Phylogenetic relationships and mitochondrial DNA sequence evolution in the African rodent subfamily Otomyinae (Muridae)

by

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ABSTRACT

Instability characterizes the taxonomy of the African rodent subfamily Otomyinae. Two genera, *Otomys* and *Parotomys*, and 14 species are currently recognized, but the generic subdivision is equivocal. Between two and five cytotypes are delimited within *O. irroratus*, which illustrate a rapid rate of karyotypic evolution, and may reflect incipient speciation. In an attempt to resolve the group's phylogeny, the mitochondrial cyt *b* (complete) and 12S rRNA (802 bp) genes were analyzed using parsimony, distance and maximum likelihood methods. Alternative models of evolutionary change were compared to determine the best-fit model for each data set. Both genes failed to resolve deeper evolutionary associations, but the cyt *b* gene proved useful in resolving relationships among closely related taxa. Robust associations consistently retrieved include: (1) the *O. irroratus* cytotypes form a

monophyletic group with *O. laminatus* basal. (2) an exclusively East African clade comprises two representatives of *O. anchietae lacustris* (not sister taxa), *O. tropicalis* with *O. denti* basal; (3) *Otomys angoniensis* and *O. maximus* from southern Africa are monophyletic sister taxa with *O. typus jacksoni* and *O. typus* successively basal, making the East African Otomyinae non-monophyletic. (4) *Parotomys brantsii* and *P. littledalei* cluster within *Otomys*, but with no clear sister relationships. These results question some of the nomenclatural divisions in current use. First, the paraphyly of *Otomys* renders the recognition of two distinct genera unsupported. Second, the strongly supported monophyletic clustering of *O. angoniensis* and *O. maximus* coupled with the comparatively small genetic distance separating them, disputes the status of *O. maximus*. Third, the paraphyletic associations shown for representatives of *O. typus* and *O. anchietae* might imply that distinct evolutionary lineages are contained within these species. *Otomys irroratus* is phylogenetically distant from other *Otomys* species previously considered conspecifics. Moreover, a sister association between the B and C cytotypes to the exclusion of A1 and A2, corroborate the delimitation of two major cytogenetic groups within *O. irroratus*. The major clades form an unresolved polytomy suggesting that the Otomyinae experienced a rapid radiation approximately 5 Myr ago. A biogeographical scenario is presented in light of palaeo-climatic changes during the late Miocene and Plio-Pleistocene epochs.

Keywords: Muridae, Otomyinae, *Otomys*, *Parotomys*, vlei rat, whistling rat, systematics, phylogeny, biogeography, sequencing, cytochrome *b*, 12S rRNA, mitochondrial DNA.

For my husband Hannes, son Hannes, daughter Moya
and my parents Faan and Annetjie Lourens, with love and appreciation.

"Education is a companion which no misfortune can depress, no crime can destroy, no enemy can alienate, no despotism can enslave. At home a friend, abroad an introduction, in solitude a solace, and in society an ornament. It chastens vice, it guides virtue, it gives, at once, grace and government to genius. Without it, what is a man? A splendid slave, a reasoning savage."

Joseph Addison
The Spectator

O Lord my God, when I in awesome wonder
consider all the works thy hands hath made,
I see the stars; I hear the mighty thunder,
thy power throughout the universe displayed.

Then sings my soul, my Saviour God to thee:
how great thou art, how great thou art!
Then sings my soul, my Saviour God, to thee:
how great thou art, how great thou art!

When through the woods and forest glades I wander,
I hear birds sing sweetly in the trees,
when I look down from lofty mountain grandeur
and hear the brook and feel the gentle breeze.

Then sings my soul, my Saviour God to thee:
how great thou art, how great thou art!
Then sings my soul, my Saviour God, to thee:
how great thou art, how great thou art!

O STORE GUD – Swedish folk melody
Text: Stuart Hine 1949

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To my Lord and Saviour, who granted me the privilege to study nature in all its beauty and complexity. I am the way He made me, the way He saw fit, I received all my talents by His Grace, and He has the Perfect Plan. Through His strength I persevered.....Hebrews 13: 5 ""I will not, I will not, I will not in any way leave you, not forsake you, nor leave you helpless without support, Assuredly not!"

Finally thanks to the RATS who taught me a valuable lesson – in the words of Dozi: ".....daar is báie myle tussen doen en sê"

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CHAPTER 1

INTRODUCTION

GENERAL INTRODUCTION TO THE SUBFAMILY OTOMYINAE

PERSPECTIVE

A challenge often posed to systematists is how best to analyze diverse data sets that produce different estimates of evolutionary relationships among taxa. This challenge is particularly well demonstrated by the African rodent subfamily Otomyinae, Thomas, 1897 (Myomorpha, Muroidea, Muridae), a rodent assemblage that has been in taxonomic disarray for a century. The subfamilial status of the group is controversial (Carleton & Musser 1984) fueled by inconsistent taxonomic classifications and contradictory topologies resulting from morphological, biochemical and molecular studies (e.g. Bohmann 1952; Taylor *et al.* 1989a, b; Dieterlen & van der Straeten 1992; Meester *et al.* 1992; Contrafatto *et al.* 1994; Taylor & Kumirai in press; Taylor *et al.* unpubl., pers. comm.). The present study employed the DNA sequences of two mitochondrial markers (mtDNA), the complete cytochrome *b* (cyt *b*) gene and a large portion of the small ribosomal subunit (12S rRNA) to address some of these taxonomic uncertainties and to assess rates and patterns of mtDNA evolution in the Otomyinae. This strategy allows for a more robust assessment of evolutionary relationships and gene evolution (Springer *et al.* 1995) and together with existing data, should improve phylogenetic resolution of the Otomyinae. Most investigations to date were limited to taxa from southern Africa. The few exceptions that included extralimital taxa are predominantly morphologically based (e.g. Bohmann 1952; Taylor & Kumirai in press). The work detailed herein includes all but one of the 14 species currently recognized in the genera *Otomys* (Cuvier, 1824; Plate 1) and *Parotomys* (Thomas, 1918; Plate 2) (Musser & Carleton 1993; Taylor *et al.* 1993).

Plate 1: *Otomys irroratus* feeding at Cape Point National Park. © Tim Jackson.

Plate 2: *Parotomys brantsii* in succulent Karoo habitat. © Tim Jackson.

THE BIOLOGY OF THE OTOMYINAE

Appearance and Dentition

The Otomyinae are referred to as laminate-toothed rats, groove-toothed rats, vlei rats or swamp rats, and are endemic to sub-Saharan Africa (Rosevear 1969; De Graaff 1981; Skinner & Smithers 1990). They have a stocky build with large ears, blunt faces, shaggy pelage and short tails relative to their body length. Their skulls are heavily built with members of the genus *Otomys* characterized by a more slender cranium than *Parotomys* species (De Graaff 1981; Meester *et al.* 1986; Skinner & Smithers 1990). Their characteristically compactly laminated molar teeth and enlarged third upper molar (M^3) distinguish the Otomyinae from other subfamilies of the Muridae (Ellerman 1941; Misonne 1974; De Graaff 1981; Meester *et al.* 1986; Skinner & Smithers 1990; Musser & Carleton 1993). The dental characteristics of the group parallel those of Holarctic voles (subfamily Microtinae) in that their M^3 and first lower molar (M_1) show considerable variation in form as well as by the presence of an enlarged M^3 (Ellerman 1941; Denys *et al.* 1987). This resemblance most likely represents a case of convergent evolution among rodent groups with similar life-styles from the two hemispheres (but see Kingdon 1974).

Geographic Distribution and Habitat

The extant Otomyinae have a largely discontinuous distribution in southern, East and Central Africa that extends from the Eastern, Western and Northern Cape Provinces in South Africa through to Ethiopia in East Africa (Misonne 1974; De Graaff 1981; Carleton & Musser 1984). Two outlying populations occur in West Africa namely, *O. occidentalis* from Cameroon and Nigeria (Dieterlen & van der Straeten 1992) and *O. tropicalis burtoni* from Cameroon. Figure 1 depicts approximate geographical ranges for 13 of the 14 species recognized by Musser and Carleton (1993) and for *O. karoensis* (*sensu* Taylor *et al.* 1993), with whom *O. saundersiae* (Musser & Carleton 1993) has been synonymized, as well as for the five cytotypes recognized within *O. irroratus* (Contrafatto *et al.* 1992a, b, 1997).

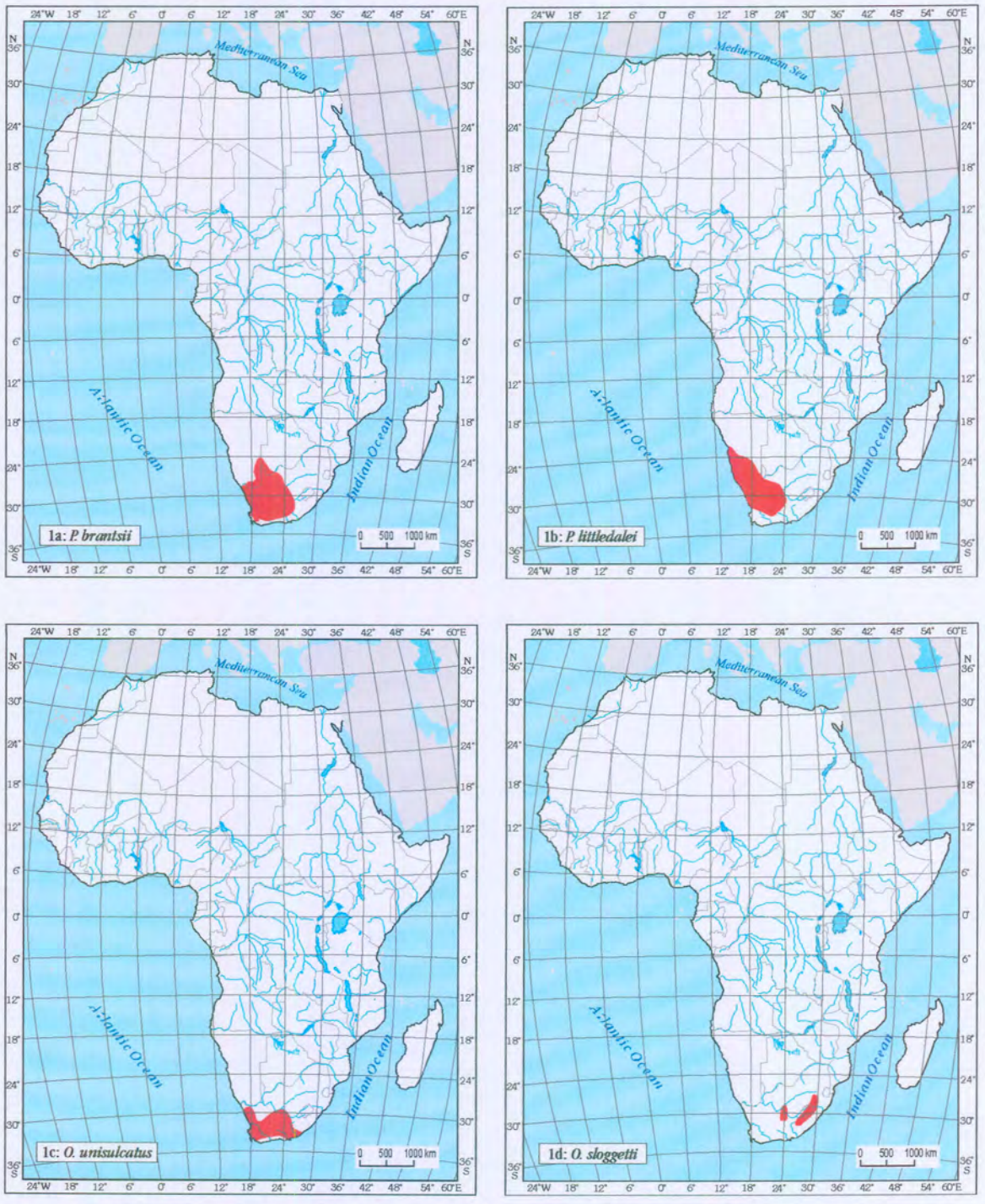
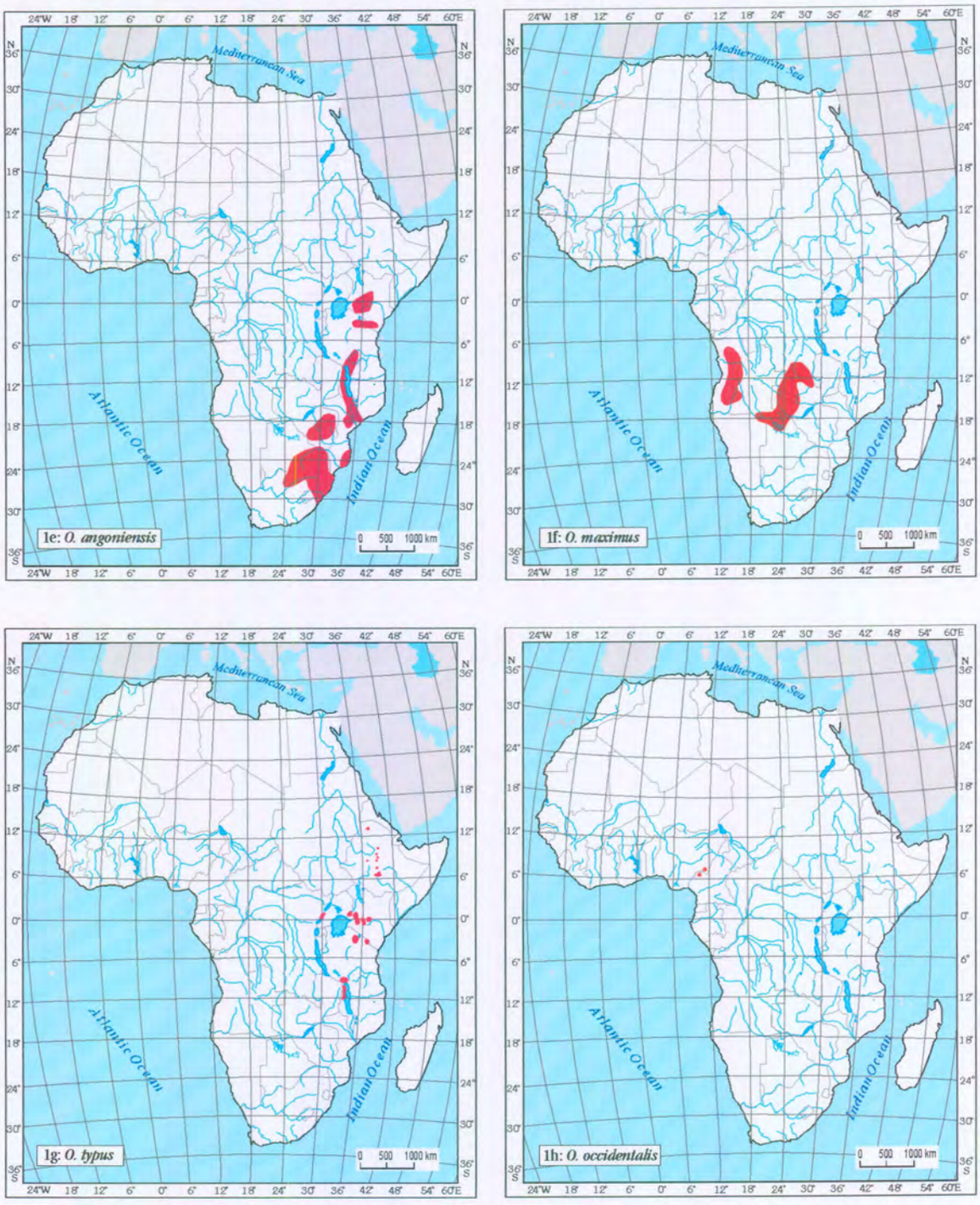


Figure 1: Distribution maps of the two species of *Parotomys* and 12 species of *Otomys* recognized in the African Otomyinae (Musser & Carleton 1993; Taylor et al. 1993; 1a - n). Also included are maps showing the distribution of the five cytotypes described within *O. irroratus* (*sensu* Contrafatto *et al.* 1992a, b, 1997) and the current political boundaries (1o, p). The maps are compiled from distribution data obtained from: Kingdon (1974), De Graaff (1981), Smithers (1983), Bronner & Meester (1988), Bronner *et al.* (1988), Skinner and Smithers (1990), Dieterlen and van der Straeten (1992), Lynch and Watson (1992), Taylor *et al.* (1993), Crawford-Cabral (1998), Taylor (1998, 2000), Taylor and Kumirai (unpubl.) and museum records from Taylor (unpubl.).



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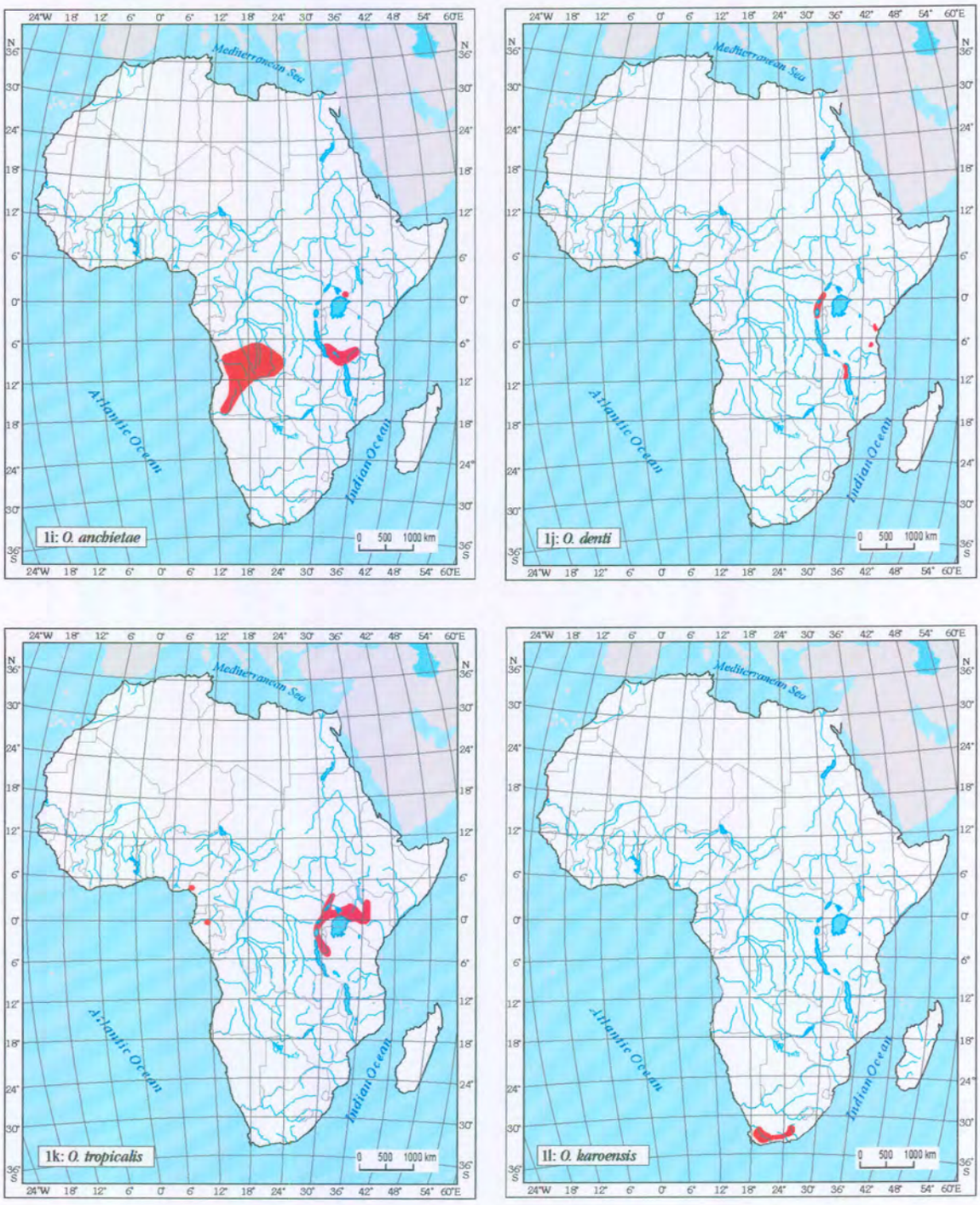

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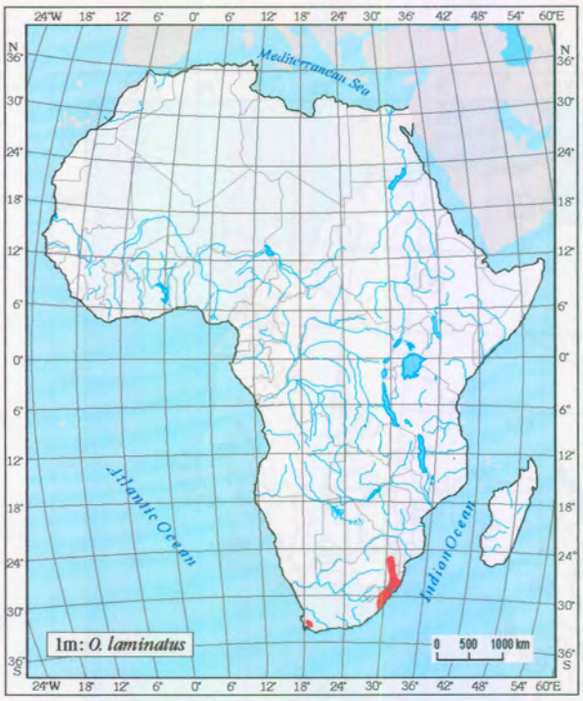

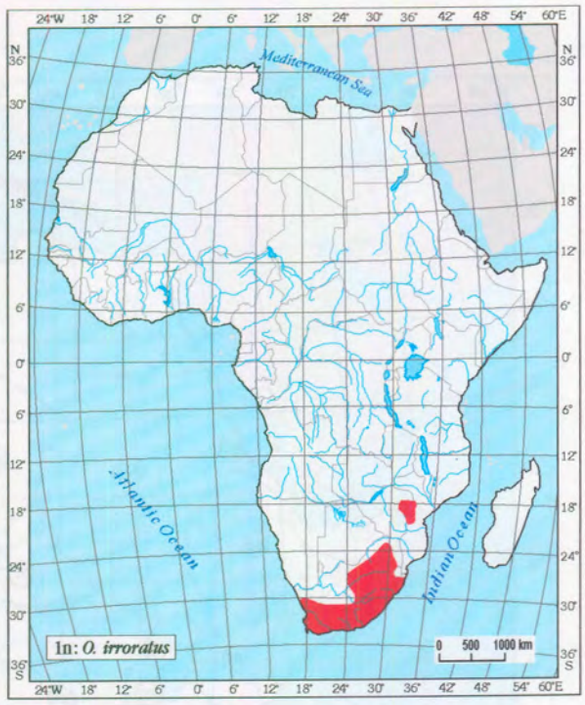

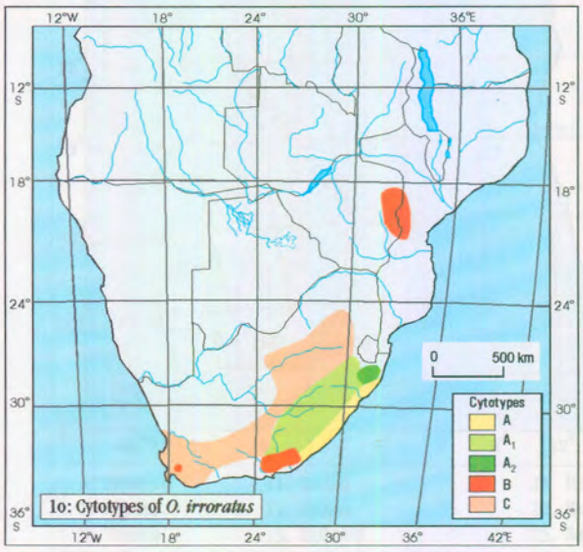

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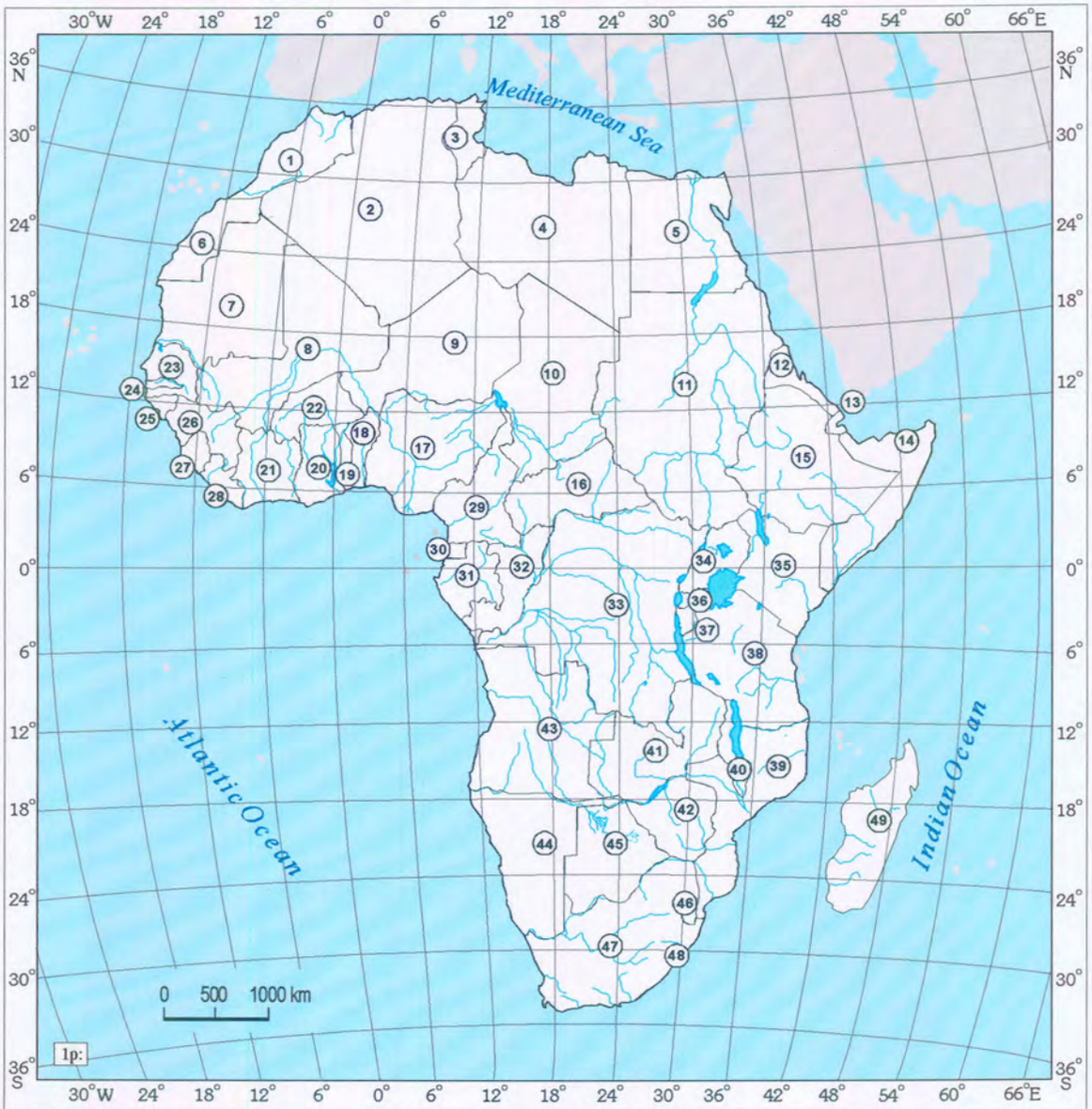

1. Morocco	11. Sudan	21. Ivory Coast	31. Gabon	40. Malawi
2. Algeria	12. Eritrea	22. Burkina Faso	32. Congo	41. Zambia
3. Tunisia	13. Djibouti	23. Senegal	33. Democratic Repu-	42. Zimbabwe
4. Libya	14. Somalia	24. Gambia	blic of Congo	43. Angola
5. Egypt	15. Ethiopia	25. Guinea Bissau	34. Uganda	44. Namibia
6. Western Sahara	16. Central African Republic	26. Guinea	35. Kenya	45. Botswana
7. Mauritania	17. Nigeria	27. Sierra Leone	36. Rwanda	46. Swaziland
8. Mali	18. Benin	28. Liberia	37. Burundi	47. South Africa
9. Niger	19. Togo	29. Cameroon	38. Tanzania	48. Lesotho
10. Chad	20. Ghana	30. Equatorial Guinea	39. Moçambique	49. Madagascar

Figure 1/continued.

Otomyinae are predominantly terrestrial but may become semi-aquatic in areas that are periodically flooded (Kingdon 1974; De Graaff 1981). They occupy habitats considered some of the wettest (mesophylic species) but also some of the driest (xerophylic species) vegetation types across 12 of the 18 major African phytogeographic regions or phytochoria (see White 1983). The subfamily is usually considered mesophylic as only three of 14 recognized species (*P. littledalei*, *P. brantsii* and *O. unisulcatus*) are from arid zones (Misonne 1974; Skinner & Smithers 1990). The genus *Parotomys* is endemic to the Karoo-Namib regional center of endemism and the Kalahari-Highveld transition zone with a marginal extension of their ranges into the Cape Region (Succulent and Nama Karoo and Fynbos biomes, Low & Rebelo 1996). In the northern areas they are associated with desert and shrubland vegetation and coastal hummocks on sand dunes. *Parotomys littledalei* also inhabits dry riverbeds, very arid gravel plains and desert flats, but are absent from the main dune system (Coetzee 1969; de Graaff 1981; Smithers 1983). In the Cape Region both species occur in areas with low mean annual rainfall (*P. brantsii*: < 300mm; *P. littledalei*: < 200mm, Jackson *et al.* in press) and are found on sandy substrates in transition areas between Cape shrubland (fynbos) and Karoo vegetation. *Otomys unisulcatus* primarily occurs in semi-arid areas of the Karoo-Namib and Cape Regions and the Kalahari-Highveld transition. In moister coastal areas of the latter two regions this species occurs in Cape shrubland, coastal bushland and thicket. In drier inland areas its range overlaps with that of *Parotomys* species in Fynbos/Karoo transition vegetation types, but it prefers habitats in the vicinity of rocky outcrops where they select for high plant cover and dense foliage (De Graaff 1981; Du Plessis 1989; Skinner & Smithers 1990). Additionally, the ranges of these three xerophylic species extend into the west-coast fog zone of southern Africa probably facilitating their extension into low rainfall areas (De Graaff 1981; Pillay *et al.* 1994).

The remaining 11 mesic adapted *Otomys* species (*O. angoniensis*, *O. irroratus*, *O. karoensis*, *O. laminatus*, *O. maximus*, *O. sloggetti*, *O. anchietae*, *O. denti*, *O. occidentalis*, *O. tropicalis* and *O. typus*) are widespread throughout southern, East and Central Africa and have a limited distribution in West Africa (Kingdon 1974; De Graaff 1981; Skinner & Smithers 1990). They occur in grassland elements of all but two of the 16 physiognomic vegetation types distributed across 10 phytochoria (Zambezian, Sudanian, Somalia-Masai, Cape, Karoo-Namib and Afromontane centers of endemism,

the Afroalpine region of floristic impoverishment, the Lake Victoria, Zanzibar Inhambane and Tongoland-Pondoland regional mosaics and the Kalahari-Highveld and Guinea-Congolian/Sudanian transitional zones, White 1983). The relevant vegetation types range in extent from regional (e.g. grasslands or savanna) to restricted distributions (e.g. woodland and scrub forest) and also include edaphic formations with distinct physiognomies (e.g. aquatic vegetation).

A variety of distinctive habitats are enclosed in the vegetation types occupied by mesophylic *Otomys spp.* (Kingdon 1974; Skinner & Smithers 1990). Certain species (e.g. *O. maximus*) are abundant in grass-covered areas in proximity to water such as wet marshes, vleis and swamps, riverine vegetation and flood plains (grass tussocks, reed beds and sedges) - (e.g. *O. anchietae* along rivers of the Congo drainage system, Crawford-Cabral 1998). Some species prefer moist grassland habitats further from water (e.g. *O. karoensis, O. typus*) and others occupy both these habitat types (e.g. *O. anchietae*, *O. angoniensis*, *O. irroratus*, *O. laminatus* and *O. sloggetti*). Since most *Otomys* species are predominantly grassland adapted, including primary and secondary types (Kingdon 1974; De Graaff 1981), the geological history of grasslands is an important variable that underpins *Otomys* distributions. White (1983) provides a comprehensive overview of the recent development of African grasslands, including the factors that shaped the present extent of primary and secondary types. These include climate (frost and altitude), edaphic factors and human mediated disturbances (fire, cultivation, grazing and deforestation).

The African distributions of the Otomyinae have a number of distinct characteristics. The five species formally recognized from East, Central and West Africa (*O. anchietae, O. denti, O occidentalis*, *O. tropicalis* and *O. typus*) have a disjunct distribution, usually restricted to higher altitude mountains of the region from above about 1500m to beyond 4000m (Allen 1939; Bohmann 1952; Kingdon 1974; Misonne 1974; Müller *et al.* 2001). This high altitude distribution is extended to southern African montane regions (*O. sloggetti* and *O. karoensis*, De Graaff 1981; Smithers 1983; Taylor *et al.* 1993; Mills & Hes 1997). These mountain habitats are of disparate ages and origins. They are included within some of the main centers of endemism in Africa such as the Drakensberg, the Cape Fold Mountains, the Equatorial Highlands, the Ethiopian Fractured Dome of which

the Simen and Bale Massifs form part, the Kivu Ridge in the Zaïre Basin, the ancient crystalline mountains of the Eastern Arc and the Cameroon Highlands (Moreau 1966; Misonne 1974; Kingdon 1990; De Jong & Congdon 1993; Lovett 1993; Wasser & Lovett 1993). These largely mesically adapted *Otomys* species are occasionally found in high altitude savannas e.g. *O. anchietae*, but most species have a preference for habitats unaffected by fire (e.g. *O. denti, O. tropicalis, O. typus*, Kingdon 1974). They therefore predominantly inhabit moist grassland mosaics (usually above 1500 - 2000m) in the Afromontane forest, Ericaceous and Afroalpine vegetation belts (see Hedberg 1951, 1955; White 1983; Kingdon 1990; Low & Rebelo 1996) found on the peaks and plateaus of these mountains (Kingdon 1974; Dieterlen & van der Straeten 1992), subject to local climate and latitude influences. For example, Afromontane forests descend to about 1200m in some tropical areas and to sea level in the Cape Fold Mountains (White 1983). Low altitude mesophylic species (*O. angoniensis, O. irroratus, O. laminatus* and *O. maximus*) typically inhabit grassland pockets interspersed among other low altitude (0 – 2500m) habitat types (Kingdon 1974; Misonne 1974; De Graaff 1981). More than one *Otomys* species may be found at a single locality, but these species are then usually associated with different altitudinal zones and/or ecological niches (Kingdon 1974; De Graaff 1981; Skinner & Smithers 1990). Detailed habitat descriptions and requirements for these mesically adapted species are discussed elsewhere (Bohmann 1952; Eisentraut 1963, 1973; Kingdon 1974; Misonne 1974; De Graaff 1981; Rowe-Rowe & Meester 1982; Smithers 1983; Meester *et al.* 1986; Ansell & Dowsett 1988; Skinner & Smithers 1990; Dieterlen & van der Straeten 1992; Lynch & Watson 1992; Taylor *et al.* 1993; Mills & Hess 1997; Crawford-Cabral 1998; Taylor 1998; Clausnitzer 1999).

Habits

The diversity of shelters used by the Otomyinae further emphasizes the different ecological niches they occupy. These range from the construction of dome nests, insulating stick nests, underground burrows and complex warren systems in the case of *Parotomys* (Shortridge 1934; De Graaff & Nel 1965; Smithers & Wilson 1979; De Graaff 1981; Vermeulen & Nel 1988; du Plessis *et al.* 1992; Coetzee & Jackson 1999; Jackson 2000). These strategies probably reflect different thermophysiological tolerances (Jackson *et al.* in press). Otomyines are strictly herbivorous, and do not drink water

except when exposed to severe droughts (Kingdon 1974; De Graaff 1981; Skinner & Smithers 1990). Additionally, the arid adapted species feed on succulent parts of xerophytic shrubs (du Plessis *et al.* 1991; Jackson 1998; Coetzee & Jackson 1999) and additional water may be derived from accumulated fog on plant surfaces (Louw & Seely 1982; Pillay *et al.* 1994). Furthermore, the renal structure of the Otomyinae conservatively reflects the relative aridity of their habitats (Pillay *et al.* 1994). *Otomys unisulcatus* and *P. brantsii* have limited urine-concentrating ability (Jackson *et al.* in press). A notable exception is *P. littledalei* that can produce urine with similar osmolarities to other herbivorous xerophylic rodents (Jackson *et al.* unpubl.). The refuge strategies, food selection and activity patterns used by these xerophylic species may contribute more to arid region survival than their physiology (Nel & Rautenbach 1974; du Plessis *et al.* 1992; Jackson 1998; Coetzee & Jackson 1999; Jackson 2000; Jackson *et al.* in press).

TAXONOMY OF THE OTOMYINAE

HIGHER ORDER RELATIONSHIPS

The Otomyinae was first described as a distinct taxonomic entity by Thomas (vernacular: 1896; scientific: 1897) and the monophyly of the group has not been questioned (Chevret *et al.* 1993). However, the phylogenetic affinities of this group to other major rodent lineages is obscured by the extremely derived dental features of fossil and extant taxa, which have lost most of the characters that can disclose their origins (Pocock 1976; Denys *et al.* 1987; Taylor *et al.* 1989a; Musser & Carleton 1993). The controversy mainly centers on the interpretation of how the otomyines developed the compactly laminate configuration of their molars and an enlarged third upper molar (M^3). Despite these "non-murid" dental features, the Otomyinae is presently ranked as a subfamily of the family Muridae, the largest mammalian family with over a quarter of all species (approximately 1 400), contained in 17 to 21 subfamilies (Steppan *et al.* 2001). The most comprehensive review of the Muridae published, lists 1 326 species in 281 genera and 17 subfamilies (Musser & Carleton 1993). The murid affiliation of the Otomyinae is in agreement with some taxonomic classifications that placed them in the Muridae together with other muroid subfamilies, including the Cricetinae (Thomas 1896; Ellerman 1941;

Ellerman *et al.* 1953; Corbet & Hill 1980; Swanepoel *et al.* 1980; Carleton & Musser 1984; Meester *et al.* 1986; Skinner & Smithers 1990). Other taxonomic treatments included them in the Muridae, but also recognized the family Cricetidae (Tullberg 1899; Miller & Gidley 1918; Simpson 1945; Schaub 1958; Misonne 1969). Furthermore, the group has variously been included as a subfamily in the family Cricetidae (Allen 1939; Davis 1965; Dieterlen 1969; Kingdon 1974; Misonne 1974; De Graaff 1981; Honacki *et al.* 1982; Smithers 1983; Lavocat & Parent 1985) as well as in the non-murid, non-cricetid family Nesomyidae (together with the Malagasy subfamily Nesomyinae; Lavocat 1973; Chaline *et al.* 1977; Lavocat 1978; Chaline & Mein 1979). It has also been placed in its own family, the Otomyidae (Roberts 1951). Palaeontological and molecular studies strongly suggest a close relationship for the Otomyinae to the Murinae (Pocock 1976; Chevret *et al.* 1993; Sénégas & Avery 1998; Verheyen *et al.* unpubl.), and recently, Michaux and Catzeflis (2000), Ducroz *et al.* (2001) and Michaux *et al.* (2001) proposed tribal rank for the group within the Murinae.

GENERIC, SPECIFIC AND SUBSPECIFIC TAXONOMY AND PHYLOGENETIC RELATIONSHIPS

The uncertainty regarding the higher order relationships of the Otomyinae is also reflected at the generic level and below. Previous taxonomic revisions are summarized in chronological order in Table 1. Additionally, De Graaff (1981), Meester *et al.* (1986) and Musser and Carleton (1993) provide more information on the subspecific taxonomy.

The latest taxonomic revision of the Otomyinae by Musser and Carleton (1993) proposes two genera, *Otomys* (Cuvier, vernacular 1823, scientific 1824; type: *Euryotis irrorata*, Brants, 1827), and *Parotomys* (Thomas, 1918; type: *Euryotis brantsii*, Smith, 1834) and 14 species. Two species, *P. brantsii* (Smith, 1834) and *P. littledalei* (Thomas, 1918) are recognized within *Parotomys*, which are endemic to southern Africa (Fig. 1a, b). Twelve species represent the genus *Otomys*, of which six are restricted to southern Africa: *O. laminatus* (Thomas & Schwann, 1905; Fig. 1m), *O. irroratus* (Brants, 1827; Fig. 1n), *O. maximus* (Roberts, 1924; Fig. 1f), *O. saundersiae* (Roberts, 1929), *O. sloggetti* (Thomas, 1902a; Fig. 1d) and *O. unisulcatus* (Cuvier, 1829; Fig. 1c).

Table 1: Chronological summary of taxonomic classifications of the subfamily Otomyinae at the genus, subgenus and species level. The original taxon descriptions and the most comprehensive revisions are included.

Original taxon description	Wroughton 1906		Dollman 1915		Thomas 1918 [c]			Broom 1937		
	Genus	Species (Section) [a]	Genus	Species (division, section, group) [b]	Genus	Subgenus	Species	Genus	Subgenus	Species
Euryotis brantsii (Smith, 1834)	*Otomys*	*brantsi* (I) (*sic*) [b]	--	--	*Parotomys*		*brantsii*	*Parotomys*		n.i.
Parotomys Liotomys littledalei (Thomas, 1918)	--	--	--	--	*Parotomys*	*Liotomys*	*littledalei*	*Parotomys*	*Liotomys*	n.i.
Otomys unisulcatus (Cuvier, 1829)	*Otomys*	*unisulcatus* (I)	n.i.	n.i.	*Myotomys*		*unisulcatus*	*Myotomys*		*unisulcatus*
Otomys unisulcatus grantii (Thomas, 1902a)	**	**	n.i.	n.i.	n.i.	n.i.	n.i.	*Myotomys*	*Metotomys*	*grantii*
Myotomys unisulcatus bergensis (Roberts, 1929)	--	--	--	--	--	--	--	*Myotomys*	*Metotomys*	*bergensis*
Otomys sloggetti (Thomas, 1902a)	*Otomys*	*sloggetti* (I)	n.i.	n.i.	n.i.	n.i.	n.i.	*Myotomys*	*Metotomys*	*sloggetti*
Otomys turneri (Wroughton, 1907)	--	--	n.i.	n.i.	n.i.	n.i.	n.i.	*Myotomys*	*Metotomys*	*turneri*
Myotomys sloggetti basuticus (Roberts, 1929)	--	--	--	--	--	--	--	*Myotomys*	*Metotomys*	*basuticus*
Myotomys sloggetti jeppei (Roberts, 1929)	--	--	--	--	--	--	--	*Myotomys*	*Metotomys*	*jeppei*
Otomys (Palaeotomys) gracilis (Broom, 1937)†	--	--	--	--	--	--	--	*Otomys*	*Palaeotomys*	*gracilis*†
Otomys robertsi (Hewitt, 1927)	--	--	--	--	--	--	--	*Otomys*	*Palaeotomys*	*robertsi*
Otomys denti (Thomas, 1906a)	**	**	*Otomys*	*denti* (A, 1)	n.i.	n.i.	n.i.	n.i	n.i	n.i
Otomys kempi (Dollman, 1915)	--	--	*Otomys*	*kempi* (A, 2)	n.i.	n.i.	n.i.	n.i	n.i	n.i
Otomys dartmouthi (Thomas, 1906a)	*Otomys*	*dartmouthi* (II)	*Otomys*	*dartmouthi* (C, I, 1)	n.i.	n.i.	n.i.	n.i	n.i	n.i
Otomys jacksoni (Thomas, 1891)	*Otomys*	*jacksoni* (II)	*Otomys*	*jacksoni* (C, I, 2)	n.i.	n.i.	n.i.	n.i	n.i	n.i
Oreomys typus (Heuglin, 1877)	*Otomys*	*typus* (II)	*Otomys*	*typus* (C, I, 3)	n.i.	n.i.	n.i.	n.i	n.i	n.i
Otomys typus fortior (Thomas, 1906b)	**	**	*Otomys*	*fortior* (C, II, 1)	n.i.	n.i.	n.i.	n.i	n.i	n.i
Otomys percivali (Dollman, 1915)	--	--	*Otomys*	*percivali* (C, I, 2)	n.i.	n.i.	n.i.	n.i	n.i	n.i
Otomys thomasi (Osgood, 1910)	--	--	*Otomys*	*thomasi* (B, I, 1)	n.i.	n.i.	n.i.	n.i	n.i	n.i
Euryotis irrorata (Brants, 1827)	*Otomys*	*irroratus* (II)	n.i.	n.i.	*Otomys*		*irroratus*	--	--	--
Otomys occidentalis (Dieterlen & van der Straeten, 1992)	--	--	--	--	--	--	--	n.i	n.i	n.i
Otomys irroratus orestes (Thomas, 1900)	**	**	*Otomys*	*orestes* (B, I, 2)	n.i.	n.i.	n.i.	n.i	n.i	n.i
Otomys irroratus tropicalis (Thomas, 1902a)	**	**	*Otomys*	*tropicalis* (B, II, 1)	n.i.	n.i.	n.i.	n.i	n.i	n.i
Otomys burtoni (Thomas, 1918)	--	--	--	--	*Otomys*		*burtoni*	n.i	n.i	n.i
Otomys rubeculus (Dollman, 1915)	--	--	*Otomys*	*rubeculus* (B, II, 3)	n.i.	n.i.	n.i.	n.i	n.i	n.i
Otomys irroratus cupreus (Wroughton, 1906)	**	**	**	**	**	**	**	n.i	**	**
Otomys irroratus angoniensis (Wroughton, 1906)	**	**	*Otomys*	*angoniensis* (B, II, 4)	n.i.	n.i.	n.i.	n.i	n.i	n.i
Otomys irroratus maximus (Roberts, 1924)	--	--	--	--	--	--	--	n.i	n.i	n.i
Otomys tugelensis (Roberts, 1929)	--	--	--	--	--	--	--	n.i	n.i	n.i
Otomys karoensis (Roberts, 1931)	--	--	--	--	--	--	--	*Otomys*		*karoensis*
Otomys tugelensis saundersiae (Roberts, 1929)	--	--	--	--	--	--	--	n.i.	n.i.	n.i.
Otomys divinorum (Thomas, 1910)	--	--	*Otomys*	*divinorum* (B, II, 3)	n.i.	n.i.	n.i.	n.i	n.i	n.i
Otomys irroratus nyikae (Wroughton, 1906)	**	**	*Otomys*	*nyikae* (B, II, 4)	n.i.	n.i.	n.i.	n.i	n.i	n.i
Otomys rowleyi (Thomas, 1918)	--	--	--	--	*Otomys*		*rowleyi*	n.i	n.i	n.i
Otomys mashona (Thomas, 1918)	--	--	--	--	*Otomys*		*mashona*	n.i	n.i	n.i
Otomys cuanzensis (Hill & Carter, 1937)	--	--	--	--	--	--	--	--	--	--
Otomys silberbaueri (Roberts, 1919)	--	--	--	--	--	--	--	*Otomys*	*Lamotomys*	*silberbaueri*
Otomys laminatus (Thomas & Schwann, 1905)	*Otomys*	*laminatus* (III)	n.i.	n.i.	*Otomys*	*Lamotomys*	*laminatus*	n.i.	n.i.	n.i
Euryotis anchietae (Bocage, 1882)	*Otomys*	*anchietae* (III)	n.i.	n.i.	*Otomys*	*Anchotomys*	*anchietae*	*Otomys*	*Anchotomys*	n.i

Note: Dashes indicate taxa not yet described at the time of a particular classification; ** - denote taxa not recognized as a valid species at the time of the revision; n.i. shows taxa not included in a particular treatment.
a - See Wroughton (1906) for a description of the sections and groups within *Otomys* based mainly on dental characters, and for subspecific taxonomy.
b - *sic* - spelt incorrectly
c - See Dollman (1915) for a description of the major divisions, sections and groups within the East African *Otomys* based on cranio-dental characters, and for subspecific taxonomy.
d - See Thomas (1918) for a description of new genera, subgenera, species and subspecies.
† - extinct taxon

Table 1 (continued)

Original taxon description	Allen 1939			Ellerman 1941 [e]				Roberts 1951 [f]		Bohmann 1952 [g]	
	Genus	Subgenus	Species	Genus	Subgenus	Species	Species group	Genus	Species	Genus	Species
Euryotis brantsii (Smith, 1834)	*Parotomys*	*Parotomys*	*brantsii*	*Parotomys*	*Parotomys*	*brantsii*	-	*Parotomys*	*brantsii*	*Otomys*	*brantsii*
Parotomys Liotomys littledalei (Thomas, 1918)	*Parotomys*	*Liotomys*	*littledalei*	*Parotomys*	*Liotomys*	*littledalei*	-	*Liotomys*	*littledalei*	*Otomys*	*littledalei*
Otomys unisulcatus (Cuvier, 1829)	*Myotomys*		*unisulcatus*	*Otomys*		*unisulcatus*	unisulcatus	*Myotomys*	*unisulcatus*	*Otomys*	*unisulcatus*
Otomys unisulcatus grantii (Thomas, 1902a)	**	**	**	**	**	**	**	**	**	**	**
Myotomys unisulcatus bergensis (Roberts, 1929)	**	**	**	**	**	**	**	**	**	**	**
Otomys sloggetti (Thomas, 1902a)	*Myotomys*		*sloggetti*	*Otomys*		*sloggetti*	unisulcatus	*Myotomys*	*sloggetti*	*Otomys*	*slogetti* (*sic*)[b]
Otomys turneri (Wroughton, 1907)	*Myotomys*		*turneri*	*Otomys*		*turneri*	turneri	**	**	**	**
Myotomys sloggetti basuticus (Roberts, 1929)	**	**	**	**	**	**	**	**	**	**	**
Myotomys sloggetti jeppei (Roberts, 1929)	**	**	**	**	**	**	**	**	**	**	**
Otomys (*Palaeotomys*) *gracilis* (Broom, 1937)[†]	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Otomys robertsi (Hewitt, 1927)	*Otomys*	*Otomys*	*robertsi*	*Otomys*		*robertsi*	not allocated	**	**	**	**
Otomys denti (Thomas, 1906a)	*Otomys*	*Otomys*	*denti*	*Otomys*		*denti*	irroratus	n.i.	n.i.	*Otomys*	*denti*
Otomys kempi (Dollman, 1915)	*Otomys*	*Otomys*	*kempi*	*Otomys*		*kempi*	irroratus	n.i.	n.i.	**	**
Otomys dartmouthi (Thomas, 1906a)	*Otomys*	*Otomys*	*dartmouthi*	*Otomys*		*dartmouthi*	typus	n.i.	n.i.	**	**
Otomys jacksoni (Thomas, 1891)	*Otomys*	*Otomys*	*jacksoni*	*Otomys*		*jacksoni*	typus	n.i.	n.i.	**	**
Oreomys typus (Heuglin, 1877)	*Otomys*	*Otomys*	*typus*	*Otomys*		*typus*	typus	n.i.	n.i.	*Otomys*	*typus*
Otomys typus fortior (Thomas, 1906b)	**	**	**	**	**	**	**	n.i.	n.i.	**	**
Otomys percivali (Dollman, 1915)	*Otomys*	*Otomys*	*percivali*	*Otomys*		*percivali*	typus	n.i.	n.i.	**	**
Otomys thomasi (Osgood, 1910)	*Otomys*	*Otomys*	*thomasi*	*Otomys*		*thomasi*	irroratus	n.i.	n.i.	**	**
Euryotis irrorata (Brants, 1827)	*Otomys*	*Otomys*	*irroratus*	*Otomys*		*irroratus*	irroratus	*Otomys*	*irroratus*	*Otomys*	*irroratus*
Otomys occidentalis (Dieterlen & van der Straeten, 1992)	--	--	--	--	--	--	--	--	--	--	--
Otomys irroratus orestes (Thomas, 1900)	*Otomys*	*Otomys*	*orestes*	*Otomys*		*orestes*	irroratus	n.i.	n.i.	**	**
Otomys irroratus tropicalis (Thomas, 1902a)	*Otomys*	*Otomys*	*tropicalis*	*Otomys*		*tropicalis*	irroratus	n.i.	n.i.	**	**
Otomys burtoni (Thomas, 1918)	*Otomys*	*Otomys*	*burtoni*	*Otomys*		*burtoni*	irroratus	n.i.	n.i.	**	**
Otomys rubeculus (Dollman, 1915)	*Otomys*	*Otomys*	*rubeculus*	*Otomys*		*rubeculus*	irroratus	n.i.	n.i.	**	**
Otomys irroratus cupreus (Wroughton, 1906)	**	**	**	**	**	**	**	*Otomys*	*cupreus*	**	**
Otomys irroratus angoniensis (Wroughton, 1906)	*Otomys*	*Otomys*	*angoniensis*	*Otomys*		*angoniensis*	irroratus	**	**	**	**
Otomys irroratus maximus (Roberts, 1924)	**	**	**	**	**	**	**	*Otomys*	*maximus*	**	**
Otomys tugelensis (Roberts, 1929)	*Otomys*	*Otomys*	*tugelensis*	*Otomys*		*tugelensis*	not allocated	*Otomys*	*tugelensis*	*Otomys*	*tugelensis*
Otomys karoensis (Roberts, 1931)	*Otomys*	*Otomys*	*karoensis*	*Otomys*		*karoensis*	not allocated	**	**	*Otomys*	*karoensis*
Otomys tugelensis saundersiae (Roberts, 1929)	**	**	**	**	**	**	**	*Otomys*	*saundersiae*	**	**
Otomys divinorum (Thomas, 1910)	*Otomys*	*Otomys*	*divinorum*	*Otomys*		*divinorum*	irroratus	n.i.	n.i.	**	**
Otomys irroratus nyikae (Wroughton, 1906)	*Otomys*	*Otomys*	*nyikae*	*Otomys*		*nyikae*	irroratus	n.i.	n.i.	**	**
Otomys rowleyi (Thomas, 1918)	*Otomys*	*Otomys*	*rowleyi*	*Otomys*		*rowleyi*	irroratus	*Otomys*	*rowleyi*	**	**
Otomys mashona (Thomas, 1918)	*Otomys*	*Otomys*	*mashona*	*Otomys*		*mashona*	irroratus	*Otomys*	*mashona*	**	**
Otomys cuanzensis (Hill & Carter, 1937)	*Otomys*	*Otomys*	*cuanzensis*	*Otomys*		**	**	n.i.	n.i.	**	**
Otomys silberbaueri (Roberts, 1919)	*Otomys*	*Otomys*	*silberbaueri*	*Otomys*		*silberbaueri*	not allocated	*Lamotomys*	*silberbaueri*		
Otomys laminatus (Thomas & Schwann, 1905)	*Otomys*	*Lamotomys*	*laminatus*	*Otomys*		*laminatus*	laminatus	*Lamotomys*	*laminatus*	*Otomys*	*laminatus*
Euryotis anchietae (Bocage, 1882)	*Otomys*	*Anchotomys*	*anchietae*	*Otomys*		*anchietae*	anchietae	n.i.	n.i.	*Otomys*	*anchietae*

e - For a review of genera, subgenera, species and species groups see Ellerman (1941).
f - See Roberts (1951) for detailed accounts of the five genera and keys to the forms recognized within each genus.
g - See Bohmann (1952) for a particularized description of the 11 species groups or "Rassenkreisen" and 64 forms recognized within a single genus, *Otomys*.

Table 1 (continued)

Original taxon description	Ellerman *et al.* 1953			Misonne 1974		De Graaff 1981		Smithers 1983		Meester *et al.* 1986	
	Genus	Subgenus	Species	Genera	Species	Genera	Species	Genera	Species	Genera	Species
Euryotis Brantsii (Smith, 1834)	*Parotomys*	*Parotomys*	*brantsi (sic)* [b]	*Parotomys*	*brantsii*	*Parotomys*	*brantsii*	*Parotomys*	*brantsii*	*Parotomys*	*brantsii*
Parotomys Liotomys littledalei (Thomas, 1918)	*Parotomys*	*Liotomys*	*littledalei*	*Parotomys*	*littledalei*	*Parotomys*	*littledalei*	*Parotomys*	*littledalei*	*Parotomys*	*littledalei*
Otomys unisulcatus (Cuvier, 1829)	*Otomys*	**	*unisulcatus*	*Otomys*	*unisulcatus*	*Otomys*	*unisulcatus*	*Otomys*	*unisulcatus*	*Otomys*	*unisulcatus*
Otomys unisulcatus grantii (Thomas, 1902a)	**	**	**	**	**	**	**	**	**	**	**
Myotomys unisulcatus bergensis (Roberts, 1929)	**	**	**	**	**	**	**	**	**	**	**
Otomys sloggetti (Thomas, 1902a)	*Otomys*		*sloggetti*	*Otomys*	*sloggetti*	*Otomys*	*sloggetti*	*Otomys*	*sloggetti*	*Otomys*	*sloggetti*
Otomys turneri (Wroughton, 1907)	**	**	**	**	**	**	**	**	**	**	**
Myotomys sloggetti basuticus (Roberts, 1929)	**	**	**	**	**	**	**	**	**	**	**
Myotomys sloggetti jeppei (Roberts, 1929)	**	**	**	**	**	**	**	**	**	**	**
Otomys (Palaeotomys) gracilis (Broom, 1937) [†]	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Otomys robertsi (Hewitt, 1927)	**	**	**	**	**	**	**	**	**	**	**
Otomys denti (Thomas, 1906a)	n.i.	n.i.	n.i.	*Otomys*	*denti*	*Otomys*	*denti*	n.i.	n.i.	n.i.	n.i.
Otomys kempi (Dollman, 1915)	n.i.	n.i.	n.i.	**	**	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Otomys dartmouthi (Thomas, 1906a)	n.i.	n.i.	n.i.	**	**	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Otomys jacksoni (Thomas, 1891)	n.i.	n.i.	n.i.	**	**	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Oreomys typus (Heuglin, 1877)	n.i.	n.i.	n.i.	*Otomys*	*typus*	*Otomys*	*typus*	n.i.	n.i.	n.i.	n.i.
Otomys typus fortior (Thomas, 1906b)	n.i.	n.i.	n.i.	**	**	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Otomys percivali (Dollman, 1915)	n.i.	n.i.	n.i.	**	**	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Otomys thomasi (Osgood,1910)	n.i.	n.i.	n.i.	**	**	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Euryotis irrorata (Brants, 1827)	*Otomys*		*irroratus*	*Otomys*	*irroratus*	*Otomys*	*irroratus*	*Otomys*	*irroratus*	*Otomys*	*irroratus*
Otomys occidentalis (Dieterlen & van der Straeten, 1992)	--	--	--	--	--	--	--	--	--	--	--
Otomys irroratus orestes (Thomas, 1900)	n.i.	n.i.	n.i.	**	**	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Otomys irroratus tropicalis (Thomas, 1902a)	n.i.	n.i.	n.i.	*Otomys*	*tropicalis*	*Otomys*	*tropicalis*	n.i.	n.i.	n.i.	n.i.
Otomys burtoni (Thomas, 1918)	n.i.	n.i.	n.i.	**	**	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Otomys rubeculus (Dollman, 1915)	n.i.	n.i.	n.i.	**	**	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Otomys irroratus cupreus (Wroughton, 1906)	**	**	**	**	**	**	**	**	**	**	**
Otomys irroratus angoniensis (Wroughton, 1906)	**	**	**	*Otomys*	*angoniensis*	*Otomys*	*angoniensis*	*Otomys*	*angoniensis*	*Otomys*	*angoniensis*
Otomys irroratus maximus (Roberts, 1924)	**	**	**	**	**	**	**	*Otomys*	*maximus*	**	**
Otomys tugelensis (Roberts, 1929)	**	**	**	**	**	**	**	**	**	**	**
Otomys karoensis (Roberts, 1931)	**	**	**	**	**	**	**	**	**	**	**
Otomys tugelensis saundersiae (Roberts, 1929)	*Otomys*		*saundersiae*	*Otomys*	*saundersae (sic)* [b]	*Otomys*	*saundersae (sic)* [b]	*Otomys*	*saundersiae*	*Otomys*	*saundersiae*
Otomys divinorum (Thomas, 1910)	**	**	**	**	**	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Otomys irroratus nyikae (Wroughton, 1906)	**	**	**	**	**	**	**	**	**	**	**
Otomys rowleyi (Thomas, 1918)	**	**	**	**	**	**	**	**	**	**	**
Otomys mashona (Thomas, 1918)	**	**	**	**	**	**	**	**	**	**	**
Otomys cuanzensis (Hill & Carter, 1937)	**	**	**	**	**	**	**	**	**	**	**
Otomys silberbaueri (Roberts, 1919)	**	**	**	**	**	**	**	**	**	**	**
Otomys laminatus (Thomas & Schwann, 1905)	*Otomys*		*laminatus*	*Otomys*	*laminatus*	*Otomys*	*laminatus*	*Otomys*	*laminatus*	*Otomys*	*laminatus*
Euryotis anchietae (Bocage, 1882)	*Otomys*		*anchietai (sic)* [b]	*Otomys*	*anchietae*	*Otomys*	*anchietae*	n.i.	n.i.	n.i.	n.i.

Table 1 (continued)

Original taxon description	Skinner & Smithers 1990		Musser & Carleton 1993 [h]	
	Genera	Species	Genera	Species
Euryotis brantsii (Smith, 1834)	*Parotomys*	*brantsii*	*Parotomys*	*brantsii*
Parotomys Liotomys littledalei (Thomas, 1918)	*Parotomys*	*littledalei*	*Parotomys*	*littledalei*
Otomys unisulcatus (Cuvier, 1829)	*Otomys*	unisulcatus	*Otomys*	*unisulcatus*
Otomys unisulcatus grantii (Thomas, 1902a)	**	**	**	**
Myotomys unisulcatus bergensis (Roberts, 1929)	**	**	**	**
Otomys sloggetti (Thomas, 1902a)	*Otomys*	*sloggetti*	*Otomys*	*sloggetti*
Otomys turneri (Wroughton, 1907)	**	**	**	**
Myotomys sloggetti basuticus (Roberts, 1929)	**	**	**	**
Myotomys sloggetti jeppei (Roberts, 1929)	**	**	**	**
Otomys (Palaeotomys) gracilis (Broom, 1937) [†]	n.i.	n.i.	n.i.	n.i.
Otomys robertsi (Hewitt, 1927)	**	**	**	**
Otomys denti (Thomas, 1906a)	n.i.	n.i.	*Otomys*	*denti*
Otomys kempi (Dollman, 1915)	n.i.	n.i.	**	**
Otomys dartmouthi (Thomas, 1906a)	n.i.	n.i.	**	**
Otomys jacksoni (Thomas, 1891)	n.i.	n.i.	**	**
Oreomys typus (Heuglin, 1877)	n.i.	n.i.	*Otomys*	*typus*
Otomys typus fortior (Thomas, 1906b)	n.i.	n.i.	**	**
Otomys percivali (Dollman, 1915)	n.i.	n.i.	**	**
Otomys thomasi (Osgood, 1910)	n.i.	n.i.	**	**
Euryotis irrorata (Brants, 1827)	*Otomys*	*irroratus*	*Otomys*	*irroratus*
Otomys occidentalis (Dieterlen & van der Straeten, 1992)	n.i.	n.i.	*Otomys*	*occidentalis*
Otomys irroratus orestes (Thomas, 1900)	n.i.	n.i.	**	**
Otomys irroratus tropicalis (Thomas, 1902a)	n.i.	n.i.	*Otomys*	*tropicalis*
Otomys burtoni (Thomas, 1918)	n.i.	n.i.	**	**
Otomys rubeculus (Dollman, 1915)	n.i.	n.i.	**	**
Otomys irroratus cupreus (Wroughton, 1906)	**	**	**	**
Otomys irroratus angoniensis (Wroughton, 1906)	*Otomys*	*angoniensis*	*Otomys*	*angoniensis*
Otomys irroratus maximus (Roberts, 1924)	**	**	*Otomys*	*maximus*
Otomys tugelensis (Roberts, 1929)	**	**	**	**
Otomys karoensis (Roberts, 1931)	**	**	**	**
Otomys tugelensis saundersiae (Roberts, 1929)	*Otomys*	*saundersiae*	*Otomys*	*saundersiae*
Otomys divinorum (Thomas, 1910)	n.i.	n.i.	**	**
Otomys irroratus nyikae (Wroughton, 1906)	**	**	**	**
Otomys rowleyi (Thomas, 1918)	**	**	**	**
Otomys mashona (Thomas, 1918)	**	**	**	**
Otomys cuanzensis (Hill & Carter, 1937)	**	**	**	**
Otomys silberbaueri (Roberts, 1919)	**	**	**	**
Otomys laminatus (Thomas & Schwann, 1905)	*Otomys*	*laminatus*	*Otomys*	*laminatus*
Euryotis anchietae (Bocage, 1882)	n.i.	n.i.	*Otomys*	*anchietae*

h - See Musser and Carleton (1993) for detailed information on the subspecies currently recognized under good species and for comments on prevalent taxonomic uncertainties at the subfamilial, generic, specific and intraspecific levels.

One species, *O. angoniensis* (Wroughton, 1906; Fig. 1e) has a range that extends from southern through to East Africa. The remaining five species are extralimital to southern Africa and include *O. anchietae* (Bocage, 1882; Angola, Tanzania, Malawi and Kenya; Fig. 1i), *O. denti* (Thomas, 1906a; Uganda, Tanzania, Malawi and Zambia; Fig. 1j), *O. tropicalis* (Thomas, 1902a; Democratic Republic of Congo, Rwanda, Burundi, Uganda, Kenya and the Cameroon; Fig. 1k), *O. typus* (Heuglin, 1877; Ethiopia, Kenya, Malawi, Tanzania, Sudan, Uganda and Zambia; Fig. 1g) and *O. occidentalis* (Dieterlen & van der Straeten, 1992; Nigeria and the Cameroon; Fig. 1h).

This classification (Musser and Carleton 1993) is adopted in the present study, although it is acknowledged that a number of species and subspecies contained therein are considered to be of uncertain taxonomic status by the authors, and that some taxonomic changes have since been suggested. *Otomys karoensis* (Roberts, 1931; Fig. 1l), previously included in *O. saundersiae* (Roberts, 1929) on morphological grounds (Roberts 1951; Ellerman *et al.* 1953; Misonne 1974; Meester *et al.* 1986), has been re-elevated to specific rank (Taylor *et al.* 1993; Table1). *Otomys saundersiae* is no longer recognized as a valid species since the populations that comprised the species, which were not identified as being part of *O. karoensis*, were synonymized with *O. irroratus*. Moreover, Dieterlen and van der Straeten (1992) and Taylor and Kumirai (in press) suggest that *O. anchietae barbouri* and *O. a. lacustris* are full species, and not subspecies of *O. a. anchietae* (*sensu* Bocage 1882), as was proposed in Musser and Carleton (1993). However, these species are not formally described. Where applicable, reference will be made to the most recent suggested taxonomy and, for purposes of clarity, concurrently to the status accorded in Musser and Carleton (1993).

The taxonomic ambiguities within the Otomyinae are caused by conserved morphological and phenotypic features (Kingdon 1974; De Graaff 1981; Smithers 1983; Musser & Carleton 1993). Their taxonomy (Table 1) has primarily been based on keys relying on dental and cranio-morphological features that are considered to be at best subtle, variable and homoplastic (see Wroughton 1906; Dollman 1915; Allen 1939; Ellerman 1941; Roberts 1951; Bohmann 1952; Ellerman *et al.* 1953; Misonne 1974; Meester *et al.* 1986; Musser & Carleton 1993). Several other taxonomic studies of murid rodents showed that their dental and cranial features are characterized by convergence, and are seemingly

unreliable characters for phylogenetic inference (Catzeflis *et al.* 1992; Chevret *et al.* 1993; Robinson *et al.* 1997; Michaux & Catzeflis 2000; Ducroz *et al.* 2001; Huchson *et al.* 2001; Michaux *et al.* 2001; Steppan *et al.* 2001). Such convergence is most likely the result of independent adaptation by diverse murid groups to similar environments.

It is not surprising, therefore, that the outcomes of numerous investigations undertaken at the generic, specific and intraspecific level in the Otomyinae are inconsistent with each other, and with those based on classical morphological criteria. These include cytogenetic studies (Robinson & Elder 1987; Contrafatto *et al.* 1992a, b; Meester *et al.* 1992; Rambau *et al.* 1997; Rambau & Robinson 1999; Rambau *et al.* 2001), allozymes (Taylor *et al.* 1989a, b; van Dyk *et al.* 1991; Taylor *et al.* 1992; Lavrenchenko *et al.* 1997; Govender 1999; Taylor *et al.* pers. comm.), restriction fragment length polymorphisms, or RFLPs (Lamb *et al.* 1996), immuno-electrotransfer analysis (Contrafatto *et al.* 1994, 1997) and mitochondrial DNA sequence analysis (Ducroz *et al.* 2001). The results of investigations dealing with comparative renal morphology (Pillay *et al.* 1994), sperm morphology (Bernard *et al.* 1991), craniometric data derived from linear measurements and qualitative cranio-dental morphology (Taylor & Kumirai in press; W.N. Verheyen pers. comm.) and geometric morphometrics (Taylor *et al.* unpubl.) also disagree to some extent with the taxonomy based on classical morphology.

Generic Level Taxonomy and Phylogenetic Relationships

Historic Generic Classifications

The Otomyinae has been considered to comprise between one (Wroughton 1906; Bohmann 1952) and five genera (Roberts 1951) based on morphological grounds and the number of subgenera too vary from one to five in the major taxonomic revisions of the group (Table 1). The diverse generic arrangements in particular reflect whether dentition or bullae development were emphasized in the diagnosis of genera (Musser & Carleton 1993). Some classifications (e.g. Thomas 1918; Ellerman 1941; Roberts 1951; De Graaff 1981) relied on additional cranial diagnostic features (nasal width, meatus and basioccipital morphology, the laminal formulae of M_1 and M^3), but these were considered unreliable by more recent taxonomists. Thomas (1918) was the first to subdivide the

Otomyinae into three genera: *Otomys*, with subgenera *Anchotomys* and *Lamotomys*, *Parotomys* with subgenus *Liotomys* and *Myotomys* with no subgenera. These subdivisions were mainly based on cranial features, but also grooves in the incisors; the latter genus was regarded as intermediate on these criteria to *Parotomys* and *Otomys*. Thomas (1918) regarded the dental features used by Wroughton (1906) and Dollman (1915) for specific and subspecific delineations (number of laminae on M^3 and M_1, incisor grooving) to be too plastic to accurately reflect true generic boundaries. However, at the subgeneric level, he considered the laminal formulae of M_1 as a reliable diagnostic character for the separation of subgenera within *Otomys* (*Anchotomys* and *Lamotomys*).

Prior to Thomas (1918), the Otomyinae was initially considered to contain two genera, *Otomys* and *Oreomys* (Heuglin, 1877). The latter was later replaced by *Oreinomys* (Trouessart, 1881), which was subsequently synonymized within *Otomys* but with no subgeneric divisions (Wroughton 1906). Likewise, Bohmann (1952) synonymized all species of *Parotomys* and *Myotomys* under *Otomys* (with no subgenera), a viewpoint echoed by Carleton and Musser (1984). The taxonomic treatments by St. Leger (1931), Broom (1937) and Allen (1939) followed the generic and subgeneric classification of Thomas (1918), but Broom (1937) described two additional subgenera (*Metotomys* and *Palaeotomys*) based on dental characters. *Metotomys* (Wroughton, 1907) was included as a subgenus in *Myotomys* and the fossil taxon *Palaeotomys*† as a subgenus in *Otomys*. *Palaeotomys*† included *O. robertsi* (Hewitt, 1927) and *Palaeotomys gracilis*†, the latter species being the extinct fossil precursor of *O. robertsi*. Broom (1937) considered *Parotomys* and *Otomys* to be clearly defined genera on the basis of dental and cranial features and retained the generic status of *Myotomys*, albeit with some reservations. This was because the dental characters of *O. robertsi* were distinctly intermediate between these two genera. Prior to Broom (1937), Roberts (1931) suggested that *Myotomys* should be reduced to subgeneric rank when he described *O. karoensis* as a species with a general appearance and cranial morphology that resembled species of *Myotomys*, but had dental characteristics similar to *Otomys* species.

Roberts (1951) recognized five genera based on dental characters and the size of the tympanic bullae and elevated the subgenera *Liotomys* and *Lamotomys* to full generic rank

in addition to *Myotomys, Otomys* and *Parotomys*. Roberts (1951) regarded the absence of interbreeding between *Otomys* and *Lamotomys* as sufficient justification for maintaining *Lamotomys* although their dentition differed only by the number of laminae on M^3. *Myotomys* (comprising *M. unisulcatus* and *M. sloggetti*) was described as being morphologically and ecologically intermediate between *Otomys* and *Parotomys*. Both *Liotomys* and *Parotomys* were distinguished from the other three genera by having inflated bullae, but the identity of each was ascertained using their upper incisor morphology.

The current tendency to recognize two genera, *Otomys* and *Parotomys* (Musser & Carleton 1993) is in agreement with some previous taxonomic treatments that did not support a proliferation of genera and subgenera in the Otomyinae (Table 1; Ellerman 1941; Ellerman *et al.* 1953; Misonne 1974; De Graaff 1981; Smithers 1983; Meester *et al.* 1986; Skinner & Smithers 1990). These authors, however, described different numbers of subgenera based on subtle morphological features (e.g. tail length, incisor grooving). Ellerman (1941) recognized *Otomys* with no subgenera and *Parotomys* with subgenera *Parotomys* and *Liotomys*. He further considered *Myotomys* an invalid genus, being associated with *Otomys* by morphologically intermediate forms, and regarded the subgenera *Anchotomys* and *Lamotomys* (*sensu* Thomas 1918) as anomalous species of *Otomys*. Ellerman *et al.* (1953) included *Lamotomys*, *Myotomys* and *Anchotomys* (*sensu* Roberts 1951) within *Otomys* (with no subgenera), but retained *Parotomys* and *Liotomys* within *Parotomys*. No subgenera were recognized within *Otomys* and *Parotomys* in more recent classifications (Misonne 1974; Swanepoel *et al.* 1980; De Graaff 1981; Smithers 1983; Meester *et al.* 1986; Skinner & Smithers 1990; Musser & Carleton 1993).

Morphology, Molecules and the Generic Taxonomy

It is obvious from the foregoing discussion that there is limited consensus among the various taxonomic treatments in respect of the generic and sub-generic divisions within the Otomyinae. Moreover, the relationships of the currently recognized southern African species that were inferred using a variety of genetic markers are discordant with the morphological affiliations. This clearly suggests that the generic boundaries are ambiguous (Taylor *et al.* 1989a, b; Contrafatto *et al.* 1994; Rambau *et al.* 1997; Govender

1999; Ducroz *et al.* 2001). The present arrangement, with *Otomys* and *Parotomys* as separate genera, rests principally on a single morphological trait, the size of the bullae (Misonne 1974; Meester *et al.* 1986; Skinner & Smithers 1990). The two species comprising *Parotomys* have enlarged bullae in comparison to most *Otomys* species; those of *O. sloggetti*, *O. unisulcatus* and *O. karoensis* are of intermediate size (Roberts 1931; Pocock 1976). Interestingly, these morphological divisions within the group were recently confirmed by the results of a geometric morphometric study that quantified the variation in the cranial size and shape of eight *Otomys* species and two *Parotomys* species (Taylor *et al.* unpubl.). It was shown that in terms of non-affine skull shape, *Otomys* and *Parotomys* are clearly distinct, and that *O. sloggetti* and *O. unisulcatus* (*Myotomys sensu* Roberts 1951) are intermediate between the strictly mesophylic *Otomys* species and xerophylic *Parotomys* species. This finding supports Pocock's (1976) observation that the generic status of *Myotomys* should be restored given that *O. sloggetti* and *O. unisulcatus* display ecological and morphological features that are intermediary to that of *Otomys* and *Parotomys*. In contrast, a phylogenetic study based on the variation in the cranio-dental morphology in the Otomyinae do not support the recognition of *Myotomys* and *Parotomys* as separate genera, but emphasize the phylogenetic closeness of *O. unisulcatus* and *O. sloggetti* to *Parotomys* (Taylor *et al.* pers. comm.). The relationships were inferred by cladistic analysis of 11 multi-state cranio-dental characters from all but two (*O. maximus, P. littledalei*) of the currently recognized Otomyinae species (*sensu* Musser & Carleton 1993; Taylor *et al.* 1993), and the subspecies *O. anchietae barbouri* and *O. a. lacustris* (*sensu* Musser & Carleton 1993). The presumed ancestral fossil species, *Euryotomys pelomyoides* (Pocock, 1976) was used as outgroup. Importantly, these authors treated *O. maximus* as being conspecific with *O. angoniensis*. Comparisons of the sperm and kidney morphology among species from southern Africa failed to separate congeneric species, although these studies have no phylogenetic relevance (Bernard *et al.* 1991; Pillay *et al.* 1994).

Similarly, the phylogenetic analyses of allozyme (Taylor *et al.* 1989a, b; Govender 1999; Taylor *et al.* pers. comm.), immuno-electrotransfer (Contrafatto *et al.* 1994) and recently, mtDNA sequence data (Ducroz *et al.* 2001) provided no clear-cut separation between species within *Otomys* and *Parotomys*, and did not support the recognition of *Myotomys* as a separate genus. In these studies, *O. unisulcatus* and occasionally, *O. sloggetti,* were

shown to be phylogenetically closer to the two *Parotomys* species than to their congeners. Additional evidence from comparative cytogenetic investigations largely underscored these relationships, but they did not include *O. sloggetti* (Rambau *et al.* 1997; Rambau 1998; Rambau & Robinson 1999). The implications of these findings for the taxonomy of the Otomyinae were two-fold. They were either taken as evidence in support of the recognition of only one genus, whereby *Parotomys* should be synonymized with *Otomys*, or, that *Otomys* as currently defined, is paraphyletic. It was further proposed that in the event of two genera being retained, *O. unisulcatus* (and *O. sloggetti* in some cases) ought to be included in *Parotomys*. However, the exact sister taxon relationships of these four taxa remain unclear (Taylor *et al.* 1989a, b; Meester *et al.* 1992; Contrafatto *et al.* 1994; Rambau *et al.* 1997; Rambau 1998).

The first protein electrophoresis study on the Otomyinae included four southern African species, *P. brantsii, P. littledalei, O. unisulcatus* and *O. irroratus* (Taylor *et al.* 1989a). The results revealed that two measures of genetic distance (D - Nei 1971 and D_R - Rogers 1972) and genetic identity values (I - Nei 1971) derived from allozyme polymorphisms (30 loci) were within the range for congeneric mammalian species given by Nevo (1978), Smith *et al.* (1978) and Thorpe (1982). *Otomys unisulcatus* and *P. brantsii* were separated by extremely low sequence divergences ($D = 0.26$, $D_R = 0.24$), whereas *O. unisulcatus* was separated from its congener, *O. irroratus* by much larger genetic distances ($D = 0.61$, $D_R = 0.46$). The distinctiveness of *O. irroratus* was confirmed by the comparatively large mean genetic distance separating it from *O. unisulcatus, P. brantsii* and *P. littledalei* ($D = 0.70$, $D_R = 0.51$). Similar patterns of genetic differentiation were reflected in the UPGMA and distance Wagner phenograms constructed from Nei's D and D_R values, respectively. *Otomys irroratus* was obviously removed from a cluster constituting *O. unisulcatus*, *P. brantsii* and *P. littledalei*, with the former two species grouping as sister taxa. Largely similar relationships among these four taxa were recovered by the phenetic and cladistic analyses of 27 allozyme loci when the species sampling was extended to include *O. angoniensis*, *O. laminatus* and *O. sloggetti* (Taylor *et al.* 1989b). In the resulting cladogram, *O. sloggetti* and *O. unisulcatus*, and the two *Parotomys* species grouped as sister taxa, respectively, and these species, in turn, formed a clade separate from a trichotomous clade formed by *O. angoniensis*, *O. laminatus* and *O. irroratus*. These associations recovered from allozyme data were for the most part

confirmed by a phylogeny of the southern African Otomyinae inferred by immuno-electrotransfer analysis (Contrafatto *et al.* 1994). The differences were that *O. sloggetti* was more closely related to *P. brantsii* and *O. unisulcatus* than to *P. littledalei* (although with poor bootstrap support), and that *O. angoniensis* and *O. irroratus* grouped as sister taxa with *O. laminatus* basal to them.

Further allozyme research included data from 15 loci of all but one (*P. littledalei*) of the currently recognized southern African species (*sensu* Musser & Carleton 1993; Taylor *et al.* 1993) and the East African *O. tropicalis*, *O. typus* and *O. a. barbouri* (Govender 1999). The branching patterns in the UPGMA phenogram inferred through cluster analyses (using Nei's D 1971) underlined the sister relationship between *O. unisulcatus* and *P. brantsii* recovered previously, but *O. sloggetti* no longer consistently grouped as sister to these two taxa. Most recently, preliminary phylogenies inferred by parsimony analyses of allozyme data (14 polymorphic loci), both separately, and combined with 11 cranio-dental characters for 10 Otomyinae species confirmed that *O. unisulcatus* and *P. brantsii* are closely related (Taylor *et al.* pers. comm.). It was further suggested that *O. sloggetti* is more closely associated with the latter two species than to the remainder of the species included (*O. a. barbouri, O. angoniensis, O. irroratus, O. karoensis, O. laminatus, O. tropicalis* and *O. typus*). It is, however, essential to mention that the choice of *P. brantsii* as outgroup for these analyses, which was based on palaeontological and morphological evidence, may be inappropriate. Previous phylogenies deduced from genetic data have suggested that *Parotomys* might be a polyphyletic genus and, further, that the position of *P. brantsii* and *P. littledalei* in relation to the species of *Otomys* is unresolved (Taylor *et al.* 1989a, b; Contrafatto *et al.* 1994).

At the chromosomal level, comparative cytogenetic studies using conventional banding techniques and a combination of the latter and molecular cytogenetics provided additional evidence in support of a closer affiliation of *O. unisulcatus* (2n = 28, Robinson & Elder 1987) with *P. brantsii* (2n = 42, Meester 1988) than with *O. irroratus* represented by specimens with a diploid number of 2n = 28 (Rambau *et al.* 1997; Rambau 1998). Initially, Robinson and Elder (1987) reported an absence of G-band homeology between the karyotypes of *O. irroratus* and *O. unisulcatus*, despite the identical diploid numbers. These authors suggested that complex intrachromosomal

restructuring of the *O. irroratus* genome resulted in dissimilar G-band patterns for the two species. Subsequently, Rambau *et al.* (1997) directed by fluorescence *in situ* hybridization (FISH) data identified six conserved chromosomal regions. Four of these were confirmed by (FISH) using a limited number of mouse chromosome specific probes. *Otomys unisulcatus* and *P. brantsii* were chromosomally most similar sharing 10 regions of G-band homeology (five confirmed by FISH, Rambau *et al.* 1997; Rambau 1998), whereas eight such regions were distinguished between *O. unisulcatus* and *P. littledalei* (Rambau & Robinson 1999). The greater karyotypic correspondence between *O. unisulcatus* and *P. brantsii* than between *O. unisulcatus* and *O. irroratus* suggested the former species are more closely related than the latter.

Recently, Ducroz *et al.* (2001) provided further support for the paraphyly of the genus *Otomys* from the phylogenetic analysis of partial 12S and 16S rRNA sequences and complete cyt *b* sequences of four species (*O. irroratus*, *O. sloggetti, P. brantsii* and *P. littledalei*). In topologies reconstructed using cyt *b* sequences and in analyses in which both 12S and 16S rRNA sequences were combined, as well as in instances where all three data sets were used (12S + 16S rRNA + cyt *b*), *O. sloggetti* was more closely related to *Parotomys* than to its congener *O. irroratus* (with weak bootstrap support). *Otomys sloggetti* grouped as the sister taxon to *P. littledalei* in the topologies recovered from the analysis of both the cyt *b* sequences and the combined 12S/ 16S rRNA/ cyt *b* data, but with variable bootstrap support (58 - 93%).

Clearly, the results of allozyme, immuno-electrotransfer, cytogenetic and molecular investigations all underscore the need for a detailed comprehensive re-evaluation of *Parotomys* as a genus separate from *Otomys*.

An Arid-Mesic Dichotomy among Lineages in Southern Africa

Irrespective of whether one or two genera are recognized in the Otomyinae, and the particular species composition of each, the existence of two discrete evolutionary lineages within southern Africa have been suggested by morphological, palaeontological (Pocock 1976; Pillay *et al.* 1994) and genetic evidence (Taylor *et al.* 1989a, b; Meester *et al.* 1992; Contrafatto *et al.* 1994). The first is essentially mesically adapted and is

thought to comprise of all the modern species of *Otomys* except for *O. unisulcatus*. These taxa are predominantly distributed in the mesic southeastern parts of the region (Pocock 1976; Fig. 1e, f, l - n). The second, primarily arid adapted lineage comprises *P. brantsii, P. littledalei* and *O. unisulcatus* (Pillay *et al.* 1994; Rambau *et al.* 1997; Rambau 1998), but some studies suggest that *O. sloggetti*, which occupies extremely high altitude, rocky habitats in both semi-arid and mesic regions, also belong to the arid lineage (Pocock 1976; Taylor *et al.* 1989a, b; Contrafatto *et al.* 1994). The geographic distributions of *P. brantsii* and *P. littledalei* extend to the predominantly arid, western side of southern Africa, whereas *O. sloggetti* and *O. unisulcatus* have intermediate distributions between the more mesic eastern and more arid western parts (Skinner & Smithers 1990; Fig. 1a - d).

The first to postulate a clear division between arid and mesic species in the southern African Otomyinae on classical morphology was Pocock (1976). The dental and cranial features of fossil and living forms suggest that the arid and mesic lineages probably diverged after being derived from *Prototomys campbelli* (approximately 3.6 Myr, Broom & Schepers 1946), the presumed mesically adapted Pliocene ancestor of all modern Otomyinae (Pocock 1976; Taylor *et al.* 1989a). Subsequent to this divergence, inflated bullae are thought to have developed in arid adapted taxa as an adaptation for survival in xeric environments. In the case of the mesophylic taxa, all species possess grooved lower incisors and completely laminate molars with a trend towards multiple laminae that probably evolved for the mastication of coarser vegetation associated with moist environments (Pocock 1976). Continued support for the two lineages emerged from preliminary parsimony analysis of 11 cranio-dental characters in the currently recognized Otomyinae species (except *P. littledalei*) and the subspecies *O. a. barbouri* and *O. a. lacustris* (*sensu* Musser & Carleton 1993). In the resulting cladogram, *P. brantsii*, *O. unisulcatus* and *O. sloggetti* were clearly separated from a well-defined clade consisting of all the mesophylic *Otomys* (Taylor *et al.* pers. comm.).

Evidence from several other sources, some of which is contradictory, also has bearing on Pocock's (1976) arid-mesic hypothesis. At a molecular level, phylogenies based on phenetic and cladistic analyses of allozyme and immuno-electrotransfer data (Taylor *et al.* 1989a, b; Contrafatto *et al.* 1994) clearly separate mesic-occurring southern African

species (*O. irroratus*, *O. angoniensis* and *O. laminatus*) from *O. sloggetti* (semi-arid/mesic), *O. unisulcatus* (semi-arid), *P. brantsii* and *P. littledalei* (arid). This trend appears to persist in the topology resulting from the parsimony analysis of allozyme data (14 polymorphic loci) when taxa representation included the mesophylic *O. a. barbouri*, *O. tropicalis* and *O. typus* from East Africa and *O. karoensis* from southern Africa (Taylor *et al.* pers. comm.).

A comparative analysis of the renal morphology of the xerophylic *O. unisulcatus*, *P. brantsii* and *P. littledalei* and the mesophylic *O. angoniensis*, *O. irroratus*, *O. sloggetti* suggests that the kidney structure of these two species groups are distinct, although neither of the kidney types approach the extremes of xeric or mesic adaptations observed in other rodents (Pillay *et al.* 1994). This finding supports genetic evidence for an arid-mesic dichotomy. Interestingly, the kidney morphology of *O. sloggetti* was most similar to that of mesophylic *O. angoniensis* and *O. irroratus*, an affiliation in conflict with the relationships inferred from allozyme and immuno-electrotransfer data that suggested the species forms part of the arid lineage (Taylor *et al.* 1989a, b; Contrafatto *et al.* 1994). Meester *et al.* (1992) attributed this to the fact that *O. sloggetti robertsi* (the subspecies used in the renal morphology study), displays modifications in kidney structure as well as other morphological and behavioural aspects that enable them to survive in cold and wet, high altitude habitats (Willan 1990; Richter 1997; Richter *et al.* 1997).

The taxonomic affinities between five southern African Otomyinae species inferred from sperm morphology do not support the existence of two evolutionary lineages (Bernard *et al.* 1991). Based on the shape of the head of the spermatozoa, *O. angoniensis*, *O. irroratus* and *O. unisulcatus* are most similar. The sperm heads that are anteriorly tightly curved with short, broad nuclei, whereas, *O. sloggetti* and *P. brantsii* are most similar with heads that have a shallow anterior curve and long, slender nuclei. It is especially the close association of the xerophylic *O. unisulcatus* to the mesophylic forms that is inconsistent with evidence from genetic and kidney morphology data. However, despite the superficial resemblance between the sperm of *O. unisulcatus*, *O. angoniensis* and *O. irroratus*, those of *O. unisulcatus* are essentially different from all other species, having a narrow midpiece with small mitochondria in the tail (Bernard *et al.* 1991). These authors suggest that should studies incorporating more species and appropriate outgroups

demonstrate that this is a derived character (autapomorphy), the conflict suggested by the sperm morphology would fall away.

Species Level Taxonomy and Phylogenetic Relationships

Diagnostic Features used for Species Identification

The two *Parotomys* species are distinguished by the groove patterns on their upper incisors, which are ungrooved in *P. littledalei*, and grooved in *P. brantsii*. Species identification within *Otomys* rests primarily on the number of laminae on the elongated M^3 (four to ten) and M_1 (four to seven). In addition, the presence or absence and number of grooves on the lower incisors are also considered important criteria as are tail length, hind foot length, the shape and width of the nasal bone and the shape of the petrotympanic foramen (Thomas 1918; Ellerman 1941; Bohmann 1952; Ellerman *et al.* 1953; Dieterlen 1968; Misonne 1974; De Graaff 1981; Petter 1982; Meester *et al.* 1986; Skinner & Smithers 1990). However, the intraspecific variation in these characters, has led to major confusion of the specific taxonomy of the group (Thomas 1918; Kingdon 1974; Carleton & Musser 1984; Taylor & Kumirai in press).

Historic Specific Classifications

A variable number of species, ranging between nine (Wroughton 1906) and 29 (Allen 1939), have been recognized (Table 1). Moreover, up to 64 forms (or subspecies) have been described in *Otomys* (Bohmann 1952), and up to eight in *Parotomys* (Ellerman *et al.* 1953). The first comprehensive revision of the Otomyinae (Wroughton 1906) included both southern and East African taxa and recognized nine species (19 forms) in a single genus *Otomys*. The revision was chiefly based on dental characters, and the author regarded the key as "far from finality", especially with respect to the West and southern African taxa. The species was provisionally grouped into three sections (I, II and III) and within sections I and II, two groups were distinguished according to the number of laminae on M_1 and M^3 and the number of grooves on the lower incisors (Wroughton 1906). Subsequently, Dollman (1915) used both cranial and dental characters to modify the classification of the East African *Otomys*. *Otomys tropicalis, O. nyikae,*

O. angoniensis, O. orestes and *O. denti* were raised to full species rank resulting in 14 species of *Otomys* (22 forms) being recognized (Table 1). Three major divisions (A, B and C) were derived from the groove patterns on the lower incisors and tail color, and two sections within divisions B and C were distinguished on differences in cranial morphology (interorbital region, nasals and skull shape). Furthermore, different groups were distinguished within the respective sections of divisions A, B and C based on the laminal formula of M^3, whereas the groups in section II of division B were delineated on nasal width and the angle of transition of nasals from broad to narrow (the original account provides an extensive report on the divisions, sections and groups). Allen (1939) recognized 29 species (64 forms) in three genera (*Myotomys, Otomys* and *Parotomys*), while Ellerman (1941) described two species (six forms) in *Parotomys* and 26 species (57 forms) within *Otomys*. The latter taxon was divided into six, possibly seven, broad species groups based on the number of laminae on M_1 and M^3, and the groove patterns on the lower incisors (*anchietae*, *irroratus*, *laminatus*, *turneri*, *typus, unisulcatus* and possibly *karoensis* groups). Bohmann (1952) described eleven species (or Rassenkreisen) and 64 forms in only one genus, *Otomys*, whereas Misonne (1974) re-instated *Parotomys* as a valid genus with two species (six forms) and recognized nine species within *Otomys* (48 forms). Taxonomic treatments that mainly dealt with southern African Otomyinae (Roberts 1951; Ellerman *et al.* 1953; De Graaff 1981; Smithers 1983; Meester *et al.* 1986; Skinner & Smithers 1990) recognized between six and 11 *Otomys* species and two *Parotomys* species (Table 1).

The Uncertain Status of Some Otomyinae Species

Current uncertainties concerning species delimitation involve two major issues. First, the recognition of *O. maximus* and *O. angoniensis* as separate species is not secure (e.g. Meester *et al.* 1986; Musser & Carleton 1993). Second, the phylogenetic relationships and status of *O. irroratus* as a species distinct from *O. anchietae, O. angoniensis, O. laminatus, O. maximus O. occidentalis, O. tropicalis* and *O. typus* has been questioned in a number of taxonomic treatments (Bohmann 1952; Misonne 1974; Petter 1982; Dieterlen & van der Straeten 1992; Musser & Carleton 1993). The validity of *O. tropicalis* as a species distinct from *O. irroratus* in particular requires confirmation (Taylor & Kumirai in press).

The status of *O. angoniensis* and *O. maximus*

Both *O. maximus* and *O. angoniensis* are considered full species by Musser and Carleton (1993). This agrees with Roberts (1951) who regarded *O. maximus* as distinct from *O. angoniensis* based on the large, relatively narrow skull of the former, and the small broader skull of the latter. This division was subsequently supported by Lundholm (1955), Davis (1962), Swanepoel *et al.* (1980), Smithers (1983) and Crawford-Cabral (1998). Davis (1974), Misonne (1974), Ansell (1978), De Graaff (1981), Crawford-Cabral (1986), Meester *et al.* (1986), Skinner & Smithers (1990), however, differ (Table 1). Crawford-Crabal (1986) adopted a supra-specific designation to include all three forms, *O. angoniensis, O. irroratus* and *O. maximus*, into one species group (the *O. angoniensis-O. irroratus* group). *Otomys angoniensis* was first described as *O. irroratus angoniensis* (Wroughton, 1906) and Dollman (1915) raised it to full species status. In subsequent classifications the taxon enjoyed distinct species status (Allen 1939; Ellerman 1941) or was synonymized with *O. irroratus* (e.g. Bohmann 1952; Ellerman *et al.* 1953). *Otomys maximus* was initially described as *O. irroratus maximus* (Roberts, 1924), a status retained by Allen (1939) and Ellerman (1941), whereas Bohmann (1952) and Ellerman *et al.* (1953) considered it to be conspecific with *O. irroratus*.

Otomys maximus and *O. angoniensis* have disjunct distributional ranges. The former is restricted to northeastern Namibia, northwestern Zimbabwe, northern Botswana, southwestern Zambia and southwestern Angola (Fig. 1f; Smithers 1971; De Graaff 1981; Smithers 1983; Crawford-Cabral 1986; Musser & Carleton 1993; Crawford-Cabral 1998). The latter is widespread in eastern and northern parts of South Africa, central and southern Mozambique, central Zimbabwe, southern to northern Malawi, central and northern Tanzania and southern Kenya (Fig. 1e; Misonne 1974; De Graaff 1981; Bronner & Meester 1988; Musser & Carleton 1993). There is considerable overlap in habitat preference, but *O. angoniensis* survives in a broader range of habitats than *O. maximus* (Smithers 1983). The former prefers savanna woodlands and secondary grassland habitats with a large component of herbaceous growth (e.g. fire-degraded grasslands) but in dry areas they occur in moist grasslands, near swamps and wet vleis and in riverine vegetation (Davis 1962, 1974; De Graaff 1981; Smithers 1983; Bronner & Meester 1988). In southern Africa its range partly overlaps with that of *O. irroratus*, especially in

wetter habitat, and with that of *O. laminatus* in low-lying and moist coastal, open and wooded grassland (De Graaff 1981; Smithers 1983). It was also noted that wherever *O. angoniensis* occurs sympatrically with *O. irroratus* and *O. tropicalis*, it prefers drier areas (Roberts 1951; Davis 1962; but see Misonne 1974). *Otomys maximus* is usually confined to riverine vegetation (reeds, sedges and tussock grass) along banks, in riverbeds and on flood plains, which form part of the Atlantic Drainage System. These include large rivers such as the Kunene (northern Namibia), the Okavango (northern Namibia and Botswana), the Chobe (northern Botswana), the upper Zambezi (northern Zimbabwe), the Cuebo, Cuebe and Cuvelai rivers (southern Angola), the high water courses of smaller rivers on the Huila highlands and northwards as far as Mt. Soque in central and eastern Angola (Roberts 1951; Crawford-Cabral 1998).

The status of *O. irroratus* and *O. tropicalis* revisited

The second taxonomic issue relevant to specific boundaries concerns *O. irroratus*. This species is viewed as having a strictly southern African distribution, south of the Zambezi River (Musser & Carleton 1993; Fig. 1n). Formerly, however, several southern, East and West African forms with a markedly discontinuous distribution were synonymized within it (Bohmann 1952; Misonne 1974; Petter 1982; Dieterlen & van der Straeten 1992). These included, *O. laminatus*, *O. maximus* from southern Africa, the extralimital *O. anchietae*, *O. tropicalis*, *O. typus*, *O. occidentalis* and one species with a wide range, *O. angoniensis* (Fig. 1). Although these taxa all currently enjoy species status (Musser and Carleton 1993), *O. irroratus* was thought by Bohmann (1952) to include *O. maximus*, *O. angoniensis* and *O. tropicalis* but he retained *O. laminatus*, *O. anchietae* and *O. typus* as separate species. Petter (1982) held quite a different view and regarded *O. angoniensis* and *O. maximus* distinct from *O. irroratus*, but incorporated *O. laminatus*, *O. anchietae*, *O. tropicalis* and *O. typus*, making it a widespread species with a range extending from the Cameroon to South Africa (Fig. 1). The taxonomies suggested by Bohmann (1952) and Petter (1982) was challenged by Misonne (1974), Meester *et al.* (1986), Dieterlen and van der Straeten (1992), and recently by Taylor *et al.* (2001) and Taylor and Kumirai (in press).

At the outset, Dieterlen and van der Straeten (1992) pointed out that some skulls studied by Petter (1982), which probably led to his description of *O. irroratus* as a polymorphic species, were misidentified and some collection localities were also confused. Their conclusion was based on quantitative and qualitative external, cranio-dental characters of 26 representatives of *Otomys* from selected East and West African mountains. These included specimens determined as *O. irroratus burtoni* (Bohmann, 1952 ≈ *O. tropicalis burtoni,* Misonne, 1974; Musser & Carleton 1993) from Mt. Cameroon and Mt. Oku (the Cameroon) and the Gotel Mountains (Nigeria), *O. tropicalis elgonis* (Allen & Lawrence, 1936 ≈ *O. barbouri*, Lawrence & Loveridge, 1953 ≈ *O. anchietae barbouri*, Musser & Carleton 1993) from Mt. Elgon (Kenya/Uganda) and *O. anchietae lacustris* (Allen & Loveridge, 1933; Musser & Carleton 1993) from the Ufipa Plateau and Poroto Mountains (southwestern Tanzania). These authors subsequently described a new species, *O. occidentalis,* from Mt. Oku and the Gotel mountains as being distinct from *O. i. burtoni* (≈ *O. t. burtoni*) from Mt. Cameroon and closely related to *O. a. barbouri* (Mt. Elgon) and *O. a. lacustris* (southwestern Tanzania), to whom they provisionally afforded specific status. The recognition of *O. occidentalis* was based on it having five laminae on M_1 and *O. irroratus* (which included *O. tropicalis*) consistently having four laminae, but also on differences in overall and cranial size and coloration.

Additional morphological evidence in contradiction of *O. irroratus* as a widespread, polymorphic species with a pan-African distribution was recently put forward when a provisional list of 13 *Otomys* species was proposed (Taylor & Kumirai in press). It was based on a discriminant analysis of 12 quantitative cranial measurements and five qualitative dental and cranial features of 285 specimens representing all but one (*O. maximus*) of the 14 Otomyinae species currently recognized (*sensu* Musser & Carleton 1993; Taylor *et al.* 1993). *Otomys maximus* was excluded because its specific status is not generally accepted (Misonne 1974; Meester *et al.* 1986). In agreement with Musser and Carleton (1993), these authors considered all the taxa that were previously treated as conspecifics of *O. irroratus* by Bohmann (1952) and Petter (1982) to be good species. In particular, compelling evidence was provided for the recognition of *O. tropicalis* as a full species, which is not commonly accepted among taxonomists. It was shown that *O. tropicalis* displayed somewhat smaller cranial proportions, but larger bullae and a shorter palatal length than *O. irroratus*; the number of laminae on M^3 and the angle of the nasal

bone also allowed for the distinction of the two (*O. tropicalis*: 7 or 8 laminae, acute angle; *O. irroratus*: 6 laminae consistently, less acute angle; also see Denys 1989). Moreover, preliminary geometric morphometric results revealed that their overall cranial size was also significantly different (Taylor *et al.* unpubl.). *Otomys tropicalis* was originally described as a subspecies of *O. irroratus* by Thomas (1902a). Wroughton (1906) retained this status, but Dollman (1915) raised it to full species rank to substitute *O. irroratus* in East Africa on account of its distinct cranial structure and M^3 morphology (Table 1). This distinction was acknowledged in classifications by Allen (1939), Ellerman (1941), Misonne (1974) and Musser and Carleton (1993), whereas *O. tropicalis* was treated as a conspecific of *O. irroratus* by Wroughton (1906), Bohmann (1952), Dieterlen (1968) and in several regional studies of East African otomyines (Kingdon 1974; Delany 1975; Petter 1982; Dieterlen & van der Straeten 1992).

Preliminary relationships inferred from as yet unpublished cranio-dental and allozyme data revealed that *O. irroratus* is phylogenetically distinct from *O. tropicalis* (falling in separate clades and not as sister taxa) and also from *O. anchietae*, *O. a. barbouri*, *O. a. lacustris*, *O. angoniensis*, *O. laminatus* and *O. typus* that were previously synonymized with it. These hypotheses will be tested in this study using mtDNA sequence data.

Uncertainties Concerning the Taxonomy and Synonymy of *Otomys* from East, Central and West Africa

In sharp contrast to the relatively clear species boundaries that are defined on morphological and genetic grounds for the majority of the southern African *Otomys* (except for *O. angoniensis*, *O. maximus* and *O. irroratus*), the taxonomy of the extralimital species is characterized by major instability (Musser & Carleton 1993). It has primarily been ascribed to the poor choice of characters used for delineating species and synonymizing taxa (Musser & Carleton 1993). Qualitative dental characters, in particular, are homoplastic and highly variable between conspecifics (e.g. Wroughton 1906; Dollman 1915; Thomas 1918; Broom 1937; Ellerman 1941; Bohmann 1952; Lawrence & Loveridge 1953; Taylor & Kumirai in press). It is, therefore, considered that several species are contained within four of the five formally recognized species (*O. anchietae, O. denti, O. tropicalis* and O. *typus*) from East, Central and West Africa

(Musser & Carleton 1993). These conclusions have been strengthened by morphological and biochemical studies investigating the relationships among *Otomys* forms that exist in high altitude Afromontane and Afroalpine habitats on isolated mountain tops in these regions (Dieterlen & van der Straeten 1992; Lavrenchenko *et al.* 1997; Taylor & Kumirai in press; J. Peterhans Kerbis, W.N. Verheyen, Taylor *et al.* pers. comm.).

In keeping with this sentiment, specific status has been suggested for *O. anchietae lacustris* from the Uzungwa, Ukinga, Poroto Mountains and the Ufipa Plateau (southwestern Tanzania) and the Abedares (Kenya) and for *O. a. barbouri* from Mt. Elgon. This was based on cranio-morphological differences distinguishing them from *O. a. anchietae* (*sensu* Bocage 1882) from Angola (Dieterlen & van der Straeten 1992; Taylor & Kumirai in press; see Fig. 1i). The decision to synonymize these East African taxa with *O. anchietae* and for placing them with *O. occidentalis* from West Africa in the "*anchietae*" species group was based on tooth laminae. These taxa commonly have five laminae in M_1 in contrast to the four or fewer in all other *Otomys*, except *O. laminatus* which usually has seven (but also see Ellerman 1941; Lawrence & Loveridge 1953; Dieterlen & van der Straeten 1992; Taylor & Kumirai in press). Dieterlen and van der Straeten (1992) considered the delimitation of *O. a. lacustris* and *O. a. barbouri* from *O. anchietae* justified, since the latter has larger cranium than *O. a. lacustris* and the shape of its nasals and external appearance are different from *O. a. barbouri* (Lawrence & Loveridge 1953). Further confirmation from craniometric data and qualitative cranio-dental characters reveal that the greatly enlarged cranium of *O anchietae* readily distinguishes it from the smaller members of the "*anchietae*" group (*O. a. lacustris*, *O. a. barbouri* and *O. occidentalis* – "*lacustris*" group, Dieterlen & van der Straeten 1992).

Otomys denti has a relic distribution in East and Central Africa on the Nyika Plateau (northern Malawi/Democratic Republic of Congo), the Uluguru and Usambara mountains (Tanzania), the Ruwenzori mountains (Uganda), the Kivu mountains (Rwanda) and the Virunga volcanoes on the border of the Democratic Republic Of Congo and Rwanda (Misonne 1974; Ansell & Dowsett 1988; Musser & Carleton 1993; Fig. 1j). *Otomys d. kempi*, one of two subspecies currently recognized (the other being *O. d. sungae*) was previously treated as a distinct species based on its slightly larger size and the number of laminae on M^3 (Dollman 1915; Thomas 1918; Ellerman 1941). Subsequently it was

reduced to subspecific rank (Bohmann 1952; Misonne 1974; Delany 1975). Specific status for *O. d. kempi* is supported from variation in cranio-dental morphology among widely separated populations, these being for example those on the Rift-edge mountains along the line of the western arm of the Great Rift Valley (e.g. Virunga Volcanoes, Kivu Mountains) and on the Ruwenzoris (J. Peterhans Kerbis pers. comm.).

Otomys tropicalis is believed to comprise two or more good species (Musser & Carleton 1993). The subspecific status of the West African *O. t. burtoni* (Misonne 1974) has clear cranio-morphological differences when compared with East African forms questioning its subspecific status (W.N. Verheyen pers. comm.). This lends support to earlier taxonomic treatments recognizing it as a full species (Thomas 1918; Allen 1939; Ellerman 1941).

Otomys typus has a markedly discontinuous distribution in East Africa (Fig.1g), and may be a complex combination of isolated forms, several of which have previously been afforded species status on the basis of distinguishing cranio-dental features (see Table 1). Specific rank has previously been awarded to: *O. t. dartmouthi*, *O. t. orestes*, *O. t. fortior*, *O. t. thomasi*, *O. t. percivali*, *O. t. uzungwensis* and *O. t. jacksoni* (Wroughton 1906; Dollman 1915; Allen 1939; Ellerman 1941; Bohmann 1952; Lawrence & Loveridge 1953). Most of these subspecies are geographically isolated from the *O. t. typus* from northern Ethiopia (type locality) by the eastern and/or western arms of the Great Rift Valley System (Werger 1978). Yalden and Largen (1992) proposed on morphological grounds, that the Ethiopian *O. typus* probably comprises a complex of two or three species, some of which may well be endemic. This was subsequently confirmed by Lavrenchenko *et al.* (1997) who found two coexisting chromosomal forms on the Bale Massif that merit specific status (species A: 2n = 56; species B: 2n = 57/58), since no hybridization apparently occurs between these forms and fixed diagnostic alleles for each population exist at four allozyme loci.

Chromosomal Variation within *O. irroratus* and the Relationships of the Major Cytogenetic Groups.

The mesic-adapted *O. irroratus* from southern Africa, has not only attracted the attention of taxonomists owing to its contentious specific boundaries (Bohmann 1952; Misonne

1974; Petter 1982; Meester *et al.* 1986; Dieterlen & van der Straeten 1992; Musser & Carleton 1993; Taylor & Kumirai in press), but it also displays a remarkable variation in diploid number. Despite absence of geographic variation in pelage, chromosomal variation among populations ranges from a diploid number of 2n = 23 - 32 (Contrafatto *et al.* 1992a, b, 1997; Rambau 1998; Rambau *et al.* 2001). Based on the number and presence or absence of heterochromatic short arms, Contrafatto *et al.* (1992a, b, 1997) described five cytotypes (A, A1, A2, B and C; Fig. 1o). Cytotype A is composed of an all-acrocentric karyotype, subgroup A1 contains a tandem fusion chromosome comprised of chromosome 7 and 12 of the *O. irroratus* standard karyotype published by Robinson and Elder (1987), and A2 lacks the tandem fusion chromosomes and contains two *de novo* autosomal pairs, a1 and a2 (see Contrafatto *et al.* 1992a). The B cytotype carried at least seven pairs (6 - 8) of large bi-armed autosomes with heterochromatic short arms whereas the C cytotype constituted four pairs of large bi-armed chromosomes with heterochromatic short arms (also see Taylor 2000). In sharp contrast, Rambau *et al.* (2001) showed that only two major chromosomal groups are discernable within *O. irroratus*. The main difference between the two is a compound chromosomal rearrangement involving three autosomes (7, 8 and 12) of the *O. irroratus* standard, and presence of cytotype specific centric fusions. The differences between these hypotheses are primarily due to disagreement on the types of chromosomal rearrangements implicated, the specific autosomes involved in these, and differences in interpretation of supernumerary chromosomes (or B chromosomes).

The role of chromosomes causing speciation is contentious (Meester 1988; Taylor 2000). Some authors believe that changes in structure may play a role in initiating speciation long before the phenotypic and genetic changes usually associated with speciation events have occurred (Cothran & Smith 1983; Capanna *et al.* 1985; Robinson & Roux 1985; Baker & Bickham 1986; Moritz 1986; Meester 1988; Taylor 2000). Others consider no causal connection between these two events (Carson 1982; Patton & Sherwood 1983; Paterson 1985; Vrba 1985c; Coyne 1994). The cytogenetic properties of *O. irroratus*, coupled with evidence for post-zygotic and pre-mating reproductive isolation between cytotypes (Pillay *et al.* 1992, 1995a, b, c) seemingly support the former hypothesis. It further suggests that incipient speciation, initiated by chromosomal rearrangements, is most likely taking place within the species (Taylor *et al.* 1994). It has been suggested

that populations carrying A1 cytotype (*sensu* Contrafatto *et al.* 1992a, b, 1997) may represent a sibling species of *O. irroratus* (Pillay *et al.* 1992, 1995a, b, c). This cytotype corresponds to the group delimited by the compound chromosome (*sensu* Rambau *et al.* 2001), which occur at high elevations in the Drakensberg (KwaZulu Natal) and in the eastern Cape Province (one population). The reduced reproductive success observed in inter-population crosses between the allopatric Hogsback (A1), Committee's Drift (B) and Karkloof (A2) populations most probably resulted from chromosomal incompatibility (a potential post-zygotic reproductive barrier) and subsequent reproductive breakdown of hybrids (Pillay *et al.* 1992, 1995b). Virtually complete hybrid sterility was observed when Hogsback individuals were involved in backcrosses. Pillay *et al.* (1995a, c) provided additional evidence of pre-mating reproductive isolation between the allopatric Kamberg (A1) and Karkloof (A2) populations resulting from specific courtship behaviour (aggression) and to a lesser extent the olfactory choice in mate recognition.

On the contrary, Taylor *et al.* (1992) intimated from allozyme data (24 loci) that extensive gene flow occurs between populations representative of all the *O. irroratus* cytotypes (*sensu* Contrafatto *et al.* 1992a, b, 1997). Cluster analysis of genetic distances (Nei's D 1978; Roger's D 1972) based on 12 polymorphic loci failed to distinguish populations consistent with the karyotypic differences between them or their geographic locality (Taylor *et al.* 1992). Immuno-electrotransfer analysis arrived at a similar conclusion (Contrafatto *et al.* 1997). This is notwithstanding the fact that the ranges of the different cytotypes are strongly correlated with climate e.g. temperature and rainfall as is suggested by their parapatric distributions in distinct bioclimatic regions of South Africa (Taylor *et al.* 1994; Taylor 2000; Fig. 1o).

Although representatives of the different cytotypes are morphologically indistinguishable, recent geometric morphometric analysis has revealed subtle, but statistically significant differences in cranial shape between climatically distinct groups of cytotypes (Taylor *et al.* unpubl.): from the east of the Drakensberg; semi-arid parts of the eastern Cape and the winter rainfall fynbos biome. In addition, between phylogeographically separated Zimbabwe and southern African populations carrying the B cytotype. Physiological differences (cold tolerance) exist between individuals of

different cytotypes at high (Hogsback – A1) and low altitudes (Alice – B). Such adaptations can result in genotypic differences amongst populations (Brown *et al.* 1997). In conclusion, chromosomal divergence among populations of *O. irroratus* may have preceded genic and morphological divergence in the species.

PALAEONTOLOGY, MOLECULAR CLOCKS AND THE EVOLUTIONARY HISTORY OF THE OTOMYINAE

Several hypotheses have been postulated from the fossil record and molecular data that are relevant to the estimated time for the origin of the Otomyinae from an ancestral stock. These and the subsequent divergence and radiation of descendent lineages are described in Bohmann (1952), Pocock (1976), Denys *et al.* (1987), Denys (1989), Taylor *et al.* (1989a), Chevret *et al.* (1993), Sénégas & Avery (1998) and Ducroz *et al.* (2001). In spite of the sparseness of the Otomyinae's fossil record (Sénégas & Avery 1998), the majority of these hypotheses were derived from morphological characters, which include the transformation of, and the similarities between the dental patterns of the presumed fossil ancestors of the group and true *Otomys* fossils (Denys *et al.* 1987; Chevret *et al.* 1993) and between the fossils of extinct and living *Otomys* species (Pocock 1976; Carleton & Musser 1984; Denys 1989). Bohmann's (1952) hypothesis was based on trends observed in the cranio-dental morphology of extant otomyine taxa. In addition, molecular time scales have been presented for the descent of the Otomyinae from the Murinae (DNA-DNA hybridization, Chevret *et al.* 1993; mtDNA, Ducroz *et al.* 2001; Verheyen *et al.* unpubl.) and for the divergence of extant lineages (allozymes, Taylor *et al.* 1989a; RFLPs, Lamb *et al.* 1996; mtDNA, Ducroz *et al.* 2001). These hypotheses collectively suggest the very late Miocene to late Pleistocene as the time frame for the Otomyinae evolution, but the fossil and molecular-based divergence dates are discrepant.

Otomyinae Relationships within the Family Muridae

Evidence from palaeontological (Pocock 1976; Denys *et al.* 1987; Denys 1989; Sénégas & Avery 1998), molecular (Chevret *et al.* 1993; Michaux & Catzeflis 2000; Ducroz *et al.* 2001; Michaux *et al.* 2001; Verheyen *et al.* unpubl.), sperm morphology (Bernard *et al.* 1991) and immunological data (Sarich 1985; Watts & Baverstock 1995) suggest that the

Otomyinae, fall within the Murinae. Pocock (1976) described the fossil *Euryotomys pelomyoides* (Langebaanweg, South Africa. Lower Pliocene, dated at between 6.0 – 4.5 Myr) to be a linking species between the African Murinae and the Otomyinae and a putative ancestor of the latter, since the dentition showed a transition from the typically murine dental pattern (e.g. cuspidated molars, reduced M^3) to a otomyine one (e.g. entirely laminate molars with no recognizable cusps, enlarged M^3). Its lower molar morphology and double-grooved upper incisors closely resembled that of the murine genus *Pelomys* (e.g. *P. fallax*), but its enlarged M^3 was similar to extant Otomyinae. On this basis, Pocock (1976) questioned the retention of the group as a distinct murid subfamily and regarded them a recent derivative of the Murinae that probably evolved from a *Pelomys*-type ancestor in the last 4 to 5 Myr (Lower Pliocene). This author further considered *Prototomys campbelli* (Taungs, South Africa, Upper Pliocene, < 3.6 Myr, Broom & Schepers, 1946) as being intermediate between *Pelomys* and the modern otomyines (see below).

Conversely, Denys *et al.* (1987) opined that fossil data coupled with biogeographical information provided no definitive evidence that the Otomyinae was more closely related to the Murinae than to other murid subfamilies. The fact that the dentition of *E. pelomyoides* resembled true murids of the *Arvicanthis* division (e.g. *Pelomys*, Pocock 1976; *Saidomys afarensis,* Sabatier 1982) and other living murids (e.g. *Dasymus, Mystromys, Mus*, *Rattus*) did not indicate to these authors that it was necessarily an ancestor to the Otomyinae. Subsequently, evidence in support of a murine origin for the group was provided by *Euryotomys bolti* (Bolt's Farm, South Africa, Lower Pliocene, dated at 5.0 - 4.0 Myr, Sénégas & Avery, 1998). This extinct species is considered the likely ancestor to all Otomyinae because it was unequivocally shown that its dental features were more derived than that of *E. pelomyoides*, but less so than the first true otomyine fossil *Otomys* cf. *gracilis* (Makapansgat, South Africa, dated at approximately 3.7 Myr, Pocock 1987).

In contrast, certain morphological properties of fossil and extant taxa were interpreted as good evidence for separating the Otomyinae from the Murinae. These include: 1) conservative middle ear features linking the subfamily to fossil cricetids, but not necessarily to modern forms (Lavocat & Parent 1985); 2) the short tail length relative to

total body length that represents a deviation from the general trend in murids (Denys *et al.* 1987); 3) the dental morphology of muroid fossils from Kenya (Fort Ternan) that did not suggest a close affinity to the Otomyinae (Tong & Jaeger 1993). Nonetheless, studies of the sperm morphology of five southern African Otomyinae species revealed that the two basic sperm morphs present in the group are also found in the Murinae and that their sperm possess a lateral eve on the acrosome, a feature previously thought to be unique to the Murinae (Bernard *et al.* 1991).

In addition, molecular evidence supported a murine origin for the subfamily, and especially indicated a close affinity to arvicanthine forms (see Misonne 1969; Musser 1987). Chevret *et al.* (1993) indicated a sister relationship between the Otomyinae (represented by *O. irroratus*) and arvicanthine murines (represented by *Arvicanthis niloticus* and *Oenomys hypoxanthus*) based on DNA-DNA hybridization data, and suggested a molecular time scale of 7.1±0.4 Myr (Upper Pliocene) for their divergence. Moreover, phylogenetic relationships inferred from partial cyt *b*, 16S and 12S rRNA mtDNA sequences placed *Otomys* in one of two African murine clades with the genera *Lemniscomys, Arvicanthis, Hybomys, Dasymus, Stochomys* and *Grammomys* (Verheyen *et al.* unpubl.). An estimated time of 5.8 Myr BP was proposed for the separation of the Otomyinae (represented by *O. tropicalis*) from arvicanthine murines (represented by *A. neumanni*). Recently, Ducroz *et al.* (2001) suggested that the Otomyinae (represented by *O. irroratus*, *O. sloggetti*, *P. brantsii* and *P. littledalei* in their study) is nested within the Murinae based on the relationships reflected in topologies retrieved from the analysis of complete cyt *b* and partial 12S and 16S rRNA sequences. The subfamily appeared to consistently group basally in a clade referred to as the "African lineage", but did not cluster as the sister group to the arvicanthine murines (*Arvicanthis, Lemniscomys, Mylomys, Pelomys, Desmomys, Rhabdomys*). Instead, it was more closely related to other murine genera (*Aethomys*, *Dasymus*, *Grammomys, Hybomys* and possibly *Golunda*), which also form part of the "African lineage" but with no clear sister taxon affiliations. Based on this evidence, tribal rank was proposed for both groups (Otomyini and Arvicanthini) within the Murinae, and it was further suggested that the Otomyinae may have been derived from an undifferentiated African murine ancestor, rather than an arvicanthine lineage as suggested by Pocock (1976). The divergence time of the "African lineage" was estimated at between 8.4 and 8.8 Myr ago (see below).

The discrepancy between these dates may be partly due to differences in species representation in the various studies. Furthermore, with the exception of the Ducroz *et al.* (2001) investigation, all the molecular clocks were calibrated using fossil-based dating for the *Mus-Rattus* split as reference point. Slightly different average estimates of the event were used in each case (DNA-DNA hybridization: 10 Myr, Jacobs & Pilbeam 1980; Jaeger *et al.* 1986; mtDNA: 12 Myr, Jacobs & Downs 1994; but see Jacobs 1978; Jacobs *et al.* 1990; Kumar & Hedges 1998; Huchon *et al.* 2000; Yoder & Yang 2000; Adkins *et al.* 2001). Ducroz *et al.* (2001) used an additional calibration based on the 16 Myr old Murinae-Gerbillinae split similarly derived from the fossil record (Tong & Jaeger 1993) to verify the 8.4 Myr estimated using the *Mus-Rattus* calibration. Interestingly, the application of the rate estimate of dental evolution in the Otomyinae (an increase of 1 lamina per 0.93 - 1.3 Myr) placed the divergence of an ancestor with a two-laminate M^3 at 5.6 - 7.8 Myr BP (Chevret *et al.* 1993), which is in good correspondence with the range predicted for the origins of the group using the independent molecular clocks.

The Fossil Record of the True Otomyinae

There is consensus among palaeontologists that the fossil data indicate an ancient origin for the Otomyinae in southern Africa, irrespective of a murid or cricetid derivation for the group (Pocock 1976; Denys *et al.* 1987; Denys 1989; Sénégas & Avery 1998). This is based on the premise that the oldest fossils exhibiting a true otomyine dental pattern (entirely laminate molars; enlarged M^3 and M_1) and their presumed fossil ancestors are known from South Africa. It is further supported by the comparatively more advanced dental pattern of the East African fossils (Denys 1989). Dental evolution in the subfamily was proposed to progress in two main directions. First, was the development of entirely laminate molar patterns from the ancestral murine cuspidated pattern. This was followed by the addition of laminae and the subsequent enlargement of M^3 and to a lesser extent M_1 (Denys 1989; Chevret *et al.* 1993).

The geological period covered by true otomyine fossils spans the Upper Pliocene to the Recent of Africa (Broom 1937; De Graaff 1981; Carleton & Musser 1984). In South

Africa, these are relatively abundant in the Plio-Pleistocene deposits of australopithecine caves in the former Transvaal (e.g. Sterkfontein, Swartkrans, Kromdraai, Taungs and Makapansgat) where they dominate rodent associations in the Pleistocene deposits, being more abundant than murids (De Graaff 1960, 1961; Denys 1989). The earliest known is *Otomys* cf. *gracilis* (Pocock 1987) that was recorded from the oldest Upper Pliocene deposits at Makapansgat (at least 3.7 - 3.5 Myr old, Partridge 1973). Subsequent fossils that appeared at this and other hominid sites belonged to two genera, *Palaeotomys* and *Prototomys* (Denys 1989). *Palaeotomys gracilis*† (Schurveberg, Upper Pliocene, Broom 1937) was later renamed as *O. gracilis* (Pocock 1987). The very rare *Prototomys campbelli* was previously only recorded from Upper Pliocene layers at Taungs (< 3.6 Myr, Broom 1939; Broom & Schepers 1946), but lately from Plio-Pleistocene deposits in Botswana (approximately 3.0 Myr old, Pickford & Mein 1988). This species is considered the closest ancestor to modern otomyines among the fossils discovered to date (Taylor *et al.* 1989a).

Few *Otomys* fossils have been documented from East Africa and they are absent from fossiliferous deposits predating 1.7 Myr (Jaeger 1979; Sabatier 1982; Denys 1989). The oldest known record from this region is *O. petteri* (Olduvai Gorge, Tanzania, Lower Pleistocene, dated at approximately 1.6 Myr, Denys 1989), which was initially erroneously described as *O. kempi* (Lavocat 1965). They are absent from well-described Upper Pliocene sites in Tanzania (e.g. Lateolil, Ndolyana beds at Laetoli), despite palaeo-environmental indications of suitable *Otomys* habitat. It is unclear whether this is due to a biostratigraphical problem, as is the case in some other Plio-Pleistocene rodent faunas (Denys & Jaeger 1986), or if it is a real characteristic of their evolutionary history (Denys 1989). The first East African records of modern *Otomys* fossils are from the Mid-Pleistocene (*O.* cf. *typus*, Melka Kunturé, Ethiopia, Denys 1989), whereas they already appeared in Lower Pleistocene deposits in South Africa (De Graaff 1960, 1961).

Detailed descriptions of the original and subsequent records of these southern and East African fossil Otomyinae and their inter-relatedness are provided elsewhere (*O.* cf. *gracilis*: Pocock 1987; Denys 1989; *Otomys (Palaeotomys) gracilis*: Broom 1937; De Graaff 1960; Carleton & Musser 1984; Pocock 1987; Denys 1989; *Prototomys campbelli*: Broom 1939; Broom & Schepers 1946; Lavocat 1978; Pickford & Mein 1988; Denys

1989; *O. petteri*: Jaeger 1979; Denys 1989; *O.* cf. *typus*: Sabatier 1978; Denys 1989; *Otomys* sp.: Brugal & Denys 1989; *O.* cf. *sloggetti*: Denys 1989; Avery 1991; *O. irroratus*, *O. laminatus* and *O. angoniensis*: Dreyer & Lyle 1931; De Graaff 1960, 1961; Bourquin & Mathias 1984; Avery 1991).

Molecular and Fossil-Based Time Scales for the Evolution within the Otomyinae.

A protein based molecular divergence of 6.4 – 5.5 Myr BP was estimated for the two major otomyine lineages (arid and mesic) (Taylor *et al.* 1989a). The clock was calibrated using the genetic distance (Nei 1971) between the most divergent of four otomyine lineages *O. irroratus, O. unisulcatus, P. littledalei* and *P. brantsii* included in their study. However, Ducroz *et al.* (2001) estimated the split of *O. irroratus* from *O. sloggetti*, *P. brantsii* and *P. littledalei* at 3.7 Myr (range: 4.1 – 3.4) and 3.8 Myr (range: 4.2 – 3.5). These divergence times were obtained using a mtDNA clock calibrated using the *Mus-Rattus* (12 Myr) and the Murinae-Gerbillinae (16 Myr) divergences, respectively. These values are remarkably similar to those derived from the fossil record for the earliest divergence among modern otomyine lineages (3.6 – 3.0 Myr BP; Broom & Schepers 1946; Pickford & Mein 1988) based on the appearance of *Prototomys campbelli*, which, on morphological grounds, seems to be ancestral to all modern Otomyinae (Taylor *et al.* 1989a).

Denys (1989) presented two alternative evolutionary hypotheses for the evolution within *Otomys* during Plio-Pleistocene times. These were based on fossil dates and the dental affinities among an apparently ancestral group of fossil (*O.* cf. *gracilis, O. gracilis*, *O. petteri*) and extant forms (*O. angoniensis, O. saundersiae*) within the true Otomyinae. The anagenetic hypothesis postulated that three lineages, *O. petteri* (East African), *O. gracilis* and *O. saundersiae* (South African), were simultaneously derived from *O.* cf. *gracilis* about 1.0 – 2.0 Myr BP. These lineages were regarded as conspecifics, from which the modern *O. angoniensis* arose approximately 0.6 Myr BP. The alternative hypothesis proposed that distinct East and southern African lineages, represented by *O. petteri* and *O. gracilis*, respectively, were derived from *O.* cf. *gracilis* about 2 Myr BP and eventually gave rise to *O. angoniensis* (from *O. petteri*, approximately 0.6 Myr BP),

and to *O. saundersiae* (from *O. gracilis*, in the last 1 Myr). The latter hypothesis is in accordance the tectonic model of rodent evolution in the Rift Valley (Denys *et al.* 1985).

MITOCHONDRIAL DNA AS A VERSATILE MOLECULAR MARKER AND PURSUING ACCURACY IN MOLECULAR PHYLOGENETIC ANALYSES

MOLECULAR DATA AND PHYLOGENETIC INFERENCE

It has been suggested that fossil data reflect the authentic evolutionary history of organisms (Barton & Jones 1983). However, an adequate fossil record is often lacking or inadequate for many animal groups. With the development of molecular techniques (Mullis & Faloona 1987; Saiki *et al.* 1988) inferring evolutionary history from patterns of genetic affinity among extant lineages became possible. Molecular phylogenies (or gene trees) inferred from appropriate homologous gene sequences at different taxonomic levels can be compared to those recovered from phenotypic characters and so provide different perspectives on the same evolutionary process (Avise 1994; Hillis *et al.* 1996). Molecular phylogenies have also proved to be invaluable for elucidating true relationships that are often obscured by convergent evolution at the morphological level (Wiens 1998b). However, some phylogenies may be unresolved with conflicting phylogenetic patterns suggested for the same organisms by different gene sequences (Moritz & Hillis 1996), probably reflecting the effects of different molecular mechanisms on nucleotide evolution in genes (Broughton *et al.* 1998).

MITOCHONDRIAL DNA

Mitochondrial DNA and Phylogenetic Inference

Animal mtDNA is a small, extra-chromosomal genome, which occurs in multiple copies as a closed circular and double-stranded DNA molecule, which, on average, is approximately 16kb in size in mammals. Apart from a small number of exceptions, all animal mtDNA genomes encode the same 37 genes: the small and large ribosomal RNA genes (12S and 16S rRNA), 22 transfer RNA genes (tRNA), 13 protein-coding genes involved in electron transport or ATP synthesis and one hypervariable, untranscribed

control region that surrounds the origin of replication of the mtDNA molecule. Several features of this cytoplasmic genome offer distinct advantages to study evolutionary biology (Barton & Jones 1983; Harrison 1989). First, a large database is available where clearly homologous sequences can be compared for the construction of universal primers (e.g. Kocher *et al.* 1989; Allard & Honeycutt 1992; Simon *et al.* 1994). Secondly, as a consequence of the maternal mode of inheritance, it is generally thought that it is not subjected to independent assortment and recombination during transmission. Moreover, the effective population size is reduced in comparison to nuclear genes (Avise & Lansman 1983; Brown 1983; Avise *et al.* 1984). The genome is, therefore, a heritable set of completely linked, multiple genetic markers that record historical events occurring along one genetic lineage and reflects matriarchal phylogenies unaffected by male dispersal (Avise 1986; Neigel & Avise 1993). However, evidence of recombination in animal mtDNA has been provided in the literature (Gyllensten *et al.* 1991; Ladoukakis & Zouros 2001a, b; Wiuf 2001; Worobey 2001). Thirdly, mtDNA has a rapid rate of evolution compared to most other vertebrate single copy nuclear genes (Brown *et al.* 1979, Curole & Kocher 1999), which makes it useful for tracing the recent evolutionary history of populations such as colonization, population bottlenecks and geographic origins.

Mitochondrial DNA has been useful for defining phylogenetic relationships across a broad range of sequence divergences since the rates of sequence evolution differ along the length of the molecule (Avise 1986; Moritz *et al.* 1987; Avise 1989; Harrison 1989; Hillis *et al.* 1996). It has been extensively used to infer evolutionary relationships among rodents at various taxonomic levels (e.g. Verheyen *et al.* 1995, 1996; Matthee & Robinson 1997a, b; Engel *et al.* 1998). Homologous sequences of the more conserved ribosomal and protein-coding genes are useful for assessing relationships among genera and species, while the more variable sequences (e.g. D-loop section of the control region) are suitable for studying recently diverged lineages e.g. conspecific populations (Harrison 1989; Hillis *et al.* 1996).

Mitochondrial Cytochrome *b* and 12S rRNA Genes

Use in Phylogenetic Analyses

The phylogenetic value of a gene is ultimately determined by the phylogenetic signal present in the data (Hillis & Huelsenbeck 1992). The gene fragment should be variable enough so that adequate phylogenetic signal can be detected in the sequences compared, but should not be so variable that the true phylogenetic patterns are obscured by random noise (Moritz *et al.* 1987). Based on these criteria, the protein-coding cyt *b* and 12S rRNA genes were selected in an attempt to determine the phylogenetic relationships in the Otomyinae and to address some of the taxonomic uncertainties in this group. In addition, the utility of these two genes for phylogenetic inferences has also been demonstrated by their extensive use in systematic studies at various taxonomic levels across diverse animal groups (Mindell & Honeycutt 1990; Hillis & Dixson 1991; Irwin *et al.* 1991; Graybeal 1993; Simon *et al.* 1994).

Cytochrome *b* is the most frequently used mitochondrial gene in animal studies (Meyer 1994) and particularly relevant to the present study has been used for resolving evolutionary relationships among murid rodents at the generic and species level (e.g. *Akodon:* Smith & Patton 1991; Patton & Smith 1992; Smith *et al.* 1992; Smith & Patton 1993; *Oligoryzomys:* Myers *et al.* 1995; *Arvicanthis*: Ducroz *et al.* 1998; *Acomys*: Barome *et al.* 1998; *Tokudaia* and *Diplothrix*: Suzuki *et al.* 2000). Below the species level, it has provided good resolution in the phylogenies of saxicolous mice, *Phyllotis xanthopygus* (Kim *et al.* 1998) and tuco-tucos, *Ctenomys mendonicus* (D'Elia *et al.* 1999).

Due to the slower rate of evolution of the 12S rRNA gene (Brown *et al.* 1982), it has proved useful in resolving divergence events over the last 10 - 20 million years in mammalian systematics (Springer & Douzery 1996). It has provided good resolution for studying higher order relationships among mammalian taxa (placentals: Springer & Kirsch 1993; orders: Lavergne *et al.* 1996; cetaceans: Douzery 1993; ungulates: Douzery & Catzeflis 1995; artiodactyls and cetaceans: Montgelard *et al.* 1997; rodents: Frye & Hedges 1995; Hänni *et al.* 1995; Rzhetsky 1995; Matthee & Robinson 1997a), but was

also useful at the generic and specific levels in the rodent families Bathyergidae (Allard & Honeycutt 1992), Leporidae (Halanych & Robinson 1999) and Muridae (Sullivan *et al.* 1995).

Structural and Functional Properties of the Cytochrome b and 12S rRNA Genes and Shortcomings for Phylogenetic Analyses

The protein-coding cyt *b* gene forms part of complex III of the mitochondrial oxidative phosphorylization system (Esposti *et al.* 1993) and is involved in electron transfer between dihydroubiquinone to cytochrome *c* and the translocation of protons across the mitochondrial inner-membrane (Hatefi 1985). The nucleotide changes in this gene are constrained according to codon position (first, second and third) and the function of the protein also imposes constraints on substitutions in the different membrane regions of the gene (Howell & Gilbert 1988; Howell 1989; Irwin *et al.* 1991; Graybeal 1993). The 12S rRNA gene produces single-stranded RNA molecules involved in the process of assembly of proteins during protein synthesis (Sullivan *et al.* 1995). The secondary structure is maintained by a complex of functional constraints regulating the rates and patterns of nucleotide substitution (van de Peer *et al.* 1993; Douzery & Catzeflis 1995; Rzhetsky 1995; Springer *et al.* 1995; Sullivan *et al.* 1995). Selective pressures are more pronounced in stems where non-independent compensational nucleotide substitutions occur to maintain complementary base pairing (Wheeler & Honeycutt 1988; Kraus *et al.* 1992; Dixon & Hillis 1993).

Despite their wide application in systematic studies, shortcomings have been identified in DNA sequences that may potentially influence the accuracy of the inferred phylogenies (Wheeler & Honeycutt 1988). These include unequal rates of evolution among different lineages (see below) and the discovery of pseudogenes in diverse animal groups (cyt *b*: Smith *et al.* 1992; Kornegay *et al.* 1993; 12S rRNA: Meyer 1994; van der Kuyl *et al.* 1995). Moreover, compositional biases and variation in the rates of evolution among different nucleotide sites can be related to the functional constraints, which control the sequence evolution of genes (Brown 1985; Wheeler & Honeycutt 1988; Mindell & Honeycutt 1990; Esposti *et al.* 1993; Meyer 1994; Miyamoto & Fitch 1995; Kumar 1996; Simon *et al.* 1996; Yang 1996a, b). Several methods have been proposed to

accommodate these shortcomings (Wheeler & Honeycutt 1988; Hillis & Dixon 1991; Albert & Mishler 1992; Dixon & Hillis 1993; Huelsenbeck *et al.* 1994). These were taken into consideration in the present study, as was the determination of realistic models of sequence evolution for the gene fragments studied (Huelsenbeck & Crandall 1997).

COMBINING DATA FOR PHYLOGENETIC ANALYSES

The prevailing trend is to combine data from different sources (e.g. morphology and molecular data, different genes) in order to obtain the most inclusive estimate of evolutionary relationships among taxa (Hillis 1987). Since homologous sequences of different gene fragments can result in discordant phylogenies it has been suggested that the analyses of multiple genes would likely result in more robust phylogenies than separate analysis of single genes (Avise 1994; Moritz & Hillis 1996; DeSalle & Brower 1997). This can be done with the provision that substitution rates are not significantly different (Farris *et al.* 1995). Several methods have been proposed as to how combined analyses should be conducted (Bull *et al.* 1993; De Quieroz 1993; Chippendale & Wiens 1994; De Quieroz *et al.* 1995; Huelsenbeck *et al.* 1996; Yang 1996b; Ballard *et al.* 1998). It has been pointed out that spurious phylogenetic associations of taxa may be recovered when different genes are combined (e.g. nuclear and mitochondrial) due to the different selection pressures and modes of inheritance (Farris *et al.* 1995; Cunningham 1997; Wiens 1998a). Intuitively, however, reliable phylogenetic relationships should be estimable from combined sequences of multiple mtDNA loci since they are linked on the same molecule and share the same evolutionary history (Brown 1985).

Given this background a decision was taken to analyze sequences from the cyt *b* and 12S rRNA genes independently as well as combined. Examples where the combination of these genes provided good resolution at the generic and specific levels include phylogenies of sigmodontine rodents (Sullivan *et al.* 1995), felids (Janczewski *et al.* 1995) as well as subfamily relationships among rodent families (Bentz & Montgelard 1999).

ACCURACY AND PRECISION IN PHYLOGENETIC ANALYSIS

Efficiency of Maximum Likelihood, Parsimony and Distance Methods in Phylogenetic Inference

Because taxonomic congruence is a powerful tool when assessing phylogenetic robustness (Hillis 1995; Hillis *et al.* 1996; Wiens 1998b), the trees recovered by maximum likelihood (ML, Felsenstein 1981a), maximum parsimony (MP, Kluge & Farris 1969) and distance-based neighbor-joining (NJ, Saitou & Nei 1987) methods were compared in this study to assess whether similar relationships were well supported across different reconstruction methods. However, a large body of literature deals with the fundamental problems of the ML, MP and NJ methods with regards to inferring accurate phylogenies using molecular data (Huelsenbeck 1995). Cognizance was taken of the advantages and limitations of each when Otomyinae relationships inferred with each method were interpreted. The major criticism of the MP method is that it is statistically unsound because it is not based on an explicit underlying model of evolutionary change (Felsenstein 1983). Maximum likelihood is an optimality criterion and has the advantage of being based on explicit evolutionary models, but the phylogenetic estimations can be affected when their assumptions are strongly violated (Swofford *et al.* 1996).

Rate Heterogeneity and Phylogenetic Inference

It has been shown that the reliability of evolutionary conclusions may be influenced if variation in substitution rates among different nucleotide sites and between disparate lineages is not taken into account in phylogenetic analysis. Inaccuracies may arise in 1) inferred phylogenetic relationships; 2) dating of divergence events and age estimations for lineages and 3) empirical parameter estimates of sequence evolution e.g. transition bias (Li *et al.* 1987; Felsenstein 1988; Jin & Nei 1990; Hillis *et al.* 1993; Huelsenbeck & Hillis 1993; Tajima 1993; Lockhart *et al.* 1994; Wakeley 1994; Sullivan *et al.* 1995; Hackett 1996; Miyamoto & Fitch 1996; Yang 1996a, b; Gu & Zhang 1997; Naylor & Brown 1998; Graur & Li 1999). Consequently, strategies have been developed to detect and correct for rate heterogeneity in phylogenetic analyses, several of which were employed in the analyses of Otomyinae sequences.

Models that have been developed to characterize the extent of among-site rate variation within genes either use a discrete (e.g. Hasegawa *et al.* 1985; Palumbi 1989) or continuous statistical distribution (e.g. gamma model, Yang 1993) to approximate the amount of variation among nucleotide sites. Although computationally demanding, the continuous ML-based gamma models were preferred in this study since a single parameter (the gamma-shape parameter, or α) describes rate variation and they are easily interpretable and versatile and have been shown to fit many data sets well (Yang 1996a). Furthermore, ML-based algorithms (e.g. Yang 1993, 1994; Gu *et al.* 1995; Kelly & Rice 1996) tend to more accurately estimate α, since parsimony-based algorithms (e.g. Holmquist *et al.* 1983; Tamura & Nei 1993; Sullivan *et al.* 1995; Tourasse & Gouy 1999) usually underestimate the amount of variation by overestimating α. This is especially so for distantly related sequences (Wakeley 1993; Yang 1996a). Yang and Kumar (1996) proposed a model, which is a combination of likelihood and parsimony, but it assumes a constant molecular clock and is therefore problematic when rates vary among lineages. Consequently, Gu and Zhang (1997) developed an alternative ML model that is presumed to be computationally as fast as the parsimony models, but has estimation accuracy similar to the likelihood models. Several models have also been developed that account for rate differences among gene regions (e.g. Gaut & Weir 1994; Hartmann & Golding 1998), distinct classes of data within genes (Muse & Gaut 1994), and heterogeneity among different genes (e.g. Farris *et al.* 1995; Yang 1995b).

On a finer scale, Zhang & Ryder (1995) showed in their study on Kirk's dik-dik (*Madoqua kirkii)*, that the rates of mtDNA evolution might not be constant among closely related taxa (conspecific populations) given that significantly different rates were detected among specimens belonging to different chromosomal races. *Otomys irroratus* mimics *M. kirkii* in terms of certain karyotypic properties. Three cytotypes have been described in Kirk's dik-dik, which demonstrate a rapid rate of chromosomal evolution (Kumamoto *et al.* 1994). Similarly, the *O. irroratus* karyotype exhibits variation in diploid number ($2n = 23 - 32$) between the two (Rambau *et al.* 2001) or, alternatively, five cytotypes described by Contrafatto *et al.* (1992a, b, 1997). Like Zhang & Ryder (1995), this investigation tested the molecular clock hypothesis in *O. irroratus* at the cytotype level.

AIMS AND KEY QUESTIONS

The present study employed mtDNA data to improve our understanding of the taxonomic and phylogenetic relationships of the Otomyinae, and had two main aims:

1. To determine the phylogenetic relationships of species comprising the African rodent subfamily Otomyinae using sequence data from the mitochondrial cyt *b* and 12S rRNA genes.
   - **a.** Primarily, this focused on the relationships within the genus *Otomys* (*O. angoniensis*, *O. irroratus*, *O. karoensis* ≈ *O. saundersiae*, *O. laminatus*, *O. maximus*, *O. sloggetti*, *O. unisulcatus*, *O. anchietae*, *O. denti*, *O. occidentalis*, *O. tropicalis* and *O. typus*).
   - **b.** The study also determined whether the southern African genus *Parotomys*, which includes the species *P. brantsii* and *P. littledalei*, has a diphyletic association with the genus *Otomys*.

2. To investigate the patterns of sequence evolution in the cyt *b* and 12SrRNA genes among otomyine species and the chromosomal races (cytotypes) of *O. irroratus*.

Key Questions:

1. Which outgroup(s) is most appropriate for phylogenetic analysis?
2. Are the phylogenetic relationships among Otomyinae taxa inferred from mtDNA sequence data concordant with previous estimates of their evolutionary history based on morphological, biochemical and molecular markers?
3. Do the southern and East African taxa form monophyletic groups?
4. What implications do the evolutionary relationships inferred from cyt *b*, 12S rRNA and the combined data have for the generic taxonomy of the group?
   - **4.1.** Do the inferred phylogenetic relationships between species of *Parotomys* and *Otomys* and the genetic distances separating them support the recognition of two distinct genera?
   - **4.2.** If *Parotomys* is retained as a valid genus, are there evolutionary grounds for including *O. unisulcatus* and/or *O. sloggetti* in this genus?

4.3. Is there evidence for the recognition of *Myotomys* as a valid genus?

5. Is there evidence for arid adapted and predominantly mesic adapted lineages in the Otomyinae, and in particular in the southern African taxa?

6. What are the phylogenetic relationships of the recognized Otomyinae species and do these relationships suggest that taxa of uncertain status represent single, or separate evolutionary lineages?

6.1. Is *O. maximus* molecularily distinct from *O. angoniensis*?

6.2. How is the southern African *O. irroratus* related to other *Otomys* species from southern, East, Central and West Africa that, previously, have been synonymized with it?

7. Do the East African *O. typus* and *O. anchietae*, for which more than one representative are included, form monophyletic assemblages?

8. What are the phylogenetic relationships of the *O. irroratus* cytotypes and what is the degree of sequence differentiation among them?

9. If the rates and patterns of sequence evolution in the otomyine cyt *b* gene justify the use of a molecular clock, what is the estimated time of divergence for the Otomyinae according to the cyt *b* clock calibrated for murid rodents by Ducroz *et al.* (1998)?

10. Do the temporal and spatial scenarios for the speciation and radiation of the group in sub-Saharan Africa correspond with previous fossil and molecular-based evolutionary hypotheses?

CHAPTER 2

MATERIAL and METHODS

MATERIAL

The 27 specimens representing all but one (*O. karoensis*) of the 14 accepted species in the subfamily Otomyinae and 11 possible outgroups included in this investigation are presented in Table 2. The ingroup comprised the two *Parotomys* species and 11 *Otomys* species. Among the latter, *O. anchietae* was represented by the subspecies *O. a. lacustris* while *O. typus* was represented by the taxon as well as representatives of one of its subspecies, *O. t. jacksoni* (see Musser & Carleton 1993). Eight specimens representing four of the five *O. irroratus* cytotypes (A1, A2, B and C *sensu* Contrafatto *et al.* 1992a, b, 1997) were also incorporated. Collection localities are given in Figure 2. The A cytotype of *O. irroratus* and *O. karoensis* were not included since material was not available. The latter species was previously synonymized with *O. saundersiae* (Meester *et al.* 1986; Taylor *et al.* 1993). *Otomys occidentalis* (represented by a single specimen) was omitted from most of the analyses due to incomplete data (only 924 bp of cyt *b* sequence was obtained from DNA extracted from teeth). However, passing reference will be made to the status of this species as inferred from phylogenetic analyses based on a truncated data set. Where possible, two or more specimens per taxon from geographically distant localities were included in a preliminary phylogenetic analysis, in an attempt to avoid long-branch attraction (Hillis *et al.* 1996; Lyons-Weiler & Hoelzer 1997). With the exception of *O. a. lacustris*, all specimens identified as representatives of the same species, subspecies or cytotype (Contrafatto *et al.* 1992a, b, 1997; Musser & Carleton 1993) formed monophyletic groups in the trees. Consequently, due to computational limitations involving the ML analyses, a single specimen per species, subspecies or cytotype was included in the core phylogenetic analyses. The only exception to this was *O. a. lacustris* for which two specimens were used resulting in a data set comprising 16 OTUs, hereafter referred to as the main ingroup taxa; indicated by * in Table 2.

Table 2: Specimens representative of the subfamily Otomyinae and outgroups examined in this investigation. The genus and species designation, geographic origin, co-ordinates, voucher specimen (or field numbers where available), collector or source, genes sequenced and GenBank accession numbers (if available) are listed.

Species	Geographic origin	Co-ordinates	Voucher specimen or field number	Collector or source	12S rRNA Accession numbers	Cyt *b* Accession numbers
1. *Otomys anchietae lacustris* *	Chita, Uzungwa Mts., Tanzania	07°36' S 35°48' E	FMNH155623	W.T. Stanley	AF492736	AF492708
2. *Otomys anchietae lacustris* *	Chingombe, Ufipa Plateau, Tanzania	07°52' S 31°43' E	RUCA13272	W.N. Verheyen	AF492737	AF492709
3. *Otomys anchietae lacustris*	Mbizi Forest, Ufipa Plateau Tanzania	07°52' S 31°41' E	RUCA13125	W.N. Verheyen	AF492738	AF492710
4. *Otomys angoniensis* *	Hlambanyati, Mkuze NR, KwaZulu Natal	27°38' S 32°14' E	DM1983	A. Beruti	AF492739	AF492711
5. *Otomys denti*	Mgahinga Gorilla NP, Uganda	01°23' S 29°38' E	FMNH157807	J.C. Kerbis	AF492740	AF492712
6. *Otomys denti* *	Bujuku River, Ruwenzori Mts., Uganda	00°22' N 29°58' E	FMNH144324	J.C. Kerbis	AF492741	AF492713
7. *Otomys irroratus* (A1)	Hogsback, Eastern Cape Province	32°35' S 26°57' E	TM46130	L. Wingate	AF492742	AF492714
8. *Otomys irroratus* (A1)	Kamberg, KwaZulu Natal	29°24' S 29°40' E	DM3628	P.J. Taylor	-	AF492715
9. *Otomys irroratus* (A2)*	Karkloof NR, Howick, KwaZulu Natal	29°18' S 30°13' E	DM1838	P.J. Taylor	AF492743	AF492716
10. *Otomys irroratus* (B) *	Alice, Eastern Cape Province	32°47' S 26°50' E	TM46132	L. Wingate	AF492744	AF492717
11. *Otomys irroratus* (B)	Baines Kloof, Western Cape Province	33°34' S 19°09' E	TM46277	A. Kumirai	AF492745	AF492718
12. *Otomys irroratus* (B)	Groendal NR, Uitenhage, Eastern Cape Province	33°45' S 25°25' E	Not available	A. Kumirai	AF492746	AF492719
13. *Otomys irroratus* (C) *	Algeria, Cederberg Mts., Western Cape Province	32°26' S 19°05' E	DM4317	P.J. Taylor	AF492747	AF492720
14. *Otomys irroratus* (C)	Tweede Tol, Baines Kloof, Western Cape Province	33°34' S 19°09' E	DM4305	P.J. Taylor	-	AF492721
15. *Otomys laminatus* *	Karkloof NR, Howick, KwaZulu Natal	29°10' S 30°05' E	DM2906	P.J. Taylor	AF492748	AF492722
16. *Otomys maximus* *	Shamvura, Rundu, Namibia	18°01' S 20°50' E	TM46056	S. Maree	AF492749	AF492723
17. *Otomys occidentalis*	Mt. Oku, Cameroon	06°15' N 10°26' E	MNHN710	M. Lamotte	-	AF492734
18. *Otomys sloggetti* *	Sani Pass, Drakensberg Mts., Lesotho	29°36' S 29°18' E	DM5027	P.J. Taylor	AF492750	AF492724
19. *Otomys tropicalis* *	Bondwa, Tanzania	07°06' S 37°42' E	RUCA14476	W.N. Verheyen	AF492751	AF492725
20. *Otomys typus* *	Beletta Forest, Jima, Ethiopia	07°32' N 36°33' E	RUCA47	L.A. Lavrenchenko	AF492752	AF492726
21. *Otomys typus jacksoni*	Mt. Rungwe, Tanzania	09°10' S 33°37' E	RUCA6273	W.N. Verheyen	AF492753	AF492727
22. *Otomys typus jacksoni* *	Arusha, Mt. Meru, Tanzania	03°22' S 36°38' E	TM46289	B. Jansen van Vuuren	AF492754	AF492728
23. *Otomys unisulcatus* *	Fish River Valley, Eastern Cape Province	31°55' S 25°25' E	DM2998	P.J. Taylor	AF492755	AF492729
24. *Parotomys brantsii* *	Kalagadi Transfrontier Park, Northern Cape Province	26°28' S 20°37' E	MMKM (HW5315)	H. Erasmus	AF492756	AF492730
25. *Parotomys brantsii*	Kleinsee, Northern Cape Province	29°41' S 17°05' E	TM46133	C.H. Scholtz	-	AF492731
26. *Parotomys littledalei* *	Goegap NR, Springbok, Northern Cape Province	29°42' S 18°02' E	TM46134	T.P. Jackson	AF492757	AF492732
27. *Parotomys littledalei*	Karub River, Hentiesbaai, Namibia	22°06' S 14°17' E	SM-M14736 (CGC6981)	C.G. Coetzee	-	AF492733
Outgroups:						
1. *Aethomys namaquensis* (Murinae)	Itala NR, KwaZulu Natal	28°31' S 31°02' E	V.V.95.64	J.-F Ducroz/L. Granjon	AF492735	-
2. *Aethomys chrysophilus* (Murinae)				Ducroz *et al.* (1998)	-	AF004587
3. *Arvicanthis somalicus* (Murinae)				Ducroz *et al.* (1998)	-	AF004574
4. *Dasymys incomtus* (Murinae)				Ducroz *et al.* (2001)	-	AF141217
5. *Mus musculus* (Murinae)				Bibb *et al.* (1981)	V00711	V00711
6. *Rattus norvegicus* (Murinae)				Gadaleta *et al.* (1989)	X14848	X14848
7. *Rhabdomys pumilio* (Murinae)				Ducroz *et al.* (2001)	-	AF141214
8. *Clethrionomys glareolus* (Arvicolinae)				F.M. Catzeflis	unpublished	unpublished
9. *Gerbillus nigeriae* (Gerbillinae)				F.M. Catzeflis	unpublished	unpublished
10. *Tatera gambiana* (Gerbillinae)				F.M. Catzeflis	-	unpublished
11. *Akodon torques* (Sigmodontinae)				Smith & Patton (1991)	-	M35700

Note: The cytotype of each *O. irroratus* specimen are indicated in brackets after the species name (*sensu* Contrafatto *et al.* 1992a, b, 1997). See introduction for a detailed description of each cytotype.
* - denote specimens used in the core phylogenetic analysis (see text).
Collection abbreviations:
CGC - C.G. Coetzee field number; DM - Natural History Museum, Durban, South Africa; FMNH - Field Museum of Natural History, Chicago, Illinois, United States of America; HW – H. Erasmus field number; MMKM - MacGregor Museum, Kimberley, South Africa; MNHN - Muséum National d'Histoire Naturelle, Paris, France; RUCA - Department of Biology, University of Antwerp, Belgium; SM – National Museum of Namibia, Windhoek, Namibia; TM - Transvaal Museum, Pretoria, South Africa; V.V. – V. Volobouev, Muséum National d'Histoire Naturelle, Paris, France.
Unpublished outgroup sequences were provided by F.M. Catzeflis, Institut des Sciences de l'Evolution de Montpellier, Université Montpellier II, France.

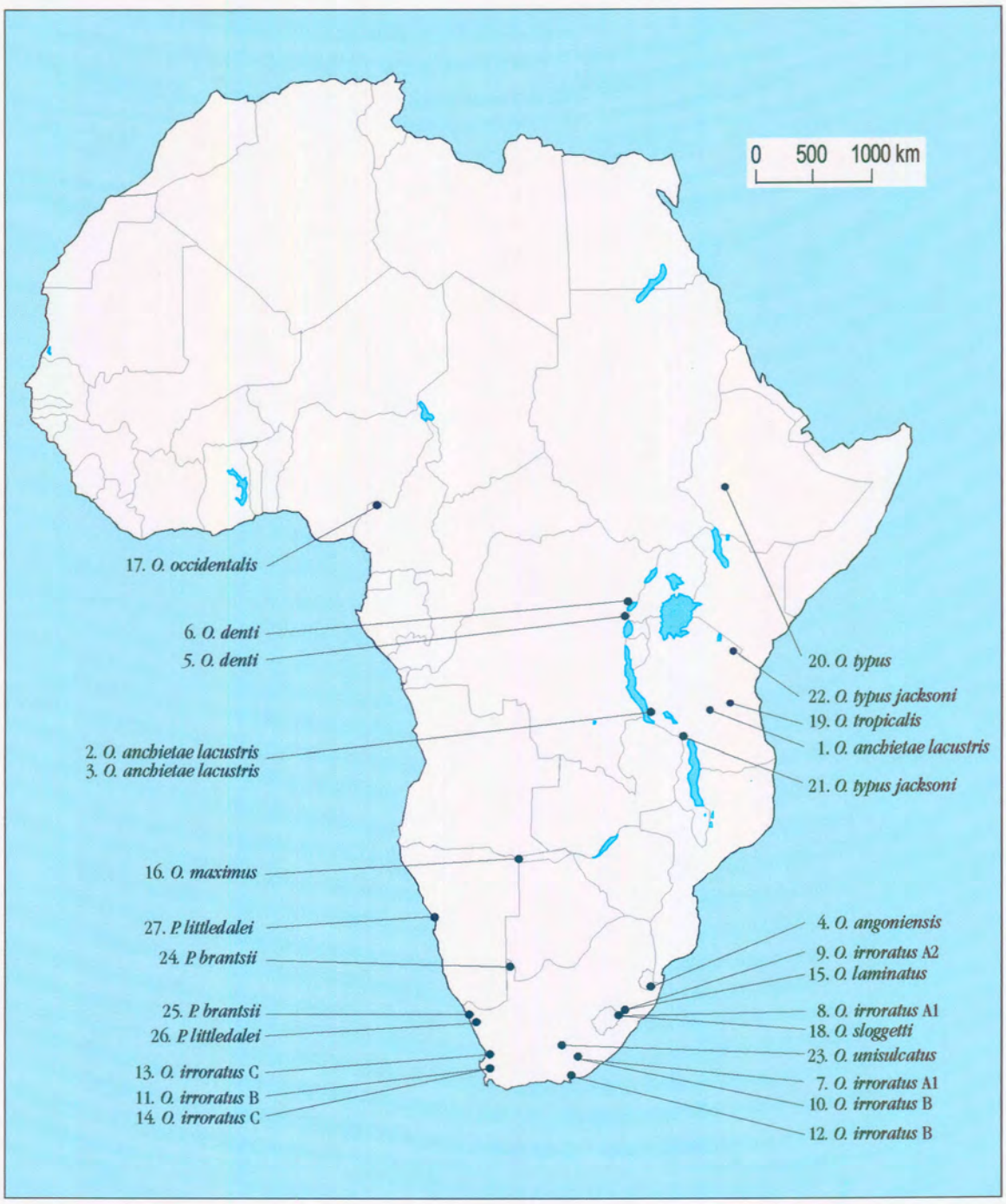

Figure 2: The collection localities for the Otomyinae taxa used in the present study. The numbers before each taxon name correspond to those in Table 2.

METHODS

GENOMIC DNA EXTRACTION, THE POLYMERASE CHAIN REACTION AND NUCLEOTIDE SEQUENCING

Total genomic DNA was extracted from frozen or preserved tissues (20% DMSO/saturated salt solution or 96% ethanol) using standard procedures involving phenol/chloroform/iso-amyl alcohol extraction and overnight precipitation in absolute alcohol (Maniatis *et al.* 1982; Amos & Hoelzel 1991). DNA was resuspended in 1 X TE (T_{10} E_5) buffer to a concentration of 1μg/μl. Since fresh material of *O. occidentalis* was unavailable, molar teeth were used as an alternative source for DNA extractions in this species. Stringent precautions, routinely used in our laboratory, were taken to minimize the risk of contamination with modern DNA or DNA from laboratory sources (Robinson *et al.* 1996; Matthee & Robinson 1999b). Multiple extractions from *O. occidentalis* samples were performed in a separate laboratory where no DNA work had previously been undertaken. Preparation of teeth with 1N HCl, 10% bleach and doubly distilled water (ddH_2O) was adapted from Pääbo (1989) to destroy any contaminating DNA on the surface. A Rotary Power Tool (Sears Best/Craftsman) fitted with flame sterilised, 0.05 mm drill-bits was used to penetrate the HCl treated teeth. The dust was collected into an Eppendorf vial and subsequent extractions followed protocols proposed by Merriwether *et al.* (1994) for ancient teeth modified by Cooper (pers. comm.). DNA was precipitated overnight at -70°C in absolute ethanol followed by an extended precipitation for one week at -20°C to ensure an optimal yield. All reagents, utensils and pipettes used for laboratory procedures were UV irradiated for 15 min prior to use, gloves were regularly changed and masks were worn at all times.

Polymerase Chain Reaction (PCR, Mullis & Faloona 1987; Saiki *et al.* 1988) was employed to amplify the complete cyt *b* gene (1143 bp) using conserved oligonucleotide end-primers situated in the flanking tRNA genes (L14724 in tRNA-Glu and H15915 in tRNA-Thr, Table 3). A fragment of approximately 800 bp at the 5' end of 12S rRNA gene was amplified using conserved primers L82 and H900 (Table 3). PCR volumes of 50 μl were carried out in a Perkin Elmer 2400 Thermocycler using approximately 50ng of template DNA, 5μl each of 10 X PCR buffer, dNTP mix (2 mM) and $MgCl_2$ (25 mM), 1μl of each primer (25 μM), 1 unit of thermostable *Taq* polymerase (Southern Cross Biotechnology) made up to 50μl with dd$H_2$0. The cycling parameters for double-stranded PCR from extractions of fresh or preserved material were: an initial denaturation cycle for 3 min at 94° C followed by 5 cycles that include denaturation for 1 min at 94°C; annealing of primers for 1 min at 45°C-52°C (primer-dependent); extension of the

Table 3: Primer sequences (5' to 3') used for PCR amplification and sequencing of the mtDNA cytochrome *b* and 12S rRNA genes of the Otomyinae. Biotinylated end-primers are marked with an asterisk.

Primer name	Primer sequence[reference]
Cytochrome *b*	
*L14724	5'-CGAAGCTTGATATGAAAAACCATCGTTG-3' [1]
L14841	5'-AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA-3' [2]
L15162	5'-GCAAGCTTCTACCATGAGGACAAATATC-3' [1]
L15408	5'-ATAGACAAAATCCCATTCCA-3' [3]
Otomys L14892	5'-TTCCCAGATGCACTAGGAG-3' [4]
BL	5'-CTCCACACATCCAAACAACGAAG-3' [5]
Otomys H14310	5'-CTGATGTGTAGTGTATGGCT-3' [4]
H15149	5'-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3' [2]
**Otomys* H14794	5'-GGAATTTTGTCTGCGTCTG-3' [4]
H15494	5'-GTGTAGTTGTCAGGGTCTCC-3' [3]
**Otomys* H15112	5'-TAGAGGGTTTGTGTAATTGG-3' [4]
*H15915	5'-AACTGCAGTCATCTCCGGTTTACAAGAC-3' [1]
12S rRNA	
*L82	5'-CATAGACACAGAGGTTTGGTCC-3' [6]
H618	5'-TATCGATTATAGAACAGGCTCC-3' [6]
L509	5'-AAAAAGCTTCAAACTGGGATTAGATACCCCA-3' [6]
*H900	5'-TGACTGCAGAGGGTGACGGGCGGTGTGT-3' [6]
Control region	
H16498	5'-CTTGAAGTAGGAACCAGAT-3' [7]

Note: L and H refer to the light and heavy strands of the double-stranded mitochondrial genome. For cyt *b*, primers are numbered with reference to the 3' nucleotide position on the *Homo sapiens* mitochondrial sequence (Anderson *et al.* 1981). For customized *Otomys* cyt *b* primers and 12S rRNA primers, the numbers correspond to the 3' nucleotide position of the mitochondrial sequence of *Mus musculus* (Bibb *et al.* 1981). The control region primer used in pseudogene assessment were originally designed for ursids (Shields & Kocher 1991) and corresponds to the complementary sequence of the L strand position 16 750 of the harbour seal (Árnason & Johnson 1992).

1 - Pääbo and Wilson (1988).
2 - Kocher *et al.* (1989).
3 - Irwin *et al.* (1991).
4 - Customized *Otomys* primers, this paper.
5 - Matthee and Robinson (1999a).
6 - Allard and Honeycutt (1992).
7 - Shields and Kocher (1991).

nucleotide chain for 90 s at 72°C and a final 25-30 cycles of 30s at 94°C; 30s at 45-52°C; 45s at 72°C; lastly the reaction included a final extension cycle of 5 min at 72°C. A negative control containing all components of the PCR reaction except DNA was routinely included in all reactions to test for contamination. DNA extracted from museum material is often highly degraded and chemically modified (Pääbo 1989; Pääbo *et al.* 1989; Austin *et al.* 1997) and it is therefore not surprising that PCR amplification of *O. occidentalis* DNA extracted from teeth using published primers that spanned long stretches of sequence failed. Consequently, internal *Otomys*-specific cyt *b* primers (Table 3) were designed for amplification of shorter stretches of DNA (< 250 bp). The PCR cycling parameters varied with the primer sets utilized. The basic cycling parameters (50 µl reactions) were: 1 cycle of 1 min at 94°C, 3 cycles of 30s at 94°C (denaturation); 30s at 52°C (primer-annealing); 30s at 72°C (extension); 37 cycles of 30s at 94°C; 20s at 55°C; 20s at 72°C; 5 min at 72°C (final extension). An additional control was included in which universal primers spanning fragments longer than 400 bp were added to test for contamination with contemporary DNA.

The double stranded PCR products were electrophoresed through 1% agarose gels (Techcomp LTD), excised and subsequently purified with a commercial purification system (Nucleotrap Extraction Kit for Nucleic Acids, Macherey-Nagel). Depending on the quality of the template, 1µl of the purified PCR product was used (diluted 20:1, 100:1 or undiluted) for sequencing. For each gene one of the end-primers and selected customized cyt *b* primers were coated with biotin (Table 3) allowing the capture of single-stranded DNA products for sequencing. Single-stranded template was attained by an initial treatment of the purified PCR product with dynabeads-streptavidin (Dynal A.S.), a magnetic particle with an affinity for biotin, and a subsequent denaturing step using 0.1M NaOH. Both the end-primers and internal primers were used for sequencing. The H and L strands of the gene fragments were sequenced to facilitate unambiguous scoring in the regions of overlap. The chain-termination sequencing method of Sanger *et al.* (1977) was employed using the Sequenase Kit version 2.0 (United States Biochemical Corporation). Nucleotides were labelled with [a-^{32}P] dATP allowing for autoradiographic detection of the nucleotides after polyacrylamide (7%) gel electrophoresis.

OUTGROUP CHOICE

Guidelines for outgroup choice suggest that multiple representatives of sister taxa successively related to the ingroup are most effective to reduce tree imbalances, break up long branches and shorten outgroup branch lengths, which may lead to the retrieval of misleading phylogenetic relationships (Maddison *et al.* 1984; Smith 1994; Milinkovitch

et al. 1996; Milinkovitch & Lyons-Weiler 1998). Furthermore, outgroups should be closely allied with the ingroup since with increasing divergence between ingroup and outgroup taxa, shared character states between them may be based on random similarity rather than a shared phylogenetic history (Wheeler 1990). However, choosing the appropriate outgroups to the Otomyinae was not simple. Subfamily groupings within the Muridae (*sensu* Musser & Carleton 1993), the interrelations among the lineages within the closely related subfamily Murinae and its relationship to the Otomyinae, are unclear (Carleton & Musser 1984; Flynn *et al.* 1985; Catzeflis *et al.* 1992, 1993; Chevret *et al.* 1993; Musser & Carleton 1993; Catzeflis *et al.* 1995; Watts & Baverstock 1995; Dubois *et al.* 1996; Robinson *et al.* 1997; Engel *et al.* 1998; Conroy & Cook 1999; Dubois *et al.* 1999; Michaux & Catzeflis 2000; Ducroz *et al.* 2001; Michaux *et al.* 2001; Steppan *et al.* 2001). Although Ducroz *et al.* (2001) recently suggested a sister taxon relationship between the Otomyinae (for whom tribal rank was proposed) and the tribe Arvicanthini within the Murinae, this association received less than 50% bootstrap support (see also Michaux & Catzeflis 2000; Michaux *et al.* 2001).

Given these uncertainties, four murid subfamilies represented by ten genera (6 murine, 2 gerbilline, 1 arvicoline and 1 sigmodontine, see Table 2) were initially included. These outgroup taxa were tested for suitability since the choice of an inappropriate outgroup can significantly decrease the phylogenetic signal present in molecular data sets (Halanych & Robinson 1999). For the preliminary 12S rRNA comparisons the murine taxa *Aethomys namaquensis*, *Mus musculus*, *Rattus norvegicus* and the more distantly related gerbil, *Gerbillus nigeriae* (Gerbillinae) and vole, *Clethrionomys glareolus* (Arvicolinae) were included. *Gerbillus nigeriae* was used as an outgroup based on the findings of Adkins *et al.* (2001). When performing the preliminary cyt *b* comparisons, *Rhabdomys pumilio*, *Arvicanthis somalicus, Dasymys incomtus* (Murinae), *Akodon torques* (Sigmodontinae) and *Tatera gambiana* (Gerbillinae) were included in addition to the taxa used in the preliminary 12S rRNA comparisons. *Akodon torques* was used as an outgroup (also see Adkins *et al.* 2001). The evaluation of the choice of outgroup species was performed using two representatives of the genus *Otomys* (*O. irroratus, O. tropicalis*) and one of *Parotomys* (*P. littledalei*). For each combination of outgroups tested, the following analyses were performed using PAUP 4.0b2a (Swofford 1999): 1) unweighted MP; 2) NJ with the HKY85 correction model (Hasegawa *et al.* 1985); 3) ML with default settings. A second set of analyses were conducted that included all the main ingroup taxa against which outgroups were examined singly and in various combinations.

SEQUENCE ANALYSIS

Sequence Alignment and Pseudogene Assessment

CLUSTAL W (Thompson *et al.* 1994) and visual inspection were used to produce preliminary multiple alignments of cyt *b* (Appendix I) and 12S rRNA (Appendix II) sequences. The individual sequences were deposited in GenBank (accession numbers: cyt *b*: AF492708 – AF492734 and 12S rRNA: 492735 – AF492757). The cyt *b* alignments were verified by converting sequences to 381 amino acids (Appendix III) using the universal mtDNA code in MacClade (version 3, Maddison & Maddison 1992). The possibility of obtaining a nuclear pseudogene during PCR amplification was assessed using criteria for a functional mtDNA protein-coding gene (Smith *et al.* 1992; Esposti *et al.* 1993; Arctander 1995; Zhang & Hewitt 1996). Pseudogenes that are transferred from the mitochondrial to the nuclear genome show an even distribution of substitutions across codon positions, whereas the majority of substitutions of functional mtDNA genes occur at third codon positions (Arctander 1995). This is because selective constraints confine substitutions to regions of the genome, which are less prone to affect the function of the protein, and therefore, 3rd codon positions accumulate changes faster than 1st and 2nd positions combined (Arctander 1995). Furthermore, functional mitochondrial protein-coding genes generally have an intrinsically higher mutation rate compared to the nuclear homologous counterparts (Arctander 1995; Lopez *et al.* 1996). Internal stop codons and substitutions at amino acid sites that are conserved across other vertebrates are further indications that a nuclear homologue may have been amplified (Esposti *et al.* 1993). A reliable indicator of a pseudogene is that double bands are generally observed on autoradiographs. The occurrence of unique amino acid substitutions in both representatives of *P. littledalei* (from Springbok and Hentiesbaai localities, Appendix III) and preliminary base composition and among-lineage rate heterogeneity analyses (see results), prompted further investigation as to whether a nuclear copy of cyt *b* may have been amplified in the species. To confirm the previously obtained sequences for the species (Appendix I) a fragment of approximately 2000 bp of sequence spanning the entire cyt *b* gene (1143 bp), tRNA-Pro, tRNA-Thr and an approximately 550 bp portion of the control region was uninterruptedly amplified with the cyt *b* L14724 (Pääbo & Wilson 1988) and the control region H16498 (Shields & Kocher 1991) primers. The internal cyt *b* H15915 (Pääbo & Wilson 1988) and BL bovid primer (Matthee & Robinson 1999a) was used for sequencing (Table 3).

Differences in the alignment of 12S rRNA sequences (Appendix II) were evaluated when various combinations of gap weights and gap length weights (10 and 1, 10 and 0.5, 20 and 0.5, 50 and 0.5) were specified in CLUSTAL W. The disagreements were negligible

and a gap weight of 10 and a gap length weight of 1 were applied. Subsequently, the 12S rRNA secondary structure model for *Mus* (Damberger & Gutell unpubl. in Sullivan *et al.* 1995) was used as a guideline to differentiate stem and loop regions, and to improve the preliminary alignments of ingroup to outgroup sequences. The model is based on the criteria of potential base-pairing (Watson-Crick and U(T):G-type interactions) and positional covariance and differs somewhat from the core mammalian secondary structure model (Springer *et al.* 1995; Springer & Douzery 1996).

Patterns and Rates of Sequence Evolution

When attempting to estimate accurate phylogenetic relationships among taxa, identifying potential noise in a data set and compensating for it, is of vital importance (Hillis & Huelsenbeck 1992 but see Philippe *et al.* 1996; Broughton *et al.* 2000 for cautionary statements). In order to achieve this, several qualitative and quantitative measures of the rates and patterns of nucleotide substitutions of the entire cyt *b* gene and the sequenced portion of 12S rRNA of the Otomyinae were employed. The various character statistics were calculated using PAUP 4.0b2a and MacClade except where otherwise indicated.

Sequence Variability and Base Composition

Estimates of sequence variability in the cyt *b* and 12S rRNA genes were calculated excluding outgroups so as to more accurately determine character evolution within the Otomyinae. In cyt *b*, sequence evolution was assessed based on the first, second and third codon positions for all 26 ingroup taxa for which complete sequences were obtained (see Table 2). Furthermore, patterns of evolution were determined for the three functional membrane domains distinguished in the structural model of the gene (Howell 1989; Irwin *et al.* 1991; Esposti *et al.* 1993). Variation among cyt *b* sequences was investigated with respect to individual nucleotides. Sequences were also translated to amino acids. Assessments of 12S rRNA sequence evolution were based on the stem-loop secondary structure model for *Mus* and calculations were based on the 22 ingroup taxa sequenced for the gene (see Table 2). The average numbers of transitions and transversions per site were calculated in MacClade using 500 random trees (Halanych 1996), since this program provides an indication of the actual numbers of each substitution type. However, empirical estimates of transition:transversion ratios (ti:tv ratios) were determined with ML in PAUP 4.0b2a, as it has been suggested that the parsimony method used by MacClade generally underestimates the substitution ratios (Halanych 1996). The total numbers of variable and parsimony informative changes, and the mean base composition of each class of data were determined in PAUP 4.0b2a. The nucleotide compositional bias was calculated following Irwin *et al.* (1991). Codon

changes in the membrane partitions of cyt *b* were scored as fast (at least two amino acid changes per site) and slow evolving sites (see Irwin *et al.* 1991, Matthee & Robinson 1997a), and the (2x2) independence test and X^2 tables were used to test whether these regions evolved at different rates.

Sequence Differentiation

Pairwise estimates of nucleotide sequence differentiation between all ingroup taxa sequenced for the cyt *b* and 12S rRNA genes were assessed using the HKY85 substitution model (Hasegawa *et al.* 1985). Uncorrected pairwise nucleotide sequence divergences (Nei 1987) were also calculated for each gene.

Rate Heterogeneity

In a microevolutionary time scale, information on substitution rate heterogeneity within and among DNA sequences represents a potentially important aspect of basic evolutionary processes and may provide insights to the factors governing genome evolution (Zhang & Ryder 1995). Furthermore, failing to account for rate heterogeneity in DNA sequences can be problematic when attempting to produce the best possible estimate of the interrelationships amongst taxa (Hillis *et al.* 1994; Sullivan *et al.* 1995; Yang *et al.* 1995; Yang 1996a, b). Therefore, several methods were employed in this study to test for differences in evolutionary rates among nucleotide sites within the cyt *b* and 12S rRNA genes and among lineages in the Otomyinae.

Among-lineage rate variation

Likelihood ratio test for equal rates of evolution

Initially ML trees were constructed using the HKY85 model of evolution (Hasegawa *et al.* 1985) assuming equal rates of among-site rate variation in PAUP 4.0b2a. Transition to transversion ratios, base frequencies, and log likelihood scores (log L) for each gene were estimated empirically without the constraint of a molecular clock. The resulting trees were subsequently used to calculate log L scores under the assumption of a molecular clock. The two scores for each gene were then compared with the likelihood ratio test (LRT) by calculating a test statistic (twice the absolute difference in log L scores between the two runs), and comparing the result to a χ^2 distribution with the degrees of freedom, n-2 where n is equal to the number of sequences in the tree (Felsenstein 1988; Kelsey *et al.* 1999). Initially, all taxa sequenced for both genes (see Table 2) were analyzed using, *M. musculus*, *A. chrysophilus* (cyt *b*) and *A. namaquensis*

(12S rRNA) as outgroup species. A second round of analysis, that involved the ingroup taxa used in the core phylogenetic analysis and the same outgroup selection subsequently followed this.

Relative rate test

As an alternative to comparing absolute rates of evolution among lineages based on the fossil record, relative rate tests can be implemented to test for deviations from a molecular clock. In these tests, the substitution rates among lineages of similar age are compared (normally within a given taxonomic group) relative to an outgroup (Sarich & Wilson 1973; Wu & Li 1985; Robinson-Rechavi & Huchon 2000). The relative rate test of Robinson *et al.* (1998) and the program RRTree (Relative-Rate Test with a tree, version 1.1.2) were used to test whether the assumption of a molecular clock in the Otomyinae was valid or not. This is considered an improvement on similar tests that compare more than three sequences (Li & Bosquet 1992; Takezaki *et al.* 1995) in that it accounts for phylogenetic relationships among taxa, corrects for biased taxonomic sampling by phylogenetic weighting, and computes the exact probabilities (P) of the observed rate differences (Robinson *et al.* 1998). Initially, pairwise comparisons were made including all otomyine lineages sequenced (Table 2). Thereafter, the most significant of these ($P < 0.01$) were re-analyzed as pairs to avoid inaccurate probability (P) values, which result from multiple testing (Rice 1989). *Aethomys namaquensis* (12S rRNA) and *A. chrysophilus* (cyt *b*) were designated as reference taxa, and *M. musculus* was excluded, considering that the results may be influenced by the introduction of homoplasy when using a relatively distant outgroup (Robinson *et al.* 1998). The test was subsequently repeated using only transversions, and in the cyt *b* comparisons, *A. chrysophilus*, *M. musculus* and *R. norvegicus* were individually used as outgroups. This was done to determine whether a cyt *b* molecular clock calibrated for murid rodents by Ducroz *et al.* (1998) could be applied in the present study for dating divergences between otomyine lineages (see below). On a finer scale, the rates of change between the cytotypes of *O. irroratus* relative to the closely related outgroup *O. laminatus*, were determined and compared with the results obtained in the larger analysis with *A. chrysophilus* as outgroup.

In the case of the cyt *b* gene, synonymous (Ks) and non-synonymous (Ka) substitution rates were computed separately, as substantial differences in evolutionary rates have been documented between these two data classes (Irwin *et al.* 1991; Li 1993; Meyer 1994). Synonymous substitutions included all third position changes and first position leucine changes and non-synonymous substitutions, all second position changes, and all non-leucine first position substitutions. Considering that cyt *b* and 12S rRNA tree topologies

varied with methods of reconstruction and data treatments (e.g. equally weighted and weighted MP employing differential weighting strategies), and that bootstrapped trees were unresolved (see below), P values were computed directly from the data without an input tree. RRTree assigns equal weights to all sequences within a group when no input tree is provided (Robinson-Rechavi & Huchon 2000).

Saturation Analysis

Saturation of sequence data generally occur as the divergence times among taxa increases (i.e. higher sequence divergences) and is thought to cause a loss in the true homologous information contained in the data because the signal is obscured by noise (Brown *et al.* 1982; Hillis 1991; Meyer 1994; Simon *et al.* 1994; Swofford *et al.* 1996; Cunningham 1997; Hassanin & Douzery 1999 but see Philippe *et al.* 1996; Vidal & Lecointre 1998; Broughton *et al.* 2000 for contradicting arguments). The extent of homoplasy was measured in the cyt *b* and 12S rRNA data by comparing the consistency and retention indices (CI and RI) resulting from unweighted parsimony analyses with and without the removal of transitions. This approach was taken because simple regression analysis is unreliable for determining the slope of saturation plots, which are often used to visualize the rates and patterns of nucleotide change in genes and gene partitions (Simon *et al.* 1996).

The Molecular Clock and Dating of Evolutionary Divergences

The use of a molecular clock in phylogenetic studies is a contentious issue (e.g. Gillespie 1986; Easteal *et al.* 1995; van Tuinen & Hedges 2001). The estimated dates of divergence among lineages are considered speculative when the fossil record is inadequate and especially when sequence divergences are high (Wilson *et al.* 1987; Vawter & Brown 1986; Martin & Palumbi 1993; Zhang & Ryder 1995; Martin 1999). Given this, and in the absence of any quantifiable estimate, a clock calibration can be useful for placing lineage origins and the associated evolutionary processes in a temporal framework (Frye & Hedges 1995; Springer 1995; Krajewski & King 1996; Ducroz *et al.* 1998; Rambaut & Bromham 1998; Matthee & Robinson 1999a; Jansen van Vuuren & Robinson 2001). Molecular clocks have often been used to approximate the divergence times of rodent lineages at various taxonomic levels. For example, the diversification among subfamilies, tribes, genera and subgenera in the African Muridae (based on complete cyt *b* and partial 12S and 16 rRNA sequences, Ducroz *et al.* 2001; partial sequences of cyt *b*, 12S and 16S rRNA, Verheyen *et al.* unpubl.), among genera in the subfamilies Sigmodontinae, tribe Akodontini (cyt *b*, Smith & Patton 1993) and Arvicolinae (cyt *b* and a portion of ND4, Conroy & Cook 1999), as well as among

species in the African murine genus *Arvicanthis* (cyt *b*, Ducroz *et al.* 1998).

With this as background an attempt was made to date the divergences of otomyine lineages using a cyt *b* molecular clock calibrated on murid data (Ducroz *et al.* 1998). By calibrating evolutionary rates using murid taxa and a murid fossil reference point (the 12 Myr old split between *Mus* and *Rattus*, Jacobs & Downs 1994), Ducroz *et al.* (1998) accounted for the acceleration of DNA rates demonstrated in murid rodents (She *et al.* 1990; Catzeflis *et al.* 1992). The clock suggests a rate of 1.53% sequence divergence of third position transversions per one million years. The earliest divergence event among lineages within the Otomyinae was estimated by using the highest interspecific sequence divergence measured in this study (the percentage uncorrected p-distance, Nei 1987, based on third position transversions) and subsequently dividing it by 1.53%. A similar approach was followed to approximate the times of the earliest divergence events within each of the major mtDNA clades that were consistently retrieved from the analyses presented herein. This methodology follows Jansen van Vuuren and Robinson (2001) who applied Matthee and Robinson's (1999a) transversions-based cyt *b* clock, which was calibrated against the fossil record for the family Bovidae, to date the earliest divergence event among contemporary lineages of duiker antelope.

PHYLOGENETIC ANALYSES

No general consensus has been reached on the best approach to phylogeny reconstruction (Flores-Villela *et al.* 2000), but it is widely accepted that congruence among topologies retrieved from different data sets and data treatments may be indicative of the robustness of phylogenetic associations (Swofford 1991; Hillis *et al.* 1993; Lockhart *et al.* 1994; Miyamoto *et al.* 1994; Hillis 1995; Hillis *et al.* 1996; Kim 1996; Härlid *et al.* 1997; Håstad & Björklund 1998; Wiens 1998b; Flores-Villela *et al.* 2000). Therefore, different methods were employed in the analysis of cyt *b*, 12S rRNA and the combined cyt *b* and 12S rRNA sequences in an attempt to identify nodes that are strongly supported across treatments and by character congruence.

Methods of Phylogenetic Reconstruction

Three different phylogenetic methods (ML, MP and NJ) were applied to cyt *b*, 12S rRNA and the combined data set using PAUP 4.0b2a. The degree of character support for each node of the resulting trees was assessed by bootstrap re-sampling analysis (Felsenstein 1985; Hillis & Bull 1993; Felsenstein & Kishino 1993). Since all characters of both genes could be unambiguously aligned, none were excluded from the phylogenetic analyses. All trees were constructed from sequences of the main ingroup taxa (Table 2)

using *M. musculus*, *A. chrysophilus* (cyt *b*) and *A. namaquensis* (12S rRNA) as outgroups.

It has been shown that outgroups can be a potential source of noise in DNA data sets which may obscure the true phylogenetic signal contained by the ingroup sequences e.g. in the lagomorph genus *Lepus* (Halanych *et al.* 1999). To assess whether the deeper branching order of taxa obtained in the trees were influenced by the inclusion of outgroup sequences, a separate set of analysis was conducted on unrooted networks of the main ingroup taxa (Table 2) using ML, unordered MP and NJ methods.

Maximum Likelihood Analysis

Parameter estimation of overall and regional transition bias and among-site rate variation and maximum likelihood tree construction

Maximum likelihood allows reliable estimates of the parameters of sequence evolution (transition bias, or κ, among-site rate variation, or α and Pinv) either empirically, or using a guide tree (e.g. Hasegawa *et al.* 1985; Yang 1993, 1994; Gu *et al.* 1995). Therefore, ML methods, as implemented in PAUP 4.0b2a were used to assess the sequence evolution of the complete cyt *b* and 12S rRNA genes and the three codon positions of cyt *b* and the stem and loop regions of 12S. In each case, the HKY85 model of nucleotide substitution (Hasegawa *et al.* 1985) was applied since it accommodates unequal equilibrium base frequencies and accounts for differences in the relative rates of accumulation of transitions and transversions in animal mtDNA (Brown & Simpson 1982; Hasegawa *et al.* 1985). Maximum likelihood trees were generated from the complete sequences of both genes while empirical parameter estimates of κ and α were simultaneously calculated (overall parameters: Tables 4, 5). These two parameters should be estimated concurrently, since estimates of κ are profoundly influenced by variation among sites (Wakeley 1994). Additionally, three alternative models of among-site rate variation were examined and the relative fit of these models to the sequence data was evaluated using the log likelihood ratio test (LRT, see below). The models were: one that assumed equal rates of substitution at all sites, a gamma-distributed rates model for all sites (Γ, Yang 1994), and a mixed distribution model (I+Γ, Gu *et al.* 1995; Waddell & Penny 1996) which combines the invariable sites (I) model of Fitch (1986) and the gamma model. When the mixed distribution model was applied, the proportion of invariable sites (Pinv) were concurrently estimated with κ and α. Six rate categories were used in the Γ and I+Γ models when estimating α. Similarly, simultaneous ML estimates of κ, α and Pinv for the three codon positions of cyt *b* and the stem and loop

Table 4: Empirical parameter estimates (overall and codon-specific) of the gamma-shape parameter (α), transition bias (κ) and proportion of invariable sites (Pinv) of the cytochrome *b* gene. Estimates were derived directly from the data in ML under three models of among-site rate variation. This was done across the main ingroup taxa incorporated into the core phylogenetic analysis (see Table 2) with outgroups *Mus musculus* and *Aethomys chrysophilus* both included and excluded.

Taxa included	Model	Data Partition											
		Overall			First position			Second position			Third position		
		α	κ	Pinv	α	κ	Pinv	α	κ	Pinv	α	κ	Pinv
In + outgroup	Equal rates	-	4.795	-	-	4.754	-	-	5.311	-	-	9.536	-
	Γ	0.180	7.977	-	0.108	8.918	-	0.005	5.532		2.160	12.461	
	I+Γ	1.645	7.916	0.596	0.326	9.704	0.549	infinity	6.046	0.930	2.901	12.521	0.019
Ingroup	Equal rates	-	7.626	-	-	8.523		-	6.028	-	-	10.504	-
	Γ	0.179	10.491	-	0.082	12.420		0.002	6.090		2.473	13.488	
	I+Γ	1.899	10.493	0.625	0.227	13.201	0.526	0.226	6.012	0.825	2.524	13.457	0.002

Abbreviations: Γ = gamma distribution model (Yang 1994); I+Γ = mixed distribution model (Gu *et al.* 1995).
"I" refers to invariable sites and "Γ" refers to the gamma-shape parameter.

Table 5: Empirical parameter estimates (overall and stem-loop-specific) of the gamma-shape parameter (α), transition bias (κ) and proportion of invariable sites (Pinv) of the 12S rRNA gene. Estimates were derived directly from the data in ML under three models of among-site rate variation. This was done across the main ingroup taxa incorporated into the core phylogenetic analysis (see Table 2) both including and excluding outgroups *Mus musculus* and *Aethomys namaquensis*.

Taxa included	Model	Data Partition								
		Overall			Stems			Loops		
		α	κ	Pinv	α	κ	Pinv	α	κ	Pinv
In + outgroup	Equal rates	-	2.727	-	-	5.183	-	-	2.390	-
	Γ	0.104	3.643	-	0.006	5.540	-	0.187	4.189	-
	I+Γ	0.410	3.959	0.610	0.546	5.673	0.701	0.778	4.238	0.544
Ingroup	Equal rates	-	4.784	-	-	14.567	-	-	4.609	-
	Γ	0.065	6.519	-	0.004	15.576	-	0.190	6.269	-
	I+Γ	0.479	6.963	0.683	0.530	17.510	0.835	0.631	6.656	0.515

Abbreviations: Γ = gamma distribution model (Yang 1994); I+Γ = mixed distribution model (Gu *et al.* 1995). "I" refers to invariable sites and "Γ" refers to the gamma-shape parameter.

regions of 12S rRNA were determined from the relevant sequence data under the same three models of among-site rate variation (Tables 4, 5). When the main ingroup taxa were analysed using cyt *b* and 12S rRNA data, midpoint rooting was applied. This was aimed at a more accurate approximation of the model of sequence evolution for the ingroup, and to assess the effect of possible saturation on the branches leading to the outgroups. Base frequencies empirically derived from the sequence data of each gene and gene partition were used during parameter estimation.

Likelihood ratio test and choice of a substitution model

The ML method provides reliable and statistically rejectable hypotheses of sequence evolution (Yang *et al.* 1994; Yang 1996a, b; Huelsenbeck & Crandall 1997; Huelsenbeck & Rannala 1997) by which one model of sequence evolution can be rejected in favor of another. Consequently, the log likelihood ratio test (LRT, Goldman 1993) was employed to evaluate the relative fit of the alternative ML models of evolutionary change (HKY+ equal rates, HKY+Γ, HKY+I+Γ) to the 12S rRNA and cyt *b* sequence data. This analysis evaluates whether more complex models explain the relevant data better than simpler models, and is an appropriate test for comparing nested models (Cunningham *et al.* 1998; Voelker & Edwards 1998; Waits *et al.* 1999). The test statistic ($\delta = 2[|\ln L_1 - \ln L_0|]$) is assumed to follow a χ^2 distribution with the degrees of freedom dependent on the difference in the number of parameters that are free to vary among models. The log L score of the ML tree derived when applying a simpler model is compared to the score of the tree derived under the progressively more complex model. The LRT test was also employed to assess whether the application of partition-specific estimates of κ, α and Pinv within each gene versus applying overall estimates, resulted in a significant increase of model fit to the data. For this purpose, an iterative search strategy was followed (Voelker & Edwards 1998). In this case, empirical codon-specific (cyt *b*) and stem-loop-specific (12S rRNA) estimates of κ, α and Pinv were specified as input values to calculate log L scores for each of the partitions under the HKY+I+Γ model using the tree derived under the same model with the overall parameters (κ, α and Pinv) specified for each gene. The log L scores for each partition were summed to estimate an overall log L score for each gene. Parameters were only retained for further phylogenetic analyses if their addition significantly improved the fit between the ML model and the data. In addition, the more sophisticated MODELTEST (version 3.06; Posada & Crandall 1998) was employed.

Parsimony Analysis

Trees were generated by executing heuristic searches (equally weighted and weighted) with tree bisection-reconnection (TBR) branch swapping. Randomization of starting options (n=100) was used to address the islands-of-trees phenomenon (Maddison 1991). A consensus tree (50% majority rule) was produced when more than one tree of minimal length was found. A total of 500 bootstrap iterations were conducted to assess nodal robustness. The numbers of unambiguous changes along the branches were calculated in MacClade.

All parsimony analyses were performed with all characters of cyt *b*, 12S rRNA and combined data sets included. The reasoning behind this approach is two-fold. First, it has been shown that phylogenetic information may be lost at some taxonomic levels by excluding certain classes of data (e.g. 3rd codon positions) or substitution types (e.g. transitions) that have been assumed to be unreliable in phylogenetic analysis (Simon *et al.* 1994; Philippe *et al.* 1996; Yoder *et al.* 1996; Vidal & Lecointre 1998; Broughton *et al.* 2000 and references therein). For example, the quantification of homoplastic and consistent transitions and transversions in mitochondrial protein-coding genes suggests that previous assumptions about the poor phylogenetic utility of transitions were probably premature (Broughton *et al.* 2000). Transitions were shown to provide substantially more useful phylogenetic information than transversions, given that the number of consistent transitions was far greater than the consistent transversions, despite the higher relative amounts of homoplasy present in the former by virtue of their faster evolutionary rate. Secondly, it has been suggested that the removal of characters to reduce homoplasy does not fundamentally increase general congruence (Phillipe *et al.* 1996; Vidal & Lecointre 1998).

Weighted parsimony

Applying weighting schemes to characters or transformation types might be useful in improving the congruence and internal robustness of phylogenetic estimates by enhancing the phylogenetic signal and reducing homoplasy in the data (Albert & Mishler 1992; Chippendale & Wiens 1994; Milinkovitch *et al.* 1996; Swofford *et al.* 1996; Edwards & Arctander 1997). However, it has also been shown that down weighting of transitions may result in a loss of valuable phylogenetic information given that these changes may contain hidden signal (see Broughton *et al.* 2000 and above). Therefore, in addition to the unweighted analyses of the cyt *b*, 12S rRNA and combined data sets, the impact of weighting on tree topology, and the congruence among topologies retrieved under different weighting schemes, was explored. This was done by incorporating *a*

posteriori weighting strategies into the analyses through the use of stepmatrices. These strategies were based on empirical estimates of the ti:tv ratio (κ) excluding the outgroups to more accurately assess relationships within the Otomyinae. Weighting schemes based on both overall and partition-specific κ estimates were alternatively applied to each data set to assess whether phylogenetic resolution is improved by accounting for differences in the dynamics of character evolution within each gene.

For cyt *b*, each codon position was initially weighted according to ingroup codon-specific κ parameters (Table 4) determined under the best-fit ML model (HKY+I+Γ, see results). The resultant tree was compared to the tree derived from κ values obtained under the best-fit model across codon positions. In addition, the topology of the tree recovered using the codon-specific weighting scheme was compared with those resulting from weighting schemes based on overall and codon-specific κ values calculated with the outgroups included (Table 4). Furthermore, sites were weighted by the inverse of their relative proportion of variable sites at each codon position of the main ingroup, standardized to the 3rd position (Huelsenbeck *et al.* 1994; Krajewski & King 1996) in combination with overall and codon-specific weighting strategies. Trees were constructed using transversions only, and by excluding leucine changes (retaining replacement substitutions and 3rd position transversions). First and 3rd position T↔C transitions were coded as Y (pYrimidine) and 3rd position A↔G transitions were coded as R (puRine; Irwin & Wilson 1993; Irwin & Arnason 1994). Leucine codons have been reported to evolve rapidly and may obscure phylogenetic relationships (Irwin *et al.* 1991). Translated amino acid sequences were analyzed with equal weights applied to all characters. Although unequal substitution rates among synonymous and non-synonymous cyt *b* sites have been reported for mammals (Irwin *et al.* 1991), these two classes of data were not analyzed separately given the small number (31) of informative, non-synonymous codons in the ingroup (also see Halanych *et al.* 1999).

In the case of the 12S rRNA parsimony analysis indels (insertions/deletions) were treated as "missing" characters, but were scored as present (1) or absent (0) as additional characters to the end of the matrix (see Appendix II). A transitional change weight equal to 1 was applied to all indels when differential weighting schemes for transitions and transversions were applied (also see Jansen van Vuuren & Robinson 1997). The empirical ingroup-specific overall κ value (7:1) estimated under the best-fit model for the entire gene (HKY+I+Γ, see results) as well as stem-loop-specific parameter estimates of κ determined under the HKY+Γ model were applied (Table 5). To accommodate the different views on character evolution in stem regions, trees were initially constructed with stems and loops weighted equally, and subsequently by down weighting stem sites

(0.61, Springer *et al.* 1995) to account for non-independent compensational changes at these sites (Wheeler & Honeycutt 1988; Kraus *et al.* 1992; Dixon & Hillis 1993). Additionally, a heavier weight (3.63) was assigned to stem sites based on the inverse of relative character variability in each region (main ingroup) so as to adjust for the slow rate of change in stems (Miyamoto *et al.* 1994). It is thought that this would almost neutralize the weighting against compensatory changes (Krajewski *et al.* 1997). Since all three weighting regimes resulted in an almost identical tree topology following bootstrap analyses (see results), stems and loops were weighted equally in all subsequent analyses. Trees were also constructed using overall and stem-loop-specific κ values; these estimates including outgroups (Table 5). Transversions were not analyzed independently since too few parsimony informative sites (22) were present in the ingroup to allow for separate analysis.

The six-parameter parsimony model of evolution (Williams & Fitch 1990) was applied to the cyt *b* and 12S rRNA data sets (main ingroup and outgroups). This weighting strategy assigns a different weight to each of the six possible transformations (A⇔C, A⇔G, A⇔T, C⇔G, C⇔T, G⇔T) that are applied to the data via *a posteriori* stepmatrices under a generalized parsimony framework (Williams & Fitch 1990; Swofford *et al.* 1996). The stepmatrices for each data set (Table 6) were based on estimates of the substitution frequencies (rate or "R" matrix) of the six substitutional classes derived under the general time-reversible (GTR) ML model (Lanave *et al.* 1984; Tavaré 1986; Rodriguez *et al.* 1990) in PAUP 4.0b2a. These R matrices were approximated from the ML trees retrieved for each gene under the best-fit model (HKY+I+Γ, see results). Subsequently, a Microsoft EXCEL spreadsheet was used to produce the stepmatrices for each gene by converting the six values of the R matrix to proportions, and calculating the natural logarithms (Felsenstein 1981b; Albert & Mishler 1992; Cunningham 1997). Unordered and unweighted parsimony analysis of the ingroup cyt *b* and 12S rRNA gene sequences was conducted using midpoint rooting, which does not permit differential weighting of transitions over transversions (overall, partition-specific, six-parameter and character weighting).

Table 6: Stepmatrices[a] used to assign a rate-specific weight to each of the six possible transformation types (A⇔C, A⇔G, A⇔T, C⇔G, C⇔T, G⇔T) in the six-parameter parsimony analysis of the cytochrome *b* and 12S rRNA data sets in PAUP 4.0b2a (Swofford 1999).

Gene	Change from	Change to			
Cytochrome *b*		A	C	G	T
	A	-	3	2	3
	C	3	-	5	0
	G	2	5	-	6
	T	3	0	6	-
12S rRNA		A	C	G	T
	A	-	3	1	3
	C	3		25	0
	G	1	25	-	5
	T	3	0	5	-

a - stepmatrices were produced by approximating the relative rates of the six substitutional classes (R matrices) using the general time-reversible model (Lanave *et al.* 1984; Tavaré 1986; Rodriguez *et al.* 1990) and the ML trees derived with the best-fit HKY+I+Γ model of evolution in PAUP 4.0b2a. R matrices were subsequently converted to proportions and log-transformed (natural) with EXCEL (Stanger-Hall & Cunningham 1998).

Distance Analysis

The NJ method was used to fit a tree to the HKY-corrected distance matrix estimated from cyt *b* and 12S rRNA alignments (Hasegawa *et al.* 1985). Among-site rate variation was incorporated into the analyses by setting values to the overall α and Pinv estimated under the best-fit model (HKY+I+Γ) for the complete genes (Table 4, 5). The influence of transition bias, in addition to α and Pinv, was also assessed using empirical overall κ estimates as input values (Table 4, 5) with the ML distance model (Felsenstein 1984). In addition the simpler Kimura two-parameter model, which assumes equal base frequencies and two substitution types (Kimura 1980), was also examined. In the case of ingroup analyses, an identical approach was followed and ingroup-specific parameter estimates of α, κ and Pinv were specified as input values (Table 4, 5).

Combined Analysis

Tests to determine whether the 12S rRNA and cyt *b* data sets could be combined were done using the partition homogeneity test in PAUP 4.0b2a (Farris *et al.* 1995). Various strategies were used to incorporate details of the dynamics of sequence evolution of the two independent genes into the phylogenetic analyses of the combined data set. These procedures were chosen after careful consideration of the literature on the treatment of combined data (e.g. Bull *et al.* 1993; De Quieroz 1993; Chippendale & Wiens 1994; Sullivan 1996; Huelsenbeck *et al.* 1996; Cunningham 1997; Ballard *et al.* 1998; Waits *et al.* 1999; Wiens 1998a; also see introduction). For the ML analyses a tree was constructed under the HKY+I+Γ model using the empirical estimation of κ, α and Pinv obtained from the combined data set. This model was chosen since it gave the best fit to the independent unpartitioned cyt *b* and 12S rRNA data sets. The resulting tree was used as the input topology to calculate log L scores for each gene fragment (also see Waits *et al.* 1999). The sum of the likelihood scores for each gene was compared to the overall log L score for the unpartitioned data set using the LRT (Goldman 1993). A similar approach was subsequently followed to evaluate whether the application of partition-specific parameter estimates (κ, α and Pinv) for the three codon positions of cyt *b* (Table 4) and for stems and loops in 12S rRNA (Table 5), resulted in a significant increase in model fit. Ingroup analyses were conducted in a similar fashion, but ingroup-specific parameter estimates for each gene and gene partition were utilized.

In the case of the parsimony analysis, the combined data were analyzed using unweighted and gene-specific and partition-specific weighting schemes. The empirical overall and partition-specific κ values inferred with the HKY+I+Γ model for the ingroup were

applied for weighting to each gene and gene partition (Tables 4, 5). These trees were compared to topologies constructed when κ values estimated from ingroup and outgroup sequences were applied as weighting schemes (Tables 4, 5). Equal weights were assigned to all characters, and gaps were treated as was discussed for the 12S rRNA analyses. An unweighted approach was followed when only the main ingroup taxa were included in the analysis.

A NJ tree was constructed from HKY-corrected distances (Hasegawa *et al.* 1985) with incorporation of the empirical α and Pinv values that were estimated for the combined data set under the HKY+I+Γ model. The resulting topology was compared to the tree derived with the ML distance model (Felsenstein 1984) and the Kimura-2-parameter model (Kimura 1980). For the analysis of the ingroup, empirical estimates of κ (9.8), α (1.087) and Pinv (0.671) derived from the main ingroup taxa were applied, using the distance models above.

Analysis of O. irroratus Cytotypes based on Cytochrome b.

A separate analysis of the cyt *b* sequences from the *O. irroratus* cytotypes (A1, A2, B and C) was conducted using ML, MP and NJ. The 12S rRNA was highly conserved between the cytotypes and was therefore not appropriate for this level of analysis. Two representatives of both the A1 and C cytotype, one representative of the A2 cytotype and three representatives of the B cytotype were included in the analysis (Table 2). Trees were rooted using *O. laminatus* since high bootstrap support was consistently obtained for a sister relationship between this taxon and the clade comprising the *O. irroratus* cytotypes in the larger phylogenetic analysis (see results). The ML tree was constructed under the best-fit ML model for cyt *b* (HKY+I+Γ, see above). In order to determine any ingroup-specific transition bias, the ML search was repeated using an unrooted network of the specimens representing the different cytotypes. A distance tree was constructed applying the HKY85 correction model to pairwise distances. To account for among-site rate variation, the empirical α and Pinv estimates were specified as input values. For parsimony analysis, the data were analyzed with characters unweighted and weighted (empirical κ values for the ingroup obtained with ML under the HKY+I+Γ model). Bootstrap analysis (100 replicates) was performed in ML and 500 iterations in MP and NJ analyses.

Statistical Evaluation of Alternative Topologies using Maximum Likelihood

Alternative evolutionary hypotheses (at various taxonomic levels) were directly compared by quantitatively testing for significant differences at the 95% level using the log likelihood comparison test of Shimodaira and Hasegawa (1999) as implemented in PAUP 4.0b2a. First, the optimal ML trees recovered under the best-fit HKY+I+Γ model for each of the cyt *b*, 12S rRNA and combined data sets were evaluated against the optimal topologies retrieved with MP and NJ. For each data set, the optimal MP trees were those recovered under the partition-specific weighting scheme derived from the empirical κ value for each gene partition (HKY+I+Γ, Table 4, 5). The optimal NJ trees were those inferred from HKY85-corrected distance matrices with incorporation of the empirical κ and Pinv values for each data set. Log likelihood scores were calculated for the optimal MP and NJ trees (recovered from cyt *b*, 12S rRNA and combined sequences, respectively), and each tree was subsequently loaded as a constrained topology with empirical estimates of κ, α and Pinv (HKY+I+Γ) specified as input values.

For each data set three different user-defined trees with topological constraints enforced were evaluated against the optimal ML tree. This was done by comparing their respective log L scores with the Shimodaira-Hasegawa test so as to determine whether any of the optimal ML topologies recovered from mtDNA sequence data were significantly better estimates of Otomyinae phylogenetic relationships than were suggested in previous hypotheses. First, the monophyly of the genus *Parotomys* (*P. brantsii* and *P. littledalei*) was constrained, since the recognition of *Parotomys* as a separate genus has been questioned (Taylor *et al.* 1989a, b; Bernard *et al.* 1991; Contrafatto *et al.* 1994; Rambau *et al.* 1997; Govender 1999; Ducroz *et al.* 2001). Second, an arid clade comprising *P. brantsii*, *P. littledalei* and *O. unisulcatus* was constrained, since a dichotomy between an arid and mesic lineage in the southern African Otomyinae has been suggested (Pocock 1976; Taylor *et al.* 1989a, b; Contrafatto *et al.* 1994). Third, the monophyly of the Central and East African Otomyinae included in this study (*O. anchietae, O. denti, O. tropicalis* and *O. typus*) was enforced to test a recent proposal based on morphological and allozyme data that the Otomyinae from Central, East and West Africa is not monophyletic (Taylor *et al.* pers. comm.).

CHAPTER 3

RESULTS

OUTGROUP CHOICE

Preliminary evaluations for the choice of outgroup species based on 12S rRNA data and using the gerbil, *G. nigeriae* as outgroup, resulted in high bootstrap support for the murine *A. namaquensis* as the closest sister taxon to the Otomyinae among the competing outgroups (ML: 92%; MP: 87%; NJ: 97%). This was followed by an unresolved dichotomous clade formed by *M. musculus* and *R. norvegicus* (ML: 78%; MP: 72%; NJ: 84%) and *C. glareolus* (Arvicolinae) that were successively basal. In the topologies inferred from cyt *b* sequences where the sigmodontine murid *A. torques* was used as outgroup, a clade comprising the three Otomyinae taxa (*O. irroratus, O. tropicalis* and *P. littledalei*) and all the murine genera (*Arvicanthis somalicus, Aethomys chrysophilus, R. pumilio, D. incomtus, M. musculus* and *R. norvegicus*) were contained in an unresolved polytomy following bootstrap analysis (ML: 57%; MP: 99%; NJ: 89%). A clade including the gerbils *G. nigeriae* and *T. gambiana* (ML: 100%; NJ: 98%) followed by the vole, *C. glareolus* were basal to the murine/otomyine clade (trees not shown). Although the higher order relationships of the Otomyinae are beyond the scope of this study, it is interesting that these results reflect the present uncertainty regarding the relationships of the Otomyinae to lineages within the Murinae, and among murine lineages.

When all the main ingroup taxa were included (see Table 2), and irrespective of the combinations of outgroups examined, inconsistencies in the deeper branching order of the ingroup were evident in the trees recovered from cyt *b* and 12S rRNA sequence data. These trees collapsed to an almost identical topology after bootstrap analysis (trees not shown). In the 12S rRNA trees, *Mus* grouped basal to *A. namaquensis* with weak to moderate bootstrap support (ML: 61%; MP: 56%; NJ: 74%), but this relationship was not supported in the cyt *b* trees. Consequently, these results and the relationships inferred from cyt *b*, 12S and 16S rRNA data by Ducroz *et al.* (2001), together with the availability of *M. musculus*, *A. namaquensis* (12S rRNA) and *A. chrysophilus* (cyt *b*) sequences, provided the basis for the choice of these taxa as the most appropriate outgroups to

polarize characters and root trees in the core phylogenetic analyses. By selecting two outgroups of successive relatedness to the ingroup, the possibility of errors arising in the polarization of characters by using a single outgroup was curtailed. Furthermore, the introduction of additional noise into the data through the inclusion of distantly related outgroups was minimized.

SEQUENCE ANALYSIS

Cytochrome *b* Alignment and Pseudogene Assessment

The 1143 bp of sequences from the 26 taxa listed in Table 2, and the incomplete segment of the *O. occidentalis* cyt *b* sequence (926 basepairs) were aligned to each other and to the outgroups. No insertions or deletions were detected in the data set (Appendix I). Sequences began at the initiation codon ATG and ended with translational termination signals produced by the polyadenylation of the processed mRNA also found in mouse and human sequences (Irwin *et al.* 1991; Esposti *et al.* 1993). Translation of the aligned codons to amino acids resulted in a functional mtDNA reading frame and 26 unique replacement substitutions within the ingroup (Appendix III). Table 7 summarizes the characteristics of these particular amino acid changes, which were all caused by single base changes. Interestingly, seven of these changes (codons 56, 158, 224, 226, 242, 307, 366) were at residues that are suggested to be conserved across some mammals (Irwin *et al.* 1991). All but two of these (codons 56, 224) also showed replacement substitutions in other rodent taxa tested as possible outgroups in this study (see Table 2).

There is some indication that a nuclear homologue of cyt *b* was possibly amplified in both representatives of *P. littledalei* (Springbok and Hentiesbaai). First, a slightly lower than average cytocine content, and a slightly higher than average thymine and guanine content (*P. littledalei* - Springbok: C = 24.5%, T = 31.9%, G = 12.8%; *P. littledalei* - Hentiesbaai: C = 24.9%, T = 32%, G = 13%; see Table 8 for mean values). Second, the amino acid sequences contained four unique amino acid changes (codons 16, 214, 300, 307) and a further two unique changes (codons 57, 241) were detected in the sequence of *P. littledalei* from Hentiesbaai (Appendix III). Third, rate heterogeneity analysis indicated a different evolutionary rate for the *P. littledalei* lineage (Hentiesbaai) in

Table 7: Characteristics of the 26 unique amino acid (aa) replacement substitutions in the translated cyt *b* sequences of the ingroup taxa (see Appendix III).

Codon number[a] fast (**)/slow (*)	Membrane domain[b]	Conserved/ variable residue[c]	Codon position, substitution type (nucleotide change)	Taxon name (locality[d])	Unique aa (abbreviation) acidic/neutral/basic, charge	Changed from aa (abbreviation) acidic/neutral/basic, charge	Change between aa classes	Change within aa classes
*7	Qi	variable	2nd, Tv (C→A)	*O. unisulcatus*	Asparagine (N) neutral, polar	Threonine (T) neutral/polar		x
*16	Qi	variable	1st, Tv (C→G)	*P. littledalei*	Aspartic acid (D) acidic, - charge	Histidine (H) basic, + charge	x	
**39	Q	variable	1st Ti (G→A); 3rd Tv (C/T →A)	*O. typus*	Methionine (M) neutral, nonpolar	Valine (V) neutral, nonpolar		x
						Isoleucine (I) neutral, nonpolar		x
*56	Qo	conserved	1st, Tv (A→T)	*P. brantsii* (Goe)	Serine (1) neutral, polar	Threonine (T) neutral, polar		x
*57	Qo	variable	1st, Ti (T→C)	*P. littledalei* (Hen)	Proline (P) neutral, nonpolar	Serine (1) neutral, polar	x	
**82	Q	variable	3rd, Ti and Tv (A→C; T→C)	*O. irroratus* C (Twe)	Isoleucine (I) neutral, nonpolar	Leucine (L) neutral, nonpolar		x
						Methionine (M) neutral, nonpolar		x
*96	Q	variable	1st, Tv (A→C/T)	*O. unisulcatus*	Methionine (M) neutral, nonpolar	Leucine (L) neutral, nonpolar		x
*158	Qo	conserved	1st, Tv (A→T)	*O. unisulcatus*	Serine (1) neutral, polar	Threonine (T) neutral, polar		x
*190	Q	variable	1st, Tv (A→T)	*O. denti*	Serine (1) neutral, polar	Threonine (T) neutral, polar		x
*193	Q	variable	2nd, Ti (T→C)	*O. denti*	Alanine (A) neutral, nonpolar	Valine (V) neutral, nonpolar		x
*214	Qi	variable	1st, Ti (G→A)	*P. littledalei*	Asparagine (N) neutral, polar	Aspartic acid (D) acidic, - charge	x	
*224	Qi	conserved	1st, Ti (T→C)	*O. typus*	Histidine (H) basic	Tyrosine (Y) neutral, polar	x	
*226	Qi	conserved	1st, Ti (A→G)	*P. brantsii*	Valine (V) neutral, nonpolar	Isoleucine (I) neutral, nonpolar		x
**232	Q	variable	3rd, Tv (T/C→A)	*O. unisulcatus*	Methionine (M) neutral, nonpolar	Leucine (L) neutral, nonpolar		x
						Isoleucine (I) neutral, nonpolar		x
						Valine (V) neutral, nonpolar		x
*234	Q	variable	3rd, Tv (C/T→A)	*O. irroratus* B (Gro)	Methionine (M) neutral, nonpolar	Isoleucine (I) neutral, nonpolar		x
**238	Q	variable	1st Ti (A→G) 1st Tv (T/C→G)	*O. tropicalis*	Alanine (A) neutral, nonpolar	Leucine (L) neutral, nonpolar	x	x
						Threonine (T) neutral, polar		x
*240	Q	variable	1st, Ti (A→G)	*O. sloggetti*	Valine (V) neutral, nonpolar	Methionine (M) neutral, nonpolar		x
**241	Q	variable	1st, Ti (A→G)	*P. littledalei* (Hen)	Alanine (A) neutral, nonpolar	Isoleucine (I) neutral, nonpolar		x
						Threonine (T) neutral, polar	x	
*242	Q	conserved	1st, Tv (C→A)	*O. typus*	Methionine (M) neutral, nonpolar	Leucine (L) neutral, nonpolar		x
*300	Q	variable	1st, Ti (A→G)	*P. littledalei*	Valine (V) neutral, nonpolar	Isoleucine (I) neutral, nonpolar		x
*303	Q	variable	1st, Ti (C→T); 3rd, Ti and; Tv (C/A→T)	*O. sloggetti*	Phenylalanine (F) neutral, nonpolar	Leucine (L) neutral, nonpolar		x
*307	Q	conserved	1st, Ti (C→T)	*P. littledalei*	Phenylalanine (F) neutral, nonpolar	Leucine (L) neutral, nonpolar		x
*327	Q	variable	1st, Ti (A→G)	*O. anchietae*	Valine (V) neutral, nonpolar	Isoleucine (L) neutral, nonpolar		x
*349	Q	variable	3rd, Tv (C/T→A)	*O. typus*	Methionine (M) neutral, nonpolar	Isoleucine (I) neutral, nonpolar		x
*366	Q	conserved	3rd, Tv (A→C)	*O. unisulcatus*	Isoleucine (I) neutral, nonpolar	Methionine (M) neutral, nonpolar		x
*376	Qi	variable	1st, Tv (C→A)	*O. a. lacustris* (Ufi)	Methionine (M) neutral, nonpolar	Leucine (L) neutral, nonpolar		x

a - Fast evolving site - at least two aa changes per site; Slow evolving site - less than two aa changes per site.
b - Membrane domains as defined in the eight domain structural model for cyt *b* (Howell 1989 adapted for mammals by Irwin *et al.* 1991). Q_o = outer-membrane; Q = trans-membrane; Q_i = inner-membrane.
c - Variable (more than two aa states) and conserved (at most two aa states) residues were evaluated according to the amino acid variability in the mammalian structural model for cyt *b* (Irwin *et al.* 1991).
d - Collection localities are given when only one of the representatives of a taxon exhibited a unique change (see Table 2); Abbreviations: Goe = Goegap, Hen = Hentiesbaai, Twe = Tweede Tol, Gro = Groendal, Ufi = Ufipa Plateau.

Table 8: Summary of the estimates of sequence variability, the sum (over sites) of average numbers of transitions and transversions (across 500 random parsimony trees), mean base composition and compositional bias of the cytochrome *b* and 12S rRNA genes and their functional partitions in the Otomyinae.

Gene/Partition	Total	Variable	Informative	Average no. transitions	Average no. transversions	A	C	G	T	Bias
Cytochrome *b*										
All (aa)	1143 (381)	361 (44)	297 (31)	1366 - 1583	255 - 329	0.31	0.28	0.12	0.29	0.173
Codon positions										
First	381	65	48	206 - 245	32 - 49	0.30	0.25	0.21	0.24	0.067
Second	381	10	9	26 - 35	2 - 4	0.20	0.25	0.13	0.42	0.227
Third	381	286	240	1110 - 1318	215 - 278	0.43	0.33	0.01	0.23	0.347
Membrane domains										
Inner (aa)	240 (80)	71 (11)	55 (9)	235 - 292	31 - 43	0.40	0.28	0.08	0.24	0.240
Trans (aa)	561 (187)	195 (26)	159 (18)	684 - 849	146 - 204	0.28	0.27	0.12	0.33	0.173
Outer (aa)	342 (114)	95 (7)	83 (4)	378 - 459	56 - 82	0.31	0.28	0.15	0.26	0.133
12S rRNA										
All	802	103 (+4)	61 (+2)	200 - 242	54 - 73	0.38	0.20	0.16	0.26	0.187
Stems	424	23	13	54 - 65	10 - 14	0.28	0.21	0.23	0.28	0.080
Loops	378	80 (+4)	48 (+2)	156 - 199	48 - 63	0.50	0.18	0.09	0.23	0.333

Note: For 12S rRNA the numbers of variable and informative gaps (see text) are shown in brackets.
For translated cytochrome *b* amino acid (aa) sequences, the total number, variable and informative aa for the complete gene and in the three membrane domains are indicated in brackets.
All estimations are based on ingroup taxa only (cytochrome *b* - 26 taxa; 12S rRNA - 22 taxa; Table 2). The incomplete *O. occidentalis* sequence was excluded from the cytochrome *b* analysis.

relation to *O. irroratus* cytotypes A1, A2, B and C, and to a lesser extent for *P. littledalei* (Goegap) in relation to the A1 cytotype (see below). However, detailed analysis of these sequences suggested that they are of mitochondrial origin and are consistent with criteria proposed for precluding pseudogene amplification (Smith *et al.* 1992; Esposti *et al.* 1993; Arctander 1995; Zhang & Hewitt 1996). Specifically, they were within the reading frame of the mtDNA genetic code, contained no stop codons, the distribution of substitutions was skewed towards predominantly 3rd codon position changes, and no double bands were observed on autoradiographs.

12S rRNA Alignment

Between 793 and 796 bp of the 12S rRNA gene were sequenced for the 22 ingroup taxa listed in Table 2. Sequence alignment (to outgroup) resulted in 802 aligned sites that fell into 94 paired regions (stems and bases involved in tertiary interaction; 424 nucleotides) and 84 loop regions (378 nucleotides); insertions or deletions were present at 12 nucleotide positions (Appendix II). Compensatory substitutions were apparent in four stem regions (4 and 4', 9 and 9', e and e', 30 and 30', see underlined nucleotides - Appendix II). Non-complementary base pairing was evident in seven stem regions, (8 and 8', 17 and 17', c and c', g and g!, 22 and 22', 34 and 34', and j and j', see italicized nucleotides - Appendix II). Six of the 12 alignment gaps were in the ingroup resulting from alignment to one or both the outgroup sequences (positions 99, 366, 444, 445, 639 and 716), one deletion was unique to *A. namaquensis* (position 286), while the remaining five (all situated in loops) involved some or all otomyine taxa. These were: 1) an insertion (A) at position 66 of *O. angoniensis, O. maximus, O. t. jacksoni*, all representatives of *O. irroratus* (except the Hogsback specimen), *O. typus* and *P. brantsii*; 2) a unique C nucleotide insertion at position 67 of *P. brantsii*; 3) an insertion (A or G) at position 285 in all the Otomyinae; 4) a deletion at position 369 of *O. sloggetti* and *A. namaquensis*; 5) a deletion at position 706 of *O. tropicalis* and all three individuals representing *O. a. lacustris* (Uzungwa Mountains and Ufipa Plateau).

Patterns and Rates of Sequence Evolution

Sequence Variability and Base Composition

Ingroup-based estimates of sequence variability, base composition and compositional bias for the complete cyt *b* gene (nucleotides and amino acids), the sequenced portion of 12S rRNA (±802 bp) and their functional partitions are given in Table 8.

Cytochrome *b* characteristics

The complete cyt *b* nucleotide data set (including all 26 ingroup taxa) revealed 361 (31.6%) variable and 297 (26%) informative sites out of 1143 nucleotide sites in the data matrix (Table 8). The least number of changes were at 2nd positions of codons (10 variable; nine informative); the majority at 3rd positions (286 variable; 240 informative) and 1st positions contained 65 variable and 48 informative sites. Nucleotide variability across codon positions (expressed as the number of changes relative to every one change that occur at 2nd positions) was 6.5:1:28.6 for all 26 ingroup taxa, and 6.3:1:28 for the 16 taxa involved in the core phylogenetic analysis (main ingroup, see Table 2). Most nucleotide changes were synonymous, and of 381 codons, 44 were variable and 31 informative. There were 26 codon changes (eight fast and 18 slow) in the trans-membrane, followed by 11 slow changes in the inner-membrane and seven slow changes in the outer-membrane (Table 8). There is no significant difference in the rate of change of the trans-membrane region compared to either the inner-membrane (Yates corrected $\chi 2 = 2.69$, $P = 0.1008$) or the outer-membrane (Yates corrected $\chi 2 = 1.41$, $P = 0.2343$) regions. The Yates correction (Zar 1984) takes the absence of fast evolving codons in the inner-membrane and outer-membrane into account. There were sixty-one leucine codons (nine inner-membrane, 43 trans-membrane, nine outer-membrane, Appendix III).

The mean base compositions of cyt *b* indicated a strong bias against Gs in the complete gene and at 2nd and 3rd positions, a clear domination of As at 3rd positions and Ts at 2nd positions, and a relatively unbiased base composition at 1st positions. A deficit of Gs was observed in the three membrane domains (most pronounced in the inner-membrane) and As were over represented in the inner-membrane (Table 8). The compositional bias

in the different gene regions should not have influenced the phylogenetic analyses, since base frequencies were not significantly variable between the taxa examined ($\chi 2$ test for homogeneity of the complete gene: $\chi 2 = 27.47$, P = 1.0; codon positions: 1st: $\chi 2 = 9.8$, P = 1.0; 2nd: $\chi 2 = 1.1$, P = 1.0; 3rd: $\chi 2 = 90.2$, P = 0.23; membrane domains: inner: $\chi 2 = 15.0$, P = 1.0; trans: $\chi 2 = 24.4$, P = 1.0; outer: $\chi 2 = 12.4$, P = 1.0).

12S rRNA characteristics

Of the 802 aligned 12S rRNA nucleotide sites in the data matrix (22 ingroup sequences), 103 (12.8%) were variable and 61 (7.6%) parsimony informative (Table 8). Both transitions and transversions were more abundant in loops than in stems. There was an overall bias in nucleotide composition against Gs and towards As in 12S rRNA. Specifically striking was the preponderance of As in loops. There was an almost balanced representation of As, Gs, Cs and Ts in stems. The G+C composition in stems (44%) was substantially higher than in loops (27%). Base frequencies were not significantly different amongst taxa (entire sequenced portion: $\chi 2 = 4.3$, P = 1.0; stems: $\chi 2 = 1.28$, P = 1.0; loops: $\chi 2 = 7.8$, P = 1.0).

Sequence Differentiation

Table 9 (cyt *b*) and Table 10 (12S rRNA) show the pairwise uncorrected (p-distances) and HKY85 corrected estimates of nucleotide differentiation between outgroups and all otomyine taxa included in the study. Table 11 summarizes the averages (and ranges) of the corrected sequence divergences separating the Otomyinae from outgroups and between otomyine taxa at different taxonomic levels and from distinct geographic regions.

Saturation Analyses

The CI and RI values of the trees resulting from the unweighted parsimony analyses of cyt *b* and 12S rRNA data were lower when transitions were included (cyt *b*: CI = 0.446, RI = 0.424; 12S rRNA: CI = 0.675, RI = 0.523) than when they were excluded (cyt *b*: CI = 0.718, RI = 0.670; 12S rRNA: CI = 0.835, RI = 0.740). For both data sets, this implies

Table 9: Observed pairwise estimates of nucleotide differentiation (transitions and transversions) of the cytochrome *b* gene between 26 ingroup taxa (excluding *O. occidentalis*) and outgroups *Aethomys chrysophilus* and *Mus musculus* (Table 2). Above diagonal: Percentage uncorrected sequence divergences (p-distances). Below diagonal: Percentage HKY85-corrected sequence divergences (Hasegawa *et al.* 1985). The cytotypes of *O. irroratus* (A1, A2, B and C) are indicated after the species name. Abbreviated collection localities are given in brackets where more than one representative per taxon was included. Abbreviations: Alg = Algeria, Ali = Alice, Aru = Arusha, Bai = Baines Kloof, Buj = Bujuku River, Chi = = Chingombe, Goe = Goegap, Gro = Groendal, Hen = Hentiesbaai, Hog = Hogsback, Kam = Kamberg, Kal = Kalagadi, Kle = Kleinsee, Kar = Karkloof, Mbi = Mbizi, Mga = Mgahinga, Run = Mt. Rungwe, Twe = Tweede Tol, Uzu = Uzungwa Mountains.

		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
1.	*M. musculus*	-	16.7	17.2	18.0	16.7	17.2	16.8	17.0	17.7	17.5	17.5	17.4	17.8	17.6	18.0	17.7	17.0	17.0	17.3	18.3	17.6	17.3	17.4	17.2	17.2	17.1	17.9	18.5
2.	*A. chrysophilus*	19.3	-	14.8	16.3	15.5	15.6	15.6	15.4	14.7	16.8	16.8	17.0	16.4	16.9	16.7	15.6	15.2	15.2	15.9	16.3	15.5	14.8	15.4	15.9	14.9	15.5	17.6	17.7
3.	*O. anchietae lacustris* (Uzu)	20.1	16.9	-	10.7	9.8	9.5	9.3	10.1	10.9	10.5	10.8	10.7	10.4	10.7	11.4	10.1	5.9	5.9	9.8	11.1	11.5	5.0	8.8	10.8	10.5	10.9	13.4	13.0
4.	*O. angoniensis*	21.1	18.9	11.9	-	10.4	11.0	9.5	9.6	8.9	9.4	9.6	9.7	9.5	9.6	6.1	6.0	11.7	11.9	10.7	2.4	11.0	11.4	8.8	11.4	11.1	11.3	13.9	13.7
5.	*O. denti* (Mga)	19.2	17.9	10.9	11.6	-	2.7	10.4	10.9	10.3	11.2	11.5	11.0	11.1	11.0	12.2	11.6	10.0	10.2	11.4	11.6	12.1	9.9	10.5	11.3	10.3	10.4	12.4	12.3
6.	*O. denti* (Buj)	20.0	18.0	10.5	12.2	2.8	-	10.5	10.9	10.8	11.8	11.9	11.6	11.7	11.8	11.6	11.3	10.2	10.3	11.3	11.7	12.1	9.5	10.3	11.7	11.5	11.5	12.9	12.8
7.	*O. irroratus* A1 (Hog)	19.3	18.0	10.1	10.6	11.6	11.7	-	1.0	4.6	6.3	6.0	6.3	6.1	6.0	9.4	8.4	9.6	9.6	8.1	9.5	11.2	9.1	8.5	11.2	10.9	10.8	11.6	11.8
8.	*O. irroratus* A1 (Kam)	19.6	17.8	11.1	10.7	12.1	12.2	1.0	-	4.3	6.4	6.3	6.4	6.4	6.4	9.8	9.0	10.2	10.2	8.5	9.5	11.5	9.9	8.2	11.6	10.7	10.5	11.8	11.9
9.	*O. irroratus* A2 (Kar)	20.6	16.6	12.1	9.8	11.4	12.0	4.8	4.5	-	7.0	6.6	7.0	6.3	6.5	9.5	9.3	11.5	11.6	8.8	9.7	10.2	10.4	8.9	11.5	10.7	10.9	12.6	12.3
10.	*O. irroratus* B (Ali)	20.4	19.6	11.6	10.3	12.5	13.3	6.7	6.8	7.5	-	1.1	0.4	1.9	2.0	10.3	9.8	10.2	10.4	8.9	10.0	11.6	10.1	8.4	11.3	11.5	11.3	12.0	11.8
11.	*O. irroratus* B (Bai)	20.3	19.6	12.0	10.6	12.9	13.4	6.4	6.7	7.0	1.1	-	1.1	2.0	2.0	10.2	9.8	10.5	10.7	9.3	10.1	11.9	9.8	8.8	11.5	11.9	11.7	11.8	11.6
12.	*O. irroratus* B (Gro)	20.2	20.0	11.9	10.7	12.3	13.1	6.7	6.8	7.5	0.4	1.1	-	2.0	2.0	10.4	9.9	10.4	10.6	9.0	10.2	11.8	10.1	8.2	11.3	11.6	11.5	12.0	11.8
13.	*O. irroratus* C (Alg)	20.7	19.1	11.5	10.4	12.4	13.2	6.5	6.8	6.7	2.0	2.0	2.0	-	1.1	9.7	9.4	10.2	10.3	9.2	10.1	11.6	9.6	8.7	10.8	11.1	10.9	11.6	11.7
14.	*O. irroratus* C (Twe)	20.4	19.7	11.8	10.6	12.3	13.3	6.3	6.8	6.9	2.1	2.1	2.1	1.2	-	10.1	9.7	10.4	10.6	9.4	10.2	11.6	9.7	9.0	10.8	11.1	11.0	12.0	11.8
15.	*O. typus jacksoni* (Aru)	21.0	19.4	12.7	6.6	13.8	13.1	10.3	10.9	10.4	11.5	11.4	11.6	10.7	11.2	-	3.4	10.9	11.1	11.0	6.0	11.1	11.1	9.2	11.6	11.3	11.8	13.6	13.4
16.	*O. typus jacksoni* (Run)	20.5	17.9	11.1	6.5	13.1	12.6	9.2	9.9	10.2	10.9	10.9	10.9	10.3	10.7	3.5	-	11.0	11.2	9.5	5.8	10.5	11.0	8.9	11.1	11.7	12.4	14.0	13.6
17.	*O. anchietae lacustris* (Chi)	19.7	17.4	6.3	13.2	11.1	11.3	10.6	11.3	12.9	11.3	11.6	11.5	11.2	11.5	12.2	12.3	-	0.2	10.7	12.0	12.8	6.4	9.7	11.2	11.6	11.3	12.6	12.4
18.	*O. anchietae lacustris* (Mbi)	19.7	17.4	6.3	13.4	11.3	11.5	10.6	11.3	13.1	11.5	11.9	11.7	11.4	11.7	12.4	12.5	0.2	-	10.7	12.2	12.8	6.4	9.7	11.2	11.6	11.3	12.6	12.4
19.	*O. laminatus*	20.1	18.3	10.8	12.0	12.8	12.8	8.9	9.3	9.6	9.8	10.3	9.9	10.1	10.3	12.5	10.5	11.9	11.9	-	10.9	11.4	11.0	9.4	11.3	12.2	12.0	12.9	12.5
20.	*O. maximus*	21.4	19.0	12.4	2.4	13.0	13.2	10.4	10.5	10.8	11.1	11.2	11.3	11.2	11.4	6.5	6.2	13.5	13.8	12.2	-	11.5	11.6	9.3	11.4	11.6	11.4	13.9	13.6
21.	*O. sloggetti*	20.4	17.9	12.9	12.3	13.7	13.7	12.6	12.9	11.4	13.1	13.5	13.4	13.2	13.1	12.5	11.7	14.6	14.6	12.8	12.9	-	11.9	11.4	11.6	11.6	11.7	13.3	12.6
22.	*O. tropicalis*	20.2	16.9	5.3	12.7	10.9	10.4	9.9	10.9	11.5	11.1	10.7	11.1	10.5	10.6	12.4	12.2	6.8	6.8	12.2	13.5	13.4	-	10.0	11.0	11.5	11.4	13.0	13.0
23.	*O. typus*	20.2	17.6	9.6	9.7	11.7	11.5	9.3	8.9	9.8	9.1	9.5	8.9	9.4	9.9	10.1	9.8	10.7	10.7	10.3	10.2	12.8	11.0	-	9.9	11.1	10.8	13.0	12.4
24.	*O. unisulcatus*	19.8	18.2	11.9	12.8	12.6	13.2	12.5	13.1	12.9	12.6	12.8	12.6	12.0	12.0	13.0	12.4	12.5	12.5	12.6	12.7	13.0	12.1	10.9	-	10.6	10.7	11.6	11.4
25.	*P. brantsii* (Kal)	20.0	17.0	11.6	12.4	11.4	12.9	12.2	11.9	11.9	12.9	13.5	13.1	12.4	12.4	12.6	13.2	13.1	13.2	13.8	13.0	13.2	12.8	12.5	11.7	-	1.5	13.3	13.2
26.	*P. brantsii* (Kle)	19.8	17.9	12.1	12.7	11.6	12.9	12.0	11.7	12.1	12.7	13.3	12.9	12.3	12.4	13.4	14.1	12.6	12.8	13.6	12.8	13.3	12.8	12.5	11.9	1.5	-	14.0	13.0
27.	*P. littledalei* (Goe)	21.0	20.7	15.5	16.2	14.2	14.8	13.0	13.4	14.4	13.6	13.4	13.6	13.0	13.6	15.7	16.3	14.4	14.4	14.8	16.3	15.5	15.0	14.9	13.0	15.4	14.9	-	2.3
28.	*P. littledalei* (Hen)	21.8	21.0	14.9	16.0	14.1	14.6	13.4	13.5	14.1	13.4	13.1	13.4	13.2	13.3	15.5	15.7	14.2	14.2	14.3	15.8	14.5	14.9	14.2	12.8	15.3	15.1	2.3	-

Table 10: Observed pairwise estimates of nucleotide differentiation (transitions and transversions) of the 12S rRNA gene between 22 ingroup taxa and outgroups *Aethomys namaquensis* and *Mus musculus* (Table 2). Above diagonal: Percentage uncorrected sequence divergences (p-distances); Below diagonal: Percentage HKY85-corrected sequence divergences (Hasegawa *et al.* 1985). The cytotypes of *O. irroratus* (A1, A2, B and C) are indicated after the species name. Abbreviated collection localities are given in brackets where more than one representative per taxon was included. Abbreviations: Alg = Algeria, Ali = Alice, Aru = Arusha, Bai = Baines Kloof, Buj = Bujuku River, Chi = Chingombe, Goe = Goegap, Gro = Groendal, Hog = Hogsback, Kal = Kalagadi, Kar = Karkloof, Mbi = Mbizi, Mga = Mgahinga, Run = Mt. Rungwe, Uzu = Uzungwa Mountains.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1.	*M. musculus*	-	7.5	6.6	6.4	5.6	5.7	5.7	5.9	5.7	5.7	5.7	5.6	6.3	6.7	6.1	6.1	5.9	6.3	6.2	6.8	5.4	5.9	8.0	5.8
2.	*A. namaquensis*	7.9	-	6.1	6.2	6.1	6.1	5.3	5.3	5.3	5.3	5.3	5.3	5.8	6.2	5.3	5.3	5.2	5.9	7.0	6.1	4.9	5.1	7.7	6.3
3.	*O. anchietae lacustris* (Uzu)	6.9	6.4	-	3.3	3.8	3.5	3.3	3.3	3.3	3.3	3.3	3.2	4.0	3.8	2.3	2.3	3.4	3.4	4.3	1.8	2.8	3.3	5.8	4.2
4.	*O. angoniensis*	6.8	6.5	3.4	-	3.1	3.4	3.0	3.0	2.8	2.8	2.8	2.9	1.1	0.5	3.1	3.1	2.8	0.4	3.8	3.9	2.4	3.1	5.7	3.7
5.	*O. denti* (Mga)	5.8	6.4	3.9	3.2	-	0.5	2.9	3.1	2.6	2.6	2.6	2.8	3.3	3.4	3.4	3.4	2.8	3.3	3.3	3.8	2.9	3.5	5.0	3.4
6.	*O. denti* (Buj)	6.0	6.4	3.7	3.5	0.5	-	2.9	2.9	2.4	2.4	2.4	2.5	3.5	3.7	3.4	3.4	2.5	3.3	3.0	3.5	2.6	3.3	5.0	3.4
7.	*O. irroratus* A1 (Hog)	5.9	5.5	3.4	3.1	3.0	3.0	-	0.3	0.8	0.8	0.8	0.9	2.9	3.3	3.2	3.2	1.6	2.9	3.4	3.3	2.0	2.4	4.2	3.1
8.	*O. irroratus* A2 (Kar)	6.2	5.5	3.4	3.1	3.2	3.0	0.3	-	0.8	0.8	0.8	0.9	2.9	3.3	3.2	3.2	1.6	2.9	3.4	3.3	2.0	2.1	4.3	3.4
9.	*O. irroratus* B (Ali)	5.9	5.5	3.4	2.8	2.7	2.4	0.8	0.8	-	0.0	0.0	0.1	2.6	3.0	2.9	2.9	1.6	2.6	3.4	3.0	2.0	2.4	4.0	2.9
10.	*O. irroratus* B (Bai)	5.9	5.5	3.4	2.8	2.7	2.4	0.8	0.8	0.0	-	0.0	0.1	2.6	3.0	2.9	2.9	1.6	2.6	3.4	3.0	2.0	2.4	4.0	2.9
11.	*O. irroratus* B (Gro)	5.9	5.5	3.4	2.8	2.7	2.4	0.8	0.8	0.0	0.0	-	0.1	2.6	3.0	2.9	2.9	1.6	2.6	3.4	3.0	2.0	2.4	4.0	2.9
12.	*O. irroratus* C (Alg)	5.8	5.5	3.2	3.0	2.8	2.6	0.9	0.9	0.1	0.1	0.1	-	2.8	3.1	2.8	2.8	1.8	2.8	3.5	3.2	2.1	2.3	4.2	3.0
13.	*O. typus jacksoni* (Aru)	6.6	6.1	4.2	1.1	3.4	3.6	3.0	3.0	2.7	2.7	2.7	2.8	-	1.1	3.3	3.3	2.6	1.0	4.2	4.0	2.8	3.3	5.4	3.7
14.	*O. typus jacksoni* (Run)	7.1	6.5	3.9	0.5	3.5	3.8	3.4	3.4	3.1	3.1	3.1	3.2	1.1	-	3.4	3.4	3.0	0.6	4.0	4.2	2.9	3.4	5.8	3.9
15.	*O. anchietae lacustris* (Chi)	6.4	5.5	2.3	3.2	3.5	3.5	3.2	3.2	3.0	3.0	3.0	2.8	3.4	3.5	-	0.0	3.4	3.0	3.7	2.5	2.5	2.4	5.1	3.7
16.	*O. anchietae lacustris* (Mbi)	6.4	5.5	2.3	3.2	3.5	3.5	3.2	3.2	3.0	3.0	3.0	2.8	3.4	3.5	0.0	-	3.4	3.0	3.7	2.5	2.5	2.4	5.1	3.7
17.	*O. laminatus*	6.2	5.4	3.5	2.8	2.8	2.6	1.7	1.7	1.7	1.7	1.7	1.8	2.7	3.1	3.5	3.5	-	2.6	3.3	3.2	1.9	2.6	4.5	3.7
18.	*O. maximus*	6.6	6.2	3.5	0.4	3.4	3.4	3.0	3.0	2.7	2.7	2.7	2.8	1.0	0.6	3.1	3.1	2.7	-	3.7	3.8	2.5	3.0	5.5	3.5
19.	*O. sloggetti*	6.5	7.3	4.5	3.9	3.4	3.1	3.5	3.5	3.5	3.5	3.5	3.6	4.3	4.2	3.8	3.8	3.4	3.8	-	4.4	3.4	3.2	4.9	3.7
20.	*O. tropicalis*	7.2	6.4	1.8	4.1	3.9	3.7	3.4	3.4	3.1	3.1	3.1	3.2	4.2	4.3	2.6	2.6	3.3	3.9	4.6	-	2.8	3.7	5.6	3.9
21.	*O. typus*	5.7	5.1	2.8	2.5	3.0	2.7	2.1	2.0	2.0	2.0	2.0	2.2	2.8	3.0	2.6	2.6	1.9	2.6	3.5	2.9	-	2.3	4.9	3.3
22.	*O. unisulcatus*	6.2	5.3	3.4	3.3	3.7	3.4	2.4	2.2	2.4	2.4	2.4	2.3	3.4	3.5	2.5	2.5	2.7	3.1	3.2	3.8	2.3	-	4.7	3.5
23.	*P. brantsii* (Kal)	8.5	8.2	6.1	6.0	5.3	5.3	4.3	4.4	4.2	4.2	4.2	4.3	5.7	6.1	5.3	5.3	4.7	5.8	5.1	5.8	5.1	4.9	-	5.2
24.	*P. littledalei* (Goe)	6.1	6.6	4.3	3.8	3.5	3.5	3.2	3.5	3.0	3.0	3.0	3.1	3.8	4.0	3.8	3.8	3.8	3.6	3.8	4.1	3.4	3.7	5.4	-

Table 11: Summary of the averages and ranges (where applicable) of the percentage sequence divergences separating the Otomyinae from outgroups *Mus musculus* (cytochrome *b* and 12S rRNA), *Aethomys chrysophilus* (cytochrome *b*), *A. namaquensis* (12S rRNA), and between the otomyine taxa at different taxonomic levels and distinct geographic regions.

	Cytochrome *b* Average % sequence divergence (range)	**12S rRNA Average % sequence divergence (range)**
M. musculus vs. Otomyinae	20.3 (19.2 - 21.4)	6.4 (5.7 - 7.2)
A. chrysophilus vs. Otomyinae	18.4 (16.6 - 21.0)	–
A. namaquensis vs. Otomyinae	–	6.0 (5.1 - 7.3)
Parotomys vs. *Otomys*	13.5 (11.4 - 16.3)	4.4 (3.0 - 6.1)
Otomys – interspecific	11.4 (2.4 - 14.6)	3.0 (0.4 - 4.6)
Parotomys – interspecific	15.2 (14.9 - 15.4)	5.4
Otomys – intraspecific	0.2 - 7.5	0.1 - 1.1
Parotomys – intraspecific	1.5 - 2.3	–
O. irroratus – intercytotype	5.4 (2.0 - 7.5)	0.6 (0.1 - 0.9)
Southern Africa – interspecific	13.1 (2.4 - 16.3)	3.6 (0.4 - 6.0)
East, Central and West Africa – interspecific	10.7 (5.3 - 13.6)	3.2 (1.8 - 3.9)
Extralimital vs. southern Africa – interspecific	12.3 (6.5 - 15.7)	3.5 (1.1 - 6.1)

that transitions show homoplasy, which may involve saturation (multiple reversals at single sites) and/or convergence among nucleotides (see Philippe *et al.* 1996). This result is not surprising given that transitions evolve at a faster rate than transversions, and, therefore, likely accumulate randomized phylogenetic signal more rapidly (Matthee & Robinson 1999a).

Among-lineage Rate Heterogeneity

Likelihood ratio test for equal rates of evolution

The null hypothesis of a molecular clock for the cyt *b* and 12S rRNA data sets was rejected ($P < 0.05$) by the likelihood ratio test (LRT, Felsenstein 1988) when all the sequenced taxa and the outgroups *M. musculus, A. chrysophilus* (cyt *b*) and *A. namaquensis* (12S rRNA) were included (Table 12). When only the main ingroup taxa and the outgroups were considered, equal rates of evolution among lineages were rejected for both genes, but at higher P values (12S: $P < 0.005$; cyt *b*: $P < 0.001$).

Relative rate test

Further investigation of among-lineage rate heterogeneity in the Otomyinae using the relative rate test (RRT, Robinson *et al.* 1998) suggested significantly different rates ($P < 0.01$) for some lineages in both cyt *b* and 12S rRNA genes when using the closely related *A. chrysophilus* (cyt *b*) and *A. namaquensis* (12S rRNA) as the reference taxa. The heterogeneity can nevertheless be considered to be at a low level, because only 11 of 378 (at non-synonymous sites) and five of 378 (at synonymous sites) pairwise cyt *b* comparisons resulted in significant differences in mutation rate. Only 14 of 253 12S rRNA comparisons showed significant rate heterogeneity. The observed rate differences can also be considered to be somewhat random, because in both cyt *b* and 12S rRNA genes, no single lineage consistently showed a significantly different rate of change relative to all the other Otomyinae lineages included. These results can, however, also indicate the differences in the selective constraints under which these genes operate. In the case of cyt *b*, both *P. littledalei* test lineages (Hentiesbaai and Springbok) as well as that of *O. irroratus* A2 (Karkloof) showed a different substitution rate relative to some

Table 12: Results of the likelihood ratio test for equal rates of evolution (Felsenstein 1988) in the cytochrome *b* and 12S rRNA genes of the Otomyinae. All sequenced taxa for each gene were examined as well as the 16 ingroup taxa used in the core phylogenetic analysis with and without the outgroups *Mus musculus*, *Aethomys chrysophilus* (cytochrome *b*) and *A. namaquensis* (12S rRNA).

Gene/taxa included	log L unconstrained[a]	log L molecular clock[b]	Test statistic[c]	No. sequences[d]	Degrees[e] freedom	P value[f]
Cytochrome *b*						
All ingroup + outgroup	-7712.132	-7731.988	39.712	28	26	$P < 0.05$
Main ingroup + outgroup	-6890.198	-6919.181	57.966	18	16	$P < 0.001$
12S rRNA						
All ingroup + outgroup	-2558.594	-2577.905	38.622	24	22	$P < 0.05$
Main ingroup + outgroup	-2500.663	-2518.988	36.650	18	16	$P < 0.005$

a - The log likelihood score estimated for the ML tree with no molecular clock enforced. The HKY85 model (Hasegawa *et al.* 1985) with equal rates of among-site rate variation assumed was used in PAUP 4.0b2a (Swofford 1999).
b - The log likelihood score estimated for the ML tree with the assumption of a molecular clock.
c - Test statistic - $\delta = 2[|\ln L_1 - \ln L_0|]$.
d - The number of sequences in the examined tree.
e - The degrees of freedom equals n-2.
f - The resulting P value when compared to a χ^2 distribution.

other otomyines (mostly representatives of the *O. irroratus* cytotypes). Only three other lineages (*P. brantsii* from Kleinsee, *O. laminatus* and *O. typus*) showed significant pairwise differences (Table 13). The *P. littledalei* lineage appears to have a different rate of non-synonymous substitutions since no rate heterogeneity was evident between the two representatives of the species. In contrast, synonymous changes evolved at a different rate in *O. irroratus* A2 (Karkloof), but not in the representatives of the A1 cytotype. Significant P values resulted from the pairwise comparisons of the 12S rRNA sequences; these mostly involved *P. brantsii* (Table 14). *Otomys sloggetti* also had a different mutation rate relative to *O. laminatus, O. typus* and *O. unisulcatus*. A different evolutionary rate for the entire *O. irroratus* lineage relative to *P. brantsii* (P = 0.000381) and *O. sloggetti* (P = 0.00921) was initially identified by specifying all the representatives of the *O. irroratus* cytotypes as a single lineage, but when the cytotypes were analyzed individually no significant rate differences were evident relative to *O. sloggetti*. No significant rate differences were identified when only transversions were considered for both genes. Likewise, no significant rate heterogeneity in cyt *b* or 12S rRNA evolution was identified among the cytotypes of *O. irroratus* relative to the closely related outgroup *O. laminatus*.

The Molecular Clock and Dating of Evolutionary Divergences

The earliest divergence event among otomyine lineages was estimated to have occurred 5.14 Myr BP, this being in respect of *O. tropicalis* and *O. t. jacksoni* (Mt. Rungwe), which are separated by the highest interspecific sequence divergence (7.87% - third position transversions) detected in this study. The estimated times for the earliest divergence among lineages within each of the major clades were: approximately 2.58 Myr BP in the *O. typus* clade (between *O. typus* and *O. t. jacksoni* from Mt. Rungwe); approximately 3.09 Myr BP in the *O. anchietae* clade (between *O. tropicalis* and the representatives of *O. denti* from Bujuku River and Mgahinga) and approximately 1.89 Myr BP in the *O. irroratus* clade (between the representatives of *O. irroratus* from Groendal, Tweede Tol and Algeria and *O. laminatus* and *O. irroratus* from Karkloof, respectively). The divergence of *O. unisulcatus* and *P. littledalei* and the earliest separation among lineages in the clade comprising the *O. irroratus* and *O. typus* clades (between the representatives of *O. irroratus* from Algeria and Tweede Tol and *O. angoniensis* and *O. typus*, respectively), were dated at ~2.74 Myr BP.

Table 13: Significant P values ($P < 0.01$) for cytochrome *b* pairwise comparisons obtained from the relative rate test (Robinson *et al.* 1998) using *Aethomys chrysophilus* as outgroup. Ka = number of non-synonymous substitutions per non-synonymous site; Ks = number of synonymous substitutions per synonymous site; P = exact probability. The cytotypes of *O. irroratus* (A1, A2, B and C) are indicated after the taxon name. Localities are given if more than one representative of a taxon was analyzed.

Taxon 1 (locality)	Taxon 2 (locality)	P values at Ka	P values at Ks
P. littledalei (Hentiesbaai)	*O. irroratus* B (Baines Kloof)	0.002373	-
P. littledalei (Hentiesbaai)	*O. irroratus* B (Groendal)	0.006343	-
P. littledalei (Hentiesbaai)	*O. irroratus* B (Alice)	0.002914	-
P. littledalei (Hentiesbaai)	*O. irroratus* A1 (Kamberg)	0.000726	-
P. littledalei (Hentiesbaai)	*O. irroratus* A2 (Karkloof)	0.001487	-
P. littledalei (Hentiesbaai)	*O. irroratus* A1 (Hogsback)	0.000720	-
P. littledalei (Hentiesbaai)	*O. irroratus* C (Algeria)	0.008051	-
P. littledalei (Hentiesbaai)	*O. laminatus*	0.002211	-
P. littledalei (Hentiesbaai)	*P. brantsii* (Kleinsee)	0.002691	-
P. littledalei (Goegap)	*O. irroratus* A1 (Kamberg)	0.006214	-
P. littledalei (Goegap)	*O. irroratus* A1 (Hogsback)	0.006178	-
O. irroratus A2 (Karkloof)	*O. irroratus* B (Baines Kloof)	-	0.004929
O. irroratus A2 (Karkloof)	*O. irroratus* B (Groendal)	-	0.005964
O. irroratus A2 (Karkloof)	*O. irroratus* B (Alice)	-	0.009988
O. irroratus A2 (Karkloof)	O. *irroratus* C (Tweede Tol)	-	0.008006
O. irroratus B (Groendal)	*O. typus*	-	0.008092

Table 14: Significant P values (P < 0.01) for 12S rRNA pairwise comparisons obtained from the relative rate test (Robinson *et al.* 1998) using *Aethomys namaquensis* as outgroup. The cytotypes of *O. irroratus* (A1, A2, B and C) are indicated after the taxon name. Localities are given if more than one representative of a taxon was analyzed. K = number of substitution (transitions and transversions) per site in non-coding sequences; P = exact probability.

Taxon 1 (locality)	Taxon 2 (locality)	P values at K
O. sloggetti	*O. laminatus*	0.005470
O. sloggetti	*O. typus*	0.001860
O. sloggetti	*O. unisulcatus*	0.001991
P. brantsii (Kalagadi)	*O. unisulcatus*	0.000346
P. brantsii (Kalagadi)	*O. typus*	0.000265
P. brantsii (Kalagadi)	*O. a. lacustris* (Mbizi)	0.003179
P. brantsii (Kalagadi)	*O. a. lacustris* (Chingombe)	0.003179
P. brantsii (Kalagadi)	*O. laminatus*	0.000631
P. brantsii (Kalagadi)	*O. irroratus* B (Baines Kloof)	0.000442
P. brantsii (Kalagadi)	*O. irroratus* B (Groendal)	0.000442
P. brantsii (Kalagadi)	*O. irroratus* B (Alice)	0.000442
P. brantsii (Kalagadi)	*O. irroratus* A2 (Karkloof)	0.000669
P. brantsii (Kalagadi)	*O. irroratus* A1 (Hogsback)	0.000669
P. brantsii (Kalagadi)	*O. irroratus* C (Algeria)	0.000442

Phylogenetic Analyses

Parameter Estimation of Overall and Regional Transition Bias and Among-site Rate Variation with Maximum Likelihood

The overall and partition-specific empirical parameter estimates of the ti:tv ratios (κ), gamma-shape parameters (α) and the proportions of invariable sites (Pinv) for the complete cyt *b* and 12S rRNA genes and their partitions are given in Tables 4 and 5. Values obtained with an equal rates, gamma-distributed and mixed distribution model of among-site rate variation for each gene and gene partition are presented. Calculations were derived from the 16 main ingroup taxa (one representative per taxon analyzed, except for *O. a. lacustris* for which two were incorporated) with the outgroups both included and excluded.

Table 4 summarizes character evolution across and within gene partitions of cyt *b*. The observed rates and patterns are largely in agreement with those documented for cyt *b* in other vertebrates (birds: Voelker & Edwards 1998; Krajewski *et al.* 1999; mammals: Waits *et al.* 1999). The gamma-shape parameters (α) derived under the HKY+Γ model of evolution suggest significant rate variation among nucleotide sites ($\alpha < 0.5$) at 1st and 2nd positions, as well as for the complete gene. At 3rd positions the pattern of substitution approached a Poisson distribution. When the HKY+I+Γ model was developed to incorporate proportions of invariable sites, a nearly uniform substitution rate was indicated among the variable sites for the complete gene, and for 3rd positions. Less extreme, but still significant, rate heterogeneity ($\alpha < 0.5$) was apparent among the 1st and 2nd variable positions. This is probably due to the skewness of the distribution of rates (α) calculated as a fraction of the variable sites, which is smaller than when all sites are included (also see Sullivan *et al.* 1995). As expected, the proportions of invariable sites, determined under the HKY+I+Γ model, was low for 3rd positions and highest at 2nd positions (Irwin *et al.* 1991). The lower overall and codon-specific ti:tv ratios (κ) calculated when outgroups were included probably reflect the greater number of transversional changes between the ingroup and the outgroups. As was expected for a protein-coding gene, the κ values for cyt *b* were lowest at 2nd positions and highest at

3rd positions. The estimates of κ across and within codon positions are higher when among-site rate variation is accounted for in the model of evolution e.g. HKY+Γ (see Yang 1996a; Yang & Kumar 1996), and more so when the model incorporates proportion of invariable sites (Wakeley 1994).

Table 5 shows estimates of the rates and patterns of nucleotide substitution in the 12S rRNA gene and in stems and loops separately. As with cyt *b*, substantial among-site rate variation ($\alpha < 0.5$) was shown across stems and loops, and within these regions when both variable and invariable sites were included (HKY+Γ model). When the fraction of invariant sites was accounted for (HKY+I+Γ model), moderate levels of rate heterogeneity among sites ($0.5 < \alpha < 1.0$) resulted within stems and loops, with only slightly higher levels over all sites ($0.4 < \alpha < 0.5$). The proportions of invariable sites were higher in stems than in loops, and the difference between transition and transversion rates (κ) were greater in the former (HKY+I+Γ). This is concordant with the general pattern for mammals, and is probably due to constraints to maintain complementary base pairing in stem regions, as well as the higher overall substitution rates (±80%) documented for mammals in loops versus stems (Springer *et al.* 1995; Springer & Douzery 1996). The κ values calculated for loops are higher than the 1.16:1 ratio reported across 18 mammalian taxa of varying relatedness (Springer *et al.* 1995), but is consistent with ratios reported for closely related mammals (Hixson & Brown 1986; Halanych & Robinson 1999).

Likelihood Ratio Test and Choice of a Substitution Model

The results of the log likelihood ratio test (LRT, Goldman 1993) showed that of the three models of sequence evolution examined herein (the complete cyt *b* and 12S rRNA data across gene partitions with HKY+equal rates, HKY+Γ, HKY+I+Γ), the most complex model (HKY+I+Γ) provides the best fit (Table 15). Moreover, a significant increase in model-fit to the data resulted from permitting a unique HKY+I+Γ model for each gene partition (cyt *b* codon positions and 12S rRNA stems and loops). This was illustrated by a significant improvement in the likelihood scores calculated from the sums of the codon-specific and stem-loop-specific likelihood values versus the overall scores (Table 15). Similar results were obtained for both genes irrespective whether outgroups were

Table 15: Comparison of log likelihood scores (log L) for the Otomyinae cytochrome *b* and 12S rRNA sequences. These were obtained using the log likelihood ratio test (Goldman 1993) under alternative models of evolutionary change (HKY+equal rates, HKY+Γ, HKY+I+Γ) and partition schemes (overall and partition-specific).

Gene	Taxa included	Partition scheme	Model	Overall log L[a]	Partial log L[b]	Test statistic $\delta = 2[\lvert \ln L_1 - \ln L_0 \rvert]$	Degrees freedom	P value[d]	Significantly better estimate?
Cytochrome *b*	In+outgroup	Overall	HKY+equal rates	-6890.198					
			HKY+Γ	-6185.160		1410.076	1	P < 0.001	Yes
			HKY+I+Γ	-6169.743		30.834	1	P < 0.001	Yes
		Codon-specific[c]	HKY+I+Γ	-5625.122		1089.242	1	P < 0.001	Yes
		First position	HKY+I+Γ		-1391.452				
		Second position	HKY+I+Γ		-671.177				
		Third position	HKY+I+Γ		-3562.493				
	Ingroup	Overall	HKY+equal rates	-5725.717			1		
			HKY+Γ	-5206.175		1039.084	1	P < 0.001	Yes
			HKY+I+Γ	-5198.254		15.842	1	P < 0.001	Yes
		Codon-specific[c]	HKY+I+Γ	-4710.009		976.490	1	P < 0.001	Yes
		First position	HKY+I+Γ		-1162.528				
		Second position	HKY+I+Γ		-596.111				
		Third position	HKY+I+Γ		-2951.370				
12S rRNA	In+outgroup	Overall	HKY+equal rates	-2500.663					
			HKY+Γ	-2355.287		290.752	1	P < 0.01	Yes
			HKY+I+Γ	-2348.624		13.326	1	P < 0.005	Yes
		Stem-loop-specific[c]	HKY+I+Γ	-2277.749		141.748	1	P < 0.01	Yes
		Stems	HKY+I+Γ		-871.877				
		Loops	HKY+I+Γ		-1405.872				
	Ingroup	Overall	HKY+equal rates	-2105.272					
			HKY+Γ	-2003.495		203.554	1	P < 0.01	Yes
			HKY+I+Γ	-1998.475		10.04	1	P < 0.005	Yes
		Stem-loop-specific[c]	HKY+I+Γ	-1923.110		150.73	1	P < 0.01	Yes
		Stems	HKY+I+Γ		-1138.931				
		Loops	HKY+I+Γ		-784.179				

Note: The ingroup comprised all Otomyinae included in the core phylogenetic analysis (Table 2). Outgroups: *Mus musculus, Aethomys chrysophilus* (cytochrome *b*) and *A. namaquensis* (12S rRNA).

a - Overall log L scores were calculated for ML trees while empirical κ, α and Pinv parameters for the complete gene were simultaneously estimated directly from the data (Table 4, 5) under the three alternative among-site rate variation models examined (equal rates, Γ and I+Γ).

b - Partial log L scores were calculated while empirical κ, α and Pinv parameters (Table 4, 5) for each partition obtained with the HKY+I+Γ model were applied, utilizing the maximum likelihood topology derived under the same model for the complete gene.

c - Total log L scores for data partitioned by codons (cytochrome *b*) and stems and loops (12S rRNA) were calculated from the sum of the partial log L scores for each partition.

d - The resulting P value when compared to a χ^2 distribution.

included or excluded. Due to limitations imposed by the available software (e.g. PAUP 4.0b2a), it was not possible to determine whether the significant improvement in model-fit of partition-specific ML models to the data was mirrored by an improvement in phylogenetic resolution or changes in tree topologies.

Cytochrome *b* phylogeny

Maximum likelihood

The optimal ML topology (log L score = -6169.743) inferred from the complete sequences of 16 main ingroup taxa and the outgroups *M. musculus* and *A. chrysophilus* after bootstrap analysis, is shown in Figure 3. The tree was constructed under the best-fit ML model for the overall gene (HKY+I+Γ) with empirical estimation of the overall α, κ and Pinv values (Table 4, 15). Four major clades were retrieved. In the first of the three well supported clades, *O. angoniensis* and *O. maximus* from southern Africa grouped as sister taxa to the exclusion of the East African *O. t. jacksoni and O. typus* (hereafter referred to as the *O. typus* clade, since it is the first described taxon in the group). The second clade comprised representatives of the B and C cytotypes of *O. irroratus* who grouped together to the exclusion of the A2 cytotype, with *O. laminatus* basal in the clade (hereafter the *O. irroratus* clade). All associations in these two clades and the sister relationship between the *O. irroratus* and the *O. angoniensis* clades were well supported by bootstrap. The third, and exclusively East African clade, included *O. a. lacustris* (Uzungwa Mountains) and *O. tropicalis* as sister taxa (weakly supported), to which successive sister taxa joined by *O. a. lacustris* (Chingombe, Ufipa Plateau) and *O. denti* (hereafter, the *O. anchietae* clade). The placement of *O. denti* in the latter clade, and the basal position of this clade in the Otomyinae phylogeny were weakly supported by bootstrap. A fourth clade that was not always well supported included taxa from *Parotomys* and *Otomys*. A sister association between *P. littledalei* and *O. unisulcatus* received 78% support with *O. sloggetti* basal in the clade (poorly supported). The position of *P. brantsii* in the phylogeny and the deeper branching order of the major clades were unresolved. An identical tree was retrieved under the sub-optimal HKY+Γ model, and when equal rates of among-site rate variation was assumed, the placement of

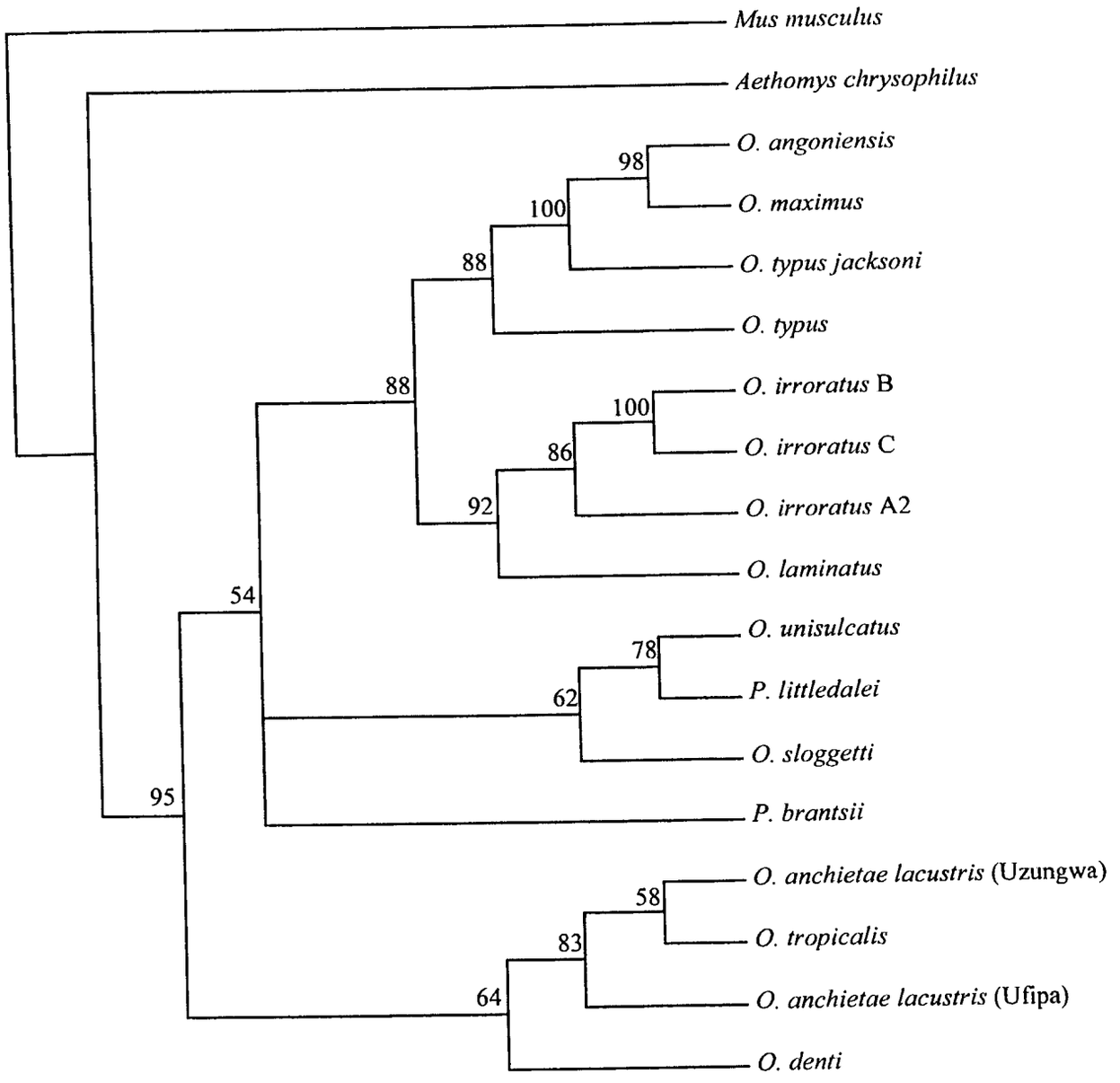

Figure 3: The optimal maximum likelihood phylogeny (log L score = -6169.743) for the Otomyinae after bootstrap analysis. The tree was retrieved from PAUP 4.0b2a (Swofford 1999) using 1143 basepairs of cytochrome *b* sequence data under the best-fit HKY+I+ Γ model of evolution (Gu *et al.* 1995), and on the overall empirical parameter estimates of κ, α and Pinv (see text). Bootstrap values > 50% for 100 maximum likelihood iterations are indicated at each node. *Mus musculus* and *Aethomys chrysophilus* were used as outgroups.

O. sloggetti was unresolved and there was weak support (53%) for *P. brantsii* basal in the tree. When the GTR (Rodriguez *et al.* 1990)+I+Γ model selected by the MODELTEST (version 3.06; Posada & Crandall 1998) was employed the resulting topology before, as well as after bootstrap analysis was similar to that derived under the HKY+I+Γ model (Fig. 3), except that the position of the *O. anchietae* was unresolved (tree not shown).

Maximum parsimony

The main ingroup cyt *b* sequences (Table 2) contained 353 variable and 254 parsimony informative sites (1st position: 63 variable, 39 informative; 2nd position: 10 variable, eight informative; 3rd: 280 variable, 207 informative). When the outgroups were included, 404 sites were variable and 294 informative (1st position: 82 variable, 50 informative; 2nd position: 17 variable, 11 informative; 3rd: 305 variable, 233 informative).

The application of the empirical weighting scheme derived from the best-fit ML model (codon-specific HKY+I+Γ, Table 15), produced a single most parsimonious tree of 3830.4 steps from 294 parsimony informative characters (CI = 0.632, RI = 0.588; Fig. 4). The empirical codon-specific ti:tv ratios for the ingroup (1st: 13.2; 2nd: 6.0; 3rd: 13.5) were applied as weights. The topology retrieved after bootstrap analysis (plain text), was identical to the optimal ML tree (Fig. 3) in terms of the robust nodes, and differences were restricted to nodes that received weak or below 50% support. These were the unresolved position of the *O. anchietae* clade, and the weakly supported position of *P. brantsii* basal in the phylogeny. Two equally parsimonious trees, one identical to the empirical tree, were retrieved at a tree length one to four steps longer (3831.4 - 3834.4 steps) than the most parsimonious solution. In the second tree, the positions of the *O. anchietae* and the clade including *O. unisulcatus* and *P. littledalei* were interchanged. Three trees of equal length, differing only in terms of the relationships of the major clades, resulted when between five and nine steps were added to the empirical tree length.

In general the weighted parsimony analyses showed that many nodes were insensitive to the weighting scheme applied. The same single most parsimonious tree (Fig. 4), resulted irrespective whether weighting was based on codon-specific or overall parameter

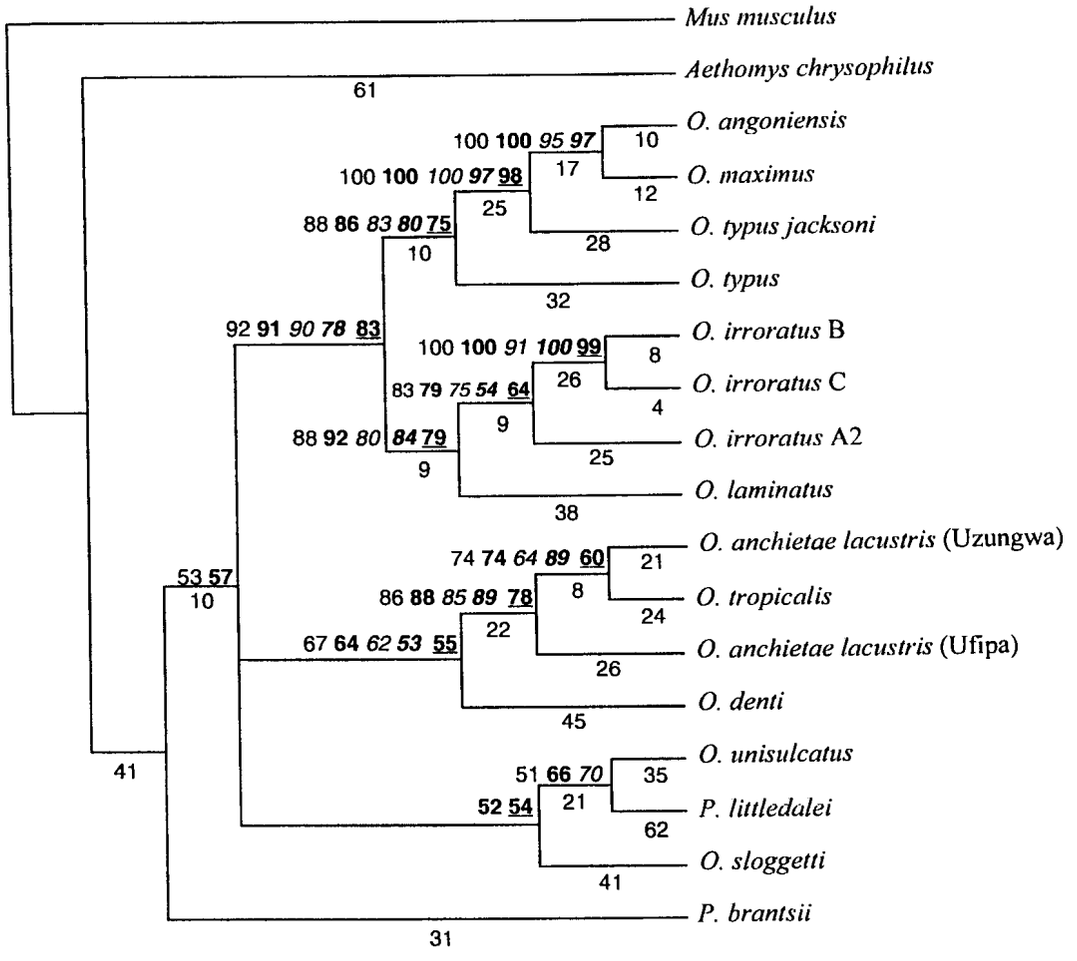

Figure 4: The most parsimonious solution for the Otomyinae (3830.4 steps) retrieved in PAUP 4.0b2a (Swofford 1999) when each of the three codon positions of cytochrome *b* were weighted using the empirical codon-specific ti:tv ratios obtained for the ingroup under the best-fit HKY+I+Γ model of evolution (see Table 4). Values above the branches show the bootstrap support for each node using 500 iterations based on the empirical ti:tv ratios (plain text) and three additional ingroup-based weighting strategies: empirical overall (bold), empirical codon-specific in combination with codons weighted according to their relative nucleotide variability (4:28:1, italics), and six-parameter parsimony (bold, italics). The nodal support based on the transversions only analysis (1:0), are underlined. Values for nodes that received less than 50% bootstrap support are not indicated and branches that received < 50% in all weighting schemes were collapsed. The number of unambiguous changes is shown beneath each branch (MacClade); no changes are indicated for branches within polytomies.

estimates of the transition bias including or excluding the outgroups (Table 4), and when character weighting (4:28:1) was used in combination with overall or codon-specific weighting schemes. There was some variation in bootstrap support among these alternative weighting strategies, but values were broadly consistent for similar nodes. The exceptions were the basal position of *P. brantsii* in the phylogeny, and the relative position of *O. sloggetti*. There was no general trend for decreasing resolution as a result of partitioning except for the node joining *O. unisulcatus* and *P. littledalei*. When six-parameter weighting was applied (Table 6), three equal-lengthed trees of 921 steps (CI = 0.700, RI = 0.607) were retrieved, which after bootstrap analysis failed to resolve the positions of *O. sloggetti, O. unisulcatus, P. littledalei* and *P. brantsii* (bold italics, Fig. 4). Therefore, although this weighting scheme (derived under the parameter-rich GTR model) was the most complex among those applied, it did not improve the resolution in terms of the internal relationships which were weakly supported when simpler weighting strategies were employed.

Several equally parsimonious trees resulted from the unweighted analysis of the complete data set (9 trees, 1167 steps, CI = 0.446, RI = 0.423, 294 informative sites) and when leucine changes were excluded (2 trees, 967 steps, CI = 0.447, RI = 0.436, 251 informative sites). The strict consensus of the equal-lengthed trees from both these analyses were virtually identical to the empirical bootstrap tree (Fig. 4), except for a basal dichotomy formed by *O. sloggetti* and *P. brantsii*, but after bootstrapping, the resolution with respect to the internal nodes were far poorer (not shown). Two equally parsimonious trees resulted from the analysis based exclusively on the more conserved, but less homoplastic transversions (216 steps, CI = 0. 718, RI = 0.670, 271 informative sites). The major clades retrieved by the other analyses were retained after bootstrapping (expectedly with poorer resolution of the closely related terminal taxa), but the deeper branching order of lineages remained unresolved (underlined, Fig. 4). This was not surprising given the few transversions in the cyt *b* gene of the Otomyinae (Table 8). Similarly, when the gene was translated to amino acid codons, few parsimony informative sites (30) remained resulting in 13 equal-length trees (118 steps, CI = 0.703, RI = 0.546, equal weights applied to characters) that collapsed to an unresolved polytomy after bootstrap analysis (not shown). The likelihood-based Shimodaira-Hasegawa test (Shimodaira & Hasegawa 1999) showed that none of the alternative topologies retrieved

in the unweighted or weighted parsimony analyses were significantly better estimates of relationships among the Otomyinae taxa ($P > 0.05$).

Distance

The phylogram in Figure 5 was generated by the NJ analysis of both HKY85 and Kimura-2-parameter-corrected distance matrices, and incorporated the empirical overall estimates of the gamma-shape parameter (1.645) and the proportion of invariable sites (0.596) inferred under the best-fit HKY+I+Γ model. The tree was identical to the empirical parsimony tree (Fig. 4) except for the position of *O. sloggetti*, but differed from the optimal ML tree (Fig. 3) in terms of the branching order of the weakly supported internal associations. After NJ bootstrap analysis, poor support emerged for some of the robust phylogenetic associations retrieved with the ML and MP analysis (Fig. 3, 4). The sister relationship of the *O. irroratus* and *O. typus* clades and the positions of *O. typus* and *O. laminatus* were not sustained. However, when the ML distance model was applied, these associations were maintained with weak support (68%, 53% and 71%, respectively), but the associations within the *O. anchietae* clade received < 50% support (not shown). The extremely short internal branch lengths observed in the phylogram may explain the poor bootstrap support at the deeper nodes and the swapping of the major clades retrieved by the other methods of analysis. Nonetheless, it is noteworthy that *P. littledalei* was distinguished by a long branch when compared to its sister *O. unisulcatus* and other terminal taxa. This finding was underscored by the large number of unambiguous changes along this branch (62) in the empirical parsimony tree (Fig. 4). Moreover, the taxon was characterized by a long branch, irrespective of which of the two representatives was included (Hentiesbaai or Springbok, see Table 2). Despite these findings, no significant rate heterogeneity was detected for *P. littledalei* (Springbok) except when compared to the A1 cytotype of *O. irroratus* by the relative rate test (Table 13; reference taxon: *A. chrysophilus*). On the other hand, this long branch may reflect the rate heterogeneity observed between *P. littledalei* (Hentiesbaai) and *P. brantsii* (Kleinsee), the *O. irroratus* cytotypes and *O. laminatus*.

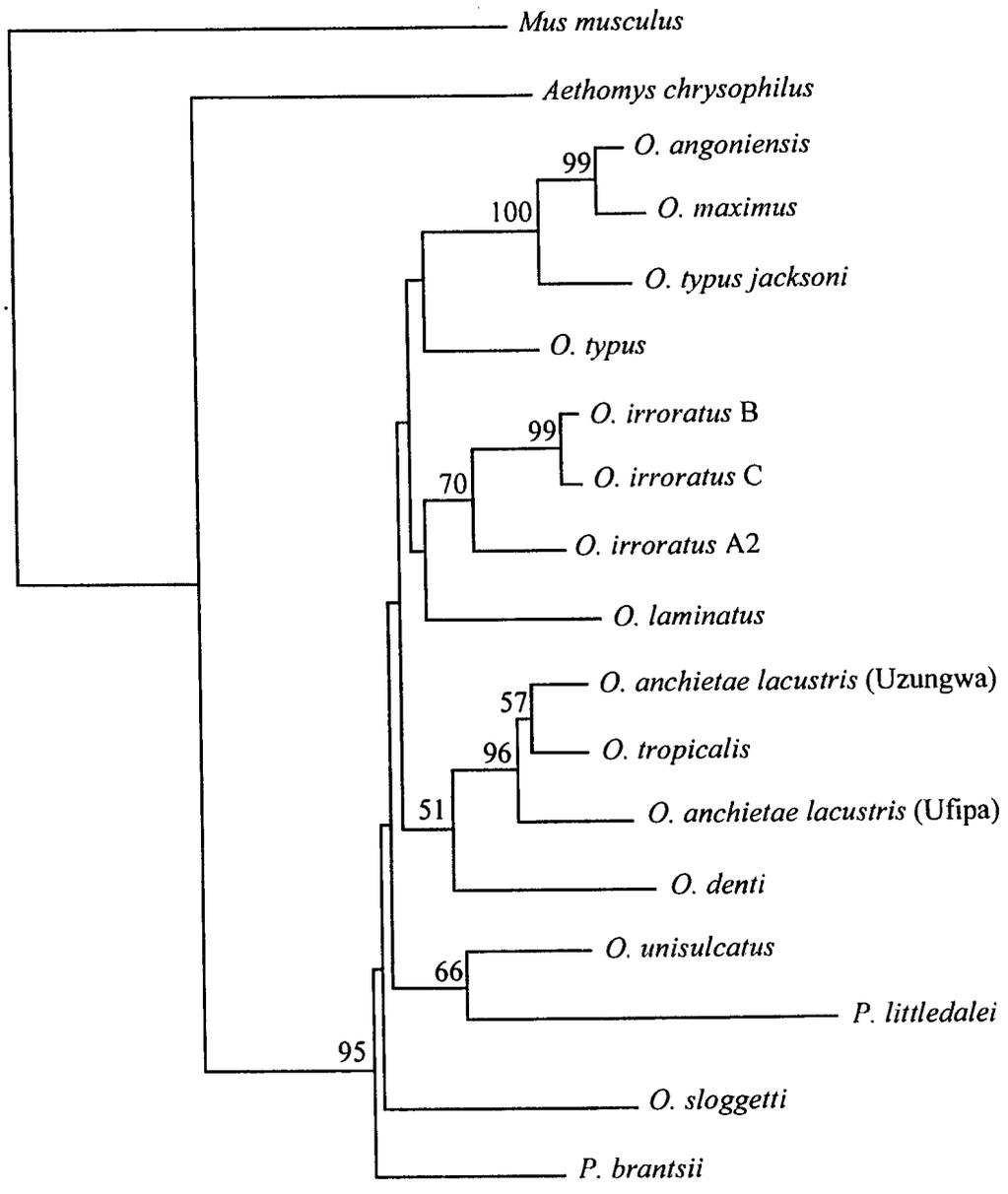

Figure 5: The cytochrome *b* neighbor-joining phylogram obtained in PAUP 4.0b2a (Swofford 1999) from the HKY85-corrected distance matrix (Hasegawa *et al.* 1985). The empirical overall α(1.645) and Pinv (0.596), estimated under the best-fit HKY+I+Γ model of evolution (Gu *et al.* 1995), were applied to account for among-site rate variation. The tree was rooted using *Mus musculus* and *Aethomys chrysophilus* and the branches were drawn proportionally to the amount of change occurring along them. Bootstrap values are indicated on nodes supported by at least 50% of 500 bootstrap replications.

The position of *O. occidentalis*

The position of *O. occidentalis* in the Otomyinae gene tree was assessed, but given the truncated sequence (only 926 bp of cyt *b* were available), the result must be viewed with some caution. Before bootstrapping the ML tree constructed under the best-fit HKY+I+Γ model (log L = -5119.952; Fig. 6) was identical to the bootstrap tree based on complete sequences (Fig. 3) and in this case, *O. occidentalis* grouped as the sister taxon to *O. unisulcatus* and *P. littledalei.* This relationship was also retrieved with NJ (HKY85 model) with the empirical $\alpha = 1.162$ and Pinv = 0.581 values applied. In the single most parsimonious tree retrieved when the empirical weighting for the reduced data set was used (6.9:1), the species grouped as the sister taxon to *P. littledalei* with *O. unisulcatus* basal. This was based on 239 informative characters. However, on bootstrapping, *O. occidentalis'* phylogenetic affiliations remained unresolved irrespective of method of analysis (Fig. 6).

Analysis of *O. irroratus* cytotypes based on cytochrome *b*

The tree in Figure 7 depicts the phylogenetic relationships of the *O. irroratus* cytotypes (A1, A2, B and C; Table 2), as inferred from cyt *b* data, using *O. laminatus* as outgroup. The pattern is essentially consistent with the larger phylogenetic analysis (Fig. 3 - 5, 8 - 10).

A close association was detected between the B and C cytotypes and the A1 and A2 cytotypes, which, respectively, form two monophyletic clades. This topology was retrieved by all three methods of analyses and under different weighting schemes (ML under the HKY+I+Γ model, $\kappa = 11.3$, α = infinity, Pinv = 0.766; unweighted parsimony analysis, 213 steps, CI = 0.798, RI = 0.768; parsimony using the empirical 12.5:1 weighting scheme, 545 steps, CI = 0.877, RI = 0.849; NJ applying the HKY85 distance correction assuming equal rates of change among variable sites). The distance parameter was chosen because when α is infinity, all sites have a relative rate of 1.0, so that an equal-rates model can be satisfied as a special case of the gamma model (Swofford *et al.* 1996). In the NJ tree, relative to the terminal branches, long internal branches lead to the clades that include the B and C cytotypes and the A1 and A2 cytotypes, respectively. This is underscored by the numbers of unambiguous changes occurring along them on the

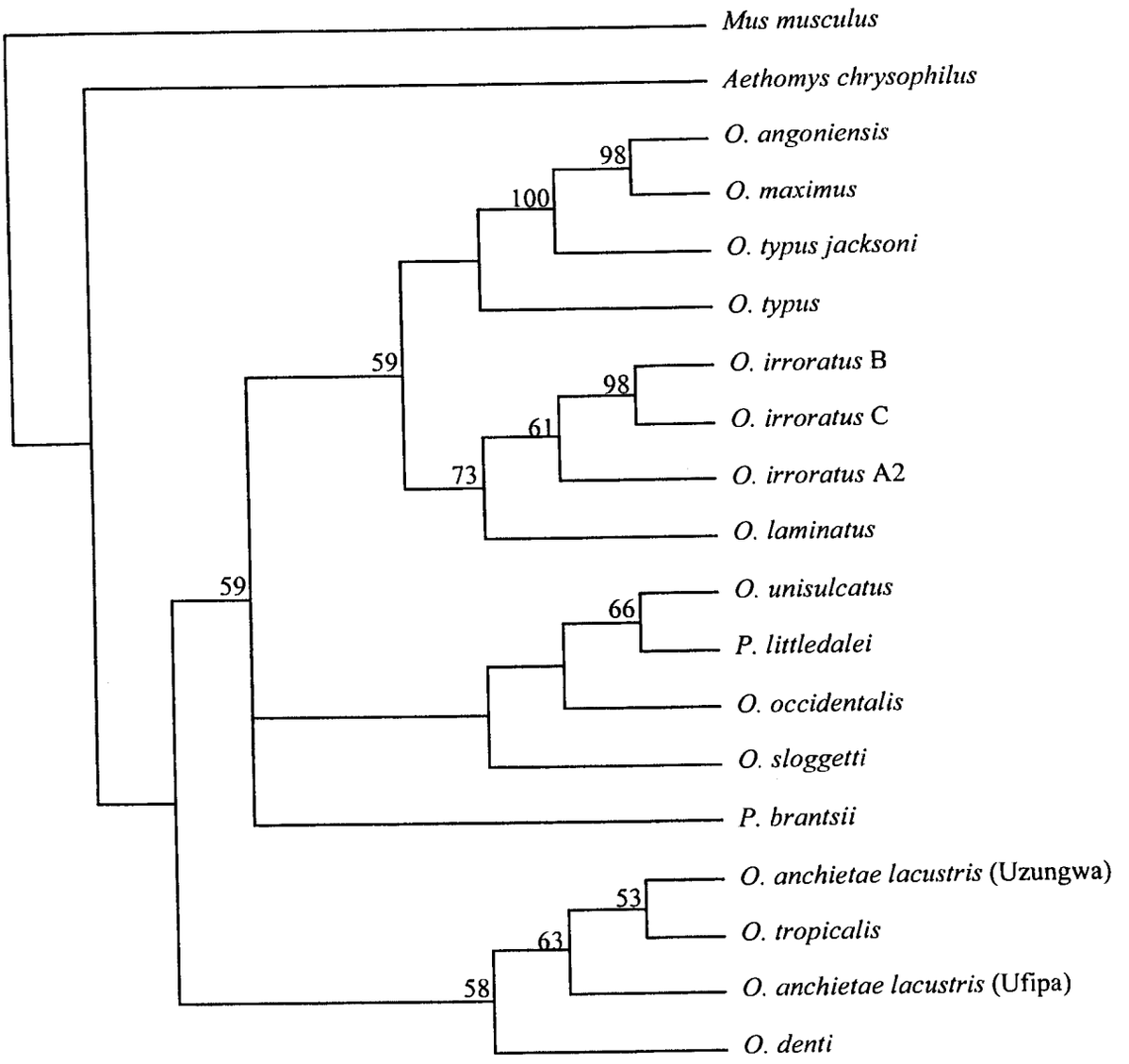

Figure 6: The maximum likelihood topology (log L score = -5119.952) inferred from the reduced cytochrome *b* data set (926 bp) to assess the phylogenetic position of *O. occidentalis* in the Otomyinae. The tree was constructed under the best-fit maximum likelihood model (HKY+I+Γ, Gu *et al.* 1995) with empirical estimation of the overall parameter estimates of gene dynamics (α = 1.162, κ = 6.863, Pinv = 0.581). Bootstrap values > 50% for 100 maximum likelihood iterations are indicated at each node. *Mus musculus* and *Aethomys chrysophilus* were used as outgroups.

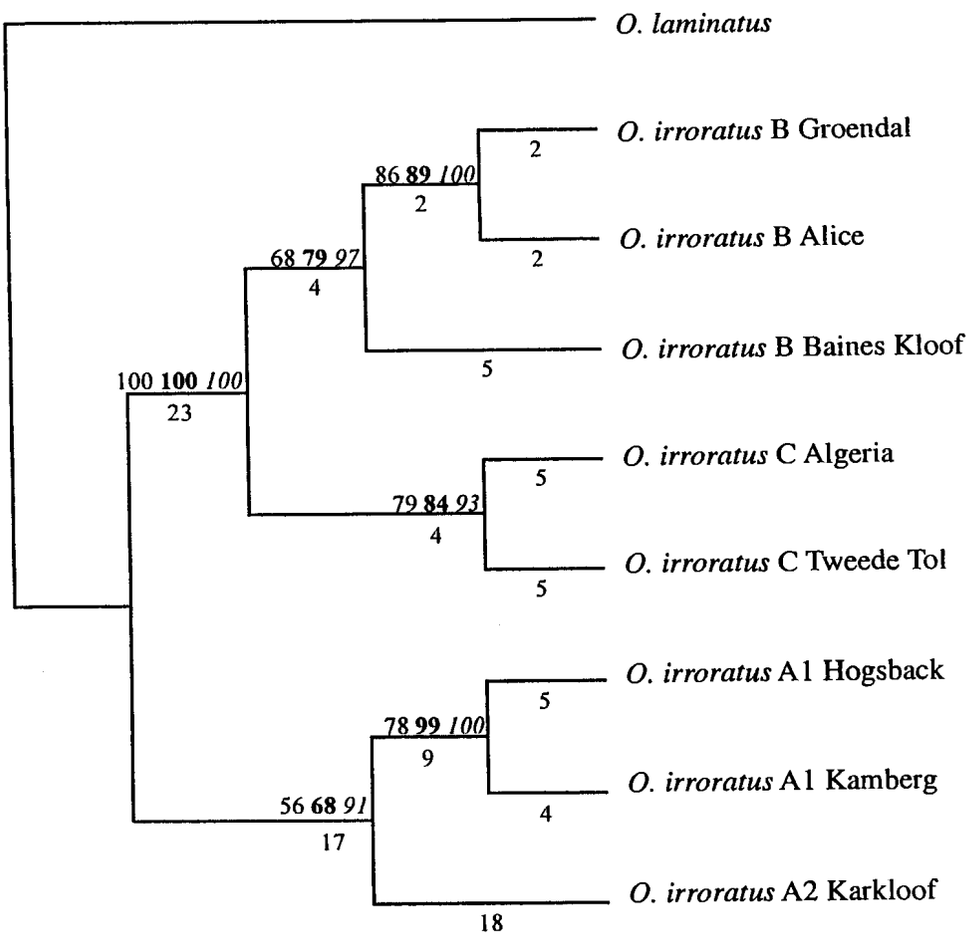

Figure 7: The topology depicting the relationships among the *O. irroratus* cytotypes (A1, A2, B and C) represented in this study (see Table 2) using *O. laminatus* as outgroup. Collection localities are indicated after the species names. The tree resulted from the ML analysis of complete cytochrome *b* sequences in PAUP 4.0b2a (Swofford 1999) under the optimal HKY+I+Γ model (Gu *et al.* 1995) with empirical parameter estimation of sequence dynamics (κ = 11.3, α = infinity, Pinv = 0.766). The numbers above nodes indicate bootstrap support > 50% for 100 ML replicates (plain text); 500 replicates each for the most parsimonious empirical tree (477.5 steps) obtained under an ingroup-based 12.5:1 weighting scheme (bold); the neighbor-joining tree using the HKY85-corrected distance matrix (Hasegawa *et al.* 1985) assuming an equal rates model (italics). The values below each branch are the number of unambiguous character changes calculated in MacClade.

empirical parsimony tree (Fig. 7). Similarly, the Karkloof lineage (A2 cytotype) is also distinguished by a long branch when compared with the amount of change occurring along the branches leading to the other cytotypes. Long branches may possibly indicate that considerable genetic change has occurred since these cytotypes last shared a common ancestor or, alternatively, it might suggest an increased mutation rate for the respective lineages. However, the relative rate test detected no significant rate heterogeneity among the cytotypes relative to the closely related *O. laminatus* (outgroup) therefore, probably precluding spurious associations caused by long-branch attraction.

12S rRNA phylogeny

The ML, MP and NJ analyses of the 12S rRNA sequences of 22 otomyine taxa using the outgroups *M. musculus* and *A. namaquensis* (Table 2) resulted in poorly resolved phylogenies (Fig. 8). The relationships that were well supported across reconstruction methods, were congruent with cyt *b* results (Fig. 3 - 5), but involved only the closely related terminal taxa. Significant branch swapping occurred at the weakly supported nodes across the various reconstruction methods and data treatments applied, and the gene did not aid in clarifying the deeper branching order of taxa or the interrelations among the major clades suggested by the cyt *b* sequences.

Maximum likelihood

Maximum likelihood analysis performed under the best-fit HKY+I+Γ model with overall rates of $\kappa = 3.959$ and $\alpha = 0.410$ and the Pinv set to 0.610, resulted in a tree with a log L score of -2348.624. After bootstrap analysis (plain text, Fig. 8) strong support emerged for a sister taxon association between *O. a. lacustris* (Uzungwa Mountains) and *O. tropicalis* and between the B and C cytotypes of *O. irroratus*, as well as for *O. a. lacustris* (Ufipa Plateau) and the A2 cytotype, respectively, basal to the sister taxa in each clade. Weak support for *O. angoniensis* and *O. maximus* as sister taxa resulted, but the basal position of *O. t. jacksoni* in the clade was well supported. An identical tree was recovered with the GTR+I+Γ model selected by the MODELTEST (Posada & Crandall 1998). As with the cyt *b* analyses, the available software did not allow an assessment of

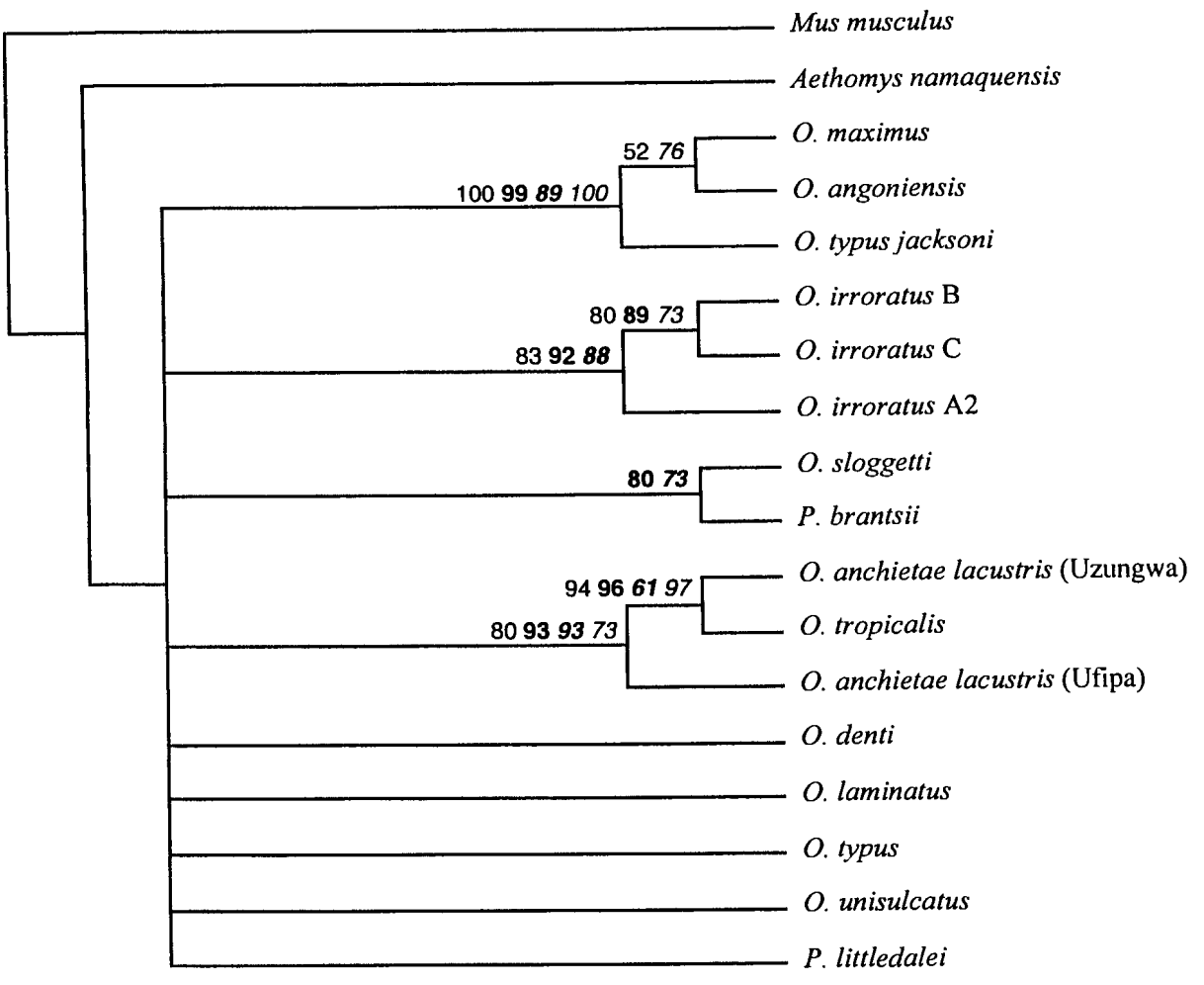

Figure 8: The consensus of the 12S rRNA phylogenies for the Otomyinae retrieved across maximum likelihood (ML), maximum parsimony (MP) and neighbor-joining (NJ) reconstruction methods in PAUP 4.0b2a (Swofford 1999). All trees were based on 802 basepairs of aligned sequences and were rooted using *Mus musculus* and *Aethomys namaquensis*. Bootstrap values (> 50%) from 100 iterations in ML and 500 iterations in MP and NJ are indicated at the nodes for the following trees: ML (plain text): the optimal phylogeny (log L score = -2348.624) recovered under the best-fit HKY+I+Γ model of evolution (Gu *et al.* 1995) with empirical estimation of the overall κ (3.959), α (0.410) and Pinv (0.610) parameters; MP (bold): weighting stems and loops according to the empirical partition-specific κ obtained for the ingroup (Table 5, HKY+I+Γ); six-parameter parsimony (bold, italics); NJ (italics): the topology inferred using the HKY85 distance correction (Hasegawa *et al.* 1985) incorporating the empirical overall α (0. 410) and Pinv (0.610) parameters derived under the HKY+I+Γ model. See text for treatment of gaps.

possible topological differences when a unique HKY+I+Γ model was adopted for the analysis of the stem and loop regions.

Maximum parsimony

The 12S rRNA data set yielded 102 variable and 52 informative characters (stems: 22 variable, nine informative; loops: 80 variable, 43 informative), when only the main ingroup taxa were considered. With the outgroups included, 132 characters were variable and 59 informative (stems: 32 variable, nine informative; loops: 100 variable, 50 informative). Coded gaps yielded two additional informative characters for the ingroup (five when the outgroups were included).

The differential weighting schemes consistently recovered well-supported associations, but resulted in changes among the weakly supported taxa. A single most parsimonious tree of 695.3 steps (CI = 0.783, RI = 0.653) was inferred under the empirical ingroup-based stem-loop-specific weighting scheme (stems: 17.5:1; loops: 6.7:1) derived with the best-fit HKY+I+Γ model. Strong bootstrap support (80%) was retrieved for a sister relationship between *O. sloggetti* and *P. brantsii* in addition to the nodes that were well supported in the optimal ML tree (bold, Fig. 8). A sister association between *O. maximus* and *O. t. jacksoni* (not retrieved in ML) was weakly supported (63%, not shown). An identical topology resulted when weighting was based on the empirical stem-loop-specific ti:tv ratio determined for the in and outgroup, and on the overall ratios both excluding and including the outgroups (Table 5). There was no tendency towards a decrease in nodal support when partitioned weighting versus the overall weighting schemes were applied. Down weighting (0.61) stems sites in combination with the partitioned and overall weighting strategies did not alter the tree's topology. However, when a heavier weight (3.63) was assigned to stem sites according to the inverse of the relative ingroup character variability, a sister relationship between *O. irroratus* clade and the clade comprising *O. sloggetti* and *P. brantsii* was supported (> 70%; not shown). No other analyses supported this association. Unweighted parsimony analyses produced seven equally parsimonious trees (255 steps, CI = 0.675, RI = 0.526, 64 informative sites). The topology after bootstrap analysis (not shown) was nearly identical to the empirical bootstrap tree (see bold, Fig. 8), but the node supporting *O. sloggetti/P. brantsii* collapsed and some support (79%) was provided for

O. angoniensis and *O. maximus* as sister taxa. With six-parameter parsimony (Table 6), 10 equally parsimonious trees (266 steps, CI = 0.808, RI = 0.683) were retrieved, which after bootstrapping, were less resolved in respect of the terminal nodes than when the empirical stem-loop-specific weighting was applied (bold italics, Fig. 8). As with cyt *b*, the Shimodaira-Hasegawa test (Shimodaira & Hasegawa 1999) test showed no significant difference among the topologies retrieved under the differential weighting schemes (transitions over transversions), or when the alternative character weighting strategies were applied to stem sites ($P > 0.05$).

Distance

The NJ tree inferred under an HKY85 model incorporating overall empirical estimates of $\alpha = 0.410$ and Pinv = 0.610 (HKY+I+Γ model) to account for among-site rate variation, was less well resolved than either the ML or MP trees after bootstrap analysis (italics, Fig. 8). An identical bootstrap tree was recovered under the Kimura-2-parameter and ML distance corrections, but branch swapping of the poorly supported lineages occurred. Similar to cyt *b*, the internal branches of the NJ trees (not shown) were short, while *P. brantsii* was characterized by an exceptionally long branch reflecting the different substitution rate detected by the relative rate test (Table 14; reference taxon: *A. namaquensis*).

Combined analysis

The appropriateness of combining the cyt *b* and 12S rRNA data sets was confirmed by the partition homogeneity test ($P = 0.452$), which detected no significant conflict in phylogenetic signal between them. Analysis of the combined data sets did not improve the phylogenetic resolution obtained in the independent analyses of the two genes (see below). Trees were constructed from 1945 bp of sequence from 22 otomyine taxa, using *M. musculus*, *A. namaquensis* (12S rRNA) and *A. chrysophilus* (cyt *b*) as outgroups.

Maximum likelihood

Figure 9 shows the ML tree (after bootstrap analysis) retrieved under the best-fit HKY+I+Γ model with no partitioning of data (κ = 6.9, α = 0.891, Pinv = 0.628). The topology was identical to the optimal cyt *b* ML tree (Fig. 3) except for the unresolved position of *O. sloggetti*. Bootstrap values were generally similar to those obtained from cyt *b* alone, except that support for the basal position of the *O. anchietae* clade improved slightly (54 - 66%), and the support for associations within this clade was noticeably higher (plain text, Fig. 9). The assessment of differences in log L scores (HKY+I+Γ model) obtained from unpartitioned versus partitioned analysis (which allows for a unique substitution model for each gene fragment or functional gene region), resulted in a lower score for the unpartitioned data (log L = -8653.784). The sum of the log L scores for data partitioned by gene (log L = -8521.628) was intermediate, and the highest score (log L = -7889.967) resulted when data were partitioned by the functional regions within each gene (codons, stems and loops). The log likelihood ratio test (Goldman 1993) indicated a highly significant increase in model fit for both partition schemes over an unpartitioned scheme (partitioned by gene: test statistic = 264.313, d.f. = 1, $P < 0.001$; partitioned by functional regions: test statistic = 1527.634, d.f. = 1, $P < 0.001$).

Maximum parsimony

The weighting of codon positions (cyt *b*) and stems and loops (12S rRNA) by their empirical ingroup-based empirical κ values derived under the best-fit HKY+I+Γ model (Table 4, 5) resulted in a single tree (4573.4 steps, CI = 0.649, RI = 0.583). Nodal support (bold, Fig. 9) was similar to that obtained in the optimal ML tree, except for the reduced support for the basal placement of the *O. anchietae* clade (ML: 66%; MP: 51%), and the *P. littledalei/O. unisulcatus* association (ML: 76%; MP: 55%). A similar topology was recovered (before and after bootstrap) using a gene-specific weighting scheme and when weighting was based on partition-specific and gene-specific empirical κ values derived with outgroups included (see Table 4, 5; values not shown). However, the internal nodes of the topologies resulting from differential weighting of ti:tv according the empirical overall ratios for the combined data set (ingroup based: 9.8:1; ingroup and outgroup based: 6.9:1), were less resolved after bootstrapping than in the

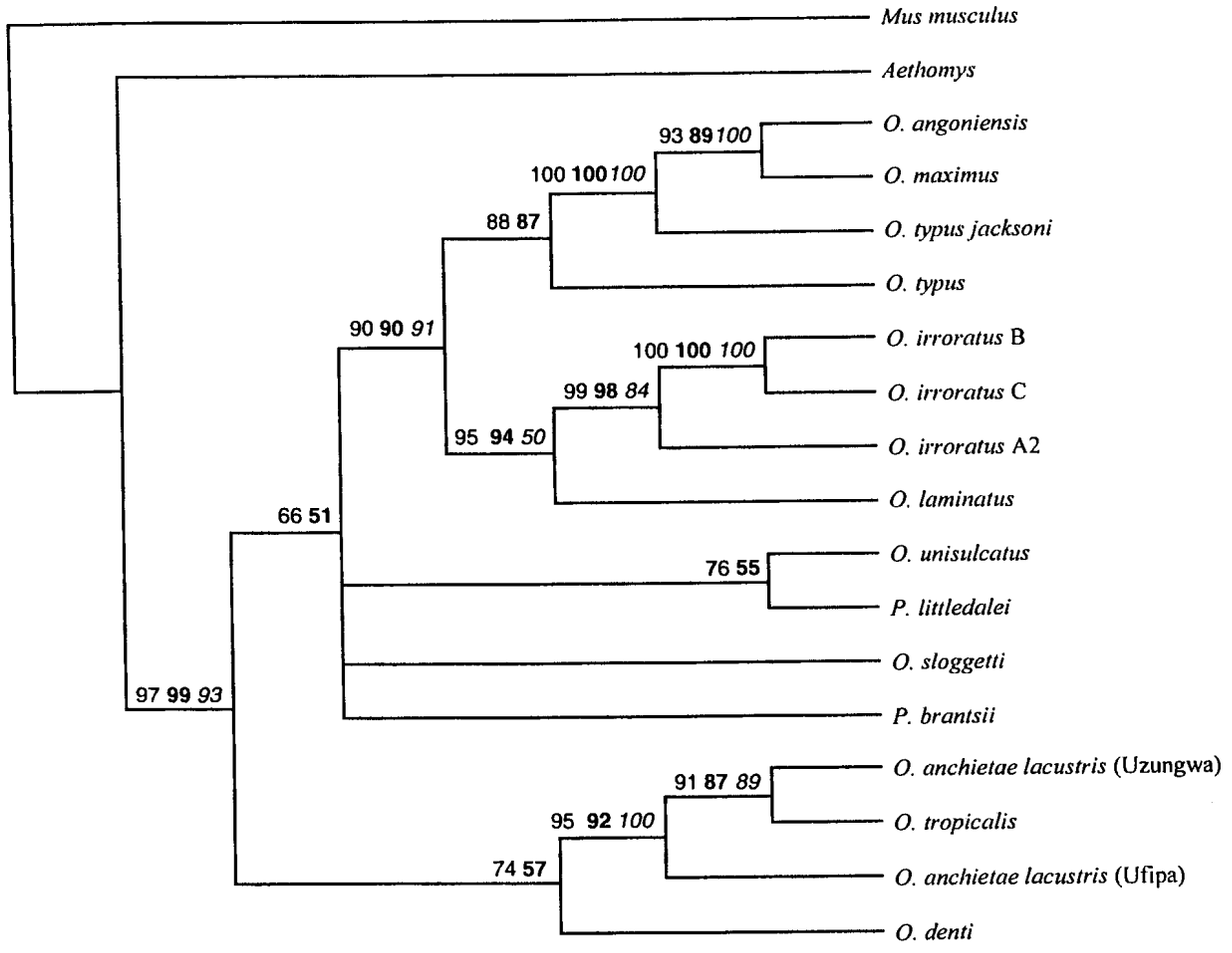

Figure 9: The optimal maximum likelihood phylogeny for the Otomyinae (log L score = -8653.784) after bootstrap analysis. The tree was inferred from 1945 basepairs of combined cytochrome *b* and 12S rRNA data under the best-fit HKY+I+Γ model (Gu *et al.* 1995) in PAUP 4.0b2a (Swofford 1999). The outgroups *Mus musculus, Aethomys chrysophilus* (cytochrome *b*) and *A. namaquensis* (12S rRNA) and 22 ingroup taxa were included in the analyses. Bootstrap values > 50% for 100 ML iterations (plain text) and 500 replicates in MP (bold) and NJ (italics) are indicated at each node for the following: MP: the most parsimonious tree (4573.4 steps) produced under the empirical ingroup-based partition-specific weighting scheme (HKY+I+Γ model, see Table 4, 5); NJ: the tree inferred from an HKY85-corrected distance matrix (Hasegawa *et al.* 1985) with the empirical overall parameter estimates of α (0.891) and Pinv (0.628) applied.

empirical tree derived under a partitioned weighting scheme (see bold, Fig. 9). *Parotomys brantsii* and *O. sloggetti* received > 75% support (values not shown). Unweighted parsimony analyses resulted in two equal-lengthed trees (1434 steps, CI = 0.483, RI = 0.428), which, after bootstrap analysis, showed poor support for internal nodes as well as basal nodes in the major clades (not shown). Reconstructing the phylogeny using only transversions (333 informative sites) produced 12 trees of equal length (298 steps, CI = 0.742, RI = 0.672). Nodes that enjoyed strong bootstrap support were the same as those in the empirical parsimony tree based on all substitutions (see bold, Fig. 9).

Distance

The tree generated by NJ from the HKY85-corrected distance matrix for the combined data with the inclusion of empirical estimates of α (0.891) and Pinv (0.628) (HKY+I+Γ model), resulted in a poorly resolved topology after bootstrap analysis (italics, Fig. 9). When a ML distance model (incorporating κ = 6.9), was applied to the pairwise distances, only the strongly supported nodes evident in the optimal ML tree (see bold, Fig. 9) were retained.

Ingroup phylogeny

Less resolution was evident at the deeper nodes of the bootstrapped topologies resulting from the phylogenetic analysis (ML, NJ and MP) of cyt *b*, 12S rRNA and the combined data sets of the 16 main ingroup taxa than was the case with the outgroups included (Fig. 10). Strong bootstrap support was, however, maintained for the robust relationships retrieved in the ML, MP and NJ trees rooted on the outgroups (Fig. 3 - 5, 8, 9). Furthermore, ML analyses of all three data sets (independently) produced trees that were better resolved than the topologies inferred by unweighted and unordered parsimony and by NJ using the of HKY85 distance correction with empirical estimates of among-site rate variation and invariable sites (see Table 4, 5). In light thereof, the discussion of the results will focus on the relationships retrieved in the respective ML trees (Fig. 10).

The best phylogenetic resolution with respect to the relationships of the main ingroup taxa was provided by the cyt *b* ML tree (log L = -5198.254) determined using the best-fit

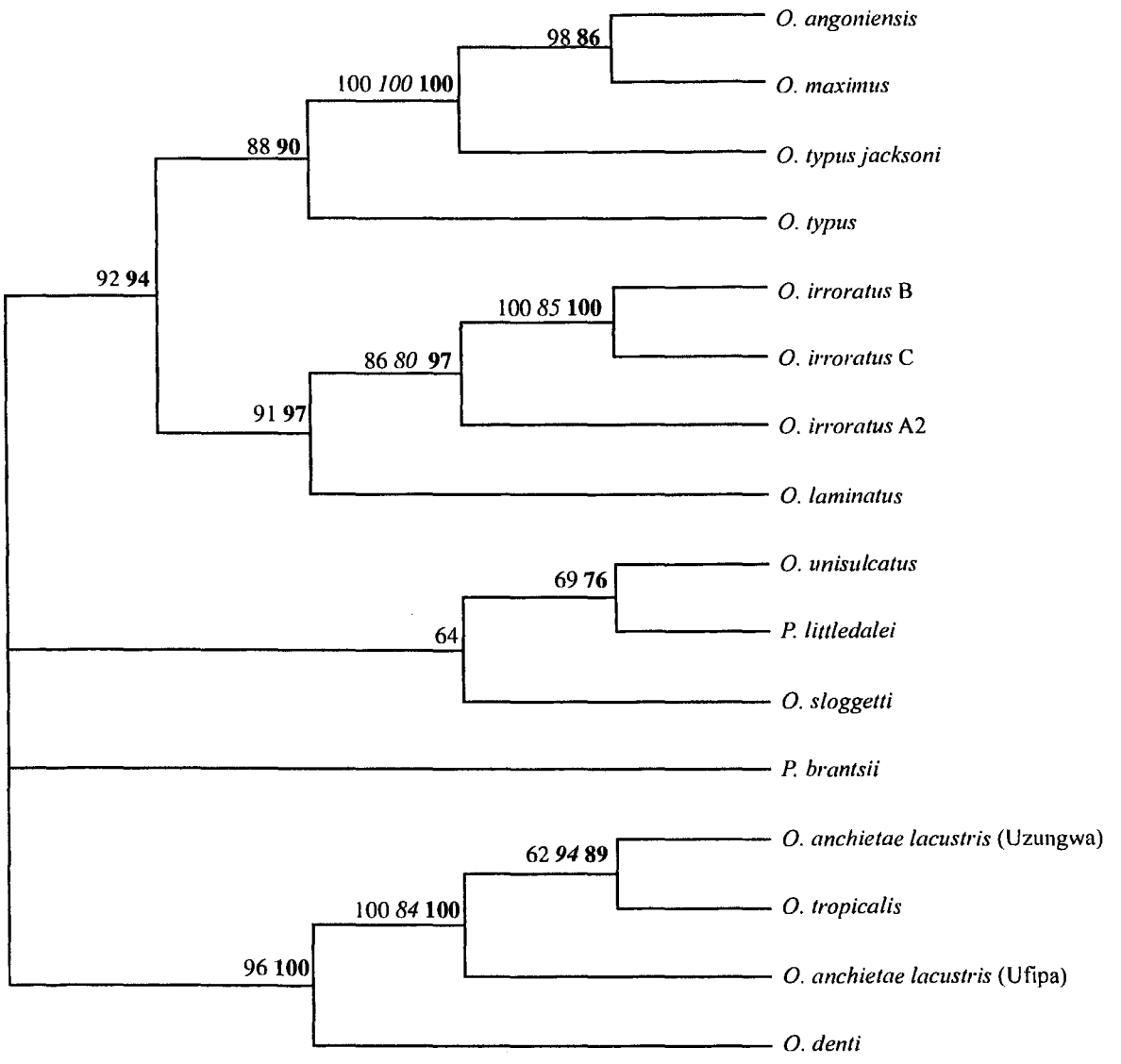

Figure 10: The optimal cytochrome *b* maximum likelihood topology (log L score = -5198.254) constructed using PAUP 4.0b2a (Swofford 1999). The tree was derived from 1143 basepairs of sequence from the main ingroup taxa using the best-fit HKY+I+Γ model of evolution (Gu *et al.* 1995), based on empirical overall parameter estimates of κ, α and Pinv. Bootstrap support (> 50%) for the nodes from 100 replications in ML are indicated. The support recovered for the respective nodes in the optimal ML trees (best-fit HKY+I+Γ model) from 802 basepairs of 12S rRNA sequences (italics) and 1945 basepairs of combined cytochrome *b* and 12S rRNA sequences (bold) is also shown.

HKY+I+Γ model with an empirical estimation of sequence characteristics (α = 1.899, κ = 10.493, Pinv = 0.625). After bootstrap analysis (plain text, Fig. 10), the three major clades (*O. typus, O. irroratus, O. anchietae* clades) and the sister association between *O. unisulcatus* and *P. brantsii* retrieved in the ML analysis rooted on the outgroups were retained (Fig. 3). A sister association between the two former clades was confirmed, but the exact relationships of the remaining clades and the position of *P. brantsii* were unresolved. The optimal ingroup-based 12S rRNA ML tree (log L = -1998.475) that was derived using the HKY+I+Γ model (α = 0.479, κ = 6.963, Pinv = 0.683) was unresolved after bootstrap analysis (italics, Fig. 10). In the case of the combined data, two optimal trees (log L = -7311.926) resulted (HKY+I+Γ model, α = 1.087, κ = 9.797, Pinv = 0.671). The same three clades were supported by higher bootstrap values (bold, Fig. 10) than was the case in the combined ML tree rooted on outgroups (plain text, Fig. 9) except for the unresolved placement of the *O. anchietae* clade. One less node (the position of *O. sloggetti*) was resolved in the combined tree than in the optimal cyt *b* tree (plain text, Fig. 10). There was a marked improvement in bootstrap support for *O. denti* as basal in the *O. anchietae* clade in ingroup ML topologies based on cyt *b* (64 – 96%) and the combined data (74 - 100%).

Statistical Evaluation of Alternative Topologies using Maximum Likelihood

The likelihood ratio test (Shimodaira & Hasegawa 1999) showed that neither the optimal ML, MP and NJ topologies inferred from cyt *b*, 12S rRNA or the combined data sets could be considered to provide a significantly better estimate of phylogenetic relationships among the Otomyinae (Table 16). The test further showed that when the topology was constrained to support the monophyly of the genus *Parotomys*, this user-defined tree was not a significantly worse estimate of the phylogeny than the optimal ML trees recovered from each data set. The existence of an arid-adapted clade comprising *P. littledalei*, *P. brantsii* and *O. unisulcatus* was rejected ($P < 0.05$) based on the comparison of the constrained and unconstrained topologies recovered from cyt *b* as well as the combined cyt *b* and 12S rRNA data. However, the constrained 12S rRNA topology was not significantly worse than the optimal ML tree (Table 16). This may be due to the limited amount of phylogenetic signal contained in the 12S rRNA data. Irrespective of this however, the monophyly of the East African taxa were consistently rejected ($P < 0.05$, Table 16).

Table 16: Results of the quantitative evaluation of alternative evolutionary hypotheses using the log likelihood comparison test of Shimodaira and Hasegawa (1999).

Hypothesis 1 vs. hypothesis 2 (constraint)	log L hypothesis 1	log L hypothesis 2	Diff. log L	P value	Significance level
Cytochrome *b*					
Best ML [a] vs. best MP [b]	-6169.743	-6169.825	0.082	0.493	N.S.
Best ML [a] vs. best NJ [c]	-6169.743	-6171.430	1.687	0.357	N.S.
Best MP [b] vs. best NJ [c]	-6169.825	-6171.430	1.605	0.196	N.S.
Best ML [a] vs. ML (*Parotomys* clade) [d]	-6169.743	-6178.269	8.526	0.095	N.S.
Best ML [a] vs. ML (arid clade) [e]	-6169.743	-6253.091	83.348	< 0.05	*
Best ML [a] vs. ML (East African clade) [f]	-6169.743	-6243.975	74.232	< 0.05	*
12S rRNA					
Best ML [a] vs. best MP [b]	-2348.624	-2354.138	5.514	0.257	N.S.
Best ML [a] vs. best NJ [c]	-2348.624	-2355.239	6.615	0.243	N.S.
Best MP [b] vs. best NJ [c]	-2354.138	-2355.239	1.101	0.481	N.S.
Best ML [a] vs. ML (*Parotomys* clade) [d]	-2348.624	-2351.893	3.269	0.253	N.S.
Best ML [a] vs. ML (arid clade) [e]	-2348.624	-2352.103	3.479	0.245	N.S.
Best ML [a] vs. ML (East African clade) [f]	-2348.624	-2381.950	33.326	< 0.05	*
Combined (cyt *b* and 12S rRNA)					
Best ML [a] vs. best MP [b]	-8653.784	-8656.387	2.603	0.306	N.S.
Best ML [a] vs. best NJ [c]	-8653.784	-8656.961	3.177	0.194	N.S.
Best MP [b] vs. best NJ [c]	-8656.387	-8656.961	0.574	0.477	N.S.
Best ML [a] vs. ML (*Parotomys* clade) [d]	-8653.784	-8664.033	10.249	0.085	N.S.
Best ML [a] vs. ML (arid clade) [e]	-8653.784	-8774,156	120.372	< 0.05	*
Best ML [a] vs. ML (East African clade) [f]	-8653.784	-8757.431	103.647	< 0.05	*

a - log L scores for the optimal maximum likelihood trees derived under the best-fit model of evolution (HKY+I+Γ) for the cytochrome *b* , 12S rRNA and combined data sets.

b - log L scores for the optimal maximum parsimony trees inferred from cytochrome *b*, 12S rRNA and combined cytochrome *b* and 12S rRNA sequences that were calculated under the best-fit HKY+I+Γ model with the empirical overall parameter estimates of κ, α and Pinv applied. The most parsimonious trees retrieved under a partition-specific weighting scheme (Table 4, 5) were loaded as topological constraints in each analysis.

c - log L scores for the optimal neighbor-joining topologies for the cytochrome *b*, 12S rRNA and the combined data sets were calculated using as loaded constraints, the topologies retrieved when the empirical α and Pinv parameters derived under the best-fit HKY+I+Γ model were applied.

d - Monophyly of the genus *Parotomys* constrained (*P. brantsii* and *P. littledalei*).

e - *Parotomys brantsii*, *P. littledalei* and *O. unisulcatus* constrained as a monophyletic "arid" clade.

f - The monophyly of the East African Otomyinae constrained (*O. anchietae lacustris*, *O. denti*, *O. tropicalis*, *O. typus* and *O. typus jacksoni*).

CHAPTER 4

DISCUSSION

The taxonomy of the African rodent subfamily Otomyinae is characterized by ambiguity at the subfamily, generic, specific and subspecific levels (Musser & Carleton 1993). This instability is due in large part to external phenotypic uniformity within the subfamily, as well as the variability and hence poor discrimination provided by the cranio-dental features on which the current taxonomy is based. The present study is novel in the sense that it is the first molecular investigation of the evolutionary relationships between all but one (*O. karoensis*) of the 14 extant species in the Otomyinae (Musser & Carleton 1993; Taylor *et al.* 1993). Representatives of four of the five cytotypes (A1, A2, B and C, Contrafatto *et al.* 1992a, b, 1997) identified on cytogenetic grounds within *O. irroratus* were included as well as putative subspecies of two of the 14 species (*O. anchietae lacustris* and *O. typus jacksoni*; see Table 2). To this end, mitochondrial cyt *b* and 12S rRNA sequences were analyzed separately and in combination using ML, MP and NJ methods. In addition, the patterns of sequence evolution among otomyine species and the *O. irroratus* cytotypes were assessed.

PATTERNS OF SEQUENCE EVOLUTION

Cytochrome *b* Characteristics

As expected for a protein-coding gene, a non-random distribution of nucleotide substitutions, inversely related to the degree of selective constraints on the different codon positions, was evident in cyt *b* (Table 8). There were six more leucine codons present than the 55 suggested in the typical cyt *b* gene of mammals (Irwin *et al.* 1991). The distribution of the codons and rates of codon changes are consistent with the mammalian structure-function model for the gene (Irwin *et al.* 1991 adapted from Howell 1989). As expected, the least number of codon changes were present in the outer-membrane, the codons of which form part of the highly conserved Q_o redox center involved in electron transfer. Most changes occurred in the more variable trans-membrane, which, together with inner-membrane are of less functional importance, except the short, conservative portion of the latter involved in the Q_i redox center (Hatefi 1985; Howell & Gilbert 1988; Esposti *et al.* 1993). The large number of nucleotide changes in the outer-membrane (95) compared to the inner-membrane (71) indicate that most outer-membrane changes were synonymous and did not result in replacement of amino acids since fewer variable sites were present in the translated amino acid sequence of the former than the latter (Table 8).

The mean base composition and compositional bias of the complete gene and the different codon positions (Table 8) coincide with trends observed in various rodents (e.g. Patton & Smith 1992; Verheyen *et al.* 1995; Matthee & Robinson 1997a; Ducroz *et al.* 1998; Conroy & Cook 1999), mammals in general, and other vertebrates (e.g. Kocher *et al.* 1989; Irwin *et al.* 1991; Patton *et al.* 1996; Halanych & Robinson 1999; Matthee & Robinson 1999a). However, the 3rd position bias (0.347) was somewhat lower than the average (0.401) calculated for mammals (Irwin *et al.* 1991) but similar to, or somewhat higher than the bias observed in other congeneric murid rodents (e.g. *Arvicanthis*: 0.308, Ducroz *et al.* 1998).

12S rRNA Characteristics

The strikingly low numbers of variable and parsimony informative nucleotide sites in 12S rRNA (Table 8) indicate the conserved nature of the gene in the Otomyinae compared to other rodents of similar evolutionary age (e.g. Sigmodontinae, *Peromyscus*, Sullivan *et al.* 1995). The lower numbers of transitions in stems compared to loops concurs with what has been reported for closely related mammals (primates, Hixson & Brown 1986; artiodactyls, Springer *et al.* 1995; Jansen van Vuuren & Robinson 2001), but is contradictory to approximately equal frequencies described when both closely and distantly related mammals are compared (Springer *et al.* 1995; Springer & Douzery 1996). The difference clearly illustrates that these ratios are sensitive to the level of phylogenetic divergence (Springer & Douzery 1996). The approximately five-fold increase in transversions in loops relative to stems is consistent with mammalian patterns, which is presumably a result of evolutionary constraints to maintain pairing in stems (Springer *et al.* 1995; Springer & Douzery 1996).

The compositional bias (against Gs and towards As) for the sequenced portion of 12S rRNA as well as stem and loop regions (Table 8) are consistent with previous reports for rodents (Nedbal *et al.* 1994, 1996; Matthee & Robinson 1997a), other mammals (Hixson & Brown 1986; Douzery & Catzeflis 1995; Springer *et al.* 1995; Springer & Douzery 1996; Halanych & Robinson 1999) and for a diverse array of non-mammalian vertebrates and invertebrate metazoans (Vawter & Brown 1993). The remarkable prevalence of As in loops probably reflects hydrophobic interactions with ribosomal proteins, since it is the least polar of the four bases (Gutell *et al.* 1985). The over-representation of Gs and Cs in stem regions (G+C stems: 44%; loops: 27%) conforms with RNA structure predictions by Turner *et al.* (1988) who linked this phenomenon to free energy considerations, since G-C pairs have less free energy than G-U or A-U associations.

Cytochrome *b*, 12S rRNA and Combined Molecular Phylogeny

The phylogenies recovered from the analyses of cyt *b* and 12S rRNA sequences, both separately and combined, consistently supported the monophyly of the subfamily. The relationships among the terminal taxa were relatively constant, but the deeper evolutionary associations were poorly resolved. Three major clades were invariably retrieved across data sets irrespective of reconstruction method, ML models, or differential weighting schemes in MP (Fig. 3 - 5, 8 - 10). The first clade comprised *O. angoniensis* and *O. maximus* as sister taxa together with the East African *O. t. jacksoni* basal. In the second clade, the B and C cytotypes of *O. irroratus* were closely related and distinct from the A2 cytotype. The third clade included the East African taxa *O. a. lacustris* (Uzungwa Mountains) and *O. tropicalis* as sister taxa with the second representative of *O. a. lacustris* (Ufipa Plateau) in the basal position. The majority of the topologies resulting from the analysis of the cyt *b* and combined data sets (Fig. 3 - 5, 9, 10) were congruent in that *O. typus* was consistently placed basal to *O. t. jacksoni* (referred to as the *O. typus* clade, see Results section), *O. laminatus* grouped basal to the *O. irroratus* cytotypes (the *O. irroratus* clade) and *O. denti* basal to *O. tropicalis* (the *O. anchietae* clade). However, the branching order of the major clades was variable in the recovered topologies, and the deeper nodes collapsed into an unresolved polytomy after bootstrap analyses (Fig. 3 - 5, 8 - 10). The only exception to this was the *O. typus* clade, which grouped as sister to the *O. irroratus* clade (with high bootstrap support) in all the cyt *b* and combined topologies (but with weak bootstrap support in the cyt *b* NJ tree; Fig. 5). The evolutionary relationships of *P. brantsii* and *O. sloggetti* remain obscure. A close relationship between *O. sloggetti*, *O. unisulcatus* and *P. littledalei* was indicated in the ML and MP topologies derived from cyt *b* data, but this association was not consistently retrieved (Fig. 3, 4, 10). *Otomys unisulcatus* and *P. littledalei* invariably grouped as sister taxa in topologies derived from the cyt *b* and combined data sets (Fig, 3 - 5, 9, 10). *Otomys occidentalis* was omitted from the definitive phylogenetic analysis (Fig. 3 - 5, 8 - 10) due to incomplete sequences but the species was none the less, placed as a sister taxon to *O. unisulcatus* and *P. littledalei* in the ML analysis of the reduced data set comprising 926 bp of cyt *b* sequences (Fig. 6).

Phylogenetic Resolution within the Otomyinae and a Rapid Radiation

The Incorporation of the Dynamics of Sequence Evolution into the Phylogenetic Analysis of the Otomyinae

The importance of using an accurate evolutionary model for phylogenetic analyses is becoming increasingly apparent, since model choice can have a profound effect on the accuracy and precision of the recovered tree topologies (Yang *et al.* 1994; Yang 1995a, 1996a, b; Miyamoto & Fitch 1996; Huelsenbeck & Crandall 1997; Huelsenbeck & Rannala 1997; Naylor & Brown 1997; Sullivan & Swofford 1997; Posada & Crandall 2001). However, the limits to which the latter can be improved by the incorporation of the dynamics of sequence evolution are a topic of an ongoing debate (Felsenstein 1988; DeSalle & Brower 1997; Yang 1997; Krajewski *et al.* 1999; Steel & Penny 2000). The fundamental question is: how much needs to be described about the underlying models of sequence evolution to facilitate the successful reconstruction of evolutionary trees? On the one hand, neglecting to include process information into the analysis of phylogenies through more complex partitioned models may result in the loss of true signal in the data and may obscure the relationships among taxa (Simon *et al.* 1994; Ballard *et al.* 1998; Naylor & Brown 1998). On the other hand, as suggested by the results of the ML analyses of mitochondrial sequences in the present study, phylogenetic resolution do not necessarily improve when more complex models are used (also see Yang *et al.* 1994; Gaut & Lewis 1995; Yang *et al.* 1995; Yang 1997; Cunningham *et al.* 1998; Steel & Penny 2000).

The likelihood ratio test (Goldman 1993) indicated that more complex partitioned models fit the data significantly better than simpler unpartitioned models (see Table 15). Parameter-rich partitioned models generally provide more accurate accounts of the sequence evolution of genes due to the recognition of unique character evolution patterns in different gene partitions, whereas simpler models assume a homogenous evolutionary pattern across partitions (Voelker & Edwards 1998; Waits *et al.* 1999). In the present investigation, the improved fit of the more complex HKY+I+Γ model was not mirrored by a significant improvement in phylogenetic resolution in topologies recovered by the ML analyses. Deeper relationships remained poorly resolved irrespective of the complexity of the models used. Shifts in tree topology could, however, only be assessed with respect to the unpartitioned models due to software limitations. The consistency of the topologies contained herein indicates the robustness of the ML model to violations of its underlying assumptions (Gaut & Lewis 1995; Huelsenbeck 1995). Moreover, it was apparent from the MP analyses that many nodes were insensitive to the weighting scheme

applied, and identical topologies emerged, irrespective whether the weighting strategies were based on partition-specific or overall parameter estimates of the ti:tv ratios.

These results show that the resolution provided by molecular sequences is not only influenced by the rates and patterns of nucleotide substitution (κ, α and Pinv) within a particular gene or gene fragment (see Huelsenbeck & Rannala 1997), but also by the nature of the topology being estimated (Yang 1994; Voelker & Edwards 1998; Waits *et al.* 1999). One of the most enduring characteristics of the topologies recovered from the phylogenetic analyses of cyt *b*, 12S rRNA and the combined data sets are the short internal branches. This indicates rapid cladogenesis in the early evolutionary history of the Otomyinae. The time intervals that separated these divergence events may have been too short for significant signal to accumulate in the data, which probably accounts for the poor resolution of the deeper nodes in the otomyine phylogenies (Fig. 3 – 5, 8, 9). Since the 12S rRNA gene is known to evolve at a slower rate than cyt *b* in mammals it ought also to contain less signal resulting in 12S rRNA phylogenies (Fig. 8) contained herein being less resolved than those derived from cyt *b* sequences (Fig. 3 – 5). Combining the 12S rRNA data with cyt *b* failed to improve the retrieval of robust deeper associations (Fig. 9). Moreover, it was initially thought that the long branches leading to the outgroups (especially to *M. musculus*) and the weak internal relationships, indicated that outgroup sequences were evolutionarily too distant, and hence a possible cause of instability in the data (see Halanych *et al.* 1999). However, when unrooted networks of the ingroup taxa were analyzed with different reconstruction methods and data treatments (Fig. 10) the deeper level associations remained inconsistent and the bootstrap support for the respective nodes did not increase.

The Geographic Origin of the Otomyinae and Mitochondrial Data

It was hoped that phylogenies derived from the mitochondrial data generated in this investigation would shed light on the origins of the Otomyinae. Several authors have suggested a Southern African genesis (Pocock 1976; Denys *et al.* 1987; Denys 1989; Sénégas & Avery 1998). This is based on the antiquity of the fossiliferous deposits in South Africa from which the presumed ancestral and the oldest *Otomys* fossils are known (*Euryotomys pelomyoides*, dated at approximately 6.0 - 4.5 Myr, *E. bolti*, at 5.0 - 4.0 Myr, *Prototomys campbelli*, <3.6 - 3.0 Myr, *O.* cf. *gracilis*, Makapansgat, at 3.7 - 3.0 Myr, *O. gracilis*, hominid caves, at 3.3 - 1.0 Myr). Further support for a southern African origin is Denys' (1989) view that the molar pattern of *O. petteri* (the oldest known East African fossil, dated at approximately 1.6 Myr) is more advanced than its contemporary fossil equivalent from South Africa, *O. cf. gracilis*. Dental evolution in the subfamily is perceived to progress towards an addition of laminae and the subsequent enlargement of

M^3 and M_1 (Denys 1989; Chevret *et al.* 1993). *Otomys petteri* has six laminae on M^3 (5 – 7), while *O.* cf. *gracilis* has five. In addition, fossils of modern *Otomys* already appear in the Lower Pleistocene of South Africa (Denys 1989), but only in the Mid-Pleistocene of East Africa (Jaeger 1979) and the highest diversity of modern forms is currently concentrated in southern Africa (De Graaff 1981; Taylor *et al.* 1989a).

However, in the present study no single taxon or clade was convincingly supported by bootstrap analysis as being the most basal in the Otomyinae phylogeny (Fig. 3 - 5, 8 - 10), although *P. brantsii* from southern Africa and the East African *O. anchietae* clade were basal in most analytical procedures (Figs 3, 4, 9). Moreover, none of the optimal topologies retrieved with NJ, ML and MP could be rejected in favour of another as being a significantly better estimate of the relationships among otomyine taxa (Shimodaira & Hasegawa 1999; Table 16). It is noteworthy that the more basal positions of *O. unisulcatus, P. brantsii* and *P. littledalei* in the molecular phylogenies (Fig. 3, 4, 9) corroborate the results of comparative cytogenetic studies based on G-banding, suggesting that these three taxa represent the older lineages in the subfamily (Robinson & Elder 1987; Rambau & Robinson 1999). These authors illustrated that their karyotypes contain several conserved whole chromosomes and chromosome arms that showed sequence homeology with chromosomes constituting the primitive murid/cricetid karyotype (Baker & Mascarello 1969; Koop *et al.* 1984). *Otomys unisulcatus* and *P. brantsii* constitute the basal lineages in cladograms reconstructed from craniometric data for 13 of the 14 recognized Otomyinae species (Musser & Carleton 1993; Taylor *et al.* 1993) using the fossil species *Euryotomys pelomyoides* as outgroup (Taylor *et al.* pers. comm.).

The Contribution of Mitochondrial DNA Analysis to the Systematics of the Subfamily Otomyinae

One of the most striking features from the present investigation is the lack of agreement between the current taxonomic divisions in the Otomyinae (*sensu* Musser & Carleton 1993; Taylor *et al.* 1993) that are primarily based on morphology, and the phylogenetic relationships recovered from the mitochondrial sequences. Likewise, there is a lack of concordance between the phylogenetic affinities obtained from mitochondrial and other genetic markers (allozymes, immunoblots and cytogenetics), which in turn, are discordant with one another. This may reflect the poor discriminating power of the cranio-dental features, or a lack of phylogenetic signal at the deeper divergence levels in the subfamily. It may, however, also emphasize that morphological and genetic data are not directly comparable at all levels of a phylogeny, and that morphological similarity is due to convergence. This phenomenon has been demonstrated in numerous murid genera

(Catzeflis *et al.* 1992; Robinson *et al.* 1997; Michaux & Catzeflis 2000; Michaux *et al.* 2001; Steppan *et al.* 2001), and especially in forms that occur on the East African mountains (*Lophuromys,* Verheyen *et al.* 1996; *Lemniscomys,* Carleton & van der Straeten 1997; *Arvicanthis*, Capanna *et al.* 1996; Ducroz *et al.* 1997; *Mastomys,* Granjon *et al.* 1997; *Apodemus*, Fadda 2000).

Lower Level Taxonomic Ambiguities within Otomyinae

The current subdivision of the Otomyinae into two genera, *Parotomys* and *Otomys* is based on a single morphological character, the size of the tympanic bullae. That this distinction is questionable has been raised by Musser & Carleton (1993) in the latest classification of the subfamily. Additional evidence probing the validity of generic subdivision has resulted from cranio-dental morphology (Bohmann 1952), allozyme data (Taylor *et al.* 1989a; Meester *et al.* 1992; Govender 1999; Taylor *et al.* pers. comm.), immuno-electrotransfer analysis (Contrafatto *et al.* 1994) and comparative cytogenetic data employing FISH and a limited number of mouse chromosome specific probes (Rambau *et al.* 1997). Jointly, these authors showed that the two *Parotomys* species were more closely allied to *O. unisulcatus* (and *O. sloggetti* in some cases) than to one another, suggesting that *Otomys*, as currently defined, is paraphyletic. It was suggested that either *Parotomys* and *Otomys* should be synonymized, or, that *O. unisulcatus* (and occasionally *O. sloggetti*) be reassigned to *Parotomys.* It has also been shown that the ecology and behaviour of the *Parotomys* species differ substantially (e.g. thermophysiological adaptations, refuge strategies, food preferences, breeding, activity patterns), and in some cases more closely resemble those of *Otomys* than they do each other (Haim & Fairall 1987; Richter *et al.* 1997; Jackson *et al.* in press, unpubl.).

The analysis of mtDNA sequence data in the present study provides additional evidence for the recognition of a single genus, *Otomys*. An interesting corollary to this finding is that *P. brantsii* and *P. littledalei* did not cluster as sister taxa, nor group in a clade distinct from *Otomys* (Fig. 3 - 5, 8 - 10). Instead, a close evolutionary association between *P. littledalei* and *O. unisulcatus* was implied (cyt *b* and combined data), and although the exact relationships of *P. brantsii* were unresolved, weak support for a sister affiliation with *O. sloggetti* was recovered in the 12S rRNA parsimony analysis (see below). Moreover, the sequence divergences between *Parotomys* and *Otomys* (cyt *b*: 11.4 - 16.3%; 12S rRNA: 3.0 - 6.1%) are similar to the interspecific distances within *Otomys* (cyt *b*: 2.4 - 14.6%; 12S rRNA: 0.4 - 4.6%). The two *Parotomys* species differ by 14.9 - 15.4%, cyt *b* and 5.4% for12S rRNA (see Table 11). These results confirm those most recently obtained from the combined analyses of partial 12S and 16S rRNA sequences and complete cyt *b* sequences of *O. irroratus*, *O. sloggetti*, *P. brantsii* and *P. littledalei*

(Ducroz *et al.* 2001). In their results, the paraphyly of *Otomys* was consistently supported (but with low bootstrap support) since *O. sloggetti* was more closely related to *Parotomys* than to *O. irroratus*.

In sharp contrast to these findings the results from the Shimodaira-Hasegawa test (1999) conducted in this study revealed that ML trees, when constrained to include a monophyletic *Parotomys* clade, were not significantly poorer estimates of Otomyinae relationships than the unconstrained ML topologies derived under the best-fit models for each data set (Fig. 3, 8, 9; Table 16). However, this result can be attributed to the limited phylogenetic signal in the data in terms of the deeper relationships. This also accounts for the inconsistent relationships evidenced for *P. brantsii, P. littledalei, O. unisulcatus* and *O. sloggetti* in the trees recovered from mtDNA sequences. These findings mirror the difficulties encountered in earlier attempts to reconcile the discrepant evolutionary associations among otomyine taxa using morphological data, and genetic markers (Taylor *et al.* 1989a, b; Meester *et al.* 1992; Contrafatto *et al.* 1994; Rambau *et al.* 1997; Ducroz *et al.* 2001; Taylor *et al.* pers. comm.). However, the branching patterns in the molecular trees do suggest that *P. brantsii, P. littledalei, O. unisulcatus* and *O. sloggetti* were all closely related. In all phylogenies derived from cyt *b* sequences and the combined cyt *b* and 12S rRNA data set (Fig. 3 - 5, 9), *P. littledalei* consistently grouped as the sister taxon of *O. unisulcatus* (51 - 78%). The 12S rRNA parsimony analyses showed *P. brantsii* and *O. sloggetti* to group as sister taxa with moderate bootstrap support when the empirical stem-loop-specific (80%) and 6-parameter parsimony (73%) weighting schemes were applied (Fig. 8). This association was, however, not supported in the ML and NJ analyses, or in any of the topologies inferred from cyt *b* or the combined sequence data, so the position of *O. sloggetti* and *P. brantsii* in the otomyine phylogeny remains to be resolved. Recently, Ducroz *et al.*'s (2001) indicated weak to high bootstrap support (58 - 93%) for *O. sloggetti* and *P. littledalei* as sister taxa, but *O. unisulcatus* was not included in their study.

It seems unlikely that the phylogenetic closeness of species of *Parotomys* and *Otomys* are attributable to long-branch attraction (Felsenstein 1978, 1988; Hendy & Penny 1989; Huelsenbeck 1995; Huelsenbeck 1998). The influence of this phenomenon is likely to have been lessened by correcting for among-site rate variation using the best-fit HKY+I+Γ model of evolution for each data set (Cunningham *et al.* 1998; Huelsenbeck 1998), and by the near complete taxon sampling of the known Otomyinae taxa (Lyons-Weiler & Hoelzer 1997). These associations are probably true reflections of shared ancestry and the relatively long branches in the cyt *b* and 12S rRNA phylogenies may be explained by the significant differences in the rates of sequence evolution in the *P. littledalei* (cyt *b*, Table 13) and the *P. brantsii* lineages (12S rRNA, Table 14) detected by

the relative rate test (Robinson *et al.* 1998). Therefore, the presence of inflated bullae in *P. littledalei* and *P. brantsii*, and the overall correspondence in cranial shape and size indicated by geometric morphometrics (Taylor *et al.* unpubl.), are more likely due to parallel evolution than common ancestry. Indeed, Pocock (1976) suggested that inflated bullae may have evolved independently in these two taxa allowing them to survive in arid environments.

The status of a third southern African genus, *Myotomys*, as described in past taxonomic treatments of the Otomyinae (Thomas 1918; Broom 1937; Allen 1939; Roberts 1951; Pocock 1976; Table 1), has been problematical. The earlier of these treatments incorporated various combinations of taxa some of which have gone extinct (e.g. *Otomys (Palaeotomys) gracilis*†), or have since been relegated to subspecific status (e.g. *O. sloggetti jeppei, O. unisulcatus grantii*). More recently, Roberts (1951) and Pocock (1976) thought it to comprise the extant *O. unisulcatus* and *O. sloggetti.* However, no molecular evidence has been found herein to support the recognition of *Myotomys*. This conclusion rests on the fact that these two species failed to form a distinct monophyletic clade in any of the phylogenies retrieved (Fig 3 - 5, 8 - 10). Moreover, the sequence evolution of these two species clearly contradicts the affinities suggested by their ecological and morphological profiles. Both taxa occupy geographic ranges that are intermediate between those of the otomyine species living in westerly arid habitats, and those in southeasterly mesic habitats in southern Africa (Skinner & Smithers 1990; Fig. 1c, d). Pocock (1976) proposed that *Myotomys* should be retained because *O. sloggetti* and *O. unisulcatus* display cranio-dental and ecological characteristics that are intermediate between *Parotomys* and *Otomys*, a finding supported by Taylor *et al.* (unpubl.) based on geometric morphometrics. However, Taylor and co-workers have concluded that the similarity in the cranial morphology (i.e. non-affine skull shape and size) of the two species may be misleading as regards their true evolutionary affinities. This may not be due to shared ancestry, but to convergence of form resulting from living in sparsely vegetated habitats which may include areas of increased aridity and/or high altitude and mountain fynbos niches.

When taken together, the bulk of evidence suggest that *Parotomys* and *Otomys* should be synonymized and that *Myotomys* should not be reinstated. The molecular phylogeny obtained herein confirms the relationships inferred with several genetic markers that consistently support the paraphyly of *Otomys*. Therefore, it is suggested that *Parotomys* be subsumed in the genus *Otomys,* which has priority (having stood for almost two centuries, Cuvier 1824).

Arid-mesic Dichotomy

Several lines of evidence (palaeontology, cranio-dental and renal morphology, ecology and genetics) suggest two discrete evolutionary lineages within the southern African Otomyinae (Pocock 1976; Taylor *et al.* 1989a, b; Meester *et al.* 1992; Contrafatto *et al.* 1994; Pillay *et al.* 1994). On one hand there is an arid adapted lineage comprising *P. littledalei, P. brantsii* and *O. unisulcatus* (some authors also include *O. sloggetti*, Taylor *et al.* 1989b, Meester *et al.* 1992, Contrafatto *et al.* 1994). On the other, a mesic adapted lineage that includes the balance of *Otomys* species from this region, *O. angoniensis* (which includes *O. maximus*), *O. irroratus, O. saundersiae* ≈ *O. karoensis* and *O. laminatus.*

Molecular data obtained in the present study do not convincingly support the hypothesis of two evolutionary lineages contradicting the morphological (Pocock 1976), allozyme (Taylor *et al.* 1989a, b) and immuno-electrotransfer analyses (Contrafatto *et al.* 1994) that are in favour of an arid-mesic dichotomy in the early evolutionary history of the subfamily. The mtDNA results also disagree with the relationships resulting from more recent cladistic analyses of allozyme and craniometric data of southern and East African otomyines (Taylor *et al.* pers. comm.). At the chromosomal level, FISH data have also indicated that the arid dwelling *O. unisulcatus* is more closely related to *P. brantsii* than to mesic adapted *O. irroratus* (Rambau *et al.* 1997). The branching patterns in the trees inferred from mitochondrial sequences failed to show a clear separation of xerophylic and mesophylic species into monophyletic clades that were well supported by bootstrap analysis. There were indications of close evolutionary relationships between *P. littledalei, P. brantsii, O. unisulcatus* and *O. sloggetti* in the MP and ML trees inferred from cyt *b* data and combined cyt *b* and 12S rRNA sequences, but these associations received poor bootstrap support (Fig. 3 – 5, 9, 10). Moreover, when the best ML topology (which did not support the dichotomy) was compared with a ML tree constrained to contain the arid clade (*P. littledalei, P. brantsii, O. unisulcatus*) using the Shimodaira-Hasegawa test (1999), the best ML trees of the cyt *b* and combined cyt *b* and 12S rRNA data sets were shown to be a significantly better estimate of the Otomyinae relationships than the constrained trees (Table 16). Neither of these topologies based on 12S rRNA data were rejected. Overall, the molecular findings mirror results of comparative analyses of the sperm morphology of southern African Otomyinae, which also failed to demonstrate a clear separation between xerophylic and mesophylic species (Bernard *et al.* 1991). Moreover, despite the fact that these two species groups were separated with respect to kidney structure (Pillay *et al.* 1994), recent thermophysiological studies of the arid occurring *P. littledalei, P. brantsii* and *O. unisulcatus* have shown that

they, to some extent, resemble mesic adapted Otomyinae (e.g. *O. irroratus* and *O. sloggetti*) (Jackson *et al.* in press).

Species Level Relationships

As a result of the variability of the dental and cranio-morphological characters on which species assignments within the Otomyinae have been based, the status of several species remains uncertain (Musser & Carleton 1993). However, it is essential to emphasize that the use of the term 'species', is dependent on the definition used. The latter continues to be one of the most contentious issues in biology (Otte & Endler 1989; Hey 2001). More than 20 species concepts have been formulated, each with different criteria (Mayden 1997; Harrison 1998), none of which are universally accepted. The most frequently cited among these include the biological species concept (Mayr 1942, 1963), the recognition species concept (Paterson 1985), the cohesion species concept (Templeton 1989), the phylogenetic species concept (Cracraft 1983, 1989; Nixon & Wheeler 1990, see also Mishler & Theriot 2000), the genealogical species concept (Baum & Shaw 1995) the evolutionary species concept (Wiley 1978) and the genotypic species cluster definition (Mallet 1995). However, the ability to differentiate species morphologically, regardless of the conceptual framework, is desirable (or essential) in many biological studies. The uncertainties surrounding the status of several species within the Otomyinae clearly illustrate some of the problems encountered when the morphological criteria used to delineate species are unreliable and limited, or when no discriminating characters have been identified (e.g. cryptic species).

It has been suggested that genetic distances (which reflects the degree of dissimilarity between the genetic composition of taxa) may be useful in species identification (e.g. Avise 2000; Bradley & Baker 2001). However, the use of genetic distance measures is contentious. Some authors consider this approach valid since genetic distance is not tied to any particular species concept, and have attempted to use it to determine divergence ranges for defining taxonomic boundaries (Ayala 1975; Avise & Aquadro 1982; Avise & Lansman 1983; Johns & Avise 1998). For example, a genetic divergence of 10% has been used as an arbitrary value for inferring the existence of distinct species (Avise *et al.* 1998). Avise (2000) has demonstrated the usefulness of mtDNA sequence variation for clarifying potential species boundaries between morphologically similar or indistinguishable taxa. Furthermore, Bradley and Baker (2001) used variation in the mammalian cyt *b* gene, for species identification under the framework of the genetic species concept (Simpson 1943; Dobzhansky 1950; Mayr 1969). However, Ferguson (2002) opines that genetic distance can only be used as an indicator of the period for which taxa have been separated, since a clear predictive value for separating species-level

differences from population-level differences has not yet emerged. Consequently, genetic distance in itself is not a suitable reference point for implying species status *per se*. This statement is qualified by the following line of reasoning: 1) Increasing genetic distance does not necessarily imply reproductive isolation (e.g. Hollocher *et al.* 1997). 2) Reproductive isolation is not necessarily a result of the gradual accumulation of genetic differences between lineages as implied by Wu & Hollocher (1998). For contradictory evidence see Coyne *et al.* (1998). 3) In conflict with suggestions by Klicka and Zink (1997), Avise *et al.* (1998) and Johns & Avise (1998) that speciation events require protracted periods of time allowing sufficient genetic divergence to characterise DNA sequences, many speciation events may be considerably shorter than the 4 Myr suggested by these authors. For opposing evidence see Vrba (1979, 1980, 1985a, b), McCune and Lovejoy (1998). 4) Finally, genetic distances as a means of identifying different species are theory-dependent with the biological species concept being used as reference point. My interpretation of genetic distances and relationships among taxa in the light of a species definition takes cognizance of the above arguments. Genetic distance was not used in isolation as a yardstick for recognizing species. Rather, a comparative approach was followed whereby the distances separating OTUs of questionable status were measured against those delimiting species of unequivocal status. While this clearly does not address the fundamental shortcomings associated with the use of distance measures for species identification it nonetheless provides hypotheses of association, which can be tested in subsequent investigations.

Clearly, the limited number of samples analyzed for some taxa (see Table 2) precludes any definitive statement on species identification, given that the molecular data do not accurately reflect the levels of interspecific versus intraspecific genetic variation in the Otomyinae. It is imperative that detailed phylogenetic analyses based on adequate sample sizes for each OTU are conducted before definitive statements on the specific status of problematic taxa can be appropriately addressed.

The relationships of *O. angoniensis* and *O. maximus*

The delimitation of *O. maximus* from *O. angoniensis*, which principally relies on the difference in physical size as well as differences in the size and shape of their skulls, is controversial (Musser & Carleton 1993). Full species rank was supported in taxonomic treatments by Roberts (1951), Lundholm (1955), Davis (1962), Swanepoel *et al.* (1980), Smithers (1983) and Crawford-Cabral (1998). Contrary to this Davis (1974), Misonne (1974), Ansell (1978), De Graaff (1981), Crawford-Cabral (1986), Meester *et al.* (1986) and Skinner & Smithers (1990) were of the opinion that no more than subspecific status was justified. In the present investigation, the two species invariably group as

monophyletic sister taxa with high bootstrap support (86 - 100%) in all phylogenetic trees recovered from the analysis of the cyt *b* data, and combined cyt *b* and 12S rRNA sequences (Fig. 3 - 5, 9, 10). There is low to moderate support for this association in the 12S rRNA phylogeny (ML: 52%, NJ: 76%; Fig. 8), but this is probably due to limited phylogenetic signal and hence its effectiveness in resolving closely related taxa. It is noteworthy that the genetic distances delimiting these taxa are the smallest detected among any of the species in the present study (cyt *b*: 2.4%; 12S rRNA: 0.4% compared to cyt *b*: 5.3 - 16.3%; 12S rRNA: 0.5 - 6.1%; Table 9, 10, 11) and are clearly more in line with intraspecific distances (cyt *b*: 0.2 - 7.5%; 12S rRNA: 0.1 - 1.1%; Table 11). The two taxa are distinct in terms of distribution (Bronner & Meester 1988; Fig. 1e, f) but occur in similar habitats (De Graaff 1981; Skinner & Smithers 1990). Both are inhabitants of moist and wooded grasslands. However, *O. maximus* prefers extremely moist habitats (riverine vegetation, flood plains), whereas *O. angoniensis* has a wider habitat tolerance and occurs in drier areas where its range overlaps with *O. irroratus* in southern Africa and with *O. tropicalis* in East Africa (Misonne 1974) and also survives in fire-degraded habitats (Kingdon 1974). A phylogeographic analysis based on extensive geographic sampling is clearly required to assess the level of variation within and among these populations before any definite conclusions can be reached.

The relationship of *O. irroratus* with *O. tropicalis*

There is disagreement among taxonomists as to whether *O. tropicalis* should be recognized as an independent species, or is conspecific with *O. irroratus* (Musser & Carleton 1993). The two have discontinuous distributions with *O. irroratus* being restricted to southern Africa south of the Zambezi River, whereas *O. tropicalis* has a relic distribution on isolated mountains in East, Central and West Africa (Misonne 1974; Musser & Carleton 1993; Fig.1k, n). Within their respective ranges *O. irroratus* appears to generally, but not exclusively, prefer low-altitude (usually < 1 500m) moist grasslands in close proximity to streams and marshes (De Graaff 1981; Smithers 1983), while *O. tropicalis* occurs at high altitudes (from 1500m but usually above 2 000m to beyond 4 000m), and has a preference for moist grassland mosaics unaffected by fire in the Afromontane forest, Ericaceous and Afroalpine vegetation belts (Kingdon 1974; Dieterlen & van der Straeten 1992).

The two species consistently group in separate clades in the phylogenies reconstructed from the cyt *b*, 12S rRNA and the combined cyt *b* and 12S rRNA data (Fig. 3 - 5, 8 - 10). *Otomys tropicalis* clustered exclusively with the East African taxa and as the closest sister to *O. a. lacustris* (Uzungwa Mountains). In contrast, *O. irroratus* has a close evolutionary association with *O. laminatus*, a species confined to southern Africa. These

relationships suggest that *O. tropicalis* and *O. irroratus* represent distinct evolutionary lineages, which might imply separate species as suggested by the Dollman (1915) classification. The latter was the first to give full specific status to *O. tropicalis* in East Africa based on differences in cranio-dental morphology. This is consistent with the treatments by Thomas (1918), Allen (1939), Ellerman (1941), Misonne (1974) and Musser and Carleton (1993) but is in sharp contrast to Thomas (1902a, 1906a), Bohmann (1952), Dieterlen (1968), Petter (1982) and Dieterlen and van der Straeten (1992), all of whom are of the opinion that the morphological differences are not substantial enough for specific separation. The results of a recent study by Taylor and Kumirai (in press) confirm that there are consistent differences in the number of laminae on their M^3 (*O. irroratus*: 6 laminae; *O. tropicalis*: 7/8 laminae) and the angle of the nasal bone. Further evidence in support of the recognition of *O. tropicalis* from *O. irroratus* is suggested by geometric morphometrics (Taylor *et al.* unpubl.), and the preliminary results from phylogenetic analyses of multi-state cranio-dental characters of 12 of the 14 recognized otomyine species (*sensu* Musser & Carleton 1993; Taylor *et al.* 1993), and these characters combined with allozyme data for 10 species (Taylor *et al.* pers. comm.). The two species fall into separate phylogenetic assemblages in the cladograms inferred by parsimony in the two latter studies and fixed allelic differences were reported at two allozyme loci (XDH, PEPLA). Moreover, the magnitude of the sequence divergences between representatives of *O. irroratus* (all cytotypes) and *O. tropicalis* (cyt *b*: 9.9 - 11.5%; 12S rRNA: 3.1 - 3.4%) is similar to those separating well-defined southern and East African *Otomys* species (e.g. *O. sloggetti* and *O. typus*: cyt *b*: 12.8%; 12S rRNA: 3.5%), and is suggestive of a relatively early radiation of the two lineages.

The relationships of *O. irroratus* with extralimital *Otomys* forms.

Potential conflict arises when the molecular data obtained in the present study are interpreted in context with the hypothesis that *O. irroratus* is a widely distributed species that include several species from southern, East, Central and West Africa (Bohmann 1952; Petter 1982). Between them, Bohmann (1952), and Petter (1982) regard *O. irroratus* as being conspecific with seven of 12 *Otomys* species recognized by Musser & Carleton (1993). Specifically, these are *O. angoniensis, O. anchietae, O. laminatus, O. maximus, O. occidentalis, O. tropicalis* and *O. typus*. Their conclusions were primarily based on the amount of overlap observed in dental characters, such as the number of laminae on M_1 and M^3, and to a lesser extent, on cranial features. On the contrary, the classification of Musser and Carleton (1993) and the results of a more recent study considering craniometric data and qualitative cranio-dental characters (Taylor & Kumirai in press), all suggest that *O. irroratus* is endemic to southern Africa. Taylor and Kumirai (in press) identified morphological landmarks that convincingly separate the East, Central

and West African species (*O. anchietae, O. tropicalis, O. occidentalis* and *O. typus*) from *O. irroratus*, and that separate *O. anchietae barbouri* and *O. a. lacustris* from *O. a. anchietae* (also see Misonne 1974; Meester *et al.* 1986; Dieterlen & van der Straeten 1992).

In the topologies recovered from the analyses of cyt *b* and the combined cyt *b* and 12S rRNA sequences, *O. irroratus* (all cytotypes) grouped as a monophyletic clade sister to the southern African *O. laminatus* (Fig. 3 - 5, 9; bootstrap: 50 - 95%). In the topologies inferred from 12S rRNA sequences alone (Fig. 8) the *O. irroratus* cytotypes always formed a monophyletic group (bootstrap: 83 - 92%), but the sister relationship with *O. laminatus* was not supported. *Otomys occidentalis* was clearly phylogenetically separated from *O. irroratus* in the ML tree inferred from the reduced cyt *b* data set (Fig. 6). These relationships suggest that *O. irroratus* represents a distinct evolutionary lineage and has a relatively distant phylogenetic association with all the species previously synonymized with it by Bohmann (1952) and Petter (1982), with the possible exception of *O. laminatus*. A similar phylogenetic pattern was reflected in the topologies inferred from cranio-dental and allozyme data for 12 and 10 Otomyinae species, respectively (Taylor *et al.* pers. comm.). These results are consistent with the proposals of Musser & Carleton (1993) and Taylor & Kumirai (in press) that *O. irroratus* warrants separate recognition as a species distinct from *O. angoniensis, O. anchietae, O. laminatus, O. maximus, O. occidentalis, O. tropicalis* and *O. typus*. Moreover, the sequence divergences (cyt *b*: 8.9 - 13.1%; 12S rRNA: 1.7 - 3.4%) that delimit *O. irroratus* from the species that were formerly synonymized with it are comparable to those separating it from well-defined *Otomys* species whose distinctiveness from *O. irroratus* has never been questioned (e.g. *O. sloggetti, O. unisulcatus, O. denti*; cyt *b*: 11.4 - 13.5%; 12S rRNA: 2.2 - 3.6%; Table 9, 10).

In southern Africa, *O. irroratus* occurs sympatrically with *O. angoniensis* and *O. laminatus* for part of its range (De Graaff 1981; Skinner & Smithers 1990; Fig. 1e, m, n). The two former species occupy distinct niches in the areas of overlap, whereas *O. irroratus* and *O. laminatus* appear to occupy similar niches (De Graaff 1981; Taylor & Kumirai in press). No hybridization has been documented between the three taxa in nature (Roberts 1951; Misonne 1974; De Graaff 1981). This would suggest an absence of gene flow and support for their recognition as separate species (Bush 1994; Schluter 1994; Dieckmann & Doebeli 1999; but see Coyne 1992; Rice & Hostert 1993). In contrast, the ranges of the extralimital *O. anchietae, O. occidentalis, O. tropicalis* and *O. typus* (all previously treated as conspecifics *of O. irroratus*) are allopatric with respect to that of *O. irroratus* (Fig.1g, h, i, k, n; Kingdon 1974; Dieterlen & van der Straeten 1992; Taylor unpubl.). It is, however, unlikely that gene flow would occur under natural

conditions, given the large geographic distances separating these populations. Therefore, the evidence presented above appears to contradict the all-inclusive definition of *O. irroratus* established by Bohmann (1952) and Petter (1982).

The placement of *O. occidentalis* within the Otomyinae

Close evolutionary relationships between *O. occidentalis*, which is only known from West Africa (Mt. Oku, Cameroon and the Gotel Mountains, Nigeria) and *O. unisulcatus*, *P. littledalei* and *O. sloggetti* from southern Africa were suggested in the topology inferred from cyt *b* sequences with ML. However there was no bootstrap support for these associations. This is in sharp contrast with morphological links between *O. occidentalis* and the East African *O. anchietae lacustris* (southwestern Tanzania) and *O. a. barbouri* (Mt. Elgon) on the basis of similarities in cranio-dental morphology (relatively small bullae, similarly shaped nasals and skulls, M_1: 5 laminae, M^3: 6 - 8 laminae; Dieterlen & van der Straeten 1992; Taylor *et al.* in press). However, *O. occidentalis* is clearly distinguished from the latter two species by consistently having 8 laminae on M_3 and a shorter skull.

The Relationships of* Otomys *from East, Central and West Africa

The analysis of the mitochondrial sequence data revealed that the *Otomys* extralimital to southern Africa do not form a monophyletic group (Fig. 3 - 5, 8 - 10) supporting Bohmann's (1952) conclusions, based largely on dental and cranial characters, and unpublished investigations using allozymes and multi-state cranio-dental characters (Taylor *et al.* pers comm.). When the ML topology recovered from the cyt *b*, 12S rRNA and combined data sets, respectively, were constrained to enforce the monophyly of the extralimital species this was statistically rejected as being a significantly worse estimate of the relationships among Otomyinae taxa, than the optimal ML topologies (Table 16). *Otomys typus* (*sensu lato* Musser & Carleton 1993) and its putative subspecies *O. t. jacksoni* failed to form part of the well-supported East African *O. anchietae* clade (*O. a. lacustris, O. tropicalis, O. denti*). Rather, *O. t. jacksoni* herein represented by a single specimen from Arusha (northern Tanzania) and *O. typus*, represented by a specimen from the Beletta Forest (Ethiopia), consistently grouped in the same clade and successively basal to *O. angoniensis* and *O. maximus* (sister taxa) in topologies retrieved by MP and ML from cyt *b* and the combined cyt *b* and 12S rRNA sequences (Fig. 3, 4, 9). The placement of the nominate *O. typus* basal in this clade was not supported in trees derived from the 12S rRNA data (Fig. 8), or the cyt *b* data analyzed with NJ (Fig. 5). Nonetheless, this paraphyletic association might imply that distinct evolutionary lineages are contained within *O. typus* and it clearly warrants further investigation. Moreover,

although the genetic distances between the representative of *O. typus* and the two of *O. t. jacksoni* (cyt *b*: 9.8 and 10.1%; 12S rRNA: 2.8 and 3.0%) do not reflect the degree of intraspecific variation within *O. typus*, it is noteworthy that they fall within the range separating other well-defined *Otomys* species (Table 11). *Otomys t. jacksoni* was recognized as a full species (*O. jacksoni*) by Thomas (1891, 1902b, 1906a), Wroughton (1906), Dollman (1915), Allen (1939) and Ellerman (1941) (see Table 1) based on substantial differences in the number of laminae on M^3 (*O. jacksoni*: 7 laminae; *O. typus*: 8 laminae), cranial morphology, fur quality and coloration. These two taxa occur at extremely high altitudes (up to 4300m), but *O. t. jacksoni* prefers drier, short and sparse vegetation (Thomas 1891; Clausnitzer 1999) whereas *O. typus* favours moist grasslands and heath vegetation (Lavrenchenko *et al.* 1997).

The close relationship suggested on morphological grounds between *O. typus* and *O. tropicalis* (Musser & Carleton 1993), both of which occur at high altitudes but in geographically distant localities in East, Central and West Africa (Fig. 1g, k) is questioned by the molecular data, which places them in different clades (Fig. 3 - 5, 8 - 10). This suggests convergence of morphological traits, mimicking similar patterns in several mountain dwelling murid genera in East Africa (Capanna *et al.* 1996; Verheyen *et al.* 1996; Carleton & van der Straeten 1997; Ducroz *et al.* 1997, 1998).

Similarly, the two representatives belonging to *O. a. lacustris* failed to cluster as monophyletic sister taxa in all the topologies retrieved from the analysis of separate and combined cyt *b* and 12S rRNA data sets (Fig. 3 - 5, 8 - 10). The specimen from the Uzungwa Mountains invariably clustered as the closest sister taxon to *O. tropicalis* (admittedly with variable bootstrap support 57 - 97%), with that from the Ufipa Plateau basal to this monophyletic clade (bootstrap: 73 - 100%). This finding suggests that these two lineages within *O. a. lacustris* are evolutionary quite distinct. However, without an assessment of the variation within *O. a. lacustris* as well as within and between populations of *O. anchietae* and *O. tropicalis*, no definitive conclusions can be drawn. Strikingly, *O. a. lacustris* (Allen & Loveridge 1933) was recently provisionally afforded specific status on the strength of cranio-dental morphology (Dieterlen & van der Straeten 1992; Taylor & Kumirai in press). The taxon was originally described as a subspecies of *O. anchietae* (Bocage 1882) from Angola and retained in subsequent taxonomic treatments by Allen (1939), Ellerman (1941), Bohmann (1952) and Misonne (1974). This rodent has a discontinuous distribution on isolated mountains in southwestern Tanzania (Fig. 1i; Musser & Carleton 1993) where it inhabits a grassland mosaic in moist forests in a relatively narrow range of altitudes that are separated by vast stretches of drier vegetation (Misonne 1974; Ansell & Dowsett 1988).

In summary, although limited samples preclude definitive statements on intraspecific relationships, the data contained herein suggest that both a rigorous specimen-based verification of taxa listed as subspecies in these regions (Bohmann 1952; Lawrence & Loveridge 1953; Misonne 1974; Dieterlen & van der Straeten 1992; Musser & Carleton 1993; Lavrenchenko *et al.* 1997; Taylor *et al.* 2001; Taylor & Kumirai in press; Taylor *et al.* pers. comm.) and a more comprehensive sampling regime are clearly called for.

Chromosomal Variation in* O. irroratus *and the Relationships of the Major Cytotypes

The discovery of extensive karyotypic variation (2n = 23 - 32) among phenotypically similar populations of *O. irroratus* (Robinson & Elder 1987; Contrafatto *et al.* 1992a, b, 1997; Rambau *et al.* 2001) coupled to evidence of partial post-zygotic and pre-mating reproductive isolation between geographically and (purportedly) chromosomally distinct populations (Pillay *et al.* 1992, 1995a, b, c) suggest that incipient speciation may be taking place within the species (Taylor *et al.* 2000). Previously, five parapatric cytotypes (A, A1, A2, B and C) were identified within *O. irroratus* (see introduction), of which the distribution corresponds closely with recognized bioclimatic zones in South Africa (Contrafatto *et al.* 1992a, b; Taylor *et al.* 1994; Contrafatto *et al.* 1997; Taylor 2000; Fig. 1o). However, more recently, comparative cytogenetic results (G and C banding) led Rambau *et al.* (2001) to the opinion that only two major cytogenetic groups exist within *O. irroratus*. The first group includes specimens with variable chromosome numbers (2n = 27 - 32) and which contain different numbers of short heterochromatic arms and a unique centric fusion; the basic diploid number is thought to be 2n = 28. The second group has a basic diploid number of 2n = 24 (range: 23 - 25) and comprises specimens with an all-acrocentric cytotype which carry a large compound chromosome involving chromosome 7, 8 and 12 of the *O. irroratus* standard (see Robinson & Elder 1987). Likewise, this group contains a centric fusion that is different from that distinguished in the 2n = 28 complex. The latter group corresponds to the A1 cytotype of Contrafatto *et al.* (1992a, b, 1997), but these authors suggest that this cytotype is fixed for a putative tandem fusion involving chromosomes 7 and 12 of the *O. irroratus* standard. This group has a high altitude (> 1 400m) distribution in montane grassland habitats in the Drakensberg (KwaZulu Natal) and in the Eastern Cape Province, with typically low annual mean temperatures and high rainfall. The former group subsumes the B and C cytotypes and has a widespread distribution at low altitudes in the Eastern and Western Cape Provinces and the Free State (Taylor *et al.* 1994; Taylor 2000). The A2 cytotype that are only known Karkloof, the Umgeni River Valley and Umvatana (KwaZulu Natal) and Stutterheim (Eastern Cape) was not included in the Rambau *et al.* (2001) study.

The phylogenetic relationships inferred from mitochondrial cyt *b* sequences among the A1, A2, B and C cytotypes (Fig. 7), are in accordance with the findings of Rambau *et al.* (2001). Two monophyletic clades were recovered, the first comprising the B and C cytotypes (included in the 2n = 27 - 32 group) and the second, the A1 cytotype (which carries the complex chromosomal rearrangement) and the A2 cytotype (not included by Rambau *et al.* 2001). The representatives of the two cytogenetic groups delimited by the latter authors are separated by sequence divergences between 6.3 to 6.8% (A1 and B: 6.4 - 6.8%; A1 and C: 6.3 - 6.8%). Within the 2n = 24 group the sequence divergence is 1%. Low genetic distances also separate the B and C cytotypes (2.0 - 2.1%) that represent the second major group (2n = 27 - 32) in this study (cytotype A was not included). Comparatively large sequence divergences also separate the A2 cytotype from the latter group (A2 and B: 7.0 - 7.5%; A2 and C: 6.7 - 6.9%). In addition, the genetic distances suggest that the 2n = 24 group (A1 cytotype *sensu* Contrafatto *et al.* 1992a, b, 1997) and the A2 cytotype are quite distinct from each other (4.5 - 4.8%). This is also reflected by the high number of unambiguous changes along the branches in the cyt *b* topology (Fig. 7), which mirror the chromosomal distinctiveness of the A2 cytotype (Contrafatto *et al.* 1992a, b). This may suggest that A2 cytotype represents a third cytogenetic group in addition to the two described by Rambau *et al.* (2001). In addition, Pillay *et al.* (1995a, c) provided circumstantial evidence for behaviourally induced pre-mating reproductive isolation between cytotype A1 (Kamberg) and A2 (Karkloof).

Several authors have suggested that *O. irroratus* is a speciating complex and that further taxonomic subdivision is probably warranted. It has been proposed that populations carrying the large compound chromosome (2n = 24 cytogenetic group, Rambau *et al.* 2001; A1 cytotype, Contrafatto *et al.* 1992a, b, 1997) represent a sibling species of *O. irroratus*, given evidence for reduced breeding success in crosses involving animals with this rearrangement (Pillay *et al.* 1992, 1995c; Taylor 2000). It is noteworthy that in the present study, the magnitude of genetic distances between representatives of the 2n = 24 group and those with 2n = 27 - 32 (B and C cytotypes) are similar to those separating closely related, but nonetheless, well-defined *Otomys* species (e.g. *O. angoniensis* and *O. t. jacksoni*: 6.5 - 6.6%). More detailed studies of genetic variation within cytotypes and appropriately designed breeding experiments taking cognizance of the results of Rambau *et al.* (2001) are clearly required.

If the molecular results hold, it will provide further supportive evidence for the hypothesis that chromosomal rearrangements may initiate the divergence of sibling species by giving rise to effective post-zygotic reproductive isolation, in the absence of marked phenotypic and/or another form of genetic differentiation between the new species and that from which it originated (Capanna 1982; Cothran & Smith 1983;

Meester 1988; King 1993; Taylor 2000 but see Carson 1982; Vrba 1985c; Paterson 1985; Coyne 1994 for conflicting arguments). Previous analyses of genetic variation based on allozyme and immuno-electrotransfer data provide no evidence of restricted gene flow among phenotypically similar, but chromosomally different populations of *O. irroratus*. In fact, these studies failed to group specimens consistently on geographic location or karyotype in the phylogenetic analyses of these data sets (Meester *et al.* 1992; Taylor *et al.* 1992, Contrafatto *et al.* 1994).

THE EVOLUTIONARY HISTORY OF THE OTOMYINAE

In this section, divergence times for the Otomyinae estimated using the cyt *b* molecular clock are interpreted in the context of climatic and geographic factors that may underlie the adaptive radiation of the subfamily (Bohmann 1952; Pocock 1976; Carleton & Musser 1984; Denys *et al.* 1987; Denys 1989; Taylor *et al.* 1989a).

The Molecular Clock, Rate Heterogeneity and Saturation

Constant nucleotide substitution rates across lineages are a prerequisite for estimating divergence times (Zuckerkandl & Pauling 1965; Kimura 1983). Consequently, given that the results of the relative rate test (Robinson *et al.* 1998) revealed a fairly uniform mutation rate of cyt *b* transversions among Otomyinae lineages relative to the outgroups *A. chrysophilus*, *M. musculus* or *R. norvegicus*, the application of a murid clock calibration (Ducroz *et al.* 1998) for dating divergences between otomyine lineages, seems reasonable. Moreover, transversions accumulate linearly with time over a broad range of evolutionary divergences in eutherian mammals (Miyamoto & Boyle 1989). Ducroz *et al.*'s (1998) calibration was derived from the *Rattus-Mus* dichotomy dated at about 12 Myr on fossil data (Jacobs & Downs 1994). This should have somewhat reduced the potential error introduced when calibrating a molecular clock for rodents against the fossil record (O'hUigin & Li 1992) given that a higher rate of molecular evolution has been suggested for muroid rodents (Wu & Li 1985; Britten 1986; Catzeflis *et al.* 1987; Li *et al.* 1990; She *et al.* 1990; Catzeflis *et al.* 1992, 1993 but see Sarich 1985; Easteal *et al.* 1985; Easteal 1998 for conflicting information on this topic). Since fossils provide a minimum approximation for divergence times among taxa (Novacek 1992; Springer 1995), an elevated mutation rate may result in overestimation of rodent divergence times. This is clearly illustrated by the controversy surrounding the *Mus-Rattus* divergence. The dates derived from molecular data for this split are substantially earlier (e.g. about 40.7 Myr BP, Kumar & Hedges 1998; about 41.9 Myr BP, Huchon *et al.* 2000; about 23 Myr BP, Adkins *et al.* 2001) than those based on fossil data (between 8 and 14 Myr; Jacobs 1978; Jacobs & Pilbeam 1980; Jaeger *et al.* 1986; Jacobs & Downs 1994).

Molecular Time Scales versus Palaeontological History for the Evolution of the Otomyinae

There are both discrepancies and similarities between the estimated time frames for the evolution of the Otomyinae derived from the fossil record (Pocock 1976; Denys *et al.* 1987; Denys 1989; Sénégas & Avery 1998) and molecular data (Taylor *et al.* 1989a; Chevret *et al.* 1993; Ducroz *et al.* 2001; Verheyen *et al.* unpubl.). The approximate estimate of 5 Myr BP (Early Pliocene) inferred for the earliest divergence among otomyine lineages in the present study concurs with the 6.4 to 5.5 Myr BP suggested by the protein molecular clock (Taylor *et al.* 1989a). However, like in the *Mus-Rattus* example given above, these molecular time scales pre-date the time frame indicated from fossil evidence, as *Prototomys campbelli*, the closest presumed fossil ancestor to the modern Otomyinae is known from Mid to Upper Pliocene deposits (about 3.6 – 3.0 Myr BP, Broom & Schepers 1946; Pickford & Mein 1988; Taylor *et al.* 1989a) and the age of the oldest known true *Otomys* fossil (*O.* cf. *gracilis*) is roughly 3.7 Myr (Pocock 1987). It is noteworthy that the dates suggested by the molecular data are in good accordance with the approximate ages of *Euryotomys bolti* (5.0 – 4.0 Myr), and *E. pelomyoides* (6.0 - 4.5 Myr), both considered intermediate fossil forms between the Murinae and modern Otomyinae (Pocock 1976; Sénégas & Avery 1998). Moreover, according to the calibration for cyt *b* applied herein, the earliest diversification among otomyine lineages marginally predates the 4 to 3 Myr suggested by Ducroz *et al.* (2001) between otomyine species. These authors used a murid reference point (*Mus-Rattus* split at 12 Myr) as well as the Gerbillinae-Murinae split (about 16 Myr) and non-saturated substitutions in the 12S and 16S rRNA (partial) and the complete cyt *b* genes to calibrate their clock. The convergence on a period 5 to 3 Myr by the various clock calibrations is encouraging.

Climatological, Geological and Vegetational Changes in the Late Miocene, Pliocene and Pleistocene of Southern and Eastern Africa and Mammalian Evolution

Biogeographical changes such as alterations in the positions of landmasses, geology and climate on a global and regional scale may indirectly drive the speciation process (e.g. Hewitt 1996; Klicka & Zink 1997; Avise *et al.* 1998). These factors may cause shifts in vegetation cover through changes in soil, drainage systems and land forms which in turn result in regional and altitudinal shifts in the distribution of suitable habitat, shelter and food resources for a particular group of organisms (Vrba 1985a; Coyne 1992). It is believed that many species originate through allopatric divergence when populations are isolated in islands of suitable habitat as a result of habitat fragmentation and consequently become adapted to local conditions influencing their habitat preferences, diet, behaviour and reproductive strategies (Mayr 1963; Coyne 1992). This is underscored by Brain

(1981) who stated that "had it not been for temperature-based environmental changes in the habitats of early hominids, we would still be secure in some warm hospitable forest, as in the Miocene of old, and we would still be in the trees". Indeed, climate has been critically important in shaping mammalian, including human evolution, particularly during the Pliocene and Pleistocene epochs when climatic oscillations linked to intensification and reduction cycles in polar glaciation led to cooler and drier, and warmer and wetter climatic cycles in Africa (Shackleton & Kennett 1975). Numerous episodes of significant global cooling, of which the most intense were at 5, 2.5 and 0.9 Myr (Shackleton & Kennett 1975; Bonnefille 1983; Lindesay 1990), accompanied pulses of speciation that represent key events in the evolutionary history of, among others, rodents and bovids (Vrba 1979, 1980, 1982, 1985a, b; Gentry 1990, 1992; Vrba 1995; Denys 1999).

In the wet Miocene (10 Myr) most of East Africa comprised tropical forests that extended to the coastal regions. From approximately 9 to 6.4 Myr the climate was warm and humid (Lovett 1993). However, after about 5 Myr a major ice sheet developed over Antarctica, which strengthened the cold Benguela current resulting in further aridification of southwest Africa. In southern Africa, the glacial phases and associated cooling of the seas during the middle Pliocene (4 - 3 Myr) resulted in the climate of southern Africa becoming generally drier. This is shown by the differentiation of grasslands and wooded grasslands in the interior and a change from palm to fynbos flora in the coastal regions (Coetzee 1986; Lindesay 1998). Denys (1999) reports that the faunal species composition (rodents, bovids and hominids) and sedimentological evidence from the NE of South Africa supports a cooler and drier climate with grasslands around 2.5 Myr (Denys 1989; Vrba 1980, 1985a, b, 1995). In the Plio-Pleistocene strata of East Africa there was a marked increase in grass pollen reflecting grassland extensions (Bonnefille 1983, 1985).

The geological history of East Africa is complex, with volcanic activity and rifting giving rise to mountainous landscapes that contrast both in altitude and vegetation with the plains. The major uplift of the Central African Plateau resulted in the development of the main rifts in the East African Rifting System (around 10 Myr) and the separation of the eastern forests by a band of dry seasonal vegetation, or arid corridor (reviewed in Axelrod & Raven 1978; Werger 1978; Tyson 1981; Potts & Behrensmeyer 1993). The resultant high lying regions created rain shadows in the northeastern parts of Africa, with the consequence that rainfall was decreased (Wasser & Lovett 1993). This arid corridor repeatedly expanded and contracted during cycles of cooler/drier and wetter/warmer climate in the Plio-Pleistocene. It extended into Namibia approximately 6 to 4 Myr ago with the northward shift of the climatic zones and the development of the winter rainfall

in the southwestern Cape (Werger 1978). It presently extends from the Horn of Africa in the northwest, through to the Namib Desert in the southeast where it reaches the coast and creates a formidable barrier to east-west migrations of organisms and to the dispersal of organisms adapted to moist environments (Lovett & Wasser 1993; Matthee & Robinson 1997b).

Proposed Speciation and Radiation of the Otomyinae in Africa

Speciation in the Otomyinae appears to be ecologically driven by geological, climatic and vegetational changes since the late Miocene and in the Plio-Pleistocene, according to the range of time frames suggested by molecular and sparse fossil data (see above). During roughly this period the various species probably evolved by vicariance in fragmented grassland habitats in southern Africa and in grassland mosaics in the Afromontane forests and Afroalpine vegetation in East, Central and West Africa (Bohmann 1952; Kingdon 1974; Robinson & Elder 1987; Taylor *et al.* 1989a; Chevret *et al.* 1993; Taylor *et al.* unpubl.).

Speciation within the Otomyinae appears to have occurred in punctuated fashion. The molecular data suggests that the subfamily originated from an ancestral stock in a rapid radiation of three main lineages that is estimated to have occurred approximately 5 Myr BP. This dating coincides remarkably well with a phase of modern Murinae radiation in Africa (from about 5.8 Myr), which was most pronounced at about 5 Myr (Misonne 1969; Chevret 1994; Denys 1999) and probably resulted from a faunal turnover after the severe arid conditions in the late Miocene. Subsequently, a second pulse of speciation occurred at approximately 3 Myr as attested to by the divergence among lineages within two of the major mesophylic clades (the *O. anchietae* clade and the clade that includes the *O. irroratus* and *O. typus* clades). Furthermore, the earliest diversification of members of the *O. typus* clade is dated at about 2.58 Myr BP, and those comprising the *O. irroratus* clade at roughly 1.89 Myr BP. This period of intense speciation in the Otomyinae is in good accordance with evolutionary pulses documented across other faunal groups in Africa including rodents, bovids and hominids (Shackleton & Kennett 1975; Vrba 1982, 1985a, b, 1995; Denys 1999; Matthee & Robinson 1999a).

If it is accepted that the Otomyinae originated in southern Africa, and that the ancestors to the modern species were mesic adapted (Bohmann 1952; Pocock 1976; Chevret *et al.* 1993; Sénégas & Avery 1998), it is possible that the gradual cooling and associated aridification of the region in the late Miocene to early Pliocene (Coetzee 1986; van Zinderen Bakker 1986) prior to the period of rapid cladogenesis about 5 Myr ago, promoted continuous populations of the presumably widespread mesophylic ancestors to

become fragmented. The isolation of the ancestral stock in refugia of suitable habitat during this period may have resulted in the divergence of ancestors of extant xerophylic species and the major mesophylic clades suggested by the molecular phylogeny. With subsequent expansions of grasslands these ancestral stocks presumably extended their ranges into areas of suitable habitat. It is probable that the subsequent diversification of species within these clades may have taken place as a result of habitat fragmentation in periods of drier climate between about 4 and 3 Myr and 2.4 and 1.8 Myr ago, during oscillations of cooler/drier and warmer/wetter periods in the Plio-Pleistocene (Shackleton & Kennett 1975; Lindesay 1990; Potts & Behrensmeyer 1993). Of the most intense periods of global cooling was around 2.5 Myr (Bonnefille 1983). These climatic oscillations invariably resulted in the opening and closing of the arid corridor (Axelrod & Raven 1978; Tyson 1981) and may have led to the ancestral stock to the xerophylic species initially becoming "trapped" on the western side of southern Africa (Balinsky 1962). In turn, this may have been followed by the divergence of *P. littledalei* and *O. unisulcatus* in this region around 2.7 Myr BP. This coincides well with a strong northward shift of the arid region and the winter rainfall regime in southern Africa (Denys 1999).

At least one mesophylic otomyine lineage may have migrated along grassland corridors into East, Central Africa during the cycles of wet and dry climate in the Plio-Pleistocene. Here they possibly moved into the Afromontane and Afroalpine vegetation belts tracking the retraction of the Afromontane forests. During periods of warming and concomitant increased rainfall, the forests would have moved down the mountains again and populations formerly isolated in the mountain top refugia could possibly have migrated along grassland corridors to neighbouring mountains. Fossil evidence pertaining to *O. denti* (Kingdon 1974) and *O. typus* (Jaeger 1979; Denys 1989) suggests that these species had a much wider distribution in moist lower lying areas in former climatic periods. Strikingly, the age of the earliest East African fossils of modern Otomyinae (*O. petteri*, about 1.6 Myr old; Denys 1989) is concordant with one of the brief periods of savanna grasslands dominance in this region (thought to have occurred at 1.7 and 1.2 Myr BP; Cerling 1992).

The geographic ranges of Otomyinae in East, Central and West Africa span several centres of endemism in Africa (Moreau 1966; Kingdon 1981, 1990) including the Cameroon highlands (*O. occidentalis* on Mt. Oku; *O. t. burtoni* on Mt Cameroon), the ancient mountains of the Eastern Arc in Kenya and Tanzania (*O. anchietae* and *O. denti*), the Ethiopian Fractured Dome (*O. typus*) and old and new mountains of the Equatorial Highlands (*O. anchietae, O. denti, O. tropicalis* and *O. typus*). In South Africa, *O. sloggetti* is associated with the Drakensberg (also Lesotho) and *O. karoensis* with the

Cape Fold Mountains, one of the oldest centres of great endemism (Kingdon 1990). Because of the great age and climatic stability of the Afromontane forests in East Africa, some species have been isolated for long periods of evolutionary time, and exist as palaeo-endemics on these mountains. During the climatic changes recent immigrants from other habitats may have subsequently migrated into these areas of higher rainfall. These populations probably speciated on one mountain peak in former climatic periods and then moved onto adjacent mountains (where they now occur as neo-endemics). This would account for the occurrence of several populations that represent subspecies of presently recognized species (e.g. within *O typus*, *O. denti*, *O. anchietae* and *O. tropicalis*) on the tops of widely separated mountain in these regions. The West African *O. occidentalis* and *O. t. burtoni* probably owe their present distribution to dispersal events across the Central African Plateau along corridors of grassland or grassland elements in forests and along rivers. Indeed, Mt Oku contains a number of endemic murids, for example *Lamottemys* and *Lophuromys* (Verheyen *et al.* 1997).

CONCLUSION

In conclusion, the phylogenetic relationships of the African Otomyinae are largely discordant with the current taxonomic subdivisions in the subfamily (Musser & Carleton 1993; Taylor *et al.* 1993). This clearly indicates that the nomenclatural subdivisions in current use do not accurately reflect the evolutionary history of the group. All methods of phylogenetic reconstruction suggest that: 1) The recognition of two genera, *Parotomys* and *Otomys* is not warranted and that a single genus, *Otomys*, should be recognized; 2) *Otomys maximus* and *O. angoniensis* are monophyletic sister taxa; 3) The representatives of *O. irroratus* (all cytotypes) form a monophyletic group that is phylogenetically clearly separated from the East African *O. tropicalis* lineage; 4) *Otomys irroratus* represents an evolutionary lineage distinct from all other southern, East and West African species that had previously been synonymized within it (*O. anchietae, O. angoniensis, O. laminatus, O. maximus, O. tropicalis, O. typus* and *O. occidentalis*); 5) The East African *O. typus* (represented by the nominate *O. typus* and *O. t. jacksoni*) and *O. anchietae* (represented by two *O. a lacustris* specimens) are both paraphyletic; 6) The representatives of the A1, A2, B and C cytotypes of *O. irroratus* (Contrafatto *et al.* 1992a, b, 1997) formed monophyletic assemblages, admittedly on very small sample sizes. Furthermore, two monophyletic clades were recovered within *O. irroratus*. The first comprised the B and C cytotypes (the 2n = 27 - 32 group, Rambau *et al.* 2001) and the second, the A1 cytotype (the 2n = 24 group, Rambau *et al.* 2001, which carries a complex compound chromosome), and the A2 cytotype (not included by Rambau *et al.* 2001). This is supportive evidence for two major cytogenetic groups existing within the species (Rambau *et al.* 2001), with the possibility that the A2 cytotype might represent a third

group. These relationships inferred from mtDNA data clearly suggest that more detailed phylogeographic studies at the population level are needed to clarify the taxonomic uncertainties at the specific and intraspecific levels. In spite of these limitations however, the results of this investigation have contributed significantly to our understanding of the evolutionary relationships within the Otomyinae *sensu lato* as well as to the more recent associations among the various cytogenetically defined subgroups within *O. irroratus*.

CHAPTER 5

GENERAL CONCLUSIONS AND FUTURE PROSPECTS

GENERAL CONCLUSIONS

The taxonomy of the African Otomyinae presents a number of inconsistencies at the subfamily and lower levels (Musser & Carleton 1993). This is attributable to the conserved external morphology among the members of this rodent subfamily, and the subtleness, variability and plasticity of the cranio-dental characters on which the current taxonomy is essentially founded. At present, the group is considered a distinct subfamily within the most diverse rodent family, the Muridae (Musser & Carleton 1993), but fossil evidence (Pocock 1976; Sénégas & Avery 1998) and genetic data have suggested that they are indeed true Murinae (Chevret *et al.* 1993; Michaux & Catzeflis 2000; Ducroz *et al.* 2001; Michaux *et al.* 2001; Verheyen *et al.* unpubl.). It is generally accepted that the subfamily comprises two genera, *Parotomys* (whistling rats) and *Otomys* (vlei rats), with 14 described species (Musser & Carleton 1993; Taylor *et al.* 1993). Numerous subspecies have been described on morphological grounds, however, the taxonomic status of several is moot, most noticeably those within the five species recognized from East, Central and West Africa (see Musser & Carleton 1993; Taylor & Kumirai in press.). It has further been suggested that incipient speciation may be taking place in the southern African *O. irroratus*, which displays extreme morphological conservatism but remarkable chromosomal variability (Taylor 2000). This species is characterized by marked variation in diploid number (2n = 23 – 32), and between two (Rambau *et al.* 2001) and five (Contrafatto *et al.* 1992a, b, 1997) major chromosomal groups have been identified within the species.

In an attempt to reconstruct the evolutionary relationships within the subfamily, sequence data of two mtDNA genes (the complete cyt *b* gene and approximately 800 bp of 12S rRNA) were analyzed using ML, MP and NJ reconstruction methods. There were essentially two major aims; the first to infer the phylogenetic relationships among the 14 recognized species (*sensu* Musser & Carleton 1993; Taylor *et al.* 1993). Of priority was to determine whether the genus *Parotomys* had a diphyletic association with *Otomys*.

The second aim was to investigate the patterns of sequence evolution in the cyt *b* and 12SrRNA genes among species within the subfamily, and among the chromosomal races (cytotypes) of *O. irroratus*. This study is unique in that sequence data obtained from all but one (*O. karoensis*) of the 14 accepted species were analyzed, including representatives of two subspecies from East African (*O. anchietae lacustris*, *O. typus jacksoni*) and four of the five *O. irroratus* cytotypes (A1, A2, B and C *sensu* Contrafatto *et al.* 1992a, b, 1997). The majority of investigations concerning the evolutionary relationships and taxonomy of the group have to date been limited to southern Africa (e.g. Taylor *et al.* 1989a, b; Contrafatto *et al.* 1992a, b; Taylor *et al.* 1992; Contrafatto *et al.* 1994, 1997; Rambau *et al.* 1997; Ducroz *et al.* 2001; Rambau *et al.* 2001). Those that did incorporate extralimital taxa have been based on morphology (Bohmann 1952; Dieterlen & van der Straeten 1992; Taylor & Kumirai in press) with the exception of one study on *O. typus*, which employed allozyme electrophoresis and comparative cytogenetic methods (Lavrenchenko *et al.* 1997).

The cyt *b* gene (and to a lesser extent 12S rRNA) proved to be useful in resolving the relationship among terminal taxa but failed to clarify deeper evolutionary branches. The limited phylogenetic signal at the deeper divergence levels was likely the result of a rapid radiation as seen in the short internal branches. These topologies could not be improved by the use of complex evolutionary models in ML and complicated weighting schemes in parsimony. The dynamics of sequence evolution were incorporated into the phylogenetic analyses by using empirical parameter estimates (transition bias, among-site rate variation and the proportion of invariable sites) derived under the best-fit ML model for each of the data sets. The 12S rRNA gene was extremely conserved among members of the subfamily and resulted in poorly resolved phylogenies. Consequently, the combination of sequences from the latter gene with cyt *b* data did not improve the phylogenetic resolution.

The most striking findings of this investigation were in the first place, strong evidence to support the monophyly of the subfamily in all the molecular topologies, secondly, the East, Central and West African species did not form a monophyletic group, and thirdly, the retrieval of three major clades (referred to by the name of the oldest taxon in the clade) that were well supported by bootstrap. The *O. typus* clade comprised *O.*

angoniensis and *O. maximus* from southern Africa and *O. t. jacksoni* and *O. typus* from East Africa; the *O. anchietae* clade incorporated the East African *O. tropicalis* and the two representatives of *O. a. lacustris* (not as sister taxa) with *O. denti* basal; the *O. irroratus* clade included the southern African *O. laminatus* and the *O. irroratus* cytotypes, which formed a monophyletic assemblage. A sister taxon association between the *O. typus* and *O. irroratus* clades was consistently retrieved with high bootstrap support. *Otomys sloggetti* and the arid dwelling *P. littledalei, P. brantsii* and *O. unisulcatus* appear to be of the older lineages in the subfamily, but their exact sister taxon affiliations remain problematic. *Otomys unisulcatus* and *P. littledalei* grouped as sister taxa (but only with moderate bootstrap support); however, the positions of *O. sloggetti* and *P. brantsii* remain equivocal. These relationships suggest that the current taxonomy of the Otomyinae does not accurately reflect the true evolutionary associations.

In the first instance, the generic status of *Parotomys* is not supported and all the species within the subfamily should be referred to a single genus, *Otomys*. The molecular phylogenies consistently reflect the paraphyly of *Otomys*, a finding which is concordant with the results of other genetic studies (Taylor *et al.* 1989a, b; Meester *et al.* 1992; Contrafatto *et al.* 1994; Rambau *et al.* 1997; Ducroz *et al.* 2001; Taylor *et al.* pers. comm.), but is in sharp contrast to those based on morphological data (Musser & Carleton 1993; Taylor *et al.* unpubl.). This, coupled to the high intergeneric genetic distances separating *Parotomys* and *Otomys* that fall within the same range as those among well-recognized *Otomys* species, strongly suggests that the generic subdivision within the group should be reconsidered. The recognition of a third genus, *Myotomys*, previously described for the Otomyinae is also unsupported. A pronounced dichotomy between arid adapted lineages with a more westerly distribution in Africa and mesic adapted lineages with a more easterly distribution is not supported.

The relationships among several Otomyinae taxa included in this investigation and the degree of sequence differentiation between them, question the current taxonomy of species in the subfamily (*sensu* Musser & Carleton 1993; Taylor *et al.* 1993). However, given the small number of specimens examined (one or two) for most of the OTUs, no definitive statements on intraspecific variation and, in some instances species status, can

be made. Therefore, the following relationships, although consistently retrieved, in the present investigation, essentially provide a basis for future investigation:

1. Without fail, *O. angoniensis* and *O. maximus* group as monophyletic sister taxa with high bootstrap support. Moreover, the sequence divergence between them is markedly smaller than those separating well-defined *Otomys* species, suggesting that the separation of the two lineages occurred rather recently. These molecular data, coupled with evidence for similar ecological preferences (De Graaff 1981; Bronner & Meester 1988) appear to suggest that the current status of *O. maximus* is questionable, and that the larger body size of the latter may possibly have erroneously influenced earlier taxonomic treatments (e.g. Roberts 1951; Swanepoel *et al.* 1980; Smithers 1983; Musser & Carleton 1993; Crawford-Cabral 1998).

2. The representatives of *O. irroratus* (all cytotypes) form a monophyletic group that is phylogenetically distant from other *Otomys* species that, in the past, have been considered conspecifics. The only potential exception to this is *O. laminatus*. Although Bohmann (1952) and Petter (1982) included *O. angoniensis* and *O. maximus* from southern Africa, *O. anchietae, O. tropicalis* and *O. typus* from East and Central Africa, and *O. occidentalis* from West Africa within *O. irroratus,* this is not supported herein. In fact, the distinctiveness on molecular grounds of *O. irroratus* is consistent with the results of recent cranio-dental and allozyme studies (Taylor *et al.* in press; Taylor *et al.* pers. comm.) as well as geometric morphometrics (Taylor *et al.* unpubl.).

3. The paraphyletic associations shown for representatives of the East African *O. typus* and *O. anchietae* (Musser & Carleton 1993) questions the taxonomy of these species. Genetic distances distinguishing *O. typus* from Ethiopia and *O. t. jacksoni* from Tanzania, as well as *O. anchietae lacustris* from the Uzungwa Mountains and the Ufipa Plateau in southwestern Tanzania are similar to those separating *Otomys* species.

4. The close phylogenetic association of the B and C cytotypes to the exclusion of the A1 and A2 cytotypes of *O. irroratus* (Contrafatto *et al.* 1992a, b, 1997) is concordant with recent evidence from comparative cytogenetic studies (Rambau *et al.* 2001), which delimit two major cytogenetic groups within the species. The monophyly of both groups

and of the A2 cytotype (which was not included by Rambau *et al.* 2001) was consistently retrieved with high bootstrap support. The one cytogenetic group (which include the B and C cytotypes) is characterized by a diploid number of 2n = 27 - 32, and the second, with 2n = 24, carries a large compound chromosome involving the fusion of chromosomes 7, 8 and 12 of the *O. irroratus* standard. The possibility that the A2 cytotype may represent a third evolutionary group is suggested by its placement in the molecular trees as well as by the relatively large genetic distances separating it from representatives of the other cytotypes.

Speciation of the Otomyinae appears to have occurred in pulses. The earliest divergence among otomyine lineages was estimated to have occurred at approximately 5.14 Myr BP (early Pliocene). It is argued that the three major mtDNA clades, originated from an ancestral stock that predates the earliest known fossil (*Prototomys campbelli*) estimated at between 3.6 and 3.0 Myr old (Broom & Schepers 1946). Subsequent speciation appears to have occurred between more or less 3.09 and 1.89 Myr ago. The present study's estimates of the rapid radiation in the subfamily coincide with the most pronounced phase of the diversification of the African Murinae, thought, on fossil evidence, to have been at approximately 5 Myr (Chevret 1994; Denys 1999).

A biogeographical scenario is presented for the speciation and radiation of the subfamily on the African continent. It is suggested that speciation may have been driven by palaeo-climatic and geological events during the late Miocene and Plio-Pleistocene epochs linked to cycles in polar glaciation (Shackleton & Kennett 1975; Bonnefille 1983, 1985; Potts & Behrensmeyer 1993). These events resulted in periods of cooler/ drier and warmer/ wetter climate, that, presumably led to range expansion and fragmentation of the ancestral stock resulting in isolated refugia of suitable grassland habitats in southern, Central and East Africa. If one accepts a southern African origin for the subfamily as indicated by fossil evidence (Denys *et al.* 1987; Denys 1989; Chevret *et al.* 1993) it seems likely that the ancestral stock probably migrated into East and Central Africa along corridors of grassland habitat. At least one lineage radiated into West Africa. The opening and closing of an arid corridor between the northeast and southwest of the continent during the Plio-Pleistocene (Axelrod & Raven 1978; Tyson 1981) may also

have been responsible for the isolation of the xerophylic *P. littledalei* and *O. unisulcatus* ancestors on the arid western side of the continent.

PRESENT PROBLEMS AND FUTURE PROSPECTS RELATED TO THE TAXONOMY OF THE OTOMYINAE

An in-depth investigation into the genetic differentiation and evolutionary relationships among species of *Otomys* that are extralimital to southern Africa is required to re-evaluate the status of taxa that are presently considered as subspecies, but previously enjoyed specific status. There is compelling morphological and genetic evidence (this study and previous work) to suggest that distinct evolutionary lineages are contained within four of the five currently recognized species of *Otomys* (*O. tropicalis*, *O. typus*, *O. denti* and *O. anchietae*) that occur on geographically isolated mountains in East, Central and West Africa (Dieterlen & van der Straeten 1992; Musser & Carlton 1993; Lavrenchenko *et al.* 1997; Taylor & Kumirai in press). Conversely, there is considerable disagreement concerning the validity of subspecific status within southern African *Otomys*. For instance, homogeneous allozyme variation has been detected over the species range of *O. unisulcatus*, questioning the recognition of several subspecies described from slight size and color variation (van Dyk *et al.* 1991). Information on the genetic diversity at the population level could potentially clarify these uncertainties.

The importance of a genetic approach for identifying cryptic species has been demonstrated in a number of studies (Capanna *et al.* 1996; Ducroz *et al.* 1997; Verheyen *et al.* 1997; Ducroz *et al.* 1998; Fadda 2000), and the utility of mtDNA analysis in this regard has been amply emphasized (Avise 2000). Population level mtDNA sequencing analysis using the cyt *b* gene or control region and microsatellites may shed further light into relationships between the different cytotypes of *O. irroratus*, the genetic diversity within, and the extent of the gene flow between them. Such investigations should include animals with known cytotypes and take cognizance of the two major cytogenetic groups delimited by Rambau *et al.* (2001). Moreover, appropriately designed breeding experiments may shed further light on the relationships among cytotypes.

"Teach thy tongue to say: 'I do not know'"

Maimonides

"The great tragedy of Science: the slaying of a beautiful hypothesis by an ugly fact"

Thomas Henry Huxley

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APPENDIX I

Alignment of the mtDNA cytochrome *b* sequences of the 27 otomyine taxa included in this study (see Table 2) and outgroups *Aethomys chrysophilus* (Ducroz *et al.* 1998) and *Mus musculus* (Bibb *et al.* 1981). • - indicates nucleotide identity to the sequence of *M. musculus*. The structural model for cytochrome *b* (Howell 1989 adapted for mammals by Irwin *et al.* 1991) distinguishes three functional domains along the length of the gene: Q_i = inner-membrane, Q_o = outer-membrane and Q = trans-membrane. The sections of sequence within each of the three domains are indicated above the nucleotides at the start and at the end of each section. Missing data in the *O. occidentalis* sequence are indicated by ?. The cytotypes of *O. irroratus* (A1, A2, B and C) are given after the species name. Abbreviations: *Mmus - Mus musculus, Achr - Aethomys chrysophilus, Oalac - O. anchietae lacustris, Oang - O. angoniensis, Oden - O. denti, Oirr - O. irroratus, Olam - O. laminatus, Omax - O. maximus, Oocc - O. occidentalis, Oslo - O. sloggetti, Otro - O. tropicalis, Otyp - O. typus, Otjac - O. typus jacksoni, Ouni - O. unisulcatus, Pbra - P. brantsii, Plit - P. littledalei*. In brackets: abbreviated collection localities of taxa represented by more than one specimen correspond to those in Table 2. Abbreviations: Uzu = Uzungwa Mountains, Chi = Chingombe, Mbi = Mbizi, Mga = Mgahinga, Buj = Bujuku River, Hog = Hogsback, Kam = Kamberg, Kar = Karkloof, Ali = Alice, Bai = Baines Kloof, Gro = Groendal, Alg = Algeria, Twe = Tweede Tol, Aru = Arusha, Run = Mt. Rungwe, Kal = Kalagadi, Kle = Kleinsee, Goe = Goegap, Hen = Hentiesbaai.

Membrane domain	Q_i →
Mmus	ATGACAAACATACGAAAAACACACCCATTATTTAAAATTATTAACCACTCATTCATTGACCTACCTGCCC
Achr	..........C.....TT.....CC..A.A...G.C.....T.....T..........C.....A.
Oalac (Uzu)	..........C.....C.....C..C.C.....C..T.....T..........CA.A.
Oalac (Chi)	..........C.....C.....C..C.C.....C.....T.....T.....T..C..CA.A.
Oalac (Mbi)	..........C.....C.....C..C.C.....C.....T.....T..C..T..C..CA.A.
Oang	..........C.....C.....CC..C.....T.....T.....C.....A.
Oden (Mga)	..........C.....T.....TC..C.C.....T.....C.....C..CA.A.
Oden (Buj)	..........C.....C.....CC..C.C.....T.....C.....T..CA.A.
Oirr A1 (Hog)	..........C.....T.....CC..C.C.....T.....T.....C..C..A.
Oirr A1 (Kam)	..........C.....T.....CC..C.C.....T.....T.....C..C..A.
Oirr A2 (Kar)	..........C.....T.....CC..C.C.....T.....T.....C..C..A.
Oirr B (Ali)	..........C.....T.....TC..C.C.....T.....T.....C..CA.A.
Oirr B (Bai)	..........C.....T.....TC..C.....T.....T.....C..CA.A.
Oirr B (Gro)	..........C.....T.....TC..C.C.....T.....T.....C..CA.A.
Oirr C (Alg)	..........C.....T.....TC.TC.C.....T.....T.....C..CA.A.
Oirr C (Twe)	..........C.....T.....TC.TC.C.....T.....T.....C..CA.A.
Olam	..........C.....C.....TC..C.C.....T.....T.....C..CA.A.
Omax	..........C.....C.....CC..C.....T.....T.....C.....A.
Oocc	..........C.....T.....C..C.C.....T..CA.A.
Oslog	..........C.....C.....C...C.....T..C..CA.A.
Otro	..........C.....C.....C..C.C.....C.....T.....A.A.
Otyp	..........T.....C.....TC..C.C.....T.....T.....C..C..A.
Otjac (Aru)	..........C.....C.....CC..C.C.....T.....T.....T..C..A.
Otjac (Run)	..........C.....C.....CC..C.C.....T.....T.....T..C..A.
Ouni	..........C.....AC.....TC.TC.C.....T..T.....T.....T..T..CA.A.
Pbra (Kal)	..........C.....T.....CC.TC.C.....C.....T..T.....C..C..A.
Pbra (Kle)	..........C.....T.....CC.TC.C.....C.....T..T.....C..C..A.
Plit (Goe)	..........C.....T.....C..C.....TG.T.....T.....T..T..CA.A.
Plit (Hen)	..........C.....T.....C..C.....TG.....T.....T..T..CA.A.
Nucleotide	10 20 30 40 50 60 70

Continued/

Membrane domain	← Q_i Q →
Mmus	CATCCAACATTTCATCATGATGA AACTTTGGGTCCCTTCTAGGAGTCTGCCTAATAGTCCAAATCATTAC
Achr	.....C..... .....C..C..TT.A.....A.T...T.....A.....C..
Oalac (Uzu)	....A....C..... .....C..A.....A.....A.T.....G..C.T.....C.C..
Oalac (Chi)	....A....C..... .....A.....A.....A.T.....C.T.....C.C..
Oalac (Mbi)	....A....C..... .....A.....A.....A.T.....C.T.....C.C..
Oang	....A....C..... .....C..C..T..A.....A.T.....T..GC.A.....C....
Oden (Mga)	....A....C..G..... .....C.....A.....A.....C...T.A.....C.C..
Oden (Buj)	....A....C.....G .....C.....A.....A.....C...T.A.....TC.C..
Oirr A1 (Hog)	....A....C..... .....T.....A.....A.....C...C.A.....C.C..
Oirr A1 (Kam)	....A....C..... .....T.....A.....A.....C...C.A.....C.C..
Oirr A2 (Kar)	....A....C..... .....T..T..A.....A.....C...C.A.....C.C..
Oirr B (Ali)	....A....C..... .....C..T..G.....A.T.....T...C.A.....C.C..
Oirr B (Bai)	....A....C..... .....C..T..G.....A.....T...C.A.....C.C..
Oirr B (Gro)	....A....C..... .....C..T..G.....A.....T...C.A.....C.C..
Oirr C (Alg)	....A....C..... .....C..T..A.....A.....T...C.A.....C.C..
Oirr C (Twe)	....A....C..... .....C..T..A.....A.....T...C.A.....C.C..
Olam	....A....C..... .....C..C..T..A.....A.T.....T...C.A.....C.C..
Omax	....A....C..... .....C..C..T..A.....A.....T..GC.G.....C....
Oocc	....A....C..... .....C.....A.....C..CC.A.....C.C..
Oslog	....A....C..... .....T..A.....A.T.....T..TC.A.....C.C..
Otro	....A....C..... .....A.....A.....A.T.....G..C.T.....C.C..
Otyp	....A....C..... .....C..C.....A.....A.A.....C...C.A.....TC.C..
Otjac (Aru)	....A....C..... .....C..C..T..A.....T...C.A.....C....
Otjac (Run)	....A....C..... .....C..C..T..A.....T...C.A.....C....
Ouni	....A..T..... .....A.....AT.....A.T.....TG.CC.A.....C....
Pbra (Kal)	....A.....G..G..... .....T.A.....A.T..T..C...C.A.....TC.C..
Pbra (Kle)	....A.....G..G..... ..T.....T.A.....A.T.....C...C.A.....TC.C..
Plit (Goe)	....G..T.....C..... .....A...T.G.....A.T.....C..CT.A.....C....
Plit (Hen)	....A..T.....C..... .....T.G.....A.T.....C..CT.A.....C.C..
Nucleotide	80 90 100 110 120 130 140

Membrane domain	← Q Q_o →
Mmus	AGGTCTTTTCTTAGCCATACACTACACA TCAGATACAATAACAGCCTTTTCATCAGTAACACACATTTGT
Achr	...C..A.....T..T... .....C....C.....A..C.....C..T..C..C
Oalac (Uzu)	...C..A..TC.....T... .....C.....A..C.....G.....C..T.....C
Oalac (Chi)	...C..A...C.....T... .....C.....A..C.....C.....C
Oalac (Mbi)	...C..A...C.....T... .....C.....A..C.....C.....C
Oang	...C..A..TC....A.....T... .....C....C.....A..C.....C..T.....C
Oden (Mga)	C..C..A..TC....T..... .....C....C.....A..C.....C..T..C..C
Oden (Buj)	C..C..A..TC..... .....C....C.....A..C.....C.....C..C
Oirr A1 (Hog)	...C..A..TC..... .....C....C.....A..C.....C..T..C..C
Oirr A1 (Kam)	...C..A..TC..... .....C....C.....A..C.....C..T..C..C
Oirr A2 (Kar)	...C..A..TC....T.....T... ..G..C....C.....A..C.....C..T..C..C
Oirr B (Ali)	.....G..TC..... .....C....C.....A..C.....C..T..C..C
Oirr B (Bai)	.....G..TC..... .....C....C.....A..C.....C..T..C..C
Oirr B (Gro)	.....G..TC..... .....C....C.....A..C.....C..T..C..C
Oirr C (Alg)	.....A..TC....T..... .....C....C.....A..C.....C..T..C..C
Oirr C (Twe)	.....A..TC....T..... .....C....C.....A..C.....C..T..C...
Olam	...C..A...C.....T... .....C....C.....A..C.....C..T..C..C
Omax	...CT.A..TC....A.....T... .....C....C.....A..C.....C..T.....C
Oocc	...CT.A...C....T..... .....C....C.....A..C.....C.....C..C
Oslog	...CT.A..TC.....T... .....C....C.....A.....G.C..T..C..C
Otro	...CT.A..TC..... .....C.....A..C.....C..T.....C
Otyp	...C..A..TC.....T... .....C....C.....A..C.....G.C..T..C..C
Otjac (Aru)	...CT.A...C....A.....T... .....C....C.....G..C.....T..C.....C
Otjac (Run)	...GT.A...C....A.....T... .....C....C.....A..C.....T..C..T.....C
Ouni	...CT.A..TC....A..... .....C....C.....A..C.....C..T.....C
Pbra (Kal)	...CT.A..TC....T.....TT.. .....C.....A..C.....C..T..C...
Pbra (Kle)	...CT.A..TC....T.....T... .....C.....A..C.....C..T..C...
Plit (Goe)	...CT.A...C..... .....C.....A.....G.C..T..C..C
Plit (Hen)	...CT.A...C.....T... C.....C.....A.....G.C..T..C..C
Nucleotide	150 160 170 180 190 200 210

Continued/

Membrane domain	← Q_o Q →
Oirr A1 (Kam)	CGAGACGTAAATTACGGGTGACTAATCCGA TATATACACGCAAACGGAGCCTCAATATTTTTTATTTGCT
Oirr A2 (Kar)	.....T..A..... .....T.....G.....C..C....
Mmus	.....T..C.....T...T.....T... ...C....T.....A.....C..C..C...C
Achr	.....T..C.....C..... ...T....T.....A.....C..C...C
Oalac (Uzu)	.....T..C.....T...T..... ...T....T.....A.....C....
Oalac (Chi)	.....T..C.....C...T..... ...T....T.....A.....C..C...C
Oalac (Mbi)	.....T..C.....C...T..... ...T....T.....A.....C..C...C
Oang	.....T..C.....T...T..... ...T....T.....A.....C..C..C....
Oden (Mga)	.....T..C.....C.....C..T... ..C.....T.....A.....C..C..TC
Oden (Buj)	.....T..C.....C.....C..T... ..C.....T.....A.....C..C..TC
Oirr A1 (Hog)	.....T..C.....C.....C..T... ...T....T.....A.....C..C...C
Oirr B (Ali)	.....C.....C.....T..T .....G..T.....A.....C..C...C
Oirr B (Bai)	.....C.....C.....C..T..T .....G..T.....A.....C..C...C
Oirr B (Gro)	.....C.....C.....T..T .....G..T.....A.....C..C...C
Oirr C (Alg)	.....C.....C.....C..T..T .....G..T.....A.....C..C...C
Oirr C (Twe)	.....C.....C.....C..T..T ..C..C..T.....A.....C..C...C
Olam	.....T..C.....C.....T..T... ..CT.....T.....A..C.....C..C..C...C
Omax	.....C.....C..... ...T....T.....A.....C..C..TC
Oocc	.....T..C.....C..... .....T.....T.....A.....C..C..C...C
Oslog	.....T..C.....C...T....T... ..CC.T..T.....A.....C..C..C...C
Otro	.....T..C..T..C...T....T... .....T.....A.....C..C...C
Otyp	.....T..C.....C.....T... ...T....T.....A.....C..C...C
Otjac (Aru)	.....T..C.....C.....C..... ...C....T.....A.....C..C
Otjac (Run)	.....T..C.....C.....C..... ...C....T.....A.....C..C..TC
Ouni	.....T..C..C.....C.....T..... ...T....T.....A.....C..C....
Pbra (Kal)	.....C.....C.....T... ..CC....T.....A.....C.....C
Pbra (Kle)	.....C.....C.....T... ..CC.G..T.....A.....C.....C
Plit (Goe)	.....T..G..C.....C.....G..... ...T.....A.....C..TC
Plit (Hen)	.....T..G..C.....C.....G..... ...T.....A.....C..TC
Nucleotide	220 230 240 250 260 270 280

Membrane domain	← Q Q_i → ← Q_i Q →
Mmus	TATTCCTTCATGTCGGACGAGGCTTATATTATGGATCA TATACATTTATAGAAACCTGA AACATTGGAGT
Achr	.....A..C.....AA....C.....T .....CC.....A... .....C.....
Oalac (Uzu)	....T..A..C..A.....A....C..... ..C.....CC.....T... .....C.....
Oalac (Chi)	.....A.....A.....A....C..... .....C.....T... ..T..C.....
Oalac (Mbi)	.....A.....A.....A....C..... .....C.....T... ..T..C.....
Oang	.....T.A.....A.....TA.....C..G..C ..C.....CC.....A... .....
Oden (Mga)	....T..A.....A.....AA.....C ..C.....C.....A... .....C.....
Oden (Buj)	....T..A.....A.....AA....C.....C ..C.....C.....A... .....C.....
Oirr A1 (Hog)	.....A.....A.....A....C.....C..C .....CC.....A... .....C.....
Oirr A1 (Kam)	.....A.....A.....A....C.....C..C .....CC.....A... .....C.....
Oirr A2 (Kar)	....T..A.....A.....T...A....C.....C..C ..C.....CC.....A... .....C.....
Oirr B (Ali)	....T..A..C..A.....A.....C .....C.....A... .....C.....
Oirr B (Bai)	....T..A..C..A.....A.....C .....C.....A... .....C.....
Oirr B (Gro)	....T..A..C..A.....A.....C .....C.....A... .....C.....
Oirr C (Alg)	....T..A..C..A.....A....C.....C .....C.....A... .....C.....
Oirr C (Twe)	....T..A..C..A.....A.....C .....C.....A... .....C.....
Olam	.....A..C..A.....A.....C ..C.....CC.....A... .....C.....
Omax	.....A.....A.....TA.....G..T ..C.....CC.....A... ..T.....
Oocc	....T..A..C..A.....A....C.....C .....CC.....A... .....C.....
Oslog	....T..A.....A.....A.....C .....CC.....A... .....
Otro	....T..A..C..A.....A....C..... ..C.....C.....T... .....C.....
Otyp	....TT.A..C..A.....A....C.....C .....C.....A... ..T..C.....
Otjac (Aru)	....TT.A.....A.....TA....C.....C ..C.....CC.....A..G ..T..C.....
Otjac (Run)	.....A.....A.....TA....C.....C ..C.....CC.....A..G .....C.....
Ouni	....TA.A..C..A.....A.....C .....CC.....A... .....
Pbra (Kal)	....T..A.....A.....A.....C.....T ..C.....CC.....A... .....
Pbra (Kle)	....T..A.....A.....A.....C.....T ..C.....CC.....A... ..T.....
Plit (Goe)	....TT.A..C..A.....A.....T .....C.....A... ..T.....
Plit (Hen)	....TT.A..C..A.....A.....T .....C.....A... ..T.....
Nucleotide	290 300 310 320 330 340 350

Continued/

Membrane domain					← Q	Q_o →	
Mmus	ACTTCTACTGTTCGCAGTCATAGCCACAGCATTTATAGGCTACGTCCTTCCA	TGAGGACAAATATCATTC					
Achr	.A.....A..TA.....T.....C.....A..T.....	.....					
Oalac (Uzu)	.A....CT.A..T..C..A.....C.....C..G.....T.....	.....C.....T					
Oalac (Chi)	GA....CT....TA.C..A.....C.....C.....T..T.....	.....C.....T					
Oalac (Mbi)	GA....CT....TA.C..A.....C.....C.....T..T.....	.....C.....T					
Oang	.A....CT.A..TA.C..A.....T.....T.....C...	.....					
Oden (Mga)	.A....T..A.....C..T.....C.....C.....T.....	.....C.....					
Oden (Buj)	.A....T..A.....C..T.....C.....C.....T.....	.....C.....					
Oirr A1 (Hog)	.A.C..CT.A..TA.C..A.....C.....C..G.....T..T.....	.....					
Oirr A1 (Kam)	.A.C..C..A..TA.C..T.....C.....C.....T..T..C...	.....					
Oirr A2 (Kar)	.A.C..T..A..TA.C..A.....C.....C.....T.....C...	.....					
Oirr B (Ali)	.A.C..C..A..T..C..A.....T..C.....C.....T..T..C...	.....T					
Oirr B (Bai)	.A.C..CT.A..T..C..A.....T..C.....C.....T..T..C...	.....T					
Oirr B (Gro)	.A.C..C..A..T..C..A.....T..C.....C.....T..T..C...	.....T					
Oirr C (Alg)	.A.C..CT.A..T..T..A.....T..C.....C.....T..T..C...	.....T					
Oirr C (Twe)	.A.C..CT.A..T..C..A.....T..C.....C.....T..T.....	.....T					
Olam	.A.C..T..A..T..C..G.....C.....T.....C...	.....					
Omax	.A....CT.A..TA.C..A.....T.....T.....C...	.....					
Oocc	.A.C..C..A.....C..A.....C.....C.....T..T.....	.....?.....?					
Oslog	.A....C..A...A.C..A.....C.....C.....T.....	.....					
Otro	.A....CT.A..TA.C..A.....T.....T..T.....	.....C.....T					
Otyp	.A....T..A..T..C..T.....T.....C.....T..T.....	.....					
Otjac (Aru)	.A....CT.A..TA.C..A.....T.....T.....	.....					
Otjac (Run)	.A....CT.A..TA.C..A.....T.....T.....	.....					
Ouni	.A....C..A...A.C..A.....C.....C.....T..T.....	.....					
Pbra (Kal)	.A....C..A...A.C..T.....T.....C.....T..T..C...	.....					
Pbra (Kle)	.A....C.....A.C..T.....T.....C.....T..T..C...	.....					
Plit (Goe)	.A....CT....TA.T..A.....T..C.....C.....T..T.....	.....					
Plit (Hen)	.A....CT.A..TA.C..A.....T..C.....C.....T..T.....	.....					
Nucleotide	**360**	**370**	**380**	**390**	**400**	**410**	**420**

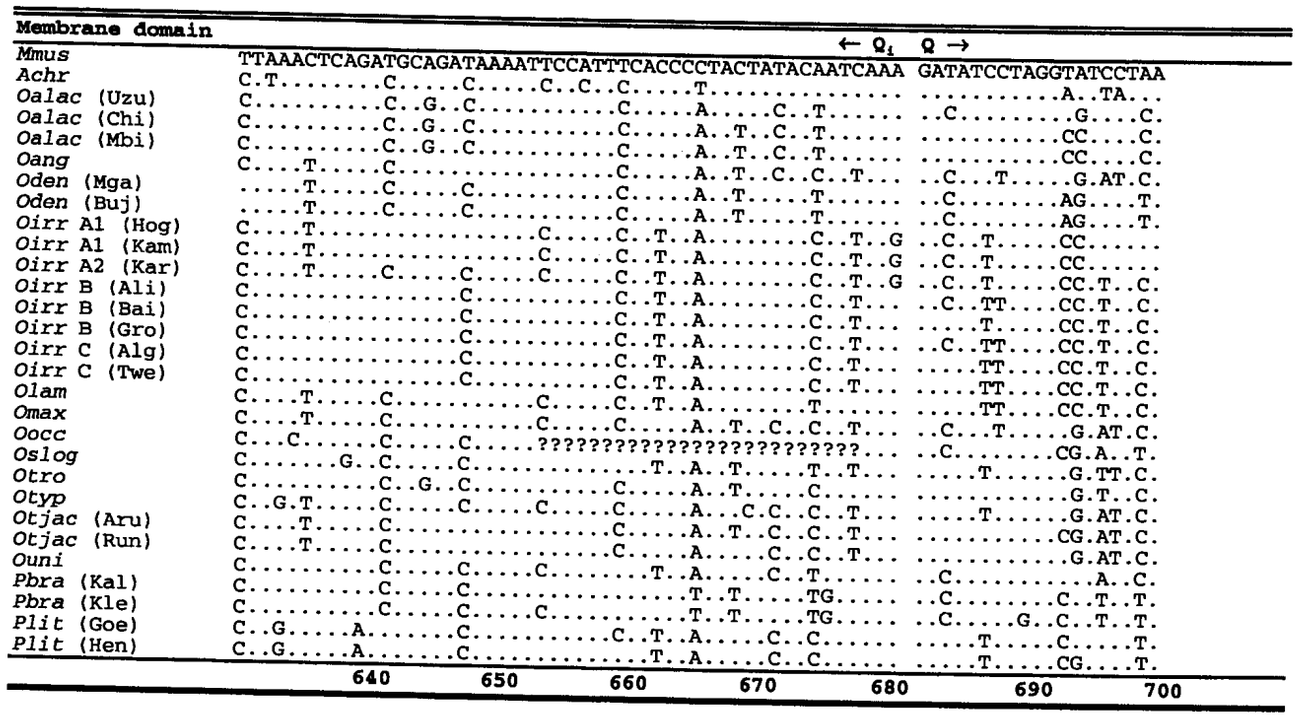
Membrane domain							
Mmus	TGAGGTGCCACAGTTATTACAAACCTCCTATCAGCCATCCCATATATTGGAACAACCCTAGTCGAATGAA						
Achr	.....A.....A.....TT.A..T.....C..C....T.....A.....						
Oalac (Uzu)	.....A..A.....C.....A..T.....C..T..C.....A.....						
Oalac (Chi)	.....A..A.....C.....T..T.....T.....C.....T..C.....A.....						
Oalac (Mbi)	.....A..A.....C.....T..T.....T.....C.....T..CG.....A.....						
Oang	.....A..A.....TT.A..T..T..T.....C..C.....C..T.....A.....						
Oden (Mga)	.....A..A..G.....C.....TT.A..C.....C..C..C..C.....A.....						
Oden (Buj)	.....A..A.....C.....T..A..C.....C..C..C..C.....A.....						
Oirr A1 (Hog)	.....A..A.....A..T..C.....T.....C..T..C.....A.....						
Oirr A1 (Kam)	.....A..A.....A..T..C.....T.....C..T..C.....A.....						
Oirr A2 (Kar)	.....A..A.....TT.A..T..C.....T.....C.....A.....						
Oirr B (Ali)	.....A..A.....C.....T.A..T..C..T..T..T.....C..C..A.....A.....G.						
Oirr B (Bai)	.....A..A.....C.....T.A..T..C..T..T..T.....C..C..A.....A.....G.						
Oirr B (Gro)	.....A..A.....C.....T.A..C..C..T..T..T.....C..C..A.....A.....G.						
Oirr C (Alg)	.....A..A.....T.A..T..C..T..T..T.....C..C..A.....A.....G.						
Oirr C (Twe)	.....A..A.....T.A..T..C..T..T..T.....C..C..A.....A.....G.						
Olam	.....A..A.....A..T..T..T..T..T.....T..C.....A.....						
Omax	.....A..A.....T..A..T..T..T.....T..C.....C..T.....A.....						
Oocc	.....A..A.....T.A..T..T..T..T..T..C.....T..T.....A.....						
Oslog	.....G..A.....T..A..T..T..T.....C.....C..CG.....A.....						
Otro	.....A..A.....C.....A..C.....T.....C..T..C.....A.....						
Otyp	.....A..A.....C..C.....A..C..T.....T..C.....T..T.....A.....						
Otjac (Aru)	.....A..A.....A..T..T..T..T..T..C.....T..T.....A.....						
Otjac (Run)	.....A..A.....T.A..T..T..T..T..T..C.....T..T.....A.....						
Ouni	.....A..A.....C..T..T.....TT.T.....A.....						
Pbra (Kal)	.....A..A.....T.A..T..T..T.....C..C..C..C...T....T.....						
Pbra (Kle)	.....A..A.....T.A..T..T..T.....C..C..C..C...T....T.....						
Plit (Goe)	.....G..A.....C.....T..A..C..T..T.....C..T..C...T....A.....G.						
Plit (Hen)	.....G..A.....C.....T..A..C..T..T.....C..T..C...T....A..G....						
Nucleotide	**430**	**440**	**450**	**460**	**470**	**480**	**490**

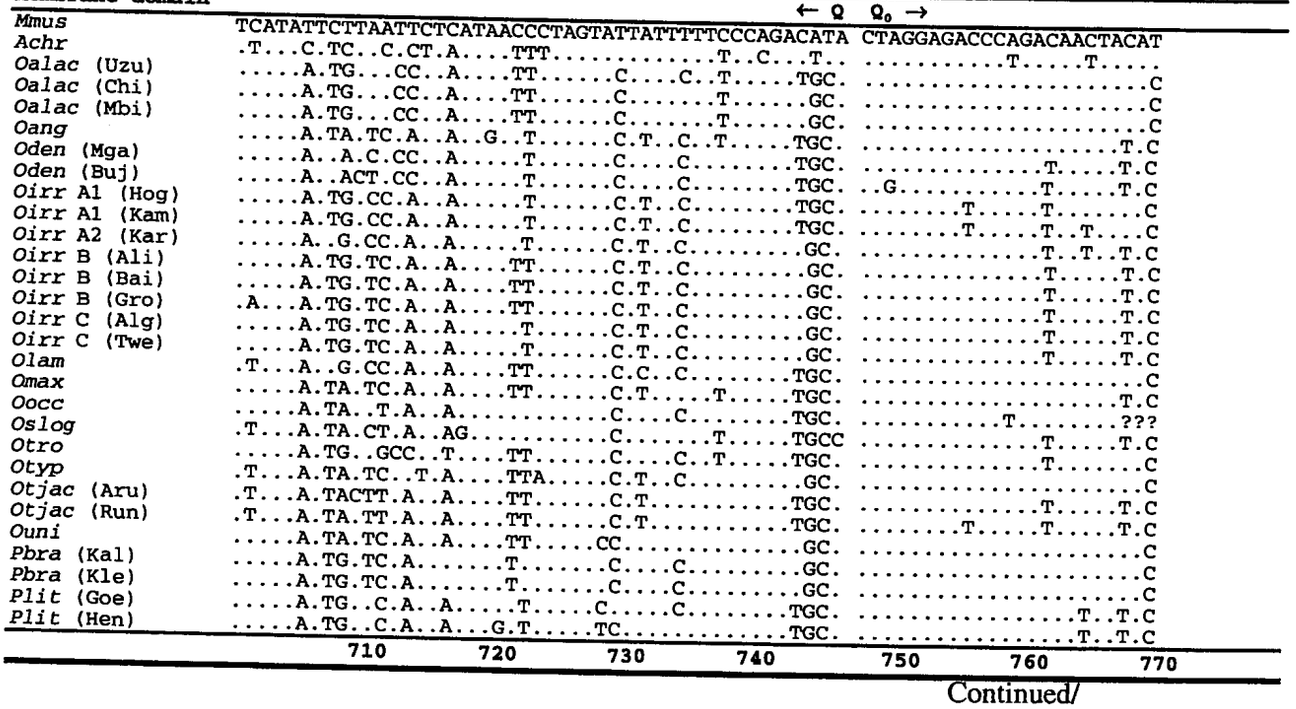
Continued/

Membrane domain	← Q_o Q →
Mmus	TTTGAGGGGGCTTCTCAGTAGACAAAGCCACCTTGACCCGATTC TTCGCTTTCCACTTCATCTTACCATT
Achr	.C.....C..A.....G..T..T..A..A..C.CT .....A..T.....
Oalac (Uzu)	.....A.....T.....T..TC.A..A..C... .....A.....T.....
Oalac (Chi)	.....T.....T.....TC.A..A..C... .....A..T.....T...C.....
Oalac (Mbi)	.....T.....T.....TC.A..A..C... .....A..T.....T.....
Oang	.C.....A..T.....T...C.A..A..C..T .....A.....T..TC.....
Oden (Mga)	.C.....A.....T.....T...C.T..A..C... .....A..T.....T..TC.....
Oden (Buj)	.C.....A.....T.....T..TC.T..A..C... ..T..A..T.....T..T.....
Oirr A1 (Hog)	.C.....T.....T.....C.A..A..C... .....A.....T..T.....
Oirr A1 (Kam)	.C.....T.....T.....C.A..A..C... .....A.....T..T.....
Oirr A2 (Kar)	.C.....A..T.....C.A..A..C... .....A.....T..TC.....
Oirr B (Ali)	.....T.....T.....C.A..A..C... .....A.....T..TC.....
Oirr B (Bai)	.C.....T.....T.....C.A..A..C... .....A.....T..TC.....
Oirr B (Gro)	.....T.....T.....C.A..A..C... .....A.....T..TC.....
Oirr C (Alg)	.....A..T.....T.....C.A..A..C... .....A.....T..TC.....
Oirr C (Twe)	.....A..T.....T.....C.A..A..C... .....A.....T..TC.....
Olam	.....G.....T.....C.A..A..C... ..T..A.....TC....G..
Omax	.C.....A..T.....G..T...C.A..A..C..T .....A.....T..T.....
Oocc	.C.....A..A.....T.....C.A..A..C... ..T..A.....T..T.....
Oslog	.....A.....A..A..C..T ..T..A..T..T..T..T.....
Otro	.C.....A.....T.....T..TC.A..A..C... .....A.....T..T.....T..
Otyp	.....A.....TC.A..A..C... .....A.....T..T.....
Otjac (Aru)	.....T.....T...C.A..A..C..T ..T..A.....T..T.....
Otjac (Run)	.....T.....T...C.A..A..C..T ..T..A.....T..T.....
Ouni	.....A.....T.....T..TC.A..T..C... .....A.....T..T.....C..
Pbra (Kal)	.C.....A.....T.....T...C.A..A..C... .....A..T.....T..T.....
Pbra (Kle)	.C.....A.....T.....C.A..A..C... .....A..T.....T..T.....
Plit (Goe)	.....A.....T.....A..A..C... ..T..A..T.....C.....
Plit (Hen)	.....A.....T.....A..A..C... ..T..A..T.....C.....
	500 510 520 530 540 550 560

Membrane domain	← Q Q_1 →
Mmus	TATTATCGCGGCCCTAGCAATCGTTCACCTCCTCTTCCTCCACGAAACAGGATCA AACAACCCAACAGGA
Achr	C.....C.....A..A..T..A..T.....C... ..T..T.....
Oalac (Uzu)	.....A.A.....T...T..C.....AT.A..T..A.....G.....C... .....C.....
Oalac (Chi)	C.....TA.A.....TG..T..C.....AT.A.....A.....C... .....C.....
Oalac (Mbi)	C.....TA.A.....TG..T..C.....AT.A.....A.....C... .....C.....
Oang	...C...A.A...T...T.....C..T..A..A.....C... .....T.....
Oden (Mga)	C.....TT.A.....T..C.....A..A.....T.....C... .....T.....
Oden (Buj)	C.....TT.A.....T.....A..A.....C... .....T.....
Oirr A1 (Hog)	.....TA.A...T...T.....C.....A..A.....A.....C... .....T.....
Oirr A1 (Kam)	.....A.A...T...T.....C.....A..A.....A.....C... .....T.....
Oirr A2 (Kar)	.....TA.A.....T.....C.....A..A.....A.....C... ..T.....T.....
Oirr B (Ali)	...C..TA.A...T...T.....C..T..A..A.....A.....C... .....T..C.....
Oirr B (Bai)	...C..TA.A...T...T.....C..T..A..A.....A.....C... .....T..C.....
Oirr B (Gro)	...C..TA.A...T...T.....C..T..A..A.....A.....C... .....T..C.....
Oirr C (Alg)	...C..TA.A...T...T.....C.....A..A.....A.....C... .....T..C.....
Oirr C (Twe)	...C..TA.A...T...T.....C..T..A..A.....A.....C... .....T..C.....
Olam	C.....TA.A.....T.....T.AT.A..T..A..... .....T..G...
Omax	...C...A.A..TT...T.....C..T..A..A.....C... .....T.....
Oocc	...C...A.A...T...T.....C.....AT.A..T..A..T.....C... .....T.....
Oslog	...C...A.A.....T.....T.AT.A.....A..T.....C... ..T.....C.....
Otro	.....TA.A.....T...T..C.....AT.A.....A.....C... .....C.....
Otyp	...C...A.A.....T...T..C.....A.....A.....C... .....T.....
Otjac (Aru)	.....TA.A...T...T.....A..A.....C... ..T.....C.....
Otjac (Run)	...C..TA.A...T...T.....AT.A..T.....C... .....T.....
Ouni	C..C..TA.A.....TC.....A..A...T.A.....C... .....C.....
Pbra (Kal)	.....TA.A.....T.....C.....T..A..T..A.....G.....C... ..T.....C.....
Pbra (Kle)	.....TA.A.....T.....C.....T..A.....A.....G.....C... ..T.....C.....
Plit (Goe)	C.....TA.A..TT...T...T.....AT.A..T..T..T.....C... ..T.....T.....
Plit (Hen)	C.....TA.A..TT...T...T.....T..AT.A..T..T.....C... ..T.....T.....
	570 580 590 600 610 620 630

Continued/


Membrane domain
← Ω_i Ω →
Mmus TTAAACTCAGATGCAGATAAAATTCCATTTCACCCCTACTATACAATCAAA GATATCCTAGGTATCCTAA
Achr C.T.....C.....C.....C..C..C.....T..... .....A..TA...
Oalac (Uzu) C.....C..G..C.....C.....A.....C..T..... ..C.....G....C.
Oalac (Chi) C.....C..G..C.....C.....A..T..C..T..... .....CC....C.
Oalac (Mbi) C.....C..G..C.....C.....A..T..C..T..... .....CC....C.
Oang C....T.....C.....C.....A..T..C..C..T... ..C...T.....G.AT.C.
Oden (Mga) .....T.....C.....C.....A..T.....T..... ..C.....AG....T.
Oden (Buj) .....T.....C.....C.....A..T.....T..... ..C.....AG....T.
Oirr A1 (Hog) C....T.....C.....C..T..A.....C..T..G ..C..T.....CC.....
Oirr A1 (Kam) C....T.....C.....C..T..A.....C..T..G ..C..T.....CC.....
Oirr A2 (Kar) C....T.....C.....C.....C..T..A.....C..T..G ..C..T.....CC.T..C.
Oirr B (Ali) C.....C.....C..T..A.....C..T... ..C..TT....CC.T..C.
Oirr B (Bai) C.....C.....C..T..A.....C..T... .....T.....CC.T..C.
Oirr B (Gro) C.....C.....C..T..A.....C..T... ..C..TT....CC.T..C.
Oirr C (Alg) C.....C.....C..T..A.....C..T... .....TT....CC.T..C.
Oirr C (Twe) C.....C.....C..T..A.....C..T... .....TT....CC.T..C.
Olam C....T.....C.....C.....C..T..A.....T..... .....TT....CC.T..C.
Omax C....T.....C.....C.....C.....A..T..C..C..T... ..C...T.....G.AT.C.
Oocc C...C.....C.....C.....??????????????????????????... ..C.....CG.A..T.
Oslog C.....G..C.....C.....T..A..T.....T..T... .....T.....G.TT.C.
Otro C.....C..G..C.....C.....A..T.....C..... .....G.T..C.
Otyp C..G.T.....C.....C.....C.....C.....A...C.C..C..T... .....T.....G.AT.C.
Otjac (Aru) C....T.....C.....C.....A..T..C..C..T... .....CG.AT.C.
Otjac (Run) C....T.....C.....C.....A.....C..C..T... .....G.AT.C.
Ouni C.....C.....C.....C.....T..A.....C..T..... ..C.....A..C.
Pbra (Kal) C.....C.....C.....T..T.....TG..... ..C.....C..T..T.
Pbra (Kle) C.....C.....C.....C.....T..T.....TG..... ..C.....G..C..T..T.
Plit (Goe) C..G.....A.....C.....C..T..A.....C..C..... .....T.....C.....T.
Plit (Hen) C..G.....A.....C.....T..A.....C..C..... .....T.....CG....T.
640 650 660 670 680 690 700


Membrane domain
← Ω Ω_o →
Mmus TCATATTCTTAATTCTCATAACCCTAGTATTATTTTTCCCAGACATA CTAGGAGACCCAGACAACTACAT
Achr .T...C.TC..C.CT.A....TTT.....T..C...T.. .....T.....T.....
Oalac (Uzu) .....A.TG...CC..A....TT.....C....C..T.....TGC. C
Oalac (Chi) .....A.TG...CC..A....TT.....C.....T.....GC. C
Oalac (Mbi) .....A.TA.TC.A..A..G..T.....C.T..C..T.....TGC. .....T.C
Oang .....A..A.C.CC..A.....T.....C....C.....TGC. .....T.....T.C
Oden (Mga) .....A..ACT.CC..A.....T.....C....C.....TGC. ..G.....T.....T.C
Oden (Buj) .....A.TG.CC.A..A.....T.....C.T..C.....TGC. .....T.....T.....C
Oirr A1 (Hog) .....A.TG.CC.A..A.....T.....C.T..C.....TGC. .....T.....T..T....C
Oirr A1 (Kam) .....A..G.CC.A..A.....T.....C.T..C.....GC. .....T..T..T.C
Oirr A2 (Kar) .....A.TG.TC.A..A....TT.....C.T..C.....GC. .....T.....T.C
Oirr B (Ali) .....A.TG.TC.A..A....TT.....C.T..C.....GC. .....T.....T.C
Oirr B (Bai) .A...A.TG.TC.A..A....TT.....C.T..C.....GC. .....T.....T.C
Oirr B (Gro) .....A.TG.TC.A..A.....T.....C.T..C.....GC. .....T.....T.C
Oirr C (Alg) .....A.TG.TC.A..A.....T.....C.T..C.....GC. .....T.....T.C
Oirr C (Twe) .T...A..G.CC.A..A....TT.....C.C..C.....TGC. C
Olam .....A.TA.TC.A..A....TT.....C.T.....T.....TGC. .....T.C
Omax .....A.TA..T.A..A.....C....C.....TGC. .....T.....???
Oocc .T...A.TA.CT.A..AG.....C.....T.....TGCC .....T.....T.C
Oslog .....A.TG..GCC..T....TT.....C....C..T.....TGC. .....T.....C
Otro .T...A.TA.TC..T.A....TTA.....C.T..C.....GC. C
Otyp .T...A.TACTT.A..A....TT.....C.T.....TGC. .....T.....T.C
Otjac (Aru) .T...A.TA.TT.A..A....TT.....C.T.....TGC. .....T.....T.C
Otjac (Run) .....A.TA.TC.A..A....TT.....CC.....GC. C
Ouni .....A.TG.TC.A.....T.....C....C.....GC. C
Pbra (Kal) .....A.TG.TC.A.....T.....C....C.....GC. C
Pbra (Kle) .....A.TG..C.A..A.....T.....C.....C.....TGC. .....T..T.C
Plit (Goe) .....A.TG..C.A..A...G.T.....TC.....TGC. .....T..T.C
Plit (Hen) 710 720 730 740 750 760 770

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Membrane domain
Mmus          ACCAGCTAATCCACTAAACACCCCACCCCATATTAAACCCGAATGATATTTCCTATTTGCATACGCCATT
Achr          G.....C..C.....C.........T..A..C..C.....A...........T.........T.........C
Oalac (Uzu)   ...C.....C.....T.....T..C..A..C..C.....A.........C...T....C..C.....T..C
Oalac (Chi)   ...C..C..C.....C.....T..C..A..C..C..G..A.........C..TT.......C.....T...
Oalac (Mbi)   ...C..C..C.....C.....T..C..A..C..C..G..A.........C..TT.......C.....T...
Oang          ...C..C..C.....T.....T..C..A.....C.....A............T....C..C.....T..C
Oden (Mga)    ...C..C.........T.....T..C..A..C..C.....A...........T.........C.....T...
Oden (Buj)    ...C..C.........T.....T..C..A..C..C.....A...........T.....C..C.....T...
Oirr A1 (Hog) ...T..C..C.....C..T..T..C..A..C..C.....A.........C...........C.....T...
Oirr A1 (Kam) ...T..C..C.....C..T..T..C..A..C..C..G..A.........C...........T.....T...
Oirr A2 (Kar) ...T..C..C.....C..T.....T..A..C.....G..A...................C..C.....T...
Oirr B (Ali)  ...T..C..C.....C..T..T..C..A.....C.....A.........C...........C..T..T...
Oirr B (Bai)  ...T..C..C.....C..T..T..C..A.....C.....A.........C.........C..C..T..T...
Oirr B (Gro)  ...T..C..C.....C..T..T..C..A.....C.....A.........C...........C..T..T...
Oirr C (Alg)  ...T..C..C.....C..T..T..T..A.....C.....A.........C...........C..T..T...
Oirr C (Twe)  ...T..C..C.....C..T..T..T..A.....C.....A.........C...........C..T..T...
Olam          ...C.....C.....T.....T..C..A..C..C.....A.....................C.....T...
Omax          ...C..C..C.....T.....T..C..A.....C.....A............T....C..C.....T..C
Oocc          ??????????????????????????????????????????????????????????????????????
Oslog         ...C.....C.....T..T.....T..A..C..C.....A...........T.........C.........C
Otro          ...C.....C..G..T..T..T..C..A..C........A.........C...T....C..C.....T...
Otyp          ...C.....C.....C.....T..C..A..C..C..G..A.........C...........C..T..T..C
Otjac (Aru)   ...C..C..C.....C..T..T..C..A........G..A...........T.........C.....T..C
Otjac (Run)   ...C..C..C.....C.....T..C..A..C..C.....A...........T.........C.....T..C
Ouni          ...C...........C.....T..T..A..C..C.....A.........C...........C..T..T..C
Pbra (Kal)    ...C..C..C..T..C.....T..T..A..C..C..G..A.........C...........C.....T..C
Pbra (Kle)    ...C.....C..G..C.....T..T..A..C..C..G..A.........C...........C.....T..C
Plit (Goe)    ...T..C.........T..T..T..T..A.....C..G..A.....................T.........
Plit (Hen)    ...C..C.........T..T..T..T..A.....C..G..A.....................T.........
                          780       790       800       810       820       830       840
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Membrane domain                    ← Q₀  Q →
Mmus          CTACGCTCAATCCCCAAT AAACTAGGAGGTGTCCTAGCCTTAATCTTATCTATCCTAATTTTAGCCCTAA
Achr          .........C.....T... ..............A....T...C....T.....C..T..T..CC.....T.CC
Oalac (Uzu)   ..T.....C.....T.... .....T.....A.........C....TC.T..C...T.............C
Oalac (Chi)   ..T.....C.....T..C .....T.....A..T......C.....C.T..C...T....C.........C
Oalac (Mbi)   ..T.....C.....T..C .....T.....A..T......C.....C.T..C...T....C.........C
Oang          ..T..T..T..T..A... ...........A...T...........C.........T..............C
Oden (Mga)    ..C.....C..T...... .....T.....A...T.......G..T.........T........G......C
Oden (Buj)    ..C.....C..T...... .....T.....A...T.......G..T.........T....C..G......C
Oirr A1 (Hog) ..T.....C..T..T... ..G..G.....A..............C.............C.....T...C
Oirr A1 (Kam) ..T.....T..T..T... ..G..G.....A..............C.............C.....T...C
Oirr A2 (Kar) ..T.....T..T..T... .....G.....A..............C.............CC....T...C
Oirr B (Ali)  ..T.....T..T...... .....G.....A..............C....C...T....C.........T
Oirr B (Bai)  ..T.....T..T...... .....G.....A..............C....C.........C.........T
Oirr B (Gro)  ..T.....T..T...... .....G.....A..............C....C...T....C.........T
Oirr C (Alg)  ..T.....T..T...... .....A.....A..............C....C...T....C.........C
Oirr C (Twe)  ..T.....T..T...... .....G.....A.........T.....C....C...T....C.........C
Olam          ..C.....C..T..T..C ...........A.........C.....C.............C.........T
Omax          ..C..T..T..T..A... ..G.........A...T...........C.........T..............C
Oocc          ?????????????????? ??????????????????????????????????????????????????
Oslog         ..T.....C..T..T..C ...........A..............TA..............C.G..TT.TC
Otro          ..T.....C.....T... .....T.....A.........C....TC.C..C...T....C.........C
Otyp          ..T.....T..T..A... ...........A.........C.....C....C...T....C.........CT
Otjac (Aru)   ..T.....C..T..A... ...........A...T...........C.........T.....C.........C
Otjac (Run)   ..C.....T..T..A... ...........A...T...........C..............C....T...C
Ouni          ........C..T.....C ...........A..............TA....C.........CC........C
Pbra (Kal)    ..T.....C..T...... ...........A..............T.........TT.....C........C
Pbra (Kle)    ..T.....C..T...... ...........A..............T.........TT.............C
Plit (Goe)    ..C.....C..T..T... ...........G.....G.........C.........T..G.C.........T
Plit (Hen)    ..C.....C..T..T... ...........A.....G.........C....C...T..G.C.........T
                          850       860       870       880       890       900       910
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Continued/

Membrane domain	← Q	Q_i →	← Q_i	Q →			
Mmus	TACCTTTCCTTCATACC	TCAAAGCAACGAAGCCTAATATTCCGCCCA		ATCACACAAATTTTGTACTGAAT			
Achr	....C.....C...	.....A.....C.....G.C.....		.....C....CCC.A.....C.			
Oalac (Uzu)	....C.....C..C..T	.....A.....C.....T..T.....		..T.....CCC.T.....G.			
Oalac (Chi)	....C.....C..C..T	.....A.....C.....T..T.....		..T.....CCC.C..T.....			
Oalac (Mbi)	....C.....C..C..T	.....A.....C.....T..T.....		..T.....CCC.C..T.....			
Oang	....C..T..C..C...	.....A.....T.....T..T..T..T...		.....CCC.C.....			
Oden (Mga)	....C..T.....C...	.....A.....C.....C..T.....		.....CCC.C.....			
Oden (Buj)	....C..T.....C...	.....A.....C.....T..T.....		.....GCCC.C.....			
Oirr A1 (Hog)	....C.....C.....	.....A.....T.....T..T..T.....		..T.....TCCC.C.....			
Oirr A1 (Kam)	.G..C.....C.....	.....A.....T.....T..T..T.....		..T.....TCCC.C.....			
Oirr A2 (Kar)	....C..T..C.....	.....A.....T.....T..T..T.....		..T.....TCCC.C.....			
Oirr B (Ali)	....C..T..C.....T	.....A.....T.....T..T..T.....		..T.....TCCC.C.....			
Oirr B (Bai)	....C..T..C.....T	.....A.....T.....T..T..T.....		..T.....TCCC.C.....			
Oirr B (Gro)	....C..T..C.....T	.....A.....T.....T..T..T.....		..T.....TCCC.C.....			
Oirr C (Alg)	....C..T..C..C..T	.....A.....T.....T..T..T.....		..T.....TCCC.C.....			
Oirr C (Twe)	....C..T..C.....	.....A.....C.....T..T..T.....		..T.....TCCC.C.....			
Olam	....C.....C...	.....A..G..T..T..T..T..T.....		..T.....CCC.C..T.....			
Omax	....CC.T..C..C...	.....A.....T.....T..T..T..T...		..T.....CCC.C.....			
Oocc	????????????????	????????????????????????????		??.....GCCC.T..T.....			
Oslog	....C..T.....	.....T.....T..T.....		..T.....C.C.C.....			
Otro	....A.....C..C...	.....A.....C.....T..C.....		..T.....GCCC.C..T.....			
Otyp	....C.....T	.....A.....T.....T..T.....		.....CCC.C.....			
Otjac (Aru)	....CC.T..C..C...	.....A.....T.....T..C.....T...		.....CCC.C.....			
Otjac (Run)	....CC.T..C..C...	.....A.....T.....T..C.....T...		.....CCC.C.....			
Ouni	....C..T.....C...	.....A.....C.....C..T.....T...		.....CCC.C.....			
Pbra (Kal)	.G..CC.A.....C...	.....A.....T.....T.....T..T		.....CCC.T.....			
Pbra (Kle)	.G..CC.A.....C...	.....A.....T.....T..T.....T..T		.....CCC.T.....			
Plit (Goe)	....C..TT....C..T	.....A.....T..T.....		..T.....CCC.C..T.....			
Plit (Hen)	....C..TT....C..T	.....A.....T..T.....		..T.....CCC.C..T.....			
	920	930	940	950	960	970	980

Membrane domain	← Q	Q_o →					
Mmus	CCTAGTAGCCAACCTACTTATCTTAACCTGAATTGGGGGCCAACCA			GTAGAACACCCATTTATTATCATT			
Achr	T.....A..T.....C...C....T.....C..A..A.....C			.....C..C..T..C			
Oalac (Uzu)	.....A.....C....T.....C..A..A.....C			.....T..C			
Oalac (Chi)	.....A.....C..TC.....A..A.....T			C			
Oalac (Mbi)	.....A.....C..TC.....A..A.....T			C			
Oang	T.....A.....T.A...C....T.....A..A..G..C			.....T.....			
Oden (Mga)	.....A.....T..CG..C.....A..A.....T			.....C.....			
Oden (Buj)	.....A.....T..CG..C....T.....C..A..A.....T			.....C..C.....			
Oirr A1 (Hog)	.....A..T.....TC....T.....C..A..A.....C			.....T...			
Oirr A1 (Kam)	T.....A..T.....TC....T.....C..A..A.....C			.....T...			
Oirr A2 (Kar)	T.....A..T.....C....T.....C..A..G.....C			.....C.....T...			
Oirr B (Ali)	T.....A.....C...C....T.....C..A.....			.....T...			
Oirr B (Bai)	T.....A.....C...C....T.....C..A..T.....			.....T...			
Oirr B (Gro)	T.....A.....C...C....T.....C..A.....			.....T...			
Oirr C (Alg)	T.....A.....C...C....T.....C..A.....			.....T...			
Oirr C (Twe)	T.....A.....C...C....T.....C..A.....			.....T...			
Olam	.....A.....C.C..T.....C..A..A.....C			.....C..T...			
Omax	T.....A.....T.A...C....T.....A..A..G..C			..G.....T.....T...			
Oocc	.....A.....A...C....T.....A..A.....C			.....AC.....T...			
Oslog	TT.....G..C....T.....C..A.....C			.....T.....C.....T...			
Otro	.....A.....C...C....T.....C..A..A.....T			.....C.....T...			
Otyp	T.....A.....C.....C..A..A.....C			.....T.....A...			
Otjac (Aru)	T....C...A.....T.A..TC....T.....A..A.....C			.....T.....T...			
Otjac (Run)	.....A.....A...C....T.....C..A.....C			.....T..C			
Ouni	T.....A..T.....C.....A..A.....T			.....T....AC.....T...			
Pbra (Kal)	T....C...A.....C....T.....A..A.....T			C			
Pbra (Kle)	T.....A.....C....T.....A..A.....T			C			
Plit (Goe)	.....A..T.....G..C....T.....A..A.....T			.....G.A.....T...			
Plit (Hen)	T.....A..T.....G..C.G..T.....A..G.....T			.....G.A.....T...			
	990	1000	1010	1020	1030	1040	1050

Continued/

Membrane domain ← Q_i Q →							← Q Qi →	
Mmus	GGC CAACTAGCCTCCATCTCATACTTCTCAATCATCTTAATTCTTATACCAATCTCAGGAATT							ATCGAAG
Achr	... .A.....TAGT.....C..T...C.TG....A.....T..A							.....A
Oalac (Uzu)	..A .....T.....TAGC.....C..T..TC.C..C..A.....							.....
Oalac (Chi)	..A .....G.....TAGC.....C..T..TC.T..C..A.....							.....
Oalac (Mbi)	..A .....G.....TAGC.....C..T..TC.T..C..A.....							.....
Oang	... ...T.....AGC.....T..T..TC.T..C..C.....T.....							.....
Oden (Mga)	..A ...T.....TAGC.....C..T..TC.T..CT.A.....T.....							.....
Oden (Buj)	..A ...T.....AGC..T.....C..T..TC.T..CT.A.....T.....							.....
Oirr A1 (Hog)	..A ...T.....AGC.....C.....TC.T.....							.....
Oirr A1 (Kam)	..A ...T.....AGC.....C.....TC.T.....							.....
Oirr A2 (Kar)	... ...T....T.....AGC.....C.....C.T.....C.....							.....
Oirr B (Ali)	..A ...T.....AGC.....C.....TC.T..C.....							.....
Oirr B (Bai)	..A ...T.....AGC.....C.....TC.T..C.....							.....
Oirr B (Gro)	..A ...T.....AGC.....C.....TC.T..C.....							.....
Oirr C (Alg)	..A ...T.....AGC.....C.....TC.T..C.....T...							.....
Oirr C (Twe)	..A ...T.....AGC.....C.....TC.T..C.....							.....
Olam	..G .....TAGC.....C.....C.C..C.....T.....							.....
Omax	..A ...T.....AGC.....T..T...C.T..C.....T.....							.....
Oocc	..A .....T.....AGC.....C..T..TC.T..C..A.....T.....C...							.....G.
Oslog	..G ...T....T.....TAGC.....C..T..TC.T.....A.....C..C							..T....
Otro	..A ...T.....TAGT.....C..T..TC.T.....A.....							.....
Otyp	..A ...T....T.....AGC.....C..T..TC.T..C.....T.....							.....
Otjac (Aru)	..A ...T....T.....AGC.....T.....TC.T..C.....G.....							.....
Otjac (Run)	..A ...T....T.....AGC.....C..T..TC.C..C.....							.....
Ouni	..A .....T...AGC.....C..T...C.C.....A..C.....T.....C							.....
Pbra (Kal)	..A .....T.....TAGC.....T..T...C.T..C..A.....							.....
Pbra (Kle)	..A .....TAGC.....T..T...C.T..CT.A.....							.....
Plit (Goe)	..A ...T.....AGC..T.....T..T..TC.T...T.A.....C							.....G.
Plit (Hen)	..A ...T.....AGC..T.....C..T..TC.C...T.A.....C							.....G.
		1060	1070	1080	1090	1100	1110	1120

Membrane domain	← Q_i		
Mmus	ACAAAATACTAAAATTATATCCA		
Achr	.T.....G.A.C???		
Oalac (Uzu)	.....C.....G.AG.T..		
Oalac (Chi)	.....G.AG.T..		
Oalac (Mbi)	.....G.AG.T..		
Oang	....TC.T.....G.AGCT..		
Oden (Mga)	.....C.....G.AG.TT.		
Oden (Buj)	.....C.....G.AG.T..		
Oirr A1 (Hog)	.....C.....G.AG.T..		
Oirr A1 (Kam)	.....C.....G.AG.T..		
Oirr A2 (Kar)	.T...C.....G.AG.T..		
Oirr B (Ali)	.....C.....G.A.CT..		
Oirr B (Bai)	.T...C.....G.A..T..		
Oirr B (Gro)	.....C.....G.A..T..		
Oirr C (Alg)	.T...C.....G.AG.T..		
Oirr C (Twe)	.T...C.....G.AG.T..		
Olam	.....C..T.....G.AG.T..		
Omax	....TC.T.....G.AGCT..		
Oocc	.....C.T.....G.AG.T..		
Oslog	.....C.....G.A.CT..		
Otro	.T...C.....G.AG.T..		
Otyp	.....C.....G.AG.T..		
Otjac (Aru)	.T..TC.T.....G.AGCT..		
Otjac (Run)	....TC.T.....G.AGCT..		
Ouni	.T...C.....G.AG..T.		
Pbra (Kal)	.....C.....G.AG.T..		
Pbra (Kle)	.....C.....G.AG.T..		
Plit (Goe)	.T...C.....G.AG.TT.		
Plit (Hen)	.T...C.....G.AG.TT.		
	1130	1140	

APPENDIX II

Alignment of the small ribosomal subunit (12S rRNA) sequences from 22 ingroup taxa (see Table 2) and outgroups *Aethomys namaquensis* and *Mus musculus* (Bibb *et al.* 1981) included in this study. The 12S rRNA secondary structure model for *Mus* (Damberger & Gutell unpubl. in Sullivan *et al.* 1995) was used as a guideline to discriminate between stem and loop regions (Watson-Crick and U:G-type base pairing were allowed). Since no stem numbers were given in the *Mus* model, the numbering (1 and 1' etc.) largely corresponds to the core mammalian model illustrated for *Bos taurus* (Springer *et al.* 1995 following Springer & Douzery 1996). Regions of sequence shown in bold show where the *Mus* model proposes a different secondary structure than that of the mammalian model. Additional stems, or stems not relatable to numbers in the mammalian model are indicated a and a' etc. Nucleotide positions where compensatory base pairing was evident are underlined. Positions where pairing was non-complementary are italicized. Lower case bases within stems indicate bulges. Tertiary interactions are denoted in upper case. Alignment gaps are indicated by dashes. Gaps were coded as present (1) or absent (0) for the parsimony analyses (see text) and are given at the end of the data matrix. The cytotypes of *O. irroratus* (A1, A2, B and C) are given after the species name. Abbreviations: *Mmus - Mus musculus, Anam - Aethomys namaquensis, Oalac - O. anchietae lacustris, Oang - O. angoniensis, Oden - O. denti, Oirr - O. irroratus, Olam - O. laminatus, Omax - O. maximus, Oslo - O. sloggetti, Otro - O. tropicalis, Otyp - O. typus, Otjac - O. typus jacksoni, Ouni - O. unisulcatus, Pbra - P. brantsii, Plit - P. littledalei*. The abbreviated collection localities for taxa represented by more than one specimen are given in brackets: Uzu = Uzungwa Mountains, Chi = Chingombe, Mbi = Mbizi, Mga = Mgahinga, Buj = Bujuku River, Hog = Hogsback, Kar = Karkloof, Ali = Alice, Bai = Baines Kloof, Gro = Groendal, Alg = Algeria, Aru = Arusha, Run = Mt. Rungwe, Kal = Kalagadi, Goe = Goegap.

Stem number	**3**	**4**	**5**	**a**	**a'**	**6**	**7**	**8**
Mmus	TT A	GAGGTAAA	ATTA CACaTGC	AA A	CCTCCA T	AGACCG	GTG	TAAAA TCCCT TAAAC ATTT
Anam	TT A	GAGGTAAG	ATTA CACaTGC	AA A	TCTCCA T	ACGCCG	GTG	TAAAA TCCCT TAAAC ATTT
Oalac (Uzu)	TT A	GAGGTAAG	ATTA CACaTGC	AA A	TTTCCA T	ACCCCG	GTG	TAAAA TCCCT TAAAC ATTT
Oalac (Chi)	TT A	GAGGTAAG	ATTA CACaTGC	AA A	TTTCCA T	ATTCCG	GTG	TAAAA TCCCT TAAAC ATTT
Oalac (Mbi)	TT A	GAGGTAAG	ATTA CACaTGC	AA A	TTTCCA T	ATTCCG	GTG	TAAAA TCCCT TAAAC ATTT
Oang	TT G	GAGGTAAG	ATTA CACaTGC	AA A	TTTCCA T	ATTCCG	GTG	TAAAA TCCCT TAAAC ATTT
Oden (Mga)	TT G	GAGGTAAG	ATTA CACaTGC	AA A	TTTCCA T	AGCCCG	GTG	TAAAA TCCCT TAAAC ATTT
Oden (Buj)	TT G	GAGGTAAG	ATTA CACaTGC	AA A	TTTCCA T	AGCCCG	GTG	TAAAA TCCCT TAAAC ATTT
Oirr A1 (Hog)	TT G	GAGGTAAG	ATTA CACaTGC	AA A	TTTCCA T	ACACCG	GTG	TAAAA TCCCT TAAAC ATTT
Oirr A2 (Kar)	TT G	GAGGTAAG	ATTA CACaTGC	AA A	TTTCCA T	GCACCG	GTG	TAAAA TCCCT TAAAC ATTT
Oirr B (Ali)	TT G	GAGGTAAG	ATTA CACaTGC	AA A	TTTCCA T	ACACCG	GTG	TAAAA TCCCT TAAAT ATTT
Oirr B (Bai)	TT G	GAGGTAAG	ATTA CACaTGC	AA A	TTTCCA T	ACACCG	GTG	TAAAA TCCCT TAAAT ATTT
Oirr B (Gro)	TT G	GAGGTAAG	ATTA CACaTGC	AA A	TTTCCA T	ACACCG	GTG	TAAAA TCCCT TAAAT ATTT
Oirr C (Alg)	TT G	GAGGTAAG	ATTA CACaTGC	AA A	TTTCCA T	ACACCG	GTG	TAAAA TCCCT TAAAT ATTT
Olam	TT G	GAGGTAAG	ATTA CACaTGC	AA A	TTTCCA T	ACCCCG	GTG	TAAAA TCCCT TAAAC ATTT
Omax	TT G	GAGGTAAG	ATTA CACaTGC	AA A	TTTCCA T	ATTCCG	GTG	TAAAA TCCCT TAAAC ATTT
Oslo	TT G	GAGGTAAG	ATTA CACaTGC	AA A	TTTCCA T	ATTCCG	GTG	TAAAA TCCCT TAAAC ATTT
Otro	TT A	GAGGTAAG	ATTA CACaTGC	AA A	TTTCCA T	ACCCCG	GTG	TAAAA TCCCT TAAAC ATTT
Otyp	TT A	GAGGTAAG	ATTA CACaTGC	AA A	TTTCCA T	ACCCCG	GTG	TAAAA TCCCT TAAAC ATTT
Otjac (Aru)	TT G	GAGGTAAG	ATTA CACaTGC	AA A	TTTCCA T	ATTCCG	GTG	TAAAA TCCCT TAAAC ATTT
Otjac (Run)	TT G	GAGGTAAG	ATTA CACaTGC	AA A	TTTCCA T	ATTCCG	GTG	TAAAA TCCCT TAAAC ATTT
Ouni	TT G	GAGGTAAG	ATTA CACaTGC	AA A	TCTCCA T	GCTCCG	GTG	TAAAA TCCCT TAAAC ATTT
Pbra (Kal)	TT G	GAGGTAGG	ATTA CACaTGC	AA A	TTTCCA T	GTTCCG	GTG	TAAAA TCCCT TAAAT ATTT
Plit (Goe)	TT G	GAGGTAAG	ATTA CACaTGC	AA A	TTTCCA T	ATTCCG	GTG	TAAAA TCCCT TAAAT ATTT
Nucleotide		**10**	**20**	**30**		**40**	**50**	**60**

Continued/

Stem number		8'		7'		9		10		10'		9'	b	
Mmus	ACTTA--	**AAAT**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	TAAA	ATaGCT	TAAGAC	ACC	TT	**GC** CTA
Anam	AATCA--	**AATC**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	TCA-	ATaGCT	CAAGAC	ACC	TT	**GC** CTA
Oalac (Uzu)	ACCCA--	**AAAT**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	TAA-	ATaGCT	TAAGAC	ACC	TT	**GC** CTA
Oalac (Chi)	ACTCA--	**AAAT**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	TAA-	ATaGCT	CAAGAC	ACC	TT	**GC** CTA
Oalac (Mbi)	ACTCA--	**AAAT**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	TAA-	ATaGCT	CAAGAC	ACC	TT	**GC** CTA
Oang	ACCCAA-	**AAGT**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	TAA-	ATaGCT	TAAGAC	ACC	TT	**GC** CTA
Oden (Mga)	ATTTA--	**AAGT**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	TAA-	ATaGCT	TAAGAC	ACC	TT	**GC** CTA
Oden (Buj)	ATTCA--	**AAAT**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	TAA-	ATaGCT	TAAGAC	ACC	TT	**GC** CTA
Oirr A1 (Hog)	ACTTA--	**AAAT**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	TAA-	ATaGCT	TAAGAC	ACC	TT	**GC** CTA
Oirr A2 (Kar)	ACTCAA-	**AAAT**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	TAA-	ATaGCT	TAAGAC	ACC	TT	**GC** CTA
Oirr B (Ali)	ATTCAA-	**AAAT**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	TAA-	ATaGCT	TAAGAC	ACC	TT	**GC** CTA
Oirr B (Bai)	ATTCAA-	**AAAT**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	TAA-	ATaGCT	TAAGAC	ACC	TT	**GC** CTA
Oirr B (Gro)	ATTCAA-	**AAAT**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	TAA-	ATaGCT	TAAGAC	ACC	TT	**GC** CTA
Oirr C (Alg)	ATTCAA-	**AAAT**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	TAA-	ATaGCT	TAAGAC	ACC	TT	**GC** CTA
Olam	ATTCA--	**AAAT**	TTA	AGGaGA	A	GGT	ATCA	AGCacAT	TAA-	ATaGCT	TAAGAC	ACC	TT	**GC** CTA
Omax	ACCCAA-	**AAAT**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	TAA-	ATaGCT	TAAGAC	ACC	TT	**GC** CTA
Oslo	ACTCA--	**AAAT**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	AAA-	ATaGCT	TAAGAC	ACC	TT	**GC** CTA
Otro	ACTCA--	**AAAT**	TTA	AGGaGA	A	GGT	ATCA	AGCacAT	TAA-	ATaGCT	TAAGAC	ACC	TT	**GC** CTA
Otyp	ACTCAA-	**AAAT**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	TAA-	ATaGCT	TAAGAC	ACC	TT	**GC** CTA
Otjac (Aru)	ACTCAA-	**AAGT**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	TAA-	ATaGCT	TAAGAC	ACC	TT	**GC** CTA
Otjac (Run)	GCCCAA-	**AAGC**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	TAA-	ATaGCT	TAAGAC	ACC	TT	**GC** CTA
Ouni	ACTCA--	**AAAT**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	TAA-	ATaGCT	CAAGAC	ACC	TT	**GC** CTA
Pbra (Kal)	TTTTTTC	**AAAT**	TTA	AGGaGA	A	GAT	ATCA	AGCacAT	AAA-	ATaGCT	TAAGAC	ATC	TT	**GC** CTA
Plit (Goe)	ACTTA--	**AAAT**	TTA	AGGaGA	G	GGT	ATCA	AGCacAT	TAA-	ATaGCT	TAAGAC	ACC	TT	**GC** CTA
Nucleotide		70		80		90		100		110		120		

Stem number	b'	6'		11		11'		5'		12		13		13'		12'
Mmus	**GC**	CAC	ACC	CC	CACG	GG	ACTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Anam	**GC**	CAC	GCC	CC	CACG	GG	ACTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Oalac (Uzu)	**GC**	CAC	ACC	CC	CACG	GG	ATTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Oalac (Chi)	**GC**	CAC	ACC	CC	CACG	GG	ATTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Oalac (Mbi)	**GC**	CAC	ACC	CC	CACG	GG	ATTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Oang	**GC**	CAC	ACC	CC	CACG	GG	ACTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Oden (Mga)	**GC**	CAC	ACC	CC	CACG	GG	ACTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Oden (Buj)	**GC**	CAC	ACC	CC	CACG	GG	ACTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Oirr A1 (Hog)	**GC**	CAC	ACC	CC	CACG	GG	ACTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Oirr A2 (Kar)	**GC**	CAC	ACC	CC	CACG	GG	ACTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Oirr B (Ali)	**GC**	CAC	ACC	CC	CACG	GG	ACTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Oirr B (Bai)	**GC**	CAC	ACC	CC	CACG	GG	ACTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Oirr B (Gro)	**GC**	CAC	ACC	CC	CACG	GG	ACTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Oirr C (Alg)	**GC**	CAC	ACC	CC	CACG	GG	ACTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Olam	**GC**	CAC	ACC	CC	CACG	GG	ACTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Omax	**GC**	CAC	ACC	CC	CACG	GG	ACTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Oslo	**GC**	CAC	ACC	CC	CACG	GG	ACTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Otro	**GC**	CAC	ACC	CC	TACG	GG	ATTCA	GCAGTG	ATAAGTA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Otyp	**GC**	CAC	ACC	CC	CACG	GG	ACTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Otjac (Aru)	**GC**	CAC	ACC	CC	CACG	GG	ACCCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Otjac (Run)	**GC**	CAC	ACC	CC	CACG	GG	ACTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Ouni	**GC**	CAC	ACC	CC	CACG	GG	ACTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Pbra (Kal)	**GC**	CAC	ACC	CC	CACG	GG	ACTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Plit (Goe)	**GC**	CAC	ACC	CC	CACG	GG	ACTCA	GCAGTG	ATAAATA	TtAAG	CAA	TAAAC	GAAA	GTTTG	A	CTAA
Nucleotide			130			140		150		160		170		180		

Stem number		4'		14	A		15		B	A'	B'		15'		14'
Mmus	G	TTaTACCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Anam	G	CTaTGCCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Oalac (Uzu)	G	CTaTGCCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Oalac (Chi)	G	CTaTACCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Oalac (Mbi)	G	CTaTACCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Oang	G	CTaTACCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Oden (Mga)	G	CTaTACCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Oden (Buj)	G	CTaTACCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Oirr A1 (Hog)	G	CTaTGCCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Oirr A2 (Kar)	G	CTaTGCCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Oirr B (Ali)	G	CTaTACCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Oirr B (Bai)	G	CTaTACCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Oirr B (Gro)	G	CTaTACCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Oirr C (Alg)	G	CTaTACCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Olam	G	CTaTGCCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Omax	G	CTaTACCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Oslo	G	CTaTACCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Otro	G	CTaTACCTC	CTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Otyp	G	CTaTACCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Otjac (Aru)	G	CTaTACCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Otjac (Run)	G	CTaTACCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Ouni	G	CTaTACCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Pbra (Kal)	G	CCaTACCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Plit (Goe)	G	CTaTACCTC	TTA	GGGTT	GGT	AAATT	TCGTG	CCA	GC C	ACC	GC	GGTCA	TACGA	TT	AACCC
Nucleotide			190		200		210		220		230			240	

Continued/

Stem number	3'			16	D		E?	17		c			18	d	
Mmus	A	AACTAATTAT	CTTC	GGC	GT	AAA	A	C	GTGT	CAAC	TA	TAAATAAA-TAAA	TAGAAT	T	AAAA
Anam	A	AATTAATTAA	TACC	GGC	GT	AAA	A	C	GTGA	TAAT	TA	ATATAAAA--TAA	TAGAAT	T	AAAA
Oalac (Uzu)	A	AACTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AAAT	TA	TTACTTAAATAAA	TAGAAT	T	AAAA
Oalac (Chi)	A	AACTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AAAT	CA	TTACTTAAATAAA	TAGAAT	T	GAAA
Oalac (Mbi)	A	AACTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AAAT	CA	TTACTTAAATAAA	TAGAAT	T	GAAA
Oang	A	AATTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AAAT	TA	TAACTAAGACAAA	TAGAAT	T	AAAA
Oden (Mga)	A	AACTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AAAT	TA	TAACTAAAACAAA	TAGAAT	T	AAAA
Oden (Buj)	A	AACTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AAAT	TA	TAACTAAAACAAA	TAGAAT	T	AAAA
Oirr A1 (Hog)	A	AATTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AAAT	TA	TAACTAAAACAAA	TAGAAT	T	AAAA
Oirr A2 (Kar)	A	AATTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AAAT	TA	TAACTAAAACAAA	TAGAAT	T	AAAA
Oirr B (Ali)	A	AATTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AAAT	TA	TAACTAAAACAAA	TAGAAT	T	AAAA
Oirr B (Bai)	A	AATTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AAAT	TA	TAACTAAAACAAA	TAGAAT	T	AAAA
Oirr B (Gro)	A	AATTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AAAT	TA	TAACTAAAACAAA	TAGAAT	T	AAAA
Oirr C (Alg)	A	AATTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AAAT	TA	TAACTAAAATAAA	TAGAAT	T	AAAA
Olam	A	AATTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AAAT	TA	TAGCTAAAACAAA	TAGAAT	T	AAAA
Omax	A	AATTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AAAT	TA	TAACTAAGACTAA	TAGAAT	T	AAAA
Oslo	A	AACTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AATT	CA	TGGCTAAAACAAA	TAGAAT	T	AAAA
Otro	A	AATTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AAAT	TA	TTACCTAAACAAA	TAGAAT	T	AAAA
Otyp	A	AATTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AAAT	TA	TAACTAAAACAAA	TAGAAT	T	AAAA
Otjac (Aru)	A	AATTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AAAT	TA	TAACTAAGACTAA	TAGAAT	T	AAAA
Otjac (Run)	A	AATTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AAAT	TA	TAACTAAGACAAA	TAGAAT	T	AAAA
Ouni	A	AACTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AAAT	TA	TAACTAGAATAAA	TAGAAT	T	AAAA
Pbra (Kal)	A	AACTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AAAT	TA	TGACTAAAGCAAA	TAGAAT	T	AAAA
Plit (Goe)	A	AATTAATTAT	TCTC	GGC	GT	AAA	A	C	GTGA	AACC	TA	TAACTAAAACAAA	TAGAAT	T	AAAA
Nucleotide		250			260				270			280	290		300

Stem number	e			f		f'		e'		d'	g		g'	18'	
Mmus	TCCAAC	TTAT	AT	GTGAAA	AT	TCATT	GTTAGG	ACCTAA	A	CTCAA	TaAC	GAAA	GTA	ATTCTA	
Anam	TCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACCTAA	A	CCCTA	TgAC	GAAA	GTA	ATTCTA	
Oalac (Uzu)	TCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACTTAA	A	CCCAA	CaAC	GAAA	GTA	ATTCTA	
Oalac (Chi)	TCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACTTAA	A	TCCAA	TaAC	GAAA	GTA	ATTCTA	
Oalac (Mbi)	TCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACTTAA	A	TCCAA	TaAC	GAAA	GTA	ATTCTA	
Oang	TCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACCTAA	A	CTCAA	CaAC	GAAA	GTA	ATTCTA	
Oden (Mga)	CCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACCTAA	A	CCCAA	TaAC	GAAA	GTA	ATTCTA	
Oden (Buj)	CCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACCTAA	A	CCCAA	TaAC	GAAA	GTA	ATTCTA	
Oirr A1 (Hog)	TCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACCTAA	A	CCCAA	TaAC	GAAA	GTA	ATTCTA	
Oirr A2 (Kar)	TCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACCTAA	A	CCCAA	TaAC	GAAA	GTA	ATTCTA	
Oirr B (Ali)	TCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACCTAA	A	CCCAA	TaAC	GAAA	GTA	ATTCTA	
Oirr B (Bai)	TCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACCTAA	A	CCCAA	TaAC	GAAA	GTA	ATTCTA	
Oirr B (Gro)	TCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACCTAA	A	CCCAA	TaAC	GAAA	GTA	ATTCTA	
Oirr C (Alg)	TCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACCTAA	A	CCCAA	TaAC	GAAA	GTA	ATTCTA	
Olam	TCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACCTAA	A	CCCAA	TaAC	GAAA	GTA	ATTCTA	
Omax	TCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACCTAA	A	CTCAA	TaAC	GAAA	GTA	ATTCTA	
Oslo	TCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACCTAA	A	CCCAA	TaAC	GAAA	GTA	ATTCTA	
Otro	TCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACTTAA	A	CCCAA	TaAC	GAAA	GTA	ATTCTA	
Otyp	TCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACCTAA	A	CTCAA	CaAC	GAAA	GTA	ATTCTA	
Otjac (Aru)	TCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACCTAA	A	CTCAA	TaAC	GAAA	GTA	ATTCTA	
Otjac (Run)	TCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACCTAA	A	CTCAA	TaAC	GAAA	GTA	ATTCTA	
Ouni	TCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACCTAA	A	CCCAA	TaAC	GAAA	GTA	ATTCTA	
Pbra (Kal)	TCCAAC	TTAT	AT	GTGAAA	AT	CCATT	GTTAGG	ACCTAA	A	CCCGA	TaAC	GAAA	GTA	ATTCTA	
Plit (Goe)	TCCAAC	TTAT	AT	GTGAAA	AT	TCATT	GTTAGG	AACTAA	A	CCCAA	TaAC	GAAA	GTA	ATTCTA	
Nucleotide		310			320			330		340		350		360	

Stem number		c'		17'		22		h	23		23'		h'	22'	
Mmus	GTCATT	TA	TAAT	ACAC	GACA	GCTAAG	ACCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Anam	AGTAT-	TA	-TTT	GCAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Oalac (Uzu)	ACCTT-	TA	CATT	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Oalac (Chi)	ATTAT-	TA	CATT	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Oalac (Mbi)	ATTAT-	TA	CATT	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Oang	ATTGC-	TA	CATT	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Oden (Mga)	ATCAC-	TA	CATT	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Oden (Buj)	ACCAC-	TA	CACT	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Oirr A1 (Hog)	ACTAT-	TT	CATC	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Oirr A2 (Kar)	ACTAT-	TT	CATC	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Oirr B (Ali)	ACTAT-	TT	CATT	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Oirr B (Bai)	ACTAT-	TT	CATT	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Oirr B (Gro)	ACTAT-	TT	CATT	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Oirr C (Alg)	ACTAT-	TT	CATT	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Olam	ACTAC-	TA	CATC	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Omax	ATTGC-	TA	CATT	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Oslo	ACCGC-	TC	-ATC	ACAC	GATA	GCTAAG	ACCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Otro	ACCAT-	TA	CATT	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Otyp	ACTAT-	TA	CATC	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Otjac (Aru)	ATTAC-	TA	CATT	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Otjac (Run)	ATTGC-	TA	CATT	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Ouni	ACTGT-	TA	CATC	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Pbra (Kal)	ACTAT-	TT	CATC	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CCTAGC
Plit (Goe)	ACCAT-	TA	TGTT	ACAC	GATA	GCTAAG	ATCC	A	AAC	TGGG	ATTAGATAC	CCCA	CTA	T	G	CTTAGC
Nucleotide			370		380		390			400		410			420

Continued/

Stem number	24	D'	24'	E'/16'	i	25	25'
Mmus	CATAAACCTA AATAATT AAATTTA	AC	AA	AACTATT	TGCC A	GAG	AACTACT AGC CATA GCT
Anam	CCTAAACCTG AATAATT TAAT--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC CACC GCT
Oalac (Uzu)	CCTAAACCTA AATAATT AAAC--A	AC	AA	AATTATT	TGCC A	GAG	TACTACT AGC AACA GCT
Oalac (Chi)	CCTAAACCTA AATAATT AAAC--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC AACA GCT
Oalac (Mbi)	CCTAAACCTA AATAATT AAAC--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC AACA GCT
Oang	CCTAAACCTA AATAATT AGAT--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC AACA GCT
Oden (Mga)	CCTAAACCTA AATAATT AAAT--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC AACA GCT
Oden (Buj)	CCTAAACCTA AATAATT AAAT--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC GACA GCT
Oirr A1 (Hog)	CCTAAACCTA AATAATT GAAT--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC GACA GCT
Oirr A2 (Kar)	CCTAAACCTA AATAATT GAAT--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC AAAA GCT
Oirr B (Ali)	CCTAAACCTA AATAATT AAAT--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC AAAA GCT
Oirr B (Bai)	CCTAAACCTA AATAATT AAAT--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC AAAA GCT
Oirr B (Gro)	CCTAAACCTA AATAATT AAAT--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC AAAA GCT
Oirr C (Alg)	CCTAAACCTA AATAATT AAAT--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC AAAA GCT
Olam	CCTAAACCTA AATAATT AAAT--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC AAAA GCT
Omax	CCTAAACCTA AATAATT AGAT--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC AATA GCT
Oslo	CCTAAACTAA AATAATT AAAT--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC AACA GCT
Otro	CCTAAACCTA AATAATT AAAC--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC AACA GCT
Otyp	CCTAAACCTA AATAATT AAAT--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC AACA GCT
Otjac (Aru)	CCTAAACCTA AATAATT AGAT--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC AACA GCT
Otjac (Run)	CCTAAACCTA AATAATT AGAT--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC AACA GCT
Ouni	CCTAAACTTA AATAATT AAAT--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC AACA GCT
Pbra (Kal)	CCTAAACCTA AATAATT AAAC--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC AACA GCT
Plit (Goe)	CCTAAACCTA AATAATT AAAT--A	AC	AA	AATTATT	TGCC A	GAG	AACTACT AGC AACA GCT
Nucleotide	430	440	450	460		470	480

Stem number	i'	2'	26	27	28	29
Mmus	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Anam	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Oalac (Uzu)	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Oalac (Chi)	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Oalac (Mbi)	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Oang	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Oden (Mga)	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Oden (Buj)	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Oirr A1 (Hog)	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Oirr A2 (Kar)	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Oirr B (Ali)	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Oirr B (Bai)	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Oirr B (Gro)	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Oirr C (Alg)	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Olam	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Omax	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Oslo	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Otro	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Otyp	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Otjac (Aru)	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Otjac (Run)	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Ouni	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Pbra (Kal)	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Plit (Goe)	TAAAA	CTC AAA GGA CT	TGGCGgTACTTTA TATCCAT	CTAGA	GG	AGCCTGTTCT ATA ATCG
Nucleotide	490	500	510	520	530	540

Stem number	29'	30	31	32	33
Mmus	ATAAACCC CGCT CTACCTCA	CCATCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Anam	ATAAACCC CGCT ATACCTCA	CCATCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Oalac (Uzu)	ATAAACCC CGCT ATACCTCA	CCACCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Oalac (Chi)	ATAAACCC CGCT ATACCTCA	CCATCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Oalac (Mbi)	ATAAACCC CGCT ATACCTCA	CCATCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Oang	ATAAACCC CGCT ATACCTCA	CCATCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Oden (Mga)	ATAAACCC CGCT ATACCTCA	CCATCCC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Oden (Buj)	ATAAACCC CGCT ATACCTCA	CCATCCC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Oirr A1 (Hog)	ATAAACCC CGCT ATACCTCA	CCATCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Oirr A2 (Kar)	ATAAACCC CGCT ATACCTCA	CCATCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Oirr B (Ali)	ATAAACCC CGCT ATACCTCA	CCATCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Oirr B (Bai)	ATAAACCC CGCT ATACCTCA	CCATCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Oirr B (Gro)	ATAAACCC CGCT ATACCTCA	CCATCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Oirr C (Alg)	ATAAACCC CGCT ATACCTCA	CCATCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Olam	ATAAACCC CGCT ATACCTCA	CCATCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Omax	ATAAACCC CGCT ATACCTCA	CCATCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Oslo	ATAAACCC CGCT ATACCTCA	CCATCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Otro	ATAAACCC CGCT ATACCTCA	CCACCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Otyp	ATAAACCC CGCT ATACCTCA	CCATCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Otjac (Aru)	ATAAACCC CGCT ATACCTCA	CCATCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Otjac (Run)	ATAAACCC CGCT ATACCTCA	CCATCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Ouni	ATAAACCC CGCT ATACCTCA	CCATCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Pbra (Kal)	ATAAACCC CGCT ATACCTCA	CCATCTC	TTGCTAATTCA GCcTATAtaCCGCCACCTT	CA	GC AA AC
Plit (Goe)	ATAAACCC CGTT ACACCTCA	CCATCTC	TTGCTAATTCA GCcTATAtaCCGCCATCTT	CA	GC AA AC
Nucleotide	550	560	570 580 590	600	

Continued/

	33	33'	33'	32	34'	34	31'	
Mmus	CCT	AAAA	AGG TATTAAA	GT AA	GC AAAAGA	AT CAAAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Anam	CCT	AAAA	AGG AATTAAA	GT AA	GC ACAAGG	AC -AAAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Oalac (Uzu)	CCT	AAAA	AGG TATTAAA	GT AA	GC ACAAGA	AT -AGAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Oalac (Chi)	CCT	AAAA	AGG TATTAAA	GT AA	GC ACAAGA	AC -AGAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Oalac (Mbi)	CCT	AAAA	AGG TATTAAA	GT AA	GC ACAAGA	AC -AGAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Oang	CCT	AAAA	AGG TATTAAA	GT AA	GC TCAAGA	AT -AGAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Oden (Mga)	CCT	AAAA	AGG TACTAAA	GT AA	GC ACAAGA	AT -AGAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Oden (Buj)	CCT	AAAA	AGG TACTAAA	GT AA	GC ACAAGA	AT -AGAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Oirr A1 (Hog)	CCT	AAAA	AGG TACTAAA	GT AA	GC ACAAGA	AT -AGAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Oirr A2 (Kar)	CCT	AAAA	AGG TACTAAA	GT AA	GC ACAAGA	AT -TAAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Oirr B (Ali)	CCT	AAAA	AGG TACTAAA	GT AA	GC ACAAGA	AT -TAAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Oirr B (Bai)	CCT	AAAA	AGG TACTAAA	GT AA	GC ACAAGA	AT -TAAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Oirr B (Gro)	CCT	AAAA	AGG TACTAAA	GT AA	GC ACAAGA	AT -TAAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Oirr C (Alg)	CCT	AAAA	AGG TACTAAA	GT AA	GC ACAAGA	AT -TAAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Olam	CCT	AAAA	AGG TATTAAA	GT AA	GC ACAAGA	AT -TAAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Omax	CCT	AAAA	AGG TATTAAA	GT AA	GC ACAAGA	AT -CAAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Oslo	CCT	AAAA	AGG TACTAAA	GT AA	GC TCAAGA	AT -AGAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Otro	CCT	AAAA	AGG TATTAAA	GT AA	GC ACAAGA	AT -AGAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Otyp	CCT	AAAA	AGG TATTAAA	GT AA	GC ACAAGA	AT -AGAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Otjac (Aru)	CCT	AAAA	AGG TATTAAA	GT AA	GC ACAAGA	AC -AGAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Otjac (Run)	CCT	AAAA	AGG TATTGAA	GT AA	GC TCAAGA	AT -AAAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Ouni	CCT	AAAA	AGG TATTAAA	GT AA	GC TCAAGA	AT -AGAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Pbra (Kal)	CCT	AAAA	AGG TATTAAA	GT AA	GC ACAAGA	AC -AAAC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Plit (Goe)	CCT	AAAA	AGG TACTTAA	GT AA	GC ACAAAA	AT -AACC	AT AAAA	ACGTTaGGTcaaGGTGTAGC
Nucleotide	610		620		630	640	650	660

Stem number	30'	28'		35	35'	j	k
Mmus	CAAT	GAAATGG	GA AGaAATGGGCT	ACATT	TTCTT ATAA	AAGAA CATTA	CT ATAC CCTTT
Anam	CAAT	GAGATGG	GA AGaAATGGGCT	ACATT	TTCTT TATA	AAGAA CATTA	CG AAAC CCTTT
Oalac (Uzu)	CAAT	GAGGTGG	AA AGaAATGGGCT	ACATT	TTCTT TT-C	AAGAA TAA-A	CG AAAC CCTTT
Oalac (Chi)	CAAT	GAGATGG	GA AGaAATGGGCT	ACATT	TTCTT TT-C	AAGAA CAA-A	CG AAAC CCTTT
Oalac (Mbi)	CAAT	GAGATGG	GA AGaAATGGGCT	ACATT	TTCTT TT-C	AAGAA CAA-A	CG AAAC CCTTT
Oang	TAAT	GAGATGG	AA AGaAATGGGCT	ACATT	TTCTT TCAC	AAGAA CAA-A	CG AAAC CCTTT
Oden (Mga)	CAAT	GGGATGG	GA AGaAATGGGCT	ACATT	TTCTT TTCC	AAGAA CAA-A	CG AAAC CCTTT
Oden (Buj)	CAAT	GGGATGG	GA AGaAATGGGCT	ACATT	TTCTT TTCC	AAGAA CAA-A	CG GAAC CCTTT
Oirr A1 (Hog)	CAAT	GAGATGG	GA AGaAATGGGCT	ACATT	TTCTT TTAC	AAGAA CAA-A	CG AAAC CCTTT
Oirr A2 (Kar)	CAAT	GAGATGG	GA AGaAATGGGCT	ACATT	TTCTT TTAC	AAGAA CAA-A	CG AAAC CCTTT
Oirr B (Ali)	CAAT	GAGATGG	GA AGaAATGGGCT	ACATT	TTCTT TTAC	AAGAA CAA-A	CG AAAC CCTTT
Oirr B (Bai)	CAAT	GAGATGG	GA AGaAATGGGCT	ACATT	TTCTT TTAC	AAGAA CAA-A	CG AAAC CCTTT
Oirr B (Gro)	CAAT	GAGATGG	GA AGaAATGGGCT	ACATT	TTCTT TTAC	AAGAA CAA-A	CG AAAC CCTTT
Oirr C (Alg)	CAAT	GAGATGG	GA AGaAATGGGCT	ACATT	TTCTT TTAC	AAGAA CAA-A	CG AAAC CCTTT
Olam	CAAT	GAGATGG	GA AGaAATGGGCT	ACATT	TTCTT TCAC	AAGAA CAA-A	CG AAAC CCTTT
Omax	TAAT	GAGATGG	AA AGaAATGGGCT	ACATT	TTCTT TCAC	AAGAA CAA-A	CG AAAC CCTTT
Oslo	CAAT	GAGATGG	GA AGaAATGGGCT	ACATT	TTCTT CTTC	AAGAA CAA-A	CG AAAC CCTTT
Otro	CAAT	GAGGTGG	GA AGaAATGGGCT	ACATT	TTCTT TT-C	AAGAA TAA-A	CG AAAC CTTTT
Otyp	CAAT	GAGATGG	GA AGaAATGGGCT	ACATT	TTCTT TTAT	AAGAA CAA-A	CG AAAC CCTTT
Otjac (Aru)	CAAT	GAGATGG	AA AGtAATGGGCT	ACATT	TTCTT TCAC	AAGAA CAA-A	CG AAAC CCTTT
Otjac (Run)	TAAT	GAGATGG	AA AGtAATGGGCT	ACATT	TTCTT TCAC	AAGAA CAA-A	CG AAAC CCTTT
Ouni	CAAT	GAGATGG	GA AGaAATGGGCT	ACATT	TTCTT TTTC	AAGAA CAA-A	CG AAAC CCTTT
Pbra (Kal)	CAAT	GAGATGG	GA AGaAATGGGCT	ACATT	TTCTT TACT	AAGAA CAA-A	CG AACC CCTTT
Plit (Goe)	CAAT	GAGATGG	GA AGaAATGGGCT	ACATT	TTCTT TTTC	AAGAA CAA-A	CG AAAC TCTTT
Nucleotide	670	680	690	700	710	720	

Stem number	k'	j'	27'	37	37'	
Mmus	ATGAAACT	AAAGG ACTA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATTGAGCAA
Anam	ATGAAACT	AAAGG ATAA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATTGAGCAA
Oalac (Uzu)	ATGAAATT	AAAGG ATAA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGTG	CTTAATT GAATAGAGCAA
Oalac (Chi)	ATGAAATT	AAAGG ATAA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATAGAGCAA
Oalac (Mbi)	ATGAAATT	AAAGG ATAA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATAGAGCAA
Oang	ATGAAATT	AAAGG ATAA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATAGAGCAA
Oden (Mga)	ATGAAACT	AAAGG ACAA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATAGAGCAA
Oden (Buj)	ATGAAACT	AAAGG ACAA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATAGAGCAA
Oirr A1 (Hog)	ATGAAATT	AAAGG ATAA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATAGAGCAA
Oirr A2 (Kar)	ATGAAATT	AAAGG ATAA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATAGAGCAA
Oirr B (Ali)	ATGAAATT	AAAGG ATAA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATAGAGCAA
Oirr B (Bai)	ATGAAATT	AAAGG ATAA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATAGAGCAA
Oirr B (Gro)	ATGAAATT	AAAGG ATAA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATAGAGCAA
Oirr C (Alg)	ATGAAATT	AAAGG ATAA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATAGAGCAA
Olam	ATGAAACT	AAAGG ATAA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATAGAGCAA
Omax	ATGAAATT	AAAGG ATAA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATAGAGCAA
Oslo	ATGAAACT	AAAGG ACAA	AG GCGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATAGAGCAA
Otro	ATGAAATT	AAAGG ATAA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATTGAGCAA
Otyp	ATGAAACT	AAAGG ATAA	AG GAGGA	TTTAG CAGTA	AATTAAG AATAGAGAG	CTTAATT GAATAGAGCAA
Otjac (Aru)	ATGAAATT	AAAGG ATAA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATAGAGCAA
Otjac (Run)	ATGAAATT	AAAGG ATAA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATAGAGCAA
Ouni	ATGAAATT	AAAGG ATAA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATAGAGCAA
Pbra (Kal)	ATGAAATT	AAAGG GTGA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATAGAGCAA
Plit (Goe)	ATGAAATT	AAAGG ACAA	AG GAGGA	TTTAG TAGTA	AATTAAG AATAGAGAG	CTTAATT GAATTGAGCAA
Nucleotide	730	740	750	760	770	780 790

Continued/

Stem number	26'	Coded gaps
Mmus	TGAAGT	0010111111111
Anam	TGAAGT	000000000011
Oalac (Uzu)	TGAAGT	000110100000
Oalac (Chi)	TGAAGT	000110100000
Oalac (Mbi)	TGAAGT	000110100000
Oang	TGAAGT	100110100010
Oden (Mga)	TGAAGT	000110100010
Oden (Buj)	TGAAGT	000110100010
Oirr A1 (Hog)	TGAAGT	000110100010
Oirr A2 (Kar)	TGAAGT	100110100010
Oirr B (Ali)	TGAAGT	100110100010
Oirr B (Bai)	TGAAGT	100110100010
Oirr B (Gro)	TGAAGT	100110100010
Oirr C (Alg)	TGAAGT	100110100010
Olam	TGAAGT	000110100010
Omax	TGAAGT	100110100010
Oslo	TGAAGT	000110000010
Otro	TGAAGT	000110100000
Otyp	TGAAGT	100110100010
Otjac (Aru)	TGAAGT	100110100010
Otjac (Run)	TGAAGT	100110100010
Ouni	TGAAGT	000110100010
Pbra (Kal)	TGAAGT	110110100010
Plit (Goe)	TGAAGT	000110100010
Nucleotide	**800**	

APPENDIX III

Sequences of the protein-coding cytochrome *b* gene translated to 381 amino acids for 27 Otomyinae taxa and the outgroups *Mus musculus* (Bibb *et al.* 1981) and *Aethomys chrysophilus* (Ducroz *et al.* 1998). • - indicates amino acid identity to the sequence of *M. musculus*. The abbreviations of the taxon names and collection localities correspond to Appendix II. Missing data are indicated by ?. The universal mitochondrial DNA genetic code in MacClade (Maddison & Maddison 1992) was used for translation. Symbols: A = Alanine, C = Cysteine, D = Aspartic acid, E = Glutamic acid, F = Phenylalanine, G = Glycine, H = Histidine, I = Isoleucine, K = Lysine, L = Leucine, M = Methionine, N = Asparagine, P = Proline, Q = Glutamine, R = Arganine, T = Threonine, V = Valine, W = Tryptophan, Y = Tyrosine, 1 and 2(S) = Serine. Sequences started with the initiation codon ATG and ended not with regular mtDNA stop codons (AGA, AGG, TAA or TAG), but with translational termination signals produced by the polyadenylation of the processed mRNA also found in mouse and human sequences (Irwin *et al.* 1991).

Amino acid	10	20	30	40	50	60	70
Mmus	MTNMRKTHPL	FKIINHIFID	LPAPINIIIW	WNFGILLGVC	LMVQIITGLF	LAMHYTIDTM	TAFIIVTHIC
Achr	...I..I...	M.V.....	....	.......I..	....	.......T..	....
Oalac (Uzu)	...I......	L.........	..T.......	.......I..	.VL..L....	....	....
Oalac (Chi)	...I......	L.........	..T.......	.......I..	..L..L....	....	....
Oalac (Mbi)	...I......	L.........	..T.......	.......I..	..L..L....	....	....
Oang	...I......	L.........	....	.......I..	..L..L....	.......T..	....
Oden (Mga)	...I......	L.........	..T.......	.......I..	..L..L....	.......T..	....
Oden (Buj)	...I......	L.........	..T.......	.......I..	..L..L....	.......T..	....
Oirr A1 (Hog)	...I......	L.........	....	.......I..	..L..L....	.......T..	....
Oirr A1 (Kam)	...I......	L.........	....	.......I..	..L..L....	.......T..	....
Oirr A2 (Kar)	...I......	L.........	....	.......I..	..L..L....	.......T..	....
Oirr B (Ali)	...I......	L.........	..T.......	.......I..	..L..L....	.......T..	....
Oirr B (Bai)	...I......	L.........	..T.......	.......I..	..L..L....	.......T..	....
Oirr B (Gro)	...I......	L.........	..T.......	.......I..	..L..L....	.......T..	....
Oirr C (Alg)	...I......	L.........	..T.......	.......I..	..L..L....	.......T..	....
Oirr C (Twe)	...I......	L.........	..T.......	.......I..	..L..L....	.......T..	....
Olam	...I......	L.........	..T.......	.......I..	..L..L....	.......T..	....
Omax	...I......	L.........	....	.......I..	..L..L....	.......T..	....
Oocc	...I......	L.........	..T.......	.......I..	.IL..L....	.......T..	....
Oslog	...I......	L.........	..T.......	.......I..	.IL..L....	.......T..	......A...
Otro	...I......	L.........	..T.......	.......I..	.VL..L....	....	....
Otyp	...I......	L.........	....	.......M..	..L..L....	.......T..	......A...
Otjac (Aru)	...I......	L.........	....	....	..L..L....	.......T..	....
Otjac (Run)	...I......	L.........	....	....	..L..L....	.......T..	....
Ouni	...I..N...	L.........	..T.......	.......I..	.VL..L....	.......T..	....
Pbra (Kal)	...I......	L.........	....	.......I..	..L..L....	.....1...T	....
Pbra (Kle)	...I......	L.........	....	.......I..	..L..L....	.......T..	....
Plit (Goe)	...I......	L....D....	..T.......	.......I..	.IL..L....	.......T..	......A...
Plit (Hen)	...I......	L....D....	..T.......	.......I..	.IL..L....	......P..T	......A...

Continued/

Amino acid	80	90	100	110	120	130	140
Mmus	RDVNYGWLIR	YMHANGA1MF	FICLFLHVGR	GLYYG1YTFM	ETWNIGVLLL	FAVMATAFMG	YVLPWGQM1F
Achr	....	....	....	.M.......L	.......I..	.T........	....
Oalac (Uzu)	....	.L........	....	.M.......L	.......I..	....	....
Oalac (Chi)	....	.L........	....	.M.......L	.......I..	.T........	....
Oalac (Mbi)	....	.L........	....	.M.......L	.......I..	.T........	....
Oang	....	.L........	....	.M.......L	.......I..	.T........	....
Oden (Mga)	....	.L........	....	.M.......L	.......I..	....	....
Oden (Buj)	....	.L........	....	.M.......L	.......I..	....	....
Oirr A1 (Hog)	....	....	....	.M.......L	.......I..	.T........	....
Oirr A1 (Kam)	....	....	....	.M.......L	.......I..	.T........	....
Oirr A2 (Kar)	....	.L........	....	.M.......L	.......I..	.T........	....
Oirr B (Ali)	....	....	....	.M.......L	.......I..	....	....
Oirr B (Bai)	....	....	....	.M.......L	.......I..	....	....
Oirr B (Gro)	....	....	....	.M.......L	.......I..	....	....
Oirr C (Alg)	....	....	....	.M.......L	.......I..	....	....
Oirr C (Twe)	....	.I........	....	.M.......L	.......I..	....	....
Olam	....	.L........	....	.M.......L	.......I..	....	....
Omax	....	.L........	....	.M.......L	.......I..	.T........	....
Oocc	....	....	....	.M.......L	.......I..	....	.........?
Oslog	....	.L........	....	.M.......L	.......I..	.T........	....
Otro	....	....	....	.M.......L	.......I..	.T........	....
Otyp	....	.L........	....	.M.......L	.......I..	....	....
Otjac (Aru)	....	.L........	....	.M.......L	.......I..	.T........	....
Otjac (Run)	....	.L........	....	.M.......L	.......I..	.T........	....
Ouni	....	.L........	.....M....	.M.......L	.......I..	.T........	....
Pbra (Kal)	....	.L........	....	.M.......L	.......I..	.T........	....
Pbra (Kle)	....	.L........	....	.M.......L	.......I..	.T........	....
Plit (Goe)	....	.L........	....	.M.......L	.......I..	.T........	....
Plit (Hen)	....	.L........	....	.M.......L	.......I..	.T........	....

Amino acid	150	160	170	180	190	200	210
Mmus	WGATVITNLL	1AIPYIGTTL	VEWIWGGF1V	DKATLTRFFA	FHFILPFIIA	ALAIVHLLFL	HETG1NNPTG
Achr	....	........I.	....	.......1..	....	....	....
Oalac (Uzu)	....	....	....	....	.........T	..V.......	....
Oalac (Chi)	....	........A.	....	....	.........T	..V.......	....
Oalac (Mbi)	....	........A.	....	....	.........T	..V.......	....
Oang	....	....	....	....	.........T	..V.......	....
Oden (Mga)	....	....	....	....	1	....	....
Oden (Buj)	....	....	....	....	1	....	....
Oirr A1 (Hog)	....	....	....	....	.........T	..V.......	....
Oirr A1 (Kam)	....	....	....	....	.........T	..V.......	....
Oirr A2 (Kar)	....	....	....	....	.........T	..V.......	....
Oirr B (Ali)	....	....	....	....	.........T	..V.......	....
Oirr B (Bai)	....	....	....	....	.........T	..V.......	....
Oirr B (Gro)	....	....	....	....	.........T	..V.......	....
Oirr C (Alg)	....	....	....	....	.........T	..V.......	....
Oirr C (Twe)	....	....	....	....	.........T	..V.......	....
Olam	....	....	....	....	.........T	..V.......	....
Omax	....	....	....	....	.........T	..V.......	....
Oocc	....	....	....	....	.........T	..V.......	....
Oslog	....	........A.	....	....	.........T	..V.......	....
Otro	....	....	....	....	.........T	..V.......	....
Otyp	....	....	....	....	.........T	..V.......	....
Otjac (Aru)	....	....	....	....	.........T	..V.......	....
Otjac (Run)	....	....	....	....	.........T	..V.......	....
Ouni	....	.......1..	....	....	.........T	..V.......	....
Pbra (Kal)	....	....	....	....	.........T	..V.......	....
Pbra (Kle)	....	....	....	....	.........T	..V.......	....
Plit (Goe)	....	....	....	....	.........T	..V.......	....
Plit (Hen)	....	....	....	....	.........T	..V.......	....

Continued/

Amino acid	220	230	240	250	260	270	280
Mmus	LN1DADKIPF	HPYYTIKDIL	GILIMFLILM	TLVLFFPDML	GDPDNYMPAN	PLNTPPHIKP	EWYFLFAYAI
Achr	....	....	..M..L.L..	I.......L.	....	....	....
Oalac (Uzu)	....	....	.V...IVT..	I.......A.	......T...	....	....
Oalac (Chi)	....	....	.L...IVT..	I.......A.	......T...	....	....
Oalac (Mbi)	....	....	.L...IVT..	I.......A.	......T...	....	....
Oang	....	....	.VF..IIL..	........A.	......T...	....	....
Oden (Mga)	....	....	.V...IIT..	........A.	......T...	....	....
Oden (Buj)	....	....	.V...ITT..	........A.	......T...	....	....
Oirr A1 (Hog)	....	....	.L...IVL..	........A.	......T...	....	....
Oirr A1 (Kam)	....	....	.L...IVL..	........A.	......T...	....	....
Oirr A2 (Kar)	....	....	.L...IVL..	........A.	......T...	....	....
Oirr B (Ali)	....	....	.L...IVL..	I.......A.	......T...	....	....
Oirr B (Bai)	....	....	.L...IVL..	I.......A.	......T...	....	....
Oirr B (Gro)	....	....	.L.M.IVL..	I.......A.	......T...	....	....
Oirr C (Alg)	....	....	.L...IVL..	........A.	......T...	....	....
Oirr C (Twe)	....	....	.L...IVL..	........A.	......T...	....	....
Olam	....	....	.L...IVL..	I.......A.	......T...	....	....
Omax	....	....	.VF..IIL..	I.......A.	......T...	....	....
Oocc	.T.....???	??????....	.V...IML..	........A.	.....?????	??????????	??????????
Oslog	....	....	.VF..IIL.V	........A.	......T...	....	....
Otro	....	....	.V...IVA..	I.......A.	......T...	....	....
Otyp	.D........	...H......	.VF..IIL..	IM......A.	......T...	....	....
Otjac (Aru)	....	....	.VF..ITL..	I.......A.	......T...	....	....
Otjac (Run)	....	....	.VF..IIL..	I.......A.	......T...	....	....
Ouni	....	....	.M...IIL..	I.......A.	......T...	....	....
Pbra (Kal)	....	.....V....	.....IVL..	I.......A.	......T...	....	....
Pbra (Kle)	....	.....V....	.....IVL..	I.......A.	......T...	....	....
Plit (Goe)	.D.N......	....	.....IVL..	........A.	......T...	....	....
Plit (Hen)	.D.N......	....	.V...IVL..	A.......A.	......T...	....	....

Amino acid	290	300	310	320	330	340	350
Mmus	LR1IPNKLGG	VLALIL1ILI	LALMPFLHT1	KQR2LMFRPI	TQILYWILVA	NLLILTWIGG	QPVEHPFIII
Achr	....	I.........	..FL......	.....V....	..T...L...	....	....
Oalac (Uzu)	....	....	...L......	.....I....	..T...V...	....	....
Oalac (Chi)	....	....	...L......	.....I....	..T.......	....	....
Oalac (Mbi)	....	....	...L......	.....I....	..T.......	....	....
Oang	....	....	...L......	.....I....	..T.......	....	....
Oden (Mga)	....	....	...L......	.....I....	..T.......	...V......	....
Oden (Buj)	....	....	...L......	.....I....	..A.......	...V......	....
Oirr A1 (Hog)	....	....	...L......	.....I....	..1.......	....	....
Oirr A1 (Kam)	....	....	...L......	.....I....	..1.......	....	....
Oirr A2 (Kar)	....	....	...L......	.....I....	..1.......	....	....
Oirr B (Ali)	....	....	...L......	.....I....	..1.......	....	....
Oirr B (Bai)	....	....	...L......	.....I....	..1.......	....	....
Oirr B (Gro)	....	....	...L......	.....I....	..1.......	....	....
Oirr C (Alg)	....	....	...L......	.....I....	..1.......	....	....
Oirr C (Twe)	....	....	...L......	.....I....	..1.......	....	....
Olam	....	....	...L......	.....I....	..T.......	....	....
Omax	....	....	...L.L....	.....I....	..T.......	....	....
Oocc	??????????	??????????	??????????	??????????	..A.......	....	......Y...
Oslog	....	.....M....	..FL......	.....I....	..T.......	...V......	....
Otro	....	....	...L......	.....I....	..A.......	....	....
Otyp	....	....	...L......	.....I....	..T.......	....	.........M.
Otjac (Aru)	....	....	...L.L....	.....I....	..T.....A.	....	....
Otjac (Run)	....	....	...L.L....	.....I....	..T.......	....	....
Ouni	....	.....M....	...L......	.....I....	..T.......	....	......Y...
Pbra (Kal)	....	....	...L.L....	.....I....	..T.....A.	....	....
Pbra (Kle)	....	....	...L.L....	.....I....	..T.......	....	....
Plit (Goe)	....	V	...L..F...	.....I....	..T.......	...V......	......Y...
Plit (Hen)	....	V	...L..F...	.....I....	..T.......	...V......	......Y...

Continued/

Amino acid	360	370	380
Mmus	GQLA1I1YF1	IILILMPI1G	IIEDKMLKLY P
Achr	......2...	...V.....	M..N....WN ?
Oalac (Uzu)	......2...	.....	.....L..W2 1
Oalac (Chi)	......2...	.....	.....W2 1
Oalac (Mbi)	......2...	.....	.....W2 1
Oang	......2...	.....	....NL..W2 1
Oden (Mga)	......2...	.....	.....L..W2 L
Oden (Buj)	......2...	.....	.....L..W2 1
Oirr A1 (Hog)	......2...	.....	.....L..W2 1
Oirr A1 (Kam)	......2...	.....	.....L..W2 1
Oirr A2 (Kar)	......2...	.....	.....L..W2 1
Oirr B (Ali)	......2...	.....	.....L..WN 1
Oirr B (Bai)	......2...	.....	.....L..WN 1
Oirr B (Gro)	......2...	.....	.....L..WN 1
Oirr C (Alg)	......2...	.....	.....L..W2 1
Oirr C (Twe)	......2...	.....	.....L..W2 1
Olam	......2...	.....	.....L..W2 1
Omax	......2...	.....	....NL..W2 1
Oocc	......2...	.....	.....L..W2 1
Oslog	......2...	.....	.....L..WN 1
Otro	......2...	.....	.....L..W2 1
Otyp	......2...	.....	.....L..W2 1
Otjac (Aru)	......2...	.....	....NL..W2 1
Otjac (Run)	......2...	.....	....NL..W2 1
Ouni	......2...	.....I....	.....L..W2 L
Pbra (Kal)	......2...	.....	.....L..W2 1
Pbra (Kle)	......2...	.....	.....L..W2 1
Plit (Goe)	......2...	.....	.....L..W2 L
Plit (Hen)	......2...	.....	.....L..W2 L