Journal of Mammalian Evolution, Vol. 6, No. 1, 1999

Molecular Phylogeny of Tuco-Tucos, Genus *Ctenomys* (Rodentia: Octodontidae): Evaluation of the *mendocinus* Species Group and the Evolution of Asymmetric Sperm

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The phylogenetic relationships among 23 individuals representing 14 species of underground hystricognath rodents of the genus *Ctenomys* were studied by analyzing variation of complete cytochrome *b* gene sequences. Maximum parsimony, neighbor joining, and maximum likelihood analyses were performed, using the octodontine genera *Octodon* and *Tympanoctomys* as outgroups. Our analyses support previous studies based on chromosomes and skull morphology that suggested a clade comprised of Argentinean and Uruguayan populations of *C. rionegrensis*. This clade is closely related to one comprised of *C. flamarioni* and the *C. mendocinus* species complex. Our analyses provide evidence that the symmetric sperm morph, which is common to other South American hystricognath rodents, is the plesiomorphic character state in *Ctenomys* and in Hystricognathi. Our analyses do not support the hypothesis that the sperm morphs define two major lineages of tuco-tuco species, because species with asymmetric sperm are diphyletic on the basis of cytochrome *b* sequences, and this morphology appears to have evolved twice in *Ctenomys*.

KEY WORDS: tuco-tuco; *Ctenomys*; systematics; cytochrome *b*; sperm evolution; Hystricognathi.

INTRODUCTION

The rodent genus *Ctenomys* (tuco-tucos) has good potential as a system to study speciation (e.g., Reig and Kiblisky, 1969), but investigations of the group have been hampered by problematic taxonomy and difficulty in identifying sister clades. These subterranean hystricognath rodents of South America underwent substantial cladogenesis beginning in the early Pleistocene (Reig and Quintana, 1992; Lessa and Cook, 1998). Although more than 50 species are recognized currently (Reig *et al.*, 1990), many named forms are in need of revision (Woods, 1993). Recent work has continued to unveil new species (e.g.,

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1064-7554/99/0300-0019$16.00/0

Kelt and Gallardo, 1994). High species diversity is accompanied by extreme chromosomal variation; *Ctenomys* diploid numbers range from $2n = 10$ to 70 (Novello and Lessa, 1986; Cook *et al.*, 1990) and the group has been mentioned in various reviews of the speciation process in mammals (e.g., White, 1978; Reig, 1989).

A well-corroborated phylogeny may provide a foundation for further investigations of the evolution of this diverse group. To date, tuco-tuco classification has been based primarily on pelage color, cranial morphology, and body size. Thomas (1916) described the subgenus *Haptomys*, comprised only of *C. leucodon*, as different from other tuco-tucos (subgenus *Ctenomys*) because incisors are extremely proodont. Later, Osgood (1946) described a third subgenus (*Chacomys*) for *C. conoveri*, a very large species (>1 kg).

Recently, another major division of the genus *Ctenomys* has been suggested based on morphological variation of the sperm. An asymmetric sperm morph is found mainly in southern species, and a simple (symmetric) form occurs in the northern species (Feito and Gallardo, 1982; Vitullo *et al.*, 1988; Vitullo and Cook, 1991). The symmetric sperm is thought to be plesiomorphic, because it is present in octodontids, the rest of South American hystricognaths, and in most mammals (Vitullo *et al.*, 1988).

Another classification (Rossi *et al.*, 1993) was based on the amount of a satellite sequence in the nuclear genome. Other major groups are based on penial morphology (Balbontin *et al.*, 1996) and karyotypes, especially diploid and fundamental numbers and C- or G-band patterns. Based on the similarity of these characters, Massarini *et al.* (1991) proposed the *C. mendocinus* group, a clade comprised of five closely related species with a diploid complement of $2n = 48$ and heterochromatic blocks in whole chromosomal arms.

Only four strictly phylogenetic analyses have been completed with species of *Ctenomys*, and these included only Bolivian and Argentinean species. The first, based on qualitative morphological features and karyotypic characters for six Bolivian species, was part of a coevolution study (Gardner, 1991). Cook and Yates (1994) studied the evolutionary relationships of seven species and an undescribed form from Bolivia (based on allelic frequencies of 21 allozymic loci). In the third cladistic analysis, Ortells (1995) studied relationships among 11 species and five karyomorphs of Argentinean *Ctenomys* based on G-band patterns and suggested the monophyly of the "Corrientes" and *C. mendocinus* groups. Finally, Lessa and Cook (1998) analyzed sequences of the mitochondrial cytochrome *b* gene of Bolivian and Argentinean species. They concluded that cytochrome *b* sequences were useful for identifying major clades of tuco-tucos. However, the initial speciation process in this group resulted in a basal polytomy. They suggested the subgenus *Chacomys* was invalid, but tentatively supported *Haptomys* (Thomas, 1916). The diphyly of the species bearing asymmetric sperm was also suggested (Lessa and Cook, 1998).

We have expanded the phylogenetic analysis of *Ctenomys*. The aims of this study are to test the monophyly of the group of species with asymmetric sperm, suggested by Vitullo *et al.* (1988) and Vitullo and Cook (1991), and examine the monophyly and relationships of the *mendocinus* species group, as defined by Freitas (1994). These two issues are related, since species of the *mendocinus* group have asymmetric sperm. We obtained sequences of the cytochrome *b* gene from species not previously studied. These were analyzed together with species previously identified within the major clades of tuco-tucos (Lessa and Cook, 1998).

MATERIALS AND METHODS

Specimens Examined

Complete sequences of the mitochondrial cytochrome *b* gene were obtained from 12 individuals representing 5 species (10 populations) of *Ctenomys: C. rionegrensis* (*N* = 5)—Uruguay: Río Negro, El Abrojal (CA 408), Los Arrayanes (CA 440), and Las Cañas (CA 392); Argentina: Entre Ríos, Ibicuy (MNCN 1716), and Paraná (MNCN 2065); *C. flamarioni* (*N* = 1)—Brazil: Río Grande do Sul, Taim (T 29); *C. pearsoni* (*N* = 1)—Uruguay: Rocha, El Potrerillo (CA 722); *C. torquatus* (*N* = 3)—Uruguay: Río Negro, El Trillo (CA 654 and CA 656); Tacuarembó, Iporá (CA 743); and *C. coyhaiquensis* (*N* = 2)—Chile: XI Región, Chile Chico (FMNH/IEEUCH 134264/3647 and FMNH/IEEUCH 134296/4436). Voucher specimens for these individuals are located in the Laboratorio de Evolución, Facultad de Ciencias, Universidad de la República, Uruguay (CA); Museo Nacional de Ciencias Naturales de Madrid, España (MNCN); Departamento de Genética, Universidad Federal do Rio Grande do Sul, Brazil (T); Division of Mammals, Field Museum of Natural History, Chicago, USA (FMNH), and Instituto de Ecología y Evolución, Universidad Austral de Chile (IEEUCH). In addition, one sequence of *C. coyhaiquensis* (Genbank accession number AF071753) was obtained from Cook and Lessa (1998). Sequences of *C. boliviensis* (AF007037 and AF007039), *C. conoveri* (AF007055), *C. frater* (AF007046), *C. haigi* (AF007063), *C. leucodon* (AF007056), *C. mendocinus* (AF007062), *C. opimus* (AF007042), *C.* sp. "minut" (AF007052), and *C. steinbachi* (AF007044), as well as of the outgroup octodontines *Octodon degus* (AF007059) and *Tympanoctomys barrerae* (AF007060), were obtained from Lessa and Cook (1998). These 14 species of tuco-tuco were from five countries (Argentina, Bolivia, Brazil, Chile, and Uruguay), including species with symmetric and asymmetric sperm, and covering the entire range of diploid variation ($2n = 10$ to $2n = 70$). New sequences have been deposited in GenBank under accession numbers AF119103—AF119114.

Data Collection

Liver tissue, either frozen in liquid nitrogen and stored at −70°C or preserved in 95% ethyl alcohol, was used as the source of DNA. Mitochondrial DNA extractions and purification from frozen samples used Wizard Minipreps (Promega) following Beckman *et al.* (1993). Total DNA extractions from alcohol-preserved samples were made with SDS/proteinase K/NaCl extraction/alcohol precipitation (modified from Miller *et al.*, 1988; Maniatis *et al.*, 1992). Amplifications (50 μl final volume) of the entire cytochrome *b* gene and partial segments were obtained by polymerase chain reaction (PCR) with the following primers: MVZ 05, 16, and 14 (Smith and Patton, 1993) and TUCO 04, 37, and 23 (Lessa and Cook, 1998). The PCR amplification conditions were 30 cycles of 1 min of denaturation at 93°C, 1 min of annealing at 45°C, and 1.5 min of extension at 72°C. Negative controls were included in all PCR experiments.

PCR products were precipitated with 30% polyethylene glycol (PEG 3350), 1.5 *M* NaCl, recovered by vacuum centrifugation and resuspended in 1X TE buffer for cycle sequencing templates utilizing a Perkin–Elmer kit (Fst-RR, 402119). The sequences were run on 2% polyacrylamide gels using an automated sequencer (ABI 373). In all cases both heavy and light DNA strands were sequenced and compared.

Sequence and Phylogenetic Analyses

Alignment of partial sequences was done by eye with the program Sequence Navigator. MEGA 1.02 (Kumar *et al.*, 1993) was used to analyze nucleotide composition. Three methods of phylogenetic reconstruction (maximum parsimony, neighbor joining, and maximum likelihood) generated hypotheses concerning relationships among the species and populations of *Ctenomys*. In all analyses, *Octodon degus* and *Tympanoctomys barrerae* were designated as outgroups.

PAUP 3.1.1 (Swofford, 1993) was utilized to generate maximum parsimony (MP) cladograms using 250 replications of heuristic searches (tree bisection and reconnection). Characters were given equal weight or weighted 2, 5, and 1 for first, second, and third codon positions respectively. These conditions were combined with two different step-matrices assigning a greater weight (5 : 1 or 10 : 1) to transversions over transitions. The phylogenetic signal was assessed by generating 10,000 random trees, and analyzing the g_1 value (Hillis and Huelsenbeck, 1992). Relative support for clades was assessed by performing 1000 bootstrap replications (allowing the branches with less than 50% support to collapse) and by estimating the decay index (Bremer, 1988) for the clades found on the shortest trees (250 replications of heuristic search with the same conditions used in the search for the most parsimonious trees).

MEGA 1.02 (Kumar *et al.*, 1993) was used to reconstruct the phylogenetic history with the neighbor joining (NJ) algorithm (Saitou and Nei, 1987) using the distance of Tamura–Nei (1993). This model takes into account differences in nucleotide frequencies, as well as different rates for transitions and transversions (Tamura and Nei, 1993; Kumar *et al.*, 1993). One thousand bootstrap replications were performed using MEGA 1.02 to assess the support of the clades obtained. DNAML from PHYLIP 3.5c (Felsenstein, 1993) was used for maximum likelihood (ML) searches. Following Lessa and Cook (1998), first, second, and third positions were defined with relative rates of 2, 5, and 1, respectively, and transversions were weighted 6 to 1 over transitions. The search was performed with five replications with the taxa added randomly.

Finally, to evaluate the strength of the phylogenetic hypotheses we compared the best maximum likelihood and parsimony trees to trees constrained to alternative hypotheses of relationships from the literature. Heuristic searches in PAUP were constrained so only trees conforming to predefined hypotheses (e.g., species with asymmetric sperm forming a monophyletic group) were retained. The Kishino–Hasegawa (1989) test, as implemented in DNAML (Felsenstein, 1993), was used to assess the statistical significance of differences among topologies.

RESULTS

Nucleotide Sequences

The mitochondrial cytochrome *b* gene of tuco-tucos has 1140 base pairs (corresponding to 379 amino acids and an AGA or AGG stop codon). Compositional bias (Table I) was similar to that previously reported for mammals (Irwin *et al.*, 1991; Lara *et al.*, 1996; Ma *et al.*, 1993) and includes a guanine deficit (12.4%) in the light strand, especially in the third codon position (2.6%).

Intrapopulation variation was analyzed in *Ctenomys coyhaiquensis* (Chile Chico

Table I. Average Nucleotide Composition of Cytochrome *b* Gene Sequences Across 23 *Ctenomys* Individuals (14 Species) and 2 Octodontine Specimens[a]

	Total	1st	2nd	3rd
A	31.3	30.4	20.3	43.2
T	30.3	27.2	40.8	22.9
C	26.0	21.5	25.3	31.3
G	12.4	20.9	13.7	2.6

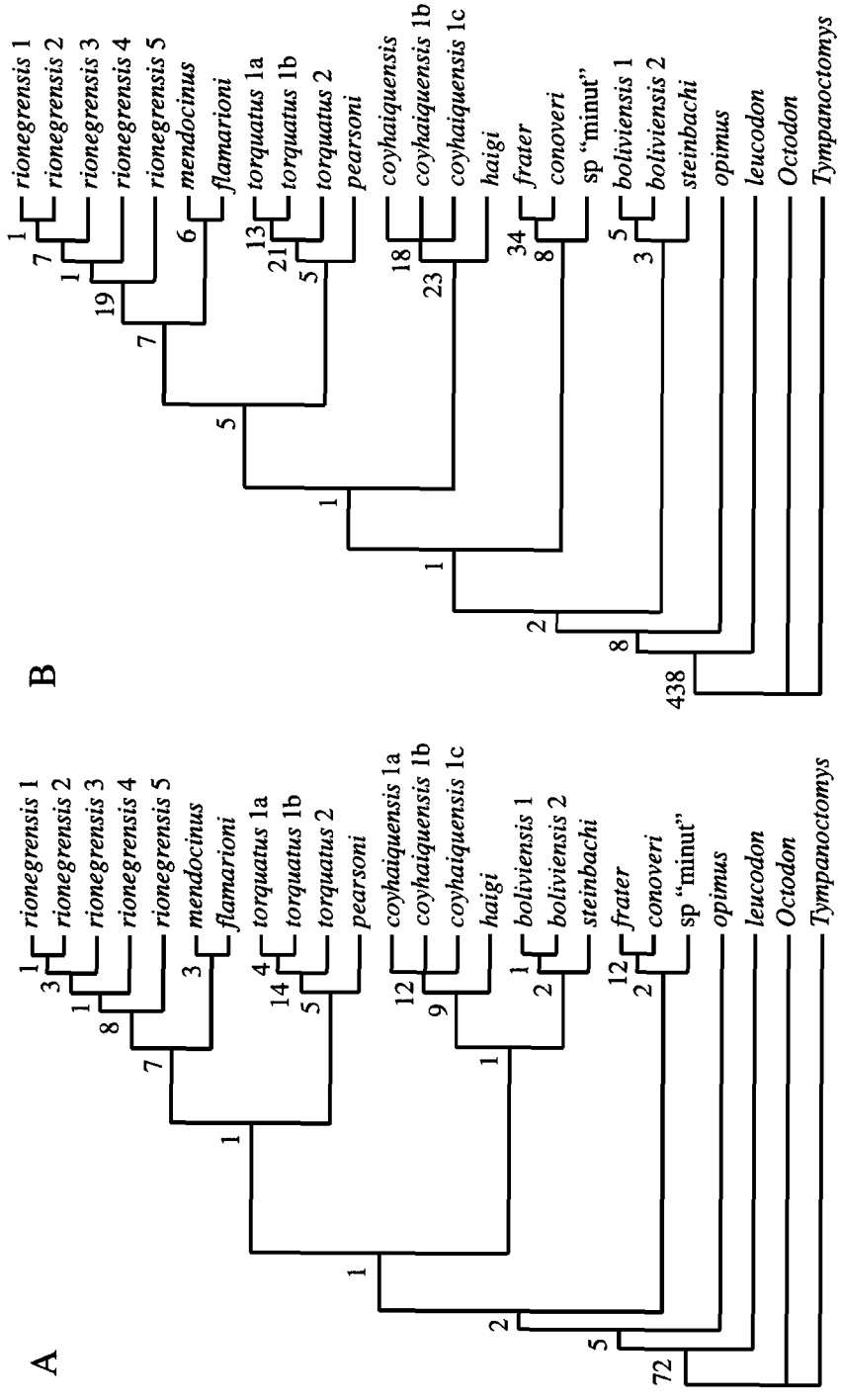
[a]Values are expressed as total percentages and discriminated by codon position. A, adenine; T, thymine; C, cytosine; G, guanine.

population) and *C. torquatus* (El Trillo population). Uncorrected observed divergence among haplotypes of the same locality ranged from 0 to 0.18% (zero to two substitutions). Comparisons among haplotypes of different populations of the same species showed an uncorrected divergence of 1.14% between populations of *C. torquatus*, 0.35 to 1.58% between populations of *C. rionegrensis*, and 5.7% between populations of *C. boliviensis* (13, 6, and 46 substitutions in first, second, and third codon positions respectively). However, the high value between these populations of *C. boliviensis* corresponds to chromosomal and morphological differences that may indicate these are distinct species (Cook, unpublished data). Observed sequence divergence between tuco-tuco species varied between 2.98% for *C. mendocinus–C. flamarioni* and 12.46% for *C. leucodon–C. conoveri*. Observed divergence between the octodontine genera (*Tympanoctomys* and *Octodon*) was 14.65%. Observed sequence divergence between the two octodontid subfamilies, Octodontinae and Ctenomyinae, ranged between 19.82% for *C. frater–Octodon* and 18.16% for the pairs *C. mendocinus–Octodon*, *C. torquatus–Octodon*, and *C. mendocinus–Tympanoctomys*.

Phylogenetic Analysis

Of the 423 variable sites, 286 (25.1% of total gene) were parsimony informative (57, 21, and 208 in first, second, and third positions, respectively). When 10,000 random trees were computed under the three weighting schemes (equally weighted; 5 : 1, 2 : 5 : 1; and 10 : 1, 2 : 5 : 1), the g_1 values (−1.002612, −3.731660, and −4.636382, respectively) were significant ($P < 0.01$) (Hillis and Huelsenbeck, 1992), indicating that the data set is phylogenetically informative (Hillis, 1991).

The most parsimonious trees (Figs. 1A–C) found under the three search conditions define six major clades: (1) a clade of 11 specimens belonging to five species located centrally in the geographic distribution of the genus. *C. mendocinus* and *C. flamarioni* are sister species, and these, together with *C. rionegrensis*, form a sister clade to a clade of *C. pearsoni* and *C. torquatus*; (2) a clade composed of species of southern distribution (*C. haigi* and *C. coyhaiquensis*); (3) a lowland Bolivian clade of *C. boliviensis* and *C. steinbachi*; (4) a clade of Chaco and intermediate elevation species including the Bolivian forms *C. frater*, *C. conoveri*, and *C.* sp. "minut"; (5) the altiplano species *C. opimus*; and (6) another Bolivian altiplano species, *C. leucodon*. The latter always appears as the sister species of the remaining tuco-tucos. These six clades occur in the consensus tree (Fig. 1D); however, the relationships among them are unstable (Figs. 1A–C), as indicated by


A
rionegrensis 1
rionegrensis 2
rionegrensis 3
rionegrensis 4
rionegrensis 5
mendocinus
flamarioni
torquatus 1a
torquatus 1b
torquatus 2
pearsoni
coyhaiquensis 1a
coyhaiquensis 1b
coyhaiquensis 1c
haigi
boliviensis 1
boliviensis 2
steinbachi
frater
conoveri
sp "minut"
opimus
leucodon
Octodon
Tympanoctomys
B
rionegrensis 1
rionegrensis 2
rionegrensis 3
rionegrensis 4
rionegrensis 5
mendocinus
flamarioni
torquatus 1a
torquatus 1b
torquatus 2
pearsoni
coyhaiquensis
coyhaiquensis 1b
coyhaiquensis 1c
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steinbachi
opimus
leucodon
Octodon
Tympanoctomys

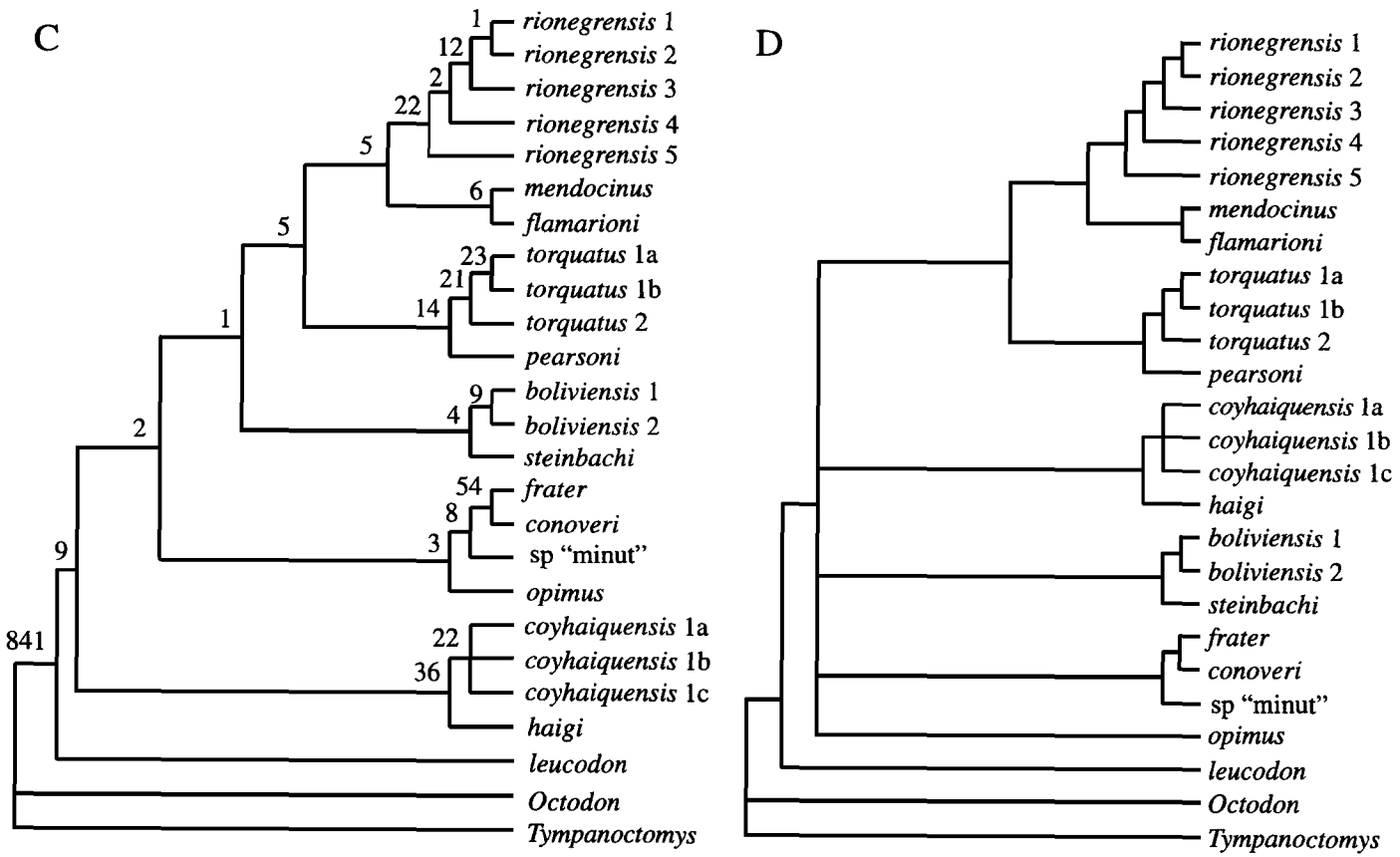


Fig. 1. Results of the maximum parsimony phylogenetic analyses of tuco-tuco species. (A) Shortest tree found with equally weighted parsimony. Tree length = 951 steps, CI = 0.720, RI = 0.676. (B) Most parsimonious tree found with a 5/1 stepmatrix for transversions/transitions and weights of 2, 5, and 1 for first, second, and third codon positions, respectively. Tree length = 2330 steps, CI = 0.671, RI = 0.636. (C) Most parsimonious tree found with a 10/1 stepmatrix for transversions/transitions and given weights of 2, 5, and 1 to first, second, and third codon positions, respectively. Tree length = 3547 steps, CI = 0.720, RI = 0.676. In the three trees, the corresponding decay indices of each clade are indicated above the branches. (D) Strict consensus tree of the most parsimonious trees found under the three different searching conditions.

the bootstrap analyses (Fig. 2), where these clades collapse to a basal polytomy (see Lessa and Cook, 1998 for a discussion of this polytomy).

Neighbor joining analysis (Fig. 3) was similar to the maximum parsimony consensus tree except that (1) *C. leucodon* is not the sister species of the other tuco-tucos, and (2) *C.* sp. "minut" is not included in the *C. frater* and *C. conoveri* clade. Similarly, the ML tree (Fig. 4) defines the same major clades. The most relevant difference is that the two populations of *C. boliviensis* and *C. steinbachi* do not constitute a monophyletic clade; this clade is not supported by maximum parsimony bootstrap values either.

DISCUSSION

Previous phylogenetic hypotheses proposed for *Ctenomys* have been based on phenetic comparisons at the chromosomal (Massarini *et al.*, 1991) and molecular level (Rossi *et al.*, 1993), and on external (Rusconi, 1928), cranial (Osgood, 1946), penial (Balbontin *et al.*, 1996), and sperm morphology (Vitullo *et al.*, 1988; Vitullo and Cook, 1991). The few cladistic studies have varied with respect to the species examined. In this study, we included species that represent several of the major clades previously proposed for *Ctenomys*. We focus on three aspects: (1) the relationship among the *C. rionegrensis* populations, (2) the validity of the *C. mendocinus* group, and (3) the evolution of sperm morphs.

Ctenomys rionegrensis Populations

The three methods of phylogenetic reconstruction agree with respect to the relationships among the *C. rionegrensis* populations. The three Uruguayan populations (*C. rionegrensis* 1, 2, 3) form a strongly supported monophyletic group (Figs. 1–4) with high bootstrap values in the MP and NJ analyses (84–91 and 93%, respectively). A tree 3–7 steps longer (0.30–0.32%) is needed to collapse this group. Kishino–Hasegawa tests carried out using maximum likelihood, however, showed that the most parsimonious topologies enforcing the polyphyly of the Uruguayan populations of *C. rionegrensis* were not significantly worse than the best maximum likelihood tree (Table II). The sister group of these Uruguayan populations is the Argentinean population of Ibicuy (*C. rionegrensis* 4), and these are sister to the Argentinean population of Paraná (*C. rionegrensis* 5) (Figs. 1, 3, and 4). This strongly supported clade (8–22 extra steps are needed to collapse it) has high bootstrap values (96–99%) in both the MP and the NJ analyses. Moreover, those trees that do not support the monophyly of *C. rionegrensis* are significantly worse than the maximum likelihood tree (Table II).

Ortells *et al.* (1990) and Massarini (1996) suggested, based on cranial and chromosomal characters, that the populations of Paraná and Ibicuy belong to *C. rionegrensis*, although they are about 250 km distant from the Uruguayan populations. Sequence divergence between the Argentinean populations (1.58%) is comparable to other pairwise comparisons between these and the Uruguayan population of Las Cañas (1.40%), the type locality of *C. rionegrensis* (Langguth and Abella, 1970). The Uruguayan populations form a less divergent group (0.35 to 0.61% observed divergence). The Argentinean populations may be peripheral isolates of a larger past distribution; this view agrees with proposed expansion and retraction cycles in the geographic range of *C. rionegrensis* (D'Elía *et al.*,

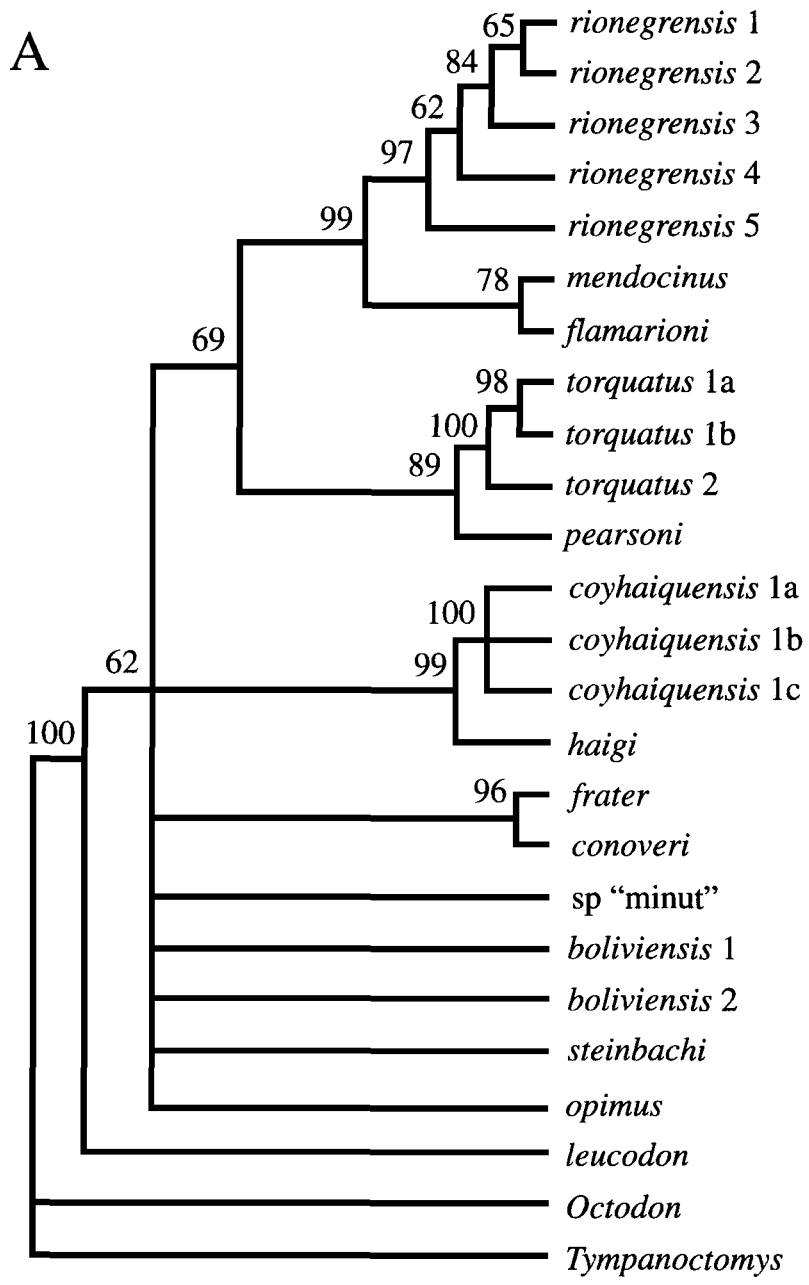
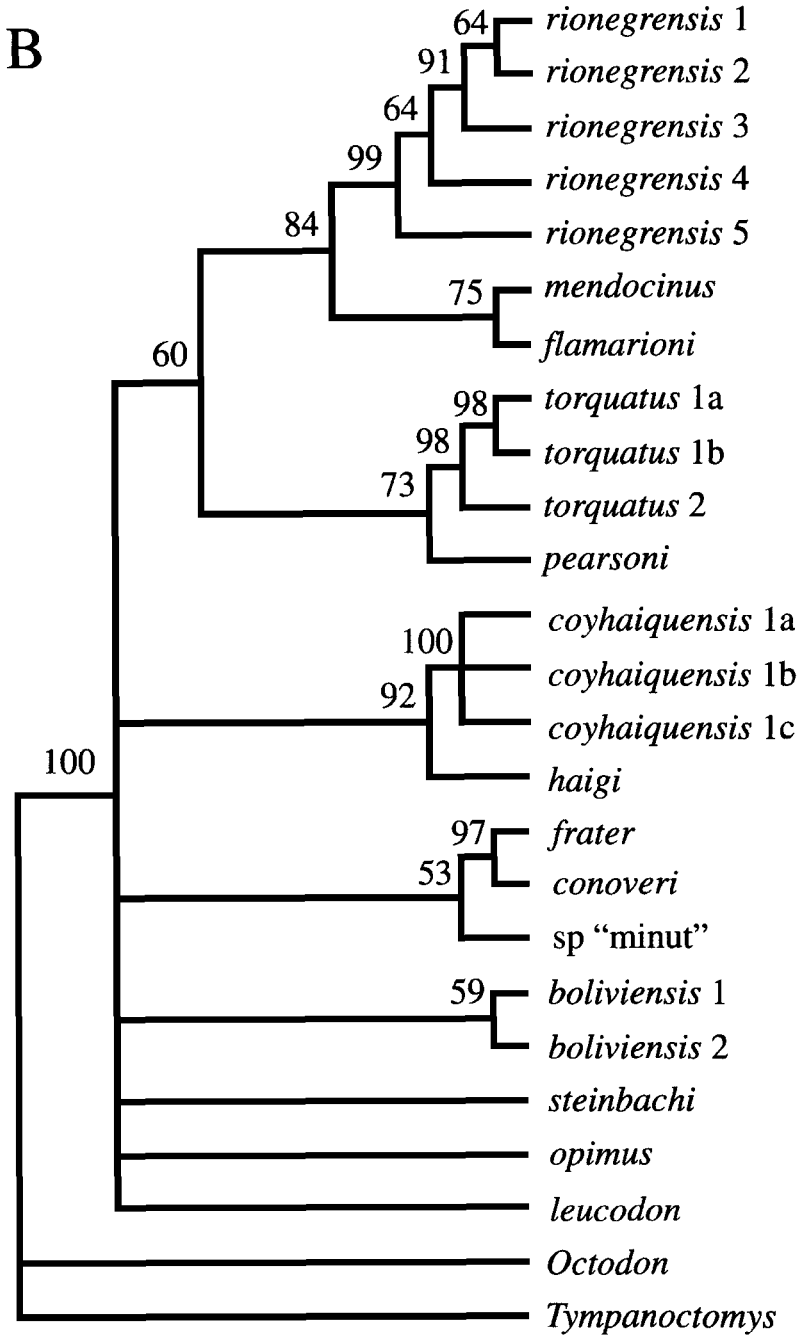


Fig. 2. Results of 1000 bootstrap pseudoreplications of unweighted parsimony analysis of *Ctenomys* species (A) and of analysis with weights of 2, 5, and 1 to first, second, and third codon positions, respectively, in combination with a 5/1 stepmatrix for transversions/transitions (B), or a 10/1 stepmatrix for transversions/transitions (C).



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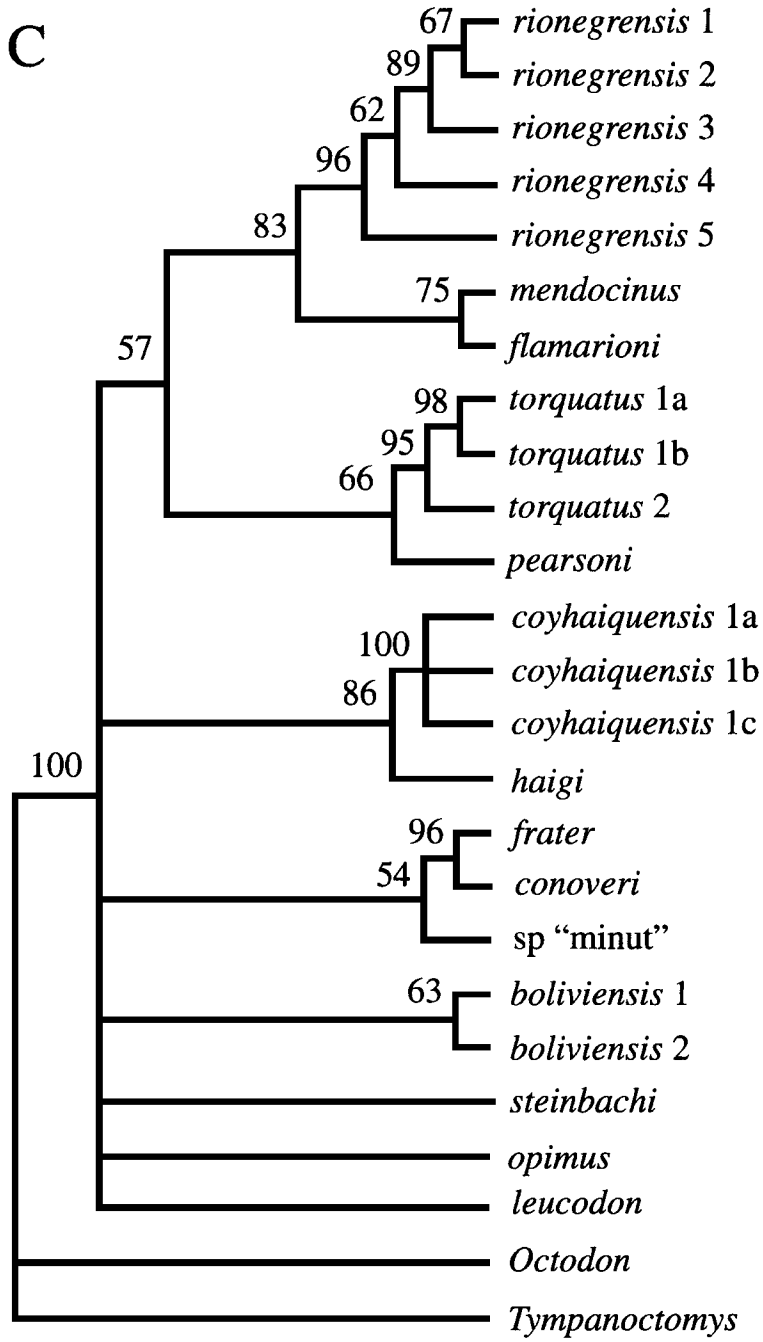

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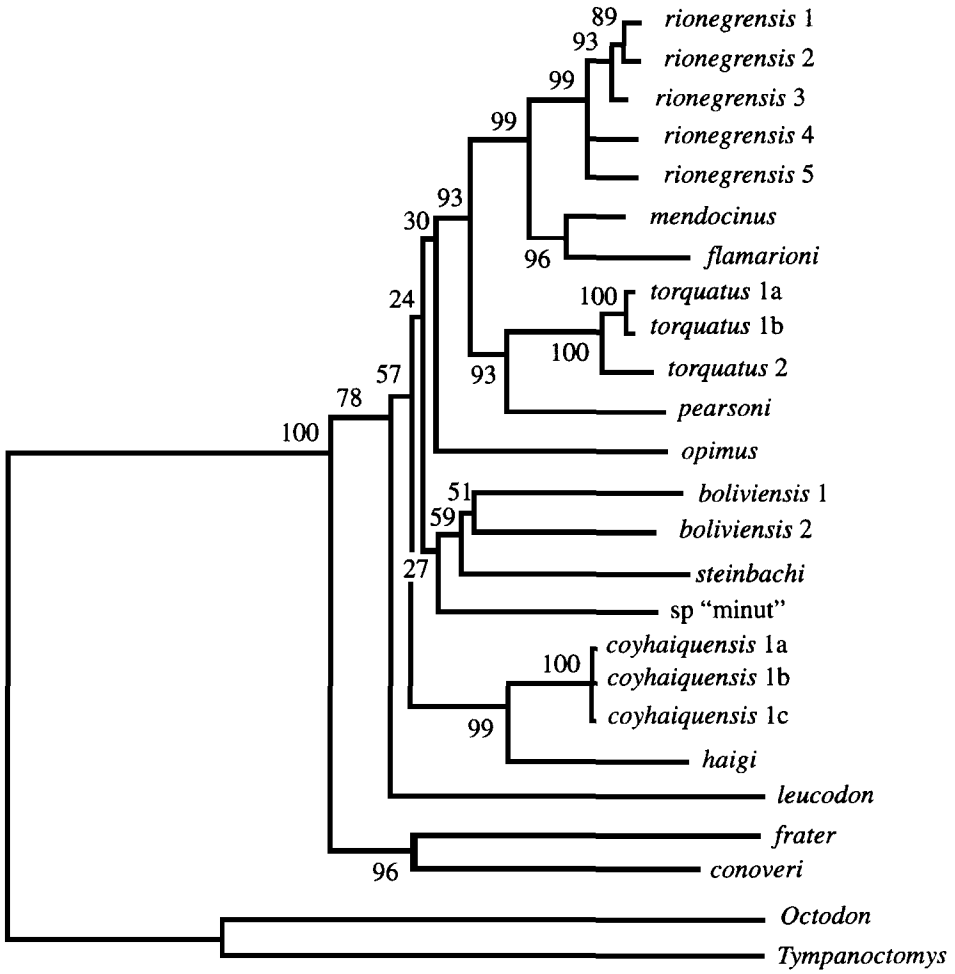

Fig. 3. Tree obtained with the neighbor joining algorithm using the distance of Tamura–Nei (1993). Results of 1000 bootstrap pseudoreplications are given at the base of each clade.

1998). These values for intraspecific divergence are among the highest estimated for tuco-tucos, but are much less than divergence observed for interspecific comparisons (2.98% for *C. mendocinus–C. flamarioni*). In general, divergence levels between tuco-tuco species are smaller than reported for haplotypes of other South American hystricognath species: 1.63–6.42% in *Mesomys hispidus* (Patton *et al.*, 1994), 2.40% in *Makalata didelphoides*, 3.25% in *Isothrix histriata* (da Silva and Patton, 1993). We conclude that the Paraná and Ibicuy populations belong to *C. rionegrensis*.

Paraná specimens have a diploid complement of $2n = 52$ (Ortells *et al.*, 1990), while those from Ibicuy have $2n = 50$ (Massarini, 1996) like the Uruguayan populations (Kiblisky *et al.*, 1977; Villar and Chiesa, 1996). This is another case of chormosomal polytypism, a relatively common phenomenon in *Ctenomys*. It has been reported for *C. bolivien-*

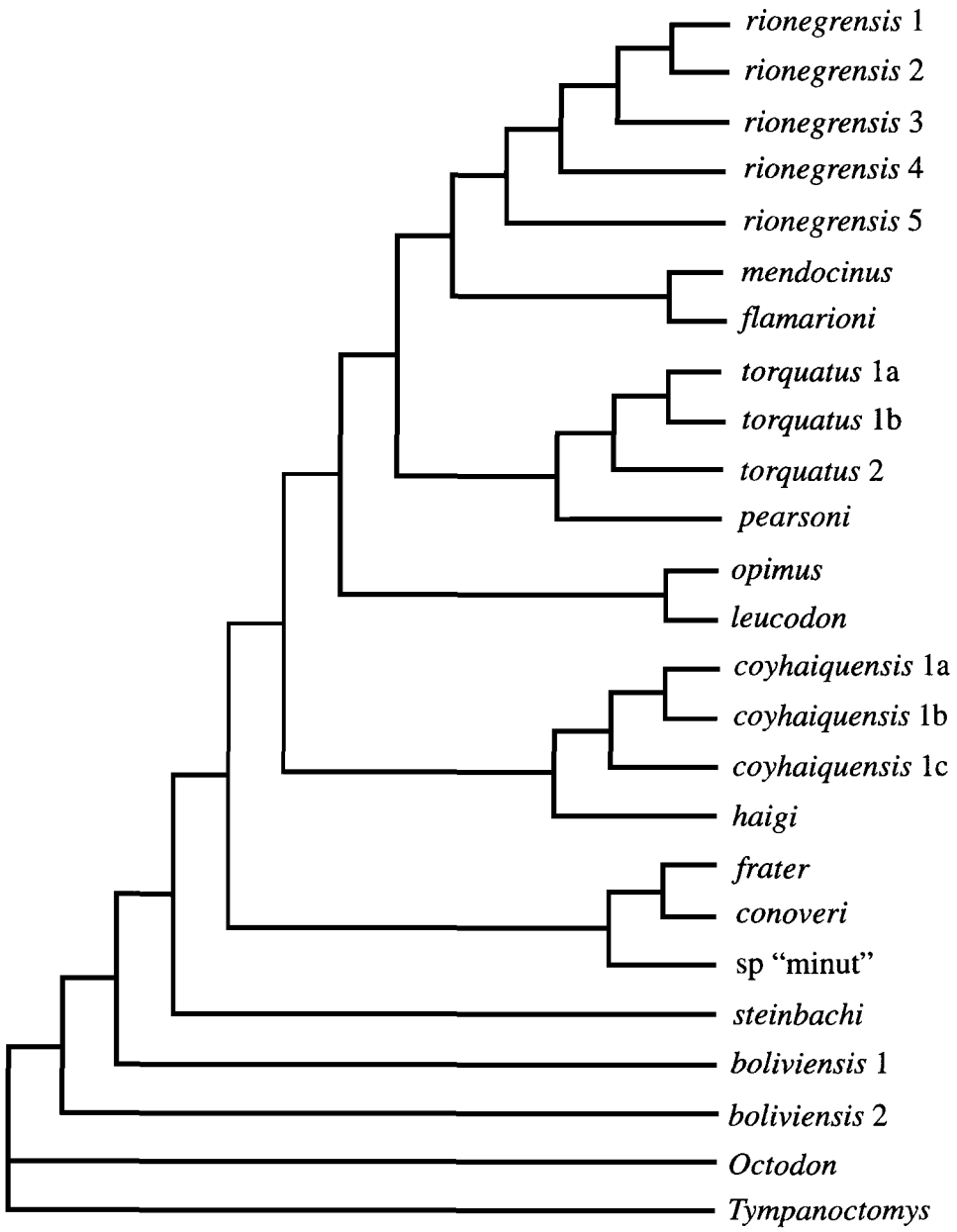

Fig. 4. Best tree obtained in the maximum likelihood analysis of cytochrome *b* gene sequences of species of *Ctenomys*. The search was performed with five replications with the taxa added randomly, transversions weighted 6 to 1 over transitions, and allowing for three equiprobable categories of characters with relative rates of 2, 5, and 1, respectively.

Table II. Results of the Kishino–Hasegawa (1989) Tests as Implemented in DNAML (Felsenstein, 1993)[a]

Hypothesis evaluated	Equally weighted parsimony	Weighted parsimony (5.1 2.5.1)	Weighted parsimony (10.1 2.5.1)
1	No[b]	No	No
2	Yes	Yes	Yes
3	Yes	No	No
4	No	No	No

[a]Twelve tests were performed to evaluate four phylogenetic hypotheses, under the three weighting schemes used in the maximum parsimony analyses (see text for details). Each test evaluates whether the shortest tree that does not support one of the four phylogenetic hypotheses of interest is significantly less likely than the most likely tree. The hypotheses evaluated were that (1) the Uruguayan populations of *Ctenomys rionegrensis* constitute a monophyletic group; (2) *C. rionegrensis* is monophyletic (five populations); (3) *C. rionegrensis* is the sister species of the clade *C. mendocinus–C. flamarioni* ("*C. mendocinus*" complex); and (4) the species bearing asymmetric sperm do not constitute a monophyletic clade.

[b]No—tree(s) not significantly less likely ($p > 0.05$) than the most likely tree. Yes—tree(s) significantly less likely ($p < 0.05$) than the most likely tree.

sis (Anderson *et al.*, 1987), *C. magellanicus* (Reig and Kiblisky, 1969), *C. torquatus* (Freitas and Lessa, 1984), *C. pearsoni* (Kiblisky *et al.*, 1977; Novello and Lessa, 1986), *C. perrensi* (Reig *et al.*, 1990), and *C. pilarensis* (Giménez *et al.*, 1977).

C. mendocinus Group

The *C. mendocinus* group includes *C. mendocinus*, *C. australis*, *C. porteousi*, *C. azarae*, and *C.* sp. from Chasicó. These species are homogeneous in diploid number and C-band patterns and have been considered closely related (Massarini *et al.*, 1991). Chromosomal similarity coincides with phenetic analyses of cranial morphology (Massarini *et al.*, 1991) and the presence of asymmetric sperm (Vitullo *et al.*, 1988).

C. mendocinus was included as representative of this group. In all reconstructions, *C. flamarioni* appears as sister to *C. mendocinus* (75–78% of bootstrap values in MP and 96% in NJ analyses). This relationship supports Freitas' (1994) proposition that the Brazilian *C. flamarioni* is a member of the *C. mendocinus* complex, based on its similar diploid number ($2N = 48$) and shared G-banding patterns. The low interspecific divergence (2.98%) was the smallest of all species comparisons. Based on diploid number and sperm morphology (Altuna *et al.*, 1985, 1986), Freitas (1994) suggested that *C. rionegrensis* also belongs to the *C. mendocinus* group. Our analyses support this hypothesis (83–99% bootstrap values in MP and NJ analyses) (Figs. 1–4). However, Kishino–Hasegawa tests gave mixed results on this issue. Only the shortest unweighted parsimony trees that do not support this hypothesis were significantly worse than the maximum likelihood tree (Table II).

Multiple Origins of the Asymmetric Sperm Morph

Species of *Ctenomys* have been divided into two major groups based on sperm morphology (Feito and Gallardo, 1976, 1982). An asymmetric sperm morph is found mainly in southern species, and a symmetric form occurs in the northern species (Feito and

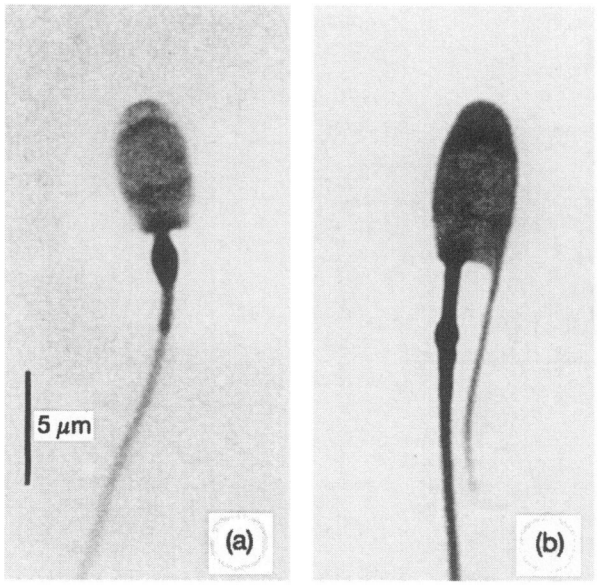

Fig. 5. Sperm morphs found in the genus *Ctenomys*: (a) symmetric and (b) asymmetric. Note the nuclear caudal extension on the asymmetric sperm. Taken and modified from Vitullo *et al.* (1988); reprinted with permission of Cambridge University Press.

Gallardo, 1982; Vitullo *et al.*, 1988; Vitullo and Cook, 1991). The asymmetric sperm, first described by Feito and Barros (1982), is characterized by a paddle-like head with a postacrosomic process that originates at the base of the head opposite the insertion of the flagellum (Fig. 5b). This process, which the symmetric sperm morph lacks (Fig. 5a), is called the nuclear caudal extension (Feito and Barros, 1982). It is not found in other caviomorphs, including other octodontoids (Berrios *et al.*, 1978), and apparently is unique among mammals (Vitullo *et al.*, 1988).

Five of the 14 species in this study have asymmetric sperm, including *C. rionegrensis* (Altuna *et al.*, 1986), *C. flamarioni* (Freitas, 1994), *C. mendocinus* (Massarini *et al.*, 1991), *C. haigi* (Reig *et al.*, 1992), and *C. coyhaiquensis* (Kelt and Gallardo, 1994). Reconstruction of the evolution of sperm morphs using parsimony agrees with the hypothesis that the asymmetric morph is the derived character state (Vitullo *et al.*, 1988; Vitullo and Cook, 1991); however, the asymmetric sperm may have evolved more than once in the history of *Ctenomys* (Fig. 6). *C. flamarioni*, *C. mendocinus*, and *C. rionegrensis* form a clade that is sister to a clade comprised of the Uruguayan species of symmetric sperm *C. pearsoni* and *C. torquatus*. *C. haigi* and *C. coyhaiquensis* form a clade (Figs. 1, 3, and 4) that was never sister to the remaining asymmetric species (the *C. mendocinus* group). Polyphyly of the asymmetric sperm species is contrary to the evolutionary scheme suggested by Feito and Gallardo (1982), Vitullo *et al.* (1988), and Vitullo and Cook (1991), who, without formally postulating the reciprocal monophyly, proposed two major lineages based on these sperm morphs. The fact that *C. haigi* is not the sister species of *C. mendocinus* supports Pearson's (1984) elevation to specific status of the former.

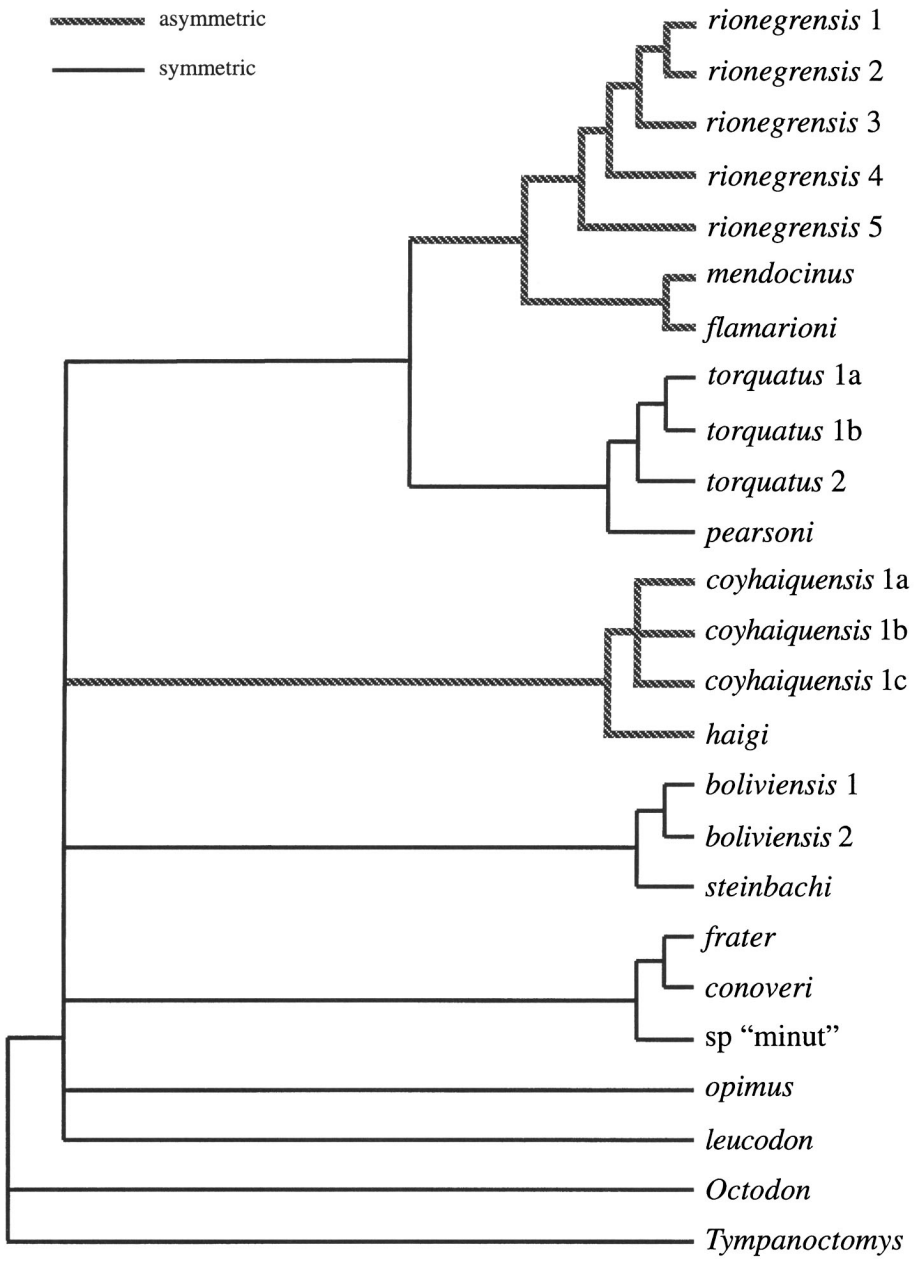

Fig. 6. Parsimony reconstruction of the evolution of tuco-tuco's sperm morphology using the strict consensus tree for cytochrome *b* from Fig. 1.

The clade formed by the *C. mendocinus* complex, *C. torquatus* and *C. pearsoni* never collapsed in the maximum parsimony analyses; however, the bootstrap values of this group in the maximum parsimony analyses are low, ranging between 57 and 69%. A tree 1–5 steps (0.11–0.14%) longer is needed to collapse this clade. However, in the distance-based tree the bootstrap value of this clade is 93%. The position of the clade formed by *C. coyhaiquensis* and *C. haigi* varied. Its closest position to the clade formed by the other three asymmetric species plus *C. torquatus* and *C. pearsoni* is in a maximum parsimony analysis (Fig. 1B). In trees at least 2 to 13 steps (0.22–0.37%) longer than the most parsimonious the asymmetric species form a monophyletic clade. In summary, the position of the *C. coyhaiquensis–C. haigi* clade varies, but they appear relatively distant from the other asymmetric species. Kishino–Hasegawa tests carried out using maximum likelihood, however, showed that the differences between topologies that enforced monophyly of the species with asymmetric sperm were not significantly worse than the most parsimonious or best maximum likelihood trees. In sum, diphyly of species with asymmetric sperm must be taken only as a working hypothesis that should be further tested.

It appears that sperm morphology may have a high degree of evolutionary plasticity in this genus. For example, *C. yolandae* has a third morph called "complex asymmetric," which consists of sperm with two nuclear caudal extensions (Vitullo *et al.*, 1988). Moreover, it has been suggested that another Argentinean species, *C. talarum*, has two sperm morphologies in different subspecies (Vitullo *et al.*, 1988); unfortunately no data supporting this statement have been published.

The diphyly of the asymmetric sperm species should be tested further with independent markers and additional taxa that represent the different sperm morphs. Alternative explanations also should be tested. Differences between species and gene trees (Nei, 1987), and horizontal transfer of mitochondrial DNA from one species to another (see Patton and Smith, 1994; Prager *et al.*, 1993) may undermine these conclusions. Studies concerning the function of the caudal nuclear process would be valuable for examining the possibility of hybridization between species of different sperm type.

ACKNOWLEDGMENTS

We thank Isabel Rey, Bruce Patterson, and Thales de Freitas for supplying DNA and tissue samples, Alejandra Chiesa for comments on an early draft of the manuscript, three anonymous reviewers for estimable suggestions, Chris Conroy, John Demboski, and especially Brandy Jacobsen for invaluable help during the laboratory work. Financial support for this research was provided by the Comisión Sectorial de Investigación Científica (CSIC), Universidad de la República; the Programa de Desarrollo de las Ciencias Básicas (PEDECIBA); and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICYT), Uruguay.

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