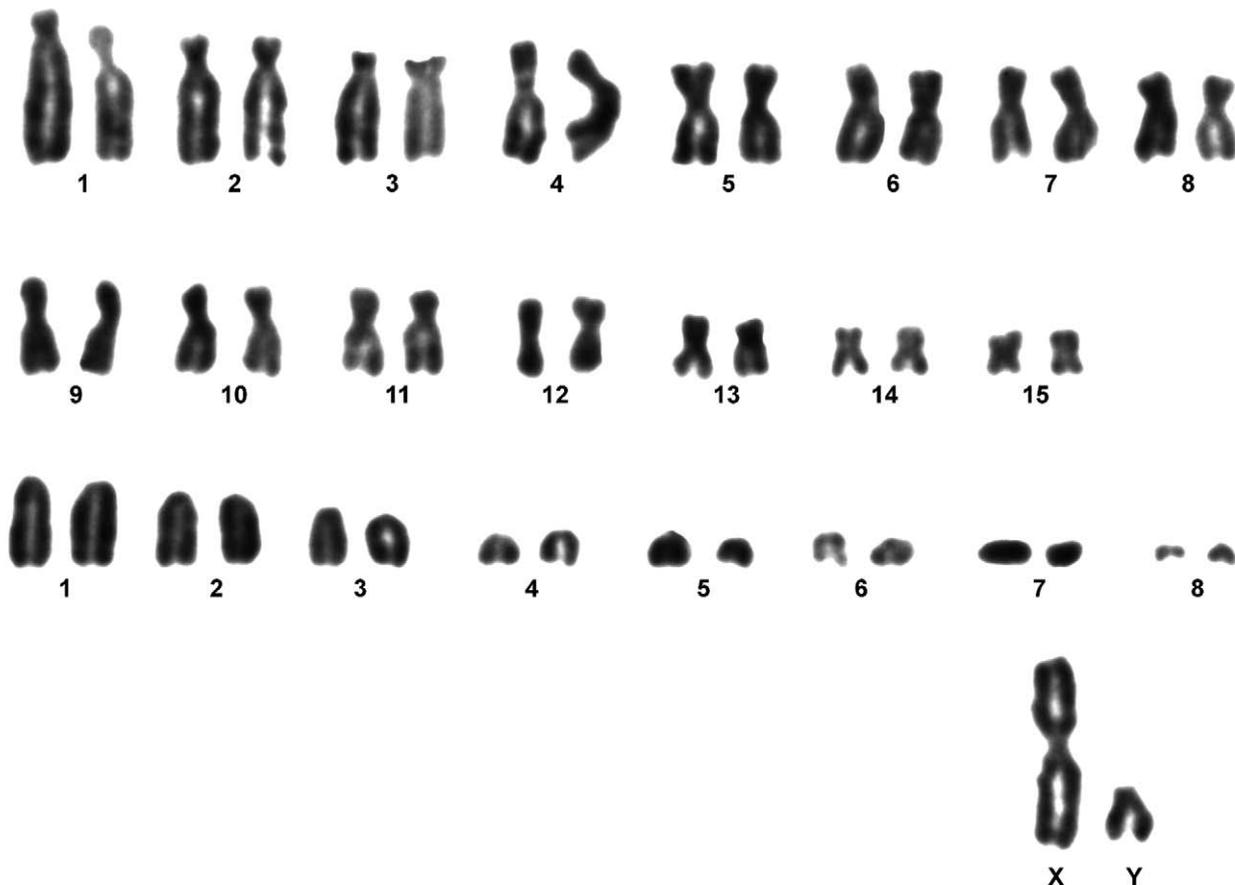


FIGURE 1. Standard Giemsa-stained karyotype of *Ctenomys mendocinus* from Ñacuñán, karyomorph $2n = 48$, FN = 76 (CMI 07227).

The branching pattern of the *Ctenomys* obtained under MP and Bayesian inference (Fig. 3) is in agreement with the topologies found in previous studies (*e.g.*, D'Elía *et al.* 1999, Castillo *et al.* 2005, Parada *et al.* 2011); therefore, for issues beyond *Ctenomys* from Ñacuñán we refer the reader to Parada *et al.* (2011) and references therein. The MP analysis retrieved 4 shortest trees of 1701 steps (Consistency Index=0.393; Retention

Index=0.556). A clade formed by *C. mendocinus* group and allies (*C. sp.* Tupungato, *C. flamarioni* Travi, 1981, and *C. rionegrensis* Langguth and Abella, 1970) is recovered with strong support (Pb=96; Bs=6; P=1), and is sister to the species group of *C. talarum* Thomas, 1898. Within the *C. mendocinus* group, haplotypes of two individuals from Ñacuñán which present different diploid numbers (CMI 07219: 2n=48; CMI 07222: 2n=50), form a clade (Pb=50; Bs=1; P=0.98) sister (Pb=56; Bs=1; P=0.87) to a clade (Pb=87; Bs=2; P=0.98) formed by an haplotype recovered from a topotype of *C. mendocinus* (Cerro de la Gloria, Mendoza; labeled *mendocinus* 1) and one gathered from a specimen of *C. mendocinus* from Las Heras, Mendoza (labeled *mendocinus* 2). The observed divergence (1.2%) among haplotypes from Ñacuñán and those recovered from specimens referred to *C. mendocinus* is comparable to those found in intraspecific comparisons (e.g. Parada *et al.* 2011). Moreover, the p-distance between haplotypes *mendocinus* 1 and CMI 07219-CMI07222 (0.62%) is smaller than that observed between haplotypes *mendocinus* 1 and *mendocinus* 2 (0.89%).

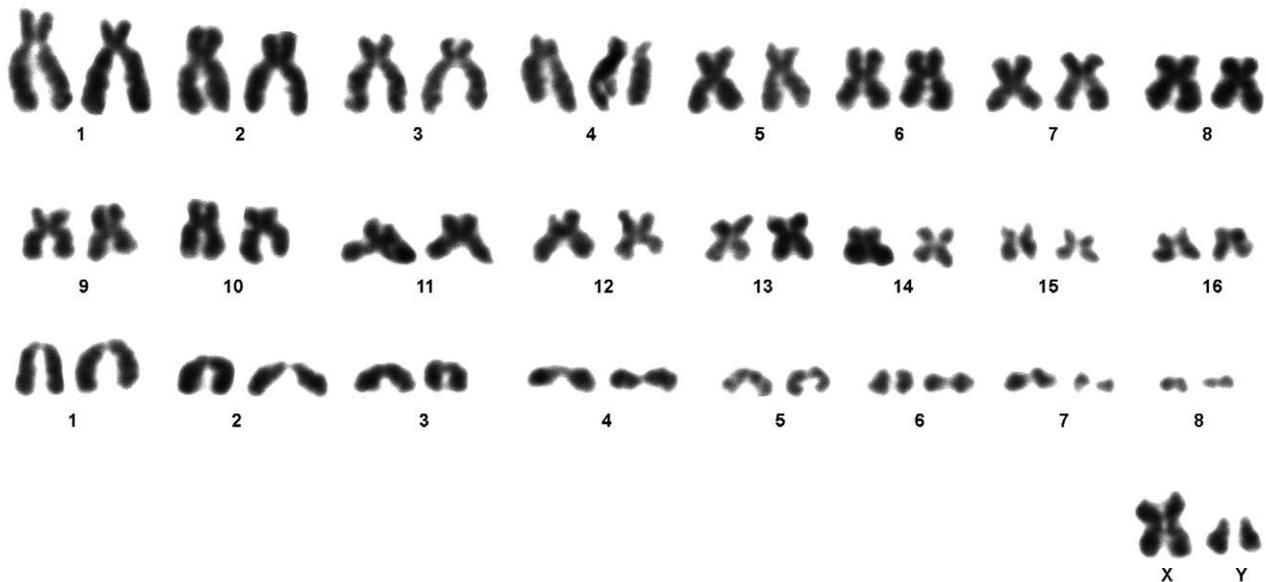


FIGURE 2. Standard Giemsa-stained karyotype of *Ctenomys mendocinus* from Ñacuñán, karyomorph 2n = 50, FN = 80 (CMI 07226).

Discussion

The taxonomic status of the population of *Ctenomys* from Ñacuñán, in Central Mendoza (Argentina) remains unclear. It has been suggested (Contreras and Roig 1975) that it represents a distinct species and as such it has been considered in several publications (e.g., Giannoni *et al.* 1996, Borghi *et al.* 2002, Rosi *et al.* 2009). However, evidence showing the specific distinctiveness of tuco-tucos from Ñacuñán is far from conclusive (Massarini *et al.* 1991a, Rosi *et al.* 2002). Tuco-tucos from Ñacuñán showed two very similar chromosome complements (2n=48 and 2n=50; Figs. 1 and 2). The 2n=48 complement, reported here for the first time, is found in populations of *C. mendocinus* (Massarini *et al.* 1991b), the species of *Ctenomys* widely distributed in west central Argentina, including the surroundings of Ñacuñán. In addition, *cyt-b* haplotypes recovered from specimens collected at Ñacuñán are remarkably similar and sister to those recovered from specimens of *C. mendocinus*, including one haplotype that is a topogenotype (sensu Chakrabarty 2010) of this taxon.

Considering the available cytogenetic and molecular evidence we suggest that the population of *Ctenomys* from Ñacuñán, referred sometimes as to *C. "eremofilus"* as well as to *C. "eremicus"* and *C. "eremophilus,"* belongs to the widespread species *C. mendocinus*. As such, there is no need to formalize a new name to encompass a presumably distinct species endemic to the Reserve of Ñacuñán. We contextualize our taxonomic hypothesis with the following two tree facts. First, Rosi *et al.* (1992) found that a sample of specimens of *Ctenomys* from Ñacuñán presents slightly smaller values for most cranial measurements than a sample of specimens referred to *C. mendocinus*. After noting that no qualitative character state has been reported to differentiate Ñacuñán specimens from *C. mendocinus*, we state that further studies, including the analysis of a larger and geographically wider

sample, are needed to contextualize the degree of differentiation in *C. mendocinus* group. In particular, the contents of the sample referred to *C. mendocinus* should be carefully amassed since several populations of *Ctenomys* from Mendoza, even some close to the type locality of *C. mendocinus*, do not belong to this species (Parada *et al.* 2011). On the other hand it is worth noting that Cicchino and Castro (1998) reported that all but one ectoparasite species found on specimens from different localities of *C. mendocinus* were also found on other species of the genus. The exception being *Phtheiropoios mendocinus*, which is also present on specimens from Ñacuñán, supporting our hypothesis that the population of *Ctenomys* from Ñacuñán belongs to *C. mendocinus*.

In his catalogue of *Ctenomys* from Argentina, Bidau (2006) listed *C. "eremophilus"* as a synonym of *C. pontifex* Thomas, 1918. The latter, is an obscure form known only from its type material, which most likely comes from the area of the Peteroa Volcano in the Argentinean-Chilean border (see Pearson and Lagiglia, 1992). The basis of Bidau (2006) taxonomic suggestion is unknown and it is somehow unexpected given the distance between Ñacuñán and the Peteroa Volcano (ca. 270 km), the different environments of both places, and in particular due to almost nothing is known about *C. pontifex*. We have not included representatives of *C. pontifex* in our study, but as *C. mendocinus* has priority over *C. pontifex*, our suggestion of *C. "eremophilus"* being synonym of *C. mendocinus* is not falsified if as stated by Bidau (2006) *C. "eremophilus"* and *C. pontifex* represent the same species.

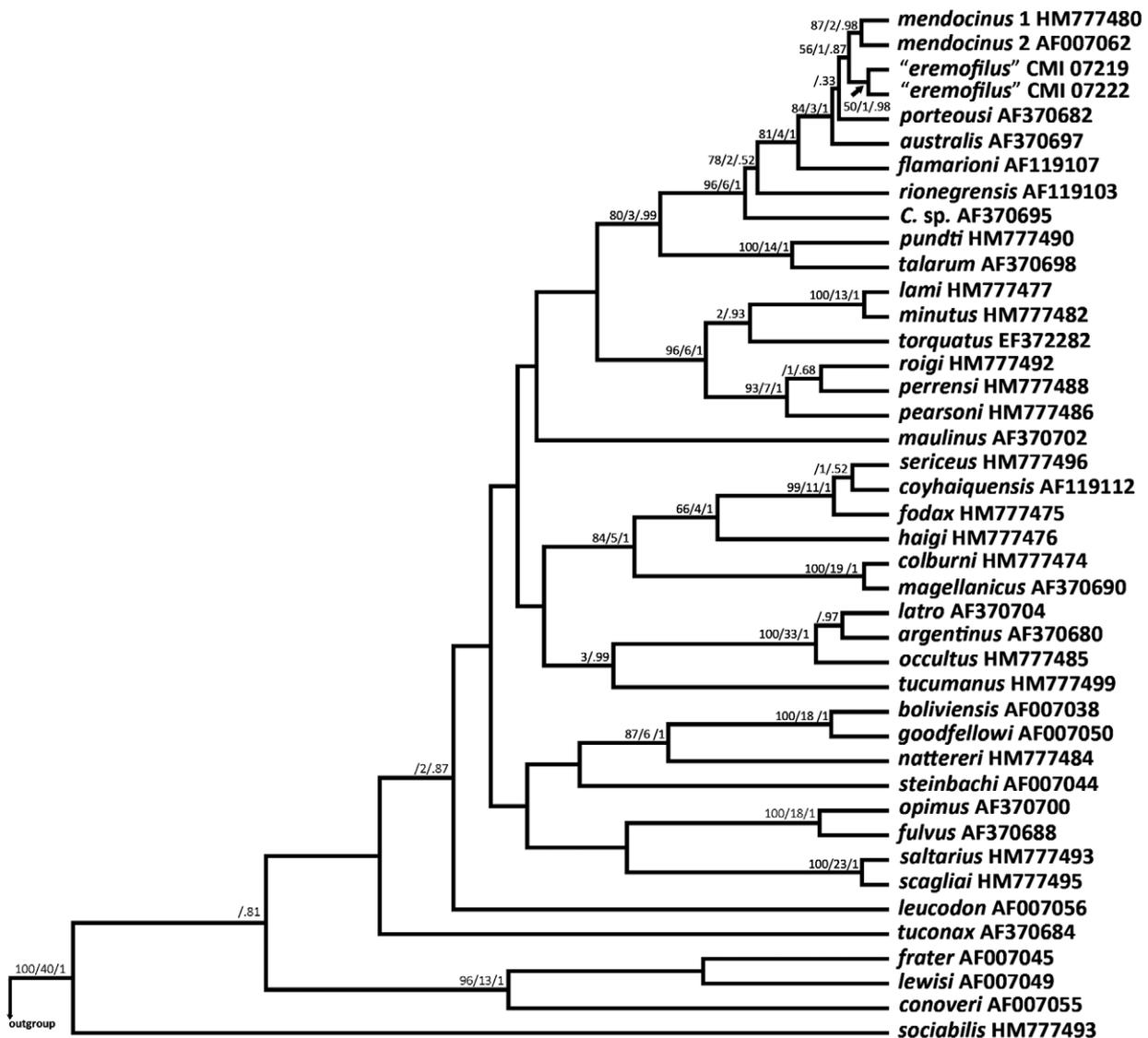


FIGURE 3. Phylogenetic tree resulting from the Bayesian analysis of the *cyt-b* gene sequences of *Ctenomys*. The outgroup is not shown. Numbers indicate support (Bootstrap [only those above 50 % are shown], Bremer support, and Posterior probabilities) of the nodes at their right (see details in text). For those sequences retrieved from GenBank, accession numbers are provided next to species labels. For those sequences gathered by us, specimen collection numbers are provided next to species label.

In a related issue, it is worth noting that the close relationship to *C. mendocinus* of *C. australis* Rusconi, 1934, *C. azarae* Thomas, 1903, and *C. porteousi* Thomas, 1916 has been discussed since their karyotypes were reported; all forms share a $2n=48$ and the same G-band pattern (Massarini et al. 1991b). These taxa (note that *C. azarae* was not included in our analysis) also show limited genetic divergence and form with *C. mendocinus* a strongly supported clade (Bp=84; Bs=3; P=1). Therefore, the available evidence cast doubts on the specific distinctiveness of these taxa; i.e., *C. australis*, *C. azarae*, and *C. porteousi* might be synonyms of *C. mendocinus* (Massarini et al. 1991, Slamovits et al. 2001, Parada et al. 2011). In this regards it is important to note that in our analysis *C. mendocinus* is not recovered paraphyletic with respect to any of these forms. As such, the formalization of the above mentioned classificatory scenario, should await the study of a larger sample of these forms, especially one including a broader geographic coverage of *C. mendocinus*, representatives of *C. azarae*, and topotypical material of all nominal forms.

To close this discussion, we remark the fact that our analyses show that *C. mendocinus* is another species of tuco-tucos (e.g., *C. lami* Freitas, 2001, *C. pearsoni* Lessa and Langguth, 1983; *C. perrensi* Thomas, 1898; see Freitas 2007, Novello and Altuna 2002, Garcia et al. 2000) variable at the karyotypic level. At least one population of *C. mendocinus*, that of Ñacuñán, is chromosomically polymorphic. This observation is of relevance given the key role that cytogenetic evidence had, and still has, in the taxonomic practice on *Ctenomys* populations. In the decade of 1960 with the pioneer and influential work of Pablo Kibliskey and Osvaldo A. Reig (e.g., Kibliskey and Reig 1966, Reig and Kibliskey 1968, 1969) attempts aimed to establishing species boundaries of tuco-tucos started using karyotypic evidence. Thereafter, a long and productive tradition of cytogenetic studies on populations of *Ctenomys* started with research groups actively working in Argentina, Brazil, Chile, and Uruguay. Behind this extended approach is the notion that chromosome differences are, in most cases, an effective reproductive barrier. For instances, based solely on chromosome evidence it has been suggested that some of the karyomorphs of *Ctenomys pearsoni* may represent undescribed biological species (Altuna et al. 1999, González 2001); the similar suggestion was advanced considering some Patagonian karyomorphs (Bidau et al. 2003).

There is a widespread notion that chromosome rearrangements, contextualized in the typical patchy population structure of *Ctenomys* (Reig et al. 1990), are a main triggering factor in the diversification of the genus; i.e., several, if not most, speciation events in the genus *Ctenomys* were cases of chromosomal speciation (Reig 1989, Ortells 1995). It remains unclear if these processes are prevalent across the whole distribution of this genus. Previously, Slamovits et al. (2001; see also Ellingsen et al. 2007) assessed changes in the copy number of the repetitive PuvII *Ctenomys* sequence (RPCS), the major *Ctenomys* satellite DNA. These authors found an association between changes in the amount of RPCS and chromosomal rearrangements. Hence, assisted with more carefully species delimitation it should be of particular interest testing if the karyotypic differentiation among certain groups of this genus has promoted speciation or if it has been just preserved as a result of phyletic branching.

In summary, in light of our results, and those of similar studies, we should call for caution on solely using karyotypic data towards the delimitation of species boundaries in *Ctenomys*, in particular when small sample sizes are analyzed and comparisons with other nominal forms are sparse. We note that this statement frames in a somehow emerging consensus on the need of integrate different lines of evidence to delimit tuco-tucos species (see Bidau et al. 2003).

In spite of recent efforts toward the clarification of species boundaries within *Ctenomys* (e.g., Fernandes et al. 2009, Gonçalves et al. 2009, Mirol et al. 2010, D'Anatro & D'Elía 2011), the alpha taxonomy of the genus is far from being resolved. Large geographic areas (e.g., Patagonia) remain mostly understudied as well as several nominal forms including *C. validus* Contreras, Roig, and Suzarte, 1977, *C. coludo* Thomas, 1920, *C. tulduco* Thomas, 1921, and *C. pontifex*, are known only from few specimens collected decades ago and have not been evaluated with current taxonomic approaches. The clarification of these and other taxonomic problems is much needed to better understand the evolutionary diversification of tuco-tucos.

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