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Original investigation

Phylogenetic analysis of sigmodontine rodents (Muroidea), with special reference to the akodont genus *Deltamys*

By G. D'ELÍA, E. M. GONZÁLEZ, and U. F. J. PARDIÑAS

The University of Michigan Museum of Zoology, Ann Arbor, Michigan, USA, Laboratorio de Evolución, Facultad de Ciencias, Montevideo, Uruguay, Museo Nacional de Historia Natural, Montevideo, Uruguay and Centro Nacional Patagónico, Puerto Madryn, Chubut, Argentina

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Abstract

We present a comprehensive phylogenetic analysis based on cytochrome b gene sequences of sigmodontine rodents. Our particular interest is to estimate the phyletic position of *Deltamys*, a taxon endemic to a small portion of the La Plata river basin in Argentina, Brazil, and Uruguay, and to assess its generic status. The three primary conclusions derived from our analyses are: (1) cotton rats (*Sigmodon*) are the sister group of the remaining sigmodontines, (2) the tribe Akodontini is monophyletic with moderate support, and (3) *Deltamys* falls outside of a clade containing all species of subgenera of *Akodon* yet examined, and thus we grant *Deltamys* status of full genus.

Key words: *Akodon*, Akodontini, Muroidea, Sigmodontinae, phylogeny

Introduction

The high diversity of muroid rodents belonging to the New World subfamily Sigmodontinae has seriously challenged researchers attempting to understand their phylogenetic relationships and to classify them accordingly. Problems range from species boundaries; to relationships among sigmodontine taxa; to the limits and contents of the subfamily, one of the most debated topics in muroid systematics (see D'ELÍA 2000).

Traditionally, sigmodontine genera have been delimited and grouped into tribes based on phenetic similarity. One of the largest of these groups is the tribe Akodontini. An akodontine concept can be traced back to THOMAS (1916, 1918) who recognised the morphologic similarity among *Akodon* and some other taxa ranked as genera by him (e. g., *Abrothrix*, *Chroeomys*, *Deltamys*, *Hypsimys*, *Necromys* (= *Bolomys*), *Thalpomys*, *Thaptomys*). Four decades later VORONTZOV (1959) coined the term Akodontini for this group. However, it was REIG (1984, 1987) who made the most significant contribution towards defining the group and understanding its evolutionary history. Following the removal of *Zygodontomys* (TATE 1932) the contents of the akodontine group have been more or less stable. The major discrepancy has revolved around *Oxymycterus* and similar genera (e. g., *Juscelinomys*,

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Lenoxus, *Blarinomys*), which have been placed in a different but closely related tribe, Oxymycterini by some (HOPPER and MUSSER 1964; HERSHKOVITZ 1966); or included within the Akodontini by others (REIG 1980, 1987), a position followed in the most recent treatise of mammal taxonomy (MCKENNA and BELL 1997).

Although a major redefinition of generic contents of the tribe Akodontini has recently been prompted by phylogenetic analyses of molecular markers (DICKERMAN 1991; SMITH and PATTON 1991, 1993, 1999; D'ELÍA et al. 2003) historically, much of the debate on akodontine taxonomy and systematics has been centered on issues at low taxonomic levels (see REIG 1987). This situation is especially true with regard to the ranking of several taxa that morphologically closely resemble *Akodon* (e. g., *Deltamys*, *Hypsimys*, *Microxus*, *Thaptomys*, *Thalpomys*). In this study we focus on *Deltamys*, a poorly studied taxon that since its original description (THOMAS 1917) has varied from being considered a subgenus or simply a synonym of *Akodon* (e. g., ELLERMAN 1941; CABRERA 1961; MASSOIA 1964; REIG 1987; MUSSER and CARLETON 1993) to a full genus (e. g., GYLDENSTOLPE 1932; MASSOIA 1980; BIANCHINI and DELUPI 1994; GONZÁLEZ and MASSOIA 1995).

A single species, *Deltamys kempi* Thomas, 1917 has been described, although two subspecies are currently recognised (GONZÁLEZ and MASSOIA 1995). *Deltamys* occupies wet environments in a small area of the La Plata river basin (Fig. 1). Its distribution ranges from northern Buenos Aires and southern Entre Ríos provinces in Argentina (MASSOIA 1964, 1983), throughout Uruguay, and extends by the Atlantic litoral of the Brazilian State of Rio Grande do Sul (GONZÁLEZ and MASSOIA 1995). The Argentinean populations correspond to the nominant subspecies, while the Uruguayan form is *D. k. langguthi*. Brazilian records have been ten-

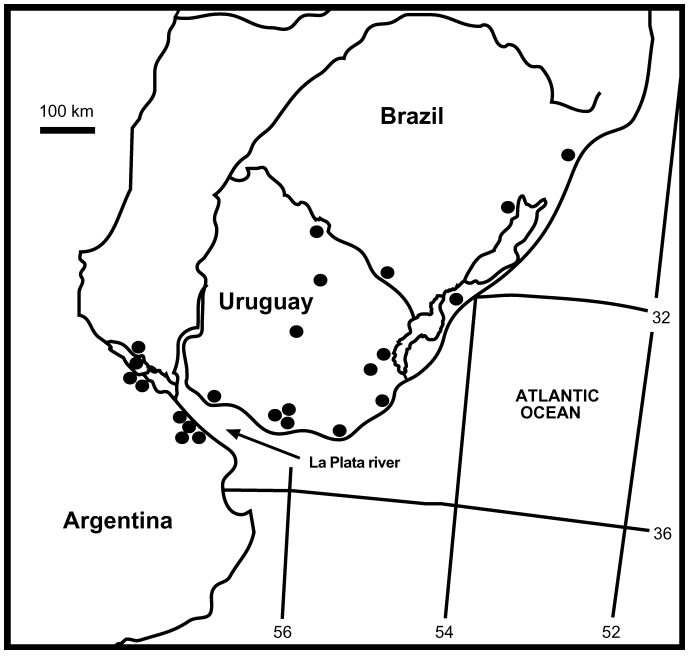


Fig. 1. Map of a portion of the La Plata river basin where the sigmodontine genus *Deltamys* is distributed. Black circles indicate recorded populations of *Deltamys* taken from GONZÁLES and PARDIÑAS (2002).

tatively assigned to the latter form (González and Massoia 1995).

The aim of this study was to assess the phylogenetic position of *Deltamys* within the sigmodontine radiation, and thus its taxonomic status. Our systematic philosophy is that taxa above the species level should be monophyletic.

Material and methods

We amplified and sequenced the cytochrome b gene sequences reported in this study (Tab. 1) in two fragments using primers located both internally and in the flanking regions of the gene (MVZ 05 – MVZ 16 and MVZ 103 – MVZ 14, da Silva and Patton 1993; Smith pers. com.). Negative controls were included in all experiments. Dye-labelled PCR products were cleaned in Sephadex columns and sequenced using an ABI 377 automatic sequencer. In all cases both heavy and light DNA strands were sequenced and compared.

The phylogenetic analysis was based on complete cytochrome b sequences, therefore it not included the partial sequences of the *Deltamys* specimens UP 65 and MNHN 4150 (Tab. 1). These haplotypes, along with the others gathered from *Deltamys* specimens were employed in population level comparisons. The phylogenetic dataset here analysed was that of D'Elía et al. (2003) with the following tree modifications. First, it was expanded with 19 specimens belonging to 18 sigmodontine species (Tab. 1). Second, the specimen representing *Akodon montensis* was changed to include one belonging to the type locality of the species. Third, one specimen of *Bibimys chacoensis* was excluded in order to have only one specimen per species. The exception to this pattern was *Deltamys kempi*, the target taxon, from which one specimen of each subspecies was included. Then, the dataset here analysed includes 134 taxa, of which 111 belong to the sigmodontine ingroup and 23 to the outgroup (Fig. 2). Outgroup taxa, which include arvicoline, cricetine, neotomine, peromyscine, *Scotinomys* and tylomyine species, were chosen based on the results of an unpublished comprehensive study of muroid phylogenetic relationships carried out by D'Elía and Weksler.

Sequence alignment was done using the program Clustal X (Thompson et al. 1997) under the default setting costs. Cytochrome b gene sequences of sigmodontine taxa vary in length (Smith and Patton 1999). Typical sequences, such as all of those reported in this study, were 1 140 base pairs long and end in a TAA or TAG stop codon. Other sequences (e. g., *Delomys*, *Chilomys*, and *Rhipidomys*) have an extra codon (i. e., 1 143 bp long) and also end in a TAA or TAC stop codon. Other sigmodontine sequences did not end with a TAA or TAG stop codon. After the last codon, these sequences have an extra T, which presumably gets polyadenylated to form a stop codon, as it was reported for the cytochrome b gene of *Mus* (Bibb et al. 1981). Then, some sequences (e. g., those of *Blarinomys*, *Lenoxus*) were 1 141 bp long and others (e. g., those of *Abrothrix*, *Irenomys*, and *Oecomys*) were 1 144 bp long. From the alignment it became clear that the position of the insertions and/or deletions responsible for the difference in gene length lies at the very end of the sequence, but it was impossible to unambiguously determine its exact position (i. e., if it either corresponded to the codon number 379, 380 or 381). To avoid this problem, we based our analysis in the first 1 134 bases of the sequences.

Maximum parsimony (MP; Farris 1983) was the optimisation criterion used to generate hypotheses of phylogenetic relationships among sigmodontine species. In all cases characters were treated as unordered and equally weighted. We employed two approaches to search for the most parsimonious tree(s). First, PAUP* 4.0b10 (Swofford 2000) was used to perform 200 replicates of heuristic searches with random addition of sequences and tree bisection-reconnection branch swapping. Second, two batches of Nixon's (1999) parsimony ratchet were performed in PAUP* using command files written with the help of the program PAUPRat (Sikes and Lewis 2001). The difference between both ratchet batches was the number of perturbed characters, 15 and 25% respectively. Each ratchet batch consisted of 20 series of 200 iterations. For each series a different command file was used. We carried out two measures of clade support. First, we performed 500 bootstrap (Felsenstein 1985) replications, each with three replicates of random sequence addition. Second, we performed 500 parsimony jackknife (Siddall 1995) replications with three addition sequence replicates each and a deletion of one third of the character data. In both bootstrap and jackknife searches the branches with less than 50% of support were allowed to collapse. Molecular synapomorphies were documented by examining PAUP* outputs and visualised using MacClade 3.05 (Maddison and Maddison 1992). Only those changes that held up irrespective of the kind of character transformation used (i. e., accelerated: ACCTRAN or delayed: DELTRAN) were taken into account.

Table 1. Specimens examined in this study (in addition to those of D'ELÍA et al. 2003; see text for details and also Fig. 2) including catalog number, and locality (for the sequences presented here) and source of their sequences. Voucher specimens for the individuals first reported in this study are or will be deposited in the following collections: Argentina, Colección de Mamíferos del Centro Nacional Patagónico (UP); Brazil, Museu de Zoologia da Universidade de São Paulo (CIT); USA, The University of Michigan Museum of Zoology (GD); and Uruguay, Museo Nacional de Historia Natural Montevideo (MNHN). Asterisks indicate sequences kindly provided by J. L. PATTON and M. F. SMITH (Museum of Vertebrate Zoology, University of California at Berkeley, USA); we have submitted these two sequences to GENBANK.

Species	Catalog number	Locality	Source
Deltamys kempi kempi	UP 42	Argentina, Buenos Aires, La Balandra (S 34°54′07″ W 57°45′56)	AY195860
D. k. kempi	UP 65	Argentina, Buenos Aires, La Balandra (S 34°54′07″ W 57°45′56)	AY195861
D. k. langutthi	MNHN 4150	Uruguay, San José, Ruta 1 sobre Arroyo Cufre (S 34°21′ W 57°06′)	AY195863
D. k. langutthi	MNHN 4151	Uruguay, San José, Ruta 1 sobre Arroyo Cufre (S 34°21′ W 57°06′)	AY195862
A. reigi	MNHN 3682	Uruguay, Lavalleja, Paso Averías (S 33°36′ W 54°19″)	AY195865
A. montensis	GD 513	Paraguay, Paraguari, Sapucái (S 25°40′ W 56°56′)	AY195864
A. paranaensis	CIT 1131	Brazil, Rio Grande do Sul, Venancio Aires (S 29°36′ W 52°12′)	AY195866
Akodon affinis *			AY196164
A. spegazzini *			AY196165
A. sp.			PARDIÑAS et al. (2003)
A. cursor			AF184051
Calomys fecundus			AF385592
C. hummelincki			AF385598
C. laucha			AF385593
C. muscullinus			AF385603
C. sorellus			AF385608
C. tener			AF385596
Sigmodon alstoni			AF293397
S. fulviventer			AF293401
S. leucotis			AF293401
S. peruanus			AF293395
S. toltecus			AF293402
Volemys kikuchii			AF348082

Results and discussion

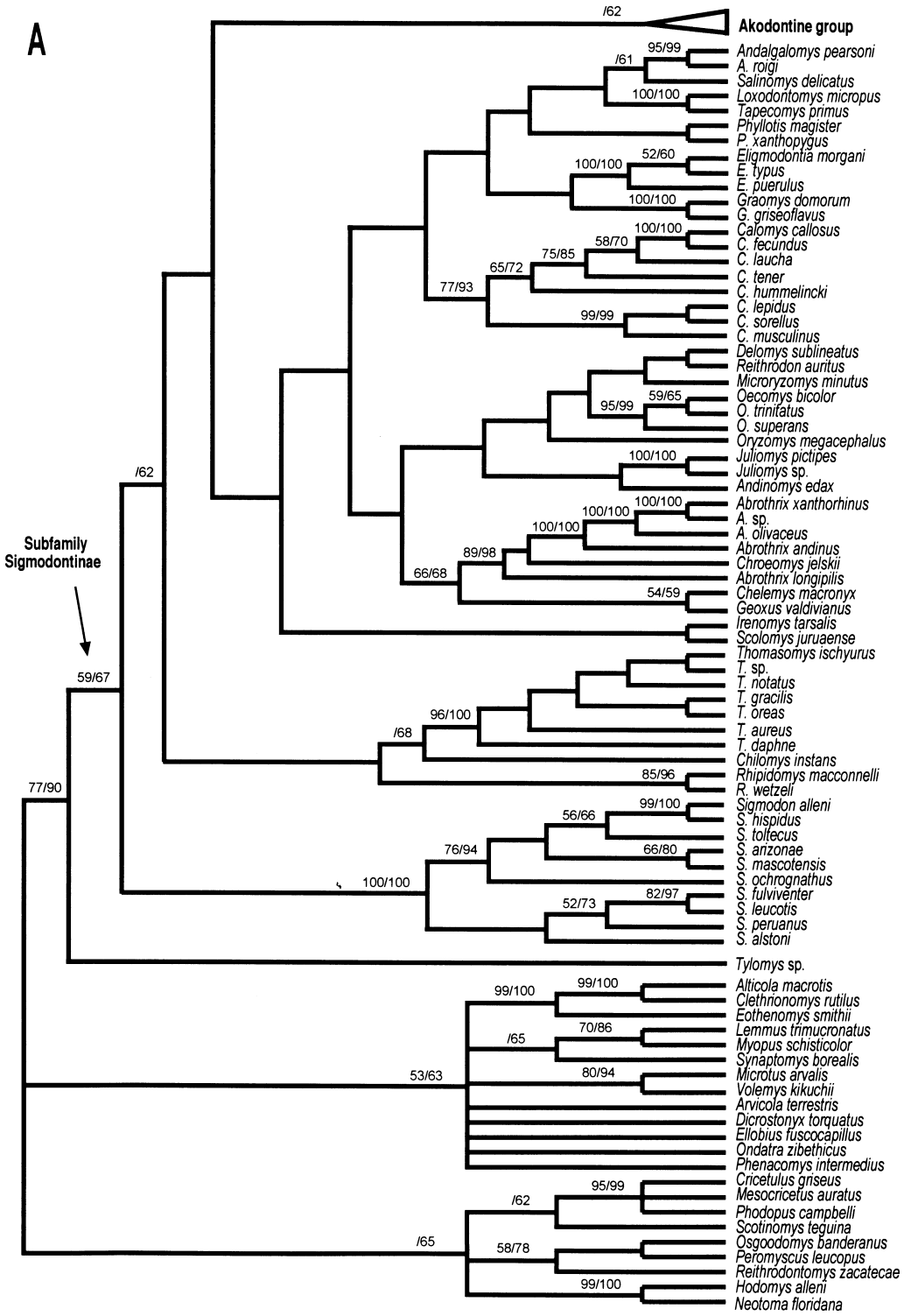
The cytochrome b gene of *Deltamys* had a length of 1 140 base pairs. The haplotypes showed a strong base compositional bias, with a marked deficit of guanine, especially in third positions. The mean base frequency across all *Deltamys* haplotypes and across all base positions are A = 0.29873, C = 0.27932, T = 0.29537, and G = 0.12658.

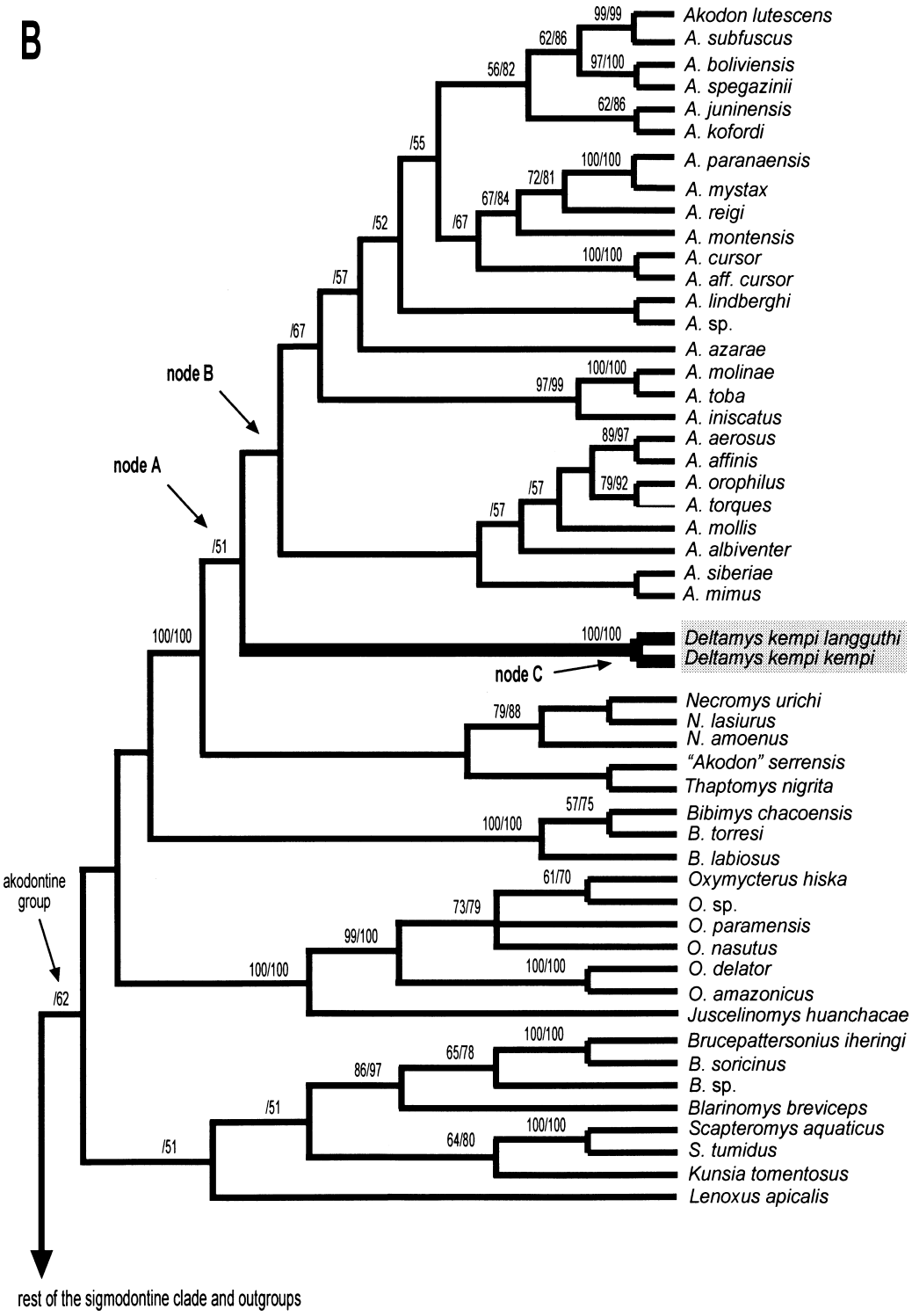
Similar compositional biases have been found in other sigmodontine rodents (e. g., Myers et al. 1995) and in mammals in general (Irwin et al. 1991). Three different cytochrome b haplotypes were found among the four sequenced specimens of *Deltamys*. Both individuals of *D. k. langguthi* shared the same haplotype. Haplotypes of the two sequenced *D. k. kempi* specimens differed in only one base pair. Interestingly, haplotypes from specimens belonging to the two described subspecies differed in only one or two positions. González and Massoia (1995) described *D. k. langguthi* based on differences in pelage colour and minor differences in the relative size of the braincase and zygomatic arch. Clearly, morphological evolution is not necessarily coupled with evolution of mitochondrial genes and different degrees of differentiation in these two systems are not surprising. However, the low level of mitochondrial differentiation is remarkable and indicates that more comprehensive morphological and molecular analyses of variation are needed to further assess the distinctiveness of the subspecies. In this respect, the study of specimens from Brazilian populations where several fixed karyotypic rearrangements have been reported (Castro et al. 1991) is much needed. The dataset analysed phylogenetically had 701 variable characters of which 582 were parsimony informative. Both search methods (replicates of heuristic searches, and two sets of iterative parsimony ratchet) employed in this study found the same four shortest trees (length of 11 690 steps). Despite the large amount of homoplasy (CI = 0.110, RI = 0.489) present in the data set, the strict consensus tree (Fig. 2) resulting from the four shortest trees shows very good resolution with only four polytomies. Three of those are in the outgroup part of the tree (Fig. 2 a). One major polytomy involves peromyscines, neotomines, and cricetines plus *Scotinomys*. A second polytomy appears within the cricetine subfamily, and a third involves different arvicoline lineages. Finally, the forth polytomy involves three lines within the sigmodontine genus *Oxymycterus* (Fig. 2 b).

The subfamily Sigmodontinae appears monophyletic, with four major clades. The most basal clade contains only the genus *Sigmodon* (10 species included), the sole member of the tribe Sigmodontini. Several authors (e. g., Hershkovitz 1972; Gardner and Patton 1976; Reig 1980, 1984; Jacobs and Lindsay 1984; Baskin 1986) have discussed the identity of the basal sigmodontine because it has direct implications for inferring the biogeographic history of the group. This is a complex subject theme that falls beyond the scope of this contribution, and we refer to D'Elía (2000). Although the basal position of *Sigmodon* has low level of bootstrap and jackknife support, it is in agreement with the results of a more reduced phylogenetic analysis of four other mitochondrial genes (Engel et al. 1998), as well as nuclear and mitochondrial DNA sequences (D'Elía 2002). It is worth noting that no sequence phylogenetic analysis has yet included representatives of the tribe Ichthyomyini. The second sigmodontine clade to branch off is the thomasomyine tribe (sensu Smith and Patton 1999). The third main sigmodontine clade is very large and includes phyllotines, oryzomyines and abrothricines and several unique sigmodontine lines (sensu Smith and Patton 1999). Relationships within this large group are weakly

▶

Fig. 2. Consensus tree of the 4 most parsimonious trees (length 11 690, CI = 0.110, RI = 0.489) obtained in the maximum parsimony analysis of 111 sigmodontine and 23 outgroup taxa. Numbers above branches indicate parsimony jackknife (right of the diagonal) and bootstrap (left to the diagonal) values of the nodes at their right. Only values above 50% are shown. For technical details of the analyses refer to the text. A) Details of the consensus tree corresponding to the outgroup taxa and the sigmodontine clade less the akodontine group. B) Part of the consensus tree corresponding to the akodontine clade. Branches leading to *Deltamys* species are drawn wider than those leading to the other taxa. Node A = common ancestor of *Akodon* and *Deltamys*. Node B = common ancestor of *Akodon*. Node C = common ancestor of the *Deltamys* clade.


A
/62
Akodontine group
95/99
/61
Andalgalomys pearsoni
A. roigi
100/100
Salinomys delicatus
Loxodontomys micropus
Tapecomys primus
Phyllotis magister
P. xanthopygus
52/60
100/100
Eligmodontia morgani
E. typus
E. puerulus
100/100
Graomys domorum
G. griseoflavus
100/100
Calomys callosus
58/70
C. fecundus
75/85
C. laucha
65/72
C. tener
77/93
C. hummelincki
C. lepidus
99/99
C. sorellus
C. musculinus
Delomys sublineatus
Reithrodon auritus
Microryzomys minutus
59/65
95/99
Oecomys bicolor
O. trinitatus
O. superans
Oryzomys megacephalus
100/100
Juliomys pictipes
Juliomys sp.
/62
Andinomys edax
100/100
Abrothrix xanthorhinus
100/100
A. sp.
100/100
A. olivaceus
Abrothrix andinus
89/98
Chroeomys jelskii
66/68
Abrothrix longipilis
54/59
Chelemys macronyx
Geoxus valdivianus
Irenomys tarsalis
Scolomys juruaense
Subfamily Sigmodontinae
Thomasomys ischyurus
59/67
T. sp.
T. notatus
T. gracilis
96/100
T. oreas
/68
T. aureus
T. daphne
Chilomys instans
85/96
Rhipidomys macconnelli
77/90
R. wetzeli
99/100
Sigmodon alleni
56/66
S. hispidus
S. toltecus
76/94
66/80
S. arizonae
S. mascotensis
100/100
S. ochrognathus
82/97
S. fulviventer
52/73
S. leucotis
S. peruanus
S. alstoni
Tylomys sp.
99/100
99/100
Alticola macrotis
Clethrionomys rutilus
Eothenomys smithii
70/86
Lemmus trimucronatus
/65
Myopus schisticolor
Synaptomys borealis
80/94
53/63
Microtus arvalis
Volemys kikuchii
Arvicola terrestris
Dicrostonyx torquatus
Ellobius fuscocapillus
Ondatra zibethicus
Phenacomys intermedius
95/99
Cricetulus griseus
Mesocricetus auratus
/62
Phodopus campbelli
Scotinomys teguina
/65
58/78
Osgoodomys banderanus
Peromyscus leucopus
Reithrodontomys zacatecae
99/100
Hodomys alleni
Neotoma floridana


B
Akodon lutescens
A. subfuscus
A. boliviensis
A. spegazinii
A. juninensis
A. kofordi
A. paranaensis
A. mystax
A. reigi
A. montensis
A. cursor
A. aff. cursor
A. lindberghi
A. sp.
A. azarae
A. molinae
A. toba
A. iniscatus
A. aerosus
A. affinis
A. orophilus
A. torques
A. mollis
A. albiventer
A. siberiae
A. mimus
Deltamys kempi langguthi
Deltamys kempi kempi
Necromys urichi
N. lasiurus
N. amoenus
"Akodon" serrensis
Thaptomys nigrita
Bibimys chacoensis
B. torresi
B. labiosus
Oxymycterus hiska
O. sp.
O. paramensis
O. nasutus
O. delator
O. amazonicus
Juscelinomys huanchacae
Brucepattersonius iheringi
B. soricinus
B. sp.
Blarinomys breviceps
Scapteromys aquaticus
S. tumidus
Kunsia tomentosus
Lenoxus apicalis
99/99
62/86
97/100
56/82
62/86
/55
100/100
72/81
67/84
/67
/52
100/100
/57
/67
100/100
97/99
89/97
79/92
/57
/57
/51
100/100
100/100
node A
node B
node C
79/88
57/75
100/100
61/70
73/79
99/100
100/100
100/100
/62
akodontine group
100/100
65/78
86/97
/51
64/80
/51
rest of the sigmodontine clade and outgroups

supported, and most lines collapse to a basal polytomy in the bootstrap and jackknife analyses. We note, as SMITH and PATTON (1993, 1999) did, that within the abrothricine clade the genus *Abrothrix* appears as paraphyletic with respect to *Chroeomys jelskii*. However, this arrangement has low levels of support. Further studies are required to test the abrothricine topology that appears in the strict consensus tree. If this topology remains stable, additional analyses will be needed to clarify if *Chroeomys* is in fact part of *Abrothrix*, or if the generic name *Abrothrix* must be applied only to *A*. longipilis (type species of the genus) while *A*. *andinus*, *A*. *olivaceus*, *A*. *xanthorrinus*, and *A*. sp. are elevated to their own genus or included in *Chroeomys*. The fourth main sigmodontine clade corresponds to the akodontine tribe (sensu SMITH and PATTON 1999).

A novel finding of our study with respect to akodontine systematics is the placement of *Akodon serrensis* well outside the *Akodon* clade (Fig. 2 b). *A*. *serrensis* appears as sister to the monotypic genus *Thaptomys*, and this clade is sister to the genus *Necromys* (of which three species were included). The sequence of *A*. *serrensis* was not generated by us and we did not have the chance to study the specimen from which it came, although we do not have any reason to suspect the specimen was misidentified. The phylogenetic position of *Akodon serrensis* is surprising because to the best of our knowledge nobody has cast doubt upon the inclusion of *A*. *serrensis* within the genus *Akodon*. Moreover, *A*. *serrensis* has always been referred to the typical subgenus of *Akodon* (REIG 1987; MUSSER and CARLETON 1993). We will not make any taxonomic judgement about this topology until further studies, including careful morphological comparisons and the sequencing of additional specimens, are carried out. However, a combined analysis of nuclear and mitochondrial DNA sequences points in the same direction (D'ELÍA 2002).

Finally, with respect to *Deltamys*, the phylogenetic analysis shows that this taxon is in fact an akodontine rodent. This result may be considered trivial since nobody has questioned the akodontine condition of *Deltamys*. However, it is important because so far this is the only study based on shared derived similarity, in opposition to overall similarity, to have evaluated the phylogenetic relationships of this taxon. *Deltamys* appears as sister group of the *Akodon* clade (27 species included). This dichotomy has low level of support. It is worth noting that cladistic analysis based on nuclear and mitochondrial DNA sequences of a more reduced dataset also place *Deltamys* outside the *Akodon* radiation (D'ELÍA 2002). The topology obtained in the present study may be taxonomically interpreted either as recognising *Deltamys* as a valid genus, or as expanding *Akodon* to include the *Deltamys* clade. The election of either taxonomic scenario is a decision for each taxonomist. We consider that the first option, granting *Deltamys* status of full genus, is the most appropriate considering the level of morphologic, and karyotypic differences between the taxa. We do not intend to fully describe *Deltamys* here, and for those interested in that we refer them to the original description by THOMAS (1917) and the review of GONZÁLEZ and PARDIÑAS (2002). Here we simply point out some of the main features that differentiate *Deltamys* from *Akodon*. *Deltamys kempi* is a small, dark coloured mouse, with conspicuously small eyes and dense velvety pelage. It is hardly distinguished externally from middle-sized species of *Akodon*. However, the epidermal scales of its tail are more conspicuous and the eyes notable smaller than in *Akodon*. The skull of *Deltamys* is narrower and appears fragile. According to THOMAS (1917) this single feature allows easy discrimination between both genera. In addition, the interparietal of *Deltamys* is very reduced, much smaller than that of most *Akodon*. The anterior border of the mesopterygoid fossa is wider than in *Akodon*, and it does not surpass the posterior margin of the M3. The upper incisors project forward, and their angle with the molar surface is between 80–83°. The lower first molar has a very reduced procingulum. Conspicuous differences between *Deltamys* and *Akodon*

are also evident in the gross morphology of their karyotypes; the karyotype of *Deltamys* lacks the small pair of metacentric chromosomes characteristic of the genus *Akodon* (Sbalqueiro et al. 1984).

In addition, the present study reveals several synapomorphies of the cytochrome b gene that diagnose the clade composed by 27 species of *Akodon* on one hand and the *Deltamys* clade on the other (Tab. 2). Eight character state transformations occurred along the line leading from the common ancestor of *Akodon* and *Deltamys* (node A in Fig. 2 b) to the common ancestor of *Akodon* species (node B in Fig. 2 b). However, these synapomorphies are obscured by the fact that in at least one case for each synapomorphy a reversal to the primitive character state has occurred within the *Akodon* clade. 74 nucleotide characters have changed state along the line leading from node A to the common ancestor of the *Deltamys* forms (node C in Fig. 2 b). 18 of these derived character states have not evolved independently in any of the 27 *Akodon* species in consideration. The other 56 derived character states of *Deltamys* also arose independently at least in one internal line of the *Akodon* clade (see Tab. 2 for details). This fact is not unexpected given the high amount of homoplasy (CI = 0.110) for the cytochrome b gene. In summary, according to the present study there are 83 molecular synapomorphies that diagnose *Deltamys* and *Akodon*. Of these shared derived character states, 18 allow unambiguously determination of *Deltamys* from *Akodon*. It must be noted that the sequencing of more specimens could potentially decrease these numbers. When DNA sequences are translated to amino acids, the changes along the line leading to the *Deltamys* clade include nine synapomorphies (Tab. 3). Of these, five independently also arose in at least one line within the *Akodon* clade. Therefore, there are four amino acid synapomorphies that allow unambiguous diagnosis of *Deltamys* with respect to *Akodon*.

The clade *Akodon-Deltamys* is sister to the clade ((*Thaptomys*-"*Akodon*" *serrensis*) *Necromys*), which together constitute a strongly supported (100% of bootstrap and jackknife support) group of akodon-like sigmodontine rodents widely distributed in South America. However, most of the branching pattern within this clade is weakly supported as indicated by the collapsing of most groups in both bootstrap and jackknife analyses. Similarly, the majority of the other suprageneric groups of sigmodontine have low values of bootstrap and jackknife support, although the latter are in general slightly higher than the former. For instance, with the exception of the abrothricine clade, all main suprageneric groups within the sigmodontine clade collapsed in the bootstrap analysis. Meanwhile, a moderate support (62%) for both the basal position of *Sigmodon* and for the akodontine tribe was obtained in the jackknife analysis. Smith and Patton (1999) earlier documented this general pattern where most sigmodontine suprageneric groups lack support when analyses are based on cytochrome b gene sequences. It is unclear at the moment if the lack of resolution of suprageneric groups of sigmodontines results from the limited resolving power of the cytochrome b gene at that taxonomic level and/or to an explosive radiation of the group following their entry into South America.

Clearly, phylogenetic analyses of other sources of evidence are needed to further evaluate the hypothesis about the generic validity of *Deltamys*, as well as those other hypotheses prompted by phylogenetic analyses of cytochrome b gene sequences (e. g., Smith and Patton 1999; D'Elía et al. 2003). Of those possible sources of evidence, the study of more slowly evolving DNA sequences not linked to the mitochondrial genome (i. e., nuclear sequences; see D'Elía 2002), as well as broad morphological analyses not limited to craniodental features, are needed. Another approach that should be pursued to test our results is to expand the taxonomic coverage of the study to include representatives of additional akodontine taxa believed to be closely related to *Akodon*, such as *Thalpomys* and *Podoxymys*. The inclusion of these taxa may also help to stabilise parts of the sigmodontine tree.

Table 2. Molecular synapomorphies of *Deltamys* (1–74) and *Akodon* (75–81) as revealed by maximum parsimony analysis of cytochrome b gene sequences. 74 fixed derived character states were found in *Deltamys*. Of these, 18 derived character states, which are indicated by asterisks, have not evolved independently in any species of *Akodon*. The remaining 56 derived states have also secondarily appeared in at least one species of *Akodon*. Numbers between parentheses indicate, respectively at both sides of the bar, the number of *Akodon* species in which the character state under consideration is present and the number of times it evolved within the *Akodon* clade. Seven synapomorphies were recovered for the *Akodon* clade. The number of species of *Akodon* that secondarily reverse to the primitive character state as well as the number of times this event has taken place are indicated between parentheses at both sides of the bar respectively.

	Nucleotide position/ Codon position	Character State			
		Akodon and *Deltamys* common ancestor	*Deltamys* common ancestor and subspecies	*Akodon* species common ancestor	*Akodon* species
1	15/3	A or G	T	A or G	A/G/T (1/1)
2	20/2	A	C*	A	A
3	66/3	A	C*	A	A
4	124/1	A	G	A	A/G (10/1)
5	129/3	C	T	C	T/C (4/4)
6	156/3	C	T	C	C/T (4/4)
7	178/1	A	G	A	A/G (1/1)
8	189/3	C	T*	C	C
9	198/3	C	T	A	C/A/T (1/1)
10	210/3	C	T	C	C/T (3/3)
11	222/3	C	T	C	C/T (4/4)
12	240/3	C	T	C	C/T (1/1)
13	261/3	C	T	C	C/T (5/3)
14	288/3	C	T	C	C/T (5/3)
15	291/3	C	T	C	C/T (10/3–6)
16	327/3	C	T	C	C/T (3/3)
17	345/3	C	T	C	C/T (5/4)
18	358/1	T	C	T	T/C (5/2–3)
19	360/3	C	G*	C	C/T
20	363/3	C or T	A	C or T	C/T/A (1/1)
21	366/3	A	T*	A	A
22	384/3	C	T	C	C/T (13/3–4)
23	396/3	A	T	A	A/G/T (1/1)
24	405/3	A	G	A	A/G (2/2)
25	417/3	C	A*	C	C/T
26	426/3	G	C*	G	A/G/T
27	447/3	C	T	C	C/T (3/2)
28	459/3	C	T	C	C/T (4/4)
29	462/3	C	T*	C	C/A
30	474/3	C	T*	C	C
31	498/3	T	G	T	A/G/T/C (2/2)
32	501/3	A or C	G	C	A/T/C/G (1/1)
33	510/3	T	C	T	T/C (9/4)
34	573/3	C	T	C	C/T (3/2)
35	574/1	T	C	T	T/C (3/3)
36	592/2	C	T	C	C/T (8/5)
37	598/2	C	T*	C	C
38	600/3	T	A*	T	T/C
39	630/3	C	T	C	C/T (1/1)
40	645/3	C	A*	C	C/T

Table 2. (continued)

	Nucleotide position/ Codon position	Character State			
		Akodon and *Deltamys* common ancestor	*Deltamys* common ancestor and subspecies	*Akodon* species common ancestor	*Akodon* species
41	669/3	C	T*	C	C
42	672/3	C	T	C	C/T (2/2)
43	684/3	T	C	T	T/C (5/4)
44	712/1	G	A	G	G/A (1/1)
45	723/3	T	C	T	T/C (1/1)
46	750/3	C	A	C	C/T/A (5/2)
47	759/3	A	T*	A	A/C
48	765/3	T	C	T	T/C (3/3)
49	768/3	T	C	T	T/C (8/6)
50	786/3	C	A	C	A/T/C (1/1)
51	789/3	C	T	C	C/T (1/1)
52	801/3	C	T*	C	C
53	813/3	A	G	A	A/G (5/3–4)
54	828/3	T	C	T	T/C (1/1)
55	831/3	C	T	C	A/C/T (3/2)
56	834/3	C	T	C	C/T (10/4–8)
57	843/3	T	A	T	T/C/A (3/1)
58	846/3	A	G*	A	A
59	858/3	C	T	C	C/T (1/1)
60	879/3	C	T	C	C/T (1/1)
61	883/1	A	G	A	A/C/G (9/3)
62	897/3	A	T	C	C/T (2/1)
63	907/1	G	A	G	G/A (7/5)
64	921/3	A	G	A	A/G (5/3–4)
65	945/3	A	G	A	G/A (1/1)
66	1014/3	T	C	T	T/C (4/3)
67	1020/3	C	T	C	G/C/T (8/8)
68	1035/3	C	T	C	C/T (9/7)
69	1053/3	A	G	A	A/G (2/2)
70	1080/3	C	A	?	C/T/A (1/1)
71	1087/1	A	G*	A	A/C
72	1093/1	C or T	A*	C or T	T/C
73	1095/3	A	T	A	C/G/T/A (6/2)
74	1113/3	T	C	T	T/C (12/6)
75	114/3	C	C	T	T/C (7/2)
76	252/3	C	C	T	T/C (9/4)
77	558/3	A	A	T	C/T/A (1/1)
78	615/3	T	T	C	C/T (7/3)
79	631/1	C	C	T	T/C (7/5)
80	837/3	T	T	C	C/T (6/4)
81	874/1	C	C	T	T/C (7/5–6)

Table 3. Amino acid synapomorphies of *Deltamys*. The translation of the DNA sequences into amino acid sequences revealed nine fixed derived character states in the *Deltamys* clade with respect to the *Akodon* clade. However, five of those character states have also independently appeared at least one time within the *Akodon* clade. Numbers between parentheses indicate, respectively, the number of *Akodon* species in which the character state under consideration is present and the number of times it has evolved within the *Akodon* clade. The remaining four *Deltamys* aminoacid synapomorphies, which are indicated by asterisks, have not evolved independently in any species of *Akodon*. Aminoacid abreviations are as follow: Asn = Asparagine, Ala = Alanine, Ileu = Isoleucine, Leu = Leucine, Met = Methionine, Phe = Phenylalanine, Thr = Threonine, Val = Valine.

	Codon number	Character state			
		Akodon and *Deltamys* common ancestor	*Deltamys* common ancestor and subspecies	*Akodon* species common ancestor	*Akodon* species
1	7	Asn	Thr*	Asn	Asn
2	42	Met	Val	Met	Met/Ileu/Val (10/1)
3	60	Thr	Ala	Thr	Thr/Ala (1/1)
4	120	Phe	Leu	Phe	Phe/Leu (5/2–3)
5	238	Ala	Thr*	Ala	Ala/Val/Ileu
6	295	Ileu	Val	Ileu	Ileu/Leu/Val (9/3)
7	303	Ala	Thr	Ala	Ala/Ileu/Thr (6/5)
8	363	Ileu	Val*	Ileu	Ileu/Leu/Thr
9	365	Leu	Ileu*	Leu	Leu

Finally, we point out the significance of the present work for conservation biology. There is general agreement that the design of conservation strategies should also take into account evolutionary diversity (Moritz 1994; Dimmick et al. 1999). The finding that *Deltamys* represents an important branch within the sigmodontine radiation (i. e., is not nested into the radiation of *Akodon*) implies that conservation efforts must be directed to preserve populations of this main sigmodontine evolutionary line for which only one species, with two subspecies, has been so far described.

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Zusammenfassung

Phylogenetische Analyse sigmodontiner Nager (Muroidea), mit besonderer Berücksichtigung der akodonten Gattung *Deltamys*

Die Autoren stellen eine umfassende phylogenetische Analyse der sigmodontinen Nagetiere mit Hilfe von Cytochrom b Gen-Sequenzen vor. Von besonderem Interesse ist dabei die phyletische Stellung und der fragliche generische Status von *Deltamys*, einem Taxon, das endemisch in einem kleinen Gebiet des La Plata Flußdeltas in Argentinien, Brasilien und Uruguay vorkommt. Die Studie lieferte drei wesentliche Ergebnisse: (1) Baumwollratten (*Sigmodon*) bilden die basale Gruppe der Unterfamilie Sigmodontinae, (2) das Tribus Akondontini ist monophyletisch, und (3) *Deltamys* fällt außerhalb einer Gruppe, die alle untersuchten Arten von *Akodon* umfaßt, und sollte daher Gattungsrang erhalten.

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Authors' addresses: GUILLERMO D'ELÍA, Laboratorio de Evolución, Facultad de Ciencias, Igúa 4225, Montevideo 11400, Uruguay (e-mail: guillermo@fcien.edu.uy); ENRIQUE GONZÁLEZ, Museo Nacional de Historia Natural. Casilla de Correo 399, 11.000 Montevideo, Uruguay; ULYSES F. J. PARDIÑAS, Centro Nacional Patagónico, Casilla de Correos 128, 9120 Puerto Madryn, Chubut, Argentina