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Application of a multidisciplinary approach to the systematics of *Acomys* (Rodentia: Muridae) from northern Tanzania

By

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Submitted in partial fulfillment of the requirements for the degree of

Master of Science (Zoology)

in the
Faculty of Natural and Agricultural Sciences
University of Pretoria
Pretoria
South Africa

December, 2006

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Dedication

This thesis is dedicated to the family of the Late Mzee Frank Mgode

General abstract

The systematic status and geographic distribution of spiny mice of the genus *Acomys* I. Geoffroy, 1838 in northern Tanzania is uncertain. This study assesses the systematic and geographic distribution of *Acomys* from northern Tanzania using a multidisciplinary approach that includes molecular, cytogenetic, traditional and geometric morphometric analyses, and classical morphology of the same individuals. The molecular analysis was based on 1140 base pairs (bp) of the mitochondrial cytochrome *b* and 1297 bp of the nuclear interphotoreceptor retinoid binding protein (IRBP) gene sequences. These data were subjected to phylogenetic analyses using Maximum likelihood, Bayesian, Maximum parsimony, and Minimum evolution analyses. The cytogenetic analysis included G-banding of metaphase chromosomes. The morphometric analyses included univariate and multivariate analyses of traditional morphometric measurements of the cranium and mandible, and of geometric morphometric two-dimensional landmarks of the dorsal, ventral, and lateral views of the cranium, and lateral view of mandible that included thin-plate spline (TPS) analysis. The classical morphology included examination of external, cranial and mandibular morphology. Results of all these multidisciplinary analyses were congruent and provide evidence for the occurrence of two sympatric species of *Acomys* in northern Tanzania, namely, the previously recorded *A. wilsoni* ($2n = 62$) and a newly recorded *A.* cf. *percivali* ($2n = 58$). These results that also represent the first reported mitochondrial cytochrome *b* and nuclear IRBP gene sequences and karyotype for *A.* cf. *percivali*, increases the number of species known to occur in Tanzania from four to five. However, the mitochondrial cytochrome *b* data that included GenBank sequences from the type locality in Kenya suggest that *A. wilsoni* may not be monophyletic. Ecologically, the two species seem to partition their niches with *A.* cf. *percivali* being found in well-covered habitats with thorn bushes, rocky and mountainous areas, and *A. wilsoni* being found in open semi-arid grasslands as well as in rice fields. The two species appear to be isolated by complex natural barriers formed by the Great East African Rift Valley whose geological features have generally been associated with active rodent speciation. However, the present results need further multidisciplinary investigation involving extensive sampling and examination of topotypical material.

Acknowledgements

First and foremost, I am so thankful and feel indebted to my supervisors Prof Chris Chimimba and Dr Amanda Bastos for their great, priceless advice and guidance which was a key to success of this thesis. I appreciate their criticism, challenges, comments and encouragement during the course of this study. These were springboards to development and successful completion of this multidisciplinary systematic study.

Secondly, I am beholden to thank the late Prof Walter Verheyen of the University of Antwerp, Belgium, for his invaluable ideological contribution and support, facilitation and permission to full access to his *Acomys* material and information in Belgium. I am very grateful to Prof Herwig Leirs of the Department of Evolutionary Biology, University of Antwerp, Belgium, for his wonderful logistic arrangements which enabled smooth execution of this study. His facilitation and invitation which allowed me to visit Belgian institutions to access *Acomys* collections and information and perform some important analyses was fantastic. My sincere appreciations are also extended to Dr Erik Verheyen of the Royal Belgian Institute of Natural Sciences, Brussels, Belgium for his wonderful support, guidance and unlimited permission to use his DNA sequencing facilities. I also owe a lot to Mrs Tine Dillen and Ms Hilde Dhuyvetter for their tireless technical support on DNA sequencing of my *Acomys* specimens. My thanks also go to Mr Wim Wendelen of the Museum for Central Africa, Tervuren, Belgium, for access to *Acomys* collections from Tanzania.

I also wish to thank the following people from the Department of Zoology and Entomology, University of Pretoria, for their diverse support: The Head of Department, Prof Sue Nicolson, Dr Bateman, Ms Ingrid Vis and Ms Babsie Potgieter. My thanks also go to Ms Shavanee Maduray, Ms Lia Rotherham, Ms Helene Brettschneider, Ms Leanne Hart, and Mr Paul Odendaal for their assistance with morphometrics; Mr. Nakedi Maputla for assistance with cytogenetics; Mr Glen Malherbe for helping with molecular protocols; and Mr Allan Hall of the Department of Microscopy and Microanalysis, University of

Pretoria for assistance on chromosome image capturing. For those not mentioned here, I value a lot their contribution to my study.

Furthermore, I highly appreciate the financial support from Sokoine University of Agriculture, Tanzania, in collaboration with the Flemish Inter-University Council of Belgium (SUA-VLIR Programme) for bearing expenses of this study. I am thankful to the Project Leader, Prof Rhodes Makundi for his effort which ensured completion of this work. I appreciate a lot the financial logistics and administrative support from Dr Peter Mtakwa and his entire team at the SUA-VLIR coordination office, Morogoro, Tanzania. I also owe thanks to the administration of Sokoine University of Agriculture, especially the Registrar and Prof Robert Machang'u (former Director of the SUA-Pest Management Centre) for their support and allowing me time to pursue this study. I truly owe much to Mr Ramadhan Iddi and Mr Hamis Ng'ombe for their tireless support and endurance they showed during field work, especially during collecting expeditions to dangerous and wild localities. I appreciate much the administrative support received from authorities such as village leaders of all collecting localities. Special thanks go to authorities of the Ikorongo Grumeti Game Reserve for understanding the importance of this study and hence granting me permission to collect *Acomys* specimens from this conservation area.

Last but not least, I am sincerely indebted to thank my family, my mom Irene Simkoko, my sisters and my brother for their moral support and encouragement at all times. Special thanks go to my beloved wife Anna and my daughter Koko for the wonderful support, endurance and tolerance when I was away for studies. I thank the Almighty God for His blessings.

Disclaimer

This thesis consists of a series of chapters that have been prepared as stand-alone manuscripts for subsequent submission for publication purposes. Consequently, unavoidable overlaps and/or repetitions may occur between chapters.

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CHAPTER ONE
GENERAL INTRODUCTION

1 Introduction

African spiny mice of the genus *Acomys* I. Geoffroy, 1838 are small to medium-sized rodents that are uniquely characterized by the possession of a spiny hair coat, especially on the dorsal part but not ventrally. The dorsal pelage ranges from grey, brown, reddish to darkish, whereas on the belly, it ranges from white, dirty white to darkish depending on the habitat. Species of *Acomys* have broad erect ears, and large round eyes. The tail is short and delicate with visible scales. Females may have four to six mammae. There is remarkable body size variation in species of *Acomys* that is mainly considered to be a response to geographic variation and habitat (Nevo 1989).

1.1 Geographic distribution

The genus *Acomys* is confined to Africa, the Near East (Arabian Peninsula) and Asia Minor (Corbet 1978; Bates 1994). In the Near East, members of the genus have been recorded from Jordan, Lebanon, Iran, Syria, Yemen, Iraq, Israel, Saudi Arabia, and Pakistan (Meester & Setzer 1971; Honacki *et al*. 1982). In Asia Minor, members of the genus have been recorded from Greece, Turkey, Crete, and Cyprus (Honacki *et al*. 1982; Musser & Carleton 1993, 2005).

The African continent is hypothesised to be the origin of the genus *Acomys* (Barome *et al*. 2000), where it has been recorded from almost all geographical regions of the continent. Out of the 14 currently recognized species of *Acomys* (Musser & Carleton 1993, 2005), only three species do not occur in Africa (Fadda *et al*. 2001). The three non-African species of *Acomys*, the so-called Asia Minor spiny mice, include *A. nesiotes*, *A. cilicicus* and *A. minous*, and are all currently listed in the IUCN Red List of threatened mammals as Critically Endangered (CR; IUCN 1994, 2006).

Within Africa, members of the genus *Acomys* have been recorded from Egypt, Libya, and Morocco in North Africa; Nigeria, Mauritania, Niger, Western Sahara, Benin, Cameroon, Burkina Faso, Togo, and Mali in West Africa; Ethiopia, Sudan, Somalia, Kenya, Uganda, and

Tanzania in East Africa; and Zambia, Malawi, and the Democratic Republic of Congo (DRC) in Central Africa (Meester & Setzer 1971; Honacki *et al*. 1982; Musser & Carleton 1993, 2005). In southern Africa, the distribution of the genus extends from Mozambique, Zimbabwe, and Botswana, south-wards to South Africa (Dippenaar & Rautenbach 1986; Janecek *et al*.1991; Musser & Carleton 1993, 2005).

Four species of *Acomys*, namely, *A*. *ignitus*, *A*. *kempi*, *A*. *spinosissimus*, and *A*. *wilsoni* have been recorded to occur in Tanzania (Musser & Carleton 1993, 2005). However, given the systematic problems within the genus, the exact number of species occurring in Tanzania is uncertain. For instance, Petter (1983) considered *A*. *ignitus* as a valid species, Ellerman (1941) related *A*. *ignitus* to *A*. *pulchellus*, *A*. *kempi*, and *A*. *montanus*. Janecek *et al*. (1991) related *A*. *ignitus* to *A*. *cahirinus*, whereas Setzer (1975) treated it as a subspecies of *A*. *dimidiatus*. A recent investigation based on chromosomal analysis (Corti *et al*. 2005) suggests a taxonomic review of the reported *A*. *cf*. *selousi* from eastern central Tanzania. The karyotype of this species has previously been related to the karyotype of *A*. *spinosissimus* (Matthey 1965) from the central and southern parts of Tanzania.

1.2 Ecology and behaviour

Spiny mice are found in dry savannas, woodlands, grassland, arid, semi-arid, and rocky habitats. Most species of *Acomys* live in or around crevices found in rocks, cracked soil, termitaries, and sometimes in burrows of other rodents (Kingdon 1974). They prefer areas with sufficient shelter and protection from predators, such as *Acacia* thorn bushes (G. Mgode pers. obs.). Species of *Acomys* feed on a variety of plant material and insects (Vesey-FitzGerald 1966; Osborn & Helmy 1980). Foraging behaviour differs among species of *Acomys*, the majority being partly nocturnal as they can be found feeding early in the morning and late afternoon such as is the case with *A*. *cahirimus*. Other species such as *A*. *russatus* are diurnal (Harrison 1972; Qumsiyeh 1996).

Acomys cahirimus and *A*. *russatus* co-exist in the hot rocky deserts of Israel with temporal partitioning of resources resulting from competition (Jones & Dayan 2000; Shargal *et al*. 2000). A study on these two common rodents of the hot rocky deserts of Israel showed that the diurnal *A*. *russatus* shifts to nocturnal mode of life in habitats where the sympatrically

occurring *A. cahirinus* has been experimentally removed or does not occur naturally (Shkolnik & Borut 1969; Shkolnik 1971). This suggests that the diurnal or nocturnal mode of life in species of *Acomys* may be due to competition (Shkolnik 1966).

A study on patterns of rodent communities in different habitats by Krasnov *et al*. (1996) showed that *A. cahirinus* and *A. russatus* occur exclusively in rocky areas throughout their geographic range. However, Osborn & Helmy (1980) and Harrison & Bates (1991) reported that unlike *A. russatus*, *A. cahirinus* occasionally occurred in other forms of habitats, suggesting that *A. cahirinus* has a wider habitat tolerance than its congener. Overlapping ecological niches accompanied by microhabitat partitioning is an efficient means of co-existence among rodents (Morris 1987; Rosenzweig 1987). For instance, *A. russatus* is more specialized with a greater foraging efficiency and is confined to boulder areas, whereas *A. cahirinus* is less specialized, having a broader geographic range with lower foraging efficiency, and only occasionally using the boulder microhabitats (Shargal *et al*. 2000).

Acomys russatus is physiologically better adapted to desert environments than *A. cahirinus* (Shargal *et al*. 2000). During severe drought and the high temperatures in the desert, the dietary salt concentration increases and the mice respond by increasing urine osmolarity and decreasing resting metabolic rate and body mass (Ron & Haim 2001). They also counter-balance the harsh desert conditions by eating more vegetative plants and preying on land snails and other invertebrates to meet their water requirements. The distributional range of *A. cahirinus* includes the whole of North Africa, East Africa, Asia Minor, Pakistan and Middle East (Harrison & Bates 1991) where high densities have been recorded in either agriculturally developed areas with abundant food resources or near human settlements (Shkolnik 1966).

Species of spiny mice are social animals where individuals of *A. cahirinus* for example, live in groups (Abramsky *et al*. 1985). Members of *Acomys* in captivity have been observed to aggregate in large numbers in a communal nest, leaving other nests empty (Shargal *et al*. 2000).

1.3 Reproduction

The reproductive pattern within the genus *Acomys* is generally undefined with overlapping seasonality. Most of the available literature on reproduction in *Acomys* is from captive animals (Grzimek 1975; Harrison 1972, Kingdon 1974; Rosevear 1969), which has generally obscured an insight into their natural reproductive patterns. Members of the genus seem to breed throughout the year provided abiotic and biotic factors are in equilibrium (Kingdon 1974). In the Middle East, *A. cahirinus* breeds throughout the year (Qumsiyeh 1996). In Malawi, it was reported to be an opportunistic breeder with the breeding season starting a few months after the first rains (Hanney 1965). *Acomys russatus* breeds between April and July (Mendelssohn & Yom-Tov 1999), whereas in South Africa, *A. subspinosus* breeds opportunistically during winter with its breeding being associated with resource availability from flowering protea (*Protea humiflora*) plants (Fleming & Nicolson 2002).

East African *Acomys* such as *A. percivali* and *A. wilsoni* from Kenya show no breeding seasonality (Neal 1983). In Tanzania, however, *A. wilsoni* and the previously designated *A. hystrella* have been reported to breed in summer and winter, respectively (Hubbard 1972). Shenbrot & Krasnov (2001) found a high positive correlation between population numbers of *A. cahirinus* and rainfall.

1.4 Systematic status of *Acomys*

The systematics of *Acomys* is uncertain at the supra-specific, specific, and subspecific levels (Denys *et al*. 1992a; Denys *et al*. 1992b; Barome *et al*. 2001). The genus has traditionally been placed within the subfamily Murinae in the family Muridae (Walker 1964; Misonne 1969; Carleton & Musser 1984; Jacobs *et al*. 1990). Based on immunological data, Sarich (1985) and Wilson *et al*. (1987) contested the inclusion of *Acomys* within the Murinae suggesting that the genus may be distantly related to the Murinae as well as to members of some subfamilies considered to be closely related to the Murinae, and even went further to suggest that *Acomys* may not be a member of the Muroidea.

Based on a cladistic analysis of morphological data, Hutterer *et al*. (1988) supported the placement of *Acomys* within the subfamily Murinae, and Denys *et al*. (1992a) further considered

the genus to be an offshoot of the subfamily's radiation. Viegas-Péquignot *et al*. (1983) showed that the karyotypes of *Acomys* and its sister taxon, *Uranomys* are perculiar and suggested that these two genera probably evolved from the ancestral stock of the Murinae. This seems to be supported by the description of a murine sub-fossil species, *Malpaisomys insularis* from the Canary Islands that is considered to be closely related to both *Acomys* and *Uranomys* (Hutterer *et al*. 1988; Denys *et al*. 1992b). G-banding chromosome analysis (Viegas-Péquignot *et al*. 1986) and isozyme data (Bonhomme *et al*. 1985) also supported the placement of *Acomys* within the subfamily Murinae, with the latter study supporting Denys *et al*. (1992b) in suggesting an isolated position of the genus *Acomys* within the subfamily Murinae.

Subsequent molecular studies strongly suggest that *Acomys* does not belong to the subfamily Murinae nor is it even related to murine rodents. DNA hybridization data showed that *Acomys* and two sister genera, *Uranomys* and *Lophuromys* are more closely related to the subfamily Gerbillinae than to the subfamily Murinae (Denys *et al*. 1992b; Chevret *et al*. 1993; Sergei & Lee 1996). In subsequent analyses of nuclear ribonuclease gene sequences (Dubois *et al*. 1999; Chevret *et al*. 2001), *Acomys*, *Deomys*, and *Uranomys* formed a clade, leading to this monophyletic assemblage of genera to be allocated to the subfamily Acomyinae. However, it has been suggested that this subfamilial designation may not be appropriate but that the subfamily name Deomyinae, that includes an *Acomys-Deomys-Uranomys-Lophuromys* clade, should be used instead because it represents the earlier available designation (Bronner *et al*. 2003; C. Denys pers. comm.)

Denys (1990) described the oldest known fossil species of *Acomys* and compared it to other members of the subfamily Murinae such as *Mus*, *Uranomys*, and *Millardia*. She concluded that the genus *Acomys* has numerous peculiar characteristics that would accord it a distinct position among murine rodents. She suggested a critical comparison between *Acomys* and the subfamily Dendromurinae which Jaeger *et al*. (1985) proposed to be a sister subfamily to the Murinae. All the above-cited studies strongly suggest a critical need to investigate phylogenetic relationships among murid subfamilies that should include the Murinae, Dendromurinae, Cricetomyinae, Cricetinae, and the Gerbillinae.

At the species level, Ellerman (1941) recognized as many as 38 species of *Acomys*, Honacki *et al*. (1982) recognized seven species, while Musser & Carleton listed 14 species (1993) and 19 species (2005) (see Table 1.1). At the subspecific level, more than 50 forms have been described over the years (Matthey 1968; Janecek *et al*. 1991; Denys *et al*. 1994; Barome *et al*. 2001).

Previous systematic studies on *Acomys* from North, West, East, central and southern Africa (Meester & Setzer 1971; Setzer 1975; Honacki *et al*. 1982; Dippenaar & Rautenbach 1986; Volobouev *et al*. 1991; Musser & Carleton 1993, 2005; Barome *et al*. 2000) revealed that several species have been recorded to occur in some areas. However, their specific status remains largely uncertain because some taxa have been variably treated as either valid species or subspecies.

For example, *A. cahirinus* from West Africa is recorded as either *A. cineraceus*, *A. airensis* or *A. johannis* and, in East Africa, as either *A. kempi* or *A. percivali* (Setzer 1975). There is also controversy regarding the taxonomic status of *A. ignitus* from Kenya and Tanzania where Petter (1983) recognized it as a valid species, Setzer (1975) treated it as a subspecies of *A. dimidiatus*, while Janecek *et al*. (1991) considered it as distinct and phylogenetically closely related to *A. cahirinus*. *Acomys kempi* from Tanzania was originally described as a subspecies of *A. ignitus* (Ellerman 1941; Hollister 1919), but Setzer (1975) considered it as a subspecies of *A. cahirinus*, while Janecek *et al*. (1991) treated it as a valid species closely related to *A. cahirinus*. These taxonomic inconsistencies are summarised in Table 1.1.

Table 1.1 List of previously and currently recognized species of *Acomys* based on the taxonomic treatments of Ellerman (1941), Honacki *et al*. (1982), and Musser & Carleton (1993, 2005).

	Ellerman (1941)	Honacki *et al*. (1982)	Musser & Carleton (1993)	Musser & Carleton (2005)
1	*Acomys russatus russatus* Wagner, 1839	*Acomys russatus*	*A. russatus*	*A. russatus*
2	*A. russatus aegyptiacus* Bonhote, 1912	*A. cilicicus*	*A. cilicicus*	*A. cilicicus*
3	*A. percivali* Dollman, 1911	-	*A. percivali*	*A. percivali*
4	*A. cahirinus* Desmarest, 1819	*A. cahirinus*	*A. cahirinus*	*A. cahirinus*
5	*A. cineraceus* Fitzinger & Heuglin, 1867	-	*A. cineraceus*	*A. cineraceus*
6	*A. dimidiatus dimidiatus* Cretzchmar, 1826	-	-	*A. dimidiatus*
7	*A. dimidiatus homericus* Thomas, 1923	-	-	
8	*A. dimidiatus flavidus* Thomas, 1917	-	-	
9	*A. dimidiatus nesiotes* Bate, 1903	-	*A. nesiotes*	*A. nesiotes*
10	*A. dimidiatus minous* Bate, 1905	*A. minous*	*A. minous*	*A. minous*
11	*A. hunteri* de Winton, 1901	-	-	
12	*A. airensis* Thomas & Hinton, 1921	-	-	*A. airensis*
13	*A. viator* Thomas, 1902	-	-	
14	*A. johannis* Thomas, 1912	-	-	*A. johannis*
15	*A. ignitus ignitus* Dollman, 1910	-	*A. ignitus*	*A. ignitus*
16	*A. ignitus montanus* Heller, 1914	-	-	
17	*A. ignitus kempi* Dollman, 1911	-	*A. kempi*	*A. kempi*
18	*A. mullah* Thomas, 1904	-	*A. mullah*	*A. mullah*
19	*A. brockmani* Dollman, 1911	-	-	
20	*A. selousi* de Winton, 1896	-	-	
21	*A. louisae louisae* Thomas, 1896	-	*A. louisae*	*A. louisae*
22	*A. louisae umbratus* Thomas, 1923	-	-	
23	*A. wilsoni wilsoni* Thomas, 1892	*A. wilsoni*	*A. wilsoni*	*A. wilsoni*
24	*A. wilsoni argillaceus* Hinton & Kershaw, 1920	-	-	
25	*A. wilsoni enid* St. Leger, 1932	-	-	
26	*A. wilsoni nubilus* Dollman, 1914	-	-	
27	*A. wilsoni ablutus* Dollman, 1911	-	-	
28	*A. subspinosus* Waterhouse, 1837	*A. subspinosus*	*A. subspinosus*	*A. subspinosus*
29	*A. chudeaui* Kollman, 1911	-	-	*A. chudeaui*
30	*A. hawashensis* Frick, 1914	-	-	
31	*A. seurati* Heim de Balsac, 1936	-	-	*A. seurati*
32	*A. intermedius* Wettstein, 1916	-	-	
33	*A. nubicus* Heuglin, 1877	-	-	
34	*A. albigena* Heuglin, 1877	-	-	
35	*A. bovonei* de Beaux, 1934	-	-	
36	*A. hystrella* Heller, 1911	-	-	
37	*A. spinosissimus* Peters, 1852	*A. spinosissimus*	*A. spinosissimus*	*A. spinosissimus*
38	*A. transvaalensis* Roberts, 1926	-	-	

A recent molecular study strongly suggested the existence of two sibling species within *A. spinosissimus* from southern Tanzania and northern Malawi (Barome *et al.* 2001). Despite limited sampling and a restricted geographic coverage, the study strongly suggested the existence of a species complex within *A. spinosissimus* and possibly in other species within the genus *Acomys*. A comprehensive study on *Acomys* (W. Verheyen *et al.* unpubl. data) covering southern, eastern, central, and other parts of Tanzania (excluding northern Tanzania) suggests that most of the previously described species of *Acomys* from these areas are probably incorrectly designated. Consequently, there is a critical need to further investigate the nature and extent of variation within the genus *Acomys* by means of a multidisciplinary approach, particularly within the under-studied northern Tanzanian region.

1.5 Aim of study

The aim of the present study is, therefore, an attempt to assess the systematic status of *Acomys* from northern Tanzania at the specific and/or subspecific levels, as well as to evaluate its geographic distribution. The study attempts to use a multidisciplinary approach that includes molecular, cytogenetic, classical morphological, as well as traditional and geometric morphometric analyses.

1.6 Research questions

The following questions will be addressed in the present investigation:

1. Which species of *Acomys* occur in northern Tanzania?
2. What is the nature and extent of variation among these species?
3. What is the geographic extent of the species of *Acomys* in northern Tanzania?

1.7 Justification

The systematic status of *Acomys* and its geographic distribution in northern Tanzania is virtually unknown. To date, there are very few multidisciplinary systematic and biogeographic studies in Tanzania and the present study may serve as a model for other similar studies in other regions in Africa. Apart from adding to a body of knowledge on small mammal systematics in Africa, the present study may assist the appropriate authorities in formulating nature conservation decisions for this genus in Tanzania.

1.8 REFERENCES

ABRAMSKY, Z., ROSENZWEIG, M.L. & BRAND, S. 1985. Habitat selection of Israel desert rodents: comparison of a traditional and new method of analysis. *Oikos* **45**:79-88.

BAROME, P.O., MONNEROT, M. & GAUTUN, J.C. 2000. Phylogeny of the genus *Acomys* (Rodentia, Muridae) based on the cytochrome *b* mitochondrial gene: implications on taxonomy and phylogeography. *Mammalia* **64**:423-438.

BAROME, P.O., LYMBERAKIS, P., MONNEROT, M. & GAUTUN, J-C. 2001. Cytochrome *b* sequence reveal *Acomys minous* (Rodentia: Muridae) paraphyly and answer the question about the ancestral karyotype of *Acomys dimidiatus*. *Molecular Phylogenetics and Evolution* **18**:37-46.

BAROME, P. O., VOLOBOUEV, V., MONNEROT, M., MFUNE, J. K., CHITAUKALI, W., GAUTUN, J-C. & DENYS, C. 2001. Phylogeny of *Acomys spinosissimus* (Rodentia, Muridae) from north Malawi and Tanzania: Evidence from morphological and molecular analysis. *Biological Journal of the Linnean Society* **73**:321-340.

BATES, P. J. J. 1994. The distribution of *Acomys* (Rodentia: Muridae) in Africa and Asia: Ecology, physiology, and systematics. *Israel Journal of Zoology* **40**:199-214.

BONHOMME, F., ISKANDER, D., THALER, L. & PETTER, F. 1985. Eletromorph and phylogeny in muroid rodents. In: *Evolutionary relationships among rodents*: A multidisciplinary approach, (eds) W.P. Luckett & J.L. Hartenberger, Pp. 671-684. Plenum, New York.

BRONNER, G. N., HOFFMANN, M., TAYLOR, P. J., CHIMIMBA, C. T., BEST, P. B., MATTHEE, C. A. & ROBINSON, T. J. 2003. A revised systematic checklist of the extant mammals of the southern African subregion. *Durban Museum Novitates* **28**:61-63.

CARLETON, M.D. & MUSSER, G.G. 1984. Muroid rodents. In: *Orders and families of recent mammals of the world*, (eds) S. Anderson & J.K. Jones, Pp. 289-379. John Wiley & Sons, New York. .

CHEVRET, P., DENYS, C., JAEGER, J. J., MICHAUX, J. & CATZEFLIS, F. 1993. Molecular evidence that spiny mouse (*Acomys*) is closely related to gerbils (Gerbillinae)

than to true mice (Murinae). *Proceedings of the National Academy of Science, USA* **90**:3433-3436.

CHEVRET, P., CATZEFLIS, F. & MICHAUX, J. R. 2001. "Acomyinae" new molecular evidences for muroid taxon (Rodentia, Muridae) In: *African small mammals*, (eds) C. Denys, L. Granjon & A. Poalet, Pp 109-125. IRD, Paris.

CORBET, G. B. 1978. *The mammals of the palaearctic region*. A taxonomic review. Pp. 314. British Museum (Natural History) & Cornell University Press, London.

CORTI, M., CASTIGLIA, R., COLANGELO, P., CAPANNA, E., BEOLCHINI, F., BEKELE, A., OGUGE, N., MAKUNDI, R.H., SICHILIMA, A.M., LEIRS, H., VERHEYEN, W. & VERHAGEN, R. 2005. Cytotaxonomy of rodent species from Ethiopia, Kenya, Tanzania and Zambia. *Belgian Journal of Zoology* **135**:197-216.

DENYS, C. 1990. The oldest *Acomys* (Rodentia, Mammalia) from the lower Pliocene of South Africa and the problem of its murid affinities. *Palaeontographica Abteilung* **210**:70-91.

DENYS, C., MICHAUX, J., PETTER, F., AGUILAR, J.P. & JAEGAR, J.J. 1992a. Molar morphology as clue to the phylogenetic relationship of *Acomys* to the Murinae. *Israel Journal of Zoology* **38**:253-262.

DENYS, C. & MICHAUX, J. 1992b. La troisieme molaire superieure chez les Muridae d' Afrique tropicale et le cas des genres *Acomys*, *Uranomys* et *Lophuromys*. *Bonner Zoologische Beitrage* **43**:367-382.

DENYS, C., GAUTUN, J-C., TRANIER, M. & VOLOBOUEV, V. 1994. Evolution of the genus *Acomys* (Rodentia, Muridae) from dental and chromosomal patterns. *Israel Journal of Zoology* **40**:215-246.

DIPPENAAR, N. J. & RAUTENBACH, I.L. 1986. Morphometrics and karyology of the southern African species of the genus *Acomys* I. Geoffroy Saint-Hilaire, 1838 (Rodentia, Muridae). *Annals of the Transvaal Museum* **34**:129-183.

DUBOIS, F. J., CATZEFLIS, F. M. & BAINTEMA, J. 1999. The phylogenetic position of "Acomyinae" (Rodentia, Mammalia) as sister group of a Murinae + Gerbillinae clade: Evidence from the nuclear ribonuclease gene. *Molecular Phylogenetics and Evolution* **13**:181-192.

ELLERMAN, J. R. 1941. *The families and genera of living rodents*. Vol. 2. Muridae,

Pp. 269-275.Trustees of the British Museum (Natural History), London.

FADDA, C., CASTIGLIA, R., COLANGELO, P., CORTI, M., MACHANG'U, R., MAKUNDI, R., SKANZANI, A., TESHA, P., VERHEYEN, W. & CAPANNA, E. 2001. The rodent fauna of Tanzania: A cytotaxonomic report from the Maasai Steppe (1999). *Rendiconti Fisiche Accademia Lincei* **12**:29-49.

FLEMING, P.A. & NICOLSON, S.W. 2002. Opportunistic breeding in the Cape spiny mouse (*Acomys subspinosus*). *African Zoology* **37**:101-105.

GRZIMEK, N.C.B.1975. *Grzimek's Animal Life Encyclopedia*. Vol. 1-5, Mammals. New York. Van Nostrand Reinhold Company.

HANNEY, P. 1965. The Muridae of Malawi (Africa: Nyasaland). *Journal of Zoology* **146**:577-633.

HARRISON, D.L. & BATES, P. 1991. *The mammals of Arabia* (2nd ed). Sevenoaks, Pp.354. Harrison Zoological Museum Publications.

HARRISON, D.L. 1972. Observations on the life histories and behaviour of some small rodents from Tanzania. *Zoologica Africana* **7**:419-49.

HOLLISTER, N. 1919. East African mammals in the United States National Museum. Part II. Rodentia, Lagomorpha, and Tubulidentata. *Bulletin of the United States National Museum* **99**:1-175.

HONACKI, J. H., KINMAN, K. E. & KOEPPL, J. W. 1982. *Mammal species of the world*: A taxonomic and geographic reference. Allen Press, Inc. & The Association of Systematics Collections, Lawrence, Kansas.

HUBBARD, C.A. 1972. Observations on the life histories and behaviour of some small rodents from Tanzania. *Zoologica Africana* **7**:419-449.

HUTTERER, R., LOPEZ-MARTINEZ, N. & MICHAUX, J. 1988. A new rodent from Quaternary deposit of the Canary Islands and its relationship with Neogene and recent Murids of Europe and Africa. *Paleovertebrata* **18**:241-262.

INTERNATIONAL UNION FOR CONSERVATION OF NATURE AND NATURAL RESOURCES (IUCN). 2006. *IUCN Red List of Threatened Species*. http://www.iucnredlist.org.

INTERNATIONAL UNION FOR CONSERVATION OF NATURE AND NATURAL RESOURCES (IUCN). 1994. *IUCN Red List of Threatened Species*.

JACOBS, L. L., FLYNN, L. J., DOWNS, W. R. & BARRY, J. C. 1990. Quovadis, Antemus? The Siwalik muroid record. In: *European Neogene Mammal Chronology*, (eds) E. H. Lindsay, V. Fahlbusch & P. Mein, Pp. 573-587. Plenum, New York.

JAEGER, J. J., TONG, H., BUFFETAUT, E. & INGAVAT, R. 1985. The first fossil rodents from the Miocene of Thailand and their bearing on the problem of the origin of the Muridae. *Review of Paleobiology* **4**:1-7.

JANECEK, L. L., SCHLITTER, D. A. & RAUTENBACH, I. L. 1991. A genic comparison of spiny mice, genus *Acomys*. *Journal of Mammalogy* **27**:542-552.

JONES, M & DAYAN, T. 2000. Foraging behaviour and microhabitat use by spiny mice, *Acomys cahirinus* and *A. russatus*, in the presence of Blanford's fox (*Vulpes cana*) odor. *Journal of Chemical Ecology* **26**:455-469.

KINGDON, J. 1974. *East African mammals*. An atlas of evolution in Africa. Vol. II (B): Hares and rodents, Pp. 655-658. Academic Press, London.

KRASNOV, B., SHENBROT, G., KHOKHLOVA, I. & IVANITSKAYA, E. 1996. Spatial patterns of rodent communities in the Ramon erosion cirque, Negev Highlands, Israel. *Journal of Arid Environments* **32**:319-327.

MATTHEY, R. 1968. Cytogenetique et taxonomie du genre *Acomys*. *A. pervicali* Dollmann et *A. wilsoni* Thomas, especes d' Abyssinie. *Mammalia* **32**:621-627.

MATTHEY, R. 1965. Cytogenetic mechanisms of speciations of mammals. In. *Invitro* **1**:1-11.

MEESTER, J. & SETZER, H. W. 1971. *The mammals of Africa*. An identification manual**.** Smithsonian Institution Press, Washington, D.C.

MENDELSSOHN, H. & YOM-TOV, Y. 1999. *Mammalia of Israel*. Jerusalem: The Israel Academy of Sciences and Humanities.

MISONNE, X. 1969. African and Indo –Australian Muridae: Evolutionary trends. *Annales Mus e Royal de l' Afrique Centrale, Sciences Zoologiques*. **172**:1-219.

MORRIS, D.W. 1987. Ecological scale and habitat use. *Ecology* **68**:362-369.

MUSSER, G. & CARLETON, M. 2005. Superfamily Muroidea. In: *Mammal species of the World*: A taxonomic and geographic reference, (eds) D. E. Wilson, D. M. Reeder, Pp. 901. The Johns Hopkins University Press. Baltimore and London

MUSSER, G. G. & CARLETON, M. D. 1993. Genus *Acomys*. In: *Mammal species of the world*: A taxonomic and geographic reference, (eds) D. E. Wilson, & D. M. Reeder, Pp. 564-567. Smithsonian Institution Press, Washington.

NEAL, B.R. 1983. The breeding pattern of two species of spiny mice, *Acomys percivali* and *A. wilsoni* (Muridae: Rodentia), in central Kenya. *Mammalia* **47**:311-321.

NEVO, E. 1989. Natural selection of body size differentiation in spiny mice, *Acomys*. *Zeitschrift für Säugetierkunde* **54**:81-99.

OSBORN, D.J. & HELMY, I. 1980. The contemporary land mammals of Egypt (including Sinai). *Fieldiana*: *Zoology* **5**:579.

PETTER, F. 1983. Elements d'une revision des *Acomys* africains. Un sous-genre nouveau, *Peracomys* Petter et Roche, 1981 (Rongeurs, Murides). *Annales Mus e Royal de l' Afrique Centrale, Sciences Zoologiques* **237**:109-119.

QUMSIYEH, M.B. 1996. *Mammals of the Holy Land*. Texas Technical University Press, Lubbock, Texas.

RON, U. & HAIM, A. 2001. How dehydration affects the thermoregulatory and osmoregulatory abilities of the golden spiny mouse *Acomys russatus*. *Israel Journal of Zoology* **47**:15-20.

ROSENZWEIG, M.L. 1987. Habitat selection as source of biological diversity. *Evolutionary Ecology* **1**:315-330.

ROSEVEAR, D.R.1969. *The rodents of West Africa*. Trustees of the British Museum (Natural History), London.

SARICH, V.M. 1985. Rodent macromolecular systematics. In: *Evolutionary relationships among rodents*, (eds) W. P. Luckett & J. L. Hartenberger, Pp. 423-452. Plenum Press.

SERGEI, I. A. & LEE, M. S. 1996. The Cairo spiny mouse *Acomys cahirinus* shows a strong affinity to the Mongolian gerbil *Meriones unguiculatus*. *Molecular Biology and Evolution* **13**:3-6.

SETZER, H. W. 1975. Genus *Acomys*. In: *The mammals of Africa*: An identification manual, (eds) J. Meester & H. W. Setzer, Pp. 1-2. Smithsonian Institution Press, Washington.

SHARGAL, E., KRONFELD-SCHOR, N. & DAYAN, T. 2000. Population biology and spatial

relationships of coexisting spiny mice (*Acomys*) in Israel. *Journal of Mammalogy* **81**:1046-1052.

SHENBROT, G. & KRASNOV, B. 2001. Rodents in desert environment: Is density dynamics really correlated with annual rainfall fluctuations? In: *Ecology of desert environments* (ed) I. Prakash, Pp. 405-421. Scientific Publishers, India.

SHKOLNIK, A. 1971. Diurnal activity in small desert rodent. *International Journal of Biometerology* **15**:115-120.

SHKOLNIK, A. & BORUT, A. 1969. Temperature and water relations in two species of spiny mice (*Acomys*). *Journal of Mammalogy* **50**:245-255.

SHKOLNIK, A. 1966. Studies in the comparative biology of Israel's two species of spiny mice (genus *Acomys*). PhD thesis. Hebrew University (in Hebrew, English summary), Israel.

VESEY-FITZGERALD, D.F. 1966. The habits and habitats of small rodents in the Congo River catchment region of Zambia and Tanzania. *Zoologica Africana* **2**:111-122.

VIEGAS-PÉQUIGNOT, E, DUTRILLAUX, B., PROD'HOMME, M. & PETTER, F.1983. Chromosomal phylogeny of Muridae: a study of 10 genera. *Cytogenetics and Cell Genetics* **35**:269-278.

VIEGAS-PÉQUIGNOT, E., PETIT, D., BENAZZOU, T., PROD'HOMME, M., LOMBARD, M., HOFFSCHIR, F., DESCAILLLEAUX, J. & DUTRILLAUX, B. 1986. Phylogenie Chromosomique chez les Sciuridae, Gerbillidae et Muridae, et etude d'especes appartenant a d'autres familes de ronguers *Mammalia* **50**:164-202.

VOLOBOUEV, V. T., TRANIER, M. & DUTRILLAUX, D. 1991. Chromosome evolution in the genus *Acomys*: Chromosome banding analysis of *Acomys cf. dimidiatus* (Rodentia, Muridiae). *Bonner Zoologische Beitrage* **42**:253-260.

WALKER, E.P. 1964. *Mammals of the world*, Vol.2. John Hopkins Press, Baltimore.

WILSON, A. C., OCHMAN, H. & PRAGER, E. M. 1987. Molecular time scale for evolution. *Trends in Genetics* **3**:241-24.

CHAPTER TWO

MOLECULAR PHYLOGENY OF *ACOMYS* FROM NORTHERN TANZANIA

2.1 ABSTRACT

The systematic status of *Acomys* I. Geoffroy, 1838 from northern Tanzania is uncertain. Four of the 19 currently recognized species within the genus have been reported to occur in Tanzania, namely, *A. ignitus*, *A. kempi*, *A. spinosissimus*, and *A. wilsoni*. However, the taxonomic status of *A. ignitus* from Tanzania and Kenya is debatable as it has variably been treated as a subspecies of *A. dimidiatus* and as closely related to *A. cahirinus* and *A. kempi*, such that the number of species occurring in Tanzania is currently uncertain. Consequently, DNA sequence analyses based on two independent genes composed of 1140 base pairs (bp) from the more rapidly evolving mitochondrial cytochrome *b* and 1297 bp from the slower evolving nuclear interphotoreceptor retinoid binding protein (IRBP) were used to assess the systematic status and distribution of *Acomys* in northern Tanzania. These data were subjected to phylogenetic analyses using Maximum likelihood (ML), Bayesian, Maximum parsimony (MP), and Minimum evolution (ME) analyses. The analysis of these data formed the basis for a multidisciplinary characterization of *Acomys* that also included parallel investigations involving cytogenetic, traditional and geometric morphometric analyses, and classical morphology of the same individuals. Results from ML, Bayesian, MP, and ME of both the mitochondrial cytochrome *b* and nuclear IRBP sequences were congruent and provide evidence for the occurrence of two sympatric species of *Acomys* in northern Tanzania, namely, the previously recorded *A. wilsoni* and a newly recorded *A.* cf. *percivali*. These results that also represent the first reported mitochondrial cytochrome *b* and nuclear IRBP sequences and the karyotype of *A.* cf. *percivali* are supported by the parallel investigations based on cytogenetic, traditional and geometric morphometric data, and classical morphology, and increase the number of *Acomys* species known to occur in Tanzania, from four to five. The minimum pairwise percent sequence divergence between the two species was found to be 19.6 % for the mitochondrial cytochrome *b* gene and 1.35 % for the nuclear IRBP gene. However, the mitochondrial cytochrome *b* data that included GenBank sequences from the type locality in Kenya suggest that *A. wilsoni* may not be

monophyletic, suggesting a need for further multidisciplinary investigation involving extensive sampling and examination of topotypical material.

2.2 INTRODUCTION

Molecular phylogenetic analysis is a powerful method for analysing data in systematic studies. DNA sequences are used to construct phylogenies and can also be used to distinguish morphologically indistinguishable or closely related taxa. DNA analysis can allow the tracing of an organism's evolutionary history and the elucidation of phylogenetic relationships as far back as the ancestral stock (Futuyma 1998). Apart from DNA sequences, other useful molecular data for inferring phylogenetic relationships (Avise 1994) although less frequently used, include: immunological distances (Ho *et al*. 1976), DNA:DNA hybridization (Sibley & Ahlquist 1987); protein electrophoresis (Goldman *et al*. 1987); restriction fragment length polymorphism analysis (Johnson *et al*. 1996), and amino acid sequences (Fitch & Margoliash 1970).

2.2.1 Mitochondrial DNA

Among the various DNA sequences, mitochondrial DNA (mtDNA) contains rapidly evolving genes but also has some highly conserved regions (Attardi 1985; Wolstenholme *et al*.1985; Moritz *et al*. 1987). The mtDNA genome is maternally inherited and becomes homogeneous within the population (Wilson *et al*. 1985; Avise *et al*. 1987; Moritz *et al*. 1987; Palmer 1986, 1987; Palmer *et al*. 1988). These characteristics allow for its utilization across a broad range of taxa (Kocher *et al*. 1989). The rate of nucleotide substitution in mammalian mtDNA is 5-10 times higher than nuclear DNA (Brown *et al*. 1979); hence mtDNA is suitable for studying intra- and inter-specific variation, population structure, and phylogenetic relationships (Page & Holmes 1998; Pesole *et al*. 1999).

Because mtDNA does not recombine and has a high substitution rate, it is very useful in assessing evolutionary relationships between closely related species and recently evolved taxa (Brown *et al*. 1982). However, mtDNA can become saturated by multiple substitutions that may impede its capacity to resolve some phylogenetic relationships (Irwin *et al*. 1991; Graybeal 1993; Meyer 1994; Krajewski & King 1995). Strong base composition bias (Collins *et al*. 1994; Perna & Kocher 1995) and saturation by multiple substitutions can result in a weak phylogenetic

signal, such as that from an ancient evolutionary history (Moore & DeFilippis 1997; DeFilippis & Moore 2000). However, mtDNA has a higher probability of producing haplotype trees that are congruent with species trees than is the case with nuclear genes (Moore 1995).

Within the mtDNA genome, the cytochrome *b* gene is a mitochrondrially encoded gene that represents a central catalytic sub-unit of the mitochondrial *complex III*, and is responsible for the oxidative phosphorylation system which is vital for most eukaryotic cell life. It is the most widely used mtDNA gene for inter-specific molecular taxonomy and for phylogenetic studies in mammals (Kocher *et al*. 1989; Montgelard *et al*. 1997; Prusak *et al*. 2004). In the cytochrome *b* gene, different taxonomic groups appear to have differential base preferences at certain codon positions (Irwin *et al*. 1991).

The cytochrome *b* gene has both conserved and variable regions or domains that make it suitable to assess both deep (Meyer & Wilson 1990; Irwin *et al*. 1991; Cantatore *et al*. 1994; Lydeard & Roe 1997; Kumazawa & Nishida 2000) as well as more recent (Sturmbauer & Meyer 1992; Rocha-Olivares *et al*. 1999; Kirchman *et al*. 2000; Lovejoy & de Araujo 2000) divergences. Other advantages of using the cytochrome *b* gene include presence of both slower and faster evolving regions (codon positions) (Irwin *et al*. 1991; Chikuni *et al*. 1995) and highly conserved sequence fragments across mammalian species that allows for the establishment of positional homology with unambiguous alignments (Irwin *et al*. 1991). Hence, analyses which include cytochrome *b* and other genes are highly recommended.

The D-loop represents the main control region of mammalian mtDNA. It has a strong rate of heterogeneity among sites, tandem repeats, high frequency of insertion/deletions in some parts and high lineage specificity (Saccone *et al*. 1991; Sbisà *et al*. 1997; Pesole *et al*. 1999). The rate of substitution of the D-loop's non-coding control region in humans is estimated to be 2.8-5 times higher than the rate of coding in other mtDNA genes (Aquadro & Greenberg 1982). D-loop sequencing is useful for distinguishing between closely related/recently diverged species, and more specifically, for population level studies.

However, the D-loop gene displays high variability within mammals such as rodents where enormous variability within members of the same genus (e.g., *Rattus norvegicus* and *R. rattus*), or even the same species (e.g., *Mus musculus*) has been recorded (Larizza *et al*. 2002). Consequently, the D-loop can obscure important evolutionary information due to back-mutation in which sites that have undergone substitutions return to their original state, as well as parallel substitution where mutations occur at the same site in independent lineages. It can also obscure phylogenetic information because of differences in the rate at which some sites undergo mutation (or rate heterogeneity) compared to other sites in the same region (http://actionbioscience.org/evolution/ingman).

2.2.2 Nuclear genes

Nuclear genes have a slower rate of mutation. The mutation rate of eukaryotic nuclear genes is about 2.2 x 10^{-9} mutations per base pair per year (Kumar & Subramanian 2002) and as a result, these genes have a lower possibility of homoplasy, and are therefore suitable for assessing higher level phylogenies (Prychitko & Moore 1997; Groth & Barrowclough 1999; Armstrong *et al*. 2001; Roca *et al*. 2001). Nuclear genes are recommended for confirming phylogenies derived from mtDNA (Curole & Kocher 1999; Takezaki & Gojobori 1999; Zardoya & Meyer 2001; Cotton & Page 2002). The commonly used nuclear genes for elucidating higher level systematics of mammals such as rodents include the single copy gene encoding interphotoreceptor retinoid binding protein (IRBP) (Stanhope *et al*. 1992, 1996, 1998; DeBry & Sagel 2001).

The IRBP represents a major soluble protein of the interphotoreceptor matrix (Adler & Severin 1981). It has a role in the exchange of 11-*cis* retinaldehyde and all-trans retinol, a parent compound of the vitamin A family, during the visual cycle, especially the regeneration of rhodopsin (Stenkamp *et al*. 1998; Pepperberg *et al*. 1993; Qtaishat *et al*. 2005). The IRBP gene is stable in all organisms with eyes, and homologous sequences from different species are readily generated to assess relationships among lineages (Borst & Nickerson 1988; Stanhope *et al*. 1992). It is highly conserved, where for example, the sequence divergence of its first exon (3173-3180 bp) between humans and bovines is 16 % (Borst *et al*. 1989; Fong *et al*. 1990). This, together with its large fragment size allows comparative sequences from diverse mammalian orders to be derived (Stanhope *et al*. 1992; Springer *et al*. 1997; Jansa & Wekler 2004).

Consequently, the present study is aimed at assessing the nature and extent of molecular variation based on the complete mitochondrial cytochrome *b* gene and exon 1 of the nuclear interphotoreceptor retinoid binding protein (IRBP) in an attempt to assess the systematic status of *Acomys* from northern Tanzania. This part of the study complements the cytogenetics (Chapter 3), classical morphology and traditional morphometric (Chapter 4) and geometric morphometric aspects (Chapter 5) of the multidisciplinary characterization of *Acomys* from northern Tanzania. Apart from elucidating the systematic status of *Acomys* from northern Tanzania, the use of both the cytochrome *b* and exon 1 of the nuclear IRBP genes in the present study also allowed a comparison of the phylogenies derived from the two independent genes with different mutation rates, an approach that is considered to provide the best estimate for inferring phylogenetic relationships (Pereira *et al*. 2002). The use of independent genes, gene regions or nuclear genes located on different chromosomes (Doyle 1992) is considered useful in avoiding the effects of hybridization, gene transfer, and lineage sorting (Maddison 1997) which may influence the elucidation of phylogenetic inferences.

2.3 MATERIALS AND METHODS

2.3.1 Specimens examined

Specimens of *Acomys* (n = 28) examined in the present study were collected from a wide range of habitats and topology in northern Tanzania. The collecting localities of all these specimens are presented in Fig. 2.1, while geographic coordinates and altitude of these localities are shown in Table 2.1. These specimens represent the same specimens used in the cytogenetics (Chapter 3), the traditional (Chapter 4) and geometric (Chapter 5) morphometrics aspects of the multidisciplinary characterization of *Acomys* from northern Tanzania.

Animals were live-trapped using Sherman traps (H.B. Sherman Traps Inc. Florida, U.S.A.) baited with a mixture of peanut butter, syrup, oat meal and fish oil. After capture, during transportation and in the laboratory, animals were kept in polyurethane cages with wood shavings as bedding, and with mouse pellets and water provided *ad libitum*. Animals were maintained under the guidelines of the American Society of Mammalogists (ASM; http://www.mammalogy.org/committees/index.asp; Animal Care and Use Committee 1998) and

as approved by the Animal Ethics Committee of the University of Pretoria, Pretoria, South Africa. Animals were subsequently sacrificed using halothane inhalation; standard external measurements were recorded, and the heart, liver, and kidney tissue was extracted and preserved in 96 % ethanol for subsequent molecular analysis. Voucher specimens were prepared using standard natural history museum procedures for mammal specimens and are deposited in the newly established small mammal reference collection of Sokoine University of Agriculture (SUA), Pest Management Centre, Morogoro, Tanzania.

2.3.2 DNA extraction

Genomic DNA for polymerase chain reaction (PCR; Saiki *et al*. 1985; Sambrook & Russel 2001) and the subsequent sequencing of the mitochondrial cytochrome *b* gene and nuclear interphotoreceptor retinoid binding protein (IRBP) was extracted from alcohol-preserved heart, liver, and kidney tissues using either the QIAamp DNA mini kit (Qiagen GmbH, Hilden, Germany) or the Roche DNA purification kit (Roche Diagnostics, Basel, Switzerland). DNA concentration was determined by spectrophotometer at two optical densities, namely, 260 nm and 280 nm wave length, with the ratio of the two providing an estimate of DNA purity. A ratio falling within 1.8-2.0 is most desirable as values above or below this range indicate contamination with RNA and proteins, respectively. DNA quality and quantity were also assessed by agarose gel electrophoresis (Sambrook & Russel 2001).

2.3.3 Genomic amplification using the polymerase chain reaction (PCR)

2.3.3.1 Amplification of the cytochrome *b* gene

The entire mitochondrial cytochrome *b* gene comprising 1140 bp was amplified by PCR using forward primer L14723 (5'-ACCAATGACATGAAAAATCATCGTT-3') and reverse primer H15915 (5'-TCTCCATTTCTGGTTTACAAGAC-3') (Irwin *et al*. 1991). PCR was performed on a Biometra T personal PCR machine (Biometra, Göttingen, Germany). The reaction mixture contained forward and reverse primers at a final concentration of 0.4 µM each, 0.2 mM dNTP, PCR Buffer with MgCl, 20-100 ng template DNA, REDTaq DNA polymerase (Sigma) 1 unit/µl (0.1 unit/µl per reaction volume), and water adjusted to a final volume of either 25 µl or 50 µl. The PCR temperature cycles consisted of initial denaturation at 94^{o} C for 3 min, 35 cycles of

denaturation at 94° C for 1 min, annealing at 52° C for 1 min, extension at 72° C for 90 seconds, and followed by a final extension step at 72° C for 10 min.

2.3.3.2 Amplification of the nuclear interphotoreceptor retinoid binding protein (IRBP) gene

IRBP exon 1 fragment comprising 1297 bp was amplified according to Stanhope *et al* (1992) using forward primer p217 (5'-ATG-GCC-AAG-GTC-CTC-TTG-GAT-AAC-TAC-TGC-TT-3') and reverse primer m1531 (5'-CGC-AGG-TCC-ATG-ATG-AGG-TGC-TCC-GTG-TCC-TG-3'). The PCR reaction mixture contained the forward and reverse primer at a final concentration of 0.4 µM, 0.2 mM dNTP, PCR Buffer with MgCl, 20-100 ng template DNA), REDTaq DNA polymerase (Sigma) 1 unit/µl (0.1 unit/µl in reaction volume), and water adjusted to a final volume of either 25 µl or 50 µl. PCR temperature cycles consisted an initial denaturation at 94° C for 3 min, 35 cycles of denaturation at 94° C for 1 min, annealing at 55° C for 1 min, extension at 72° C for 80 seconds, and followed by a final extension at 72° C for 10 min.

2.3.4 Purification of PCR products

The quantity and quality of the cyt-b and IRBP PCR products were determined by electrophoresis on a 1.5 % agarose gel stained with ethidium bromide. PCR products of the correct size were purified using either the GFX PCR DNA and Gel purification kit (code 27-9602-01; Amersham Biosciences, GmbH, Freiburg, Germany) or the Roche High pure PCR purification kit (Roche Diagnostics, Basel, Switzerland) according to the manufacturers' specifications. The DNA concentration of the purified PCR product was assessed by electrophoresis of 2µl of purified PCR product against 25 ng of lambda DNA. The volume of the purified PCR product was adjusted for cycle sequencing so that between 25 ng and 100 ng of template was present in the reaction.

2.3.5 Cycle sequencing

The cycle sequencing of the cytochrome *b* gene was performed with the forward (L14723) and reverse (H15915) primers separately. Sequencing of the exon 1 fragment of IRBP gene was similarly performed with both the forward primer (p217) and reverse primer (m1531) separately. Big Dye® Terminator sequencing kit, version 3.1 (ddNTP, dNTP, and enzyme) (product number 4336915, Applied Biosystems, Foster City, USA) was used for cycle sequencing on a Biometra

T gradient Thermoblock (Biometra, Göttingen, Germany). The cycle sequencing reaction consisted of 2.5X Ready reaction premix (2 µl), 5X Big Dye sequencing buffer (1 µl), 3.2 pmol of primer, 25-100 ng of purified PCR product template, and water adjusted to a final volume of 10 µl.

The cycle sequencing thermal profile consisted of an initial denaturation (rapid thermal ramp to 96° C, held at 96° C for 1 min), 25 cycles of denaturation at 96° C for 10 seconds, rapid thermal ramp to 50° C, annealing at 50° C for 5 seconds, rapid thermal ramp to 60° C and extension at 60° C for 4 min, after which the products were held at 4° C. Ethanol-precipitated cycle sequencing products were run on a Genetic Analyser 3130 (Applied Biosystems, Foster City, USA), and automatic analysis of the sequences using ABI DNA Sequencing Analysis Software version 5.2 (Applied Biosystems, Foster City, USA).

2.3.6 Sequence analysis

The forward and reverse sequences were visually inspected using Chromas version 3.2 (http://www.technelysium.com.au/chromas.html) and ambiguous sequence fragments, located primarily at the terminal ends, were removed. The complete gene sequences of both the Cyt *b* (1140 bp) and the IRBP (1297 bp) genes were obtained by alignment of the forward and reverse complement sequences.

Phylogenetic relationships among species of *Acomys* from northern Tanzania were inferred with Maximum Likelihood (ML; Muse & Gaut 1994; Nei & Kumar 2000), Bayesian analysis (Yang *et al*. 1995; Yang & Rannala 1997; Nei & Kumar 2000), Minimum Evolution (ME) and Maximum Parsimony (MP) methods. The ML analysis was undertaken using PAUP version 4.0b10 (Swofford 1998), Bayesian analysis was performed with MrBayes version 3.1.1, ME and MP analyses were run in MEGA version 3 (Kumar *et al*. 2004) using representatives of *Uranomys* and *Tatera* as outgroups.

The Tamura-Nei model of sequence evolution was used to infer an ME tree in MEGA version 3 (Kumar et *al*. 2004). Pairwise deletion of missing data/gaps and a gamma distribution shape parameter of 1.9128 for cytochrome *b* and 0.4732 for IRBP were specified. Bootstrap

resampling (1000 replicates) was performed in order to assess nodal support. In ML analysis, 100 bootstrap replicates were performed for cytochrome *b* and 250 replicates for IRBP sequences. Bayesian analysis involved running 100,000 generations with burn-in set at 300 for both the cytochrome *b* and IRBP sequences.

2.4 RESULTS

2.4.1 Sequence statistics

2.4.1.1 Mitochondrial cytochrome *b* gene sequences

Complete cytochrome *b* gene sequences (1140 bp) generated for the specimens of *Acomys* from northern Tanzania were complemented with GenBank sequences resulting in a final data set of 51 taxa. The data set comprised 418 (36.66 %) parsimony informative sites, 512 (44.91 %) variable sites, and 629 (55.17 %) conserved sites. Cyt *b* base frequencies were: A = 0.3227, G = 0.1044, T = 0.2584, and C = 0.3145.

The GTR+I+G model was selected in Modeltest version 3.06 (Posada 2000) under the Akaike Information Criterion (AIC) as the model of sequence evolution that best fitted the cytochrome *b* data. A gamma distribution shape parameter of 1.9128 and proportion of invariant sites (I) of 0.5005 was obtained. Empirically determined transition:transversion ratios (Ti/Tv) at the three codon positions (MEGA 3.0) were 1.8, 3.0, and 1.8, respectively, indicating a transitional bias, particularly at 2nd base positions.

2.4.1.2 Nuclear interphotoreceptor retinoid binding protein (IRBP) gene sequences

Nucleotide sequences of the exon 1 segment (1297 bp) of this highly conserved and slowly evolving nuclear gene were generated for 23 specimens of *Acomys* from northern Tanzania and were complemented with a further five sequences from the Genbank database. This data set of 28 taxa consisted of 76 (5.85 %) parsimony informative sites, 248 (19.12 %) variable sites, and 1024 (78.95 %) conserved sites, with IRBP base frequencies being: A = 0.2179, G = 0.2921, T = 0.2045, and C = 0.2855. The GTR+G model was selected in Modeltest version 3.06 (Posada 2000) under the Akaike Information Criterion (AIC) as the model of sequence evolution that best fitted the nuclear IRBP gene. A gamma distribution shape parameter of 0.4732 was obtained.

Transition:transversion ratio (Ti/Tv) at the three codon positions were 3.8, 1.2, and 1.4, respectively, indicating transitional bias at the 1st base position, in particular.

2.4.2 Phylogenies derived from Maximum Likelihood analysis

Maximum Likelihood analysis of the complete cytochrome *b* gene sequences (1140 bp) of species of *Acomys* from northern Tanzania (Fig. 2.2) shows the presence of two species of *Acomys*, namely, *A. wilsoni* and *Acomys* cf. *percivali*). There is a poor relationship among GenBank sequences of *A. wilsoni* such that specimens from Shimba Hills (GenBank accession number AJ010560) and Machakos (accession number AJ010561) that is near the type locality, *versus* a specimen from Marich Pass (accession number Z96045) appear to be paraphyletic (Fig.2.2). *Acomys wilsoni* from northern Tanzania is linked to the specimens from near the type locality, but with low bootstrap support (53 %) (Fig. 2.2).

Phylogenetic relationships among species of *Acomys* as inferred from the nuclear IRBP gene (exon-1 fragment 1297 bp) using ML are essentially similar to that obtained from the mitochondrial cytochrome *b* gene sequences (Fig. 2.3). However, there were no IRBP sequences for *A. wilsoni* from Kenya or elsewhere in GenBank to allow a comparison of the paraphyletic relationship observed with cytochrome *b* gene sequences.

2.4.3 Phylogenies derived from Bayesian analysis

Bayesian analysis of the mitochondrial cytochrome *b* (Fig. 2.4) and nuclear IRBP genes (Fig. 2.5) generated trees of similar topology. Both gene trees also support the occurrence of two species of *Acomys* in northern Tanzania. The two clades depicting the two species of *Acomys* are supported by high posterior probabilities (Fig. 2.4 & Fig. 2.5).

2.4.4 Phylogenies derived from Minimum evolution (ME) analysis

Four trees with only slight differences at the terminal branches that involved swapping of specimens of the same species and locality, were recovered following ME analysis of cytochrome *b* data, with the sum of the branch length (SBL) of all trees being 1.5875 (Fig. 2.6). A single tree was produced from IRBP sequences with an SBL of 0.3035 (Fig. 2.7). Both

analyses by the ME method similarly confirmed the occurrence of two species *Acomys* in northern Tanzania (Figs 2.6 & 2.7).

2.4.5 Phylogenies derived from Maximum parsimony (MP) analysis

Nine equally parsimonious trees with only slight differences at the terminal branches that involved swapping of specimens of same species and locality were produced from Cyt *b* sequences. Tree length was 1659 (Fig. 2.8), and consistency (CI) and retention (RI) indices were 0.459 and 0.810, respectively. This analysis similarly supports the occurrence of two species of *Acomys* in northern Tanzania. 110 equally parsimonious trees were produced from the IRBP gene data (Fig 9), with a tree length of 304, CI and RI of 0.878 and 0.836 respectively. The consensus tree similarly supports the existence of two species of *Acomys* from northern Tanzania (Fig. 9).

2.4.6 Geographic distributions

All the analyses in this study strongly suggest the presence of two species of *Acomys* in northern Tanzania, namely, *Acomys wilsoni* and *Acomys* cf. *percivali*. Collation of all these data suggest that the two species occur sympatrically at locality 4 (Longido) (Fig. 2.10). Longido is located on western side of Mount Kilimanjaro, which forms an arbitrary natural barrier between this site and other collection sites on the eastern side of Kilimanjaro.

2.5 DISCUSSION

The molecular study strongly suggests the occurrence of two species of *Acomys* in northern Tanzania, namely, *Acomys wilsoni* Thomas, 1892 and a species probably belonging to *A. percivali* Dollman, 1911, referred hereto as *Acomys* cf. *percivali*. CF is abbreviation for Latin word "*conferatur*" = "probably belongs to the identified species". The principle of nomenclature of the International Code of Zoological Nomenclature (ICZN 1999) is applied to the present study. The percent sequence divergence between *A. wilsoni* and *A.* cf. *percivali* is 19.6 % for mtDNA cytochrome *b* and 1.35 % for nuclear interphotoreceptor retinoid binding protein (IRBP) gene. The percent sequence divergence within *A. wilsoni* is 1.1 % $\pm$ 0.1 for mtDNA Cyt *b* and 0.57 % $\pm$ 0.12 for nuclear IRBP gene, while the percent sequence divergence for populations of *A.* cf. *percivali* is 1.4 % + 0.2 for mtDNA Cyt *b* and 0.5 % $\pm$ 0.12 for nuclear IRBP gene. These

findings show that the *Acomys* specimens from the present study belong to two different species, and the evolutionary species concept (Mayden 1997) fits well to the present molecular systematic report. This is also supported by findings from the cytogenetic aspect (Chapter 3) which show that these *Acomys* present two different diploid chromosome numbers (karyotype), hence fitting also to the biological species definition (Mayr 1949a, 1949b) which is based on non-interbreeding between populations such as these *Acomys* with different karyotypes (Chapter 3) which cannot interbreed (see also Coates & Shaw 1982).

Phylogenies derived from mtDNA Cyt *b* and IRBP sequence analyses using Maximum Likelihood, Bayesian analysis, Maximum parsimony and Minimum evolution strongly correspond with findings of cytogenetic analysis (Chapter 3), classical morphology and traditional morphometrics (Chapter 4) and geometric morphometrics (Chapter 5). All these analyses congruently show that two *Acomys* species (*A. wilsoni* and *A.* cf. *percivali*) occur in northern Tanzania. The present molecular findings are strongly supported by significant bootstrap values (>70 %) for the two clades recovered, with the high sequence divergence values being indicative of two distinct species. The phylogenies derived from Bayesian analysis are supported with high posterior probabilities (e.g. Fig. 2.4 and Fig. 2.5).

The two species occur sympatrically at Longido (Fig. 2.10), which is located on the Great East African Rift Valley. Delany & Neal (1966) and Dollman (1911, 1914) also respectively reported on the sympatric occurrence of these two species of *Acomys* in Karamoja in Uganda and Chanler Falls in Kenya.

Based on molecular analyses in the present study that included sequences from GenBank, the specimens from Rombo Alleni Chini, Lower Moshi and Longido plain correspond to those of *A. wilsoni*. These specimens are also morphologically similar to the type specimen of *A. wilsoni* from Kenya (Dollman 1911, 1914) with their tail length, head and body length, ear length and hind foot length dimensions being typical of *A. wilsoni*, and typically characterized by a short tail (Dollman 1911, 1914; Allen & Lawrence 1936; Delany & Neal 1966; Petter 1983). *Acomys wilsoni* has also been recorded from Tanga, Tanzania (Allen & Loveridge 1942) and more recently from other parts of northern Tanzania (Fadda *et al*. 2001; Corti *et al*. 2005). Tanga is

closer to Rombo Alleni Chini and Lower Moshi, and borders Mombasa, Kenya the type locality of *A. wilsoni*.

Apart from the morphological and biogeographical support alluded to above, the molecular analysis of cytochrome *b* gene supports the allocation of the above specimens to *A. wilsoni*. Their sequences correspond with GenBank sequences of *A. wilsoni* from Kenyan localities that include Marich Pass (Z96045), Shimba Hills near Mombasa (AJ010560) and Machakos (AJ010561). However, the phylogenetic relationship among these specimens is weakly supported by bootstrap analysis and their relationship appears not to be monophyletic, particularly when the Genbank sequence of what is purported to be *A. wilsoni* from Marich Pass is included (Fig. 2.2, Fig. 2.4, Fig. 2.6 and Fig. 2.8).

For example, while the relationships between *A. wilsoni* from Shimba Hills and Machakos have a strong bootstrap support (100 %), there is a weak support (35 %) between these two specimens and the '*A. wilsoni*' from Marich Pass. This raises questions on the systematic status of this latter GenBank entry. It is possible that the Marich pass specimen in GenBank may actually represent a specimen of a species that is closely related to *A. wilsoni*. Members of this genus are difficult to distinguish morphologically. For example, *A. nubilus* resembles *A. wilsoni* in body size but has a slightly longer tail (65-67 mm) (Dollman 1914), while *A. ablutus* also resembles *A. wilsoni* but is slightly smaller in body size and has a less rufous pelage colouration (Dollman 1911).

An additional influence on nodal support may be because the cytochrome *b* sequences of *A. wilsoni* from Marich Pass, Shimba Hills and Machakos differ vastly from each other in base pair sequence length. For example, the base pair sequence length of the sample from Marich Pass is short (356 bp) while Shimba Hills and Machakos are 961 bp and 1014 bp, respectively. Unlike the shorter sequence, the longer sequences fall within clades that has high bootstrap support (100 %). Such length variations may have constrained the phylogenetic analyses in the present study.

Together these results suggest the need for an in-depth, geographically encompassing multidisciplinary analysis of *A. wilsoni* that should also include specimens from the type locality.

In the interim, it may be more appropriate to exclude the Marich Pass GenBank sequence entry until its specific affiliation has been established and a full-length gene sequence generated. However, despite this questionable sequence, the general conclusions in the present study are unequivocal for the samples of this study, particularly given the congruence between phylogenies inferred from mitochondrial cytochrome *b* and nuclear IRBP genes that all strongly support the allocation of these northern Tanzanian specimens to *A. wilsoni*.

The other species of *Acomys* considered to occur in northern Tanzania may most likely be *A.* cf. *percivali*. These specimens examined in the present study are morphologically similar to the type specimen of *A. percivali* from Chanler Falls, Guaso Nyiro, Kenya (Dollman 1911, 1914), particularly in the external morphology. The specimens of *A.* cf. *percivali* in the present study emanate from a wide range of localities ranging from Tingatinga, Kilimamoja Karatu, Gelai Mountain, Longido Mountain and Ikorongo–Grumeti Game Reserve. With the exception of Ikorongo-Grumeti, all these localities are located within the Great East African Rift Valley system.

Chanler Falls, Guaso Nyiro, Kenya, the type locality *A. percivali* also falls within the Great East African Rift Valley. More importantly, *Acomys* cf. *percivali* occurs sympatrically with *A. wilsoni* at Longido, which lies approximately on the same longitude as the type locality of *A. percivali* where the two species are also reported to occur in sympatry (Dollman 1911, 1914). In addition, while *A.* cf. *percivali* occurs on the western side of Mt. Kilimanjaro, *A. wilsoni* primarily occurs on the eastern side of Mt. Kilimanjaro, which is closer to Mombasa, Kenya, its type locality. If indeed the occurrence of *A.* cf. *percivali* in northern Tanzania is valid, it represents the first record of the species in Tanzania in particular, and the first molecular data of the species ever sequenced (specifically, the mtDNA Cyt *b* and nuclear IRBP).

Ecologically, *A.* cf. *percivali* was captured in well-covered habitats with thorn bushes, rocky and mountainous areas, whereas *A. wilsoni* was mainly captured in open semi-arid grasslands as well as rice fields in Lower Moshi plains. The two species appear to be arbitrarily isolated by the complex natural barriers formed by the Great East African Rift Valley (King

1970; Dawson 1970; Quennell 1982) whose geological features have generally been associated with active rodent speciation in the region (Colangelo *et al*. 2005).

If the findings in the present study are valid, the number of species of *Acomys* recognized to occur in Tanzania increases from four to five (Musser & Carleton 1993, 2005). However, there is a need for a further multidisciplinary characterization of the putative *A*. cf. *percivali* and *A*. *wilsoni* involving extensive sampling and the examination of topotypical material.

2.6 REFERENCES

ADLER, A.J. & SEVERIN, K.M. 1981. Proteins of the bovine interphotoreceptor matrix: tissues of origin. *Experimental Eye Research* **32**:755-69.

ALLEN, G.M. & LAWRENCE, B. 1936. Scientific results of an expedition to rain forest regions in Eastern Africa. III. Mammals. *Bulletin of the Museum of Comparative Zoology* (*Harvard*) **79**:31-l26.

ALLEN, G. M. & LOVERIDGE, A. 1942. Scientific results of a fourth expedition to forested areas in East and Central Africa. I. Mammals. *Bulletin of the Museum of Comparative Zoology* (*Harvard*) **89**:205-206.

ANIMAL CARE AND USE COMMITTEE. 1998. Guidelines for the capture, handling and care of mammals as approved by the American Society of Mammalogists. *Journal of Mammalogy* **79**:1416-1431.

AQUADRO, C.F. & GREENBERG, B.D. 1982. Human mitochondrial DNA variation and evolution: Analysis of nucleotide sequences from seven individuals. *Genetics* **103**:287-312.

ARMSTRONG, M.H., BRAUN, E.L. & KIMBALL, R.T. 2001. Phylogenetic utility of avian ovomucoid intron G: A comparison of nuclear and mitochondrial phylogenies in Gallifomes. *Auk* **118**:799-804.

ATTARDI, G. 1985. Animal mitochondrial DNA. An extreme example of genetic economy. *International Review of Cytolology* **93**:93-145.

AVISE, J.C. 1994. *Molecular markers, natural history, and evolution*. Chapman and Hall, New York.

AVISE, J.C., ARNOLD, J., BALL, R.M., BERMINGHAM, E., LAMB, T., NEIGEL, J.E., REEB, C.A. & SAUNDERS, N.C. 1987. Intraspecific phylogeography: the mitochondrial bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* **18**:489-522.

BORST , D.E. & NICKERSON, J.M. 1988. The isolation of a gene encoding interphotoreceptor retinoid binding protein. *Experimental Eye Research* **47**:825-838.

BORST, D.E., REDMOND, T.M., ELSER, J.E., GONDA, M.A., WIGGERT, B., CHADER,

G.J. & NICKERSON, J.M. 1989. Interphotoreceptor retinoid binding protein: Gene characterization, protein repeat structure, and its evolution. *Journal of Biological Chemistry* **264**:1115-1123.

BROWN, W.M., GEORGE, M.Jnr & WILSON, A.C. 1979. Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Science*, USA **76**:1967-1971.

BROWN, W.M., PRAGER, E.M., WANG, A. & WILSON, A.C. 1982. Mitochondrial DNA sequences of primates: tempo and mode of evolution. *Journal of Molecular Evolution* **18**:225-239.

CANTATORE, P., ROBERTI, M., PESOLE, G., LUDOVICO, A., MILELLA, F., GADALETA, M.N. & SACCONE, C. 1994. Evolutionary analysis of cytochrome *b* sequences in some Perciformes: Evidence for a slower rate of evolution than in mammals. *Journal of Molecular Evolution* **39**:589-597.

CHIKUNI, K., MORI, Y., TABATA, T., SAITO, M., MONMA, M. & KOSUGI-YAMA, M. 1995. Molecular phylogeny based on the k-casein and cytochrome *b* sequences in the mammalian suborder Ruminantia. *Journal of Molecular Evolution* **41**:859-866.

COATES, J.D. & SHAW, D.D. 1982. The chromosomal component of reproductive isolation in the grasshopper *Caledia captive*. *Chromosoma (Berlin)* **86**:509-531.

COLANGELO, P., CORTI, M., VERHEYEN, E., ANNESI, F., OGUGE, N., MAKUNDI, R.H. & VERHEYEN, W. 2005. Mitochondrial phylogeny reveals differential modes of chromosomal evolution in the genus *Tatera* (Rodentia: Gerbillinae) in Africa. *Molecular Phylogenetics and Evolution* **35**:556-568.

COLLINS, T., WIMBERGER, P.H. & NAYLOR, G.J.P. 1994. Compositional bias, character-State bias, and character-state reconstruction using parsimony. *Systematic Biology* **43**:482-496.

CORTI, M., CASTIGLIA, R., COLANGELO, P., CAPANNA, E., BEOLCHINI, F., BEKELE, A., OGUGE, N., MAKUNDI, R.H., SICHILIMA, A.M., LEIRS, H., VERHEYEN, W. & VERHAGEN, R. 2005. Cytotaxonomy of rodent species from Ethiopia, Kenya, Tanzania and Zambia. *Belgian Journal of Zoology* **135**:197-216.

COTTON, J.A. & PAGE, R.D.M. 2002. Going nuclear: gene family evolution and vertebrate phylogeny reconciled. *Proceedings of the Royal Society of London* B **269**:1555-1561.

CUROLE, A.P. & KOCHER, T.D. 1999. Mitogenomics: digging deeper with complete mitochondrial genomes. *Trends in Ecology and Evolution* **14**:394-398.

DAWSON, J.B. 1970. Neogene rift tectonics and volcanism in northern Tanzania. *Proceedings of the Geological Society of London* **1663**:151-153.

DeBRY, R.W. & SAGEL, R.M. 2001. Phylogeny of Rodentia (Mammalia) inferred from the nuclear-encoded gene IRBP. *Molecular Phylogenetics and Evolution* **19**:290-301.

DeFILIPPIS, V.R. & MOORE, W.S. 2000. Resolution of phylogenetic relationships among recently evolved species as a function of amount of DNA sequence: an empirical study based on woodpeckers (Aves: Picidae). *Molecular Phylogenetics and Evolution* **16**:143-160.

DELANY, M.J. & NEAL B.R. 1966. A review of the Muridae (Order Rodentia) of Uganda. *Bulletin of the British Museum (Natural History) Zoology* **13**:334-335.

DOLLMAN, G. 1911. New and interesting mammals from East Africa. *Annals and Magazine of Natural History* **8**:125-128.

DOLLMAN, G. 1914. On the spiny mice of British East Africa, with a description of a new species from Magadi. *Annals and Magazine of Natural History* **8**:485-488.

DOYLE, J.J. 1992. Gene trees and species trees: Molecular systematics as a one-character taxonomy. *Systematic Botany* **17**:144-163.

FADDA, C., CASTIGLIA, R., COLANGELO, P., CORTI, M., MACHANG'U, R., MAKUNDI, R., SKANZANI, A., TESHA, P., VERHEYEN, W. & CAPANNA, E. 2001 The rodent fauna of Tanzania: A cytotaxonomic report from the Maasai Steppe (1999). *Rendiconti Fisiche Accademia Lincei* **12**:29-49.

FITCH, W. M. & MARGOLIASH, E. 1970. The usefulness of amino acid and nucleotide sequences in evolutionary studies. *Evolutionary Biology* **4**:67-110.

FONG, S.-L., FONG, W.-B., MORRIS, T.A., KEDZIE, K.M. & BRIDGES, C.D.B. 1990. Characterization and comparative structural features of the gene for human interstitial retinol-binding protein. *Journal of Biological Chemistry* **265**:3648-3653.

FUTUYMA, D.J. 1998. *Evolutionary biology*, 3rd edition. Sinauer Associates, Inc. Sunderland, Massachusetts.

GOLDMAN, D., GIRI, P.R. & O'BRIEN, S.J. 1987. A molecular phylogeny of the hominoid primates as indicated by two-dimensional protein electrophoresis.

Proceedings of the National Academy of Science, USA **84**:3307–3311.

GRAYBEAL, A. 1993. The phylogenetic utility of cytochrome *b*: lessons from bufonid frogs. *Molecular Phylogenetics and Evolution* **2**:256-269.

GROTH, J.G. & BARROWCLOUGH, G.F. 1999. Basal divergence in birds and the phylogenetic utility of the nuclear RAG-1 gene. *Molecular Phylogenetics and Evolution* **12**:115-123.

HO, C.Y.-K., PRAGER, E., WILSON, A.C., OSUGA, D.T. & FEENEY, R.E. 1976. Penguin evolution: comparisons demonstrate phylogenetic relationship to flying aquatic birds. *Journal of Molecular Evolution* **8**:271–282.

INGMAN: Investigating human evolution: internet website: http://www.actionbioscience.org/evolution/ingman.html

INTERNATIONAL COMMISSION ON ZOOLOGICAL NOMENCLATURE (ICZN). 1999. International Code of Zoological Nomenclature, 4th edition. The International Trust for Zoological Nomenclature, London

IRWIN, D.M., KOCHER, T.D. & WILSON, A.C. 1991. Evolution of the cytochrome *b* gene of mammals. *Journal of Molecular Evolution* **32**:128-144.

JANSA, S.A. & WEKSLER, M. 2004. Phylogeny of muroid rodents: relationships within and among major lineages as determined by IRBP gene sequences. *Molecular Phylogenetics and Evolution* **31**:256-276

JOHNSON, W. E., DRATCH, P. A., MARTENSON, J. S. & O'BRIEN, S. J. 1996. Resolution of recent radiations within three evolutionary lineages of Felidae using mitochondrial restriction fragment length polymorphism variation. *Journal of Mammalian Evolution* **3**:97-120.

KING, B.C. 1970. Vulcanicity and rift tectonics in East Africa. In: *African Magmatism and Tectonics*, (eds) T.N. Clifford & I.G. Gass, Oliver & Boyd, Pp. 263-267, 269-275, 281-283. Edinburgh.

KIRCHMAN, J.J., WHITTINGHAM, L.A. & SHELDON, F.H. 2000. Relationships among cave swallow populations (*Petrochelidon fulva*) determined by comparisons of microsatellite and cytochrome *b* data. *Molecular Phylogenetics and Evolution* **14**:107-121.

KOCHER, T.D., THOMAS, W.K., MEYER, A., EDWARDS, S.V., PAABO, S.,

VILLABLANCA, F.X. & WILSON, A.C. 1989. Dynamics of mitochondrial DNA evolution in mammals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Science*, USA **86**:6196-6200.

KRAJEWSKI, C. & KING, D.G. 1995. Molecular divergence and phylogeny: rates and patterns cytochrome *b* evolution in cranes. *Molecular Biology and Evolution* **13**:21-30.

KUMAR, S., TAMURA, K. & NEI, M. 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Briefings in Bioinformatics* **5**:150-163.

KUMAR, S. & SUBRAMANIAN, S. 2002. Mutation rates in mammalian genomes. *Proceedings of the National Academy of Science, USA* **99**:803-808.

KUMAZAWA, Y. & NISHIDA, M. 2000. Molecular phylogeny of Osteoglossoids: a new model for Gondwanian origin and plate tectonic transportation of the Asian arowana. *Molecular Biology and Evolution* **17**:1869-1878.

LARIZZA, A., PESOLE, G., REYES, A., SBISÀ, E., SACCONE, C. 2002. Lineage specificity of the evolutionary dynamics of the mtDNA D-Loop region in rodents. *Journal of Molecular Evolution* **54**:145-155.

LOVEJOY, N.R. & de ARAUJO, M.L.G. 2000. Molecular systematics, biogeography, and population structure of Neotropical freshwater needle fishes of the genus *Potamorrhaphis*. *Molecular Ecology* **9**:259-268.

LYDEARD, C. & ROE, K.J. 1997. The phylogenetic utility of the mitochondrial cytochrome *b* gene for inferring relationships among Actinopterygian fishes. In: *Molecular systematics of fish*, (eds) T.D. Kocher & C.A. Stepien, Pp. 285-303. Academic Press, New York.

MADDISON, W.P. 1997. Gene trees and species trees. *Systematic Biology* **46**:523-536.

MAYDEN, R. L. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. In: *Species*: *The Units of Biodiversity*, (eds) M.F, Claridge, H.A. Dawah and M.R. Wilson, Pp. 381-424. Chapman and Hall Ltd., London.

MAYR. E . 1949a. "The Species Concept: Semantics Versus Semantics," *Evolution* **3**:371-372.

MAYR. E. 1949b. "Speciation and Systematics," In: *Genetics, Paleontology, and Evolution*, (eds) G.L. Jepsen, G.G. Simpson, and E. Mayr, Pp. 281-298. Princeton University Press, Princeton.

MEYER, A. 1994. Shortcomings of the cytochrome *b* gene as a molecular marker.

Trends in Ecology and Evolution **9**:278-280.

MEYER, A. & WILSON, C. 1990. Origin of tetrapods inferred from their mitochondrial DNA affiliation to lungfish. *Journal of Molecular Evolution* **31**:359-364.

MONTGELARD, C., CATZEFLIS, F.M. & DOUZERY, E. 1997. Phylogenetic relationships of artiodactyls and cetaceans as deduced from comparison of cytochrome *b* and 12S rRNA mitochondrial sequences. *Molecular Biology and Evolution* **14**:550-559.

MOORE, W.S. & DeFILIPPIS, V.R. 1997. The window of taxonomic resolution for avian phylogenies based on mitochondrial cytochrome *b* DNA sequences. In: *Avian molecular and systematics,* (ed) D.P. Mindell, Pp. 83-119. Academic Press, San Diego.

MOORE, W.S. 1995. Inferring phylogenies from mtDNA variation: mitochondrial gene trees versus nuclear gene trees. *Evolution* **49**:718-726.

MORITZ, C., DOWLING, T.E. & BROWN, W.M. 1987. Evolution of animal mitochondrial DNA: implications for population biology and systematics. *Annual Review of Ecology and Systematics*. **18**:269-292.

MUSE, S. V. & GAUT, B. S. 1994. A likelihood approach for comparing synonymous and nonsynonymous nucleotide substitution rates, with application to the chloroplast genome. *Molecular Biology and Evolution* **11**:715-724.

MUSSER, G. & CARLETON, M. 2005. Superfamily Muroidea. In: *Mammal species of the World*, (eds) D. E. Wilson & D. M. Reeder. The John Hopkins University Press, Baltimore and London.

MUSSER, G. G. & CARLETON, M. D. 1993. *Genus Acomys*. In: *Mammal species of the world,* (eds) D. E. Wilson, & D. A. M. Reeder, Pp. 564-567. Smithsonian Institution Press, Washington.

NEI, M. & KUMAR, S. 2000. *Molecular evolution and phylogenetics*, Pp. 207-209, Oxford University Press, UK.

PAGE, R.D.M. & HOLMES, E.C. 1998. *Molecular evolution*. A phylogenetic approach. Blackwell Science Ltd, UK.

PALMER, J.D. 1986. Chloroplast DNA and phylogenetic relationships. In: *DNA systematics*, (ed) S. K. Dutta, Pp. 63-80. CRC Press, Boca Raton, FL.

PALMER, J.D, 1987. Chloroplast DNA evolution and biosystematic uses of chloroplast DNA variation. *American Naturalist* **130**:S6-S29.

PALMER, J.D., JANSEN, R.K., MICHAELS, H.J., CHASE, M.W. & MANHART, J.R. 1988. Chloroplast DNA and plant phylogeny. *Annals of the Missouri Botanical Garden* **75**:1180-1206.

PEPPERBERG, D.R., OKAJIMA, T.L., WIGGERT, B., RIPPS, H., CROUCH, R.K. & CHADER, G.J.1993. Interphotoreceptor retinoid-binding protein (IRBP). Molecular biology and physiological role in the visual cycle of rhodopsin. *Molecular Neurobiology* **7**:61-85.

PEREIRA, S.L., BAKER, A.J. & WAJNTAL, A. 2002. Combined nuclear and mitochondrial DNA sequences resolve genetic relationships within the Cracidae (Galliformes, Aves). *Systematic Biology* **51**:946-958.

PERNA, N.T. & KOCHER, T. 1995. Unequal base frequencies and estimation of substitution rates. *Molecular Biology and Evolution* **12**:359-361.

PESOLE, G., GISSI, C., De CHIRICO, A. & SACCONE, C. 1999. Nucleotide substitution rate of mammalian mitochondrial genomes. *Journal of Molecular Evolution* **48**:427-434.

PETTER, F. 1983. Elements d'une revision des *Acomys* africains. Un sous-genre nouveau, *Peracomys* Petter et Roche, 1981 (Rongeurs, Murides). *Annales Mus e Royal de l'Afrique Centrale, Sciences Zoologiques* **237**:109-119.

POSADA, D. 2000.Testing models of evolution - Modeltest Version 3.06. Brigham Young University,USA.

PRUSAK, B., GRZYBOWSKI, G. & ZIEBA, G. 2004. Taxonomic position of *Bison bison* (Linnaeus, 1758) and *Bison bonasus* (Linnaeus, 1758) based on analysis of C*yt b* gene. *Animal Science Papers and Reports* **22**:27-35.

PRYCHITKO, T.M. & MOORE, W.S. 1997. DNA sequences of an intron from β-fibrinogen gene in phylogenetic analysis of woodpeckers (Aves: Picidae). *Molecular Phylogenetics and Evolution* **8**:193-204.

QTAISHAT, N.M., WIGGERT, B., PEPPERBERG, D.R. 2005. Interphotoreceptor retinoid-binding protein (IRBP) promotes the release of all-trans retinol from the isolated retina following rhodopsin bleaching illumination. *Experimental Eye Research* **81**:455-463.

QUENNELL, A.M. 1982. Rift Valleys. Afro-Arabian. Hutchinson Ross Publishing Company, Stroudsburg, Pennsylvania.

ROCA, A.L., GEORGIADIS, N., PECON-SLATTERY, J. & O'BRIEN, S.J. 2001. Genetic

evidence for two species of elephant in Africa. *Science* **293**:1473-1477.

ROCHA-OLIVARES, A., ROSENBLATT, R.H. & VETTER, R.D. 1999. Molecular evolution, systematics, and zoogeography of rockfish subgenus *Sebastomus* (*Sebastes*, Scorpaenidae) based on mitochondrial cytochrome *b* and control region sequences. *Molecular Phylogenetics and Evolution* **11**:441-458.

SACCONE, C., PESOLE, G. & SBISÀ, E. 1991. The main regulatory region of mammalian mitochondrial DNA: structure-function model and evolutionary pattern. *Journal of Molecular Evolution* **33**:83-91.

SAIKI, R. K., SCHARF, S., FALOONA, F., MULLIS, K. B., HORN, G. T., ERLICH, H.A. & ARNHEIM, N. 1985. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* **230**:1350-1354.

SAMBROOK, J. & RUSSEL, D.W. 2001. *Molecular cloning*: *A laboratory manual*, 3rd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

SBISÀ, E., TANZARIELLO, F., REYES, A., PESOLE, G. & SACCONE, C.1997. Mammalian mitochondrial D-loop region structural analysis: identification of new conserved sequences and their functional and evolutionary implications. *Gene* **205**:125-140.

SIBLEY, C.G. & AHLQUIST, J.E. 1987. DNA Hybridization evidence of hominoid phylogeny: results from an expanded data set. *Journal of Molecular Evolution* **26**:99-121.

SPRINGER, M.S., BURK, A., KAVANAGH, J.R., WADDELL, V.G. & STANHOPE, M.J. 1997. The interphotoreceptor retinoid binding protein gene in therian mammals: implications for higher level relationships and evidence for loss of function in the marsupial mole. *Proceedings of the National Academy of Science, USA* **94**:13754-13759.

STANHOPE, M.J., CZELUSNIAK, J., SI. J.-S., NICKERSON, J. & GOODMAN, M. 1992. A molecular perspective on mammalian evolution from the gene encoding interphotoreceptor retinoid binding protein, with convincing evidence for bat monophyly. *Molecular Phylogenetics and Evolution* **1**:148-160.

STANHOPE, M.J., MADSEN, O., WADDELL, V.G., PORTER, C.A., SHIVJI, M.S. & GOODMAN. M. 1996. Mammalian evolution and the interphotoreceptor retinoid binding protein (IRBP) gene: Convincing evidence for several superordinal clades. *Journal of Molecular Evolution* **43**:83-92.

STANHOPE, M.J., MADSEN, O., WADDELL, V.G., CLEVEN, G.C., de JONG, W.W. &

SPRINGER, M.S. 1998. Highly congruent molecular support for a diverse superordinal clade of endemic African mammals. *Molecular Phylogenetics and Evolution* **9**:501-508.

STENKAMP, D.L., CUNNINGHAM, L.L., RAYMOND, P.A. & GONZALES-FERNANDEZ, F. 1998. Novel expression pattern of interphotoreceptor retinoid – binding protein (IRBP) in adult and developing zebrafish retina and RPE. *Molecular Vision* **4**:26-39.

STURMBAUER, C. & MEYER, A. 1992. Genetic divergence, speciation, and morphological stasis in lineage of African cichlid fishes. *Nature* (London) **359**:578-581.

SWOFFORD, D. L. 1998. PAUP*: Phylogenetic analysis using parsimony (and other methods). Version 4. Sinauer Associates, Sunderland, Mass.

TAKEZAKI, N. & GOJOBORI, T. 1999. Correct and incorrect vertebrate phylogenies obtained by the entire mitochondrial DNA sequences. *Molecular Biology and Evolution* **16**:590-601.

WILSON, A.C., CANN, R.L., CARR, S.M., GEORGE, M. Jnr, GYLLENSTEN, U.B., HELM-BYCHOWSKI, K., HIGUCHI, R.C., PALUMBI, S.R., PRAGER, E.M., SAGE, R.D. & STONEKING, M. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. *Biological Journal of the Linnean Society* **26**:375-400.

WOLSTENHOLME, D.R., CLARY, D.O., MacFARLANE, J.L., WAHLEITHNER, J.A. & WILCOX, L. 1985. Organization and evolution of invertebrate mitochondrial genomes. In: *Achievements and perspectives of mitochondrial research*, (eds) E. Quagliariello, E.C. Slater, F. Palmieri, C. Saccone & A.M. Kroon, Pp. 61-69. Elsevier Press, Amsterdam.

YANG, Z., KUMAR, S. & NEI, M. 1995. A new method of inference of ancestral nucleotide and amino acid sequences. *Genetics* **141**:1641-1650.

YANG, Z. & RANNALA, B. 1997. Bayesian phylogenetic inference using DNA sequences: A Markov chain Monte Carlo method. *Molecular Biology and Evolution* **14**:717-724.

ZARDOYA, R. & MEYER, A. 2001. Vertebrate phylogeny: limits of inference of mitochondrial genome and nuclear rDNA sequence data due to an adverse phylogenetic signal/noise ratio. In: *Major events in early vertebrate evolution*, (ed) P.E. Ahlberg, Pp.135-156. Taylor and Francis, London.

2.7 FIGURE LEGENDS

Fig. 2.1 Map of Tanzania showing collecting localities of *Acomys* examined in this study: 1 = Lower Moshi (Kahe Chekeleni); 2 = Rombo–Alleni Chini; 3 = Tingatinga; 4 = Mt. Longido and Longido plains; 5 = Mt. Gelai-Olikisima; 6 = Ikorongo–Grumeti Game Reserve; 7 = Kilimamoja view-point, Lake Manyara National Park. The dotted line denotes Mt. Kilimanjaro which separates some of these localities, while the insert indicates the geographic position of Tanzania in relation to the African continent.

Fig. 2.2 Cytochrome *b* gene tree for species of *Acomys* from northern Tanzania inferred from complete gene sequences (1140 bp) using the Maximum Likelihood method and GTR+G + I model of evolution. Clade A corresponds to *Acomys wilsoni* and clade B corresponds to *Acomys* cf. *percivali*. The bootstraps support for clade A comprising animals from northern Tanzania alone is 99 % whereas clade B is supported by 100 % of the bootstrap replicates.

Fig. 2.3 Relationships of species of *Acomys* from northern Tanzania inferred from exon-1 fragment (1297 bp) of interphotoreceptor retinoid binding protein (IRBP) gene using the Maximum Likelihood method with GTR+G model of sequence evolution. Clade A (100 % bootstrap support) indicates *Acomys wilsoni* and clade B (86 % bootstrap support) corresponds to *Acomys* cf. *percivali*.

Fig. 2.4 Cytochrome *b* gene tree of species of *Acomys* from northern Tanzania obtained from Bayesian analysis of complete nucleotide gene sequences (1140 bp). Values at each internal node correspond to posterior probabilities. Clade A corresponds to *A. wilsoni* and clade B corresponds to *A.* cf. *percivali*.

Fig. 2.5 Interphotoreceptor retinoid binding protein (IRBP) gene tree of species of *Acomys* from northern Tanzania obtained from Bayesian analysis of nuclear IRBP gene (1297 bp). The values

at each internal node correspond to posterior probabilities. Clade A corresponds to *Acomys wilsoni* and clade B represents *Acomys* cf. *percivali*.

Fig. 2.6 A representative cytochrome *b* gene tree showing the relationships between species of *Acomys* from northern Tanzania inferred from minimum evolution (ME) analysis with Tamura-Nei model of sequence evolution. Clade A corresponds to *Acomys wilsoni* and clade B to *Acomys* cf. *percivali*. Four equally good trees were produced, with a sum of branch length (SBL) = 1.5875. Bootstrap support for each clade is 100 %.

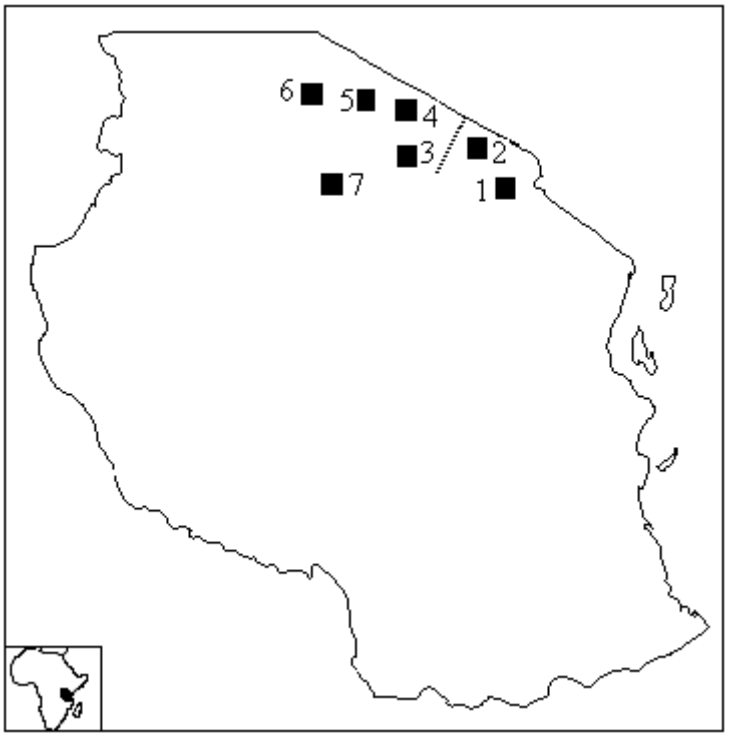
Fig. 2.7 Interphotoreceptor retinoid binding protein (IRBP) gene tree of species of *Acomys* from northern Tanzania based on ME analysis of exon 1 fragment (1297 bp) of nuclear IRBP gene with the Tamura-Nei model of sequence evolution. Clade A corresponds to *Acomys wilsoni* and clade B represents *Acomys* cf. *percivali*. A single tree was produced with a sum branch length (SBL) = 0.3035. The bootstrap support for clade A is 95 % and clade B is 73 %.

Fig. 2.8 Maximum parsimony phylogenetic tree of species of *Acomys* from northern Tanzania based on analysis of the mitochondrial Cyt *b* gene. Clade A corresponds to *Acomys wilsoni* and clade B represents *Acomys* cf. *percivali*. Nine equally good trees were produced with tree a length of 1659, CI = 0.459 and RI = 0.810. The two clades A and B show a strong bootstrap support of 99 % each.

Fig. 2.9 Bootstrap consensus phylogenetic tree of species of *Acomys* from northern Tanzania based on maximum parsimony analysis of nuclear IRBP gene data. Clade A corresponds to *Acomys wilsoni* and clade B corresponds to *Acomys* cf. *percivali*. 110 equally good trees were produced with a tree length of 304, CI = 0.878 and RI = 0.836. The bootstrap support for clade A is 99 % and for clade B is 81 %.

Fig. 2.10 The geographic distribution of *Acomys wilsoni* and *A*. cf. *percivali* in northern Tanzania based on specimens analysed in the present study using molecular data. The two species occur sympatrically at locality 4 (Longido). Locality 1 = Lower Moshi; 2 = Rombo Alleni Chini; 3 = Tingatinga; 4 = Mt. Longido and Longido plain; 5 = Mt. Gelai-Olikisima; 6 =

Ikorongo-Grumeti Game Reserve; 7 = Kilimamoja view point–Karatu. Localities 1 and 2 are closer to *A*. *wilsoni* type locality (Mombasa, Kenya), whereas localities with *A*. cf. *percivali* are located south of type locality of *A*. *percivali* (Chanler Falls, Guaso Nyiro, Kenya). Dotted line indicates an arbitrary boundary between localities with *A*. *wilsoni* on eastern side of Mount Kilimanjaro and localities with *A*. cf. *percivali* on the western side.



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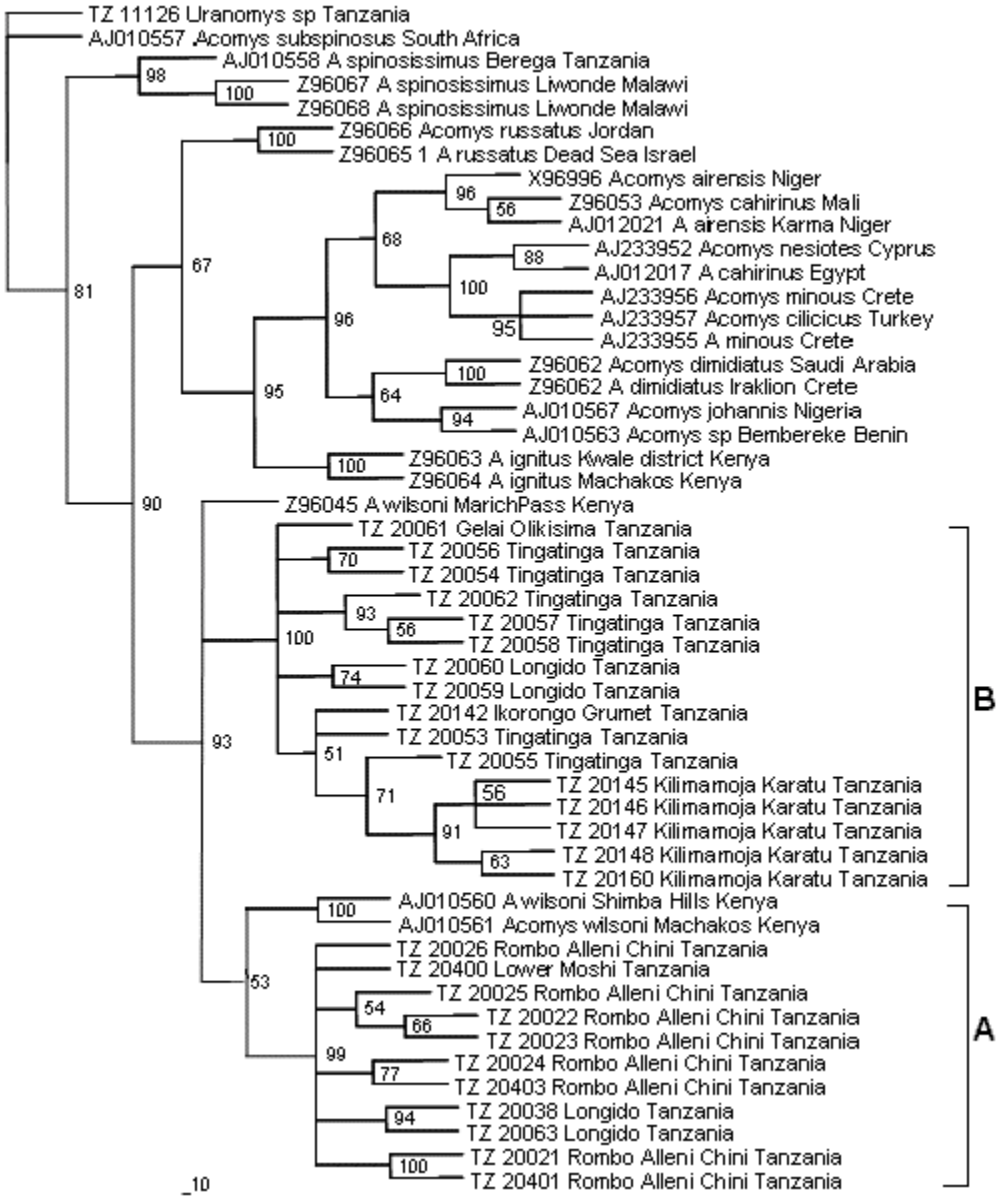
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Fig. 2.2

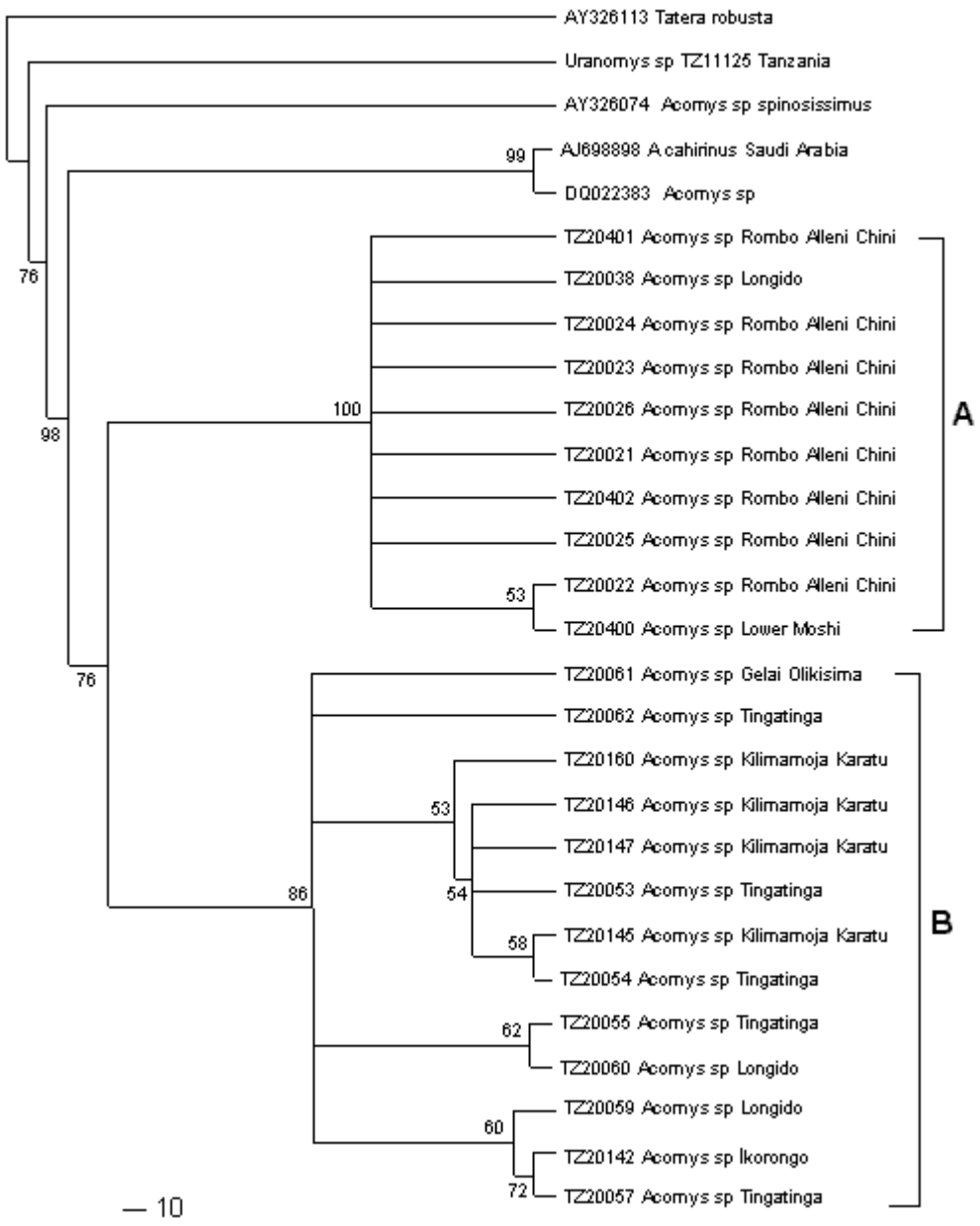
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Fig. 2.3

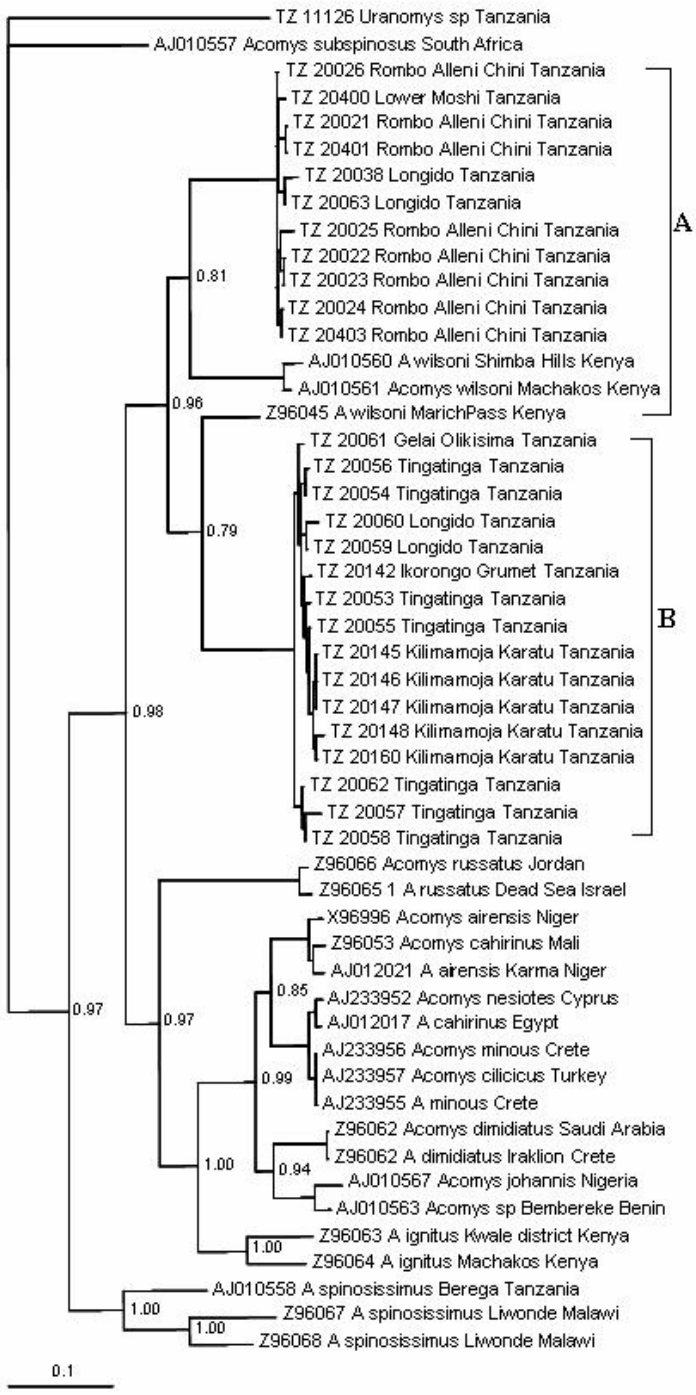
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Fig. 2.4

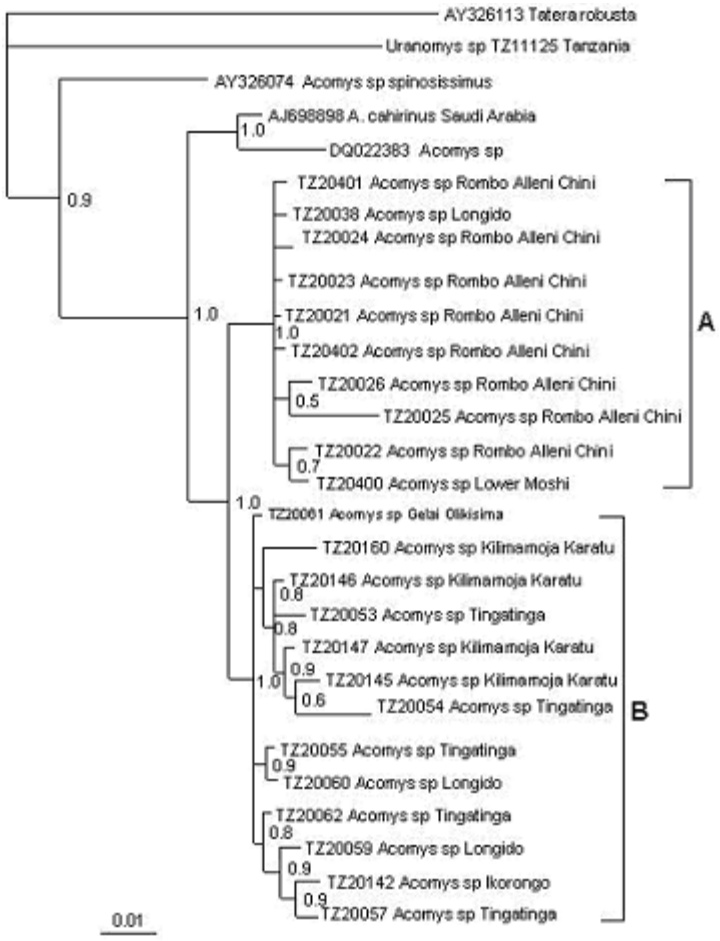
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Fig. 2.5

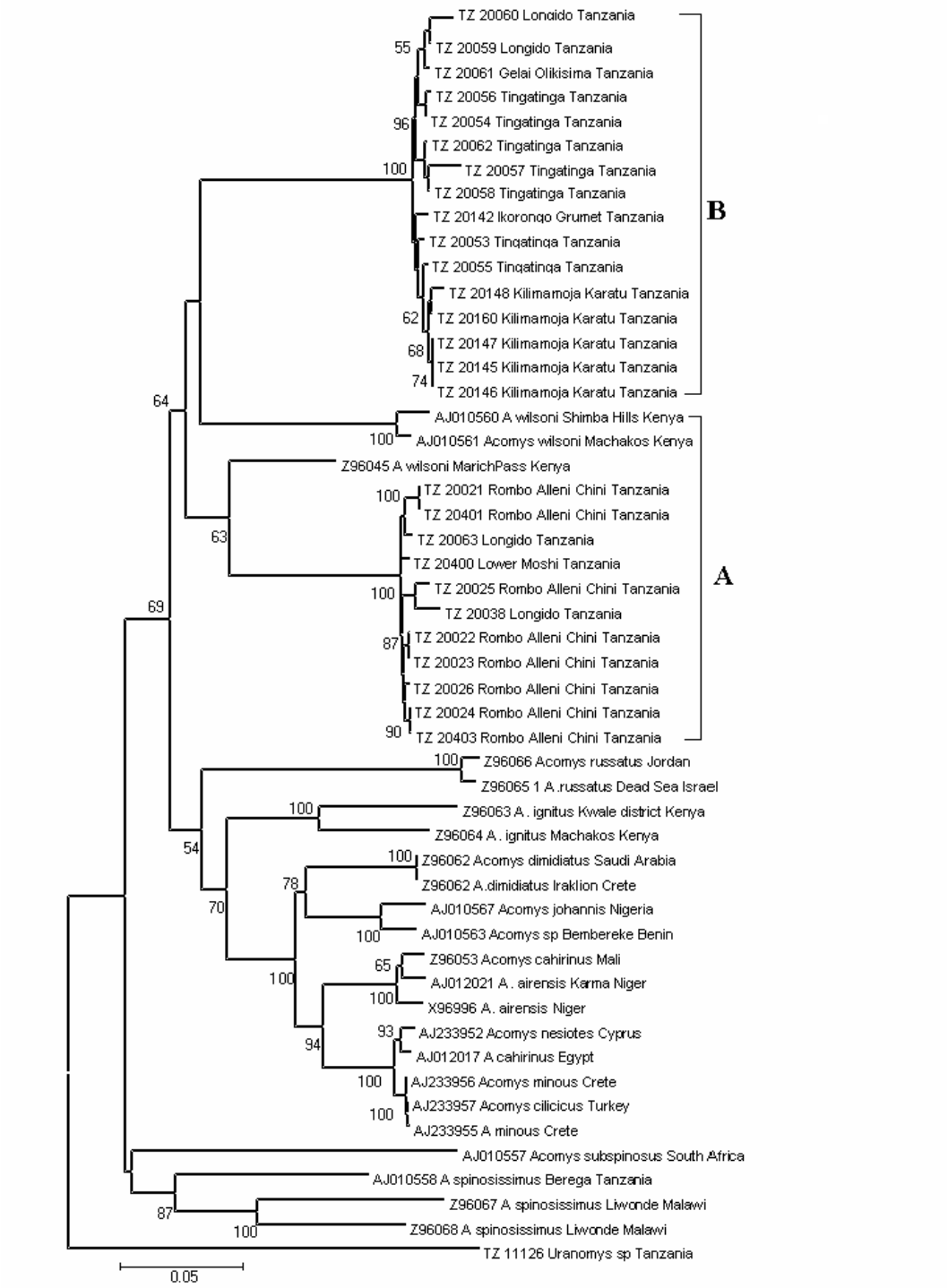
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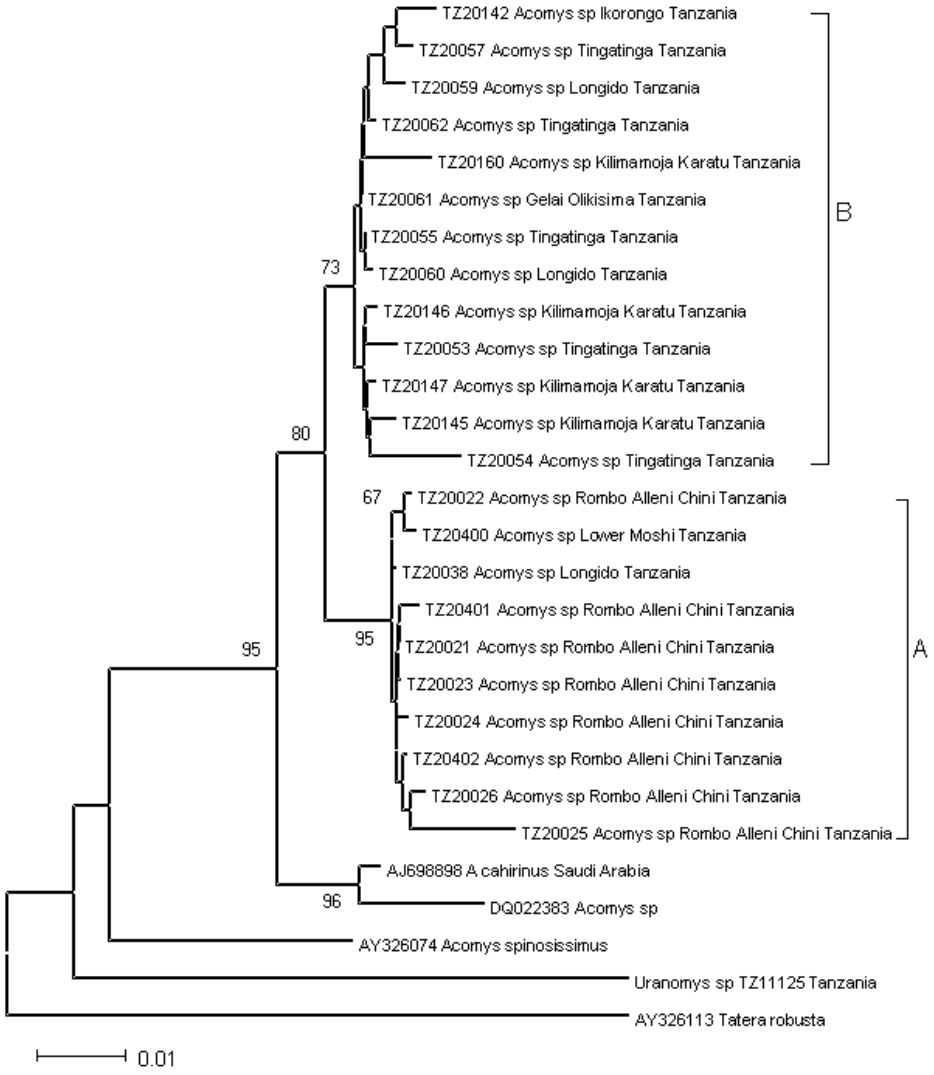
Fig. 2.6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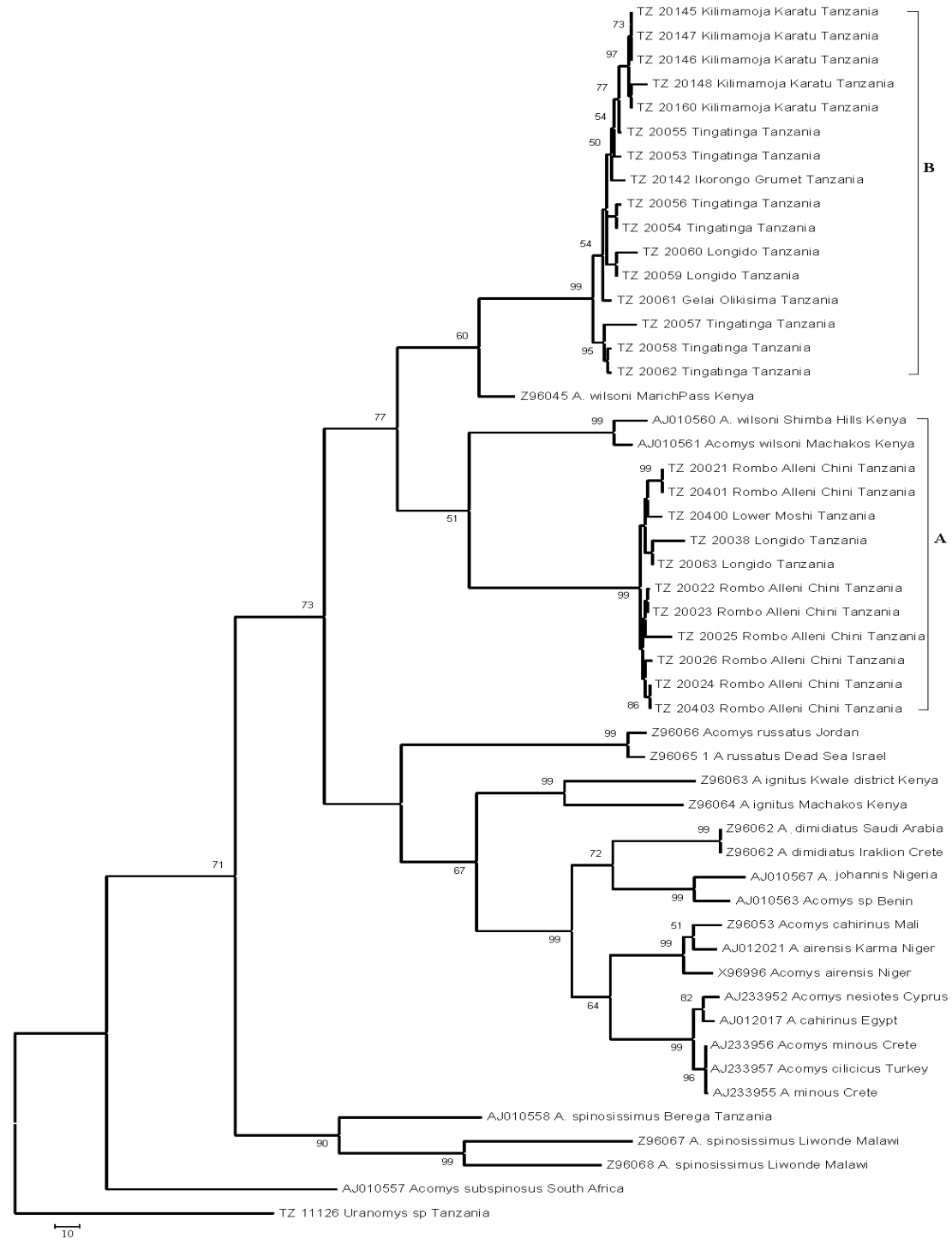
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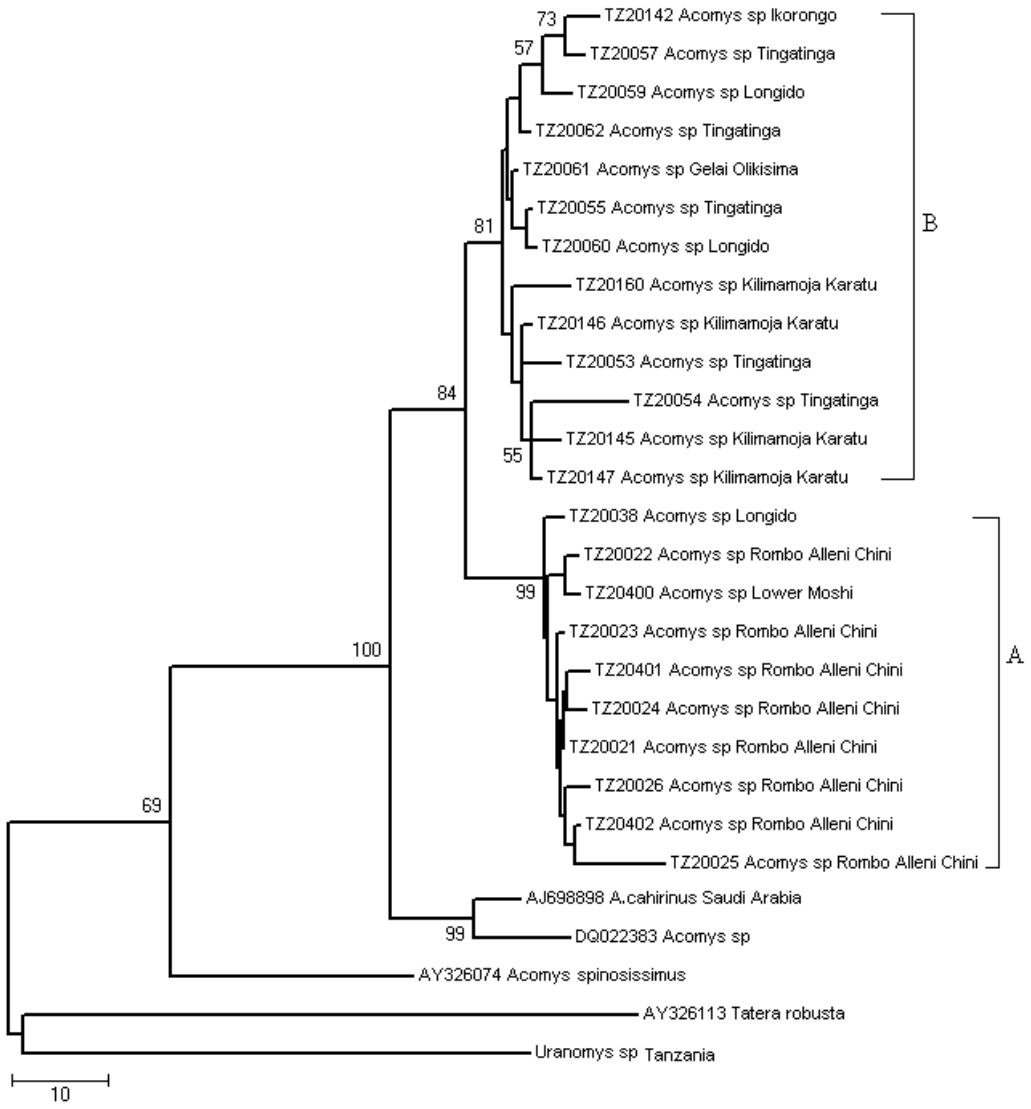
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100
69
99
B
A
10

Fig. 2.9

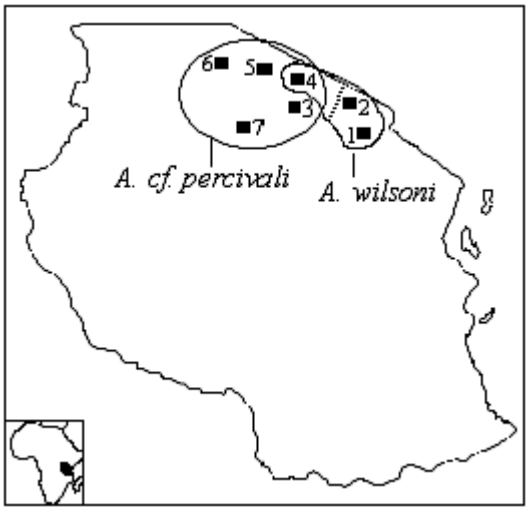

Fig. 2.10

Table 2.1 Collecting localities, a gazetteer and altitude of specimens of *Acomys* from northern Tanzania examined in the present study. Numbers correspond to localities in Fig. 2.1.

	Locality	Geographic Coordinates and Altitude
1	Lower Moshi, eastern Kilimanjaro	03.41S; 37.37E; 750 m a.s.l
2	Rombo–Alleni Chini, eastern Kilimanjaro	03.24081S; 037.68139E; 1037 m a.s.l.
3	Tingatinga, western Kilimanjaro	02.96169S & 02.96810S; 036.95261E & 036.94566E; 1210 & 1215 m a.s.l
4	Longido Mountain, east of Lake Natron, western Kilimanjaro	02.71276S; 036.68261E; 1398 m a.s.l
	Longido plain–Irmibokuni–Engosipa or Olekibari on rift valley east of Lake Natron, western side of Mt. Kilimanjaro	02.70563S, 036.60299E; 457 m a.s.l
	Longido plain–Parmunyan east of Lake Natron, western Kilimanjaro	02.70693S; 036.54184E; 1447 m a.s.l.
5	Mt. Gelai-Olikisima – East of Lake Natron	02.70367S; 036.11255E; 1427 m a.s.l.
6	Ikorongo–Grumeti Game Reserve, west of Serengeti National Park	02.10707S; 034. 47665E; 1414 m a.s.l.
7	Kilimamoja view point, Lake Manyara National Park along the Great East African Rift valley	03.38696S; 035.82093E; 1214 m a.s.l.

CHAPTER THREE

CYTOGENETIC ANALYSIS OF *ACOMYS* FROM NORTHERN TANZANIA

3.1 ABSTRACT

Trypsin-Giemsa-Banding of metaphase chromosomes was used to assess the systematic status and geographic distribution of *Acomys* from northern Tanzania. Currently, four species of *Acomys*, namely; *A. spinosissimus* ($2n$ = 60), *A. wilsoni* ($2n$ = 62), *A. ignitus* ($2n$ = 36) and *A. kempi* have been reported to occur in Tanzania. However, the results of the Trypsin-G-Banding provide evidence for the occurrence of two sympatric species of *Acomys* in northern Tanzania, namely, the previously recorded *A. wilsoni* with a diploid number of $2n$ = 62 and a newly recorded *A.* cf. *percivali* with a diploid number of $2n$ = 58. These results that also represent the first reported karyotype of *A.* cf. *percivali* from Tanzania are supported by the parallel investigations based on molecular, traditional and geometric morphometric data, and classical morphology of the same individuals, and increases the number of recognized species of *Acomys* known to occur in Tanzania from four to five. However, the karyotype of *A.* cf. *percivali* ($2n$ = 58) suggest that its northern Tanzanian populations may have lost a pair of chromosomes via Robertsonian translocation, leading to karyotypic differences with the only reported karyotype of *A. percivali* from Ethiopia with a diploid number of $2n$ = 60. Karyotypic variation attributed to Robertsonian translocation has also previously been reported in other *Acomys* species such as *A. cahirinus* from the Middle East which has a karyotype of either $2n$ = 36 or $2n$ = 38. However, the results in the present study need further multidisciplinary investigation which incorporates other cytogenetic techniques such as fluorescence *in situ* hybridization (FISH) and also involving extensive sampling and the examination of topotypical material.

3. 2 INTRODUCTION

Cytogenetics is the study of chromosomes that includes determining the diploid number or karyotype, morphology (i.e., shape and size), behaviour, and activity status, which may be useful for inferring evolutionary processes (Bickham & Baker 1979; Key 1968; White 1978b), and is governed by the conventions of the International System for Human Cytogenetic Nomenclature (ISCN 1985, 1995). The range of chromosome number or karyotype is very wide. For example,

the lowest recorded haploid chromosome number is 2 reported in the iceryine coccid insect (*Steatococcus tuberculatus*) while the highest is 220 recorded in the small blue lycaenid butterfly (*Lysandra atlantica*) (Macgregor 1993).

It has been established that organisms with high chromosome number have smaller chromosomes and smaller genomes, while those with relatively few chromosomes have larger genomes such that an increase in chromosome number corresponds with a decrease in genome size (Grant 1963; Macgregor 1993). On the other hand, a reduction in chromosome size in a species correlates with an increase in specialisation (Grant 1963). Consequently, primitive species such as plants have fewer chromosomes and are less specialised (Grant 1963). Animals with more telocentric karyotypes such as rodents are also considered to be ancestral species (Volobouev *et al*. 1988; Castiglia *et al*. 2002).

3.2.1 Variation in chromosome number

Chromosome number may vary within a species due to several factors (Fukuda & Chanell 1975). For example, the octodontid rodent, *Ctenomys talarum talarum* has nine karyotypes (Massarini *et al*. 2002) while some mole rat species of the family Spalacidae have over 30 karyotypes (Savic & Nevo 1990; Yuksel 1984; Nevo *et al*. 1994). Variation in karyotypes within a species indicates either chromosomal evolution or active speciation and adaptation of a species to various conditions (Wahrman *et al*. 1969; White 1973, 1978b; Nevo *et al*.1994; Coskun 2004; Colangelo *et al*. 2005).

Karyotypes evolve through chromosomal pericentric and paracentric inversions, translocations, deletions, and duplications (Macgregor 1993). Chromosomal inversions arise when the chromosome breaks into two parts with a subsequent 180^{o} turn or through the switching of the segments where the chromosome breaks. Inversions may lead to reproductive isolation (Coates & Shaw 1982) and speciation as has been reported to underlie the formation of two rodent species, *Peromyscus crinitus* and *P. leucopus* (Arrighi *et al*. 1976).

Translocations, the most common form of karyotypic evolution in mammals, involve the fusion or exchange of genetic material between chromosomes arising from either the physical

removal or the breakage of a segment in one chromosome and its attachment to the end of either another homologous or non-homologous chromosome (Hsu 1979). It results in the reduction of the diploid number but not the fundamental number or NF, an abbreviation derived from the Latin words "*nombre fundamentale*". If the breakage of the long arm occurs away from the centromere, the centromere part may survive with the diploid number remaining the same but the fundamental number increases (Hsu & Mead 1968).

Robertsonian translocations are considered to be the cause of karyotypic variation within species of rodents (Matthey 1965; Yuksel 1984; Savic & Nevo 1990; Nevo *et al*. 1994; Massarini *et al*. 2002). For example, the African pygmy mouse of the genus *Leggada* has a diploid number (2*n*) of between 18–36 bi-armed and acrocentric chromosomes, which decrease in different populations with a parallel increase in the number of bi-armed chromosomes (Matthey 1965). Similarly, in Alpine valley *Mus musculus* and the Tobacco mouse, *M. poschiavinus* have 2*n* = 40 telocentrics, but due to translocations, *M. poschiavinus* for example, has 2*n* = 22 and 2*n* = 26 (Gropp *et al*.1970; Capanna *et al*. 1973).

Deletions involve the loss of either the whole or part of a chromosome. There are terminal deletions that involve a breakage and loss of the end of a chromosome and interstitial deletions in which a chromosome loses its material from the region between the ends. Some deletions are considered to be beneficial and these include those that involve non-coding gene sequences or multi-gene families. However, deletions involving structural or coding genes such as the deletion associated with the loss of the short arm of chromosome 5 in humans, and which causes the *Cri du chat* syndrome, are fatal (Lejeune *et al*. 1963).

Duplications occur when a fragment or a sequence in the chromosome, usually a non-coding sequence is presented more than once (or duplicated) yielding additional genetic material while the chromosome number remains the same. Hence, karyotypes with duplications are recognized by the presence of repetitive DNA sequences. Duplication is mainly due to unequal crossing-over between two chromatids and the breakage of chromosomes resulting in one chromatid gaining excess genetic material and other losing genetic material (= deletion). Duplication induces karyotypic evolution especially when it involves either non-coding or non-

structural genes. Kondrashov *et al*. (2002) have recently reported that duplicated genes have a greater chance of evolving faster than unduplicated genes.

Cytogenetics is useful for distinguishing between morphologically similar taxa. For example, five species of rodents within the genus *Taterillus*, which were traditionally considered a single species based on morphology, are currently considered to represent separate species based on karyotypic analysis (Dobigny *et al*, 2002a, 2002b). Similarly, some species of rodents within the genus *Tatera* were conventionally separated into different forms because of difficulties in differentiating them due to morphological similarity (Bates 1985; Davis 1975). Their taxonomic status has recently been clarified using cytogenetic data (Colangelo *et al*. 2005).

In addition, the available evidence has shown that the genus *Acomys* has undergone an active karyotype evolution which has led to complications in morphologically differentiating some of the species within the genus (Zahavi & Wahrman 1956; Matthey 1963; Volobouev *et al*. 1996a, 1996b), strongly suggesting that karyotypic analysis is necessary for this group of rodents. For example, on morphological grounds, *Acomys dimidiatus* ($2n$ = 38; NF = 70) from Arabia and *A. airensis* ($2n$ = 42; NF = 68) from Niger have previously been treated as subspecies (Setzer 1975), but recent cytogenetic analysis supports their taxonomic recognition as two valid species (Volobouev *et al*. 1991).

3.2.2 Cytogenetic methods

Common cytogenetic methods include Giemsa (G-) banding (Sumner *et al*. 1971; Patil *et al*. 1971), Centromeric (C-) banding (Evans *et al*. 1973), Quinacrine (Q-) banding (Caspersson *et al*. 1969, 1970; Paris conference 1971), Reverse (R-) banding (Dutrillaux & Lejeune 1971), and Fluorescence *in situ* hybridization (FISH; Pardue & Gall 1970; Trask 1991; Korenberg *et al*. 1992). G-banding is the most commonly used method (Sumner *et al*.1971; Patil *et al*.1971; Drets & Shaw 1971) and forms the basis of the internationally agreed system for human cytogenetic nomenclature (ISCN 1985) which also includes information from Q-, R-, and C-banding. It involves the staining of a chromosome's metaphase preparation using Giemsa solution where dark G-bands of the stained chromosome indicate a high A-T content, and negative or unstained regions of the chromosome indicate a high G-C content (Hsu 1979). G-banding is considered to

be the most superior and reliable method for mammalian karyotypes (Macgregor 1993). It allows the precise identification of all chromosome arms as well as specific and very short segments of chromosome arms. However, although it works well with mammalian chromosomes, it has been shown to be less useful for the chromosomes of other organisms (Macgregor 1993).

Centromeric (C-) banding involves the denaturing of a chromosome on a flame, incubating in saline and staining with Giemsa (Sumner *et al*. 1971; Patil *et al*. 1971). This results in each centromere being differentially stained to allow the differentiation of chromosomes based on the amount of densely staining material in the centromere region (Evans *et al*. 1973).

Quinacrine dihydrochloride is used to treat metaphase chromosomes which are then visualized under fluorescent microscope. The metaphase chromosomes show characteristic fluorescent segments or bands of various degrees of brightness (Caspersson *et al*. 1969, 1970; Paris Conference 1971). Q-banding is the quickest method of differentiating the extra Y chromosome and an extra chromosome 21 in humans, which are morphologically similar and indistinguishable with conventional staining (Macgergor 1993). The long arm of Y chromosome fluoresces intensely unlike the extra 21 chromosome.

Reverse (R-) banding involves the heating of chromosome preparations in a hot phosphate buffer and staining with Giemsa to induce chromosome cross-banding leading to a banding pattern that is the reverse of that obtained by G-banding (Dutrillaux & Lejeune 1971; Paris conference 1971). In essence, a darkly stained region in a G-band preparation is lightly stained in an R-band preparation, and vice versa. R-banding is considered to be useful for resolving unclear chromosomal terminal segments of human karyotypes after using G- and Q-banding (Hsu 1979). Modified R-banding makes use of Acridine Orange fluorescence treatment to slides that have been treated in phosphate buffered solution (Macgregor 1993). This modified R-banding technique uses the constant and asynchronous pattern of replication of chromosomes therefore allowing a high resolution and the identification of chromosomes in species with chromosomes that may not stain well with G-banding, as has been shown in the African clawed frog, *Xenopus laevis* (Macgregor 1993).

Fluorescence *in situ* hybridization (FISH) is an advancement of *in situ* hybridization (Pardue & Gall 1970) where a fluorescently labelled DNA probe is hybridized *in situ* to the DNA of either an interphase nucleus or a metaphase chromosome fixed on the slide. The fluorescent DNA probe that involves either a labelled DNA sequence or segments binds to the complementary sequence on the target chromosome and becomes visible as a fluorescing body (Trask 1991; Korenberg *et al*. 1992). FISH yields the highest specificity, sensitivity, resolution and greatest chance of uncovering chromosomal rearrangements (Trask 1991; Korenberg *et al*. 1992). Although the technique is expensive, it is considered to be relatively economical in terms of time (Korenberg *et al*. 1992; Trask 1991; Macgregor 1993).

Among the various cytogenetic techniques outlined above, the present investigation made use of G-banding to assess the nature and extent of chromosomal variation in an attempt to assess the systematic status of *Acomys* from northern Tanzania. This part of the study complements the molecular (Chapter 2), traditional (Chapter 4) and geometric (Chapter 5) morphometrics aspects of the multidisciplinary characterization of *Acomys* from northern Tanzania. The basis for choosing G-banding method in the present study included availability of reagents, cost- and time effectiveness of the method and the ease of comparisons with previous cytotaxonomic studies on *Acomys* in Tanzania and elsewhere. Currently, there are only four karyotypes of *Acomys* species described from Tanzania, namely; *A. spinosissimus* ($2n = 60$), *A. wilsoni* ($2n = 62$), *A. ignitus* ($2n = 36$) and *A.* cf. *selousi* ($2n = 59$) (Fadda *et al*. 2001; Corti *et al*. 2005). Of these species, the systematic status of *A.* cf. *selousi* described on the basis of chromosomal analysis is controversial (Corti *et al*. 2005) and further supports a need for multidisciplinary assessment of the systematic status of *Acomys*.

3.3 MATERIALS AND METHODS

3.3.1 Specimens examined

Specimens of *Acomys* ($n = 22$) examined in the present study were collected from a wide range of habitats, topology, and climate in northern Tanzania. The collecting localities of all these specimens that included Lower Moshi ($n = 1$), Rombo Alleni Chini ($n = 7$), Tingatinga ($n = 5$), Longido ($n = 3$), Mt. Gelai-Olikisima ($n = 1$), and Kilimamoja view point ($n = 5$) are presented in Fig. 3.1, while geographic coordinates and altitude of these localities are shown in Table 3.1.

Apart from specimens from Ikorongo–Grumeti Game Reserve (Locality 6) that were not available for the cytogenetic analysis in this study, all these specimens represent the same specimens used in the molecular (Chapter 2), the traditional (Chapter 4) and geometric (Chapter 5) morphometrics aspects of the multidisciplinary characterization of *Acomys* from northern Tanzania.

Animals were live-trapped using Sherman traps (H.B. Sherman Traps Inc. Florida, U.S.A.) baited with a mixture of peanut butter, syrup, oat meal and fish oil. After capture, during transportation and in the laboratory, animals were kept in polyurethane cages with wood shavings as bedding, with mouse pellets and water provided *ad libitum*. Animals were maintained under the guidelines of the American Society of Mammalogists (ASM; http://www.mammalogy.org/committees/index.asp; Animal Care and Use Committee 1998) and as approved by the Animal Ethics Committee of the University of Pretoria, Pretoria, South Africa. Animals were subsequently sacrificed using halothane inhalation, and standard external measurements were recorded. Heart, liver, and kidney tissue was dissected out and preserved in 96 % ethanol for subsequent molecular analysis (see Chapter 2). Voucher specimens were prepared for morphometric analysis and classical morphological examination (see Chapters 4 and 5) using standard natural history museum procedures for mammal specimens and were deposited in the newly established small mammal reference collection of Sokoine University of Agriculture (SUA), Pest Management Centre, Morogoro, Tanzania.

3.3.2 Preparation of metaphase chromosomes

Karyotypes in this study were prepared from bone marrow using the splash technique (Hsu & Patton 1969; Hills & Moritz 1990) with slight modifications. Mitotic metaphase chromosomes were induced by injecting live animals with an active yeast suspension and were arrested using anti-mitotic Velbe (vinblastine sulphate) (Tjio & Levan 1956; Macgregor & Varley 1983; Watt & Stephen 1986). This included the yeast suspension being prepared by mixing 3 g of dry yeast (*Saccharomyces cerevisiae*; S.I. Lesaffre 59703 Marcq France), 2 g of glucose (dextrose monohydrate B.P; Chemi & Cotex, DSM, Tanzania), and 12 ml sterile distilled water.

Live specimens of *Acomys* were sub-cutaneously injected with an active yeast dosage of 0.1 ml for every 25 g of body weight. Twenty-four hours later, the animals were injected with 0.01 ml of anti-mitotic Velbe (Lylly, France) per every 2 g of body weight, with metaphase arresting occurring in the 30-45 min post-inoculation period, following which the animals were sacrificed using halothane inhalation. Hind limb bones were aseptically removed and placed in petri dishes containing 5-7 ml of pre-warmed (37^{o} C) hypotonic Potassium Chloride solution (0.075M KCl) where they were crushed and incubated at 37^{o} C for 30-40 min. While avoiding bone and tissue particles, the cell suspension was pipetted into clean centrifuge tubes, centrifuged at 2000-2900 rpm for 10 min, and the supernatant carefully removed without disturbing the pellet.

5-7 ml of ice-cold 3:1 fixative (3 parts methanol and 1 part glacial acetic acid) was slowly added through a slanted tube wall to avoid disturbing the pellet. Fixation was carried out in a freezer for 20 min. The pellet was then broken by pipetting up and down and centrifuged at 2000-2900 rpm for 10 min. The supernatant was discarded and fresh ice-cold fixative (5-7 ml) added to dissolve the pellet. The suspension was re-centrifuged at 2000-2900 rpm for 10 min, with this step being repeated twice. Finally, 3 ml of fresh ice-cold fixative was added to the final pellet and dispensed into small aliquots and kept frozen at -20^{o} C until used. Cell density was checked by microscopic examination of splash slides prepared using absolute ethanol-cleaned ice-cold slides. Slide preparation involved placing slides on a flat bench and 2-4 drops of fixed chromosome suspension being splashed onto slides, in duplicate, from a height of about 0.5 m. The slides were air-dried and stored in a refrigerator for later use.

3.3.3 Trypsin-Giemsa-banding

Chromosomal G-banding was enhanced with trypsin treatment. The slides containing chromosome preparations were aged at either 60^{o} C for 24 hrs or at 95^{o} C for 20 min. Slides were immersed in fresh trypsin working solution (0.025 %) for 15 seconds and trypsinisation was stopped by rinsing the slides with phosphate buffered saline (PBS, pH 6.8). The slides were then flooded with Giemsa working solution (4 ml of Gurr's Giemsa stock solution and 96 ml of phosphate buffered saline, pH 6.9) for 10 min (Gustashaw 1991), rinsed in PBS, drained and dried at 60^{o} C. Chromosomes were examined at x1000 magnification under the transmitted light

microscope (Nikon Optiphot, Tokyo, Japan) and photographs of the metaphase chromosomes taken using a digital camera (Nikon DXM1200, Tokyo, Japan) linked to a desktop computer.

3.3.4 Karyotyping

The karyotype (diploid number) of the 22 specimens examined in this study was established manually by arranging chromosome pairs based on banding patterns and length in descending order. An average of five metaphase spreads were examined for each specimen and representative spreads compared.

3.4 RESULTS

Chromosomal G-banding analyses showed that two species of *Acomys*; *A. wilsoni* and *A.* cf. *percivali* occur in northern Tanzania with diploid numbers of $2n = 62$ and $2n = 58$, respectively (Figs. 3.2 and 3.3). Nine of the 22 specimens of *Acomys* examined had a diploid number of $2n = 62$ (Table 3.2). This includes all specimens from Lower Moshi (Locality 1; see Fig. 3.1), Rombo Alleni Chini (Locality 2) and some specimens from Longido (Locality 4) that were identified as *A. wilsoni* by the molecular and morphometric analyses as well as classical morphology (Chapters 2, 4, 5).

The karyotype of the remaining 13 specimens of *Acomys* was $2n = 58$ (Table 3.2). This includes all specimens from Tingatinga (Locality 3; see Fig. 3.1), Mt. Gelai-Olikisima (Locality 5) and Kilimamoja (Locality 7), and some specimens from Longido (Locality 4), that were similarly identified as *Acomys* cf. *percivali* by the molecular, morphometric and classical morphological approaches (Chapters 2, 4, 5).

Specimens examined from Longido had both cytotypes represented ($2n = 58$, $n = 2$; $2n = 62$, $n = 1$) (Table 3.2). These results confirm that the two species are sympatric in this region of northern Tanzania. The sympatric occurrence of the two species together with their karyotypic differences is confirmed by the molecular (Chapter 2) and morphometric analyses (Chapters 4 and 5) as well as classical morphology (Chapter 4). However, it was not possible in the present investigation to assess the chromosomal status of each species such as chromosomal pericentric

and paracentric inversions, translocations, deletions, and duplications due to the lack of clarity in the derived karyotypes.

3.5 DISCUSSION

The cytogenetic analysis in the present study suggest the occurrence of two species of *Acomys* in northern Tanzania, namely *A. wilsoni* and *A.* cf. *percivali*. The karyotype identified to represent *A. wilsoni* ($2n = 62$) was typical in specimens from Lower Moshi, Rombo Alleni Chini and a specimen from Longido. A karyotype of $2n = 62$ has also been reported in specimens identified to represent *A. wilsoni* from adjacent localities in Same district, Kilimanjaro, Tanzania (Fadda *et al.* 2001; Corti *et al.* 2005). The cytogenetic identity of these specimens as representing specimens of *A. wilsoni* is supported by the results of the molecular (Chapter 2), the traditional (Chapter 4) and geometric (Chapter 5) morphometrics as well classical morphological aspects of the multidisciplinary characterization of *Acomys* from northern Tanzania.

The karyotype identified as representing *A.* cf. *percivali* ($2n = 58$) was typical in specimens from Tingatinga, Gelai-Olikisima, and Kilimamoja-Karatu, located south of Chanler Falls, Guaso Nyiro, Kenya, the type locality of *A. percivali* (Dollman 1911, 1914). Similarly, the cytogenetic identity of these specimens as representing specimens of *A.* cf. *percivali* was supported by molecular analyses, traditional and geometric morphometrics, as well as classical morphology (Chapters 2, 4 and 5).

The published karyotype of *Acomys percivali* based on a specimen from Omo River, Ethiopia (Matthey 1968), which is located north of Chanler Falls, Guaso Nyiro, Kenya, the type locality of *A. percivali* has been reported to be $2n = 60$. Besides the possibility of Ethiopian ($2n = 60$) and Tanzanian ($2n = 58$) karyotypes representing different species, this study indicated that it may also be karyotypic variation within *A. percivali*. Of particular relevance is that the northern Tanzanian localities investigated in the present study and Omo River, Ethiopia, are all located within the Great East African Rift Valley that has been associated with rodent speciation in East Africa. This suggests that the observed karyotypic variation could be due to either chromosomal evolution or active speciation and/or adaptation to different environmental conditions (see

Wahrman *et al*. 1969; White 1973, 1978b; Nevo *et al*. 1994; Coskun 2004; Colangelo *et al*. 2005).

One possible mechanism for the observed decrease in diploid number of chromosomes from $2n = 60$ to $2n = 58$ could involve a single chromosomal change through a Robertsonian translocation which has been reported to be common in rodents (Yuksel 1984; Savic & Nevo 1990; Nevo *et al*. 1994; Massarini *et al*. 2002). Differences in chromosome diploid numbers that have been attributed to a Robertsonian event have for example, been reported in other species of *Acomys* such as in *A*. *cahirinus* which has a diploid number of either $2n = 38$ for Israel or $2n = 36$ for Sinai populations (Wahrman & Goitein 1972). However, such chromosomal changes or events could not be evaluated due to a lack of clarity in the derived karyotypes indicating a need for further investigation using alternative techniques such as FISH.

The cytogenetic analysis in the present study indicates that two species of *Acomys* from northern Tanzania occur in sympatry at the Longido locality (Fig. 3.1) where specimens with both the $2n = 62$ and $2n = 58$ karyotypes, and *A*. *wilsoni* and *A*. cf. *percivali* mitotypes were found. This sympatric occurrence is also evident in the traditional (Chapter 4) and geometric (Chapter 5) morphometric analyses as well classical morphology based on the same specimens that were analysed cytogenetically. The sympatric occurrence of *A*. *wilsoni* and *A*. *percivali* has been reported at localities in the neighbouring Kenya and Uganda (Dollman 1911, 1914; Delany & Neal 1966; Matthey 1968).

Apart from their sympatric occurrence, the two species seem to partition their niches. For example, while *A*. *wilsoni* was captured in open savanna grassland in Longido Plain, *A*. cf. *percivali* was captured *ca*. 13 km away on Longido Mountain. Furthermore, while *A*. *wilsoni* was captured in semi-arid grasslands in Rombo Alleni Chini and rice fields in Lower Moshi, *A*. cf. *percivali* was collected from well-covered habitats such as thorn bushes, and rocky and mountainous areas. In overgrazed areas such as Tingatinga, *A*. cf. *percivali* was collected around patches of thorny trees, wild sisal species, and thorny fences surrounding grassy areas reserved for grazing cattle calves. Such habitat partitioning corroborates previous findings with other species of *Acomys* (Shkolnik 1966; Shkolnik & Borut 1969; Shkolnik 1971; Jones & Dayan

2000; Shargal *et al*. 2000). For example, the sympatric occurrence of *A*. *wilsoni* and *A*. *percivali* in Longido corresponds with findings of the same two species at Chanler Falls, Guaso Nyiro, Kenya, the type locality of *A*. *percivali* (Dollman 1911, 1914). Both the type locality and Longido are located on the Great East African Rift Valley.

If indeed the occurrence of the two species in northern Tanzania is valid, then the number of species reported to occur in Tanzania increases from four (Musser & Carleton 1993, 2005) to five and these include: *A*. *spinosissimus*, *A*. *wilsoni*, *A*. *ignitus*, *A*. *kempi*, and *A*. cf. *percivali*. Nevertheless, there is a critical need for a further multidisciplinary characterization of the putative *A*. cf. *percivali* and *A*. *wilsoni* involving extensive sampling and the examination of topotypical material.

3.6 REFERENCES

ANIMAL CARE AND USE COMMITTEE. 1998. Guidelines for the capture, handling and care of mammals as approved by the American Society of Mammalogists. *Journal of Mammalogy* **79**:1416-1431.

ARRIGHI, F.E., STOCK, A.D. & PATHAK, S. 1976. Chromosomes of *Peromyscus* (Rodentia, Cicetidae). V. Evidence of pericentric inversions. In: *Chromosomes Today*, (eds) P.L, Pearson & K.R, Lewis, Pp. 323-329. John Wiley & Sons, New York.

BATES, P.J.J. 1985. Studies of gerbils of genus *Tatera*: the specific distinction of *Tatera robusta* (Cretzschmar, 1826), *Tatera nigricauda* (Peters, 1978) and *Tatera phillipsi* (De Winton, 1898). *Mammalia* **49**:37-52.

BICKHAM, J.W. & BAKER, R.J. 1979. Canalization model of chromosome evolution. In: *Models and methodologies in evolutionary theory*, (eds) J.H. Swartz & H.G. Rollins. *Bulletin of Carnegie Museum of Natural History* **13**:70-84.

CAPANNA, E., CIVITELLI, M.V. & CRISTALDI, M. 1973. Chromosomal polymorphism in an Alpine population of *Mus musculus* Linnaeus. *Bulletin of Zoology* **40**:379-383.

CASPERSSON, T., ZECH, L., MODEST, E.J., FOLEY, G.E. & WAGH, U. 1969. Chemical differentiation with fluorescent alkylating agents in *Vicia faba* metaphase chromosomes. *Experimental Cell Research* **58**:128-140.

CASPERSSON, T., ZECH, L., & JOHANSON, C. 1970. Differential banding of alkylating fluorochromes in human chromosomes. *Experimental Cell Research* **60**:315-319.

CASTIGLIA, R., FADDA, C., CORTI, M., SCANZANI, A., VERHEYEN, W. & CAPANNA, E.2002. Chromosomal evolution in the African Arvicanthine rats (Murinae, Rodentia): comparative cytogenetics of *Lemniscomys* (*L. zebra*, *L. rosalia*, *L. striatus*) and *Arvicanthis dembeensis*. *Journal of Zoological Systematics and Evolutionary Research* **40**:223-231.

COATES, J.D. & SHAW, D.D. 1982. The chromosomal component of reproductive isolation in the grasshopper *Caledia captive*. *Chromosoma (Berlin)* **86**:509-531.

COLANGELO, P., CORTI, M., VERHEYEN, E., ANNESI, F., OGUGE, N., MAKUNDI, R.H. & VERHEYEN, W. 2005. Mitochondrial phylogeny reveals differential modes of chromosomal evolution in the genus *Tatera* (Rodentia: Gerbillinae) in Africa. *Molecular Phylogenetics and Evolution* **35**:556-568.

CORTI, M., CASTIGLIA, R., COLANGELO, P., CAPANNA, E., BEOLCHINI, F., BEKELE, A., OGUGE, N., MAKUNDI, R.H., SICHILIMA, A.M., LEIRS, H., VERHEYEN, W. & VERHAGEN, R. 2005. Cytotaxonomy of rodent species from Ethiopia, Kenya, Tanzania and Zambia. *Belgian Journal of Zoology* **135**:197-216.

COSKUN, Y. 2004. A new chromosomal form of *Nannospalax ehrenbergi* from Turkey. *Folia Zoologica* **53**:351-356.

DAVIS, D.H.S. 1975. Genera *Tatera* and *Gerbillurus*. In: *The mammals of Africa*: An identification manual, (eds). J. Meester & H.W. Setzer, Parts 1-15.1971-1977. Smithsonian Institution Press, Washington.

DELANY, M.J. & NEAL B.R. 1966. A review of the Muridae (Order Rodentia) of Uganda. *Bulletin of the British Museum (Natural History) Zoology* **13**:334-335.

DOBIGNY, G., BAYLAC, M., & DENYS, C. 2002a. Geometric morphometrics, neutral networks and diagnosis of sibling *Taterillus* (Rodentia, Gerbillinae) species. *Biological Journal of the Linnean Society* **77**:319-327.

DOBIGNY, G., ANISKIN, V. & VOLOBOUEV, V. 2002b. Explosive chromosome evolution and speciation in the gerbil genus *Taterillus* (Rodentia, Gerbillinae): a case of two new cryptic species. *Cytogenetics and Genome Research* **96**:117-124.

DOLLMAN, G. 1911. New and interesting mammals from East Africa. *Annals and Magazine of Natural History* **8**:125-128.

DOLLMAN, G. 1914. On the spiny mice of British East Africa, with a description of a new species from Magadi. *Annals and Magazine of Natural History* **8**:485-488.

DRETS, M.E. & SHAW, M.W. 1971. Specific banding patterns of human chromosomes. *Proceedings of the National Academy of Science,* USA **68**:2073-2077.

DUTRILLAUX, B. & LEJEUNE, J. 1971. Sur une nouvelle technique d'analyse du caryotype humain. *Comptes Rendus de l'Academie des Sciences de Paris* **272**:2638-2640.

EVANS, H.J., BUCKLAND, R.A. & SUMNER, A.T. 1973. Chromosome homology and heterochromatin in goat, sheep and ox, studied by banding techniques. *Chromosoma* (Berlin) **42**:383-402.

FADDA, C., CASTIGLIA, R., COLANGELO, P., CORTI, M., MACHANG'U, R., MAKUNDI,

R., SKANZANI, A., TESHA, P., VERHEYEN, W. & CAPANNA, E. 2001. The rodent fauna of Tanzania: A cytotaxonomic report from the Maasai Steppe (1999).*Rendiconti Fisiche Accademia Lincei* **12**:29-49.

FUKUDA, I. & CHANELL, R. B. 1975. Distribution and evolutionary significance of chromosome variation in *Trillium ovatum*. *Evolution* **29**:257-266.

GRANT, V. 1963. *The origin of adaptation*. Columbia University Press, New York, London.

GROPP, A., TETTENBORN, U. & LEHMANN, E. von. 1970. Chromosomenvariation vom Robertsonischen Typusber der Tabakmaus *M. poschiavinus*, und ibren Hybriden mit der Laboratoriumsmaus. *Cytogenetics* **9**:9-23.

GUSTASHAW, K.M. 1991. Chromosome stains. In: *The ACT cytogenetics laboratory Manual*, (ed) M. J. Barch, 2nd edition. The Association of cytogenetic technologists, Raven Press Ltd., New York.

HILLS, D. M. & MORITZ, C. 1990. *Molecular systematics*. Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts.

HSU, T.C. & PATTON, J.L. 1969. Bone marrow preparation for chromosomes studies. In: *Comparative mammalian cytogenetics*, Pp.454-460. Springer, Berlin.

HSU, T.C. & MEAD, R.A. 1968. Mechanisms of chromosomal changes in mammalian speciation. In: *Comparative Mammalian Cytogenetic,* (ed) K. Benirschke, Pp.8-17. Springer-Verlag, New York.

HSU, T.C. 1979. *Human and mammalian cytogenetics*. an historical perspective. Springer Verlag, New York.

ISCN. 1985. An International System for Human Cytogenetic Nomenclature. Birth Defects. *Original Article Series* **21**:1.

ISCN. 1995. An International System for Human Cytogenetic Nomenclature, (ed) F. Mitelman. S. Karger, Basel.

JONES, M. & DAYAN, T. 2000. Foraging behaviour and microhabitat use by spiny mice, *Acomys cahirinus* and *A. russatus*, in the presence of Blanford's fox (*Vulpes cana*) odor. *Journal of Chemical Ecology* **26**:455-469.

KEY, K.H.L. 1969. The concept of stasipatric speciation. *Systematic Zoology* **17**:14-22.

KONDRASHOV, F. A., ROGOZIN, I. B., WOLF, Y. I. & KOONIN, E.V. 2002. Selection in the evolution of gene duplications. *Genome Biology* **3**:1–9.

KORENBERG, J.R., YANG-FENG, T., SCHRECK, R. & CHEN, X.N. 1992. Using fluorescence in situ hybridization (FISH) in genome mapping. *Trends in Biotechnology* **10**:27-32.

LEJEUNE, J., LAFOUREADE, J., BERGE, R., VAILATTE, J., BOESWILLWALD, M.,SERINGE, P. & TURPIN, R. 1963. Trois cas de deletion partielle du bras court d'un chromosome 5. *Comptes Rendus Hebdomadaire des Seances de l'Academie des Sciences* **257**:3098-3102.

MACGREGOR, H.C. 1993. *An introduction to animal cytogenetics*. Chapman & Hall, London.

MACGREGOR H. & VARLEY, J. 1983. *Working with animal chromosomes*. Wiley, New York.

MASSARINI, A., MIZRAHI, D., TIRANTI, S., TOLOZA, A., LUNA, F. & SCHLEICH, C.E. 2002. Extensive chromosomal variation in *Ctenomys talarum talarum* (Rodentia: Octodontidae) from the Atlantic coast of Buenos Aires province, Argentina. *Journal of Neotropical Mammalogy* **9**:199-207.

MATTHEY, R. 1968. Cytogenetique et taxonomie du genre *Acomys*. *A. pervicali* Dollman et *A*. *wilsoni* Thomas, especes d' Abyssinie. *Mammalia* **32**:621-627.

MATTHEY, R. 1965. Cytogenetic mechanisms of speciations of mammals. In: *Invitro* **1**:1-11.

MATTHEY, R. 1963. Polymorphismes chromosomique intraspecifique et intraindividuel chez *A*. *minous* Bate (Mammalia, Rodentia, Muridae). *Chromosoma* **14**:468-497.

MUSSER, G. & CARLETON, M. 2005. Superfamily Muroidea. In: *Mammal species of the World*, (eds) D. E. Wilson & D. M. Reeder. The Johns Hopkins University Press. Baltimore and London.

MUSSER, G. G. & CARLETON, M. D. 1993. Genus *Acomys*. In: *Mammal species of the world*, (eds) D. E. Wilson, & D. A. M. Reeder, Pp. 564-567. Smithsonian Institution Press, Washington.

NEVO, E., FILIPPUCCI, M.G. & BEILES, A. 1994. Genetic polymorphism in subterranean mammals (*Spalax ehrenbergi* superspecies) in the neareast revisited. Patterns and theory. *Heredity* **72**:65-487.

PARDUE, M.L. & GALL, J.G. 1970. Chromosomal localization of mouse satellite DNA. *Science* **168**:1356-1358.

PARIS CONFERENCE 1971. Standardization in human cytogenetics. Birth defects original article series **8**:1-46. The National Foundation-March of Dimes, New York.

PATIL, S.R., MERRICK, S. & LUBS, H.A. 1971. Identification of each human chromosome with a modified Giemsa stain. *Science* **173**:821-822.

SAVIC, I. & NEVO, E. 1990. The Spalacidae: Evolutionary history, speciation, and population biology. In: *Evolution of subterranean mammals at the organismal and molecular levels,* (eds) E. Nevo & O.A. Reig, Pp. 129-143. Alan R. Liss, Inc. New York.

SETZER, H. W. 1975. Genus *Acomys*. In: *The mammals of Africa*: An identification manual, (eds) J. Meester & H. W. Setzer, Pp 1-2. Smithsonian Institution Press, Washington.

SHARGAL, E., KRONFELD-SCHOR, N. & DAYAN, T. 2000. Population biology and spatial relationships of coexisting spiny mice (*Acomys*) in Israel. *Journal of Mammalogy* **81**:1046-1052.

SHKOLNIK, A. 1966. Studies in the comparative biology of Israel's two species of spiny mice (genus *Acomys*). PhD thesis, Hebrew University (in Hebrew, English summary), Israel.

SHKOLNIK, A. & BORUT, A. 1969. Temperature and water relations in two species of spiny mice (*Acomys*). *Journal of Mammalogy* **50**:245-255.

SHKOLNIK, A. 1971. Diurnal activity in small desert rodent. *International Journal of Biometerology* **15**:115-120.

SUMNER, A.T., EVANS, H.J. & BUCKLAND, R.A. 1971. A new technique for distinguish between human chromosomes. *Nature New Biology* **232**:31-32.

TJIO, J. H. & LEVAN, A. 1956. The chromosome number of man. *Hereditas* **42**:1-6.

TRASK, B.J. 1991. Fluorescence in situ hybridization: application in cytogenetics and gene mapping. *Trends in Genetics* **7**:149-154.

VOLOBOUEV, V. T., TRANIER, M. & DUTRILLAUX, D. 1991. Chromosome evolution in the genus *Acomys*: Chromosome banding analysis of *Acomys* cf. *dimidiatus* (Rodentia, Muridiae). *Bonner Zoologische Beitrage* **42**:253-260.

VOLOBOUEV, V.T., GAUTUN, J-C, SICARD, B. & TRANIER, M. 1996a. The chromosome complement of *Acomys* spp. (Rodentia, Muridae) from Oursi, Burkina Faso-the ancestral karyotype of *cahirinus-dimidiatus* group? *Chromosomal Research* **4**:526-530.

VOLOBOUEV, V.T., GAUTUN, J-C. & TRANIER, M. 1996b. Chromosome evolution in the genus *Acomys* (Rodentia, Muridae): chromosome banding analysis of *Acomys cahirinus*. *Mammalia* **60**:217-222.

VOLOBOUEV, V., VIEGAS-PÉQUIGNOT, E., LOMBARD, M. PETTER, F., DUPLANTIER,

J.M. & DUTRILLAUX , B. 1988. Chromosomal evidence for a polytypic structure of *Arvicanthis niloticus* (Rodentia, Muridae). *Journal of Zoological Systematics and Evolutionary Research* **26**:276-285.

WAHRMAN, J., GOITEIN, R. & NEVO, E. 1969. Geographic variation of chromosome forms in *Spalax*, a subterranean rodent of restricted mobility. In: *Comparative mammalian cytogenetics*, (ed) E. Benirschke, Pp. 30-48. Springer Verlag, New York.

WAHRMAN, J. & GOITEIN, R. 1972. Hybridization in nature between two chromosome forms of spiny mice. *Chromosomes Today* **2**:228-237.

WATT, J. L. & STEPHEN, G. S. 1986. Lymphocyte culture for chromosome analysis. In: *Human cytogenetics*, (eds) D. E. Rooney & B. H. Czepulkowski, Pp.39-55. IRL Press, Oxford.

WHITE, M.J.D. 1978a. *Modes of speciation*. W.H. Freeman & Co., San Francisco.

WHITE, M.J.D. 1978b. Chain processes in chromosomal speciation. *Systematic Zoology* **27**:285-298.

WHITE, M.J.D. 1973. *Animal cytology and evolution*. Ch. 16-20. The University Press, Cambridge.

YUKSEL, E. 1984. Cytogenetic study in *Spalax* (Rodentia, Spalacidae) from Turkey. *Communications, de la Faculte des Sciences de l'Universite d'Ankara, Serie C*: *Biologie Tome* **2**:1-12.

ZAHAVI, A. & WAHRMAN, J. 1956. Chromosome races in the genus *Acomys* (Rodentia, Murinae). *Bulletin of Research Council of Israel* **5**:316.

3.7 FIGURE LEGENDS

Fig. 3.1 Map of Tanzania showing collecting localities of *Acomys* examined in this study: 1 = Lower Moshi (Kahe Chekeleni); 2 = Rombo–Alleni Chini; 3 = Tingatinga; 4 = Mt. Longido and Longido plains; 5 = Mt. Gelai-Olikisima; 6 = Ikorongo–Grumeti Game Reserve; 7 = Kilimamoja view point, Lake Manyara National Park. The dotted line denotes Mount Kilimanjaro which separates some of these localities, while the Africa map insert in the lower left indicates the geographic position of Tanzania in relation to the African continent. Apart from a specimen from Ikorongo–Grumeti Game Reserve (Locality 6) that was not available for the cytogenetic analysis in this study, all specimens analysed are from the same localities used in the molecular (Chapter 2), the traditional (Chapter 4) and geometric (Chapter 5) morphometrics aspects of the multidisciplinary characterization of *Acomys* from northern Tanzania.

Fig. 3.2 G-banded karyotype ($2n = 62$) of a female *Acomys wilsoni* (TZ20401) from northern Tanzania as a representative karyotype of the nine specimens from Lower Moshi ($n = 1$), Rombo Alleni Chini ($n = 7$), and Longido ($n = 1$) that were also identified as *A. wilsoni* by molecular analyses, traditional and geometric morphometrics, as well as classical morphology.

Fig. 3.3 G-banded karyotype ($2n = 58$) of a male *Acomys* cf. *percivali* (TZ20146) from northern Tanzania as a representative karyotype of the 13 specimens from Tingatinga ($n = 5$), Longido ($n = 2$), Mt. Gelai-Olikisima ($n = 1$), and Kilimamoja–Karatu ($n = 5$) that were also identified as *Acomys* cf. *percivali* by molecular analyses, traditional and geometric morphometrics, as well as classical morphology.

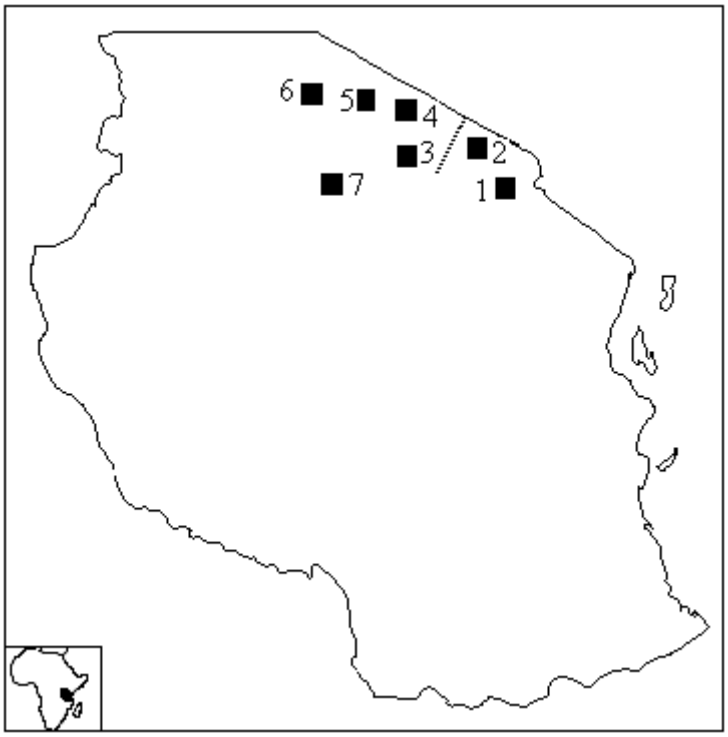

Fig. 3.1

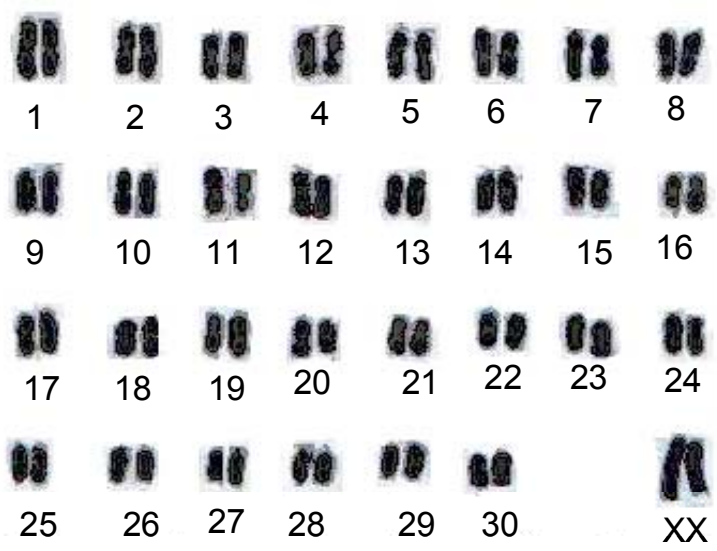
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Fig. 3.2

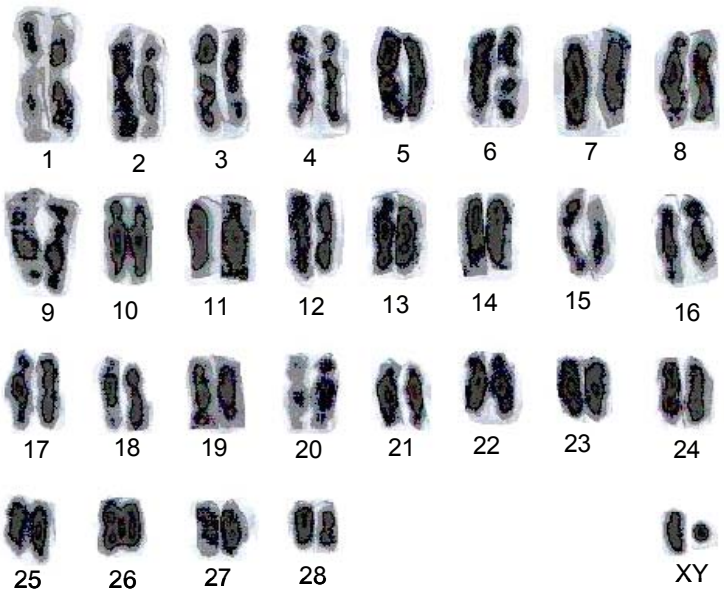
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Fig. 3.3

Table 3.1 Collecting localities, a gazetteer and altitude of specimens of *Acomys* from northern Tanzania examined in this study. Numbers correspond to localities in Fig. 3.1.

	Locality	Geographic Coordinates and Altitude
1	Lower Moshi, eastern Kilimanjaro	03.41S; 37.37E; 750 m a.s.l.
2	Rombo–Alleni Chini, eastern Kilimanjaro	03.24081S; 037.68139E; 1037 m a.s.l.
3	Tingatinga, western Kilimanjaro	02.96169S & 02.96810S; 036.95261E & 036.94566E; 1210 & 1215 m a.s.l.
4	Longido Mountain, east of Lake Natron, western Kilimanjaro	02.71276S; 036.68261E; 1398 m a.s.l.
	Longido plain–Irmibokuni–Engosipa or Olekibari on Rift Valley east of Lake Natron, western side of Mt. Kilimanjaro	02.70563S, 036.60299E; 457 m a.s.l.
	Longido plain–Parmunyan east of Lake Natron, western Kilimanjaro	02.70693S; 036.54184E; 1447 m a.s.l.
5	Mt. Gelai-Olikisima – East of Lake Natron	02.70367S; 036.11255E; 1427 m a.s.l.
7	Kilimamoja view-point, Lake Manyara National Park along the Great East African Rift Valley	03.38696S; 035.82093E; 1214 m a.s.l.

Table 3.2 Karyotypes of individual specimens (n = 22) of two species of *Acomys*, *A. wilsoni* ($2n$ = 62) and *A.* cf. *percivali* ($2n$ = 58) from six localities in northern Tanzania that were identified in the present study. The karyotypes were found to occur in sympatry at Longido (Locality 4), and specimens examined represent the same specimens that were also examined by molecular analyses, traditional and geometric morphometrics, as well as classical morphology.

	Specimen Number	**Locality**	**Geographic coordinates**	**Observed karyotype ($2n$)**	**Species**
1.	TZ-20400	Lower Moshi	03.41S; 037.37E 750 m a.s.l.	$2n$ = 62	*A. wilsoni*
2.	TZ-20021	Rombo-Alleni Chini	03.24081S; 037.68139E 1037 m a.s.l.	$2n$ = 62	*A. wilsoni*
3.	TZ-20022			$2n$ = 62	*A. wilsoni*
4.	TZ-20023			$2n$ = 62	*A. wilsoni*
5.	TZ-20024			$2n$ = 62	*A. wilsoni*
6.	TZ-20025			$2n$ = 62	*A. wilsoni*
7.	TZ-20026			$2n$ = 62	*A. wilsoni*
8.	TZ-20401			$2n$ = 62	*A. wilsoni*
9.	TZ-20055	Tingatinga	02.96169S; 036.95261E 1210 m a.s.l.	$2n$ = 58	*A.* cf. *percivali*
10.	TZ-20056			$2n$ = 58	*A.* cf. *percivali*
11.	TZ-20057		02.96810S; 036.94566E 1215 m a.s.l.	$2n$ = 58	*A.* cf. *percivali*
12.	TZ-20058			$2n$ = 58	*A.* cf. *percivali*
13.	TZ-20062			$2n$ = 58	*A.* cf. *percivali*
14.	TZ-20059	Longido Mountain	02.71276S; 036.68261E 1398 m a.s.l.	$2n$ = 58	*A.* cf. *percivali*
15.	TZ-20060			$2n$ = 58	*A.* cf. *percivali*
16.	TZ-20063	Longido plain - Irmibokuni-Engosip or Olekibari	02.70563S; 036.60299E 1457 m a.s.l.	$2n$ = 62	*A. wilsoni*
17.	TZ-20061	Mt.Gelai-Olikisima	02.70367S; 036.11255E 1427 m a.s.l.	$2n$ = 58	*A.* cf. *percivali*
18.	TZ-20145	Kilimamoja-Karatu	03.38696S; 035.82093E 1214 m a.s.l.	$2n$ = 58	*A.* cf. *percivali*
19.	TZ-20146			$2n$ = 58	*A.* cf. *percivali*
20.	TZ-20147			$2n$ = 58	*A.* cf. *percivali*
21.	TZ-20148			$2n$ = 58	*A.* cf. *percivali*
22.	TZ-20160			$2n$ = 58	*A.* cf. *percivali*

CHAPTER FOUR
TRADITIONAL MORPHOMETRIC ANALYSIS OF *ACOMYS* FROM NORTHERN TANZANIA

4.1 ABSTRACT

Traditional morphometric analyis of 10 linear measurements of the cranium and mandible, and classical morphological examination of external, cranial and mandibular morphology were used to assess the systematic status and geographic distribution of *Acomys* from northern Tanzania. Currently, four species of *Acomys*, namely; *A. spinosissimus* ($2n = 60$), *A. wilsoni* ($2n = 62$), *A. ignitus* ($2n = 36$) and *A. kempi* have been reported to occur in Tanzania. However, the results of multivariate analyses that included Unweighted-pair group arithmetic average (UPGMA) cluster, principal components (PCA), and canonical variates (disciminant) (CVA) analyses, and classical morphology were congruent and provide evidence for the occurrence of two sympatric species of *Acomys* in northern Tanzania, namely, the previously recorded *A. wilsoni* and a newly recorded *A.* cf. *percivali*. These results are supported by the parallel investigations based on molecular, cytogenetic, and geometric morphometric data of the same individuals, and increases the number of species of *Acomys* known to occur in Tanzania from four to five. Quantitatively, *A. wilsoni* and *A.* cf. *percivali* differ in the breadth of braincase, height of rostrum, and greatest height of skull that reflect either the lateral flatness or the curved nature of the cranium. Externally, the length of the tail also differs between these two species. These phenetic differences are also reflected in univariate standard descriptive statistics of the cranial, mandibular, and external measurements. Qualitatively, *A. wilsoni* and *A.* cf. *percivali* differ in gross cranial, and maxillary and mandibular molar configurations. However, the results in the present study need further multidisciplinary investigation involving extensive sampling and the examination of topotypical material.

4.2 INTRODUCTION

Morphometrics is the study of shape variation and its covariation with other variables (Bookstein 1991; Dryden & Mardia 1998), and derives its name from the Greek words: "*morphe*" = shape and "*metron*" = measurement. Historically, the identification of organisms based on classical morphology is the oldest approach that has been used to name organisms. For example, measurements of morphological features were recorded and summarized as mean values for comparisons among organisms (Bumpus 1898). Subsequently, these morphology-based studies have largely been conducted using statistical methods such as analysis of variance (ANOVA; Fisher 1935), principal components (PCA) (Pearson 1901; Hotelling 1933), discriminant, canonical variates (CVA), factor, and cluster analyses.

Both earlier and modern-day taxonomists have translated these morphology-based studies into biological classifications where hierarchical groupings of organisms are fundamentally based on morphology. This type of classification has developed rapidly to match scientific advancement, and has survived challenges from other methods of classification. This is best exemplified by the change in the recording of morphometric data from linear measurements used in traditional morphometrics to the capturing of geometric information such as coordinates and landmarks used in geometric morphometrics (Rohlf & Marcus 1993). However, some of the morphological features used in morphometrics are prone to geographic and environmental influences (see Nevo 1989; Chimimba 1997, 2000a, 2000b) and need to be interpreted with caution. The morphology-based methods include classical morphology and morphometrics.

Classical morphology includes the use of external morphological features such as head and body, ear, tail, and hind foot lengths, body weight and pelage colour, as well as gross cranial and mandibular morphology such as size and shape of the cranium, mandible and teeth. Such features are widely used in rodent taxonomy and provide a basis for grouping species as has previously been applied to species within the genus *Acomys* (Dollman 1911, 1914; Heller 1911; Thomas 1923; Allen & Lawrence 1936; Allen & Loveridge 1942; Hanney 1965; Delany & Neal 1966; Demeter & Topal 1982; Petter 1983).

These external morphological features are still useful despite their potential short-comings associated with plasticity especially in distinguishing closely related species when using

characters such as pelage colour and body size that are susceptible to the potential influence of geographical factors as well as climate (Nevo 1989). This problem can be minimized by including qualitative external morphological features as well as cranial measurements which are considered more useful for rodent taxonomy (Thomas 1906; Osgood 1909; Goldman 1910). Generally, classical morphology may be useful in the preliminary grouping of organisms to at least the higher levels of classification. Consequently, the classical morphological approach is used in the present study as a preliminary attempt to identify specimens of *Acomys* from northern Tanzania.

Among other uses, morphometrics is useful as a systematic tool to quantify morphological differences both *within* and *among* taxonomic units (Sneath and Sokal 1973), where joint relationships in character complexes are assessed simultaneously by the reduction of large character sets to a few dimensions (James and McCulloch 1990). This can be achieved by linear/orthogonal measurement-based traditional morphometrics and/or unit-free landmark/outline-based geometric morphometrics (Marcus 1990; Reyment 1991; Rohlf and Marcus 1993), where the generated data are in turn subjected to a series of both univariate and multivariate statistical analyses.

These morphometric methods are in turn used to address biological questions on for example, systematics, growth and evolutionary changes in an organism. Morphometrics is useful for studying differences in shape and size due to age, sexual dimorphism and differences between taxa. Rodent morphometric studies are usually based on the cranium, mandible and teeth which are used to assess the nature and extent of morphological variation and have for example, previously been used to address systematic questions in southern African species of *Acomys* by Dippenaar & Rautenbach (1986) and are similarly applied in the present study.

Consequently, the present study is aimed at assessing the nature and extent of morphological variation and is based on both traditional morphometrics as well as classical morphology in an attempt to assess the systematic status of *Acomys* from northern Tanzania. This part of the study complements mitochondrial cytochrome *b* gene and exon 1 of the nuclear interphotoreceptor retinoid binding protein (IRBP) data (Chapter 2), cytogenetics (Chapter 3),

and geometric morphometrics (Chapter 5) aspects of the multidisciplinary characterization of *Acomys* from northern Tanzania.

4.3 MATERIALS AND METHODS

Specimens of *Acomys* examined in the present study (n = 28) were collected from a wide range of habitats, topology and climate in northern Tanzania. The collecting localities of all these specimens are presented in Fig. 4.1, while geographic coordinates and altitude of these localities are shown in Table 4.1. For direct comparison with the genetic data, the 28 specimens represent the same specimens that were genetically/chromosomally-identified in Chapters 2 and 3 and were also used in the geometric morphometrics (Chapter 5) aspects of the multidisciplinary characterization of *Acomys* from northern Tanzania.

Animals were live-trapped using Sherman traps (H.B. Sherman Traps Inc. Florida, U.S.A.) baited with a mixture of peanut butter, syrup, oat meal and fish oil. After capture, during transportation and in the laboratory, animals were kept in polyurethane cages with wood shavings as bedding, with mouse pellets and water provided *ad libitum*. Animals were maintained under the guidelines of the American Society of Mammalogists (ASM; http://www.mammalogy.org/committees/index.asp; Animal Care and Use Committee 1998) and as approved by the Animal Ethics Committee of the University of Pretoria, Pretoria, South Africa. Animals were subsequently sacrificed using halothane inhalation and standard external measurements were recorded. Voucher specimens were prepared using standard natural history museum procedures for mammal specimens and are deposited in the newly developed mammal reference collection of Sokoine University of Agriculture (SUA), Pest Management Centre, Morogoro, Tanzania.

4.3.1 Ageing of specimens and sexual dimorphism

To limit the confounding effect of age variation, only adult specimens as defined by Dippenaar & Rautenbach (1986), were used for analysis. Following Dippenaar and Rautenbach (1986), animals were classified into five relative age classes based on the degree of maxillary tooth-wear. Specimens of toothwear classes I and II which were considered to be either juvenile or subadult were excluded from all subsequent morphometric and classical morphological analyses.

Similarly, very old specimens of toothwear V which normally are deformed due to old age (Dippenaar & Rautenbach (1986) were also excluded from analysis such that all subsequent morphometric and classical morphological analyses were based on specimens of toothwear classes III and IV only. The lack of sexual dimorphism in *Acomys* revealed by a previous study (Dippenaar & Rautenbach 1986) allowed for the pooling of sexes in all subsequent morphometric and classical morphological analyses.

4.3.2 Morphometric measurements

Ten cranial measurements adopted from Dippenaar & Rautenbach (1986) were recorded to the nearest 0.05 mm using a pair of Mitutoyo® digital callipers (Mitutoyo American Corporation, Aurora, Illinois, U.S.A.) and are indicated in Figure 4.2. These measurements defined and illustrated by Dippenaar & Rautenbach (1986) included: 1) greatest length of skull; 2) interorbital breadth; 3) breadth of braincase; 4) greatest length of longest palatal foramen; 5) crown length of maxillary toothrow; 6) height of rostrum; 7) length of diastema; 8) greatest height of skull; 9) height of mandible from ventral edge of angular process to horizontal plane through bullae; and 10) length of mandible from posterior condylar process to anteroventral edge of alveolus of incisor.

4.3.3 Morphometric analysis

The generated traditional morphometric data were subjected to a series of multivariate morphometric analyses to identify phenetic groupings in which no *a priori* sub-divisions of samples were presumed based on Unweighted pair-group arithmetic average (UPGMA) cluster analysis and principal components analysis of standardized variables (Sneath & Sokal 1973; Marcus 1993). Cluster analysis is a multivariate method used to group entities *a priori* based on distances with sets arranged hierarchically and represented in a phenogram (or dendrogram) in which similar entities are clustered together. Among the various clustering methods, UPGMA cluster analysis is recommended in systematics (Sneath & Sokal 1973) because being a cross-averaging algorithm, it conserves space by minimizing input and output distances leading to a distribution of Operational Taxonomic Units (OTUs; Sneath & Sokal) into a reasonable number of groups (Belbin 1989; James & McCulloch 1990). The UPGMA cluster analysis was based on both Euclidean distances and correlation coefficients among groups (Sneath & Sokal 1973).

PCA is also an *a priori* data reduction method in which variables or components of linear combinations of original data responsible for much of the variation in the data set are shown (Jolliffe 1986). PCA projects points from the original data on two dimensions with axes corresponding to the two most essential components. Minimal information is lost during its computation and it is recommended for analyzing morphometric data. The PCA was based on product-moment correlation coefficients among variables (Sneath & Sokal 1973).

The groups obtained *a priori* were further subjected to *a posteriori* analyses that assess the integrity of designated groups by maximizing the variation *between* groups while minimizing variation *within* groups, and for the classification of unknown specimens (Sneath & Sokal 1973). This included canonical variates (discriminant) analysis (CVA; Sneath & Sokal 1973) that was always followed by a multivariate analysis of variance (MANOVA; Zar 1996) in order to test for statistically significant differences between the designated group centroids.

Other analyses in the study included the generation of standard descriptive univariate statistics (Zar 1996) for each delineated phenetic group. All morphometric analyses were based on the 10 cranial measurements, and were undertaken using algorithms in STATISTICA version 7.0 (StatSoft, Inc. 2004).

4.4 RESULTS

4.4.1 Traditional Morphometrics

4.4.1.1 Cluster analysis

The results of a UPGMA cluster analysis based on both Euclidean distances and correlation coefficients were similar, and are best exemplified by the results of the Euclidean distances (Fig. 4.3). The phenogram revealed two distinct clusters, designated A & B (Fig. 4.3) that correspond with the two groups identified by the mitochondrial cytochrome *b* gene and exon 1 of the nuclear interphotoreceptor retinoid binding protein (IRBP) data (Chapter 2) and cytogenetics (Chapter 3) that equate to *A. wilsoni* and *A.* cf. *percivali*, respectively. Cluster A comprised specimens from Rombo Alleni Chini (Locality 2; Fig. 4.1) and Longido Plain (Locality 4), while cluster B

consists of specimens from Tingatinga (Locality 3), Longido (Locality 4), Gelai (Locality 5), Ikorongo-Grumeti Game Reserve (Locality 6), and Kilimamoja view point (Locality 7), with the two phenetic groups shown to co-exist at Longido (Locality 4).

4.4.1.2 Principal components analysis

Similarly, the PCA revealed two distinct clusters, designated A & B (Fig. 4.4) that also correspond with the two groups identified by the mitochondrial cytochrome *b* gene and exon 1 of the nuclear interphotoreceptor retinoid binding protein (IRBP) data (Chapter 2), cytogenetics (Chapter 3), and the UPGMA cluster analysis above (Fig. 4.3). This correspondence also includes specimens within clusters as well as specimens occurring in sympatry.

The second PCA axis that explained 21.09 % of the total variance, along which the two clusters of specimens separate, has both positive and negative measurement loadings that suggest shape-related variation (Table 4.2). Measurements with relatively high loadings, regardless of mathematical sign, on the second axis include breadth of braincase, height of rostrum, greatest height of skull, and height of mandible from ventral edge of angular process to horizontal plane through bullae.

4.4.1.3 Canonical variates (discriminant) analysis

A CVA of the two delineated phena produced a 100 % *a posteriori* classification, and the standardized coefficients generated by the CVA that are useful in the classification of unknown specimens are presented in Table 4.3. The derived associated CVA scores of individual specimens presented as a histogram (Fig. 4.5) also show two distinct groups that correspond with the two groups identified by the mitochondrial cytochrome *b* gene and exon 1 of the nuclear interphotoreceptor retinoid binding protein (IRBP) data (Chapter 2), cytogenetics (Chapter 3), and the UPGMA cluster analysis above (Fig. 4.3). A subsequent MANOVA indicated a statistically significant cranial morphological difference between the centroids of the two phenetic groups delineated ($F_{(10,7)} = 5.50$; $P < 0.05$). The phenetic differences shown by the UPGMA cluster, PCA and CVA are also shown by the mean values in the standard descriptive

external and cranial measurements given in Table 4.4, where the tail length between the two phenetic groups is diagnostic with no overlap in their observed ranges.

4.4.2 Classical morphology

Given the congruence between the results obtained from the mitochondrial cytochrome *b* gene and exon 1 of the nuclear interphotoreceptor retinoid binding protein (IRBP) (Chapter 2), cytogenetic (Chapter 3), and the results from the present traditional morphometric analyses, all specimens were re-examined for qualitative morphological differences. By so doing, qualitative morphological differences were identified in gross cranial configuration as well as in maxillary and mandibular molar morphology.

4.4.2.1 Morphological differences in gross cranial configuration

An examination of gross cranial configuration revealed that specimens from Lower Moshi (Locality 1; Fig. 4.1), Rombo Alleni Chini (Locality 2) and Longido Plains (Locality 4) that have been designated as *A. wilsoni* by the mitochondrial cytochrome *b* gene and exon 1 of the nuclear interphotoreceptor retinoid binding protein (IRBP) (Chapter 2), cytogenetic (Chapter 3), and the results from the traditional morphometric analyses in the present study are relatively small with shorter skulls, and laterally, have a characteristic smooth outward curve from the anterior (nasal bone region) to the posterior end after interparietal bone (Fig. 4.6a). Specimens from Tingatinga (Locality 3), Longido (Locality 4), Gelai (Locality 5), Ikorongo-Grumeti (Locality 6) and Kilimamoja (Locality 7) that have been designated as *A.* cf. *percivali* are relatively large with longer skulls, and laterally, have a characteristic depression in the middle of the frontal bone, resulting in a flattening of the skull when viewed laterally as well as an uneven curving in the region approximately mid-way between the anterior and posterior part of the cranium (Fig. 4.6b).

4.4.2.2 Morphological differences in maxillary molar morphology

An examination of maxillary morphology revealed that specimens from Lower Moshi (Locality 1; Fig. 4.1), Rombo Alleni Chini (Locality 2) and Longido Plains (Locality 4) that have been designated as *A. wilsoni* have relatively small maxillary teeth, with their general morphology and the cusps on M^1, M^2 and M^3 being similar to that of the type specimen of *A. wilsoni* specimen as described by Petter (1983) (Fig. 4.7). In addition, specimens from these localities have a

constriction on the cusp on M^1 (Fig. 4.7a) and an anterolabial cusp on M^2 that is relatively larger than the anterolingual cusp (Fig. 4.7a). They also show four distinct cusps on M^3 (see Fig. 4.7a and the type specimen of *A. wilsoni*). Specimens from Tingatinga (Locality 3), Longido Mountain (Locality 4), Gelai (Locality 5), Ikorongo-Grumeti (Locality 6) and Kilimamoja (Locality 7) that have been designated as *A.* cf. *percivali* have relatively large maxillary teeth. In addition, specimens from these localities lack the constriction on the cusp on M^1 (Fig. 4.7b) that is present in *A. wilsoni* and have an accessory anterolingual cusplet (ac) on M^1 that is absent in *A. wilsoni*. The anterolabial cusp on M^2 of *A.* cf. *percivali* is approximately equal in size to the anterolingual cusp (Fig. 4.7b). The cusps on M^3 of this species are not as distinct as in *A. wilsoni* where there are four distinct cusps present (Fig. 4.7a and 4.7b).

4.4.2.3 Morphological differences in mandibular molar morphology

An examination of mandibular molar morphology revealed that specimens from Lower Moshi (Locality 1; Fig. 4.1), Rombo Alleni Chini (Locality 2) and Longido Plains (Locality 4) that have been designated as *A. wilsoni* (Fig. 4.8a) have relatively small mandibular teeth that also resemble those of the type specimen of *A. wilsoni* (Fig. 4.8). Specimens from these localities are also characterised by an anterolabial cusplet on M_1, which in an advanced toothwear condition, fuses with the hypoconid cusp, while M_2 has peripheral labial cusp-like accessories present (Fig. 4.8a). Additionally, these specimens have a constriction between the anterior cusps on M_3 which slightly separate the two cusps (see Fig. 4.8a and the type specimen of *A. wilsoni*). Specimens from Tingatinga (Locality 3; Fig. 4.1), Longido Mountain (Locality 4), Gelai (Locality 5), Ikorongo-Grumeti (Locality 6) and Kilimamoja (Locality 7) that have been designated as *A.* cf. *percivali* have relatively large mandibular teeth that do not exhibit a complex cusp pattern (Fig. 4.8b) as observed in specimens from Lower Moshi (Locality 1; Fig. 4.1), Rombo Alleni Chini (Locality 2), and Longido Plains (Locality 4) that have been designated as *A. wilsoni* (Fig. 4.8a). The M_3 of *A.* cf. *percivali* does not have a constriction between anterior cusps which in *A. wilsoni* and in the type specimen of *A. wilsoni* the two cusps are separated.

4.5 DISCUSSION

The results of both traditional morphometric analyses and classical morphology in the present study suggest the occurrence of two species of *Acomys* in northern Tanzania, namely *A. wilsoni*

and A. cf. *percivali*. The occurrence of these two species in northern Tanzania is supported by the results of mitochondrial cytochrome *b* gene and exon 1 of the nuclear interphotoreceptor retinoid binding protein (IRBP) (Chapter 2) and cytogenetic (Chapter 3) data. The results in the present study represent the first report of the occurrence of *A*. cf. *percivali* in Tanzania, and increases the number of species reported to occur in Tanzania from four (Musser & Carleton 1993, 2005) to five. In addition to *A. cf. percivali*, the other species so far reported to occur in Tanzania include: *A. spinosissimus*; *A. wilsoni*; *A. ignitus*; and *A. kempi*.

Although the two species occur sympatrically at Longido (Locality 4, Fig. 4.1) on the western side of Mt. Kilimanjaro, *A. wilsoni* is more confined to the eastern side of Mt. Kilimanjaro, and confirms previous reports on the distribution of *A. wilsoni* in eastern East Africa, with its type locality being in Mombasa, Kenya. Dollman (1911, 1914) also reported on the sympatric occurrence of *A. wilsoni and A. percivali* at Chanler Falls, Guaso Nyiro, central Kenya, which is the type locality of *A. percivali*. Longido, the locality where the two species have been found to occur in sympatry in the present study and Chanler Falls, the type locality of *A. percivali* (Dollman 1911, 1914) have similar topography and are both located along the Great East African Rift Valley. Delany & Neal (1966) also reported on the sympatric occurrence of the two species in Karamoja in north-eastern Uganda.

Although the two species have unequivocally been reported to be sympatric in both previous and the present study, they seem to differ ecologically. Specimens of *A. cf. percivali* in the present study were mainly captured in well-covered habitats with thorn bushes, rocky and mountainous areas (G. Mgode pers. obs.). *Acomys wilsoni* on the other hand, was in the present study, mainly captured in open semi-arid grasslands and in rice fields in the Lower Moshi Plains (G. Mgode pers. obs.), suggesting that there may be some ecological segregation between the two species.

The results in the present study suggest that *A*. cf. *percivali* may be the common species along the Great East African Rift Valley since some localities in the present study such as Tingatinga (Locality 3; Fig. 4.1), Gelai (Locality 5), Ikorongo–Grumeti (Locality 6) and Kilimamoja (Locality 7) are associated with natural barriers in northern Tanzania. These natural

barriers form part of the Great East African Rift Valley which is known for its unique volcanoes and rift tectonics dating back from the early Miocene to the present (Dawson 1970; King 1970; Quennell 1982). These geological features have been associated with active rodent speciation in this region (Colangelo *et al*. 2005).

The two species show remarkable variation in morphology, especially in body size. Of particular relevance in the present study is that specimens of *A*. *wilsoni* from Rombo Alleni Chini (Locality 2; Fig. 4.1) have a characteristically shorter tail length (*ca*. 45 mm) that is similar to *A*. *wilsoni* from neighbouring Kenya including Mombasa, the type locality of *A*. *wilsoni* (Allen & Lawrence 1936; Delany & Neal 1966). However, Allen & Loveridge (1942) reported on a specimen of *A*. *wilsoni* from Siga caves near Tanga, Tanzania with a tail length of 52 mm, which slightly exceeds the tail length range reported in this study ($\overline{X}$ = 50.4 mm; range = 45-60 mm; *n* = 5; Table 4.4). Specimens of *A*. *wilsoni* examined in the present study were medium-sized, with a greyish to darker pelage, especially in young individuals, and had a head and body length range (see Allen & Lawrence 1936; Allen & Loveridge 1942; Delany & Neal 1966) that is within that of *A*. *wilsoni*. In addition, hind foot dimensions were also similar to that of *A*. *wilsoni*.

Although the ear lengths of specimens from Rombo (Locality 2; Fig. 4.1) are longer than the average length of a typical *A*. *wilsoni*, the general external morphological features of specimens from Rombo suggest that they may be that of *A*. *wilsoni* and do not correspond with those of other species of *Acomys* from East Africa (Dollman 1911, 1914; Allen & Lawrence 1936; Allen & Loveridge 1942; Delany & Neal 1966). A single specimen of *A*. *wilsoni* from Lower Moshi (Locality 1; Fig. 4.1), which is near Rombo Alleni Chini (Locality 2), had a relatively longer tail (60 mm) than reported for a typical *A*. *wilsoni* (Allen & Loveridge 1942), but its tail length was nevertheless closer to that of *A*. *wilsoni* than to other longer-tailed species of *Acomys* from East Africa.

Of particular interest in the present study is that the specimen from Lower Moshi (Locality 2; Fig. 4.1) is similar to *A*. *nubilus* from neighbouring Kenya in body dimensions. *Acomys nubilus* is almost similar in body size dimensions to *A*. *wilsoni* but has a longer tail. *Acomys nubilus* is considered difficult to classify and has been reported to be intermediate

between the large, long-tailed *A. ignitus* and small, short-tailed *A. wilsoni* (Dollman 1914). However, the allocation of the specimen from Lower Moshi to *A. wilsoni* is similarly confirmed by multidisciplinary data (Chapters 2 and 3) data.

The second species, *A.* cf. *percivali* reported to occur in northern Tanzania in the present study is relatively large in body size dimensions and with its other external morphological features being similar to *A. percivali* from Kenya (Dollman 1911). Most specimens of *A. percivali* are dorsally darker and ventrally white. One old individual of this species from Tingatinga (Locality 3; Fig. 4.1) was exceptionally reddish in pelage colour which is common for *A. percivali*. Another *A.* cf. *percivali* from Ikorongo-Grumeti Game Reserve (Locality 6) was more greyish dorsally. Although such pelage variation seems common within *A. percivali*, its pelage is mostly dark brown, with reddish grey colour being associated with the rufous tinting of the spines (Dollman 1911). Although the occurrence of *A. percivali* is supported by multidisciplinary data (Chapters 2 and 3) data, there is a range of pelage colouration within *A. percivali* from East Africa. This suggests that this characteristic is too variable in the species to be useful for taxonomic purposes, particularly when not used in combination with alternative datasets.

Of particular relevance in this study is that all aspects assessed in the present study are supported by the results of mitochondrial cytochrome *b* gene and exon 1 of the nuclear interphotoreceptor retinoid binding protein (IRBP) (Chapter 2) and cytogenetic (Chapter 3) data. These include occurrence in sympatry, pelage colour, and tail, head and body, foot, and ear dimensions. Although these results suggest the occurrence of two species of *Acomys* in northern Tanzania, these results however, need to be investigated further particularly with additional samples from Kenya in order to improve on our current understanding of the systematics and the distribution of this genus in East Africa. For a robust systematic and biogeographic perspective, such studies should also include type specimens, topotypical material, as well specimens in museum holdings that have not necessarily been positively identified.

4.6 REFERENCES

ALLEN, G.M. & LAWRENCE, B. 1936. Scientific results of an expedition to rain forest regions in Eastern Africa. III. Mammals. *Bulletin of the Museum of Comparative Zoology (Harvard)* **79**:31-l26.

ALLEN, G. M. & LOVERIDGE, A. 1942. Scientific results of a fourth expedition to forested areas in East and Central Africa. I. Mammals. *Bulletin of the Museum of Comparative Zoology* (*Harvard)* **89**:205-206.

ANIMAL CARE AND USE COMMITTEE. 1998. Guidelines for the capture, handling and care of mammals as approved by the American Society of Mammalogists. *Journal of Mammalogy* **79**:1416-1431.

BELBIN, L. 1989. 'PATN analysis package. Users' and technical reference manuals'. CSIRO Division of Wildlife and Ecology: Canberra.

BOOKSTEIN, F. L. 1991. *Morphometric tools for landmark data*: *geometry and biology*. Cambridge University Press, Cambridge.

BUMPUS, H. C. 1898. The elimination of the unfit as illustrated by the introduced sparrow *Passer domesticus*. Biological Lectures, Marine Biological Laboratory, Woods Hole **11**:209-226.

CHAPMAN R.W., STEPHENS, J.C. LANSMAN, R.A & AVISE, J.C. 1982. Models of mitochondrial DNA transmission genetics and evolution in higher Eucaryotes. *Genetic Research* **40**:41-57.

CHIMIMBA, C. T. 1997. *A systematic revision of southern African Aethomys* Thomas, 1915 (Rodentia: Muridae). PhD thesis, University of Pretoria, Pretoria.

CHIMIMBA, C. T. 2000a. Geographic variation in *Aethomys chrysophilus* (Rodentia: Muridae) from southern Africa. *Zeitschrift fuer Saugetierkunde* **65**:157-171.

CHIMIMBA, C. T. 2000b. Infraspecific morphometric variation in *Aethomys namaquensis* (Rodentia: Muridae) from southern Africa. *Journal of Zoology (London)* **253**:191-210.

COLANGELO, P., CORTI, M., VERHEYEN, E., ANNESI, F., OGUGE, N., MAKUNDI, R.H. & VERHEYEN, W. 2005. Mitochondrial phylogeny reveals

differential modes of chromosomal evolution in the genus *Tatera* (Rodentia: Gerbillinae) in Africa. *Molecular Phylogenetics and Evolution* **35**:556-568.

DAWSON, J.B. 1970. Neogene rift tectonics and volcanism in northern Tanzania. *Proceedings of the Geological Society of London* **1663**:151-153.

DELANY, M.J. & NEAL B.R. 1966. A review of the Muridae (Order Rodentia) of Uganda. *Bulletin of the British Museum (Natural History) Zoology* **13**:334-335.

DEMETER, A. & TOPAL, G. 1982. Ethiopian mammals in the Hungarian Natural History Museum. *Annales Historico-Naturales Musei Nationalis Hungaric* **74**:331-349.

DIPPENAAR, N. J. & RAUTENBACH, I.L. 1986. Morphometrics and karyology of the Southern African species of the genus *Acomys* I. Geoffroy Saint-Hilaire, 1838 (Rodentia, Muridae). *Annals of the Transvaal Museum* **34**:129-183.

DOLLMAN, G. 1911. New and interesting mammals from East Africa. *Annals and Magazine of Natural History* **8**:125-128.

DOLLMAN, G. 1914. On the spiny mice of British East Africa, with a description of a new species from Magadi. *Annals and Magazine of Natural History* **8**:485-488.

DRYDEN, I. L. & MARDIA, K. V. 1998. Statistical shape analysis. John Wiley & Sons, New York.

FISHER, R. A. 1935. The logic of inductive inference. *Journal of the Royal Statistical Society* **98**:39-82.

GOLDMAN, E.A. 1910. Revision of the wood rats of the genus *Neotoma*. *North American Fauna* **31**:1-124.

HANNEY, P. 1965. The Muridae of Malawi (Africa: Nyasaland). *Journal of Zoology* **146**:577-633.

HELLER, E. 1911. New species of rodents and carnivores from Equatorial Africa. *Smithsonian Miscellaneous Collections* **17**:1-16.

HOTELLING, H. 1933. Analysis of a complex of statistical variables into principal components. *Journal of Educational Psychology* **24**:417-441, 498-520.

JAMES, F. C. & McCULLOCH, C. E. 1990. Multivariate analysis in ecology and systematics: Panacea or Pandora's Box? *Annual Review of Ecology and Systematics* **21**:129-166.

JOLLIFFE, I.T. 1986. *Principal component analysis*. Springer-Verlag.

KING, B.C. 1970. Vulcanicity and rift tectonics in East Africa. In: *African Magmatism and Tectonics*, (eds) T.N. Clifford & I.G. Gass, Pp.263-267, 269-275, 281-283. Oliver & Boyd, Edinburgh.

MARCUS, L. F. 1990. Traditional morphometrics. In *Proceedings of the Michigan morphometrics workshop*, (eds) F.J. Rohlf & F. L. Bookstein. Special Publication Number 2, Pp.77-122, University of Michigan Museum of Zoology, Ann Arbor.

MARCUS, L. F. 1993. In: *Contributions to morphometrics*, (eds) L. F. Marcus, A, Valdecasas & E. Bello, Madrid Museum of Natural History, Madrid.

MUSSER, G. & CARLETON, M. 2005. Superfamily Muroidea. In: *Mammal species of the World,* (eds) D. E. Wilson & D. M. Reeder. The Johns Hopkins University Press, Baltimore and London.

MUSSER, G. G. & CARLETON, M. D. 1993. Genus *Acomys*. In: *Mammal species of the world*, (eds) D. E. Wilson & D. A. M. Reeder, Pp.564-567. Smithsonian Institution Press, Washington.

NEVO, E. 1989. Natural selection of body size differentiation in spiny mice, *Acomys*. *Zeitschrift fuer Saugetierkunde* **54**:81-99.

OSGOOD, W.H. 1909. Revision of the mice of the American genus *Peromyscus*. *North American Fauna* **28**:1-285.

PEARSON, K. 1901. On lines and planes of closest fit to systems of points in space. *Philosophical Magazine Series* **6**:559-572.

PETTER, F. 1983. Elements d'une revision des *Acomys* africains. Un sous-genre nouveau, *Peracomys* Petter et Roche, 1981 (Rongeurs, Murides). *Annales Mus e Royal de l' Afrique Centrale, Sciences Zoologiques* **237**:109-119.

QUENNELL, A.M. 1982. *Rift Valleys*. Afro-Arabian. Hutchinson Ross Publishing Company, Stroudsburg, Pennsylvania.

REYMENT, R. A. 1991. *Multidimensional paleobiology*. Pergamon Press, New York.

ROHLF, F.J. & MARCUS, L. F.. 1993. A revolution in morphometrics. *Trends in Ecology and Evolution* **8**:129-132.

SNEATH, P. H. A. & SOKAL, R.R. 1973. *Numerical taxonomy*. W. H. Freeman, San Francisco.

STATSOFT.2004. *Statistica for Windows* version 7.0, Statsoft Inc. Tulsa, Oklahoma.

THOMAS, O. 1906. Notes on South American rodents. *Annals and Magazine of Natural History* **7**:442-448.

THOMAS, O. 1923. Notes on some spiny mice (*Acomys*). *Annals and Magazine of Natural History* **9**:173-174.

ZAR, J. 1996. *Biostatistical Analysis*. New Jersey: Prentice Hall.

4.7 FIGURE LEGENDS

Fig. 4.1 Map of Tanzania showing collecting localities of *Acomys* examined in this study: 1 = Lower Moshi (Kahe Chekeleni); 2 = Rombo–Alleni Chini; 3 = Tingatinga; 4 = Mt. Longido and Longido Plains; 5 = Mt. Gelai-Olikisima; 6 = Ikorongo–Grumeti Game Reserve; 7 = Kilimamoja view-point, Lake Manyara National Park. The dotted line denotes Mt. Kilimanjaro which separates some of these localities, while the insert indicates the geographic position of Tanzania in relation to the African continent.

Fig. 4.2 Reference points of cranial measurements adopted from Dippenaar and Rautenbach (1986) used in the present traditional morphometric analyses of *Acomys* from northern Tanzania: 1) Greatest length of skull; 2) Interorbital breadth; 3) Breadth of braincase; 4) Greatest length of longest palatal foramen; 5) Crown length of maxillary toothrow; 6) Height of rostrum; 7) Length of diastema; 8) Greatest height of skull; 9) Height of mandible from ventral edge of angular process to horizontal plane through bullae; and 10) Length of mandible from posterior condylar process to anteroventral edge of alveolus of incisor.

Fig. 4.3 A Euclidean distance phenogram from an Unweighted pair-group arithmetic average (UPGMA) cluster analysis of *Acomys* from northern Tanzania. A = specimens designated to be *A. wilsoni*, while B = specimens designated to be *A.* cf. *percivali*. Numbers correspond to locality numbers in Fig. 4.1 and Table 4.1.

Fig. 4.4 Axes I and II from a principal components analysis of specimens of *Acomys* from northern Tanzania. Minimum convex polygons enclose genetically and karyologically-identified specimens. A = specimens designated to be *A. wilsoni*, while B = specimens designated to be *A.* cf. *percivali*. Numbers correspond to locality numbers in Fig. 4.1 and Table 4.1.

Fig. 4.5 A histogram of discriminant scores from a two-group canonical variates analysis of specimens of *Acomys*. (■) = specimens designated to be *A. wilsoni*, while (▨) = specimens designated to be *A.* cf. *percivali*.

Fig. 4.6 The lateral view of the cranium of species of *Acomys* from northern Tanzania of specimens designated to be *A. wilsoni* (A; TZ20026) *A.* cf. *percivali* (B; TZ20056) showing the extent of the curvature on top of the cranium.

Fig. 4.7 The occlusal view of the right maxillary molar toothrow of *Acomys* from northern Tanzania of specimens designated to be *A wilsoni* (A) and *A.* cf. *percivali* (B) and the type specimens of *A wilsoni* (after Petter 1983) showing differences and similarities in molar cusp pattern: ac) is an accessory cusplet present in *A.* cf. *percivali*; i) constriction on M^1 cusp; ii) M^2 anterolingual cusp; iii) M^2 anterolabial cusp; iv) M^3 anterior anterolingual cusp; v) M^3 rear cusp.

Fig. 4.8 The occlusal view of the left mandibular molar toothrow of *Acomys* from northern Tanzania of specimens designated to be *A wilsoni* (A) and *A.* cf. *percivali* (B) and the type specimens of *A wilsoni* (after Petter 1983) showing differences and similarities in molar cusp pattern: i) anterolabial cusplet on M_1 which may fuse with hypoconid cusp; ii) peripheral anterolabial cusp-like accessories; iii) a constriction between M_3 anterior cusps which seems to slightly separate the two cusps in *A. wilsoni* and type specimen but which is not obvious in *A.* cf. *percivali*.

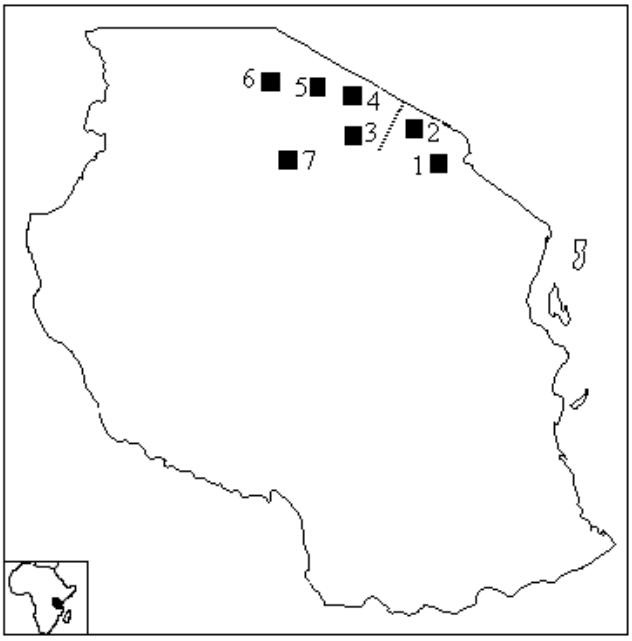


Fig. 4.1

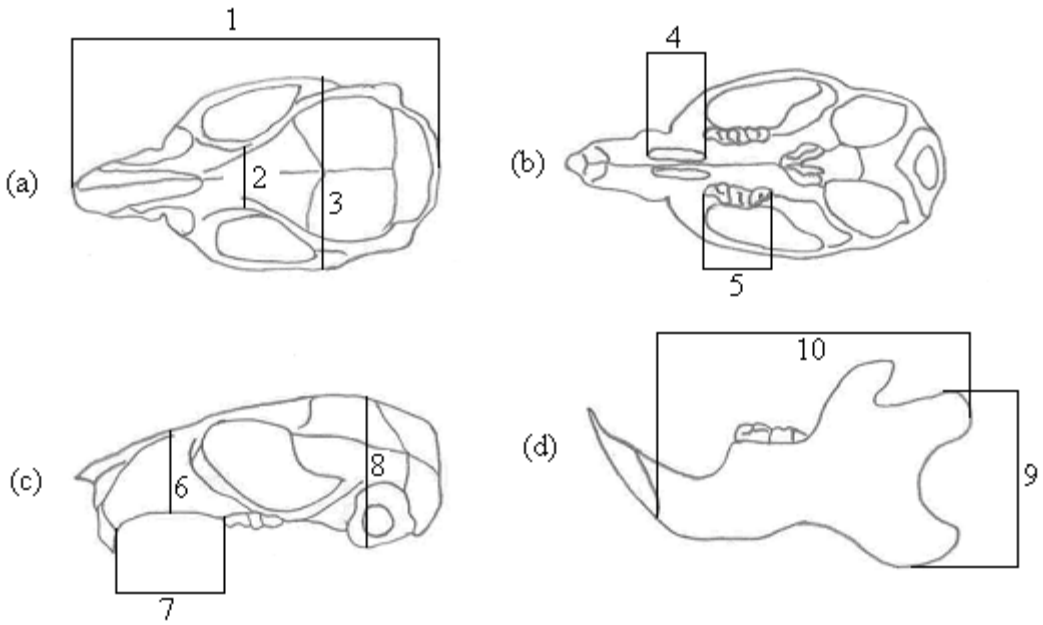
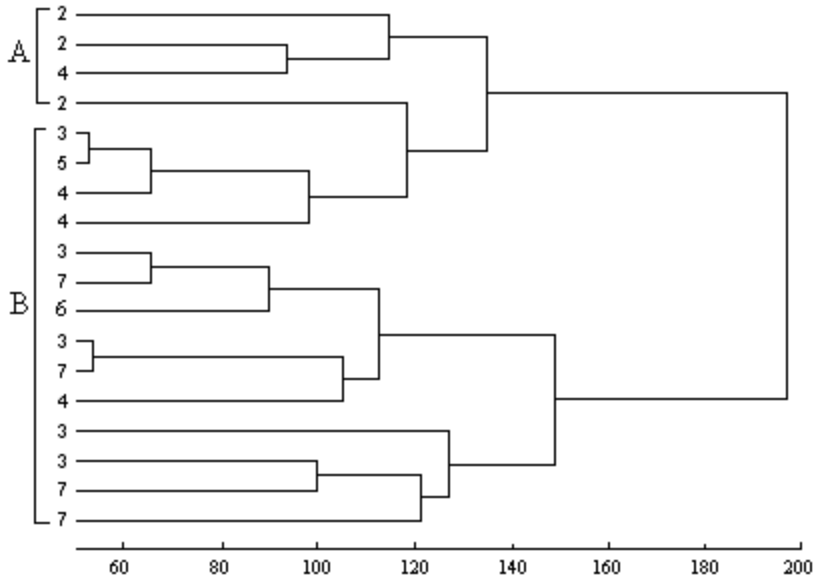


Fig. 4.2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200

Fig. 4.3

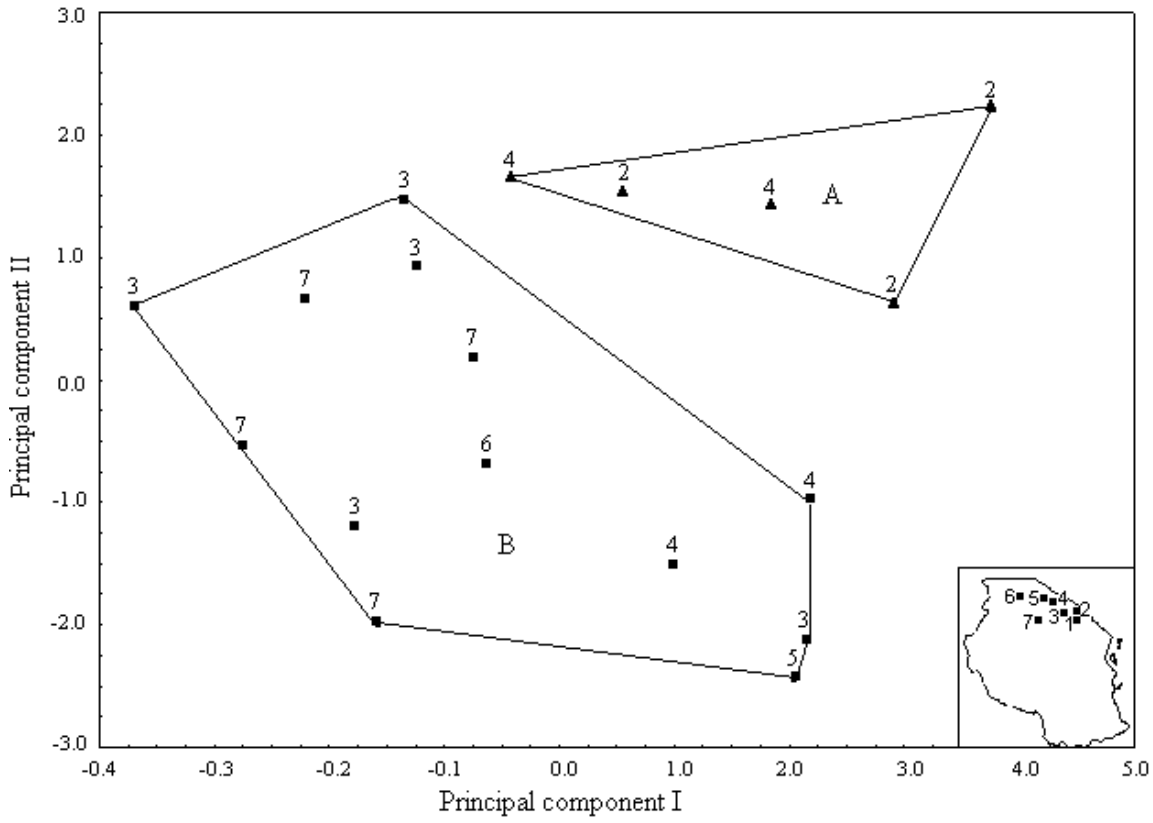

Principal component II
Principal component I
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
-0.4
-0.3
-0.2
-0.1
0.0
1.0
2.0
3.0
4.0
5.0
A
B

Fig. 4.4

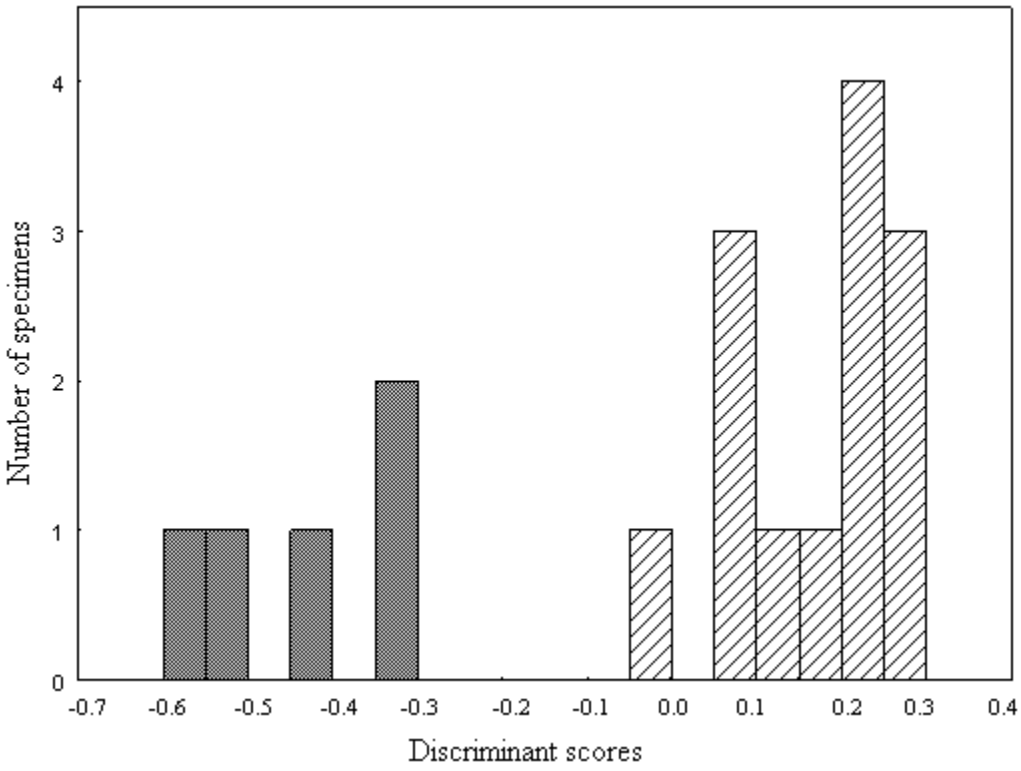

Number of specimens
0
1
2
3
4
-0.7
-0.6
-0.5
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
0.4
Discriminant scores

Fig. 4.5

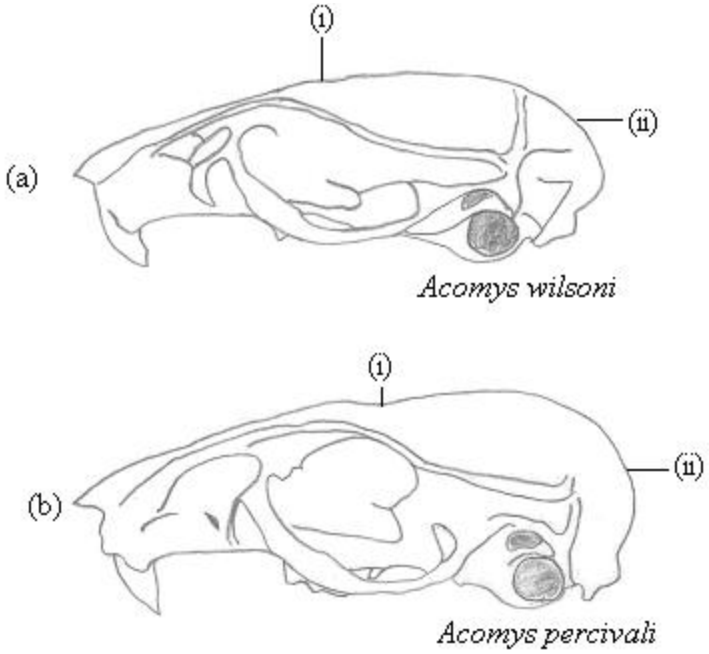

(i)
(ii)
(a)
Acomys wilsoni
(i)
(ii)
(b)
Acomys percivali

Fig. 4.6

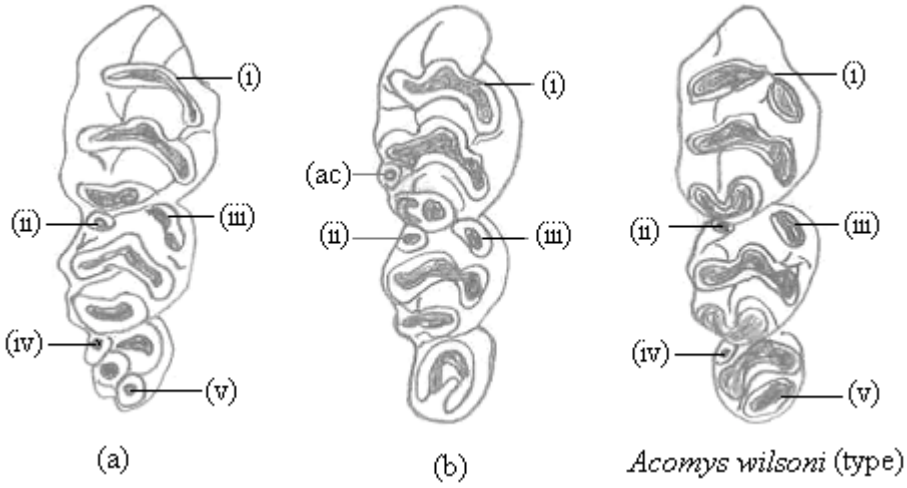

(i)
(ii)
(iii)
(iv)
(v)
(a)
(i)
(ac)
(ii)
(iii)
(b)
(i)
(ii)
(iii)
(iv)
(v)
Acomys wilsoni (type)

Fig. 4.7

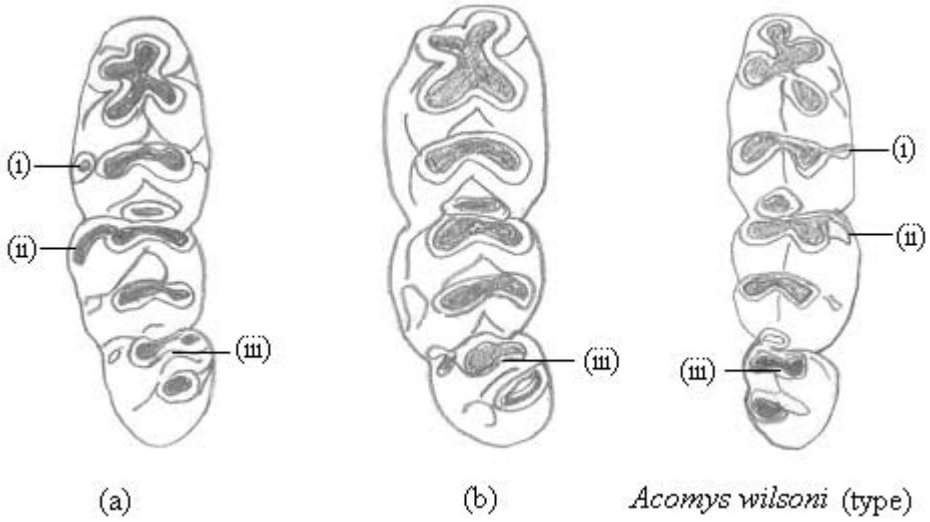


Fig. 4.8

Table 4.1 Collecting localities, a gazetteer and altitude of specimens of *Acomys* from northern Tanzania examined in this study. Numbers correspond to localities in Fig. 4.1.

	Locality	Geographic Coordinates and Altitude
1	Lower Moshi, eastern Kilimanjaro	03.41S; 37.37E; 750 m a.s.l.
2	Rombo–Alleni Chini, eastern Kilimanjaro	03.24081S; 037.68139E; 1037 m a.s.l.
3	Tingatinga, western Kilimanjaro	02.96169S & 02.96810S; 036.95261E & 036.94566E; 1210 & 1215 m a.s.l.
4	Longido Mountain, east of Lake Natron, western Kilimanjaro	02.71276S; 036.68261E; 1398 m a.s.l.
	Longido plain–Irmibokuni–Engosipa or Olekibari on Great East African Rift Valley, east of Lake Natron, western Mt. Kilimanjaro	02.70563S, 036.60299E; 457 m a.s.l.
	Longido plain–Parmunyan, east of Lake Natron, western Kilimanjaro	02.70693S; 036.54184E; 1447 m a.s.l.
5	Mt. Gelai-Olikisima, east of Lake Natron	02.70367S; 036.11255E; 1427 m a.s.l.
6	Ikorongo–Grumeti Game Reserve, west of Serengeti National Park	02.10707S; 034. 47665E; 1414 m a.s.l.
7	Kilimamoja view-point, Lake Manyara National Park along the Great East African Rift Valley	03.38696S; 035.82093E; 1214 m a.s.l.

Table 4.2 Loadings of variables on the first and second principal components from a principal components analysis of *Acomys* from northern Tanzania.

Variable	Principal components	
	I	II
Greatest length of skull	-0.88	0.01
Interorbital breadth	-0.84	-0.42
Breadth of braincase	-0.69	-0.50
Greatest length of longest palatal foramen	-0.76	-0.08
Crown length of maxillary toothrow	-0.17	-0.43
Height of rostrum	-0.70	0.51
Length of diastema	-0.61	0.22
Greatest height of skull	-0.37	-0.79
Height of mandible from ventral edge of angular process to horizontal plane through bullae	-0.64	0.66
Length of mandible from posterior condylar process to anteroventral edge of alveolus of incisor	-0.70	0.30
% trace	45.28 %	21.09 %

Table 4.3 Loadings of variables on the first canonical variate and standardized coefficients from a canonical variate analysis of two groups of *Acomys* from northern Tanzania. The standardized coefficients generated by the canonical variates analysis may be used to identify unknown individual specimens (Chapman *et al*. 1982). A discriminant score for an unknown specimen is calculated by subtracting the overall means from the corresponding measurements of the unknown, multiplying the results with the corresponding standardized coefficients, and summing the resulting values.

Variable	Canonical variate I	Overall means	Standardized coefficients
Greatest length of skull	0.03	26.26	2.73
Interorbital breadth	-0.12	4.71	-1.65
Breadth of braincase	-0.02	11.57	-0.35
Greatest length of longest palatal foramen	0.03	5.95	0.93
Crown length of maxillary toothrow	-0.01	3.93	-0.20
Height of rostrum	-0.05	4.27	-0.93
Length of diastema	-0.04	7.19	-1.41
Greatest height of skull	0.10	8.64	2.19
Height of mandible from ventral edge of angular process to horizontal plane through bullae	-0.01	6.22	-0.45
Length of mandible from posterior condylar process to anteroventral edge of alveolus of incisor	0.03	13.03	1.24

Table 4.4 Standard statistics of external and cranial measurements (in mm) of *Acomys* species from northern Tanzania. $\bar{X}$ = arithmetic mean; SD = standard deviation; *n* = sample size; CV = coefficient of variation; Range = observed range.

Variable	Species	
	Acomys wilsoni	*Acomys cf. percivali*
Length of head and body		
n	12	16
$\bar{X}$	84.66	96.81
SD	9.43	7.2
CV	11.13	7.43
Range	70-98	87-111
Length of tail		
n	5	11
$\bar{X}$	50.4	77.36
SD	5.63	6.98
CV	11.17	9.02
Range	45-60	68-87
Length of hind foot (without claw)		
n	12	16
$\bar{X}$	12.88	14.38
SD	0.51	0.66
CV	3.95	4.58
Range	12.05-13.82	13.3-15.4
Length of ear		
n	11	15
$\bar{X}$	13.46	13.79
SD	1.25	1.08
CV	9.28	7.83
Range	11.7-15-5	10.3-14.8
Greatest length of skull		
n	5	13
$\bar{X}$	25.64	26.51
SD	0.56	0.78
CV	2.19	2.97
Range	24.83-26.39	25.48-27.75

Table 4 (*continued*)

Variable	Species	
	Acomys wilsoni	*Acomys percivali*
Interorbital breadth		
n	5	13
$\bar{X}$	4.52	4.77
SD	0.19	0.11
CV	4.26	2.3
Range	4.26-4.75	4.64-4.98
Breadth of braincase		
n	5	13
$\bar{X}$	11.24	11.7
SD	0.12	0.22
CV	1.13	1.95
Range	11.11-11.39	11.32-12.09
Greatest length of longest palatal foramen		
n	5	13
$\bar{X}$	5.71	6.03
SD	0.26	0.27
CV	4.54	4.57
Range	5.37-5.96	5.7-6.66
Crown length of maxillary toothrow		
n	5	13
$\bar{X}$	3.84	3.96
SD	0.2	0.18
CV	5.41	4.63
Range	3.6-4.08	3.76-4.34
Height of rostrum		
n	5	13
$\bar{X}$	4.27	4.27
SD	0.07	0.19
CV	1.85	4.44
Range	4.2-4.39	3.87-4.54
Length of diastema		
n	5	13
$\bar{X}$	7.14	7.21
SD	0.25	0.35
CV	3.62	4.88
Range	6.97-7.6	6.73-8.02

Table 4 (*continued*)

Variable	Species	
	Acomys wilsoni	*Acomys percivali*
Greatest height of skull		
n	5	13
$\bar{X}$	8.3	8.77
SD	0.2	0.2
CV	2.5	2.38
Range	8.11-8.64	8.4-9.19
Height of mandible from ventral edge of angular process to horizontal plane through bullae		
n	5	13
$\bar{X}$	6.32	6.18
SD	0.39	0.39
CV	6.26	6.3
Range	5.77-6.77	5.64-6.72
Length of mandible from posterior condylar process to anteroventral edge of alveolus of incisor		
n	5	13
$\bar{X}$	12.83	13.11
SD	0.45	0.48
CV	3.52	3.69
Range	12.11-13.36	12.33-13.88

CHAPTER FIVE

GEOMETRIC MORPHOMETRIC ANALYSIS OF *ACOMYS* FROM NORTHERN TANZANIA

5.1 ABSTRACT

Geometric morphometric analysis of two-dimensional landmarks of the dorsal, ventral, and lateral views of the cranium, and the lateral view of the mandible were used to assess the systematic status and geographic distribution of *Acomys* from northern Tanzania. Currently, four species of *Acomys*, namely; *A. spinosissimus* ($2n = 60$), *A. wilsoni* ($2n = 62$), *A. ignitus* ($2n = 36$) and *A. kempi* have been reported to occur in Tanzania. However, the results of multivariate analyses that included Unweighted-pair group arithmetic average (UPGMA) cluster analysis, the principal components analysis (PCA) equivalent-relative warp analysis (RW), and canonical variates (disciminant) (CVA) analysis were congruent and provide evidence for the occurrence of two sympatric species of *Acomys* in northern Tanzania, namely, the previously recorded *A. wilsoni* and a newly recorded *A.* cf. *percivali*. These results are supported by the parallel investigations based on molecular, cytogenetic, and traditional morphometric data, and classical morphology of the same individuals, except for the morphologically damaged specimens that were excluded from analysis, and increases the number of species of *Acomys* known to occur in Tanzania from four to five. Thin plate splines (TPS) from the geometric morphometric data show that these two species differ in the lateral views of the cranium and mandible, especially the saggital crest region and posterior edge of the cranium dorso-laterally, as well as the posterior part of the mandible that to an extent, are also reflected in the traditional morphometric data.

However, the results in the present study need further multidisciplinary investigation involving extensive sampling and the examination of topotypical material.

5.2 INTRODUCTION

Among other uses, morphometrics is useful as a systematic tool to quantify morphological differences both *within* and *among* Operational Taxonomic Units (OTUs; Sneath and Sokal 1973), where joint relationships in character complexes are assessed simultaneously through the reduction of large character sets to a few dimensions (James and McCulloch 1990). This can be achieved by either linear/orthogonal measurement-based traditional morphometrics and/or unit-free landmark/outline-based geometric morphometrics (Marcus 1990; Reyment 1991; Rohlf and Marcus 1993), where the generated data are in turn subjected to a series of both univariate and multivariate statistical analyses.

Geometric morphometrics is analytically more superior in the partitioning of organismic shape variation than traditional morphometrics, and uses coordinate data such as landmarks to assess organismic shape differences (Corti *et al*. 1996; Marcus *et al*. 1996). It has been applied in studies of mammalian morphological variation where variation in shape is examined by the analysis of digitized landmarks, and in rodents, it is usually based on an assessment of different views of the cranium, mandible and teeth (Voss *et al*. 1990). These anatomical structures may contain useful information for understanding phylogenetic and adaptive processes. The mandible for example has been used in rodent taxonomy to differentiate populations (Davis 1983; Schriven & Bauchau 1992), chromosomal races (Corti & Thorpe 1989), and in distinguishing sibling species (Vogl *et al*. 1993; Demeter *et al*. 1996). Apart from being useful in the analysis of both intra- and inter-specific morphological variation, geometric morphometrics (as well as traditional morphometrics) can also be used to address questions relating to microevolution, environmental and genetic effects, sexual dimorphism, and developmental stages of organisms, among others.

The digitized landmarks, captured either in two- or three-dimensions are analysed using for example, the thin-plate spline technique (TPS) (Rohlf & Marcus 1993). TPS allows the visualization of shape change of landmark configuration and the construction of deformations in

relation to their original positions. The TPS series of programs also generate data such as procrustes distances, relative warps, and relative weights that are in turn used in multivariate analyses such as in the *a priori* cluster analysis and principal components analysis (PCA) and the *a posteriori* canonical variates (discriminant) analysis (CVA) (Rohlf & Marcus 1993).

Procrustes distances of digitized landmarks are used to cluster specimens based on distances with sets arranged hierarchically and represented in the form of a phenogram (or a dendogram) in which similar specimens are grouped together. Relative warps represent principal components of variation in shape generated as relative warp scores that can be presented in the form of a PCA scatterplot (Bookstein 1989, 1991; Rohlf 1993). Relative weight matrices provide information that is useful in the *a posteriori* CVA of predefined groups that can in turn be used to test groups obtained *a priori* by either cluster analysis or PCA. The CVA is used to maximize the variance **among** *a posteriori*-defined group while minimizing the variation **within** groups, and can also be used to classify unknown specimens (Sneath & Sokal 1973; Marcus 1993).

To this end, the aim of the present study is to assess the nature and extent of morphological variation based on geometric morphometric analysis of the dorsal, ventral, and lateral views of the cranium, and the lateral view of the mandible in an attempt to assess the systematic status of *Acomys* from northern Tanzania. This part of the study complements mitochondrial cytochrome *b* gene and exon 1 of the nuclear interphotoreceptor retinoid binding protein (IRBP) data (Chapter 2), cytogenetics (Chapter 3), and traditional morphometrics (Chapter 4) aspects of the multidisciplinary characterization of *Acomys* from northern Tanzania.

5.3 MATERIALS AND METHODS

5.3.1 Specimens examined

Specimens of *Acomys* examined in the present study (n = 28) were collected from a wide range of habitats, topology and climate in northern Tanzania. The collecting localities of all these specimens are presented in Fig. 5.1, while geographic coordinates and altitude of these localities are shown in Table 5.1. For direct comparison with the genetic data, the 28 specimens represent the same specimens that were genetically/chromosomally-identified in Chapters 2 and 3, and that

were also used in the traditional morphometrics (Chapter 4) aspects of the multidisciplinary characterization of *Acomys* from northern Tanzania.

Animals were live-trapped, transported, maintained and sampled as described in Chapter 4, with all voucher specimens being deposited in the newly developed mammal reference collection of Sokoine University of Agriculture (SUA), Pest Management Centre, Morogoro, Tanzania.

5.3.2 Ageing of specimens and sexual dimorphism

To limit the confounding effects of age variation, only adult specimens as defined by Dippenaar & Rautenbach (1986) were used for analysis. Following Dippenaar and Rautenbach (1986), animals were classified into five relative age classes based on the degree of maxillary toothwear. Specimens of toothwear classes I and II which were considered to be either juvenile or subadult were excluded from all subsequent geometric morphometric analyses. Similarly, very old specimens of toothwear class V which normally are deformed due to old age (Dippenaar & Rautenbach (1986) were also excluded from analysis such that all subsequent geometric morphometric analyses in the present study were based on specimens of toothwear classes III and IV only. The lack of sexual dimorphism in *Acomys* as revealed by a previous study (Dippenaar & Rautenbach 1986) allowed for the pooling of sexes in all subsequent geometric morphometric analyses.

5.3.3 Geometric morphometric data

Geometric morphometrics (Corti and Marcus 1996), which is considered much more superior in assessing organismal shape differences in morphology than traditional morphometrics, was used to assess shape differences between specimens of *Acomys* from northern Tanzania. A Canon® PowerShot A95 digital camera attached to a tripod stand was used to capture two-dimensional images of the dorsal, ventral, and lateral views of the cranium, as well as lateral views of the mandible of each specimen (Fig. 5.2). To standardize the image capturing process, each

specimen was placed on a marked graph paper, with all images being captured by one observer (GM).

A Thin Plate Spline (TPS; Rohlf 2004a) sub-routine, TPSDig, was used to digitize landmarks, each with an (x,y) two-dimensional coordinate, on each of the four views for each specimen (Fig. 5.2). Landmarks of the different views illustrated in Fig. 5.2 were captured and their respective specific positions in the configuration of the cranium and mandible included: 1) tip of nasal bone; 2) posterior edge of interparietal bone; 3) base of M^3; 4) tip of I^1; 5) anterior region of crista; 6) anterior edge of bulla; 7) sagittal crest region; 8) posterior edge of bulla; 9) tip of condylar occipital bone; 10) base of I^1; 11) base of M^1; 12) posterior edge of nasal bone; 13) anterior base of zygomatic bone; 14) mid-posterior base of zygomatic bone (= inter-temporalis); 15) tip of I_1; 16) exterior base of I_1; 17) mid-region between base of I_1 and M_1; 18) tip of M_1; 19) base of M_3; 20) tip of coronoid process; 21) dorsal edge of condylar process; 22) posterior edge of condylar process; 23) ventral edge of condylar process; 24) mid-region between ventral edge of condylar process and edge of angular process; 25) tip (edge) of angular process; 26) anterior base of angular process–ascending ramus region; 27) ascending ramus region; 28) alveolar process parallel to base of M_3; 29) region between alveolar process (parallel to base of M_3 and base of I_1); 30) posterior base of I_1; 31) base of M_2; 32) tip of M_2); 33) mid-I_1 alveolar region parallel to exterior base of I_1; and 34) anterior base of M_1.

5.3.4 Digitizing error

In order to assess the degree of landmark digitizing error (DE), the degree of error was expressed as a percentage (%DE) of the total variability due to *within*-individual variation (Pankakoski *et al*. 1987; Bailey and Byrnes 1990). Percent DE analysis was based on three independent data sets of repeated digitized landmarks derived by GM on three separate occasions. Because these analyses revealed very low %DE values that ranged from 0.01 % to 1.56 %, averages of landmarks were computed and used in all subsequent geometric morphometric analyses.

5.3.5 Geometric morphometric analyses

A TPS sub-routine, tpsSpline (Rohlf 2004b), was used to compute splines in order to compare each specimen to a consensus configuration in order to detect any subtle differences in cranial

and mandibular shape morphology (Corti and Marcus 1996). The generated geometric morphometric data were subjected to a series of multivariate morphometric analyses to identify phenetic groupings in which no *a priori* sub-divisions of samples were presumed based on Unweighted pair-group arithmetic average (UPGMA) cluster analysis and principal components analysis of standardized variables (Sneath & Sokal 1973; Marcus 1993). Cluster analysis is a multivariate method used to group entities *a priori* based on distances with sets arranged hierarchically and represented in a phenogram (or dendrogram) in which similar entities are clustered together. Among the various clustering methods, UPGMA cluster analysis is recommended in systematics (Sneath & Sokal 1973) because being a cross-averaging algorithm, it conserves space by minimizing input and output distances leading to a distribution of Operational Taxonomic Units (OTUs; Sneath & Sokal 1973) into a reasonable number of groups (Belbin 1989; James & McCulloch 1990). The UPGMA cluster analysis of the geometric morphometric data was based on procrustes distances of landmarks generated from the TPS sub-routine, tpsSmall (Rohlf 2004d).

PCA is also an *a priori* data reduction method in which variables or components of linear combinations of original data responsible for much of the variation in the data set are shown (Jolliffe 1986). PCA projects points from the original data on two dimensions with axes corresponding to two most essential components. Minimal information is lost during its computation and is recommended for analyzing morphometric data. The PCA of the geometric morphometric data was based on a weighted matrix generated from the TPS subroutine tpsRelw (Rohlf 2004c) that was used to perform a relative warp analysis that is equivalent to a PCA.

The phenetic and/or genetically/chromosomally-identified assemblages obtained *a priori* were further subjected to *a posteriori* analyses that assess the integrity of designated groups by maximizing the variation **between** groups while minimizing variation **within** groups, and for the classification of unknown specimens (Sneath & Sokal 1973). This included canonical variates (discriminant) analysis (CVA; Sneath & Sokal 1973). The CVA was based on relative weight matrices of landmarks generated from the TPS subroutine tpsRegr (Rohlf 2004c) that was always followed by a multivariate analysis of variance (MANOVA; Zar 1996) in order to test for statistically significant differences between the designated group centroids. All geometric

morphometric analyses in this study were accomplished using sub-routines in the TPS (Rohlf 2004) series of programmes and algorithms in Statistica version 7.0 (STATSOFT 2004).

5.4 RESULTS

Although analyses based on mitochondrial cytochrome *b* gene and exon 1 of the nuclear interphotoreceptor retinoid binding protein (IRBP) (Chapter 2), cytogenetic (Chapter 3), traditional morphometrics and classical morphological (Chapter 4) data were based on the same specimens (n = 28), eleven and fourteen of these specimens were excluded from the cranial and mandibular geometric morphometric analyses, respectively, due to damage that precluded the digitization of a complete suite of landmarks for analysis. While the geometric morphometric analyses of *Acomys* from northern Tanzania were based on the dorsal, ventral, and lateral views of the cranium, and the lateral view of the mandible, geometric morphometric patterns from UPGMA cluster analysis, PCA, and CVA, were discernible only in the lateral views of the cranium and the mandible, and are therefore, presented below.

5.4.1 Geometric morphometrics of the lateral view of the cranium

5.4.1.1 UPGMA cluster analysis of the lateral view of the cranium

The phenogram from a UPGMA cluster analysis (n = 17) based on procrustes distances of landmarks of the lateral view of the cranium revealed two distinct clusters, designated A & B (Fig. 5.3) that broadly correspond with the two groups identified by the mitochondrial cytochrome *b* gene and exon 1 of the nuclear interphotoreceptor retinoid binding protein (IRBP) data (Chapter 2), cytogenetics (Chapter 3), and traditional morphometric (Chapter 4) data that equate to *A. wilsoni* and *A.* cf. *percivali*, respectively. Cluster A comprised specimens from Rombo Alleni Chini (Locality 2; Fig. 5.1) and Longido Plain (Locality 4). Cluster B consists of specimens from Tingatinga (Locality 3), Longido Mountain (Locality 4), Mount Gelai-Olikisima (Locality 5), Ikorongo-Grumeti Game Reserve (Locality 6), and Kilimamoja (Locality 7). The only exception to the general pattern involved two specimens (indicated by an asterisk in Fig. 5.3), one from Kilimamoja (Locality 7) and the other from Longido (Locality 4). Like in all other multidisciplinary techniques outlined above, the UPGMA cluster analysis of the lateral view of the cranium also showed the two phenetic groups to be sympatric at Longido (Locality 4).

5.4.1.2 Principal components analysis of the lateral view of the cranium

The PCA (n = 17) of the lateral view of the cranium based on a weighted matrix revealed two distinct clusters, designated A & B (Fig. 5.4) that also correspond with the two groups identified by the mitochondrial cytochrome *b* gene and exon 1 of the nuclear interphotoreceptor retinoid binding protein (IRBP) data (Chapter 2), cytogenetics (Chapter 3), traditional morphometrics (Chapter 4) data, and the UPGMA cluster analysis above (Fig. 5.3). This separation is particularly evident on the first (28.97 % variance) rather than the second (19.72 % variance) relative warps (RW). The PCA's correspondence with the other multidisciplinary techniques outlined above also includes specimens within clusters as well as specimens occurring in sympatry.

5.4.1.3 Canonical variates (discriminant) analysis of the lateral view of the cranium

The CVA (n = 17) of the lateral view of the cranium based on relative weight matrices of landmarks, and presented as a histogram (Fig. 5.5) also show two distinct groups that correspond with the two groups identified by the mitochondrial cytochrome *b* gene and exon 1 of the nuclear interphotoreceptor retinoid binding protein (IRBP) data (Chapter 2), cytogenetics (Chapter 3), traditional morphometric (Chapter 4) data, and the UPGMA cluster analysis above (Fig. 5.3). A MANOVA indicated a highly statistically significant cranial morphological difference between the centroids of the two delineated phenetic groups ($F_{8,8}$ = 56.64, $P < 0.05$). The CVA's correspondence with the other multidisciplinary techniques outlined above also includes specimens within clusters as well as specimens occurring in sympatry.

5.4.1.4 Splines of changes in the lateral view of the cranium

The changes in the position of landmarks with reference to a consensus configuration (splines) of the lateral view of the cranium derived from tpsSpline (Fig. 5.6) show that the differences between the specimens within phenetic clusters A and B is linked to the saggital crest region and posterior edge of the cranium. These differences are also reflected in traditional morphometric analysis (Chapter 4) where the PCA highlighted measurements with relatively high loadings to include breadth of braincase, height of rostrum, and greatest height of skull, that reflect either the lateral flatness or the curved nature of the cranium to be important morphological differences between the two phenetic groups. These differences are more pronounced in the assessment of

qualitative morphology (Chapter 4), which clearly shows either a cranial curvature or a depression dorsolaterally.

5.4.2 Geometric morphometrics of the lateral view of the mandible

The geometric morphometric analysis of the lateral view of the mandible was based on 14 of the 28 specimens due to mandibular damage that precluded the digitization of a complete suite of landmarks for analysis. Similarly, limited sample size necessitated data to be analysed by only a *priori* (UPGMA cluster analysis and PCA), the results of which are presented below, rather than *a posteriori* (CVA) analysis because of insufficient cell sizes, particularly of phenetic group A that was represented by lateral mandibular data of two specimens only from Rombo Alleni Chini (Locality 2).

5.4.2.1 UPGMA cluster analysis of the lateral view of the mandible

A UPGMA cluster analysis (n = 14) of the lateral view of the mandible revealed two distinct clusters (A & B (Fig. 5.7) that also broadly correspond with the two groups identified by the mitochondrial cytochrome *b* gene and exon 1 of the nuclear interphotoreceptor retinoid binding protein (IRBP) data (Chapter 2), cytogenetics (Chapter 3), and traditional morphometric (Chapter 4) data that equate to *A. wilsoni* and *A.* cf. *percivali*, respectively. Cluster A comprised specimens from Rombo Alleni Chini (Locality 2; Fig. 5.1). Cluster B consists of specimens from Tingatinga (Locality 3), Longido (Locality 4), and Kilimamoja (Locality 7). Similar to the UPGMA cluster analysis of the lateral view of the cranium (5.4.1.1), a single specimen (indicated by an asterisk in Fig. 5.7) from Kilimamoja (Locality 7) did not conform to the general pattern. As with all the multidisciplinary techniques applied to these northern Tanzanian specimens, the UPGMA cluster analysis of the lateral view of the mandible similarly revealed the presence of two phenetic groups of *Acomys*.

5.4.2.2 Principal components analysis of the lateral view of the mandible

Similarly, the PCA (n = 14) of the lateral view of the mandible revealed two distinct clusters, designated A & B (Fig. 5.8) that also correspond with the two groups identified by the mitochondrial cytochrome *b* gene and exon 1 of the nuclear interphotoreceptor retinoid binding protein (IRBP) data (Chapter 2), cytogenetics (Chapter 3), traditional morphometric (Chapter 4)

data, and the UPGMA cluster analysis above (Fig. 5.3). This separation is particularly evident on the third (13.18 % variance) rather than the first (32.37 % variance) relative warps (RW).

5.4.2.3 Splines of changes in the lateral view of the mandible

The changes in the position of landmarks with reference to a consensus configuration (splines) of the lateral view of the mandible (Fig. 5.9) show that the differences between the specimens within phenetic clusters A and B is linked to the posterior part of the mandible. These differences are also reflected in traditional morphometric analysis (Chapter 4) where the PCA highlighted measurements with relatively high loadings to include the height of the mandible from the ventral edge of the angular process to horizontal plane through bullae.

5.5 DISCUSSION

Geometric morphometric analyses in the present study strongly suggest the occurrence of two species of *Acomys* in northern Tanzania, namely, *A. wilsoni* and *A.* cf. *percivali* that have in the present study, been shown to be sympatric at Longido (Locality 4; Fig. 5.1). The occurrence of these two species in northern Tanzania and their co-existence at Longido is supported by mitochondrial cytochrome *b* gene and exon 1 of the nuclear interphotoreceptor retinoid binding protein (IRBP) (Chapter 2), cytogenetic (Chapter 3), and traditional morphometric and classical morphological (Chapter 4) data.

These results represent the first record of the occurrence of *A. percivali* in Tanzania which together with previously reported records of *A. spinosissimus*, *A. ignitus*, *A. kempi*, and *A. wilsoni* (Musser & Carleton 1993; 2005) increases the number of species of *Acomys* so far reported to occur in Tanzania from four to five. Similar to previous reports on geographic distribution (Allen and Loveridge 1942; Delany and Neal 1966; Fadda *et al.* 2001, Corti *et al.* 2005) and the multidisciplinary datasets indicated above (Chapters 2, 3, 4), the geometric data in the present study also suggest that *A. wilsoni* is distributed on the eastern part of Mt. Kilimanjaro, with its type locality in Mombasa, Kenya.

Although the present study found *A. wilsoni* and *A.* cf. *percivali* to be sympatric at Longido (Locality 4; Fig. 5.1), other instances of sympatry have also previously been reported at

Chanler Falls, Guaso Nyiro, central Kenya, the type locality of *A. percivali* (Dollman 1911, 1914) and in Karamoja, north-eastern Uganda (Delany & Neal 1966). Of particular relevance is that Longido and Chanler Falls are of similar topography and are both within the Great East African Rift Valley. Although sympatric, it is possible that the two species may be ecologically segregated since *A.* cf. *percivali* was in the present study, mainly captured in well-covered habitats with thorn bushes, rocky and mountainous areas, while *A. wilsoni* was mainly captured in open semi-arid grasslands and in rice fields in the Lower Moshi Plains (G. Mgode pers. obs.).

All results from present study and the other multidisciplinary characterization of the genus in northern Tanzania suggest that *A. percivali* may be the common species along the Great East African Rift Valley because some localities in northern Tanzania are associated with natural geological barriers. These geological barriers form part of the Great East African Rift Valley (Dawson 1970; King 1970; Quennell 1982), which has been associated with active rodent speciation (Colangelo *et al*. 2005).

Of particular relevance in the present study is that the geometric morphometric data showed differences in the saggital crest region and posterior edge of the cranium dorsolaterally. These differences are also reflected in traditional morphometric analysis where breadth of braincase, height of rostrum, and greatest height of skull, that reflect either the lateral flatness or the curved nature of the cranium to be important morphological differences between the two phenetic groups (Chapter 4). These differences are more pronounced in the assessment of qualitative morphology (Chapter 4), which clearly shows either a cranial curvature or a depression dorsolaterally. Other differences are linked to the posterior part of the mandible and are also reflected in traditional morphometric analysis (Chapter 4) where the height of the mandible from the ventral edge of the angular process to horizontal plane through bullae was shown to be an important measurement in the PCA. These parts of the cranium and mandible may be useful in future studies of the genus, particularly if an identification key for East African *Acomys* is to be derived. Of particular relevance is that these differences are further corroborated by data on type material (Chapter 4) and by mitochondrial cytochrome *b* gene and exon 1 of the nuclear interphotoreceptor retinoid binding protein (IRBP) (Chapter 2), cytogenetic (Chapter 3) data, and differences in the length of the tail (Chapter 4).

The traditional morphometric data (Chapter 4) indicated a specimen of *A. wilsoni* to have similar body dimensions with *A. nubilus* which characteristically has a longer tail. Although the latter species is considered difficult to classify because of its intermediate characteristics between the large, long-tailed *A. ignitus* and small, short-tailed *A. wilsoni* (Dollman 1914), the geometric data in the present study allocated the specimen to *A. wilsoni*. This allocation is further supported by the mitochondrial cytochrome *b* gene and exon 1 of the nuclear interphotoreceptor retinoid binding protein (IRBP) (Chapter 2) and the cytogenetic (Chapter 3) data.

Despite the suggested two species of *Acomys* in northern Tanzania in the present study, there is a need for further investigations of *Acomys* in East Africa in order to improve on our current understanding of the systematics and the distribution of the genus in East Africa. These future studies should focus on additional sampling from Kenya and specimens in museum holdings that have not necessarily been positively identified, as well as the examination of both type specimens and topotypical material.

5.6 REFERENCES

ALLEN, G. M. & LOVERIDGE, A. 1942. Scientific results of a fourth expedition to forested areas in East and Central Africa. I. Mammals. *Bulletin of the Museum of Comparative Zoology* (*Harvard*) **89**:205-206.

BAILEY, R. C. & BYRNES, J. 1990. A New, old method for assessing measurement error in both univariate and multivariate morphometric studies. *Systemati Zoology* **39**:124-130.

BELBIN, L. 1989. 'PATN analysis package. Users' and technical reference manuals'. CSIRO Division of Wildlife and Ecology: Canberra.

BOOKSTEIN, F. L. 1989. Principal warps: thin-plate splines and the decomposition of deformations. IEEE *Transactions on pattern analysis and machine intelligence* **11**:567-585.

BOOKSTEIN, F. L. 1991. *Morphometric tools for landmark data*: *geometry and biology*. Cambridge University Press, Cambridge.

COLANGELO, P., CORTI, M., VERHEYEN, E., ANNESI, F., OGUGE, N., MAKUNDI, R.H. & VERHEYEN, W. 2005. Mitochondrial phylogeny reveals differential modes of

chromosomal evolution in the genus *Tatera* (Rodentia: Gerbillinae) in Africa. *Molecular Phylogenetics and Evolution* **35**:556-568.

CORTI, M., CASTIGLIA, R., COLANGELO, P., CAPANNA, E., BEOLCHINI, F., BEKELE, A., OGUGE, N., MAKUNDI, R.H., SICHILIMA, A.M., LEIRS, H., VERHEYEN, W. & VERHAGEN, R. 2005. Cytotaxonomy of rodent species from Ethiopia, Kenya, Tanzania and Zambia. *Belgian Journal of Zoology* **135**:197-216.

CORTI, M. & MARCUS.L.F. 1996. Overview of the new or geometric morphometrics. In: *Advances in Morphometrics*, (eds) M. Corti, A. Loy, L.F. Marcus, G.J.P. Naylor and D.E. Slice. Pp.1-13. Plenum Press, New York.

CORTI, M., FADDA, C., SIMSON, S. & NEVO. E. 1996. Size and shape variation in the mandible of the fossorial rodent *Spalax ehrenbergi*. A Procrustes analysis of three dimension. In: *Advances in morphometrics*, (eds) L.F. Marcus, M. Corti, A. Loy, G.J.P. Naylor & D.E. Slice. Pp. 303-320. Plenum Press, New York & London.

CORTI, M. & THORPE, R.S. 1989. Morphological clines across a karyotypic zone of house mice incentral Italy. *Journal of Evolutionary Biology* **2**:253-264.

DAVIS, S.J.M. 1983. Morphometric variation of populations of house mice *Mus domesticus* in Britain and Faroe. *Journal of Zoology* (London) **199**:521-534.

DAWSON, J.B. 1970. Neogene rift tectonics and volcanism in northern Tanzania. *Proceedings of the Geological Society of London* **1663**:151-153.

DELANY, M.J. & NEAL B.R. 1966. A review of the Muridae (Order Rodentia) of Uganda. *Bulletin of the British Museum (Natural History) Zoology* **13**:334-335.

DEMETER, A., RACZ, G. & CSORBA, G. 1996. Identification of house mice, *Mus musculus*, and mound-building mice *M. spicilegus*, based on distance and landmark data. In: *Advances in morphometrics*, (eds) L.F. Marcus, M. Corti, A. Loy, G.J.P. Naylor & D.E. Slice. Pp.359-369. Plenum Press, New York & London.

DIPPENAAR, N. J. & RAUTENBACH, I.L. 1986. Morphometrics and karyology of the Southern African species of the genus *Acomys* I. Geoffroy Saint-Hilaire, 1838 (Rodentia, Muridae). Annals of the Transvaal Museum **34**:129-183.

DOLLMAN, G. 1911. New and interesting mammals from East Africa. *Annals and Magazine of Natural History* **8**:125-128.

DOLLMAN, G. 1914. On the spiny mice of British East Africa, with a description of a new

species from Magadi. *Annals and Magazine of Natural History* **8**:485-488.

FADDA, C., CASTIGLIA, R., COLANGELO, P., CORTI, M., MACHANG'U, R., MAKUNDI, R., SKANZANI, A., TESHA, P., VERHEYEN, W. & CAPANNA, E. 2001. The rodent fauna of Tanzania: A cytotaxonomic report from the Maasai Steppe (1999). *Rendiconti Fisiche Accademia Lincei* **12**:29-49.

JAMES, F. C. & McCULLOCH, C. E. 1990. Multivariate analysis in ecology and systematics: Panacea or Pandora's Box? *Annual Review of Ecology and Systematics* **21**:129-166.

JOLLIFFE, I.T. 1986. *Principal* components *analysis*. Springer-Verlag.

KING, B.C. 1970. Vulcanicity and rift tectonics in East Africa. In: *African Magmatism and Tectonics*, (eds) T.N. Clifford & I.G. Gass. Pp.263-267, 269-275, 281-283. Oliver & Boyd, Edinburgh.

MARCUS, L. F., CORTI, M., LOY, A., NAYLOR, G. J. P. & SLICE, D.E. 1996. *Advances in Morphometrics*. Plenum Press. New York.

MARCUS, L. F. 1993. In: *Contributions to morphometrics,* (eds) L. F. Marcus, A, Valdecasas & E. Bello, Madrid Museum of Natural History, Madrid.
Thomas, especes d' Abyssinie. *Mammalia* **32**:621-627.

MARCUS, L. F. 1990. Traditional morphometrics. In: *Proceedings of the Michigan morphometrics workshop*, (eds) F.J. Rohlf & F. L. Bookstein. Special Publication Number 2. Pp.77-122, University of Michigan Museum of Zoology, Ann Arbor.

MUSSER, G. & CARLETON, M. 2005. Superfamily Muroidea. In: *Mammal species of the World,* (eds) D. E. Wilson & D. M. Reeder. The Johns Hopkins University Press, Baltimore and London.

MUSSER, G. G. & CARLETON, M. D. 1993. Genus *Acomys*. In: *Mammal species of the world,* (eds) D. E. Wilson & D. A. M. Reeder. Pp.564-567. Smithsonian Institution Press, Washington.

PANKAKOSKI, E., VAISANEN, R. A. & NURMI, K. 1987. Variability of muskrat skulls: measurement error, environmental modification and size allometry. *Systematic Zoology* **36**:35–51.

QUENNELL, A.M. 1982. *Rift Valleys*. Afro-Arabian. Hutchinson Ross Publishing Company, Stroudsburg, Pennsylvania.

REYMENT, R. A. 1991. *Multidimensional paleobiology*. Pergamon Press, New York.

ROHLF, F.J. & MARCUS, L. F.. 1993. A revolution in morphometrics. *Trends in Ecology and Evolution* **8**:129-132.

ROHLF, F.J. 2004a. tpsDig, version 1.39. Department of Ecology and Evolution, Stony Brook, New York.

ROHLF, F.J. 2004b. tpsSpline, version 1.39. Department of Ecology and Evolution, Stony Brook, New York.

ROHLF, F.J. 2004c. tpsRelw, version 1.39. Department of Ecology and Evolution, Stony Brook, New York.

ROHLF, F.J. 2004d. tpsSmall, version 1.39. Department of Ecology and Evolution, Stony Brook, New York.

ROHLF, F. J. 2004. Thin-Plate Spline programs. Department of Ecology and Evolution, State University of New York, Stony Brook, New York.

ROHLF, F.J. 1993. TPSRW-Thin-plate spline relative warp. Department of Ecology and Evolution, State University of New York, Stony Brook, New York.

SCHRIVEN, P.N. & BAUCHAU, V. 1992. The effect of hybridization on mandible morphology in an island population of house mouse. *Journal of Zoology* (London) **226**:573-583.

SNEATH, P. H. A. & SOKAL, R.R. 1973. *Numerical taxonomy*. W. H. Freeman, San Francisco.

STATSOFT.2004. *Statistica for Windows* version 7.0, Statsoft Inc. Tulsa, Oklahoma.

VOGL, C., ATCHLEY, W.R., COWLEY, D.E., CRENSHAW, P., MURRAY, J.D. & POMP, D. 1993. The epigenetic influence of growth hormone on skeletal development. *Growth, Development and Aging* **57**:163-182.

VOSS, R. S., MARCUS, L. F. & ESCALANTA, P. 1990. Morphological evolution in muroid rodents. Conservative patterns of craniometric covariance and their ontogenic basis in the Neotropical genus *Zygodontomys*. *Evolution* **44**:1568-1587.

ZAR, J. 1996. *Biostatistical Analysis*. Prentice Hall, New Jersey.

5.7 FIGURE LEGENDS

Fig. 5.1 Map of Tanzania showing collecting localities of *Acomys* examined in this study: 1 = Lower Moshi (Kahe Chekeleni); 2 = Rombo–Alleni Chini; 3 = Tingatinga; 4 = Mt. Longido and Longido Plains; 5 = Mt. Gelai-Olikisima; 6 = Ikorongo–Grumeti Game Reserve; 7 = Kilimamoja view-point, Lake Manyara National Park. The dotted line denotes Mt. Kilimanjaro which separates some of these localities, while the insert indicates the geographic position of Tanzania in relation to the African continent.

Fig. 5.2 Landmarks of the dorsal (a), ventral (b), and lateral (c) views of the skull, and the lateral view of the mandible (d) used in geometric morphometric analyses of *Acomys* from northern Tanzania.

Fig. 5.3 An Unweighted-pair group arithmetic average (UPGMA) cluster analysis from a geometric morphometric analysis of the lateral view of the cranium of *Acomys* from northern Tanzania. A = specimens designated to be *A. wilsoni*, while B = specimens designated to be *A.* cf. *percivali* Numbers correspond to locality numbers in Fig. 5.1 and Table 5.1. Specimens that do not conform to the general pattern are indicated by an asterisk (*).

Fig. 5.4 A scattergram of relative warps (RW) I and II from a principal components analysis (PCA) of geometric morphometric data of the lateral view of the cranium used to assess morphometric variation in *Acomys* from northern Tanzania. Minimum convex polygons enclose genetically and karyologically-identified specimens. A = specimens designated to be *A. wilsoni*, while B = specimens designated to be *A.* cf. *percivali*. Numbers correspond to locality numbers in Fig. 5.1 and Table 5.1.

Fig. 5.5 A histogram of discriminant scores from a two-group canonical variates analysis of specimens of *Acomys* from northern Tanzania. (■) = specimens designated to be *A. wilsoni*, while (▨) = specimens designated to be *A. percivali*.

Fig. 5.6 Changes in the position of landmarks with reference to a consensus configuration (splines) (a & b) of the lateral view of the cranium derived from TPSSpline (Rohlf 2004a) are indicated for the specimens designated to be *A.* cf. *percivali* (c & d) and specimens designated to be *A. wilsoni* (e & f).

Fig. 5.7 An Unweighted-pair group arithmetic average (UPGMA) cluster analysis from a geometric morphometric analysis of the lateral view of the mandible of *Acomys* from northern Tanzania. A = specimens designated to be *A. wilsoni*, while B = specimens designated to be *A.* cf. *percivali*. Numbers correspond to locality numbers in Fig. 5.1 and Table 5.1. Specimens that do not conform to the general pattern are indicated by an asterisk (*).

Fig. 5.8 A scattergram of relative warps (RW) I and III from a principal components analysis (PCA) of geometric morphometric data of the lateral view of the mandible used to assess morphometric variation in *Acomys* from northern Tanzania. Minimum convex polygons enclose genetically and karyologically-identified specimens. A = specimens designated to be *A. wilsoni*, while B = specimens designated to be *A.* cf. *percivali*. Numbers correspond to locality numbers in Fig. 5.1 and Table 5.1.

Fig. 5.9 Changes in the position of landmarks with reference to a consensus configuration (splines) (a & b) of the lateral view of the mandible derived from TPSSpline (Rohlf 2004a) are indicated for the specimens designated to be *A.* cf. *percivali* (c & d) and specimens designated to be *A. wilsoni* (e & f).

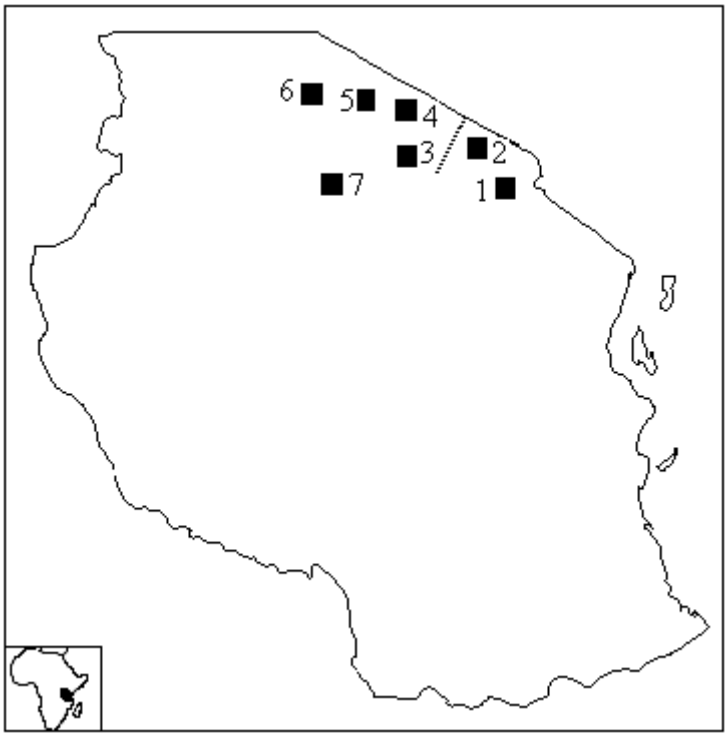
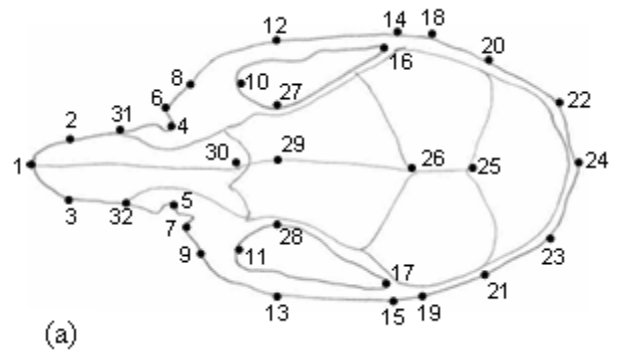


Fig. 5.1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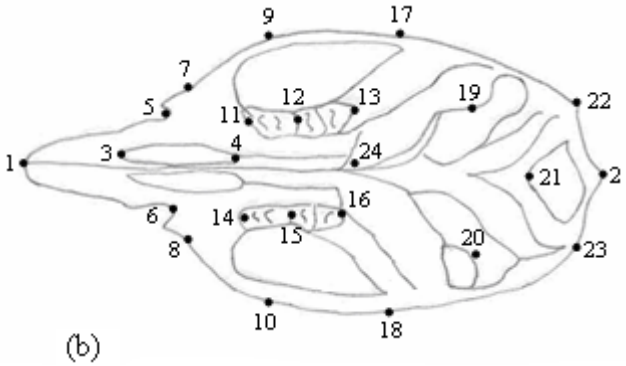
(a)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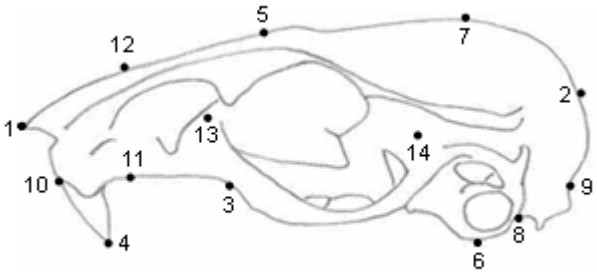
(b)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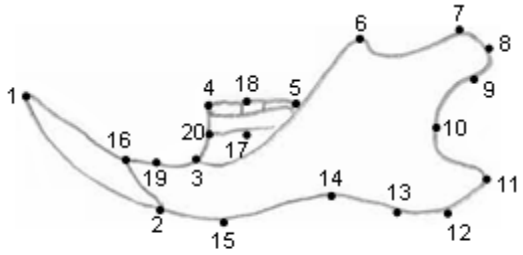
(c)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(d)

Fig. 5.2

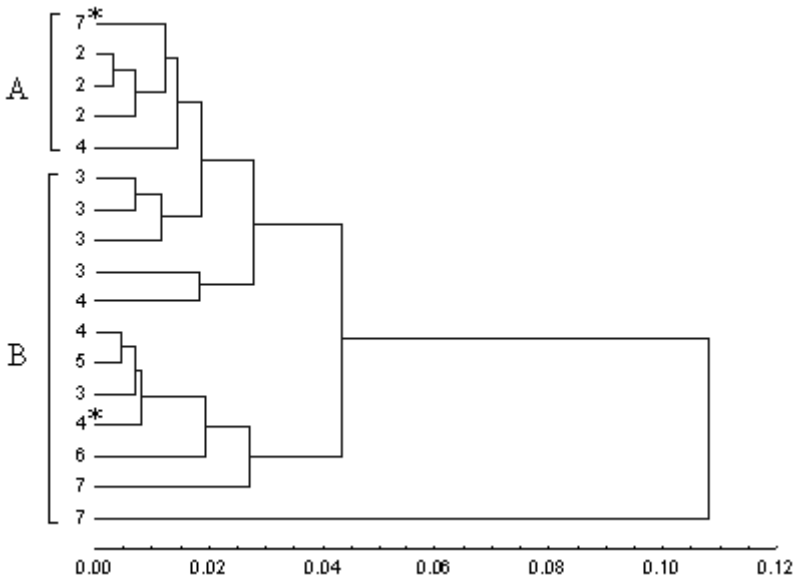

Fig. 5.3

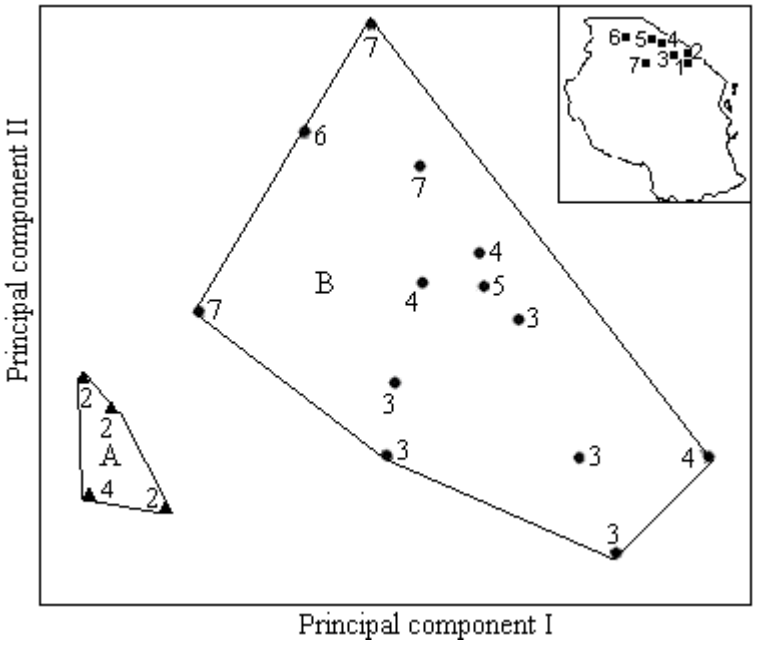
Principal component II
Principal component I
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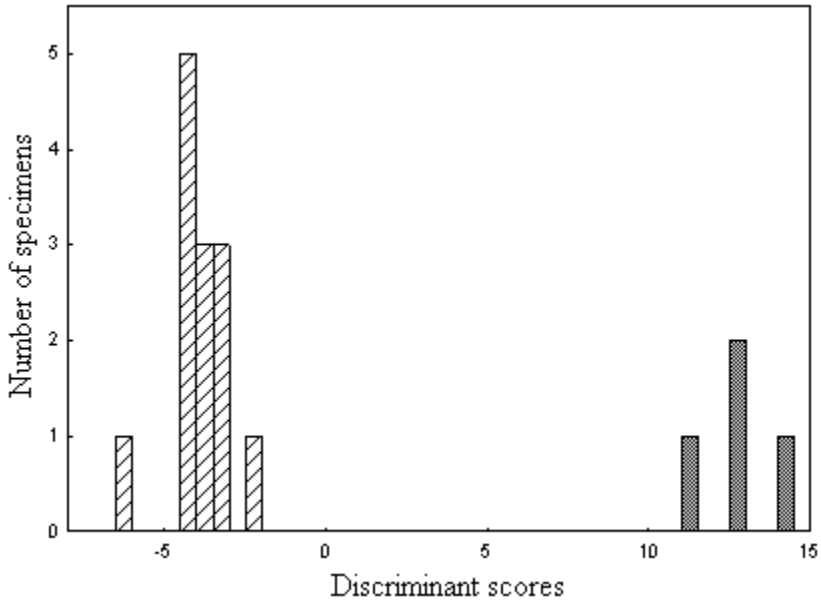
Fig. 5.4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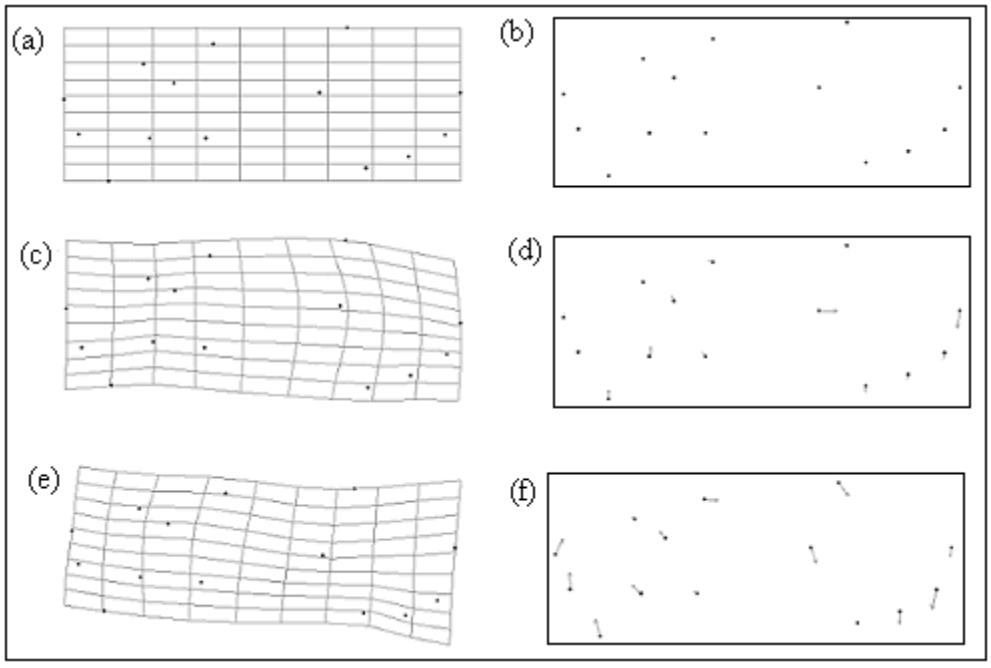
Discriminant scores

Fig. 5.5


(a)
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(f)

Fig. 5.6

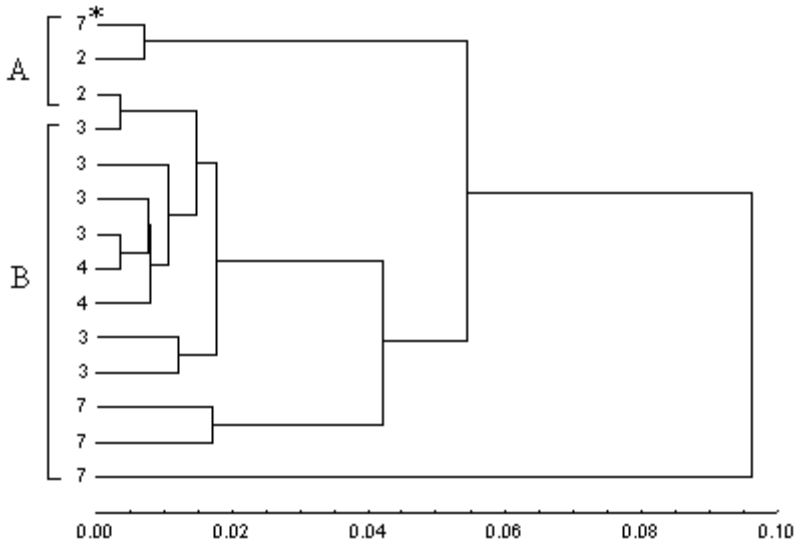

Fig. 5.7

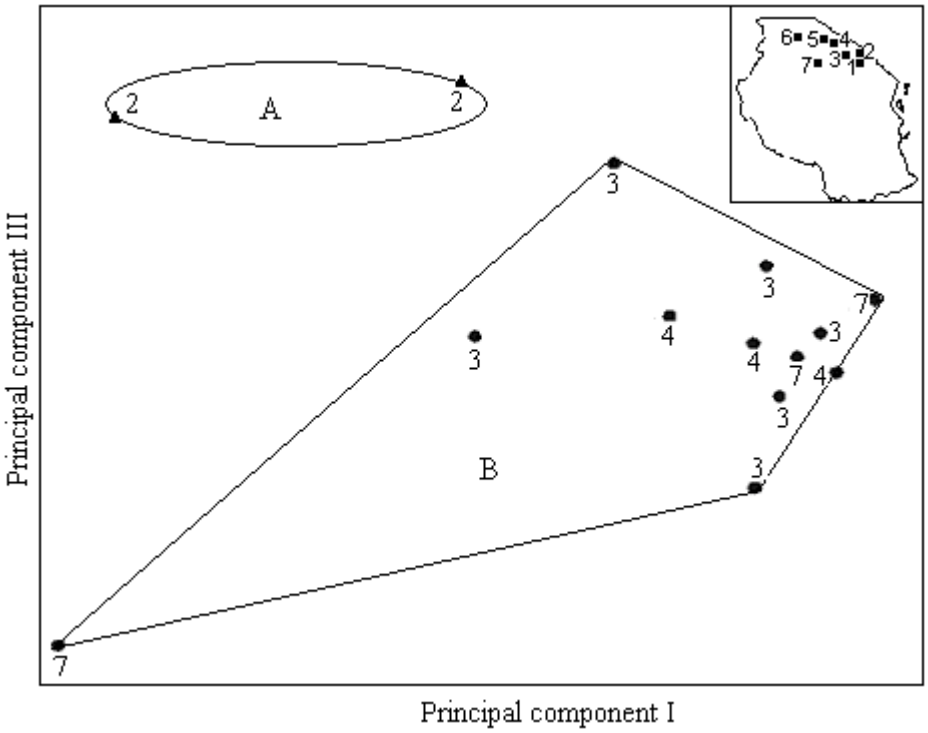
Principal component III
Principal component I
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Fig. 5.8

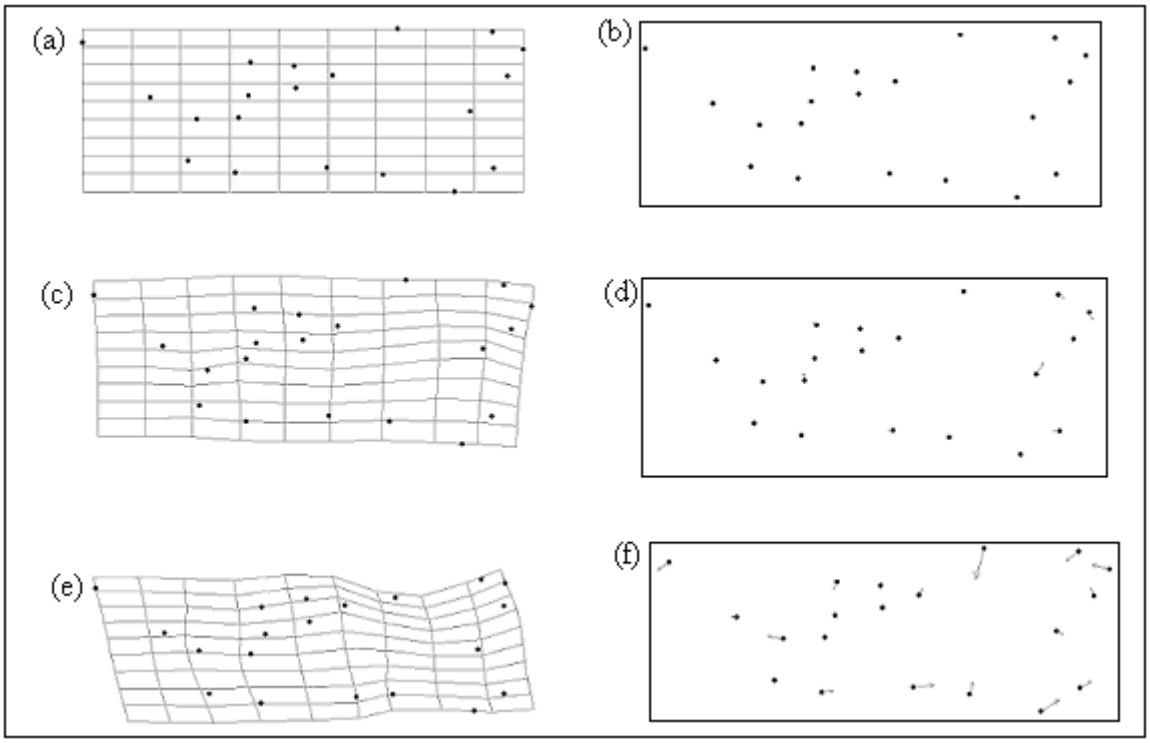

Fig. 5.9

Table 5.1. Collecting localities, a gazetteer and altitude of specimens of *Acomys* from northern Tanzania examined in this study. Numbers correspond to localities in Fig. 5.1.

	Locality	Geographic Coordinates and Altitude
1	Lower Moshi, eastern Kilimanjaro	03.41S; 37.37E; 750 m a.s.l.
2	Rombo–Alleni Chini, eastern Kilimanjaro	03.24081S; 037.68139E; 1037 m a.s.l.
3	Tingatinga, western Kilimanjaro	02.96169S & 02.96810S; 036.95261E & 036.94566E; 1210 & 1215 m a.s.l.
4	Longido Mountain, east of Lake Natron, western Kilimanjaro	02.71276S; 036.68261E; 1398 m a.s.l.
	Longido plain–Irmibokuni–Engosipa or Olekibari on Great East African Rift Valley, east of Lake Natron, western Mt. Kilimanjaro	02.70563S, 036.60299E; 457 m a.s.l.
	Longido plain–Parmunyan, east of Lake Natron, western Kilimanjaro	02.70693S; 036.54184E; 1447 m a.s.l.
5	Mt. Gelai-Olikisima, east of Lake Natron	02.70367S; 036.11255E; 1427 m a.s.l.
6	Ikorongo–Grumeti Game Reserve, west of Serengeti National Park	02.10707S; 034. 47665E; 1414 m a.s.l.
7	Kilimamoja view-point, Lake Manyara National Park along the Great East African Rift Valley	03.38696S; 035.82093E; 1214 m a.s.l.

CHAPTER SIX

6.1 GENERAL DISCUSSION

Evidence presented in the present study strongly suggests the occurrence of two species of *Acomys* in northern Tanzania, namely, *A. wilsoni* and *A.* cf. *percivali*. These findings are supported by mtDNA cytochrome *b* and nuclear interphotoreceptor retinoid binding protein (IRBP) sequences, cytogenetics, traditional and geometric morphometrics, and classical morphological data. More importantly, the two species occur in sympatry at Longido on the Great East African Rift Valley (King 1970; Dawson 1970).

Within this sympatric zone, the two species seem to partition their niches. For example, while *A. wilsoni* was captured in open savanna grassland in the Longido Plain, *A.* cf. *percivali* was captured *ca.* 13 km away on Longido Mountain. This niche partitioning was observed elsewhere. For example *A. wilsoni* was captured in semi-arid grasslands in Rombo Alleni Chini and rice fields in Lower Moshi, whilst *A.* cf. *percivali* was collected from well-covered habitats such as thorn bushes, and rocky and mountainous areas (G. Mgode pers. obs.). In overgrazed areas such as Tingatinga, *A.* cf. *percivali* was collected around patches of thorny trees, wild sisal species, and thorny fences surrounding grassy areas reserved for grazing cattle calves (G. Mgode pers. obs.). Such habitat partitioning corroborates similar findings with other species of *Acomys* (Shkolnik 1966; Shkolnik & Borut 1969; Shkolnik 1971; Jones & Dayan 2000; Shargal *et al.* 2000). For example, the sympatric occurrence of *A. wilsoni* and *A.* cf. *percivali* in Longido corresponds with findings at Chanler Falls, Guaso Nyiro, Kenya, the type locality of *A. percivali* (Dollman 1911, 1914). Both the type locality and Longido are located on the Great East African Rift Valley. The sympatric occurrence of *A. wilsoni* and *A. percivali* has also been reported in Karamoja in north-eastern Uganda (Delany & Neal 1966).

Although evidence of the occurrence of *A. wilsoni* in northern Tanzania is supported by the multidisciplinary analyses in the present study that also incorporated GenBank sequences of specimens from Kenya, the phylogenetic analysis does not support a monophyletic origin of

Acomys species. This suggests a critical need for further multidisciplinary investigations that include material from Mombasa, Kenya, the type locality of *A. wilsoni*.

The suggested future multidisciplinary analyses should also include an extensive investigation of external morphology especially the tail length. For example, while shorter tails (45–50 mm) seem to be typical of *A. wilsoni* (Dollman 1911, 1914; Allen & Lawrence 1936; Delany & Neal 1966), a specimen from Lower Moshi had a relatively longer (60 mm) tail than is typical for *A. wilsoni* (Allen & Loveridge 1942). Despite this relatively longer tail, this specimen was allocated to *A. wilsoni* rather than *A. nubilus* which is similar in body size to *A. wilsoni*, except for a longer tail (65–67 mm). Given that the taxonomic status of *A. nubilus* is uncertain, often considered intermediate between the large and long-tailed *A. ignitus* and the small and short-tailed *A. wilsoni* (Dollman 1914); further investigations incorporating external morphology are critically needed.

Of particular relevance is the evidence of the occurrence of the putative *A.* cf. *percivali* in the present study. It is essentially based on the morphological similarity between specimens examined in this study with the type specimen of *A. percivali* from Kenya (Dollman 1911, Delany & Neal 1966). Specimens of *A.* cf. *percivali* examined in the present study are larger with a longer tail (68–87 mm, which falls within the tail length range of the type specimen). In addition, specimens of *A.* cf. *percivali* from this study were dark dorsally and white ventrally. However, one old individual from Tingatinga was exceptionally reddish in colour which is common in *A. percivali*, while another individual from Ikorongo-Grumeti Game Reserve was more greyish dorsally. Although colour variation is common in *A. percivali*, the species is mostly dark-brown in colour, with the reddish–grey colour being associated with a rufous tinting of the spines (Dollman 1911). This colour variation should be investigated further with reference to an analysis based on a wide range of systematic techniques as well as a comprehensive geographic coverage.

Prior to the present study there were no published mtDNA cytochrome *b* and nuclear IRBP sequences for *A. percivali* in GenBank. The only available GenBank sequence of this species is from the D-loop gene, a genomic region that is mainly used for population studies due

to its hypervariability (Barreto *et al*. 1996). If the results of the cytogenetics, traditional and geometric morphometric, and external morphological data in the present study indeed support the occurrence of *A*. *percivali*, then this represents the first ever reported mtDNA cytochrome *b* and nuclear IRBP sequences for this species. These findings will however, need to be confirmed by sequencing material from Chanler Falls, Guaso Nyiro, Kenya, the type locality of the species.

Based on the findings of the present study, the number of *Acomys* species occurring in Tanzania increases from four (Musser & Carleton 1993, 2005) to five, although the systematic status of one of these four species, *A*. *ignitus*, is controversial. Fadda *et al*. (2001) confirmed previous reports of the occurrence of four species of *Acomys* in Tanzania, namely, *A*. *spinosissimus*, *A*. *wilsoni*, *A*. *ignitus*, and *A*. *kempi* but they included a cautionary note on the systematic status of *A*. *ignitus*. For example, Petter (1983) considered *A*. *ignitus* to be a valid species, Ellerman (1941) related it to *A*. *pulchellus*, *A*. *kempi*, and *A*. *montanus*, Janecek *et al*. (1991) related it to *A*. *cahirinus*, while Setzer (1975) treated it as a subspecies of *A*. *dimidiatus* (see Chapter 1, Table 1.1).

Apart from these taxonomic uncertainties, the *A*. cf. *percivali* recognized in the present study shows neither genetic nor morphological resemblance to any of these four species, strongly suggesting the need for further multidisciplinary systematic studies of *Acomys* from East Africa. The need for further studies is enhanced further by the suggestion that Africa may be the origin of genus *Acomys* (Barome *et al*. 2000). This hypothesis is supported by the occurrence in Africa of 11 of the 19 recognized species of *Acomys*, four of which were reported from Tanzania (Musser & Carleton 1993, 2005). Northern Tanzania, in particular is an important region from an evolutionary perspective as it forms part of the complex East African Rift Valley (King 1970; Dawson 1970; Quennell 1982), a region that has generally been associated with active rodent speciation (Colangelo *et al*. 2005). The results of this study are therefore likely to contribute to, and be incorporated within future broader-based geographical studies that address the diversity and origins of members of the genus *Acomys* in Africa.

6.2 REFERENCES

ALLEN, G. M. & LOVERIDGE, A. 1942. Scientific results of a fourth expedition to forested areas in East and Central Africa. I. Mammals. *Bulletin of the Museum of Comparative Zoology* (*Harvard*) **89**:205-206.

ALLEN, G.M. & LAWRENCE, B. 1936. Scientific results of an expedition to rain forest regions in Eastern Africa. III. Mammals. *Bulletin of the Museum of Comparative Zoology* (*Harvard*) **79**:31-l26.

BARRETO, G., VAGO, A.R., GINTHER, C., SIMPSON, A.J. & PENA, S. D. 1996. Mitochondrial D-loop ‘‘signatures’’ produced by low-stringency single specific primer PCR constitute a simple comparative human identity test. *American Journal of Human Genetics* **58**:609-616.

BAROME, P.O., MONNEROT, M. & GAUTUN, J.C. 2000. Phylogeny of the genus *Acomys* (Rodentia, Muridae) based on the cytochrome *b* mitochondrial gene: implications on taxonomy and phylogeography. *Mammalia* **64**:423-438.

COLANGELO, P., CORTI, M., VERHEYEN, E., ANNESI, F., OGUGE, N., MAKUNDI, R.H. & VERHEYEN, W. 2005. Mitochondrial phylogeny reveals differential modes of chromosomal evolution in the genus *Tatera* (Rodentia: Gerbillinae) in Africa. *Molecular Phylogenetics and Evolution* **35**:556-568.

DAWSON, J.B. 1970. Neogene rift tectonics and volcanism in northern Tanzania. *Proceedings of the Geological Society of London* **1663**:151-153.

DELANY, M.J. & NEAL, B.R. 1966. A review of the Muridae (Order Rodentia) of Uganda. *Bulletin of the British Museum (Natural History) Zoology* **13**:334-335.

DOLLMAN, G. 1911. New and interesting mammals from East Africa. *Annals and Magazine of Natural History* **8**:125-128.

DOLLMAN, G. 1914. On the spiny mice of British East Africa, with a description of a new species from Magadi. *Annals and Magazine of Natural History* **8**:485-488.

ELLERMAN, J. R. 1941. *The families and genera of living rodents*. Vol. 2. Muridae, Pp. 269-275. Trustees of the British Museum (Natural History), London.

FADDA, C., CASTGLIA, R., COLANGELO, P., CORTI, M., MACHANG’U, R., MAKUNDI, R., SKANZANI, A., TESHA, P., VERHEYEN, W. & CAPANNA,

E. 2001. The rodent fauna of Tanzania: A cytotaxonomic report from the Masai Steppe (1999). *Rendiconti Fisiche Accademia Lincei* **12**:29-49.

JANECEK, L. L., SCHLITTER, D. A. & RAUTENBACH, I. L. 1991. A genic comparison of spiny mice, genus *Acomys*. *Journal of Mammalogy* **27**:542-552.

JONES, M. & DAYAN, T. 2000. Foraging behaviour and microhabitat use by spiny mice, *Acomys cahirinus* and *A. russatus*, in the presence of Blanford's fox (*Vulpes cana*) odor. *Journal of Chemical Ecology* **26**:455-469.

KING, B.C. 1970. Vulcanicity and rift tectonics in East Africa. In: *African magmatism and tectonics*, (eds) T.N. Clifford & I.G. Gass, Oliver & Boyd, Pp. 263-267, 269-275, 281-283. Edinburgh.

MUSSER, G. & CARLETON, M. 2005. Superfamily Muroidea. In: *Mammal species of the World*: A taxonomic and geographic reference, (eds) D. E. Wilson & D. M. Reeder, The Johns Hopkins University Press. Baltimore and London.

MUSSER, G. G. & CARLETON, M. D. 1993. Genus *Acomys*. In: *Mammal species of the world*, (eds) D. E. Wilson & D. M. Reeder, Pp. 564-567. Smithsonian Institution Press, Washington.

PETTER, F. 1983. Elements d'une revision des *Acomys* africains. Un sous-genre nouveau, *Peracomys* Petter et Roche, 1981 (Rongeurs, Murides). *Annales Mus e Royal de l' Afrique Centrale, Sciences Zoologiques* **237**:109-119.

QUENNELL, A.M. 1982. *Rift Valleys*. Afro-Arabian. Hutchinson Ross Publishing Company, Stroudsburg, Pennsylvania.

SETZER, H. W. 1975. Genus *Acomys*. In: *The mammals of Africa*: An identification manual, (eds) J. Meester & H. W. Setzer, Pp. 1-2. Smithsonian Institution Press, Washington.

SHARGAL, E., KRONFELD-SCHOR, N. & DAYAN, T. 2000. Population biology and spatial relationships of coexisting spiny mice (*Acomys*) in Israel. *Journal of Mammalogy* **81**:1046-1052.

SHKOLNIK, A. 1971. Diurnal activity in small desert rodent. *International Journal of Biometerology* **15**:115-120.

SHKOLNIK, A. & BORUT, A. 1969. Temperature and water relations in two species of spiny mice (*Acomys*). *Journal of Mammalogy* **50**:245-255.