SYSTEMATICS OF SPECIES OF THE GENUS *AKODON* (RODENTIA: SIGMODONTINAE) IN SOUTHEASTERN BRAZIL AND IMPLICATIONS FOR THE BIOGEOGRAPHY OF THE *CAMPOS DE ALTITUDE*

PABLO RODRIGUES GONÇALVES, PHILIP MYERS, JÚLIO FERNANDO VILELA AND JOÃO ALVES DE OLIVEIRA

MISCELLANEOUS PUBLICATIONS

MUSEUM OF ZOOLOGY, UNIVERSITY OF MICHIGAN, NO. 197

Ann Arbor, January, 2007
ISSN 0076-8405

P U B L I C A T I O N S O F T H E
MUSEUM OF ZOOLOGY, UNIVERSITY OF MICHIGAN NO. 197

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COVER ILLUSTRATION —*Campos de altitude*, Brazil

SYSTEMATICS OF SPECIES OF THE GENUS *AKODON* (RODENTIA: SIGMODONTINAE) IN SOUTHEASTERN BRAZIL AND IMPLICATIONS FOR THE BIOGEOGRAPHY OF THE *CAMPOS DE ALTITUDE*

Pablo Rodrigues Gonçalves[1, 3], Philip Myers[2], Júlio Fernando Vilela[1] and João Alves de Oliveira[1]

ABSTRACT

Faunal inventories of the highest peaks of the Atlantic forests of South America, in the Caparaó and Itatiaia mountain ranges in Southeastern Brazil, have revealed a new community of small mammals. The species making up this community appear to be restricted to the highest altitudinal zones and are found in close association with scattered montane grasslands (*campos de altitude*). Their phylogenetic relationships can provide insights into speciation in mountaintop communities of the Atlantic forest. In this paper we review the taxonomic identity and systematic relationships of *Akodon mystax*, a high-altitude endemic described from Caparaó. We demonstrate that populations from Itatiaia previously assigned to *mystax* are morphologically, cytogenetically and genetically distinct from that species and appear to represent a northern isolate of *Akodon paranaensis*, and that *Akodon mystax* is closely related to *Akodon lindberghi* from the Central Brazilian grasslands. Phylogenetic relationships among these populations demonstrate that differentiation of the mountaintop endemics of Caparaó and Itatiaia is not solely attributable the isolation of *campos de altitude* from southern grasslands of Brazil and Uruguay as grasslands retreated southward following glaciation. Instead, phylogenetic analyses of some groups suggest connections between the *campos de altitude* and the grasslands of Central Brazil.

Key words: Rodentia, Sigmodontinae, *Akodon paranaensis*, *Akodon mystax*, *Akodon lindberghi*, *Akodon sanctipaulensis*, *Akodon reigi*, *Akodon cursor*, *Akodon philipmyersi*, *Akodon azarae*, biogeography, *campos de altitude*, Brazil

INTRODUCTION

Akodon is the most diverse genus of the tribe Akodontini, one of the major subdivisions of Neotropical rodents of the subfamily Sigmodontinae. Species diversity within the genus is remarkably high in the Andean habitats of western South America, from which roughly 70% of the living species of *Akodon* have been described (Reig, 1987). Accordingly, efforts to clarify and revise the taxonomy of the members of the genus have focused mostly on western South American forms, resulting in the definition of groups of genetically and morphologically similar species, such as the *varius*, *fumeus* and *boliviensis* groups (Myers, 1989; Myers *et al*., 1990). These species-group taxonomic treatments have provided a valuable initial framework for hypotheses concerning the evolutionary history (Smith & Patton, 1993) and biogeography (Patton *et al*., 1990; Patton & Smith, 1992) of the Andean fauna. Taxonomic treatments of *Akodon*, however, have rarely included species from eastern South America, and several of these eastern species are known only from their original descriptions.

Lack of a clear understanding of the phyletic relationships of eastern South American *Akodon* is especially unfortunate because some species appear to be closely linked with or restricted to the Cerrado, Atlantic forest, and Southern grasslands biomes (Fonseca *et al.*, 1996). This suggests an association with the evolution of the biotas of these regions, and thus, a window for studying how diversification has proceeded in these unique areas.

Hershkovitz (1990) recently attempted to include all species of *Akodon* in a sweeping taxonomic arrangement, creating two groups based on body size, the *boliviensis* (small) and *mollis* (large) groups. Hershkovitz's goal was apparently to pave the way for his descriptions of new species from Brazil rather than to provide an evolutionary hypothesis or a detailed revision of the genus.

Despite the lack of broad revisionary treatments of *Akodon*, the number of recognized species within the genus has experienced a steady increase in the last decade, especially in eastern South America. Intensified genetic studies have unraveled cryptic species complexes, such as the cytogenetically diverse *cursor* group (Fagundes *et al*., 1997; Fagundes *et al.*, 1998; Geise *et al*., 2001). As well, new inventories have led to the discovery of new species (Hershkovitz, 1998; Gonzalez *et al.*, 1998; Christoff *et al*., 2000; Pardiñas *et al*., 2005). These studies have greatly improved the knowledge of the diversity of *Akodon*.

One of the species resulting from this recent wave of descriptions is *Akodon mystax* Hershkovitz 1998, described from the Caparaó, a major coastal mountain in the Atlantic forest of southeastern Brazil. This small mouse is restricted to the high elevation grass-dominated habitats called *campos de altitude,* which occur between 1800-2890m and cover the highest summits of Caparaó and other Atlantic forest mountain ranges (Bonvicino *et al*., 1997; Safford, 1999a). Little is known about the ecology and evolution of this unique high-elevation environment and its endemics, and the phylogenetic patterns of populations of *A. mystax* on different peaks

[1]Departamento de Vertebrados, Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.
[2]Mammals Division and Dept. Ecology and Evolutionary Biology, University of Michigan Museum of Zoology, Ann Arbor, MI, USA.
[3]contact: prg@acd.ufri.br.

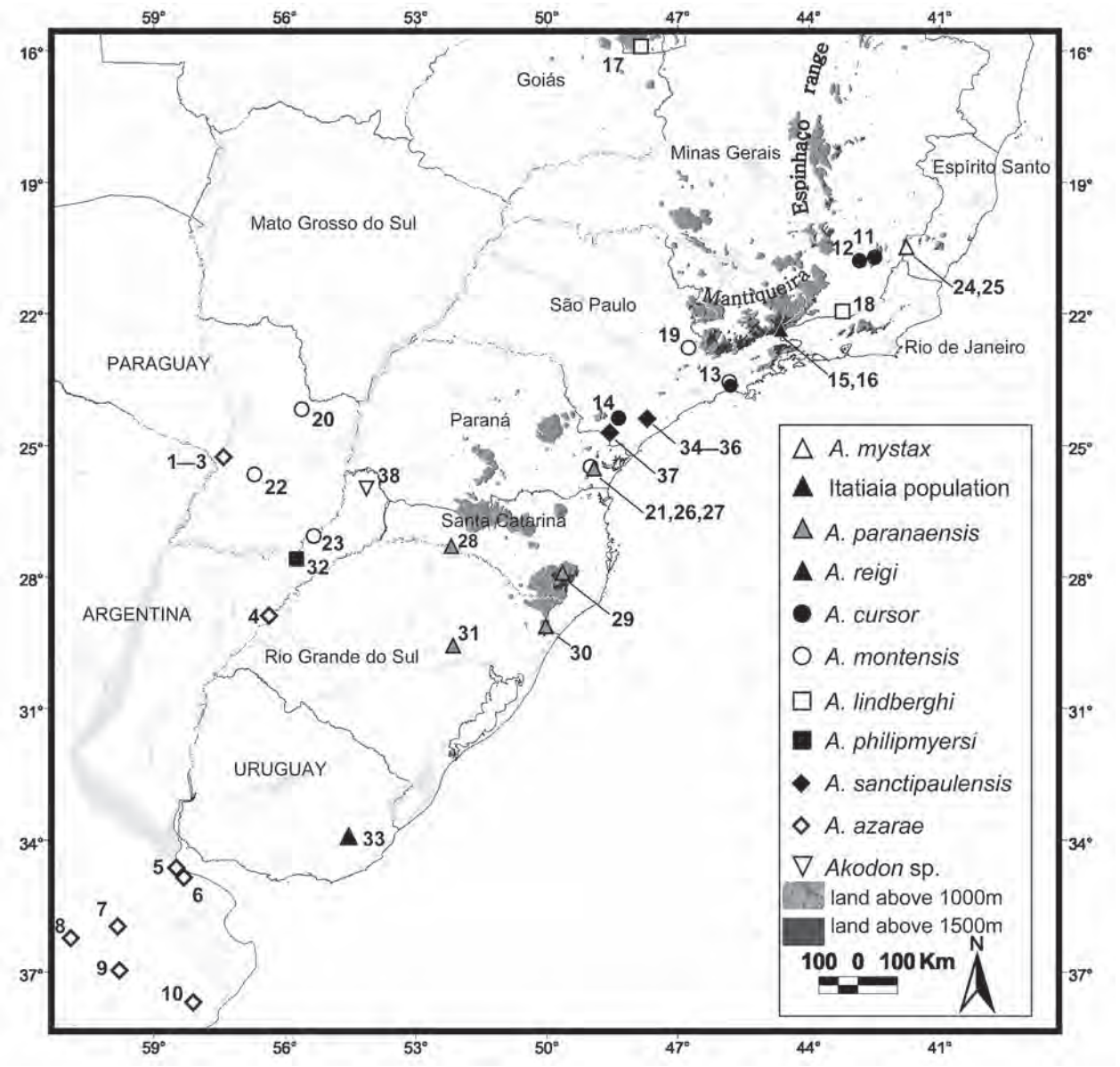

Fig. 1. Map showing major mountain ranges in southeastern and southern Brazil and samples of eastern species of the genus *Akodon* analyzed in this study. Localities: [1] 24km NW Villa Hayes, Presidente Hayes: [2] 15.5km NW Chaco, Presidente Hayes, [3] 83.2km NW Puerto Falcon, Presidente Hayes; [4] Pirayui, Capital, Corrientas; [5] Hurlingham, Buenos Aires, [6] Ezeiza, 20km S Buenos Aires, [7] Capital Federal, Nunez, Costa do Rio La Plata; [8] Torrecita (Urdampilleta), Buenos Aires [9] 35km Sierra Azul, Buenos Aires; [10] INTA, Balcarce, Buenos Aires; [11] Fazenda Neblina, Pq. Est. Serra do Brigadeiro, Fervedouro, Minas Gerais; [12] Mata do Paraíso, Viçosa, Minas Gerais; [13] Estacao Ecologica Boraceia, Salesopolis, Sao Paulo [14] Fazenda Intervales, Capão Bonito, Sao Paulo; [15] Brejo da Lapa, Itatiaia, Itamonte, Minas Gerais; [16] Campos do Itatiaia, Abrigo Rebouças, Pq. Nac. Itatiaia, Rio de Janeiro; [17] Matosa, Parque Nacional de Brasília, Brasília, Distrito Federal; [18] Sitio Maglandia, Simao Pereira, Minas Gerais; [19] Pedreiras, Sao Paulo; [20] Estancia Felicidad, Canindeyu; [21] Mananciais da Serra, Piraquara, Paraná; [22] Sapucai; [23] Caraguatai, Misiones; [24] Arrozal, [25] Terreirão, Parq. Nac. Caparaó, Alto Caparaó, Minas Gerais; [26] Estacao Ecologica de Canguiri, Piraquara, Parana; [27] Roca Nova, Parana; [28] Três Barras, margins of Uruguay river, Aratiba, Rio Grande do Sul; [29] Urubici, Santa Catarina [30] Parq. Nac. Aparados da Serra, Rio Grande do Sul; [31] Venâncio Aires, Rio Grande do Sul. [32] Estáncia Santa Inés, Posadas, Misiones, Argentina [33] Paso Averías, Lavalleja, Uruguay; [34] Morretinho, São Paulo; [35] Primeiro Morro, São Paulo; [36] Quadro Penteado, São Paulo [37] Iporanga, São Paulo.

may provide valuable insights into how communities in these regions have evolved. However, the reconstruction of the relationships of this high-altitude endemic rodent has proven controversial. Hershkovitz (1998), in the description of *A. mystax*, suggested that this species would be most closely related to other small-bodied eastern forms such as *A. azarae, A. lindberghi* or *Akodon sanctipaulensis*. Recent molecular phylogenetic analyses, however, have repeatedly placed *A. mystax* close to *A. paranaensis* and *Akodon reigi*, two large-sized species

from the southern Brazil and Uruguay (D'Elía *et al.*, 2003; Pardiñas *et al.*, 2003, 2005; Smith & Patton, in press).

Sequence data for these studies of *Akodon mystax* have not come from type material, but from specimens collected in the montane habitats of the Itatiaia Mountain, in Rio de Janeiro state (Geise *et al.*, 2001; Geise *et al.*, 2004). Itatiaia and Caparaó share *campos de altitude*, but these mountains are separated by more than 370 km of lowland forested habitats, and the Itatiaia specimens have never been compared carefully with type material of *A. mystax*. Nevertheless, lacking additional samples from Caparaó, authors subsequent to Hershkovitz (1998) have considered sequence data derived from specimens from Itatiaia as representative of *A. mystax*. Using genetic samples from Itatiaia Mountain, Pardiñas *et al.* (2003) suggested close genetic similarities among *A. mystax*, *A. paranaensis*, *A. reigi* and an unidentified sample from Misiones, Argentina. However, they remarked on the morphological heterogeneity of this clade, noting that *A. mystax* departs widely from the morphology exhibited by *A. paranaensis* and *A. reigi*. D'Elía *et al.* (2003), Pardiñas *et al.* (2005) and Smith and Patton (*in* press) added additional support to the *mystax—paranaensis—reigi* clade, which they repeatedly recovered in phylogenetic analyses with more dense taxonomic sampling of the genus. This clade is apparently also supported by cytogenetics; *paranaensis*, *reigi*, and "*mystax*" from Itatiaia Mountain share a common diploid number of 44 chromosomes (Bonvicino *et al.*, 1997; Gonzalez *et al.*, 1998; Christoff *et al.*, 2000). Yet all phylogenetic conclusions made so far about *A. mystax* are critically based on the assumption that the Itatiaia population represents *A. mystax*. Confusion over the identity of these Itatiaia specimens is also suggested by their referral to *A. reigi* by Geise *et al.* (2004a, b).

Recent collections of sigmodontines made by us at Caparaó and Itatiaia allowed us to reexamine the *Akodon* species from the high-elevation habitats of these two mountain ranges. Our morphological and cytogenetic comparisons of topotypes and type series of *A. mystax* with specimens from Itatiaia revealed differences between these two samples, stimulating a reappraisal of the molecular phylogenetics of *A. mystax* using confidently identified material. In this paper we clarify the systematic relationships of *Akodon mystax*, discuss its morphological and cytogenetic similarities with other species of the genus, and reassess its phylogenetic position in an analysis that includes cytochrome-*b* sequence data derived from topotypes. Finally, we address the biogeographic implications that stem from the phylogenetic patterns of this *campos de altitude* endemic, especially those regarding the historical interconnections of these unique high-altitude communities of southeastern Brazil with other South American biomes.

MATERIAL AND METHODS

SAMPLES. Detailed morphological comparisons were made within a restricted set of species selected to include candidates for close relationship to *Akodon mystax* as suggested by previous morphological (Hershkovitz 1998) and molecular (*e.g.*, D'Elía, 2003; Pardiñas *et al.*, 2005) studies. This set comprised (1) *A. mystax* (collected at or near the type locality in the Caparaó *campos de altitude*), (2) the 2n = 44 population from Itatiaia referred originally by Geise *et al.* (2001) to *A. mystax* (hereafter referred to as the Itatiaia population); (3) *A. cursor*; (4) *A. montensis*, (5) *A. paranaensis*, (7) *A. azarae*, (8) *A. lindberghi*, (9) *A. sanctipaulensis* and (10) *A. philipmyersi* (localities shown in Fig.1). Molecular phylogenetic analyses included these and several additional species that were represented solely by sequence data (see Molecular Analyses). One of the latter group is a specimen from Misiones, Argentina, designated *Akodon* sp2. by Pardiñas *et al.* (2003) (*Akodon* sp. in Fig. 1)

The series of *Akodon mystax* analyzed by us included the original type series plus additional specimens we collected at the type locality in the Parque Nacional do Caparaó, in the *campos de altitude* of Terreirão (2500-2700m; loc. 24 in Fig. 1), and at Pico da Bandeira (2600-2700m, loc. 25 in Fig.1), totaling 55 specimens. Morphological and molecular analyses also included the sample from Itatiaia (Brejo da Lapa, 2000m, loc. 15 in Fig. 1) with 44 chromosomes reported by Geise *et al.* (2001), and 17 specimens (also 2n = 44) collected by us at the Campos do Itatiaia (2300-2400m; 6km SE from Brejo da Lapa), Parque Nacional do Itatiaia, Rio de Janeiro. *Akodon paranaensis*, *A. reigi* and members of the *cursor* group (*A. montensis* and *A. cursor*) are morphologically cryptic and no diagnostic craniodental characters have been found that reliably discriminate them. Therefore, *a priori* taxonomic identifications of samples from these taxa relied on examination of type or topotypical material (for *A. paranaensis*, *A. sanctipaulensis* and *A. lindberghi*) and karyotypes or sequence data (*A. montensis* and *A. cursor*) analyzed by us or by other authors that clearly referenced genetic data to voucher specimens we examined (see Christoff *et al.*, 2000; Geise *et al.*, 2001; Pardiñas *et al.*, 2003; D'Elía *et al.*, 2003). In this way, we avoided the inclusion of non-karyotyped or non-sequenced samples that could include more than one species and therefore provide misleading morphological discrimination patterns. Localities of samples and material examined are listed in Appendix I.

MORPHOLOGICAL ANALYSES. Quantitative analyses of morphological variation among and within samples were based on 16 craniodental measurements taken with a digital caliper accurate to 0.01mm. The

measurements were as follows: greatest skull length (GSL), condyloincisive length (CIL), nasal length (NL), length of diastema (LD), length of palatal bridge (LPB), length of maxillary molar series (LM), breadth of 1st upper molar (BM1), length of incisive foramina (LIF), breadth of rostrum (BR), depth of rostrum (DR), breadth of palatal bridge (BPB), breadth of zygomatic plate (BZP), least interorbital breadth (LIB), breadth of braincase (BB), and depth of braincase (DB, measured at the midline with caliper tips placed at the basioccipital-basisphenoid suture and along the suture connecting left and right parietal). These measurements are as defined by Voss (1991), Carleton & Musser (1993) and Myers *et al.* (1990) with the exception of DB. Head and body, tail, hindfoot (HF) and ear lengths, recorded from skin tags, were also considered in morphological comparisons, although they were not included in multivariate statistical analyses. Multivariate patterns of phenotypic similarity among taxa were assessed by Principal Components and Canonical Variate Discriminant Analyses, both relying on the covariance-variance matrices of log-transformed craniometric characters. Principal Component Analysis (PCA) was used primarily to summarize the general trends of shape and size variation among taxa. The relative contribution of each character to the multivariate patterns portrayed by the Principal Components was interpreted directly from the standardized vector correlation coefficients. Canonical Variate Discriminant Analysis was used on selected comparisons of *a priori* identified samples to test if morphometric characters could consistently discriminate among closely related species. Contributions of craniometric characters to each discrimination axis were evaluated by Pearson product-moment correlation coefficients between log-transformed character values and discriminant scores (Strauss 1985). We restricted the age-interval of specimens used in statistical analyses to adults (molars completely erupted and worn, with M3 presenting no trace of enamel islands or flexi on its occlusive surface) in order to ensure that the morphometric differences obtained reflected genetic rather than ontogenetic variation. We made exceptions only in the case of *A. sanctipaulensis*, due to absence of adults in the small sample available. Statistical significance of morphometric discrimination patterns were assessed by univariate (ANOVA) and multivariate (MANOVA) analyses of variances. Descriptive statistics for external and craniodental measurements are provided in Appendix 2.

Qualitative comparisons of craniodental characters were made following the terminology used by Reig (1977), Voss (1988, 1991) and Myers *et al.* (1990). In addition to providing a summary of shared similarities among species, qualitative and morphometric character distributions served as potential markers for species limits and diagnosis between closely related forms.

CYTOGENETIC ANALYSES. Chromosome preparations of specimens collected by us were obtained from short-term bone marrow cultures. Cell suspensions were cultivated for two hours at 37°C in RPMI 1640 culture medium supplemented with 20% fetal calf serum, ethidium bromide (5μg/ml) and colchicine (10^{-6}M). Karyotypes were determined on Giemsa-stained slides prepared following standard protocols (Ford & Hamerton, 1956). 2n refers to the diploid number of chromosomes while FN refers to the fundamental number of chromosomal arms excluding sex chromosomes.

MOLECULAR ANALYSES. DNA was isolated from ethanol-preserved liver tissues using either the Dneasy extraction kit (QIAGen) or following the proteinase K/chloroform/ethanol procedure described by Sambrook (1989). A fragment of 1140 bases representing the complete mitochondrial cytochrome *b* gene was amplified using primers MVZ05 and MVZ14 (Smith & Patton, 1993) in PCR reactions of 35 cycles of denaturation at 94C for 20s, annealing at 50C for 1 min. and extension at 72C for 1.5 min. Negative controls were always included in reactions, and size and quality of all PCR products were confirmed on 1% agarose gels prior to purification and sequencing reactions. Purification of PCR products was done using the QIAquick DNA purification kit (QIAgen). Sequencing of the entire 1140bp fragment was accomplished using primers MVZ05 (Smith & Patton, 1993) and MEU1 (5' ACAACCATAGCAACAGCATTCGT3'). Sequences were entered, assembled and manually aligned in Chromas Pro (Technyelsium Inc.).

In addition to the specimens sequenced in this study, sequences from all species of *Akodon* available on GenBank were included in the phylogenetic analyses (Appendix 1). Most of the *Akodon* cytochrome *b* sequences available in the GenBank dataset were generated or have been updated by Smith & Patton (in press). Other sequences were generated by Smith & Patton (1993), Geise *et al.* (2001), D'Elía (2003), D'Elía *et al.* (2003), Pardiñas *et al.* (2003, 2005). Representatives of other akodontine genera such as *Deltamys*, *Thaptomys, Bolomys*, *Oxymycterus, Brucepattersonius, Blarinomys* and *Lenoxus* were also included to insure that new sequences fell within the genus *Akodon*.

Cytochrome b sequences were subjected to maximum parsimony analyses conducted on PAUP 4.0 (Swofford, 1999). Parsimonious trees were recovered using a heuristic search with 200 random sequences of taxa addition and tree-bisection-reconnection branch-swapping algorithm. Consistency of nodes was evaluated by 1000 bootstrap replicates (Felsenstein, 1985), conducted by heuristic searches with only 10 random sequences of taxa addition to ensure faster computational times. Trees were rooted

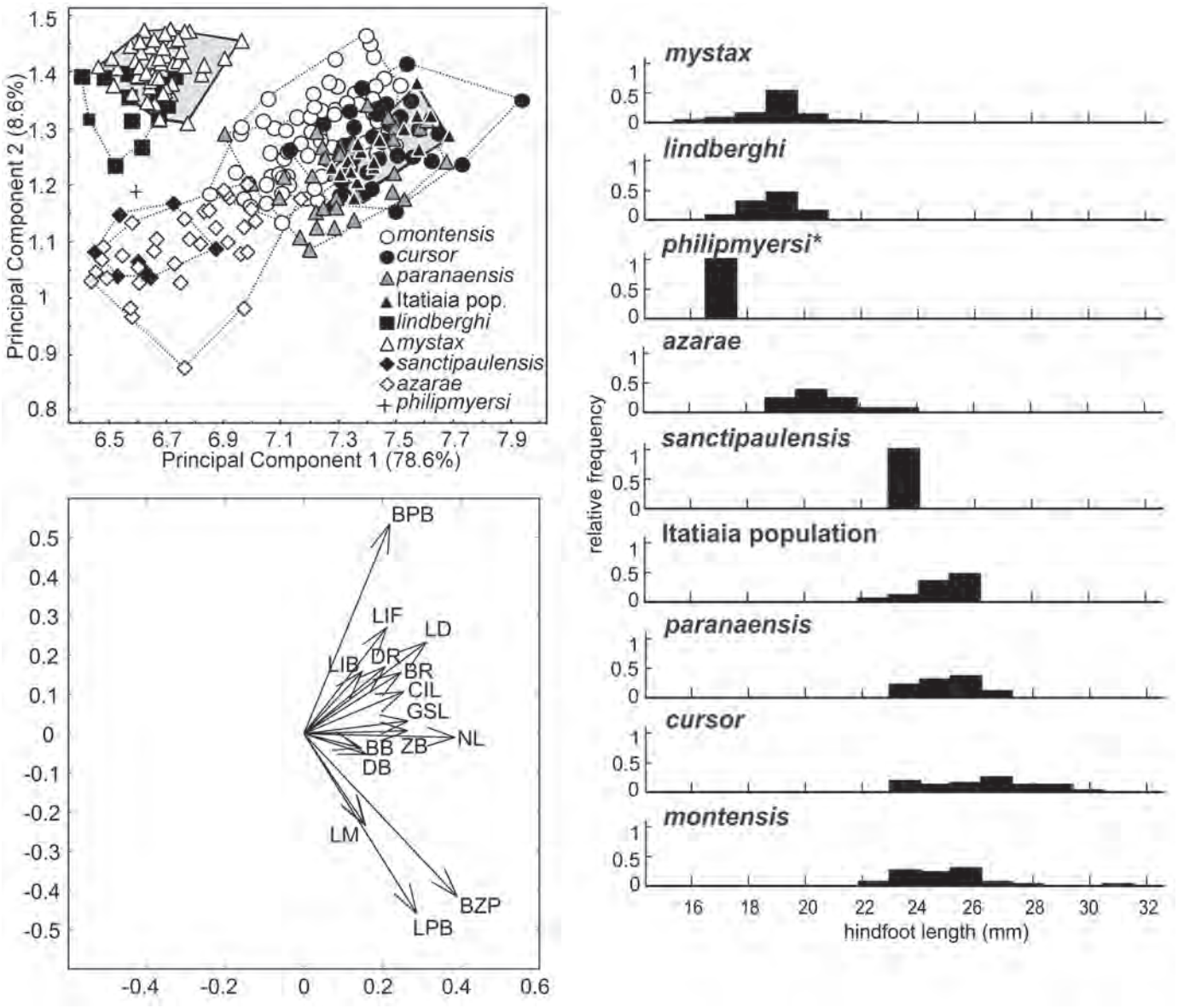

Fig. 2. Morphometric variation among eastern species of the genus *Akodon*: (a) – multivariate individual scores for the first and second principal components (population multivariate spaces of *A. mystax* and the Itatiaia population are in gray); (b) – vector correlation of craniometric characters with first and second principal components; (c) – histogram of distributions of hindfoot length across species (in millimeters).

using the abrothrichines *Geoxus valdivianus* and *Abrothrix andinus* as outgroups.

RESULTS

Morphological analyses

PRINCIPAL COMPONENT ANALYSIS. Most variation (78.6%) is explained by the 1st principal component (Fig. 2a). All morphometric characters are positively correlated with this axis (Fig. 2b), suggesting that it reflects cranial size (Voss *et al.*, 1992). The correlations of characters with other axes vary in sign, indicating that those axes reflect shape variation. The 1st and 2nd principal component axes together summarize 87.2% of the total craniometric variation, and scatter along these two axes reveals three clusters (Fig. 2a). One cluster ("group 1" below), includes *Akodon cursor*, *A. montensis*, *A. paranaensis*, and members of the Itatiaia population. It consists of distinctively larger individuals with long rostra (long nasals, diastema, and palatal bridge) and broad zygomatic plates. Two clusters of smaller mice (groups 2 and 3) are clearly separated on PC2. Members of group 2, including *A. mystax* and *A. lindberghi*, have high values on this axis, indicating relatively short and broad palates, long incisive foramina and diastemas, narrow zygomatic plates, short maxillary

Table 1. Vector correlation coefficients between log-transformed craniometric characters and principal components (PC1 and PC2) extracted in an analysis of *Akodon mystax*, the Itatiaia population and selected eastern *Akodon* species.

Characters	Principal Components	
	PC1	PC2
GSL	0.260	0.035
ZB	0.268	0.018
CIL	0.253	0.084
NL	0.370	0.003
LD	0.311	0.231
LPB	0.292	-0.483
LM	0.155	-0.210
BM1	0.169	-0.213
LIF	0.204	0.276
BR	0.255	0.147
DR	0.215	0.141
BPB	0.235	0.526
BZP	0.379	-0.434
LIB	0.163	0.178
BB	0.150	-0.033
DB	0.158	-0.035

toothrows, and narrow M1s. Members of group 3 include *A. azarae* and *A. sanctipaulensis*. They have low values on both axes and are characterized by relatively longer and narrower palates, shorter incisive foramina and diastemas, broader zygomatic plates, larger maxillary toothrow and broader M1s compared to group 2. *Akodon philipmyersi*, represented by a single specimen, occupies an intermediate position between groups 2 and 3.

HIND FOOT LENGTH. Length of hind foot is highly correlated with general size (PC1) among samples ($r = 0.77$, $P < 0.001$, $n = 270$), and the small and large-sized groups of species discriminated by the first principal component (Fig. 2c) are similar to groups suggested by hind foot length. The only disagreement is in populations of *Akodon sanctipaulensis*, which are grouped with a small-sized group by PCA but have hindfoot lengths similar to those of large-sized species (Fig. 2c). The *A. sanctipaulensis* included here are mostly young individuals with lightly worn teeth, comprising young adults or subadults. Young animals typically have disproportionately large feet, and the small overall size of the crania (and the resulting placement of *A. sanctipaulensis* with *A. azarae*) may result from the age distribution of specimens.

Members of the Itatiaia population have large hind feet (23-26 mm), placing them in the group of large-sized species (*paranaensis*, *cursor*, *montensis*).

QUALITATIVE TRAITS. Qualitative similarities in cranial characteristics suggest similar patterns of groupings of species populations (Figs 3, 4). We first describe traits that appear to separate the three groups identified by principal component analysis, then we describe and compare the members of each group separately. Our focus is on groups 1 and 2, which include *A. mystax* and the Itatiaia population.

The shape of the rostral region is especially informative. Members of the first group (the Itatiaia population, *Akodon paranaensis*, *A. cursor*, and *A. montensis*) have pronounced, stocky and broadened rostra, in which the nasals are not expanded laterally to the extent seen in group 2 (Fig. 4). The premaxillaries are conspicuous in dorsal view and not hidden beneath the nasals throughout the rostrum extent.

Members of the second group, *Akodon mystax* and *A. lindberghi*, are characterized by elongated rostra that taper gradually toward their distal limits. *Akodon philipmyersi* has an equally widened and robust rostrum, but the diastema and rostrum are shorter in this species than in *A.mystax* or *A. lindberghi* (Fig 3). Nasals of *A. mystax*, *A. lindberghi* and *A. philipmyersi* are noticeably expanded laterally and occupy almost the entire dorsal surface of the rostrum; consequently, in dorsal view the premaxillaries are restricted to slim lateral stripes that are completely hidden beneath the nasals distally.

In the third group, *Akodon azarae* also presents a relatively long rostrum. The nasals of *A. azarae* are less expanded than those of *A. mystax*, *A. lindberghi* and *A. philipmyersi*; this is especially obvious at the level of the nasolacrimal capsules, where in dorsal view the premaxillaries appear distinctively broad. The rostrum of *Akodon sanctipaulensis* is abruptly and distinctively tapered distally, giving it a narrow and strongly pointed appearance.

Other regions of the cranium also suggest a similar pattern of relationship. The interorbital regions of *Akodon mystax*, *A. lindberghi* and *A. philipmyersi* are relatively broad (LIB/ZB averages 38%). The lateral margins of the supraorbital region converge to the interorbital constriction in a nearly straight line, resulting in broad frontals with approximately squared supraorbital margins. In contrast, crania of *A. azarae*, *A. paranaensis*, *A. cursor, A. montensis*, and members of the Itatiaia population all have comparatively narrower (LIB/ZB averages 35%) and typical bi-concave or hourglass-shaped interorbital regions with supraorbital edges generally rounded, except in old adults, where these edges also become squared.

The interorbital region of *Akodon sanctipaulensis* is noticeably expanded (LIB/ZB averages 39%); the impression of breadth is strengthened by the narrow and pointed rostrum (Fig. 3d). Young specimens of *A. montensis* and *A. cursor* also present similarly broadened interorbital regions, and it is likely that the condition seen in *A. sanctipaulensis* reflects the young age of the specimens examined.

The incisive foramina of *Akodon mystax* and *A. lindberghi* are wide and long (LIF/LD averages 97%),

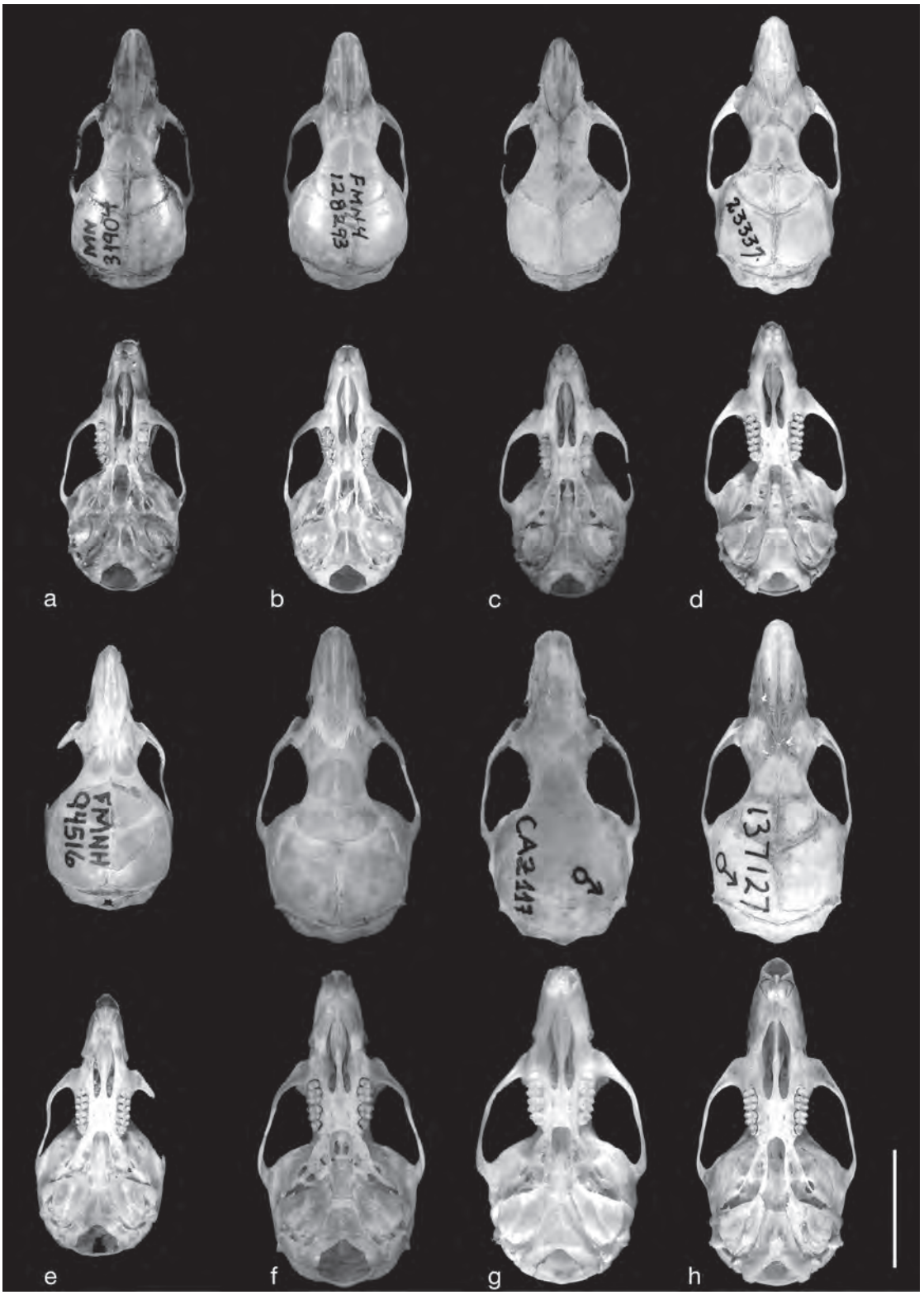

Fig. 3. Dorsal, ventral and lateral views of skulls of (a) *Akodon mystax* (topotype: Terreirão, Parque Nacional do Caparaó), (b) *A. lindberghi* (paratype: Matosa, Parque Nacional de Brasília, Brazil), (c) *A. philipmyersi* (paratype: Estancia Santa Inés, Misiones, Argentina), (d) *A. sanctipaulensis* (holotype: Primeiro Morro, São Paulo, Brazil) and (e) *A. azarae* (Torrecita, Buenos Aires, Argentina). Scale bar = 10mm.

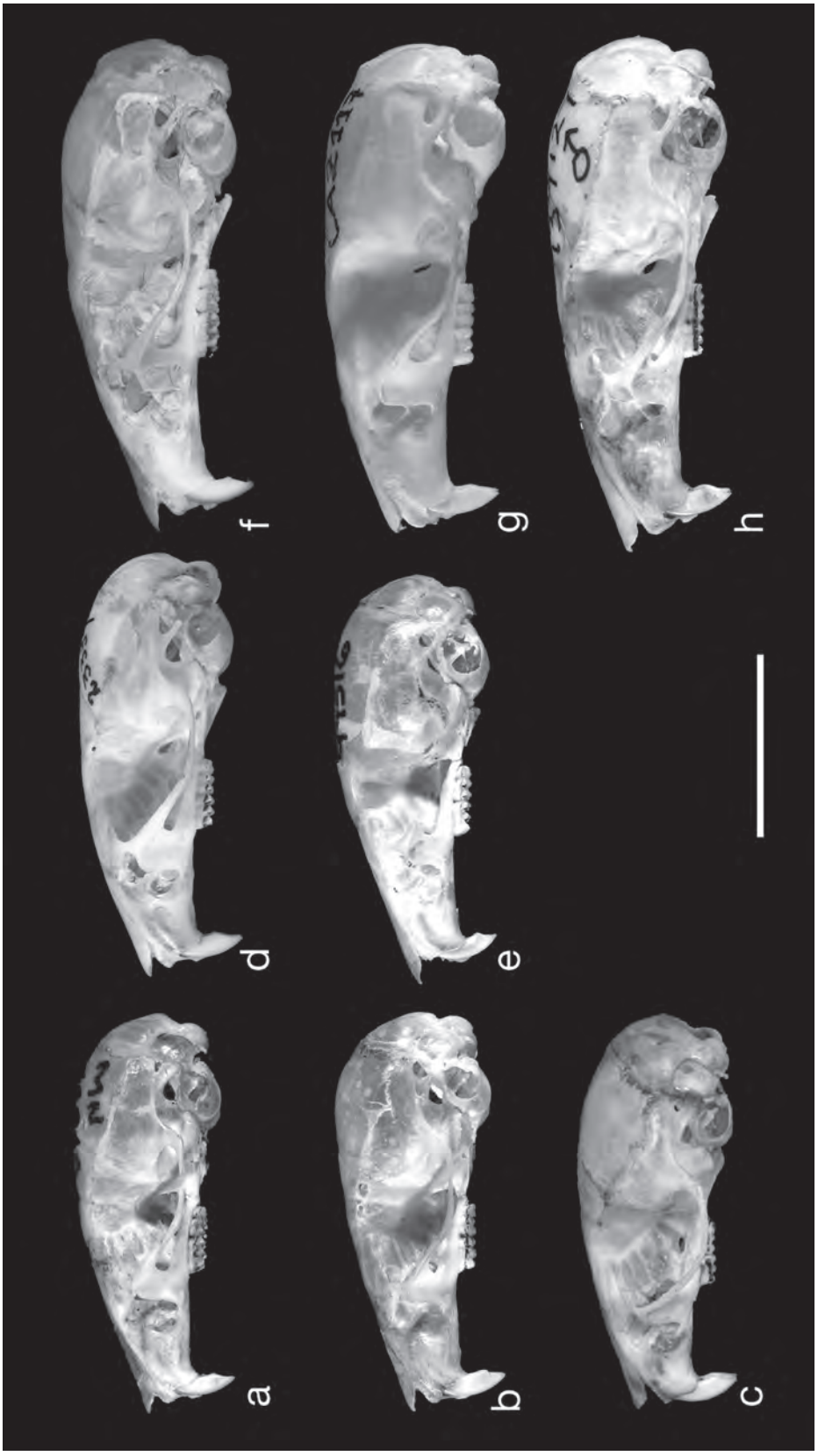

Fig. 4. Dorsal, ventral and lateral views of skulls of (a) the Itatiaia population (Campos do Itatiaia, Parque Nacional do Itatiaia, Rio de Janeiro, Brazil), (b) *Akodon paranaensis* (holotype: Piraquara, Paraná, Brazil), (c) *A. montensis* (Parque Nacional Ybycuí, Paraguarí, Paraguay) and (e) *A. cursor* (Estação Biológica de Boracéia, São Paulo, Brazil). Scale bar = 10mm.

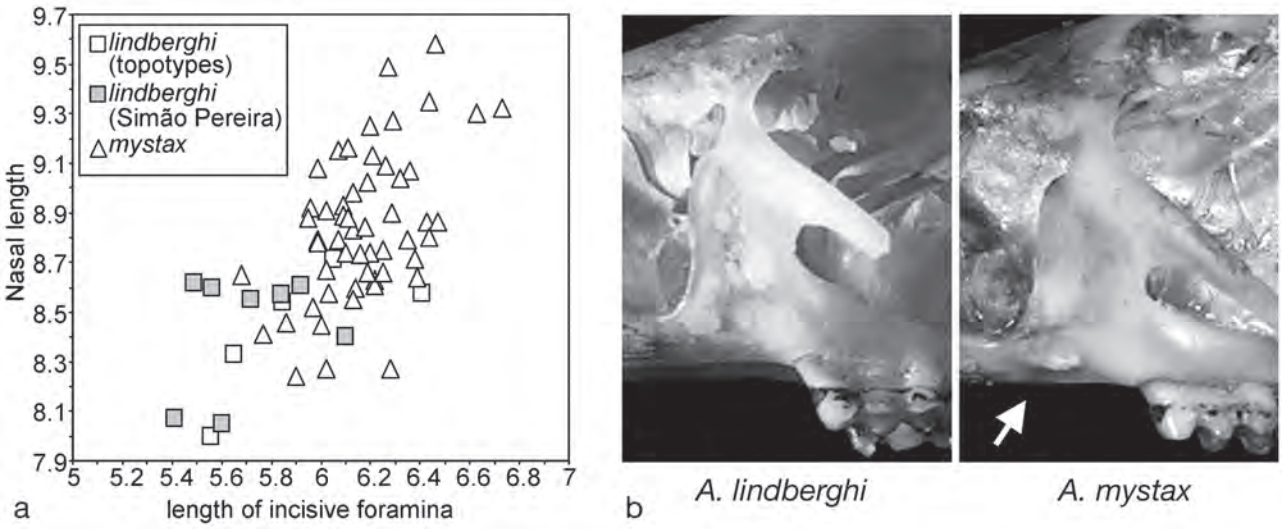

Fig. 5. Morphological variation between *Akodon mystax* and *A. lindberghi*: (a) distributions of incisive foramina and nasal lengths of specimens (in millimeters); (b) lateral view of zygomatic plates showing the masseteric tubercle (indicated by the arrow).

extending posteriorly beyond the protocone of the first upper molar (M1), generally to the level of the M1 hypoflexus (Fig. 3a, b). This results in a shortened palatal bridge. In addition, the external margins of the posterior portions of the incisive foramina are conspicuously angled outwards. Incisive foramina in *A. philipmyersi*, *A. azarae,* the Itatiaia population, *A. paranaensis*, *A. cursor* and *A. montensis* are generally shorter (LIF/LD averages 89%), and usually do not extend posteriorly further than the M1 protocone. Their external borders are generally straight (Figs. 3c-e and 4). There are some exceptions, however; we noted that 6 of 36 specimens of *A. paranaensis* have incisive foramina that extend beyond the M1 protocone with lateral margins resembling the expanded condition seen in *A. mystax* and *A. lindberghi*.

Akodon mystax, *A. lindberghi* and *A. philipmyersi* also share narrow zygomatic plates. The plates have straight anterior margins that barely project in front of the antorbital bridge (Fig. 3). Consequently, zygomatic notches are shallowly incised and inconspicuous when viewed from above, particularly in *A. mystax* and *A. philipmyersi*. The remaining species of *Akodon* present wide zygomatic plates that project well anterior to the antorbital bridge; their zygomatic notches are considerably deeper and more conspicuous.

Variation in the hamular process of the squamosal can also be used to differentiate among these groups of species. *Akodon mystax*, *A. lindberghi* and *A. philipmyersi* present relatively stocky hamular processes, which delimit minute subsquamosal foramina posterodorsally (Fig. 3a-c), while the remaining species have slender hamular processes with comparatively broader subsquamosal foramina (Figs. 3d-e and 4).

Morphological variation among species thus suggests strong divergence between *Akodon mystax* and the Itatiaia population. Together, the results of quantitative and qualitative morphological analyses suggest closer resemblance between *A. mystax* and *A. lindberghi* than between *A. mystax* and the Itatiaia population, *A. paranaensis*, or members of the *cursor* group. *Akodon azarae* and *A. sanctipaulensis* are well distinct from these two sets of species. The young age of specimens of *A. sanctipaulensis* currently available for study, however, may largely account for the morphological divergence of *A. sanctipaulensis* compared to the large-sized species.

Comparison of *A. mystax* and *A. lindberghi*

These two species are very similar in cranial structure. Qualitative characters mentioned by Hershkovitz (1998) as diagnostic for *Akodon mystax*, such as the narrow zygomatic plate, wide and rounded mesopterygoid fossa and long incisive foramina, are shared by individuals of *A. lindberghi*. The craniometric discrimination between the species is highly significant, (MANOVA results: $F_{(1, 65)} = 6.0436$, Wilk's lambda = 0.3408, $P<0.001$), with *A. mystax* generally larger for most dimensions, despite the overlap between their measurement ranges. *Akodon mystax* crania generally have longer nasals and incisive foramina than those of *A. lindberghi*, but these measurements do overlap, with roughly 17% of the individuals of *A. mystax* included within the range of *A. lindberghi* (Fig. 5a). The sole cranial character that consistently separates the two species is the presence of a masseteric tubercle located near the base of the zygomatic plate, immediately anterior to M1, in *A. mystax*. This tubercle serves as an insertion point for the superficial masseter (Voss 1988) and is represented in adult specimens of *A. mystax* as a small but distinct crest

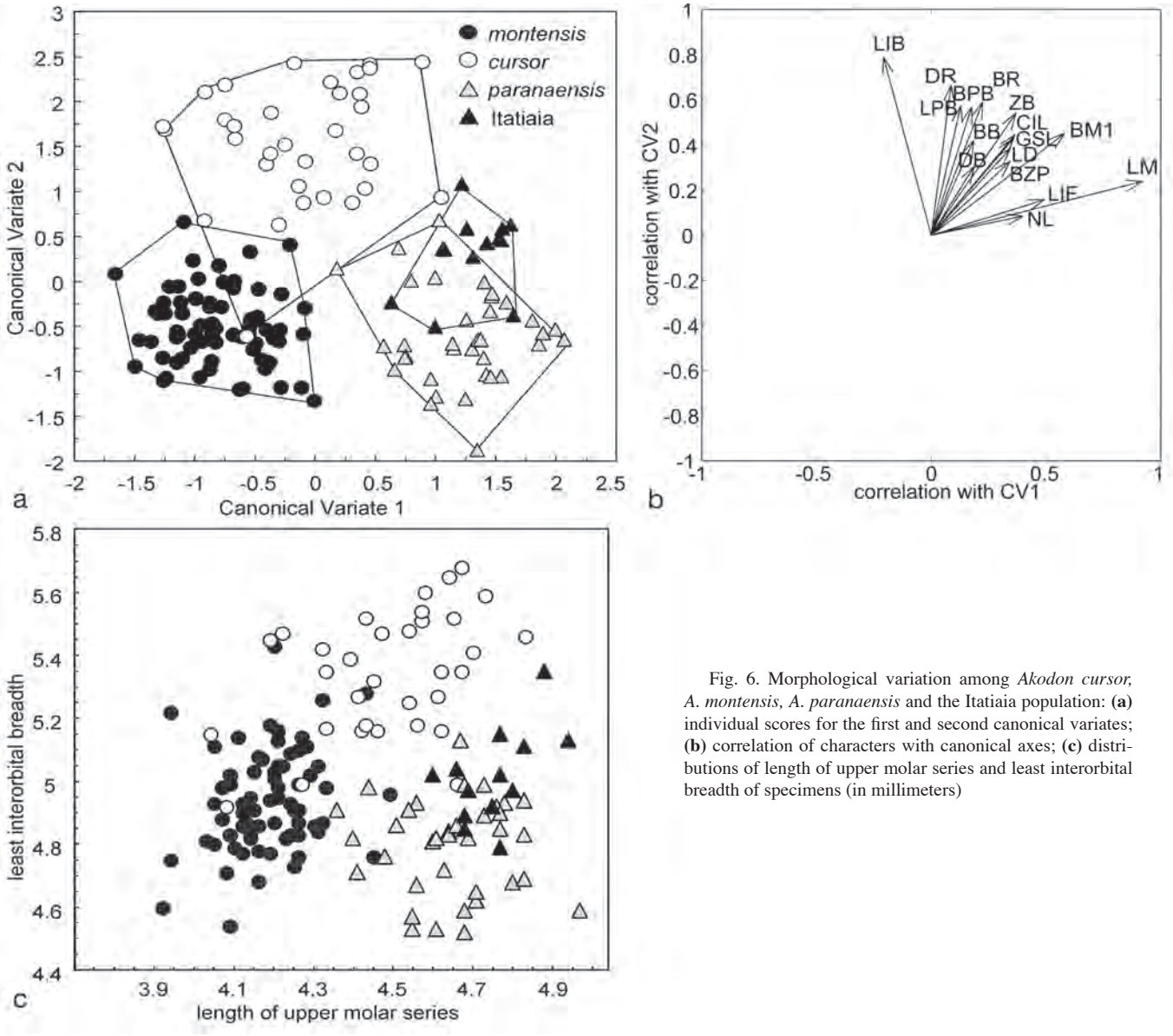

Fig. 6. Morphological variation among *Akodon cursor*, *A. montensis*, *A. paranaensis* and the Itatiaia population: **(a)** individual scores for the first and second canonical variates; **(b)** correlation of characters with canonical axes; **(c)** distributions of length of upper molar series and least interorbital breadth of specimens (in millimeters)

easily visible in lateral view (Fig. 5b). Comparably aged adults of *A. lindberghi* show no trace of this tubercle, presenting instead a much smoother maxillary surface.

Pelage color also differs slightly between the species. Both are brownish on the dorsum, but *Akodon mystax* appears paler and grayer due to pale yellow pheomelanic bands on the dorsal hairs, while *A. lindberghi* is more strongly reddish-brown in dorsal color due to more vividly orange pheomelanic bands. Hershkovitz (1998), when describing *A. mystax*, noted that many males and a few females have a patch of blackish hairs extending from the tip of the rostrum to each eye region (Hershkovitz 1998: Fig. 24). He referred to this trait as the "thin dark rostral band," and this "moustache" is the basis of the specific epithet of this species. We note that under magnification, a small patch of minute blackish hairs is visible just dorsal to the upper rhinarium. However, in specimens examined by us this dark band of hairs is restricted to the snout and does not extend to reach the eye region. Further, some specimens of *A. lindberghi* share this same patch of darkened hairs near the rhinarium, which suggests that this trait may not reliably separate the two species.

Comparison of members of the Itatiaia population, *A. paranaensis* and the *cursor* group

In a Canonical Variate Analysis (CVA) of members of this group, the first and second canonical axes, which summarize 97.3% of the discriminatory variation (Fig. 6a), clearly differentiate three clusters. The first cluster consists of members of the Itatiaia population plus *Akod-*

Table 2. Pearson product-moment correlation coefficient between log-transformed craniometric characters and canonical variates (CV1 and CV2) extracted in a discriminant analysis among the Itatiaia population, *Akodon cursor, A. montensis* and *A. paranaensis*.

Characters	Canonical Variates CV1	CV2
GSL	0.246	0.315
ZB	0.382	0.600
CIL	0.272	0.395
NL	0.303	-0.018
LD	0.193	0.307
LPB	0.190	0.579
LM	0.861	0.369
BM1	0.276	0.653
LIF	0.300	-0.031
BR	-0.029	0.540
DR	0.093	0.633
BPB	-0.067	0.491
BZP	0.225	0.246
LIB	-0.287	0.818
BB	0.260	0.625
DB	0.487	0.485

on paranaensis, while the other two comprise *A. montensis* and *A. cursor*. Dimensions related to molar series (BM1 and LM) are especially important for discrimination on CV1, while the other characters contribute more to segregation along CV2 (Fig. 6b; Table 2). Members of the *paranaensis*-Itatiaia cluster appear to have disproportionately large teeth compared to the other species, but in comparisons with *A. cursor*, members of this cluster diverge due to their relatively narrow interorbits, short and narrow palates and low rostra (Fig. 6; Tab. 2). *Akodon cursor* and *A. montensis* are also consistently discriminated throughout both discriminant axes, with *A. cursor* being generally larger in all dimensions.

Their contribution to CV1 and CV2 identifies least interorbital breadth (LIB) and length of upper molars (LM) as the most efficient measurements for discriminating these species (Fig. 6c). In a plot of LIB vs. LM, a single specimen of *A. cursor* (3% of the *Akodon cursor* sample) is included within the *A. paranaensis* cluster, and just two individuals of *A. montensis* (2.8%) lie within the *A. paranaensis* cluster. Separation of specimens of *A. cursor* and *A. montensis* based on these two dimensions is, however, less consistent, as approximately 10% of the *A. cursor* specimens are included within the *A. montensis* cluster.

The CVA did not reliably differentiate between members of the Itatiaia population and *Akodon paranaensis*. The Itatiaia population slightly diverges from *A. paranaensis* along CV2, but 70% of the specimens from Itatiaia are included within the morphological variability of *A. paranaensis*.

In summary, with the resolution provided by the morphological characters analyzed it is possible to recognize 3 units within this set of large-sized forms – *Akodon cursor*, *A. montensis* and *A. paranaensis*, with the Itatiaia population included within the *A. paranaensis* cluster.

No qualitative aspect of morphology was discovered that provides any discrimination between these taxa. All are exceedingly similar in external appearance; the dorsal pelage of each has the same dark olivaceous-agouti color, and external measurements overlap broadly (Appendix 2). *Akodon paranaensis* and the Itatiaia population generally have zygomatic plates with anterodorsal margins produced as sharp corners, while the zygomatic plates in *A. cursor* and *A. montensis* have smoothly round anterodorsal margins. These qualitative differences, however, are blurred when a large number of individuals are considered, as many appear to be intermediate.

Cytogenetics

All specimens of *Akodon mystax* collected by us at the type locality had 2n = 42 chromosomes and FN = 42 arms (Fig. 7). The autosomal complement consists of a large pair of acrocentric chromosomes followed by 18 progressively smaller pairs of acrocentric / subtelocentrics and a minute submetacentric pair of chromosomes. The sexual pair consists of a medium-sized, acrocentric X and a small acrocentric Y. *Akodon mystax* and *A. lindberghi* share the same diploid and fundamental numbers (Table 3), including the same large acrocentric and minute metacentric pairs. *Akodon philipmyersi* shares with *mystax* and *lindberghi* the fundamental number of 42, but diverges by presenting a diploid number of 36 (Pardiñas *et al.* 2005).

The karyotype of *Akodon mystax* described here differs from that suggested by Bonvicino *et al.* (1997), who reported a 2n = 44 karyotype for this species based on specimens from the original series. We did not detect this karyotype in specimens collected by us at the type locality, but at the same time we have not analyzed the cytogenetic material obtained by Bonvicino *et al.* (1997). The only voucher specimen (MN31904) associated with the 2n = 44 karyotype by Bonvicino *et al.* (1997: Table 2) was identified by us as an *A. mystax*.

Cytogenetic polymorphisms involving diploid numbers have been reported in *Akodon molinae*, *A. dolores*, *A. simulator, A. puer* and *A. cursor* (Table 3) and may be revealed in more intensive studies of cytogenetically poorly studied species such as *A. mystax*. Unfortunately, no information other than the diploid number was reported for the specimens karyotyped by Bonvicino *et al.* (1997), preventing further inference about the mechanisms involved (Robertsonian translocations, supernumerary chromosomes) in the presumptive

Table 3. Cytogenetic data for species of *Akodon* and bibliographical sources consulted.

Species	Diploid number (2n)	Fundamental number (FN)	Sources
A. mystax	42, 44	42	this paper; Bonvicino *et al.* (1997)
A. lindberghi	42	42	Svartman (1994); Geise *et al.* (1996)
A. philipmyersi	36	42	Pardiñas *et al.* (2005)
A. azarae	38	42	Vitullo *et al.* (1986)
A. cursor	14, 15	18-21	Fagundes *et al.* (1998); Geise *et al.* (1998)
A. aff. *cursor*	16	not available	Fagundes *et al.* (1998); Geise *et al.* (1998); Geise *et al.* (2001)
A. montensis	24	42	Yonenaga *et al.* (1975)
Itatiaia population	44	44	this paper; Geise *et al.* (2001)
A. paranaensis	44	44	Christoff *et al.* (2000)
A.reigi	44	44	Gonzalez *et al.* 1998
A. boliviensis	40	40-42	Myers *et al.* (1990)
A. juninensis	40	40	Myers *et al.* (1990)
A. subfuscus	40	40	Myers *et al.* (1990)
A. puer	34-40	40	Barquez *et al.* (1980); Vitullo *et al.* (1986); Myers *et al.* (1990)
A. kofordi	40	40	Myers & Patton (1989)
A. dolores	34, 35, 37, 38, 40	44	Bianchi *et al.* (1979)
A. molinae	42-44	44	Bianchi *et al.* (1973)
A. neocenus	40	40	Bianchi *et al.* (1971)
A. simlulator	41, 42	42	Barquez *et al.* (1980)
A. toba	40	40	Gardner & Patton (1976)

cytogenetic polymorphism of *A. mystax*.

Molecular phylogenetics

Akodon mystax is represented here by 3 haplotypes recovered from 6 individuals captured at the type locality (loc. 24 in Fig. 1). In pairwise comparisons these 6 individuals diverge in only 2-5 substitutions (0.2-0.4% uncorrected sequence divergence). In contrast, *A. mystax* diverges from *A. lindberghi* by 1.7-1.9% uncorrected sequence divergence (*p*-distance) and from *A. philipmyersi* by 10.1-10.6% (Table 4). The Itatiaia population is represented here by 5 haplotypes that include the 2 (MN48041, 48070 from loc. 15 in Fig. 1) previously obtained by other authors (Geise *et al.* 2001; D'Elía 2003) and those recovered from our sample of 2n = 44 individuals (MN69686, MN69700, MN69726 from loc. 16 in Fig. 1). Pairwise divergence among haplotypes from Itatiaia is minimal; these individuals differ by only 2-4 substitutions (0.2-.35% uncorrected sequence divergence, including comparisons between recent and previously obtained haplotypes). The Itatiaia haplotypes diverge from the southern haplotypes of *A. paranaensis* by 1.1-2.5% sequence divergence and from the southernmost *A. reigi* by 5.3-5.4% sequence divergence (Table 4). Haplotypes of *A. paranaensis* and *A. reigi*, including those recovered from or near the type localities of both species, diverge by 5.5-5.7% *p*-distance. Even the southernmost samples of *A. paranaensis* diverge from *A. reigi* by 5.6-5.7% *p*-distance. Divergence between *A. mystax* and Itatiaia haplotypes is high, varying from 9.1 to 9.9% *p*-distance. High divergence levels (7.7 to 8.1%) are also found between the Itatiaia population and the 801bp haplotype from Misiones (MMP-Ma 2421), which was considered to be closely related to the Itatiaia specimens by Pardiñas *et al.* (2003).

Analysis of the entire cytochrome *b* sequence dataset available for *Akodon*, including the new sequences reported here, revealed 539 variable characters, of which 448 are informative under the parsimony criterion. Heuristic searches recovered six equally parsimonious trees of 2779 steps, which we have summarized in a strict consensus phylogram (Fig. 8). *Akodon mystax* is consistently grouped with *A. lindberghi* with a high bootstrap support (100%). *Akodon philipmyersi* appears as sister to *A. mystax* and *A. lindberghi* in all six most parsimonious trees, although bootstrap values do not strongly support this relationship. The Itatiaia population and the Misiones specimen are grouped in a moderately supported clade with relationships not fully resolved and represented as an internal polytomy. The Misiones haplotype appears highly autapomorphic as suggested by the exceedingly long branch depicted in the parsimony phylogram (Fig. 8). Consequently,

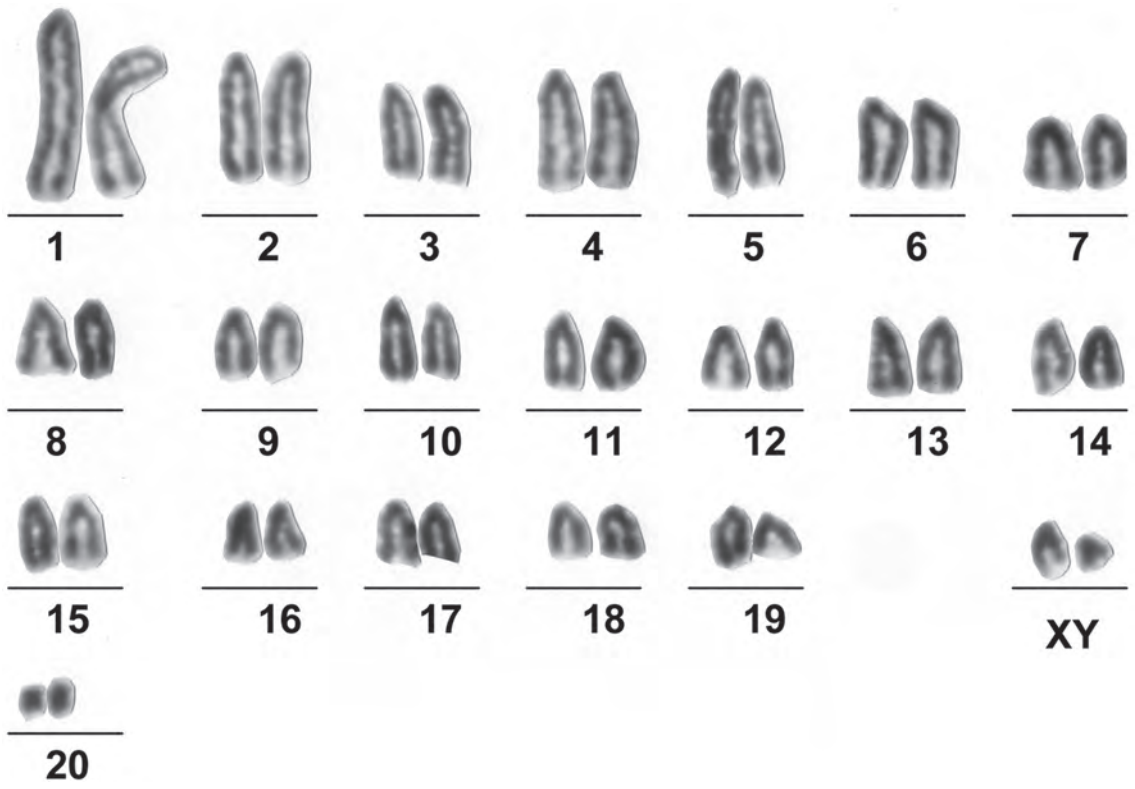

Fig. 7. Karyotype of *Akodon mystax* (MN69628) after Giemsa staining: 2n = 42, FN = 42.

divergence levels become considerably inflated when this sequence is included in comparisons with other groups of haplotypes.

The Itatiaia population and the Misiones specimen are grouped with the southern *Akodon paranaensis* in a well supported clade (95% bootstrap support) that is sister to *A. reigi.* The Itatiaia population, the Misiones specimen, the southern populations of *A. paranaensis* and the Uruguayan *A. reigi* form a highly supported group that is sister to a paraphyletic *cursor* group, which includes *A. montensis* as the immediate sister lineage, followed by *A. cursor* and *A. aff. cursor*. Members of the *boliviensis* group are the sister group to this eastern clade of *Akodon*, although statistical support is weak. *Akodon azarae* appears as a lineage immediately basal to the eastern *Akodon* and the *boliviensis* group, but bootstrap values supporting this arrangement are low, and *A. azarae* doesn't seem closely related to any of the other sequenced *Akodon* species. Other monophyletic groups that were revealed in the maximum parsimony analyses and that have already been reported by previous molecular assessments (Smith and Patton *in press*; Pardiñas *et al.* 2005) include the Andean group of species represented by *A. mollis, A. affinis, A. aerosus, A. siberiae, A. orophilus, A. torques, A. albiventer*, and the members of the *varius* group (*sensu* Myers, 1989) represented by *A. toba, A. molinae, A. dolores and A. dayi.* We refer the reader to Smith and Patton (in press) for a more detailed discussion of the molecular phylogenetics of these species groups. The genus *Akodon*, as currently understood, was not recovered as a monophyletic assemblage due to the placement of *A. serrensis* outside *Akodon*. A similar arrangement was revealed by analyses of nuclear genes (D´Elía 2003) and in other cytochrome *b* phylogenetic assessments (Smith and Patton, in press). A formal generic delimitation awaits a comprehensive phylogenetic analysis of morphological, cytogenetic, and molecular characters with broad taxonomic sampling of the genus and potential outgroups.

TAXONOMIC IMPLICATIONS

Relationship and taxonomic status of *Akodon mystax*

Morphological, cytogenetic and molecular comparisons strongly support the hypothesis that *Akodon mystax* and *A. lindberghi* are sister species, in agreement with the suggestion by Hershkovitz (1998) that small species from eastern South America may be more closely related among each other than to large-sized forms. These data also refute a close association of *A. mystax* with the 2n = 44 specimens from the Itatiaia region

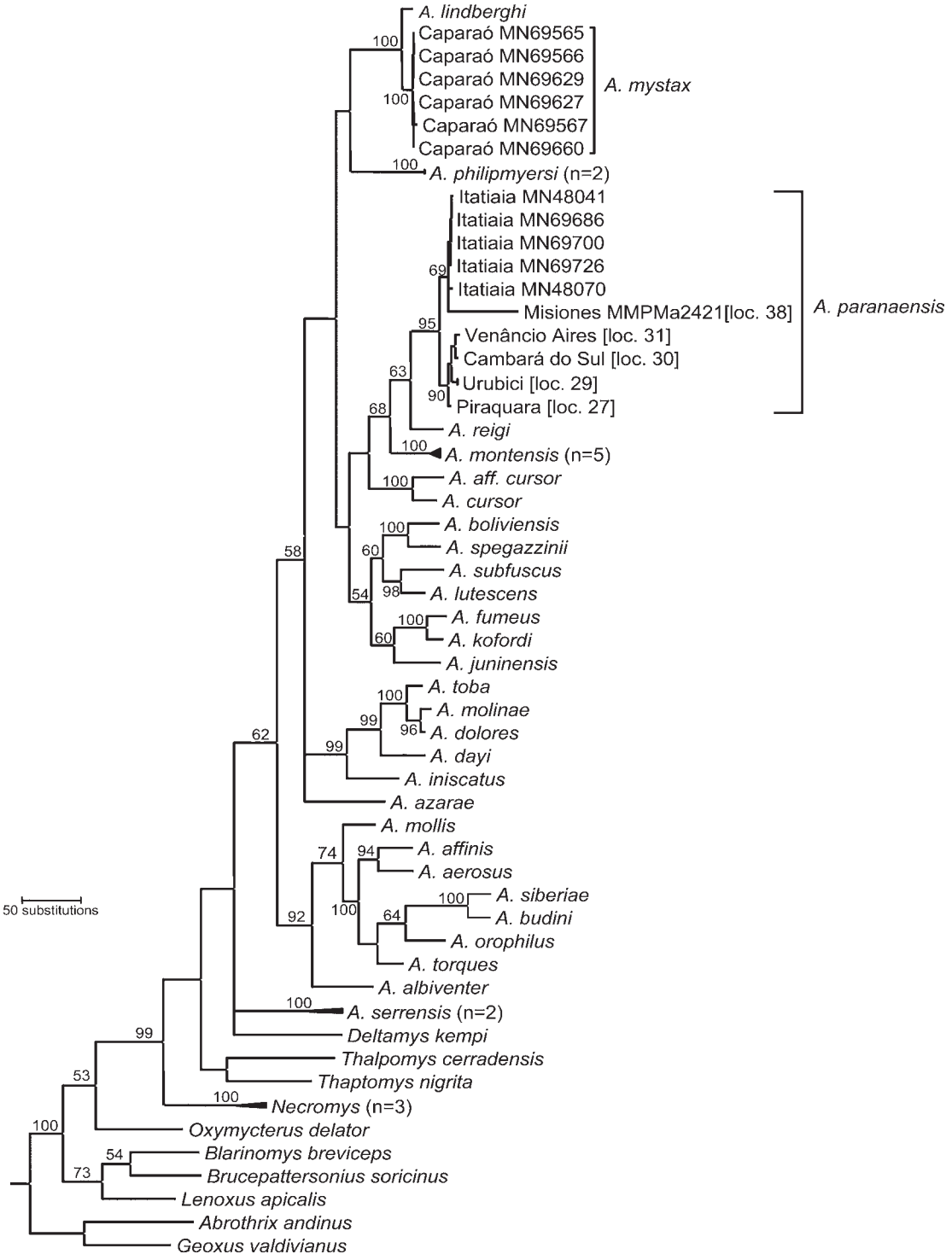

Fig. 8. Most parsimonious tree recovered under maximum-parsimony analysis of sequences of the entire cytochrome b gene (2605 steps, 445 parsimony-informative characters, consistency index = 0.2987, retention index = 0.6321). Numbers above branches indicate bootstrap support values of nodes.

reported by Geise *et al.* (2001) or with the southern large-sized *A. paranaensis* and *A. reigi*, which will be discussed in a separate section below.

Akodon mystax and *A. lindberghi* are so similar that a reevaluation of their taxonomic status is needed. Only 12 complete adult specimens (skin and skulls) of *A. lindberghi* are available in museums, representing just 2 localities. This is clearly a small sample for taxonomic inference. The first locality is the source of the type series (Hershkovitz, 1990). These animals were collected at Brasília, Brazilian Federal District (Fig. 1), in wet grasslands typical of the Cerrado biome. The second (Geise *et al.,* 1996) was discovered in Simão Pereira, Minas Gerais state, 850km east of Brasília in grassland habitats bordered by Atlantic forest (Fig.1). No populations of *A. lindberghi* intermediate to these two have been reported so far, leaving a large gap in the distribution of this species.

Members of the two geographically distant samples of *Akodon lindberghi* are morphologically more similar to each other than to *A. mystax* from the type series of that species, which was collected just 220km away from the Simão Pereira sample of *lindberghi* (Fig. 5). This pattern indicates that morphological cohesion clearly is not related to distance and corroborates the assignment of the two samples currently called *lindberghi* to the same species despite the large distance between them, as suggested by Geise *et al.* (1996).

While specimens of *Akodon lindberghi* and *A. mystax* are similar morphologically, morphological differences can usually be used to separate the two species. Most characters, however, are quantitative and overlapping. In this study, nasal length combined with incisive foramen length, and the occurrence of a masseteric crest, provided the best basis for discrimination, but these differences should be interpreted with caution until larger samples of *A. lindberghi* become available.

In agreement with their morphological similarity, the two species show relatively little divergence between their cytochrome *b* sequence divergence (p-distance < 2%), suggesting that they separated very recently.

Akodon philipmyersi seems to be the likeliest candidate for a sister relationship to the *mystax-lindberghi* complex. This is suggested by both molecular evidence and shared morphological similarities such as small size, relatively broad interorbit, narrow zygomatic plates and squamosal with stocky hamular process. Nevertheless, as previously noted by Pardiñas *et al.*(2005) and evidenced by the low support levels shown in Fig. 7, cytochrome-*b* data are not conclusive regarding this relationship, and analyses of additional genes are required to further test the association of *A. philipmyersi* with *A. mystax* and *A. lindberghi*. Moreover, the unique 2n = 36/FN = 42 karyotype of *A. philipmyersi*, and its shorter rostrum and less expanded incisive foramina, suggest a relatively deep divergence between that species and (*A. mystax* + *A. lindberghi*).

Differentiation within eastern large-sized *Akodon* and identity of the Itatiaia population

Previous molecular studies have suggested that specimens bearing a 2n = 44 karyotype from Itatiaia are closely related to *Akodon paranaensis* (D`Elía, 2003; Pardiñas *et al.*, 2003; Pardiñas *et al.*, 2005), and our results indicate a clear difference between these specimens and *A. mystax*. *Akodon reigi* is immediately sister to the clade uniting the Itatiaia population, the Misiones specimen and *A. paranaensis*; together, these species comprise a monophyletic assemblage sharing the same 2n = 44 karyotype.

Based on molecular analyses, the species most closely related to this 2n = 44 assemblage are members of the *cursor* group (*Akodon cursor*, *A. aff. cursor* and *A. montensis* -- Rieger *et al.*, 1995; Geise *et al.,* 2001). Together these lineages form a morphologically cohesive group of large *Akodon* with narrow and biconcave interorbits, pronounced and robust rostra, and deeply incised zygomatic notches. No thorough morphological analysis has been published comparing all the species of this group, and the investigation of species limits within these large sized *Akodon* using morphological evidence is clearly difficult. Boundaries among these species have been delineated following an almost strictly cytogenetic approach; morphological features, when discussed at all, have been dismissed as diagnostic characters. Gonzalez *et al.* (1998), when diagnosing *A. reigi*, relied on the 2n = 44 diploid number to differentiate it from *A. cursor* and *A. montensis*. Christoff *et al.* (2000) performed morphological comparisons among *A. paranaensis*, *A. serrensis* and *A. sanctipaulensis,* but did not include closely related taxa, such as the geographically close *A. reigi* or any member of the *cursor* group. Ample cytogenetic and molecular evidence are generally available supporting the specific status of most species of large-sized *Akodon* in eastern South America, such as *A. cursor* and *A. montensis*, but these data are obviously uninformative when applied to the hundreds of non-karyotyped specimens deposited in museums (Geise *et al.*, 2005). The first initiative in testing morphological data for species limits in synmorphic *Akodon* species was taken by Geise *et al.* (2004a, 2005), who demonstrated relevant morphological divergence between *A. cursor* and *A. montensis* characterized by different gall bladder conditions and craniometric dimensions.

The morphological analyses presented here provided resolution not only for *Akodon cursor* and *A. montensis*, but also for the recognition of *A. paranaensis*

as a divergent unit identifiable by interorbital and upper molar dimensions. These characters were mentioned in the diagnosis of the species by Christoff *et al.* (2000), but that study did not include quantitative comparisons with *A. cursor* and *A. montensis*, precluding the identification of specimens that had not been karyotyped. Morphological evidence, however, provides no support for the recognition of the Itatiaia population as a distinct taxon, and when considered together with cytogenetic information, morphological data suggest that this sample and *A. paranaensis* should be treated as a single species. Molecular levels of divergence are generally low in comparisons between the Itatiaia and the southern cytochrome *b* haplotypes (p-distance <2.5%), in agreement with the pattern of morphological similarity between these samples.

Pardiñas *et al.* (2003), while identifying species of *Akodon* from Misiones, Argentina, reported that based on cytochrome *b* sequence comparisons, one specimen from Parque Provincial Islas Malvinas (MMP-Ma 2421) appeared to be closely related to members of the Itatiaia sample (regarded by them as *A. mystax*). Because this specimen was morphologically distinct from the published description of *mystax*, however, they left it unidentified (*Akodon* species 2). A diploid number of 44 chromosomes was also reported for the same specimen by Liascovich and Reig (1989), which prompted Christoff *et al.* (2000) to identify it as *A. paranaensis*. With the clarification of the Itatiaia sample as a northern representative of *A. paranaensis* and its dissociation from *A. mystax*, the allocation of the Misiones specimen now becomes congruent with morphological, molecular and chromosomal data. The haplotype recovered by Pardiñas *et al.* (2003) from dry muscle of specimen MMP-Ma 2421 falls within the Itatiaia population clade in the phylogenetic analysis presented here. The cranial measurements of this specimen are also well within the range of our *A. paranaensis* samples, supporting its inclusion in this species as previously suggested by Christoff *et al.* (2000). The phylogenetic affinity between Misiones and Itatiaia haplotypes implies a disjunct pattern of distribution for *A. paranaensis*, given that intermediate 2n = 44 populations have not been reported from the Atlantic forest of São Paulo. There is no resolution, however, as to whether the Misiones and Itatiaia populations are reciprocally monophyletic. Most of the genetic differentiation of the Misiones specimen is due to an unusually high number of autapomorphies, hindering clear resolution of its relationships of this specimen.

Akodon reigi is also a member of the 2n = 44 group. Morphological measurements (from Gonzalez *et al.*, 1998) generally fall within the range of variation reported here for *A. paranaensis*. Molecular analyses, however, reveal moderate levels of sequence divergence between *A. paranaensis* and *A. reigi* (5.6%), even when the divergent Misiones haplotype is excluded from comparisons. Further, the southernmost population of *A. paranaensis* (loc. 31 in Fig. 1), although geographically closer to Uruguayan *A. reigi* (loc. 33), is genetically much more similar to distant *paranaensis* populations from Itatiaia or Paraná state. Thus, the deep divergence between Brazilian and Uruguayan populations is uncorrelated with geographic distance, Given this moderate genetic differentiation between populations of *A. paranaensis* and *A. reigi* and the lack of sequence data from intermediate samples (*e.g.*, Christoff *et al.*, 2000), we consider that combining the two species into a single entity would be premature. Therefore, we maintain the species distinction of the Brazilian and Uruguayan 2n = 44 populations until further samples become available for study. We expect that the morphometric characters provided here will help in the discovery of additional overlooked non-karyotyped 2n = 44 populations, improving the knowledge of geographic limits of this species complex.

BIOGEOGRAPHIC IMPLICATIONS

The *campos de altitude* are cool-humid non-forest formations that generally cover the summits and plateaus of the Brazilian Atlantic forest above 1800m. They comprise a mosaic of shrubs and bamboo immersed in a matrix of tall grassland, which is supported by shallow hygrophilic soils dissected by small streams. These formations manifest extensively in many mountain groups throughout Southeastern Brazil, but they are most notable in the Caparaó and Itatiaia mountain ranges (Safford 1999a), the respective localities of *Akodon mystax* and *A. paranaensis* populations discussed here.

The physiognomic and climatic similarities between the *campos de altitude* and the high humid paramos of the Andean cordillera are surprising and have resulted in special designations to these Atlantic forest habitats such as "Brazilian paramos" or "pseudoparamos" (Safford, 1999a, 1999b). Temperatures in these mountaintop environments are relatively low for tropical habitats, with minima varying from -6°C to -10°C at altitudes above 2000m (Segadas-Vianna & Dau, 1965; Safford, 1999b). Morphological adaptations to cold and chilly conditions such as sclerophylly are common among plant species, making the general landscape even more similar to the Andean paramos.

The flora of the *campos de altitude* is remarkably diverse, with approximately 550 species of vascular plants recorded on a single summit, and levels of endemism are impressively high, with roughly 21% of the species restricted to the *campos de altitude* vegetation type (Safford ,1999a). Some genera have cross-continental

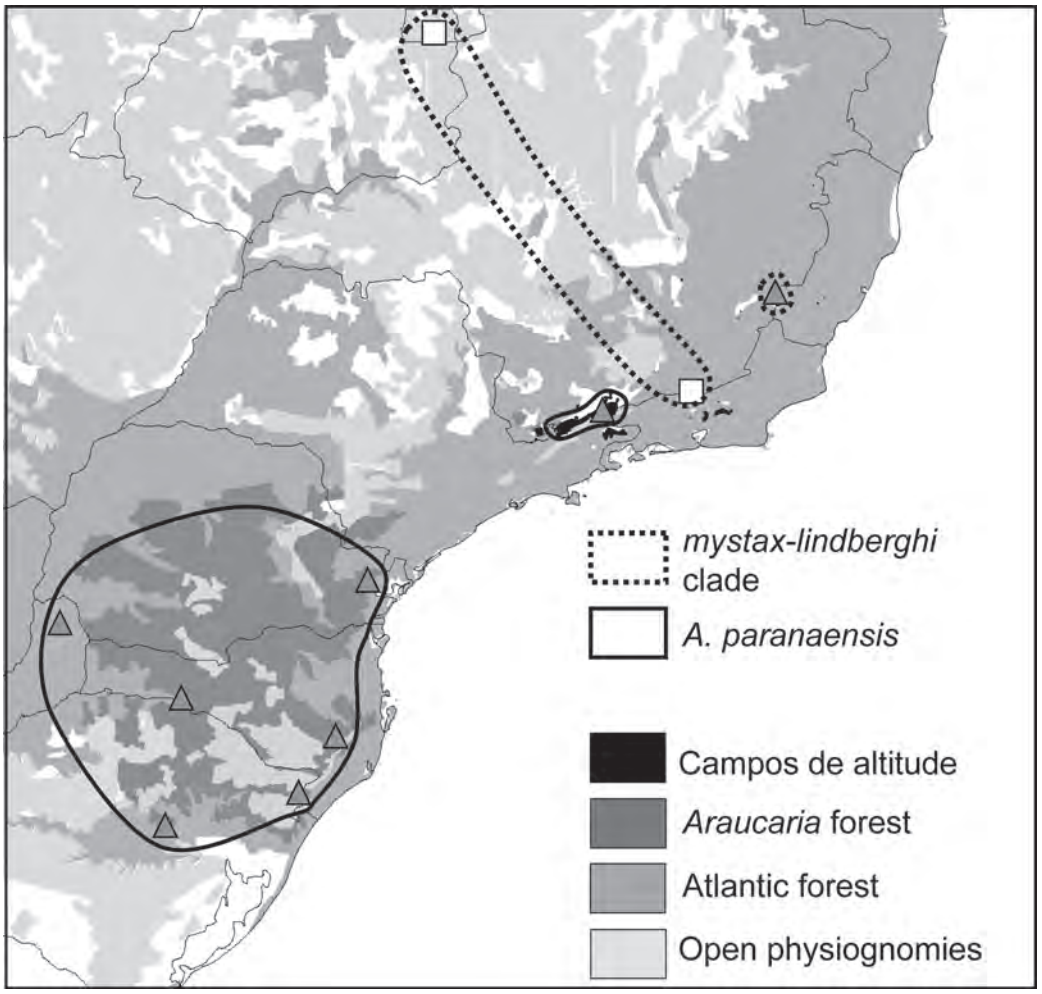

Fig. 9. Distribution of clades of *Akodon* containing lineages restricted to the campos de altitude. Distributions of Atlantic forest, areas of open physiognomy (Cerrado and Southern Grasslands) and campos de altitude were adapted from Rizzini (1979) and Safford (1999a).

disjunct distribution patterns, with species in both the Atlantic mountain ranges and the Andean Cordillera (Clark, 1992). Despite the importance of the *campos de altitude* as endemism centers in the Atlantic forest, little is known about the ecology and evolution of these high-altitude habitats (Safford, 1999a, 1999b).

The study of the mammals from the *campos de altitude* is still in its infancy, as only recently have mammalogists begun to survey these habitats systematically. Nevertheless, in a short time these surveys have revealed a diverse and interesting array of species of sigmodontine rodents closely associated with montane habitats, including a number that are new to science (Hershkovitz, 1998; Oliveira & Bonvicino, 2002). Furthermore, extensive surveys in the Caparaó and Itatiaia mountain ranges have also demonstrated that the new species are restricted to the *campos de altitude* and highest montane zones, while their congeners are more broadly distributed throughout slopes and Atlantic forest lowlands (Bonvicino *et al.* 1997; Geise *et al.*, 2004b). Although the ecological information about these mammalian communities is still far from complete, the altitudinal distribution pattern and the strong association of some sigmodontines with the *campos de altitude* suggest that the phylogenetic patterns of these species could be used to track signals of historical processes that determined the uniqueness of these montane biotas.

Palynological data suggest that the distribution of the present scattered *campos de altitude* can be attributed largely to Pleistocene-Holocene climate shifts that occurred mainly following the Last Glacial Maximum (LGM, ca. 25,000-10,000 years before present). Palynofloras of southeastern and southern Brazil indicate that conditions during the LGM were drier and cooler. Humid forests retracted and were replaced by grasslands and open savanna-like formations that expanded their distributions north and eastward (Ab'Saber, 1977; Behling, 1998, 2002). During this cold period, conditions on the highest summits of southeastern Brazil were periglacial (Clapperton, 1993) and probably unsuitable

for supporting the humid habitats that cover them today. Pollen records from nearby the Itatiaia mountains indicate that the *campos de altitude* were found at much lower altitudes, probably as low as 1800m (Behling, 1997). Likewise, records near the Caparaó mountains indicate that grasslands were dominant even in altitudes down to 700m (Behling, 1998, 2002). The onset of warmer and humid climates in the early and middle Holocene (10,000 – 3000 *ybp*) initiated the re-expansion of forests into higher altitudes and latitudes, while grasslands and open formations retreated southward and upward on mountain slopes (Behling, 2002; Behling *et al.*, 2005). At this phase the conditions atop the highest summits probably offered "glacial refugia" for these grassland communities. They became increasingly isolated as the coldest zones shifted to even higher altitudes following the increasing temperatures of late Holocene (Rizzini, 1979).

Further detail on the evolution of the *campos de altitude* is not available and many questions remain. For example, it is not known whether the splitting of formerly connected grasslands in glacial refugia favored the differentiation of all endemic lineages in these areas. Even if a vicariant event involving these biotas consistently occurred, different organisms may have responded in different ways as a consequence of their unique ecological and life history characteristics (*e.g.*, see Costa, 2003). Additionally, multiple climatic cycles occurred during the entire Pleistocene and it is possible that different endemic lineages formed at different episodes of disjunction. We do not know which grassland communities effectively maintained contact with the *campos de altitude* during these cycles and what was the timing of those connections.

These questions cannot be answered by the palynological evidence alone, as pollen records from more remote periods (>100,000 *ybp*) are seldom available for eastern South America. Instead, they must be approached through investigation of the patterns of diversification of the endemic components of the *campos de altitude* and the phylogenetic relationships of those endemics to species in other forest and nonforest communities of South America.

The phylogenetic data for *Akodon mystax* and *A. paranaensis* can be used to test the power of the proposed evolutionary scenario to explain the current distributional and phylogenetic patterns of these high-altitude sigmodontines. These distributional and evolutionary patterns can be summarized by simply mapping the phylogenetic relationships of these two species onto geography (Fig. 9). If a vicariant scenario of glacial refugia is correct, two predictions can be made concerning phylogenetic patterns:

First, mountaintop isolates of different mountain groups should appear as sister lineages in a cladogram. That is, they should be more closely related to each other than to other populations and species from other areas. This prediction is readily refuted for *Akodon mystax* and *A. paranaensis* given that these species are not sisters and cannot be placed in any monophyletic unit more exclusive than the genus *Akodon*. In fact, the remote relationship between these two species suggests that the Caparaó and Itatiaia mountain ranges may harbor some very unique and historically disassociated components in their biotas. This idea is also partially supported by the fact that many mono- and ditypic plant genera of the *campos de altitude* are exclusively found on single mountaintops (Safford, 1999a). Although some connectivity among *campos de altitude* may have persisted during drier and cooler periods typical of glacial maxima, *A. mystax* and *A. paranaensis* apparently failed to cross the "glacial corridor" separating the Itatiaia and Caparaó ranges, or if they were originally broadly distributed, each became extinct in the mountain range now occupied by the other species following the onset of altitudinal isolation.

The second prediction from the glacial refugia model concerns the relationships between mountaintop and southern taxa. If the *campos de altitude* resulted from the shrinking of formerly vast grasslands that extended from southern to southeastern South America during glacial maxima, then we would expect close relationships between mountaintop isolates and austral components. This prediction is supported by the disjunct distribution presented by *Akodon paranaensis*, which typically inhabits the southern grasslands and cool-moist *Araucaria* forests of Paraná, Santa Catarina and Rio Grande do Sul states in Brazil (Christoff *et al.*, 2000) and Argentina (Misiones; Pardiñas *et al.*, 2003), but also persists as an isolated population restricted to the *campos de altitude* of Itatiaia. Considering that the Itatiaia population is restricted to the highest altitudinal zones (2100-2450m: Geise *et al.* 2004b and our results) and that the gap between the Itatiaia mountains and the southern grasslands (São Paulo state) has been relatively well sampled, the current distribution pattern is highly suggestive of a mountaintop-southern grassland past connection. Lack of reciprocal monophyly between the Itatiaia and the Misiones populations indicates that this connection may have persisted until recently. The climatic oscillations throughout the Pleistocene, in that case, did not produce taxonomically recognizable diversification but probably shaped the current distributional pattern of *A. paranaensis*.

On the other hand, *Akodon mystax* provides no evidence of a mountaintop-southern connection, as its nearest relative, *A. lindberghi*, inhabits grasslands from the Cerrado of central Brazil and transitional areas of Atlantic forest (Hershkovitz 1990; Geise *et al.*

1996). The phylogenetic patterns of *A. mystax* in fact exemplify a second type of interchange, one that took place between the *campos de altitude* and the savannas of central Brazil. The genetic differentiation between *A. mystax* and *A. lindberghi* is very low, indicative of very recent contact between components of the Cerrado and *campos de altitude*. In addition, the wide gap between the western and eastern populations of *A. lindberghi*, whether a sampling artifact or not, is highly suggestive of former broadly distributed grasslands throughout the western border of Atlantic forest. The presence on the mountaintops of many xerophytic plant genera typical of the Cerrado flora (Safford 1999a) also provides evidence that the grasslands of central Brazilian and the campos de altitude may have been connected.

In summary, the phylogenetic patterns of the sigmodontines presented here suggest that the history of diversification of the *campos de altitude* may be more complex than suggested by the classical interpretations of palynological and paleoclimatological data. These communities may represent mosaics of lineages shaped by different historical events. The analysis of these sigmodontines indicates that differentiation among these communities, mainly at the species level, is not solely attributable to episodes of disjunction between *campos de altitude* and austral grassland or cool-humid habitats. Instead, contact with the central Brazilian savannas probably also helped to determine the community composition of the montane habitats of the Atlantic forests. Floristic and physiognomic similarities also suggest past connections between the *campos de altitude* and the Andean páramos (Clark, 1992; Safford, 1999a). However, signals of this connection are not recorded in the phylogenetic patterns of the sigmodontines we examined, probably because this interchange took place before speciation in the species groups analyzed here.

The biogeographic inferences made here are still very preliminary and represent a first step in exploring the evolution of the fauna of the *campos de altitude*. Many other rodent and marsupial genera, such as *Brucepattersonius*, *Delomys*, *Juliomys*, *Oxymycterus*, and *Monodelphis*, include species with close association to these montane habitats; most have been poorly studied and could serve as models for comparative biogeographic analyses. We hope that the information introduced here stimulates future studies focusing on the zoogeography of the *campos de altitude*, as only the cumulative description of biogeographic and historical patterns concerning this biota will improve our view of these important Atlantic forest ecosystems.

ACKNOWLEDGEMENTS

We are grateful to L. M. Tiepolo, F. Casado D. da Silva, M. O. Garcia Lopes and C. R. Bonvicino for assistance in field work. L. Tiepolo kindly shared valuable tissue samples used in this work. J. Patton (MVZ), B. Patterson (FMNH), Gisele Lessa (MZUFV) and Mario de Vivo (MZUSP) granted access to specimens deposited in collections under their care. IBAMA provided collecting permits to conduct field work at Parque Nacional do Caparaó (license 070/2003), Parque Nacional do Itatiaia (lic. 143/2004, process 02022.000776/04-39), Parque Nacional São Joaquim (lic. 121/2004, proc. 02001.001499/2004), Parque Nacional Aparados da Serra (lic. 098/2004, proc. 02001.001499/04-20) and at areas in Southeastern and Southern Brazil (lic. 084/2005), and also authorized transportation and analyses of genetic samples (proc. 02001.002943/2004). J. Patton and G. D'Elía provided insightful comments on a previous version of this manuscript. P. R. Gonçalves was supported by the Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico (CNPq) through a split-fellowship while conducting work as a visiting scholar at the University of Michigan Museum of Zoology, Ann Arbor, and a Collection Visiting Grant during studies at the Field Museum of Natural History, Chicago.

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APPENDIX 1 Specimens examined:

Morphological and genetic data were derived from voucher-specimens deposited in the following institutions: Museu Nacional, Rio de Janeiro (MN, VA, PRG, JAO, EDH, LMT); Museu de Zoologia da Universidade de São Paulo (MZUSP, CIT); Museu de Zoologia "Joao Moojen de Oliveira", Viçosa (MZUFV, BRG, SLM); Museo Nacional de Historia Natural, Montevideo (MNHN); Centro Nacional Patagónico, Puerto Madryn (CNP); Museo de Ciencias Naturales y Tradicional de Mar del Plata "Lorenzo Scaglia" (MMP-Ma); Field Museum of Natural History, Chicago (FMNH); Museum of Natural Science, Louisiana State University (LSUMZ); Museum of Vertebrate Zoology, Berkeley (MVZ); Michigan State University, Lansing (MSU); University of Michigan Museum of Zoology, Ann Arbor (UMMZ). Numbers in brackets preceding the localities names refer to their mapping in Fig. 1. Numbers in parentheses refer to GenBank accession code. Sources of sequence data for *Akodon* are indicated by the following superscripted symbols: *–this study, *a*–Smith and Patton (1993), *b*–Geise *et al.* (2001), *c*–D'Elía (2003), *d*–D'Elía *et al.* (2003), *e*–Pardiñas *et al.* (2003), *f*–Pardiñas *et al.* (2005), *g*–Smith and Patton (in press).

Akodon aerosus – PERU: 72km NE Paucartambo, Cuzco: MVZ 171679 (M 35703[g]).

A. affinis – COLOMBIA: Corregimiento La Florida, Risaralda (AY 196164[d])

A. aff. cursor – BRAZIL: Estação Experimental Djalma, CEPLAC, Una, Bahia: EDH 30 (AF 184053[b]).

A. albiventer – CHILE: Parinacota, ca. 72 km E Arica, 10 km S Chapiquina, 22 km S Putna, Tarapaca,: FMNH 129978 (AY494838[g]).

A. azarae – PARAGUAY: [1] 24km NW Villa Hayes, Presidente Hayes: UMMZ 133969, [2] 15.5km NW Chaco, Presidente Hayes: UMMZ 126070; 5.8km by road NE Pilar, Neembucu: UMMZ 134443 (U 03529[a]); [3] 83.2km NW Puerto Falcon, Presidente Hayes: UMMZ 137561, ARGENTINA: [4] Pirayui, Capital, Corrientas, Argentina: MVZ 166102-103, 173730-731, [5] Hurlingham, Buenos Aires: UMMZ 109223, 109226, 111009, [6] Ezeiza, 20km S Buenos Aires: MVZ 134234-236; [7] Capital Federal, Nunez, Costa do Rio La Plata: UMMZ 111010, [8] Torrecita [Urdampilleta], Buenos Aires: FMNH 23327-23343; [9] 35km Sierra Azul, Buenos Aires: MVZ 134230-233; [10] INTA, Balcarce, Buenos Aires: MSU 16695.

A. boliviensis – PERU: 12km S Santa Rosa, Puno: MVZ 171607 (M 35691[g]).

A. budini – BOLIVIA: Rinconada del Bufete, Chuquisaca (AY 605060[g])

A. cursor – BRAZIL: [11] Fazenda Neblina, Pq. Est. Serra do Brigadeiro, Fervedouro, Minas Gerais: BRG 12, 13, 17, 20, 21, 30, 33, 34, 39, 43, 46, 52, 67, 73-75, 85, 88, 91, 92, 111; [12] Mata do Paraíso, Viçosa, Minas Gerais: PRG 208, 293, 298, 327, 378, 416, 927, 933, SLM 190, 214, 219, 228, 258, 260; [13] Estação Ecológica Boracéia, Salesópolis, São Paulo: MVZ 182780-783, 183026-030, MZU SP29257 (AF 184051[g]); [14] Fazenda Intervales, Capão Bonito, São Paulo: MVZ 182072, 182073, 182784, 183017-025, 183249, 183251.

A. dayi – BOLIVIA: El Refugio, Parque Nacional Noel Kempff Mercado, Santa Cruz (AY 605059[g]).

A. dolores – ARGENTINA: Papagayos, San Luis (AY 273904[c]).

A. fumeus – BOLIVIA: Rinconada del Bufete, Chuquisaca (AY 605061[g]).

A. iniscatus – ARGENTINA: 10km S Comallo, Rio Negro: MVZ 182655 (AY 273917[c]).

A. juninensis – PERU: 22km N La Oroya, Junin: MVZ 173038 (M 35698[g]).

A. kofordi – PERU: Agualani, Puno: MVZ 171665 (M 35697[g]).

A. lindberghi – BRAZIL: [17] Matosa, Parque Nacional de Brasília, Brasília, Distrito Federal: FMNH 128292-298; [18] Sitio Maglandia, Simão Pereira, Minas Gerais: MN 33681-686, 33703, 48026 (AF 184057[g]).

A. lutescens – PERU: 12km S Santa Rosa, Puno: MVZ 171612 (M 35710[g]).

A. molinae – ARGENTINA: Ñacuñan MaB Reserve, Santa Rosa, Mendonza (AY 494839[g]).

A. mollis – PERU: "Machete" on Zapalache Carmen trail, Piura: LSUMZ 27007 (U 03546[a]).

A. montensis – BRAZIL: [13] Estação Ecológica Boracéia, Salesópolis, São Paulo: FMNH 141602 (AF 184055[g]); [18] Pedreiras, São Paulo: MN 50239, 50256-269, 50271-278, 50280, 50301-304, 53668-63104; [21] Mananciais da Serra, Piraquara, Parana: JAO 965, 966, 973, 974, 977, 984, 990, LMT 425 (EF101873*), 428 (EF 101874*). PARAGUAY: [20] Estancia Felicidad, Canindeyu, Paraguay: UMMZ 174920-922, 174924, 174926, 174928, 174929, 174931, 174935-937, 174940-942, 174919, 174969 (AY 273905[c]); Parque Nacional Ybycuí, Paraguarí: UMMZ 133948-133952, 133954, 133955, 137113-137116, 137118, 137122, 137126-137128, 137130-137133 [22] Sapucay: UMMZ 57075-075; ARGENTINA [23] Caraguatay, Misiones, Argentina: FMNH 26817, 26832-838, 26842, 26845-847.

A. mystax – BRAZIL: [24] Arrozal: MN 31910-912, PH 10427,

10430, 10431, [25] Terreirão, Parq. Nac. Caparaó, Alto Caparaó, Minas Gerais: MN 31896-909, 31913, 59113, 69565 (EF 101875*), 69566 (EF 101876*), 69567 (EF 101877*), 69568-576, 69585-587, 69589, 69590, 69596, 69599, 69602, 69605, 69606, 69609, 69613, 69623, 69627 (EF 101878*), 69628, 69629 (EF 101879*), 69644, 69645, 69660 (EF 101880*), 69664, 69665, PH 10241.

A. orophilus – PERU: 16km NNE Palca, Junin: MVZ 173057 (M 35699[g]).

A. paranaensis – BRAZIL: [15] Brejo da Lapa, Itatiaia, Itamonte, Minas Gerais: MN48041 (AF 184054[b]), 48067, 48070 (AF 273907[b]), 63110; [16] Campos do Itatiaia, Abrigo Rebouças, Pq. Nac. Itatiaia, Rio de Janeiro: MN 69677, 69679, 69681, 69682, 69685, 69686 (EF 101886*), 69695, 69700 (EF 101887*), 69710, 69719, 69724, 69726 (EF 101888*), 69745; [26] Estação Ecológica de Canguiri, Piraquara, Parana: MZUSP 29088-118 ; [27] Roça Nova, Piraquara, Parana: LMT 405 (sequence to be deposited); [28] Tres Barras, margins of Uruguay river, Aratiba, Rio Grande do Sul: MZUSP 29119-126; [29] Urubici, Santa Catarina: LMT 301(EF 101883*), LMT 304 (EF 101884*); [30] Parq. Nac. Aparados da Serra, Rio Grande do Sul: LMT 270 (EF 101881*), LMT 294 (EF 101882*); [31] Venâncio Aires, Rio Grande do Sul: CIT 1131 (AY 195866[e]); [38] Parque Provincial Islas Malvinas: MMP-Ma2421 (AY 702968[e]).

A. philipmyersi – ARGENTINA: [32] Estancia Santa Inés, Posadas, Misiones, Argentina: CNP 739 (AY 702965[f]), UMMZ 176194 (AY 702967[f]).

A. reigi – URUGUAY: [33] Paso Averías, Lavalleja, Uruguay: MNHN 3682 (AY 195865[e]).

A. sanctipaulensis – BRAZIL: [34] Morretinho, São Paulo: FMNH 94520 [35] Primeiro Morro, São Paulo: 94514-519, [36] Quadro Penteado, São Paulo: FMNH 94522 [37] Iporanga, São Paulo: FMNH 94521.

A. serrensis – BRAZIL: Vale das Antas, Parque Nacional da Serra dos Órgãos, Rio de Janeiro: VA 1 (AY 273908[c]); Urubici, Santa Catarina: LMT 436 (EF 101889*).

A. siberiae – BOLIVIA: 28km by road W Comarapa, Cochabamba (AY 273909[c]).

A. spegazzinii – ARGENTINA: Pampa de Achala, Córdoba (AY 196165[g]).

A. subfuscus – PERU: 15km S Callali, Arequipa: MVZ 174109 (M 35695[g]).

A. toba – PARAGUAY: 9km NE Juan de Zalazar, Presidente Hayes: UMMZ 133965 (U 03527[g]).

A. torques – PERU: 32km NE Paucartambo, Cuzco: MVZ 17120 (M 35700[g]).

Deltamys kempi – URUGUAY: San José: Ruta 1 sobre Arroyo Cufre: MNHN 4151 (AY 195862).

Necromys lasiurus – BRAZIL: Telêmaco Borba, Paraná: JAO 1692. PARAGUAY: 8 km NE Juan de Zalazar, Presidente Hayes: UMMZ 134431 (U 03528).

Necromys temchucki – ARGENTINA: Estancia Santa Inés, Misiones: UP 22 (AY 273914).

Thalpomys cerradensis – BRAZIL: Jaborandi, Bahia: MN 59503 (AY 310356).

Thaptomys nigrita – BRAZIL: Estação Biológica de Boracéia, Salesópolis, São Paulo: MVZ 183044 (AF 108666).

Blarinomys breviceps – BRAZIL: Estação Experimental Djalma, Una, Bahia: UFMG-MAS 17 (AF 108668).

Brucepattersonious soricinus – BRAZIL: Estação Biológica de Boracéia, Salesópolis, São Paulo: MVZ 186036 (AY 277486).

Lenoxus apicalis – PERU: 14 km W Yanahuaya, Puno: MVZ 171752 (U 03541).

Oxymycter delator – PARAGUAY: 13.3 km by road N Curuguaty, Canendiyu: UMMZ 137077 (AF 454766).

Abrothrix andinus – PERU: 2 km W Sumbay, Arequipa: MVZ 174066 (AF 108671).

Geoxus valdivianus – ARGENTINA: 43 km SSW Bariloche, Rio Negro: MVZ 154601 (U 03531).

APPENDIX 2

External and craniodental measurements of selected Akodon species from eastern South America. Summary statistics: sample size (n), mean followed by standard deviation, and observed range (in parentheses).

Characters	*A. mystax* (*n* = 54)	*A. lindberghi* (*n* = 13)	*A. philipmyersi* (*n* = 1)	*A. azarae* (*n* = 34)	*A. sanctipaulensis* (*n* = 8)	Itatiaia population (*n* = 17)	*A. paranaensis* (*n* = 36)	*A. nontensis* (*n* = 70)	*A. cursor* (*n* = 33)
HBL	85 ±8 (101-66)	83 ±8 (93-67)	--	94 ±9 (113-75)	81 ±9 (100-73)	108 ±7 (125-100)	106 ±9 (125-88)	107 ±19 (131-63)	104 ±10 (128-80)
HFL	19 ± 1 (22-16)	19 ±1 (20-17)	--	20 ±1 (23-19)	23 ±1 (24-23)	25 ±1 (26-22)	25 ±1 (26-23)	25 ±2 (31-22)	26 ±2 (30-22)
Ear	13 ±1 (18-11)	14 ±1.4 (16-11)	--	14 ±1 (16-12)	14 ±2 (16-12)	18 ±1 (19-17)	17 ±1 (19-13)	19 ±1 (22-17)	18 ±1 (21-14)
TL	68 ±6 (76-59)	65 ±7 (74-49)	--	67 ±8 (82-54)	65 ±5 (75-58)	97 ±6 (110-90)	79 ±8 (92-60)	89 ±9 (102-63)	93 ±9 (118-72)
GSL	24.33 ±0.56 (25.58-22.62)	23.79 ±0.6 (24.67-22.78)	23.13	24.66 ±1.25 (27.28-22.7)	23.49 ±1.1 (25.58-22.24)	29.23 ±0.85 (31.02-28.27)	28.94 ±1.18 (31.41-25.67)	28.30 ±1.19 (30.43-25.86)	29.32 ±1.35 (33.46-26.61)
ZB	12.03 ±0.3 (12.75-11.47)	11.88 ±0.3 (12.28-11.35)	11.77	12.31 ±0.57 (13.45-11.31)	11.80 ±0.42 (12.7-11.45)	14.92 ±0.49 (15.89-14.28)	14.44 ±0.84 (16.16-11.64)	13.83 ±0.51 (14.94-12.74)	14.96 ±0.59 (16.49-14.09)
CIL	22.52 ±0.57 (24.17-21.28)	21.84 ±0.65 (22.71-20.72)	21.36	22.61 ±1.33 (25.24-20.47)	20.97 ±1.05 (22.99-20.14)	26.78 ±0.94 (28.66-25.64)	26.26 ±1.26 (29.01-23.23)	25.53 ±1.71 (27.49-23.26)	26.82 ±1.28 (30.9-24.76)
NL	8.84 ±0.29 (9.58-8.24)	8.43 ±0.24 (8.71-8)	8.39	8.98 ±0.60 (10.28-7.67)	8.91 ±0.62 (9.75-7.88)	11.62 ±0.55 (13.03-10.92)	11.61 ±0.65 (12.84-9.62)	11.19 ±0.72 (12.56-9.28)	11.25 ±0.75 (13.07-9.55)
LD	6.36 ±0.23 (7.22-5.9)	6.08 ±0.22 (6.53-5.74)	5.85	6.06 ±0.52 (7.24-5.1)	5.87 ±0.35 (6.54-5.54)	7.82 ±0.4 (8.54-7.26)	7.49 ±0.52 (8.38-6.32)	7.35 ±0.49 (8.25-6.25)	7.69 ±0.46 (8.89-6.8)
LPB	2.75 ±0.15 (3.21-2.53)	2.78 ±0.18 (3.04-2.29)	3.13	3.31 ±0.28 (4.07-2.8)	3.22 ±0.23 (3.65-2.85)	3.68 ±0.14 (4.09-3.5)	3.49 ±0.30 (4.19-2.75)	3.38 ±0.22 (3.87-2.94)	3.77 ±0.27 (4.32-3.15)
LM	3.89 ±0.10 (4.1-3.63)	3.84 ±0.13 (4.1-3.61)	3.78	4.09 ±0.13 (4.41-3.75)	4.29 ±0.18 (4.5-3.99)	4.7 ±0.16 (4.93-4.21)	4.64 ±0.14 (4.96-4.35)	4.19 ±0.11 (4.49-3.92)	4.48 ±0.19 (4.83-4.04)
BM1	1.09 ±0.04 (1.16-0.96)	1.11 ±0.06 (1.23-1)	1.06	1.17 ±0.05 (1.29-1.05)	1.22 ±0.04 (1.29-1.15)	1.32 ±0.05 (1.39-1.21)	1.25 ±0.06 (1.39-1.12)	1.22 ±0.05 (1.31-1.06)	1.33 ±0.08 (1.49-1.18)
LIF	6.17 ±0.2 (6.73-5.68)	5.78 ±0.28 (6.41-5.41)	5.27	5.8 ±0.35 (6.59-5.07)	5.23 ±0.36 (5.61-4.54)	6.99 ±0.32 (7.67-6.59)	6.8 ±0.37 (7.52-6.07)	6.64 ±0.38 (7.42-5.79)	6.62 ±0.35 (7.54-6.12)
BR	4.48 ±0.21 (5.18-3.89)	5.55 ±0.18 (4.76-4.22)	4.5	4.45 ±0.32 (5.18-3.94)	4.34 ±0.24 (4.88-4.2)	5.35 ±0.28 (5.9-4.84)	5.07 ±0.33 (6.03-4.6)	5.12 ±0.31 (5.68-4.19)	5.49 ±0.27 (6.08-4.98)
DR	4.94 ±0.16 (5.56-4.69)	4.84 ±0.19 (5.21-4.61)	5.08	4.89 ±0.37 (5.82-4.3)	4.51 ±0.24 (4.88-4.2)	5.64 ±0.31 (6.25-5.18)	5.42 ±0.33 (6.23-4.78)	5.33 ±0.27 (6.11-4.76)	5.87 ±0.42 (7.35-5.28)
BPB	2.82 ±0.12 (3.1-2.58)	2.62 ±0.15 (2.88-2.36)	2.42	2.46 ±0.22 (2.99-2.04)	2.37 ±0.1 (2.5-2.19)	3.15 ±0.15 (3.4-2.9)	2.91 ±0.25 (3.36-2.45)	2.98 ±0.24 (3.5-2.57)	3.21 ±0.20 (3.84-2.76)
BZP	1.82 ±0.06 (2.01-1.7)	1.78 ±0.08 (1.89-1.61)	2	2.24 ±0.18 (2.63-1.87)	1.94 ±0.14 (2.15-1.64)	2.54 ±0.12 (2.9-2.4)	2.5 ±0.18 (2.88-2.09)	2.42 ±0.18 (2.86-1.93)	2.53 ±0.19 (3.1-2.11)