
Phylogeny of the African murid tribe Otomyini (Rodentia), based on morphological and allozyme evidence

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Based on a cladistic analysis of 45 morphological (craniodental) and 46 binary allozyme characters, previous systematic treatments of the African murid tribe, Otomyini (lamine-toothed rats), are reviewed. Cladistic analysis of the craniodental data, involving eight outgroup taxa, confirmed the monophyly of the Otomyini, and suggested *Pelomys* to represent the sister genus of the Otomyini. Craniodental synapomorphies provided strong support for certain basal relationships among Otomyini rodents, reinforcing available palaeontological evidence. However, poor statistical (Bremer decay index) support was obtained for terminal relationships. The data presented revealed a 'mesic clade' of southern and eastern African species, with *Otomys sloggetti* basal to this group. The arid-adapted, southern Africa-endemic species, *Parotomys littledalei*, *P. brantsii* and *O. unisulcatus*, were all placed basal to the 'mesic clade', but did not form a separate 'arid clade', as suggested by earlier biochemical studies. Two allozyme synapomorphies supported the existence of the 'mesic clade', separate from arid-adapted southern African species. A strict cladistic interpretation of the present data did not support the existence of two genera in the tribe, and the two species of *Parotomys* (whistling rats) should be transferred to *Otomys*. At the species level, specific identity of *O. lacustris* and *O. barbouri*, distinct from *O. anchietae*, was supported by several autapomorphies, and *O. tropicalis burtoni* was shown to be included in *O. angoniensis* rather than *O. tropicalis*, extending the range of the former species into West Africa.

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Introduction

Two genera, *Parotomys* Thomas 1918 and *Otomys* Cuvier 1823, and 14 extant species are currently recognized within the murid tribe Otomyini (lamine-toothed rats) (Musser & Carleton 1993). However estimates of the actual number of species vary, and this number is probably underestimated due to the likely existence of numerous undescribed relict populations adapted to high altitude, moist, fire-less regimes occurring throughout mountainous regions of central, East and West Africa (Bohmann 1952; Misonne 1974; Petter 1982; Denys 1989; Dieterlen & Van der Straeten 1992; Taylor *et al.* 1993; Lavrenchenko *et al.* 1997; Taylor & Kumirai 2001; Maree 2002).

Palaeontological data support a South African origin and early Pliocene radiation of the tribe (Pocock 1976; Denys 1989, 1990, 1999, 2003; Sénégas & Avery 1998; Sénégas

2001). Two fossil species of *Euryotomys* from South Africa, *E. pelymoides* (3.7–5 Myr) (Pocock 1976; Denys *et al.* 1989) and *E. boliti* (4–5 Myr; Sénégas & Avery 1998; Sénégas 2001) provide evidence for the murine origins of Otomyini. The oldest true *Otomys* fossils are not known before 3–3.7 Myr in South Africa (cf. *gracilis* and cf. *sloggetti*), and 1.5–2.0 Myr in East Africa (*O. petteri* and cf. *petteri*; Denys 1989, 1990, 2003), despite excellent fossil records from older sequences in East Africa.

Previous phylogenetic analyses of morphological characters of the Otomyini were based on an intuitive approach, and on the use of a few craniodental characters that were highly polymorphic and/or prone to ecological convergence (Bohmann 1952; Petter 1982; Dieterlen & Van der Straeten 1992; Taylor & Kumirai 2001) (Fig. 1A). Palaeontological studies involving molar morphology have shown that within

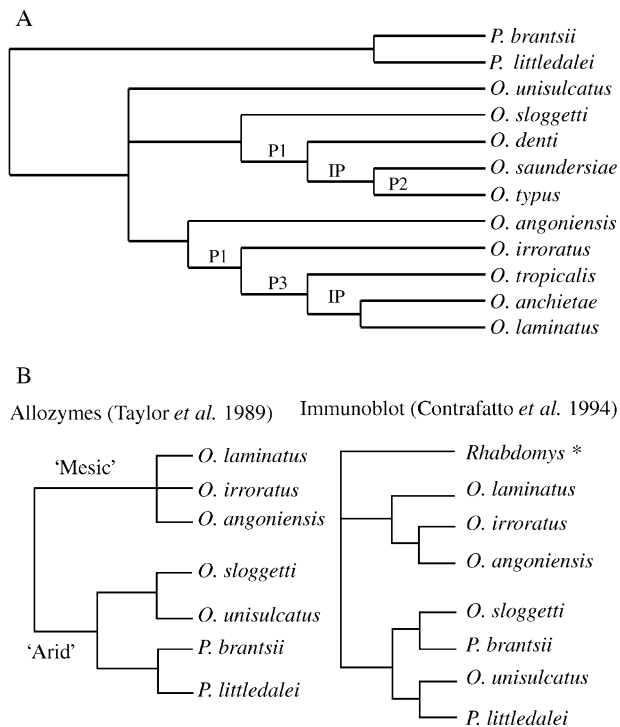


Fig. 1 A, B. Previous phylogenies obtained for Otomyini rodents. —A. Intuitive analysis of Bohmann (1952) based on morphological and ecological data (IP, 'interpluvials'; P, 'pluvials'). —B. Phenetic and cladistic analyses of allozyme and immunoblot data (Taylor *et al.* 1989b; Contrafatto *et al.* 1994). Outgroup indicated with an asterisk.

the subfamily there is an evolutionary trend towards an increasing number of laminae in the third upper molar, from four in the ancestral *E. pelymoides*, to five or six in the earliest *Otomys* cf. *gracilis* fossils, to five to 10 in modern *Otomys* (Chevret *et al.* 1993; Denys *et al.* 1989; Sénégas 2001). But none of these studies provided any analysis of all the morphological features based on cladistics and none of these studies considered all members of the tribe Otomyini including fossil taxa.

Earlier phylogenetic analyses of southern African laminate-toothed rats based on allozyme and immunological characters suggested the existence of distinct arid and mesic lineages in southern Africa (Taylor *et al.* 1989a,b; Meester *et al.* 1992; Contrafatto *et al.* 1994) (Fig. 1B). These studies failed to support the monophyly of either recognized genus, and consistently tended to group the bush rat, *O. unisulcatus*, occurring in semiarid regions of South Africa, and, sometimes Sloggett's rat, *O. sloggetti*, occurring at high altitudes in the Drakensberg Range of South Africa, with *Parotomys* rather than with *Otomys*. In support of these results, data on kidney morphology (Pillay *et al.* 1994) and chromosome banding and fluores-

cent *in situ* hybridisation (FISH) results (Robinson & Elder 1987; Rambau *et al.* 1997; Rambau & Robinson 1999) indicated that *O. unisulcatus* shares greater affinity with *Parotomys* than with its congener, *O. irroratus*. On the other hand, data on sperm morphology (Bernard *et al.* 1991) found *O. unisulcatus* to be distinct from all other Otomyini, although closer to *O. irroratus* and *O. angoniensis* than to *Parotomys*.

Maree (2002) used sequence data from two mitochondrial DNA genes, cytochrome *b* and 12S RNA, to model the phylogeny of the Otomyini based on representatives of all but one recognized species of the subfamily (*O. saundersiae*). While several terminal nodes were consistently supported, deeper nodes remained largely unresolved, probably due to the very rapid evolutionary radiation of the group. Based on the cytochrome *b* molecular clock, the earliest split in the subfamily was estimated to have occurred around 6.3 Myr BP, an estimate that accorded closely with the protein molecular clock (Taylor *et al.* 1989a) but that predates the earliest fossil records of *Euryotomys*.

Cladistic analysis of a large set of independent morphological attributes can be extremely useful, not only in defining species boundaries of problematic taxa (e.g. Lecompte *et al.* 2001; Taylor & Kumirai 2001), but also in providing valuable synapomorphies and resolving phylogenetic relationships (e.g. Barome *et al.* 2001; Lecompte *et al.* 2001; Masters & Brothers 2002). For this reason, we have undertaken a thorough examination of various morphological features of skull and dental morphology.

This study reports on the first such cladistic analysis of an initial set of 45 craniodental characters, obtained by independent inspection of dorsal, lateral and ventral views of the crania and mandibles of representatives of all known Otomyini taxa and eight outgroup taxa. Included in this set were four supplementary characters which have been used extensively by previous workers: the number of laminae in the upper third and lower first molars, lower incisor grooving, and the shape of the petrotympanic foramen. This study further reports on the cladistic analysis of new allozyme data for 10 species, including both eastern and southern African species. Previous allozyme studies have considered only southern African species (Taylor *et al.* 1989a,b, 1992; Van Dyk *et al.* 1991).

Materials and methods

Material examined

Morphology. Table 1 indicates the number of specimens of each species examined, comprising a total of 140 specimens representing 19 ingroup and eight outgroup taxa, from the following museum collections: Durban Natural Science Museum; Museum National d'Histoire Naturelle, Paris; Staatliches Museum für Naturkunde, Stuttgart; and the Natural History Museum, London. Wherever possible, at least

Table 1 Taxa and sample sizes examined for morphological and allozyme analyses of Otomyini rodents, geographical origin, and museums where voucher specimens are lodged.

Taxa	<i>n</i> (morphology)	<i>n</i> (allozymes)	Origin	Museums
<i>Arvicanthis niloticus</i>	6	—	Senegal, Mauritania	MNHN
<i>Tatera guineae</i>	5	—	Senegal	MNHN
<i>Mastomys natalensis</i>	6	—	Cameroon	MNHN
<i>Mystromys albicaudatus</i>	6	—	South Africa	MNHN, NHM
<i>Saccostomus campestris</i>	5	—	Zimbabwe	MNHN
<i>Acomys spinosissimus</i>	5	—	Zimbabwe	MNHN
<i>Pelomys fallax campanae</i>	3	—	Malawi, Congo DRC	MNHN, NHM
<i>Rhabdomys pumilio</i>	5	3	Uganda, South Africa, Zimbabwe	DM
<i>Parotomys brantsii</i>	8 (type)	2	South Africa	DM, NHM
<i>P. littledalei</i>	4 (type)	—	South Africa, Namibia	DM, NHM
<i>Otomys sloggetti</i>	7 (type)	2	South Africa, Lesotho	DM, NHM
<i>O. unisulcatus</i>	8	2	South Africa	DM, NHM
<i>O. saundersiae</i>	6	2	South Africa	DM
<i>O. laminatus</i>	4 (type)	2	South Africa	DM
<i>O. lacustris</i>	2	—	Tanzania	NHM
<i>O. anchietae</i>	3	—	Angola	MNHN, NHM
<i>O. occidentalis</i>	4 (type)	—	Mt Oku (Nigeria)	MNHN, SM
<i>O. denti</i>	4 (type)	—	Congo DRC, Ruwenzori Mts	NHM, SM
<i>O. irroratus</i>	7	2	South Africa, Zimbabwe	DM, NHM
<i>O. typus typus</i>	8	—	Ethiopia	MNHN, SM
<i>O. typus jacksoni</i>	2	2	East Africa	DM, NHM
<i>O. tropicalis</i>	14 (type)	1	Central, East Africa, Sudan	MNHN, NHM, SM
<i>O. tropicalis burtoni</i>	5 (type)	—	Mt Cameroon	NHM, SM
<i>O. angoniensis</i> (SA)	7	2	South Africa, Botswana	DM, MNHN, SM
<i>O. angoniensis</i> (EA)	5 (type)	—	Kenya	NHM
<i>O. a. maximus</i>	2	—	Congo DRC	SM
<i>O. barbouri</i>	4	5	Mt Elgon (Uganda)	DM
Totals	140	25		

DM, Durban Natural Science Museum; MNHN, Museum National d'Histoire Naturelle, Paris; NHM, The Natural History Museum, London; SM, Staatliches Museum für Naturkunde, Stuttgart. Wherever species type specimens were examined this is indicated in parentheses.

five unbroken, adult skulls were selected per taxon. In few instances, the rarity of the taxon concerned and the presence of large numbers of broken or juvenile skulls meant that only two or three specimens could be included. In any event, low levels of intraspecific variation in the characters used minimized the possibility of bias due to a small sample size. Ingroup taxa included all described Otomyini taxa in addition to certain geographically isolated subspecies or populations that have previously been considered candidates for species status (Dieterlen & van der Straeten 1992; Taylor & Kumirai 2001; Maree 2002): *O. tropicalis burtoni*, *O. typus jacksoni*, *O. angoniensis maximus*, *O. lacustris* and *O. barbouri*, and both southern and eastern African populations of *O. angoniensis*. In order to minimize errors due to age variation in characters, specimens were selected having an intermediate degree of tooth wear, i.e. excluding juveniles and very old specimens. Characters were selected after detailed morphological comparisons of the dorsal, ventral and lateral views of the cranium and mandible of a representative selection of ingroup and outgroup taxa. Terminology of cranial foramina and other cranial structures followed Carleton & Musser (1989), Voss

(1988) and Wahlert (1985). Morphological characters and character states are described in the Appendix.

Allozymes. Data were obtained from 22 specimens of 10 Otomyini species, and three specimens of the outgroup, *Rhabdomys pumilio*, reported in Govender (1999) (Table 1). Although most of the specimens were obtained from earlier studies (Taylor *et al.* 1989a,b; Meester *et al.* 1992), specimens of *O. saundersiae* were collected in the western Cape during January 1998, by P. J. Taylor, G. Campbell and A. Kumirai, while specimens of *O. tropicalis*, *O. typus* and *O. anchietae barbouri* were collected at Mt Elgon (Uganda) by P. J. Taylor and A. Kumirai in February and March 1999. Regardless of whether specimens had been previously analysed by allozyme electrophoresis, the present study re-assessed specimens of all 10 species simultaneously on the same gel. Kidney and liver samples from each sacrificed animal were frozen at -20°C in the field, and at -190°C in the laboratory of the School of Life and Environmental Science at the University of Natal, until processed by starch gel electrophoresis. All specimens were deposited in the Durban Natural Science Museum.

Morphological analysis. Of the initial set of 45 craniodental characters, seven were excluded due to non-variance, extreme polymorphism, subjectivity of determination, or assumed non-independence from other characters. The majority of characters were not polymorphic in the species samples examined. However, for those that were, character states were coded using the 'majority' option of Wiens (1995). When alternative character states were both present at 50% frequency in a particular species, the character was scored as 'missing'. Initially, a maximum parsimony (MP) analysis was conducted, using PAUP version 4.0b10 (Swofford 2001), on the full data set of ingroup and outgroup taxa. The use of multiple outgroups successively distantly related to the ingroup is advisable, as it allows a test of the monophyly of the ingroup, and may allow conclusions about the higher-order relationships of the ingroup (Maddison *et al.* 1984; Barriel & Tassy 1998). Because the position of outgroups in the matrix can affect the topology of ingroup taxa (Barriel & Tassy 1998), analyses were repeated with different outgroup taxa placed as the 'primary outgroup' (first listed in the matrix). As this did not materially affect ingroup relationships, no attempt was made to obtain a consensus of different permutations, as suggested by Barriel & Tassy (1998).

Robustness and node support for the trees obtained was tested by means of bootstrap analysis (Felsenstein 1985) as well as the Bremer decay index (Källersjö *et al.* 1992). Because bootstrap analysis assumes character independence (Purvis 1994), and because morphological characters are often interdependent, the bootstrap approach is not well suited to morphological data. For this reason, although both bootstrap and Bremer indices were shown on presented trees, only the latter are discussed in the text. Bremer values exceeding 5 are generally considered to represent robust and well-supported relationships. Nevertheless, caution should be exercised when using this index, as it is affected not only by the level of homoplasy but also by the number of characters, so low Bremer decay indices may suggest either homoplasy or a small character set.

In order to allow some assessment of the higher-order relationships of Otomyini, a MP analysis was also performed with all murid taxa treated as the ingroup, and *Mystromys albicaudatus*, the only cricetid representative, treated as the outgroup. Further analyses were performed with only two outgroups, *Pelomys* (which was invariably the closest sister species to Otomyini in all preceding analyses) and *Arvicanthis* (which is accepted by many authors to be representative of the 'arvicanthine' group of rodents to which Otomyini has been associated on molecular grounds: Chevret *et al.* 1993; Ducroz *et al.* 2001), and excluding two ingroup taxa represented by small samples ($n = 2$) and/or a high proportion of missing data, *O. a. lacustris* and *O. typus* (East Africa), as this arrangement provided optimal resolution of the ingroup

taxa. Analyses were repeated with and without four multistate dental and skull shape characters (2, 38, 39 and 41 in the Appendix) that had been widely used in previous taxonomic assessments. These same four characters were ordered; in the case of the number of laminae in upper M3 (character 38) and lower M1 (character 39), a numerical increase with time is well documented in the fossil record (Denys 1989). Furthermore, progressive expansion of the nasal bone (character 2) and grooving of the lower incisors (character 41) can be considered to be ordered as they are represented in the extreme states in taxa which can be considered to be relatively 'derived', based on molar laminae number. All other characters were binary or multistate and unordered. The full morphological data matrix is shown in Table 2.

Allozyme analysis. Using starch gel electrophoresis, Govenader (1999) presented allozyme data for 15 polymorphic structural protein and enzyme loci and three monomorphic loci, for 10 species of Otomyini rodents and one outgroup, *Rhabdomys pumilio*. For the purpose of the present study, these data were modified slightly based on overlapping data obtained for larger samples of two ingroup taxa, *O. irroratus* and *O. saundersiae* (Mukerjee 1999), resulting in a final choice of three monomorphic loci: malate dehydrogenase, sorbitol dehydrogenase and peptidase (LG substrate); and 14 polymorphic loci: aspartate aminotransferase, albumin, β -galactosidase, α -glycerol-3-phosphate dehydrogenase, glucose 6-phosphate dehydrogenase, haemoglobin, isocitrate dehydrogenase (IDH), L-lactate dehydrogenase, malic enzyme, phosphogluconate dehydrogenase (PGD), phosphoglycerate mutase, purine-nucleoside phosphorylase, xanthine dehydrogenase, and superoxide dismutase. Allele frequencies were calculated for each species sample, standard genetic distances (D ; Nei 1972) were calculated between all pairs of taxa, and UPGMA and neighbour-joining trees were computed using the program DISPAN (Ota 1993). Using the independent alleles model, alleles were coded as present or absent, resulting in 46 binary characters (Table 3), which were analysed by MP using the heuristic search option of PAUP. Because the outgroup *Rhabdomys* was found to group genetically within the ingroup (D -values less than for some ingroup taxa comparisons), analyses were run including *Rhabdomys* as the outgroup and using midpoint rooting, without *Rhabdomys*.

Phylogenetic analysis of allozyme data may have limited utility where alternative alleles segregating at a locus are shared extensively between taxa, particularly when data are based on small species samples, as in the present study. However, previous studies of intraspecific and interspecific genetic variation in the Otomyini, and in the outgroup, *Rhabdomys pumilio*, based on relatively large ($n > 10$) samples of at least some species (e.g. *O. irroratus*, *O. saundersiae*, *O. unisulcatus*, *O. sloggetti*, *O. angoniensis*), have indicated that

Table 2 Morphological data matrix. An explanation of the morphological characters can be found in the Appendix.

Species	Character number																																															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45			
<i>Arvicanthis</i>	0	0	1	1	1	1	1	1	0	1	0	0	?	0	1	0	1	0	0	0	0	0	1	0	1	1	0	1	1	1	1	1	1	0	1	1	1	0	0	0	0	0	0	0	?	1		
<i>Tatera</i>	0	0	0	1	0	1	0	0	1	0	0	0	?	0	0	1	1	1	1	1	0	0	1	0	1	1	0	1	1	1	0	0	0	1	1	1	1	0	0	0	1	0	0	0	?	1		
<i>Mystromys</i>	0	0	1	0	0	1	1	0	1	1	1	0	?	0	0	0	0	1	1	1	0	0	1	0	1	1	0	1	0	1	2	0	0	0	1	1	0	0	0	0	0	0	0	1	0	?	1	
<i>Mastomys</i>	0	0	0	1	0	1	1	1	0	1	0	0	?	0	0	0	0	1	0	?	0	0	0	0	1	1	1	1	1	1	2	0	1	1	1	1	1	0	0	0	0	0	1	0	?	1		
<i>Saccostomus</i>	0	0	1	1	0	1	1	0	0	1	0	0	?	1	?	0	1	1	0	?	0	0	1	0	1	1	0	1	0	1	2	0	0	1	1	1	0	0	0	0	0	1	1	0	1			
<i>Acomys</i>	0	0	0	1	0	1	0	1	0	1	0	0	?	0	1	1	0	0	1	?	0	0	1	1	1	0	1	1	1	2	0	0	0	1	1	0	0	0	1	1	0	0	0	1	1	0	1	
<i>Rhabdomys</i>	0	0	0	1	0	1	0	0	1	1	0	0	?	0	1	0	1	1	1	1	0	0	1	0	1	1	1	1	1	0	1	1	?	0	1	0	0	0	0	0	0	0	1	0	?	1		
<i>Pelomys</i>	0	0	0	1	1	1	0	1	1	0	2	0	?	0	0	0	1	1	1	1	0	0	1	0	1	1	1	1	1	1	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	1	
<i>P. brantsii</i>	1	1	0	1	1	1	0	1	1	0	2	1	0	0	1	0	2	0	1	0	1	1	1	0	1	1	1	1	1	0	1	1	1	0	0	0	0	1	0	1	1	1	0	0	1	0	1	
<i>P. littledalei</i>	1	1	0	1	1	1	1	0	1	0	2	1	0	0	0	0	2	1	0	0	1	1	1	0	1	1	1	1	0	1	1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	1	0	1
<i>O. sloggetti</i>	1	1	0	1	1	1	1	2	1	0	2	1	1	1	?	1	0	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	0	0	0	1	0	2	1	1	1	0	1	0	1	0	1	
<i>O. unisulcatus</i>	1	1	0	1	1	1	0	1	0	1	2	1	1	0	0	1	1	1	1	1	0	1	1	1	0	1	1	0	1	1	1	0	0	1	1	0	0	0	1	0	1	0	1	0	1	0	1	
<i>O. saundersiae</i>	1	2	0	1	?	1	0	1	0	0	2	1	0	0	1	1	0	1	0	?	1	0	1	0	1	1	1	1	0	0	1	1	0	0	0	1	0	0	1	0	3	1	1	1	0	1	1	1
<i>O. a. lacustris</i>	1	2	0	1	?	1	0	?	1	0	2	1	0	0	1	1	?	1	?	?	?	0	1	0	1	1	1	0	0	0	1	1	0	0	0	1	0	4	2	1	1	1	1	1	1	1		
<i>O. laminatus</i>	1	3	0	1	1	1	1	0	1	0	2	1	1	0	1	1	1	1	1	0	1	0	1	0	1	1	1	0	0	0	1	1	0	0	0	0	0	0	6	3	1	1	0	1	1	1	1	
<i>O. anchietae</i>	1	2	0	1	1	1	1	0	1	0	2	1	0	0	1	1	1	1	1	0	1	1	1	0	1	1	1	0	0	0	1	1	0	0	0	1	0	5	2	1	1	0	1	1	1	1		
<i>O. irroratus</i>	1	3	0	1	1	1	1	0	1	0	2	1	0	0	1	0	1	1	1	0	1	0	1	0	1	1	1	0	0	0	1	1	0	0	0	0	0	4	1	1	1	0	1	1	1	1		
<i>O. occidentalis</i>	1	3	0	1	0	1	?	0	1	0	2	1	1	?	?	1	1	1	1	1	0	0	1	1	1	1	0	0	0	1	1	0	0	0	1	0	6	2	1	1	1	0	1	1	1	1		
<i>O. denti</i>	1	2	0	1	?	1	1	0	1	0	2	1	1	0	?	0	1	1	0	?	1	0	1	0	1	1	1	0	?	0	1	1	0	0	0	0	0	3	1	1	1	0	1	1	1	1		
<i>O. typus</i> Ethiopia	1	3	0	1	0	1	1	0	1	0	2	1	1	0	1	1	0	1	1	1	1	0	1	0	1	1	1	0	0	0	1	1	0	0	0	1	0	6	1	1	2	0	1	1	1	1		
<i>O. typus</i> EA	1	3	0	1	0	1	0	1	1	0	2	1	0	0	1	1	0	1	1	1	?	0	1	0	1	1	1	0	0	0	1	1	0	0	0	0	0	5	1	1	2	0	1	1	1	1		
<i>O. tropicalis</i>	1	2	0	1	1	1	1	0	1	0	2	1	0	0	1	1	1	1	1	0	1	0	1	0	1	1	1	0	0	0	1	1	0	0	0	0	0	5	1	1	1	0	1	1	1	1		
<i>O. t. burtoni</i>	1	2	0	1	0	1	0	0	1	0	2	1	0	0	1	1	0	1	1	0	1	0	1	1	1	1	1	0	0	0	1	1	0	0	0	0	5	1	1	1	0	1	1	1	1			
<i>O. angoniensis</i> SA	1	2	0	1	1	1	0	1	1	0	2	1	0	0	0	1	1	1	1	0	1	0	1	1	1	1	1	0	0	0	1	1	0	0	0	1	0	5	1	1	1	0	1	1	1	1		
<i>O. angoniensis</i> EA	1	2	0	1	0	1	0	1	0	1	2	1	0	0	0	1	1	1	1	0	1	0	1	1	1	1	1	0	0	0	1	1	0	0	0	1	0	5	1	1	1	0	1	1	1	1		
<i>O. a. maximus</i>	1	2	0	1	0	1	1	0	1	0	2	1	?	0	1	1	1	1	1	?	0	0	1	1	1	1	1	0	0	0	1	1	0	0	0	1	0	5	1	1	1	0	1	1	1	1		
<i>O. a. barbouri</i>	1	3	0	1	0	1	0	0	1	0	2	1	0	1	?	1	1	1	0	?	1	0	1	0	1	1	1	1	0	0	1	1	0	0	0	1	0	5	2	1	1	0	1	1	1	1		

Table 3 Binary allozyme data matrix.

	Genetic loci and alleles													
	AAT	ALB	GAL	GPD	G6PD	HB	IDH	LDH	ME	PGD	PGM	PNP	XDH	SOD
	ab	ab	abc	abc	ab	abcde	abcde	abcde	abc	abcd	ab	abcd	abcd	ab
<i>R. pumilio</i>	01	01	010	100	10	00001	00100	00001	010	0010	10	0000	0010	01
<i>P. brantsii</i>	11	01	001	011	01	00100	00010	01100	110	0100	10	0100	0100	10
<i>O. sloggetti</i>	01	01	010	001	01	0000?	00100	10000	001	0001	10	0001	0100	10
<i>O. unisulcatus</i>	01	01	011	001	01	00100	00001	00010	010	1000	10	1000	0100	10
<i>O. saundersiae</i>	01	01	010	001	01	00100	00100	00100	100	0001	11	0001	0100	01
<i>O. laminatus</i>	01	01	011	101	01	10000	01100	00100	010	0001	10	0001	1001	01
<i>O. irroratus</i>	11	01	011	001	01	01000	00100	10000	110	0001	10	0001	0100	01
<i>O. typus</i>	01	01	011	001	01	01000	00100	10000	110	0001	10	0001	0100	01
<i>O. tropicalis</i>	01	01	011	001	01	01000	00100	10000	010	0001	10	0001	0100	01
<i>O. angoniensis</i>	01	10	010	100	01	00100	00100	10000	010	0001	10	00?0	0100	01
<i>O. barbouri</i>	10	01	100	001	01	01000	00100	10000	100	0001	10	0001	0100	11

AAT, aspartate aminotransferase; ALB, albumin; GAL, β -galactosidase; GPD, α -glycerol-3-phosphate dehydrogenase; G6PD, glucose 6-phosphate dehydrogenase; HB, haemoglobin; IDH, isocitrate dehydrogenase; LDH, L-lactate dehydrogenase; ME, malic enzyme; PGD, phosphogluconate dehydrogenase; PGM, phosphoglycerate mutase; PNP, purine-nucleoside phosphorylase; XDH, xanthine dehydrogenase; SOD, superoxide dismutase.

allozyme differences between species are invariably qualitative (species- or group-specific alleles) rather than quantitative (sharing of two or more alleles at a particular locus), making them more amenable to cladistic analysis (Taylor *et al.* 1989a,b, 1992; Van Dyk *et al.* 1991; Contrafatto *et al.* 1992; Mahida *et al.* 1999; Mukerjee 1999). Thus, at least for some of the species, the 'common' alleles indicated in the present study, based on just two specimens per species, could be verified to be the same alleles present in wider samples studied previously (data available on request from PJT).

Combined morphology and allozyme data. Finally, a MP analysis was conducted on the combined allozyme + morphological data set (84 characters), with *Pelomys* and *Rhabdomys* as outgroups. The analysis was limited to species for which both allozyme and morphological data were available, with the exception of the outgroup, *Pelomys*, for which only morphological data were available (allozyme characters scored as missing for this species).

Results and discussion

Morphology

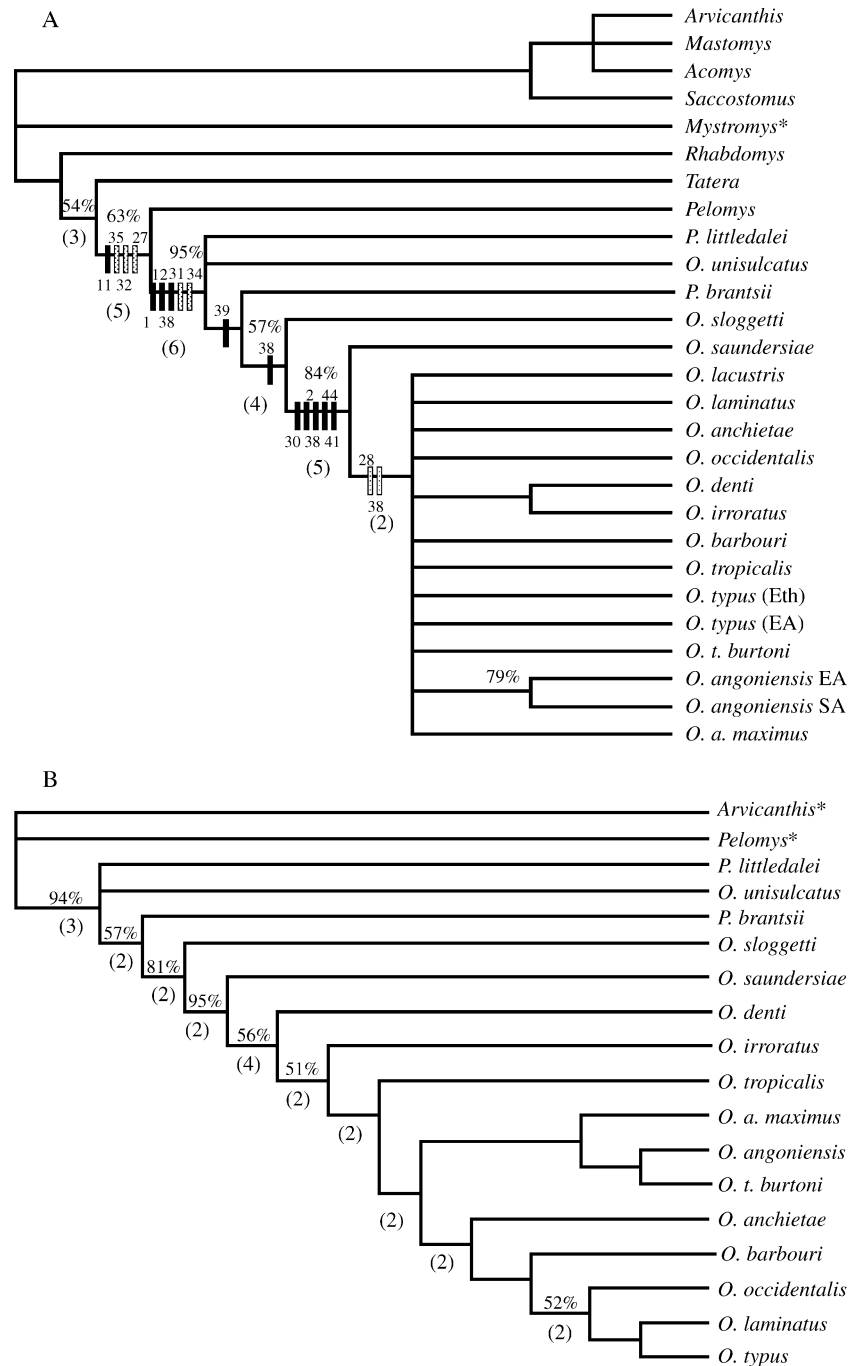
Figure 2A shows the strict consensus tree resulting from MP analysis of all ingroup and outgroup taxa, treating all murids as ingroup taxa, and the cricetid, *Mystromys*, as the sole outgroup (consistency index = 0.41, retention index = 0.724, length = 122). An identical topology for Otomyini taxa was obtained with eight outgroup taxa (i.e. treating Otomyini only as the ingroup). However, in the latter case, relationships among outgroups were poorly resolved, and the sister taxon of the Otomyini could not be ascertained due to a basal trichotomy (tree not shown). The tree in Fig. 1A indicates *Pelomys* to be the sister genus to Otomyini, with a Bremer

decay index of 5 supporting this relationship. One unreversed synapomorphy supports this node: posteriorly diverging interdental width (character 11); as do three ambiguous synapomorphies, showing single reversals or convergences: thick bridge separating optic and sphenopalatine foramina (character 27) (reversal in *O. unisulcatus* and convergently present in *Rhabdomys*); squamosal part of zygomatic arch robust (character 32) (also convergently present in *Arvicantthis*) and absence of distinct masseteric knob of mandible (character 35) (convergently present in *Rhabdomys*).

High support (Bremer value of 6) and three unreversed synapomorphies support the monophyly of the Otomyini: inflation of the nasal bones (character 1), posterior palatine foramina partially obscured by folded palate (character 12), and upper M3 with more than two laminae (character 38). Additionally, the horizontal and linear (as opposed to sloping and curved) shape of the crest connecting the articular and coronoid processes of the mandible (character 34) is found in all Otomyini (but also in *Mystromys* and *Acomys*). Furthermore, in all Otomyini (but also *Rhabdomys* and *Arvicantthis*), the maxillary masseteric tubercle is replaced by a roughened attachment surface (character 31).

The monophyly of the 'mesic' *Otomys* lineage (excluding the arid-adapted *Parotomys* species and *O. unisulcatus*) is supported by a Bremer value of 4 and by one synapomorphy: five or more laminae in upper M3 (character 38). Among the three arid species, the more recent branching order of *P. brantsii* relative to the other two species is not well supported. Good support (Bremer value of 5) and five synapomorphies show *O. sloggetti* to be the sister species to the remaining species of the 'mesic' lineage: angle of expansion of nasals > 115° (character 2); disposition of zygomatic plate in lateral view short, closing infraorbital foramen dorsally (character 30);

Fig. 2 A, B. Maximum parsimony analysis of morphological data for rodents of the tribe Otomyini. —A. Strict consensus tree from 25 trees produced by maximum parsimony based on 34 parsimony-informative craniodental characters, showing the phylogeny of Otomyini taxa in relation to eight outgroup taxa. All murid taxa were treated as ingroup taxa, and the tree was rooted with only *Mystromys* as the outgroup (indicated with an asterisk). Synapomorphies are indicated as solid rectangles; ‘near synapomorphies’ (character state changes involving a single reversal or convergence) are indicated by stippled rectangles. Bootstrap values > 50 ($n = 100$ replicates) are shown. Bremer decay indices > 1 are shown in parentheses. Length = 122; consistency index = 0.41; retention index = 0.724. The same topology of ingroup taxa was obtained when the tree was rooted with all eight outgroups. However, a basal trichotomy was obtained involving: (1) a monophyletic group of all outgroups except *Pelomys*; (2) *Pelomys*; (3) Otomyini. —B. Strict consensus tree from two trees produced by maximum parsimony based on craniodental characters, showing phylogeny of Otomyini rodent taxa in relation to two outgroups (indicated with asterisks). Weighting ($\times 2$) was applied to upper and lower molar laminar numbers (characters 38 and 39). Bootstrap values > 50 ($n = 100$ replicates) are shown. Bremer decay indices > 1 are shown in parentheses. Length = 96; consistency index = 0.563; retention index = 0.720.



modal number of six or more laminae in upper M3 (character 38); lower incisors grooved (character 41) and rostral perforation large (character 44).

In subsequent analyses, only two outgroups (*Pelomys* and *Arvicanthis*) were considered (Fig. 2B). After several preliminary analyses, the following three taxon or character changes were found to provide significant improvements in the

consistency index (final consistency index = 0.563, retention index = 0.72, length = 96) and in the level of resolution of consensus trees obtained; (1) ingroup taxa with small sample sizes and a high level of polymorphism or missing data were excluded (*O. typus* from East Africa and *O. anchietae lacustris*); (2) character 31 (lower incisor grooving) was re-coded to allow for four as opposed to three character states (see

Appendix); and (3) laminar number characters (38 and 39) were weighted ($\times 2$), based on their evolutionary importance as underlined by excellently documented palaeontological data (Denys 1989, in press; S n gas 2001). The resulting strict consensus tree, from two trees obtained, is shown in Fig. 2B. In this case, the same basal nodes within Otomyini were retrieved that were retrieved by the more inclusive analysis with all outgroup and ingroup taxa. Bremer support is 3 for the monophyly of the Otomyini, 2 for the monophyly of the ‘mesic lineage’ and 2 for the basal position of *O. sloggetti* relative to other ‘mesic’ species. Additionally, Bremer values of 2 or 4 support some more terminal nodes in the tree, suggesting relatively primitive status for *O. denti*, *O. saundersiae*, *O. irroratus* and *O. tropicalis* within *Otomys*, and monophyly of the group comprising *O. occidentalis*, *O. laminatus* and *O. typus* (Fig. 2B). The above results largely reflect the preferential weighting ($\times 2$) of molar laminar number characters (38 and 39). When laminar number characters were weighted equally compared with all other characters, 32 trees were obtained, and the consensus tree was poorly resolved (results not shown).

While most of the more terminal nodes are not supported by bootstrap values $> 50\%$ or Bremer values > 1 , it is noteworthy that *O. tropicalis burtoni* and *O. angoniensis* are shown to be sister species to the exclusion of *O. angoniensis maximus* (usually taken as synonymous with *O. angoniensis*) and *O. tropicalis* (Fig. 2B). This apparent close relationship between *O. angoniensis* and *O. tropicalis burtoni* is partly due to the shared presence of a slit-shaped petrotympanic (stapedial) foramen in these two species; this foramen is clearly round in shape in *O. tropicalis* and in all other Otomyini, with the exception of *O. sloggetti* and *O. occidentalis* (Table 2). As specific separation of *maximus* and *angoniensis* was not upheld by mitochondrial sequence data (Maree 2002), it may be best to include the West African isolated population, *burtoni*, as a valid subspecies of *O. angoniensis*, and not as a subspecies of *O. tropicalis*, as previously suggested by Dieterlen & van der Straeten (1992).

The synonymy of *O. lacustris* and *O. barbouri* under *O. anchietae* (Musser & Carleton 1993), based only on shared possession of five laminae in the lower M1, is clearly not supported by the trees in Fig. 2. Among other character differences, a unique autapomorphy within the Otomyini appears to validate recognition of *O. lacustris* as a distinct species: the possession of a squamosal foramen (also present in the outgroups, *Mastomys*, *Acomys*, *Mystromys*, *Saccostomus*). However, in view of the small sample size of *O. lacustris* examined, wider sampling is necessary to validate the potential usefulness and constancy of this character as a diagnostic species trait. Apart from its much larger skull size (Taylor & Kumirai 2001), *O. anchietae* is distinguished from both *O. lacustris* and *O. barbouri* in having a thickened anterodorsal edge of the external

auditory meatus (character 22); more dorsally located parietal crests (character 7); and a conspicuous supraorbital process (character 5). In *O. barbouri*, the nasal bones are more acutely expanded than in *O. anchietae* and *O. lacustris*, approximating a 90° angle (character 2); the optic foramen is larger (character 27); and the foramen ovale failed to penetrate the wall of the pterygoid (character 14), a very rare condition among the rodents assessed in this study (in two out of three skulls examined for this character; the third skull was too damaged to make an assessment).

Allozyme analysis

Figure 3 shows the results of a MP analysis of 46 binary, unordered allozyme characters, using both outgroup (*Rhabdomys*)

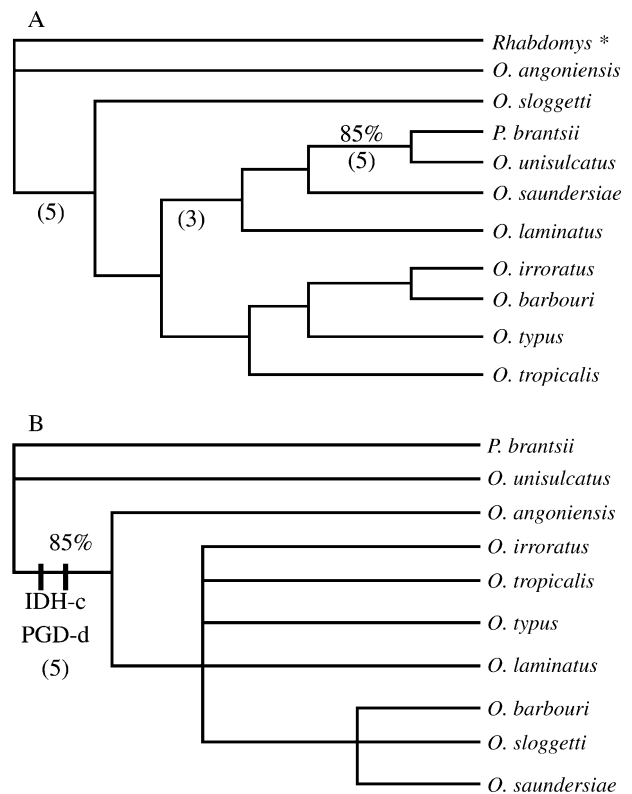


Fig. 3 A, B. Maximum parsimony analysis of allozyme data for 10 species of Otomyini rodents (14 polymorphic loci, 46 binary characters). —A. A single tree produced by maximum parsimony analysis of allozyme characters, with *Rhabdomys* as the outgroup (indicated by an asterisk). Bootstrap values > 50 ($n = 100$ replicates) are shown. Bremer decay indices > 1 are shown in parentheses. Length = 76; consistency index = 0.566; retention index = 0.175. —B. Strict consensus tree from three trees produced by maximum parsimony analysis with *Rhabdomys* excluded, and using midpoint rooting. Length = 62; consistency index = 0.592; retention index = 0.219. Bootstrap values > 50 ($n = 100$ replicates) are shown. Bremer decay indices > 1 are shown in parentheses.

Table 4 Nei's (1972) genetic distances showing the extent of genetic divergence between 10 species of Otomyini rodents and *Rhabdomys pumilio*, based on 17 genetic loci analysed by allozyme electrophoresis.

	Rha	Pbr	Osl	Oun	Osa	Oirr	Ola	Oty	Otr	Oan	Oba
<i>R. pumilio</i>	—										
<i>P. brantsii</i>	0.899	—									
<i>O. sloggetti</i>	0.734	0.671	—								
<i>O. unisulcatus</i>	0.798	0.330	0.542	—							
<i>O. saundersiae</i>	0.673	0.611	0.308	0.596	—						
<i>O. irroratus</i>	0.618	0.584	0.241	0.516	0.222	—					
<i>O. laminatus</i>	0.519	0.418	0.399	0.448	0.266	0.199	—				
<i>O. typus</i>	0.629	0.510	0.272	0.475	0.272	0.003	0.170	—			
<i>O. tropicalis</i>	0.562	0.526	0.267	0.442	0.308	0.026	0.148	0.005	—		
<i>O. angoniensis</i>	0.531	0.631	0.511	0.562	0.462	0.323	0.328	0.334	0.288	—	
<i>O. barbouri</i>	0.955	0.592	0.307	0.595	0.403	0.105	0.407	0.144	0.197	0.571	—

(Fig. 3A) and midpoint (Fig. 3B) rooting. Based on Nei's (1972) genetic distances calculated between all pairs of taxa (Table 4), it was found that *Rhabdomys* grouped within the ingroup particularly with *O. angoniensis*, rendering *Rhabdomys* unsuitable as an outgroup to the Otomyini. Whether the observed genetic similarity between *Rhabdomys* and *Otomys* spp. was due to random similarity resulting from 'saturation' of electrophoretically detectable allelic changes at genetic loci, or to some form of 'biochemical convergence' (*Rhabdomys pumilio* invariably coexists in identical mesic grassy habitats to *Otomys* spp.) could not be established. Nevertheless, it is clear from the midpoint-rooted tree (Fig. 3B) that the 'arid' Otomyini taxa represented (*P. brantsii* and *O. unisulcatus*) are at least genetically separate to the remaining *Otomys* species (bootstrap value 85%; Bremer decay index 5), a result which agrees strongly with the morphological data. Two synapomorphies support the monophyly of the 'mesic clade' in Fig. 3B: IDH allele c and PGD allele d. Unlike in previous allozyme (Taylor *et al.* 1989a,b) and immunoblot (Contrafatto *et al.* 1997) studies, *O. sloggetti* does not group with the arid species.

Phenetic (UPGMA; Fig. 4A) and neighbour-joining (Fig. 4B) analysis of Nei's (1972) genetic distances (Table 4) revealed similar results to the MP analysis, with slightly improved bootstrap support for certain relationships. The UPGMA tree (Fig. 4A) reinforces the hypothesis of sister-group status of *P. brantsii* and *O. unisulcatus*, but also reveals strong bootstrap support (85%) for a group comprising *O. irroratus* and two East African taxa, *O. typus* and *O. tropicalis*. This result is based on the very low genetic distances between these three species ($D = 0.003\text{--}0.026$), compared with other species ($D > 0.105$), and it contradicts the conclusions based on mitochondrial sequences of a sister-species relationship between *O. laminatus* and *O. irroratus* (Maree 2002). The outgroup-rooted neighbour-joining tree (Fig. 4B) again supports a close relationship between *O. irroratus* and East African *Otomys*. A weak bootstrap value (61%) suggests a monophyletic

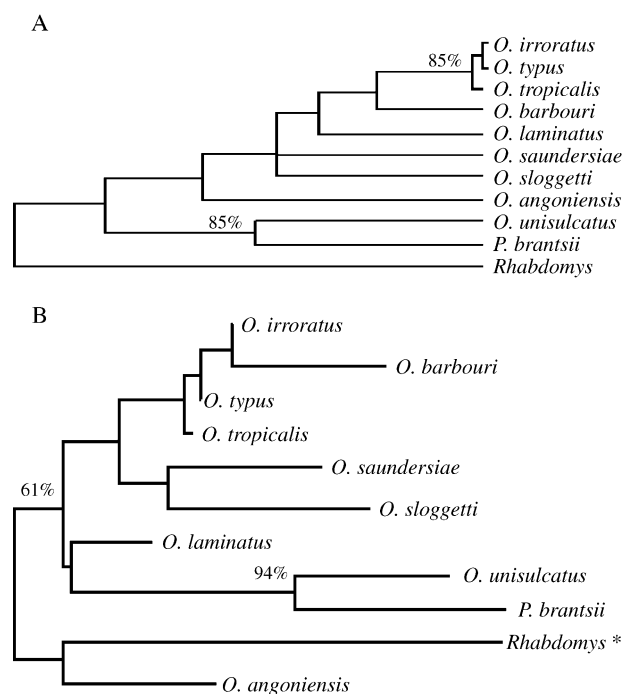


Fig. 4 A, B. UPGMA —A and neighbour-joining —B trees based on allele frequency data. Bootstrap values > 50 ($n = 1000$ replicates) are shown. The neighbour-joining tree was rooted using *Rhabdomys* as the outgroup.

group comprising all Otomyini except for *O. angoniensis*, which groups instead with the outgroup. A monophyletic clade of 'arid' (*Parotomys* species and *O. unisulcatus*) species is very strongly supported (94%).

Combined morphology and allozyme data

When craniodental and allozyme character matrices were combined, the MP analysis resulted in a single well-resolved tree having a relatively high consistency index of 0.662 (Fig. 5). The resulting tree supports the monophyly of the

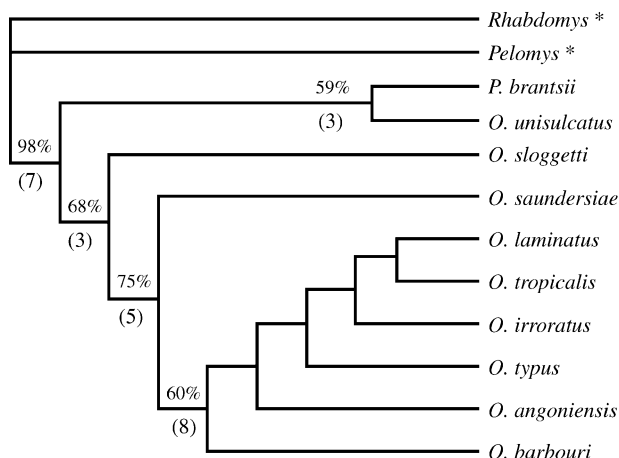


Fig. 5 A single tree produced from maximum parsimony analysis of combined morphological (character 38) and allozyme (character 46) characters, with *Pelomys* and *Rhabdomys* as outgroups (indicated with asterisks). Length = 130; consistency index = 0.662; retention index = 0.621. Bootstrap values > 50 ($n = 100$ replicates) are shown. Bremer decay indices > 1 are shown in parentheses. The percentage of total incongruence between the two data sets was 22.5%, according to the iMF metric of Mickevich & Farris (1981).

Otomyini (98% bootstrap support, and Bremer decay index of 7), monophyly of the ‘mesic lineage’ (bootstrap support 68%; Bremer value 3), sister-group status of *P. brantsii* and *O. unisulcatus* (i.e. an ‘arid clade’) (bootstrap support 59%; Bremer value 3), and the basal position of *O. sloggetti* (Bremer value 5) and *O. saundersiae* (Bremer value 8), relative to other mesic *Otomys* species. Less basal relationships are not supported by bootstrap values > 50% or Bremer values > 1.

General discussion and conclusions

Methodological discussion

Morphological trees involving all outgroup and ingroup taxa initially showed a high degree of homoplasy (consistency index = 0.41) and an unresolved polychotomy involving most of the ingroup taxa (Fig. 2A). Homoplasy was reduced (consistency index = 0.53) by ordering and weighting ($\times 2$) the upper and lower molar laminae characters, based on palaeontological evidence, and by removing all but two outgroups, as well as removing ingroup taxa and populations which suffered from sample sizes of two or fewer specimens, and/or five or more characters with missing values (Fig. 2B). Cladistic analyses of both allozyme and morphological matrices appeared to resolve basal, but not terminal, relationships among species of Otomyini. Despite low genetic distances between the chosen outgroup for the allozyme data, *Rhabdomys*, and certain ingroup taxa, congruence between allozyme and morphological data was high (22.5% incongruence,

based on the iMF metric of Mickevich & Farris 1981). For example, two allozyme synapomorphies and five craniodental synapomorphies support the existence of the same mesic *Otomys* clade (excluding *Parotomys*, *O. unisulcatus* and *O. sloggetti*). Thus, it is not surprising that MP analysis of the combined matrix resulted in a well-resolved tree with a higher consistency index (Fig. 5; 0.662) compared with either allozyme (Figs 3, 4) or craniodental (Fig. 2) data alone. Comparison of cladistic analyses of the present morphological (Fig. 2) and previous mitochondrial sequence data (Maree 2002) reveal different results with respect to statistical support at different phylogenetic levels; bootstrap and Bremer support are high for basal relationships and poor for terminal relationships in the case of morphological data, and poor for basal relationships but high for terminal relationships in the case of molecular data. This situation bodes well for the possibility that a future combined analysis of morphological and molecular data will provide a robust tree, which adequately resolves relationships spanning the entire time span of the evolutionary radiation of the Otomyini.

Higher-level relationships of Otomyini

Recent molecular and palaeontological studies suggest that laminate-toothed rats are best classified as a murid tribe, Otomyini, grouping close to the ‘arvicanthine division’ (Ducroz *et al.* 2001; Sénégas 2001; Denys 2003). The present morphological study suggests *Pelomys* rather than *Arvicanthus* as a sister to the Otomyini; this genus also belongs to the arvicanthine division, and has striking pelage and ecological similarities to typical species of *Otomys*. On palaeontological and morphological grounds, Pocock (1976) considered *Pelomys* to be the closest living relative of *Otomys* within the Muridae, while Denys *et al.* (1987) considered the extinct *Saidomys afarensis* to be the closest putative ancestor of the Otomyini. Although *Pelomys* is not presently located in South Africa, where Otomyini originated, it has been located in Pleistocene microfossil deposits in the KwaZulu-Natal province of South Africa (Avery 1991), as well as in 3.3 Myr deposits at Makapansgat in South Africa (Denys 1999), and may have once been widespread in South Africa. *Saidomys* has so far only been recorded from Plio-Pleistocene sites in East Africa (Denys 1999).

Generic relationships and taxonomic implications

A strict cladistic interpretation of the present data does not support the existence of two monophyletic genera in the tribe Otomyini, and species of *Parotomys* (whistling rats) should be transferred to the genus *Otomys*. Previous biochemical (Taylor *et al.* 1989a,b; Contrafatto *et al.* 1997) and molecular (Maree 2002) data provide additional support for the non-monophyly of the current genera with some molecular support for a sister-species relationship between *Parotomys* and

O. unisulcatus (Maree 2002), a relationship supported by the combined data analysis (Fig. 4). The finding that specimens of *tropicalis* invariably possess a round petrotympanic foramen provides strong evidence for including this isolated West African subspecies in *O. angoniensis* rather than in *O. tropicalis* as currently supposed (Fig. 2B).

Relationships within Otomyini and biogeographical scenarios

Figure 1 summarizes some previous intuitive and cladistic hypotheses of Otomyini phylogeny. Largely on the basis of the reduced number of laminae in the upper and lower molars, Bohmann (1952) considered *Parotomys* (endemic to southern Africa) to be ancestral to *Otomys*, hence inferring a southern African origin for the Otomyini. Based largely on observed trends showing progressive increases in the number of laminae in upper M3, he surmised that diversification of laminate-toothed rats occurred through fragmentation of the South African range of the immediate ancestor of *Otomys*, leading to three major lineages: *O. unisulcatus* in semiarid Karroid habitats, *O. angoniensis* in savannah habitats, and *O. sloggetti* in montane habitats (Fig. 1A). Subsequent radiation within these three basal lineages was speculated to be triggered by Pleistocene periods of cooling and warming. During the former regimes, temperate afro-montane forest-grassland mosaics would have occurred continuously from southern Africa to central, eastern and western Africa, leading to faunal radiations from south to north. For example, the first such Pleistocene event would have resulted in the expansion of the range of the ancestor of *O. sloggetti* throughout much of southern, central and East Africa. With the return of drier conditions, favourable temperate habitats would have been confined again to higher altitudes, effectively fragmenting the range of *O. sloggetti*, giving rise to the allopatric speciation of *O. denti* in East Africa and *O. saundersiae* in South Africa. A subsequent cooling event would have triggered the northerly dispersal of *O. saundersiae* to give rise to *O. typus* in East Africa.

The above scenario, based primarily on morphological data and an intuitive approach, has been refined to a great extent by recent palaeontological and palaeoclimatic data (Denys 1989, 1990, 1999, 2003). Denys (1990, 2003) described an evolutionary scenario invoking an initial event of dispersal from southwest Africa (*Parotomys*) to the former Northern Transvaal (*Otomys* cf. *gracilis*, the earliest known fossil of *Otomys*) between 5 and 3 Mya and then, during a cooler period between 2.5 and 1.6 Mya, a northwards dispersal (along the Rift corridor) of the *Otomys* genus into East Africa, with a subsequent late dispersal to Ethiopia (*O. typus*) in the middle Pleistocene (0.6 Mya). Denys (2003) suggested that *O. angoniensis* evolved in East Africa from a *O. petteri*-like ancestor, as the oldest known cf. *angoniensis* fossils (0.8–0.3 Mya) are known from East Africa, and are predated by fossils

of *O. petteri* and cf. *petteri* (1.5–2 Mya) which are also known only from East Africa. Denys suggested that cf. *gracilis*, an immediate South African ancestor of *O. saundersiae*, radiated northwards from South Africa, possibly associated with savannah-like vegetation associations which would have been favoured by a known period of significant cooling, around 2.4 Mya. The presence of *Otomys* sp. fossils at 2.3 Mya in Zaire suggests that the northwards migration of *Otomys* from South Africa first reached the western part of the Rift Valley, only reaching the eastern part of the Rift Valley in Tanzania at 1.8 Mya (as cf. *petteri*).

Denys' scenario contrasts with that of Bohmann in supposing a single dispersal event from South to East Africa, followed by further speciation events occurring independently in South and East Africa, rather than multiple south–north invasions associated with speciation events. Thus, for *angoniensis* (the only species of *Otomys* to occur both in East and South Africa), Bohmann suggested a southern African centre of origin, while Denys supported an East African origin.

The above scenarios generally maintain the monophyly of *Otomys*. In contrast, allozyme and immunoblot data for southern Africa support a 'diphyle hypothesis' involving an arid clade comprising *Parotomys* and *O. unisulcatus* (and sometimes *O. sloggetti*) and a mesic clade comprising all other *Otomys* (Taylor et al. 1989a,b; Contrafatto et al. 1997) (Fig. 5B). This hypothesis was originally suggested by Pocock (1976) on palaeontological grounds.

In contrast to the above hypotheses, Maree (2002) suggested, based on mitochondrial sequence data, a scenario which consisted of the almost simultaneous (in geological terms) origin of four species complexes.

The morphological and allozyme data presented here strongly support a mesic *Otomys* clade, with the arid-adapted *P. littedalei*, *P. brantsii* and *O. unisulcatus*, and the montane-adapted *O. sloggetti*, basal to this clade. This hypothesis is supported by the relative age (3–3.5 Myr) of described *O. cf. sloggetti* fossils from South Africa (Denys 1989); fossils of *Parotomys* and *O. unisulcatus* have not yet been described. Due to poor Bremer decay indices and bootstrap support values for the more terminal nodes, the present data are less convincing when it comes to testing hypotheses concerning the relationships between southern and East African taxa, i.e. did East African taxa undergo multiple speciation events following multiple invasions from southern African species (*sensu* Bohmann), or did speciation of all or most East African species occur *in situ* following a single dispersal event of *O. cf. gracilis* (*sensu* Denys)? Once again, combining the molecular data of Maree (2002) with the present data should provide a more robust tree, which resolves both basal and terminal relationships with high bootstrap support, allowing more critical tests of the competing biogeographical hypotheses discussed above.

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Appendix

Morphological characters and character states

1 NAS: nasal rectilinear (0); nasal inflated anteriorly (1). (Note: redundant with number 2, but used in place of number 2 in preliminary analyses with all outgroups.)

2 NAS_ANG: nasal rectilinear (0); nasal expanded but lateral borders not extending to full width of maxilla (nasal width < maxilla width); angle of expansion not very acute, ~146–170° (1); nasal expanded, lateral borders extending to width of maxilla (nasal width ~ maxilla width); angle of expansion acute, ~116–145° (2); nasal expanded, lateral borders extending to width of maxilla (nasal width ~ maxilla width); angle of expansion approaching a right angle, ~90–115° (3).

3 FR_SH: frontal crests converge anteriorly in interorbital region (0); frontal crests parallel anteriorly in interorbital region (1).

4 FR_PR: frontal crests absent (0); frontal crests present (1).

5 SO_PR: supraorbital process/extension absent (0); supraorbital process present (1). (Note: highly polymorphic; excluded in final analyses.)

6 PA_PR: parietal crests absent (0); parietal crests present (1). (Note: proved to be constant.)

7 PA_PO: parietal crest situated laterally on wall of braincase (0); parietal crest situated dorsally on roof of braincase (1).

8 PA_SH: parietal crest linear (0); parietal crest curved (1); parietal crest sinuous (2).

9 ZY_SH: zygomatic shape with anterior maximum curvature level with infraorbital foramen (0); zygomatic shape with posterior maximum curvature level with interorbital region (1).

10 IF_LEN: incisive foramen short, not reaching beyond anterior root of upper M1 (0); incisive foramen longer, penetrating onto palate between molar rows (1).

11 ID_SH: interdental width equal anterior and posterior (0); interdental width greater anteriorly than posteriorly (converging posteriorly) (1); interdental width greater posteriorly than anteriorly (diverging posteriorly) (2).

- 12** PP_PR: posterior palatine foramen apparent on unfolded palate (0); posterior palatine foramen partially obscured by folded palate (1).
- 13** PAL_SH: when folded, medial crest short, ending at level of second lamina of M3 (0); medial crest longer, extending just before end of, or beyond level of, palate (1).
- 14** FO_SHP: foramen ovale penetrating wall of pterygoid fossa (0); foramen ovale not penetrating wall of pterygoid fossa (1).
- 15** FO_SZV: foramen ovale small (0); foramen ovale large (1).
- 16** PC_SH: periotic capsule flat (0); periotic capsule inflated (1).
- 17** FM_SH: foramen magnum round (width = height) (0); foramen magnum flat (width > height) (1); foramen magnum triangular (height > width) (2).
- 18** SOC_SH: supraoccipital reaching anterior edge of occipital condyle (0); supraoccipital not reaching anterior edge of occipital condyle (1).
- 19** MP_SH: mastoid process oblique in lateral view (0); mastoid process vertical in lateral view (1).
- 20** MP_SH2: tip of mastoid process oblique in posterior view (0); tip of mastoid process vertical in posterior view (1). (Note: highly polymorphic and subjective; excluded from final analyses.)
- 21** MP_LEN: mastoid process short (0); mastoid process long (1).
- 22** EAM_SH: anteriodorsal edge of external auditory meatus normal (0); anteriodorsal edge of external auditory meatus thickened (1).
- 23** PT_PR: petrotympanic (= stapedial) foramen visible/present (0); petrotympanic foramen invisible/absent (1).
- 24** PT_SH: petrotympanic foramen, if present, round in shape (0); petrotympanic foramen, if present, slit-like in shape (1).
- 25** ALC_PR: anterior opening of alisphenoid canal absent (0); anterior opening of alisphenoid canal present (1). (Note: proved to be constant.)
- 26** OF_SH: optic foramen separated from sphenopalatine vacuity by horizontal bar (0); optic foramen separated from sphenopalatine vacuity by oblique bar (1); optic foramen separated from sphenopalatine vacuity by vertical bar (2). (Note: proved to be constant.)
- 27** OF_SH2: bar mentioned above thin (0); bar mentioned above thick (1).
- 28** OF_SZ: optic foramen small (0); optic foramen large (1).
- 29** SPH_SH: sphenopalatine foramen round (0); sphenopalatine foramen slit-like (1).
- 30** ZPL_SH: viewed laterally, zygomatic plate appears short, with infraorbital foramen closing dorsally (0); viewed laterally, zygomatic plate appears longer, with infraorbital foramen closing ventrally, allowing space for an expanded vertical fissure (1).
- 31** MTB_PR: masseteric tubercle absent (0); masseteric tubercle replaced by roughened surface (1); masseteric tubercle present (2).
- 32** SQU_SH: squamosal part of zygomatic arch thin, delicate (0); squamosal part of zygomatic arch thick, robust, with obvious trace of masseteric attachment surface (1).
- 33** MNB_SH: in ventral view angular part of mandible inflected medially (0); in ventral view angular part of mandible straight (1).
- 34** CAR_SH: crest connecting the coronoid and articular processes horizontal and linear (0); crest connecting the coronoid and articular processes curved and slightly oblique (1).
- 35** MK_PR: masseteric knob of mandible absent (0); masseteric knob of mandible present (1).
- 36** MMC_SH: foramen in line with mandibular masseteric crest (0); foramen anteroventral to mandibular masseteric crest (1).
- 37** MMC_NO: only one foramen associated with mandibular masseteric crest (0); two foramina, one larger than the other (1).
- 38** M3LAM: upper M3 with only two laminae (0); upper M3 with modal number of four laminae (1); upper M3 with modal number of five laminae (2); upper M3 with five or six laminae, modal number of six, but sometimes with five (3); upper M3 with modal number of six laminae, never with five (4); upper M3 with modal number of seven laminae (5); upper M3 with modal number of more than seven laminae (6).
- 39** M1LAM: lower M1 with three laminae (0); lower M1 with modal number of four laminae (1); lower M1 with modal number of five laminae (2); lower M1 with modal number of more than five laminae (3).
- 40** UIG: upper incisors ungrooved (0); upper incisor grooved (1).
- 41** LIG: lower incisors ungrooved or very faintly grooved (0); lower incisors grooved; one deep groove, with or without extra shallow groove (1); lower incisors grooved; two deep grooves (2). (Note: for some analyses, character state (1) was divided into two further states: (I) lower incisors grooved; one deep groove only, i.e. no shallow groove; (II) lower incisors grooved; one deep groove and one shallow groove.)
- 42** SQF_PR: squamosal foramen absent posterior to postglenoid foramen (0); squamosal foramen present posterior to postglenoid foramen (1). (Note: this is the 'mastoid strut' character of *Mastomys*; Meester *et al.* 1986.)
- 43** RP_PR: absence of rostral perforation posterolateral to incisive foramen (0); rostral perforation present (1).
- 44** RP-SZ: rostral perforation small (0); rostral perforation large (1).
- 45** PGL: postglenoid fossa small (0); postglenoid fossa large (1). (Note: proved to be constant.)