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Aspects of the biology of the East African root rat, *Tachyoryctes splendens* (Family: Spalacidae) from Tanzania

by

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Declaration

I, **Jestina Venance Katandukila** declare that this thesis which I hereby submit for the degree of Doctor of Philosophy in Zoology at the University of Pretoria is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature: 

Date: 8th April 2014

Disclaimer

This thesis consists of a series of chapters that have been prepared as stand-alone manuscripts, some of which have been published and others submitted for publication. It is therefore possible that some unavoidable repetition may occur between chapters.

Thesis Abstract

This thesis investigates a number of aspects of the biology of the East African root rat, *Tachyoryctes splendens* (Rüppell, 1835) and provides important information currently depauperate in the literature. These aspects include the general burrow architecture with respect to fractal dimension (i.e. exploration efficiency), locomotory activity patterns in relation to specific light cycles, the pattern of reproduction, age structure and population growth characteristics based on craniometrics and body mass data, and genetic population structure based on molecular data.

Assessment of burrow architecture revealed higher fractal dimension during the wet compared to the dry season as a consequence of increased soil moisture content promoting efficient excavation. Female burrow systems exhibited greater fractal dimensions than males as a result of increased energy costs for provisioning when female has pup(s).

Locomotory activity patterns of the captive East African root rat subjected to different lighting schedules maintained under a constant temperature demonstrated that captive *T. splendens* displays a higher percentage of activity during the dark than the light phase of all light-dark and dark-light cycles, suggesting that their activity is entrained by light. Under constant darkness, the species concentrated a higher percentage of its activity during subjective night intimating that *T. splendens* is able to run their activities at the absence of light stimulus (i.e. possesses an endogenous circadian rhythm).

An investigation of reproductive biology of the species as determined from post-mortem examination of the gonads and hormone concentrations of specimens collected on a monthly basis in the field revealed a bimodal pattern of reproduction that mirrored the pattern of rainfall.

The peaks of both male and female indicators of reproductive markers coincided with peaks of rainfall implying that precipitation is the major factor influencing reproductive activities since rainfall water facilitates the flush of vegetation and enhance food production in the form of forbes, grasses and underground storage organs of geophytes.

An assessment of ontogenetic variation and sexual dimorphism based on craniometric data from five relative age classes revealed overall increase in cranial dimensions with increasing age and that males were larger than females from age class 2, a trend that was also reflected in body mass.

The molecular results revealed the low genetic distances using cytochrome b (cyt-b) across the sampling range, implying that amongst distinct populations only a single species occurs across the sampled range. The greatest sequence differences at the mitochondrial DNA D-loop was observed both within and among geographically neighbouring populations, while the same haplotypes were sometimes shared across the sampling range that indicate a high molecular diversity within the species.

Key words: activity pattern, burrow architecture, fractal dimension, histology, hormone, maxillary molar, molecular genetics, ontogeny, radioimmunoassay, sexual dimorphism, *Tachyoryctes splendens*, Tanzania

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Dedication

This work is the fruitful effort of my parents, **Venance Benard Katandukila** and **Fridah Fanuel Machungi** for their love and care. Although they are not with me at this moment, I believe that I am excelling because of their compassionate parenting. I dedicate this work to my lovely husband, **Mohamed Mwinyihaji Jumbe** for accepting loneliness and caring of our children alone while I was busy with my PhD studies; you really "love" me. I also dedicate this work to my lovely sons, **Innocent** and **Isiack** as well as to my angel daughter, **Mariam**.

Ahsante Mwenyezi Mungu kwa kunibariki!!

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Chapter 1: General Introduction

1.1 Study Animal

The East African root rat, *Tachyoryctes splendens* (Rüppell, 1835) is a subterranean rodent of the family Spalacidae within the subfamily Rhizomyinae (Jansa *et al.* 1999; Michaux *et al.* 2001; Jansa & Weksler 2004). The genus comprises Old World fossorial muroid rodents (Kingdon 1974; Corbert 1984). *Tachyoryctes splendens* is a rodent with a stocky body and it resembles both a mole and a rat with a tail approximately twice the length of the hind feet. The body of *T. splendens* is covered by a soft thick pelage that varies in colour from black and pale grey through to chocolate brown and they possess tactile stiff bristles around the face.

The root rat superficially resembles members of the family Bathyergidae and thus they are often mistaken for bathyergid mole-rats. The characteristic feature that distinguishes this genus from bathyergid mole-rats is the distinct yellow-orange colour of their thick extra-buccal incisors. They also possess small claws and short powerful legs. These mammals use visual, tactile, olfactory, taste and auditory cues in their burrow environment and they rap on the roof of

the tunnel with their upper incisors as a form of territorial behaviour (Poor 2005). Although sexual dimorphism has been reported in East African root rats (Poor 2005), the nature and extent of its variation remains unknown. Because of the paucity of information, the current study adopted a multifaceted approach involving classical morphology and morphometrics to evaluate the nature and extent of sexual dimorphism in *T. splendens*.

1.1.1 The geographic distribution

The geographic distribution of spalacids ranges from the Ukraine in Europe through to the Balkans and north-eastern Africa and as far west as Libya (Topachevski 1969; Nowak 1999). In Asia, spalacids occur from western China, south to Sumatra and north to southern Siberia (Corbert 1984). Despite the wide distributional range of spalacids, *T. splendens* is known to be native to eastern and central Africa including Tanzania, Kenya, Rwanda, Burundi, Ethiopia, Somalia and the eastern part of the Democratic Republic of Congo (DRC) (Jarvis & Sale 1971; Kingdon 1974; Carleton & Musser 1984; Lacey *et al.* 2000; Musser & Carleton 2005; Kokiso & Bekele 2008; Schlitter *et al.* 2008).

1.1.2 Taxonomy, systematics and evolution

The first fossil record of representatives of the genus *Tachyoryctes*, *T. pliocaenicus* dating back to the Mio-Pliocene (5 MYA) and Miocene (5.8 MYA) was discovered in Ethiopia although its relationship with the extant members of the genus remains unknown (Sabatier 1978; Yalden 1985; Haile-Selassie *et al.* 2004). Fossil data suggest that the extant East African members of the genus *Tachyoryctes* have been around since the Lower Pleistocene (2.5 MYA) (Kingdon 1974;

Sabatier 1978; Flynn 1982; Haile-Selassie *et al.* 2004). Taxonomically, the genus was originally placed within the family Rhizomyidae and included other extant genera such as *Rhizomys* and *Cannomys* (Miller & Gidley 1918; Kowalski 1968). *Tachyoryctoides*, an ancestral rhizomyid has been recorded in Eurasian deposits dating back to the Oligocene (37-24 MYA) (Bohlin 1937; Kingdon 1974; Flynn 1982). Phylogenetic analyses based on molecular data revealed that members of the families Rhizomyidae and Spalacidae are monophyletic and suggested the re-allocation of the former family into the latter family (Michaux *et al.* 2001; Jansa & Weksler 2004; Norris *et al.* 2004; Steppan *et al.* 2004) as was proposed earlier by Tullberg (1899) based on plesiomorphic characters.

As proposed by Major (1897), differences in dentition, masticatory apparatus, infra-orbital canal and the zygomatic plate have all been used taxonomically to re-allocate members of the genus *Tachyoryctes* from the subfamily Rhizomyinae to the subfamily Tachyoryctinae comprising the genera *Tachyoryctes* and *Brachyuromys* (Ellerman 1940, 1941; Simpson 1945; Chaline *et al.* 1977; Lavocat 1978; Flynn 1990). Although members of the genera *Tachyoryctes* and *Brachyuromys* were considered monophyletic, phylogenetic analyses based on molecular data revealed that members of the two genera are paraphyletic and hence the re-allocation of the genus *Brachyuromys* to the subfamily Nesomyinae (Jansa *et al.* 1999; Michaux *et al.* 2001; Jansa & Weksler 2004).

The taxonomy of the genus *Tachyoryctes* is uncertain following placement of the genus to both subfamilies Tachyoryctinae and Rhizomyinae by different systematists (Jansa *et al.* 1999; Norris *et al.* 2004; Potapova & Vorontsov 2004; Steppan *et al.* 2004). However, phylogenetic analyses based on molecular data revealed that members of both subfamilies Tachyoryctinae and Rhizomyinae are sister taxa and suggested the re-allocation of the genus *Tachyoryctes* to the

latter subfamily (Michaux *et al.* 2001; Jansa & Weksler 2004) and this taxonomic treatment is followed in the present study. Conversely, palaeontological data suggests that the subfamilies Tachyoryctinae and Rhizomyinae diverged during the Miocene approximately 17 MYA, although they had functional convergence of subterranean lifestyle (Black 1972; Flynn & Sabatier 1984; Flynn 1990; Mein *et al.* 2000; Patnaik 2001). Furthermore, osteological fragments of the early fossil records of members of the two subfamilies indicate that the early forms were not subterranean (Flynn 1990; Endo *et al.* 2001) and that, specialized subterranean adaptations in members of the Rhizomyinae and Tachyoryctinae appear to have evolved around 8.5 and 7.8 MYA, respectively (Flynn, 1990; Musser & Carleton, 2005).

Based on classical morphology, Allen (1939) and Ellerman (1941) recognized 14 species within the genus *Tachyoryctes*. However, a taxonomic revision of the genus by Misonne (1974) and subsequently Corbert & Hill (1991) based on craniometric data recognized only two species namely, *T. macrocephalus* and *T. splendens*. *Tachyoryctes macrocephalus* was retained due to its relatively large skull while the remaining 13 species were synonymized with *T. splendens*. Bekele (1986) and Baskevich *et al.* (1993) however, argued that the classification of the genus should not only be based on craniometric data but, instead other criteria should also be used. Subsequently, Beolchini & Corti (2004) recognized 2 species based on geometric morphometrics data supporting the taxonomy suggested by Missone (1974) and Corbert & Hill (1991). Recently, Musser & Carleton (2005) recognized 13 species within the genus *Tachyoryctes* with *T. macrocephalus* being retained.

Musser & Carleton (2005) however, indicated that the description of some species such as *T. ankoliae*, *T. naivashae* and *T. ruandae* were based on their type localities with poorly supported taxonomic data. The taxonomic treatment of members of the genus *Tachyoryctes*

based on their type localities should be treated as tentative because the same species may occur allopatrically. However, T. *naivashae*, an IUCN-listed endangered species, *T. ruddi*, *T. daemon and T. ibeanus* have also been reported to occur in Tanzania (Schlitter *et al.* 2004; Musser & Carleton 2005). Therefore, the present multidisciplinary analysis encompassing molecular analyses was used to investigate the occurrence of *Tachyoryctes* species across a distributional range in Tanzania.

1.1.3 Ecology

As with other subterranean rodents, *T. splendens* prefers to excavate in deep and moist soils particularly in areas rich in food such as agricultural fields and small holdings (Jarvis & Sale 1971; Kokiso & Bekele 2008). They mostly prefer open grassland and upland savannah (Lacey *et al.* 2000). Generally, East African root rats are known to construct underground burrow systems (Jarvis & Sale 1971; Kingdon 1974). The burrow architecture has been used to interpret ecological characteristics such as food availability, atmospheric temperature, soil texture and moisture of the habitat (Jarvis & Sale 1971; Davies & Jarvis 1986; Lovegrove & Knight-Eloff 1988; Genelly 1995; Barnett *et al.* 2003). Furthermore, burrow architecture is useful in gaining insights into the behavioural ecology of burrow occupant(s) such as their reproductive potential, home range size, foraging efficiency, social structure and predator-avoidance (Spinks *et al.* 2000; Herbst & Bennett 2006; Sichilima *et al.* 2008; Thomas *et al.* 2009).

Ecological characteristics and behavioural ecology can be influenced by seasonal variation (Martin 2001; Püttker *et al.* 2008; Previtali *et al.* 2009) that triggers the animals to interact differently between and within a species (Davies & Jarvis 1986; Lovegrove 1989; Spinks *et al.* 2000; Šumbera *et al.* 2003; Herbst & Bennett 2006; Sichilima *et al.* 2008). Although

several studies have been conducted on burrow architecture with respect to seasonal variation and sexes effects in architecture in a number of African mole-rats (Jarvis & Sale 1971; Hickman 1977; Davies & Jarvis 1986; Spinks *et al*. 2000; Šumbera *et al.* 2003; Herbst & Bennett 2006; Sichilima *et al.* 2008; Thomas *et al.* 2009), little is known about *T. splendens*.

Burrows and the burrowing behaviour of *T. splendens* have been studied (see Jarvis & Sale 1971; Hickman 1983; Kokiso & Bekele 2008). Root rats dig with their incisors, pushing back soil with their forefeet, kicking it behind them with their hind feet, and using their head to push soil out of their tunnels. The burrow system of *T. splendens* has been reported to consist of foraging tunnels, a bolt hole and multi-functional nest possessing food store chamber, latrine and sleeping area which is lined with grasses. They occupy a burrow singly except when females have pups (Jarvis & Sale 1971) but the burrow architecture with respect to fractal dimension on seasonal variation and sexes remains largely unknown, and consequently, attempts were made in the present study to investigate these aspects of the biology of *T. splendens.*

East African root rats are known to be diurnal (Jarvis 1973a) and are active throughout the year, but less active during the dry season when they are believed to burrow deeper below the surface (Flynn 1990). *Tachyoryctes splendens* however, can survive cold temperatures at high elevations since fermenting faeces and nesting material in their burrows raises the temperature in their underground chambers (Jarvis & Sale 1971; Flynn 1990; Nevo 1999; Nowak 1999). Although a subterranean rodent, the East African root rat also forages above-ground on some occasions without venturing too far from the burrow entrance, especially during the day and early evening in their natural habitat (Jarvis 1973a). This intimated that they may perceive light which could be used as a *zeitgeber* for their locomotory rhythm. However, there was a lack of general information on their activity pattern under controlled laboratory conditions where food

was supplied *ad libitum*. It was also unknown whether theyare able to free run their activities in absence of external stimuli (i.e. light and temperature), the circadian rhythm of activity (i.e. endogenous circadian rhythm). Consequently, the present study also attempted to investigate the locomotory activity pattern of *T. splendens* under controlled laboratory conditions as has been carried out in other subterranean rodents (Hickman 1984; Benedix 1994: Tobler *et al.* 1998; Oosthuizen *et al.* 2003; Tomotani *et al.* 2012).

Members of the genus *Tachyoryctes* are solitary, with adult males and females coming together briefly to mate (Flynn 1990; Nowak 1999). Polygynous mating has been documented for all members of the genus *Tachyoryctes* with males initiating mating by visiting the burrows of females (Flynn 1990). They are reported to have a litter size of 2 annually with a gestation period of 38.5 days (Jarvis 1973b) but it was unknown whether post-partum oestrous occurs, or if they breed parallel to preferable climatic cues. Consequently, the present study investigated the reproductive pattern of *T. splendens* encompassing a wide range of post-mortem characteristics such as the histology of gonads and steroid hormones. Field observations were also incorporated in the present study in order to assess reproductive characteristics that include presence of foetuses from post-mortem dissection of females, visible teats, perforated vagina, and the presence of infants, juveniles as well as sub-adults.

A number of studies have grouped populations of *T. splendens* into juveniles, sub-adults and adults (Jarvis 1973b; Kokiso & Bekele 2008) although the criteria used on categorization were unclear. The present study aged populations of *T. splendens* based on maxillary eruption and wear patterns on the molariform teeth since this method has been used successfully in the age determination for small mammals (Taylor *et al.* 1985; Bennett *et al.* 1990; Van Rensburg *et al.* 2004; Abdel-Rahman 2005; Hart *et al.* 2007; Chimimba *et al.* 2010). The age grouping is

basis towards investigation and assessment of various biology aspects such as reproductive status, ontogenetic variation and population recruitment as has previously been used in other African subterranean rodents (Van Rensburg *et al.* 2004; Hart *et al.* 2007; Chimimba *et al.* 2010).

Tachyoryctes is herbivorous feeding on roots, rhizomes, bulbs, tubers, and grass (Jarvis & Sale 1971; Kokiso & Bekele 2008). Small invertebrates including larvae and adult flies, mites, beetles and other dipterans are known to shelter in the burrows of East African root rats, but it is not known if these animals form part of prey items for the species. However, an on-going analysis of various tissues based on stable isotopes may allow insights into the diet of these root rats. East African root rats are important bio-engineers with their extensive digging activities that influence the distribution of nutrients, the composition of gases and the humidity of the soil which is important for soil turn-over and therefore, impacting greatly on plant diversity (Zhang *et al.* 2003). *Tachyoryctes splendens* has been reported to destroy crops by cutting roots, the hollowing-out of tubers and the consumption of bulbs during feeding as well as burrow construction resulting in a decreased yield on agricultural small-holdings (Kokiso & Bekele 2008; ASDP 2009). The conspicuous destruction of vegetation including crops, steppe and pastures by *T. splendens* occurs when their population is relatively high (more than two burrows per acre: Farmers pers. comm.). Eradication methods are currently not successful due to paucity of information on reproductive patterns with farmers knowing little on when to capture to keep the population of *T. splendens* under control. Therefore, reproduction and burrow architecture information from the present study may allow insights into the timing for reducing reproduction potential of individuals.

East African root rats are important in ecosystem functioning, consuming a variety of plant species, but they are also the prey of numerous avian and mammalian predators (Flynn 1990). Natural predators of East African root rats include snakes, jackals, hawks and owls but they can avoid predation to some degree by living underground (Flynn 1990; Nowak 1999). East African root rats are also hunted for food by indigenous people (Kingdon 1974; Nowak 1999) or given to domestic carnivores such as cats and dogs.

1.2 Research problem Statement

There is a paucity of information on the systematics and other biological aspects of *T. splendens* (Carleton & Musser 1984, Musser & Carleton 1993, 2005; Jansa *et al.* 1999; Lacey *et al.* 2000; Michaux *et al.* 2001; Huchon *et al.* 2002; Beolchini & Corti 2004). *Tachyoryctes splendens* is known to feed on both the aerial portion and underground storage organs of vegetation and/or geophytes (Jarvis & Sale 1971; Kokiso & Bekele 2008). The East African root rat is reported to be responsible for the loss of tons of crops from agricultural small-holdings and farms annually (Kokiso & Bekele 2008; ASDP 2009), but there is little information on their general ecology and physiology that may have implications on their biological control in order to enhance food security. Of particular relevance with systematics studies of the genus *Tachyoryctes* is that they may allow insights into the taxonomic status of the genus in Tanzania.

1.3 Objectives

General objective

The present study is intended to broaden the knowledge base on the systematics as well as the ecological and physiological aspects of the *T. splendens* from Tanzania.

Specific objectives and Research Questions (RQ)

Ecology

1. To assess the burrow architecture of *T. splendens* from Tanzania in relation to season and sex.

   RQ. Does the burrow architecture of *T. splendens* differ between seasons and sexes?

Physiology

2. To investigate the locomotory activity patterns of *T. splendens* under laboratory conditions and examine whether they able to free run their activities in absence of light stimuli (i.e. constant darkness) conditions.

   RQ. 1: Does the locomotory activity pattern of *T. splendens* change under laboratory conditions and if so, what are the factors that may influence those changes?

   RQ.2: Does *T. splendens* show a locomotory activity under constant conditions?

3. To assess whether *T. splendens* from Tanzania is a seasonal or an aseasonal breeder.

   RQ. Is *T. splendens* a seasonal or an aseasonal breeder and what are the environmental variables that may influence its reproduction?

Systematics

4. To assess craniometric ontogenetic variation and sexual dimorphism in *T. splendens* from Tanzania.

   RQ. What is the nature and extent of craniometric sexual dimorphism and ontogenetic variation in *T. splendens* from Tanzania?

5. To investigate phylogenetic relationships of *T. splendens* from Tanzania.

   RQ. What are the phylogenetic relationships of *T. splendens* from Tanzania based on molecular data?

1.4 Justification of the study

The rationale for undertaking the present study was to increase knowledge on the general paucity of information on various aspects of the biology of *T. splendens*. A very quick glance of all major textbooks covering African mammals or mammals of the world reveals that the literature pertaining to information on the African Rhizomyinae is data deficient (Lacey *et al.* 2000). In this thesis, I attempted to address this deficiency of information by exploring a number of aspects on the biology of the subterranean East African root rat.

East African root rats are subterranean and as a consequence occur in a burrow which has moulded the behaviour and biology of this rodent mole. In this thesis, I explore the overall architecture of the burrow system with more emphasis on the extent of habitat exploration and examine if the foraging effectiveness changes with the sex of an occupant and season over an entire calendar year.

Currently, there is no formal study which investigated the role of light on the locomotor activity of *T. spendens* under controlled laboratory conditions. Moreover, the present study allowed the assessment of whether the East African root rat exhibits an endogenous circadian rhythm of locomotor activity which is independent of light stimulation.

The reproductive biology of *T. splendens* has been reported from a population from Kenya but was predominantly involved in assessing the various reproductive parameters of

females (Jarvis 1973b). Furthermore, the involvement of external environmental factors that may potentially trigger reproduction in the East African root rat was unknown until the inception of this study. The current study investigated the reproductive status of *T. splendens* in both sexes from a single population in order to gain insights into the underlying patterns of reproduction and to assess the environmental factors that may be associated with onset of reproductive activities. This study is probably the most thorough post-mortem examination of reproduction in a population from the family Spalacidae that has ever been attempted.

There is currently no information on the ontogenetic variation and sexual dimorphism in populations of East African root rats. The current study assessed the nature and extent of ontogenetic variation and sexual dimorphism in three populations (i.e., Marangu, Keni-Aleni and Uru-Shimbwe) of the species. In this assessment I established relative age classes occurring within these three populations of *T. splendens* to determine the age structure and investigate age specific sexual dimorphism.

Finally, the taxonomic confusion of how many species occurs within the genus *Tachyoryctes* is in critical need of investigation. Taxonomy based on classical morphology and both traditional and geometric morphometrics (Allen 1939; Ellerman 1941; Misonne 1974; Corbert & Hill 1991; Beolchini & Corti 2004) and type locality data (Musser & Carleton 1993, 2005) have been inconclusive in the taxonomic treatment of species within the genus *Tachyoryctes*. There was therefore a critical need for molecular studies to gain additional insights into the taxonomy and the phylogeography of the East African root rat across its distributional range in Tanzania.

1.5 Contribution of the study

The aspects of biology detailed in this thesis provides more information on natural history and population biology of less studied rodents mole that were recently missing on literature, and consequently affluent biodiversity of the subregion. The information generated from investigations, examinations and assessments of various biological characteristics of *T. splendens* revealed convergence and divergence pattern of biology to ecologically similar and taxonomically different species which accentuate its conservation. These biology aspects are as follows;

An assessment of the burrow architecture of the East African root rats over an entire calendar year revealed how the species changes its behaviour with the annual rainfall pattern. This allowed an investigation of how the burrow structure of *T. splendens* changes both with season and the sex of the occupant. The locomotory activity studies under laboratory condition revealed that *T. splendens* is able to perceive light and change its locomotor pattern of activity using this environmental cue. Consequently, root rats may prove to be a useful animal model for future research into light studies in microphthalmic organisms. The finding that reproduction is enhanced during the rainfall period has important implications for agriculture because the litter size of 2.2 animals per adult female per year suggests that *T. splendens* may not necessarily be an agricultural pest on small holding farms. The relative age classes of the population may provide insights into growth characteristics through life expectancy of these animals. In addition, the ontogenetic variation and sexual dimorphism exhibited by *T. splendens* may provide data that may be useful for ecological models for assessment of the population dynamics for the conservation of the species.

The molecular data revealed that the phylogeographic structure of *Tachyoryctes splendens* populations in Tanzania is relatively recent. Given that the greatest genetic differences occurred within rather than between neighbouring regions, conservation of the species across its entire distributional range in Tanzania is recommended. Although Tanzania has diverse agricultural pests, there was no niche overlaps between *T. splendens* and that of members of the family Bathyergidae. This absence of niche overlap suggests that *T. splendens* may competitively exclude other subterranean rodents from the niche and that the species may venture into habitats where bathyergids cannot because of its more catholic diet.

1.6 Study area

The study was conducted at various localities within Tanzania, East Africa (Figure 1) along the shores of the Indian Ocean, between latitude 1° and 12° South and longitude 29° and 40° East (Agrawala *et al.* 2003). Tanzania borders Kenya and Uganda to the north, Rwanda, Burundi and the Democratic Republic of Congo (DRC) to the west, Zambia, Malawi and Mozambique to the south, while the island of Zanzibar lies to the east (Kashaigili *et al.* 2003). The country has a land area of approximately 945,087 km^2 of which 3% represents arable land, 1% permanent crops, 40% permanent pastures and 38% forests together with other woodlands (Agrawala *et al.* 2003).

Tanzania lies to southern part of equator, it has mean annual temperature of 23° C (range = 17-27 ° C) however in the highlands such as areas near Mt. Kilimanjaro and Rungwe temperature may drop to 4° C (Agrawala *et al.* 2003). The country has two rainfall patterns, unimodal and bimodal (Mwandosya *et al.* 1998; Agrawala *et al.* 2003). The unimodal rainfall pattern occurs between late November to early April, and it prevails to the southern, south-western, central, and western areas of the country and the highest humidity in these areas is

between December and March (Agrawala *et al.* 2003). The bimodal pattern has two rainfall seasons; heavy rainfall *"masika"* occurs between late February and May with the highest humidity between March and April (58%), while short rainfall *"vuli"* occurs between September and early November with the highest humidity from September to October (30%) (Mwandosya *et al.* 1998; Kashaigili *et al.* 2003). Tanzania has a diverse number of biomes that support a high diversity of flora and fauna with high species endemism (NRI 2001). These biomes include the Eastern Arc Montane Forests, East African Coastal Forests, Albertine Rift Highland Forests, East African Highland Forests, Zambezian Woodlands & Savannas, East African Acacia Savannas, East African Moorlands, Zambezian Montane Savannas & Woodlands, Rift Valley Lakes, East African Mangroves, and East African Marine Ecosystems (Oslon & Dinerstein 1998).

The drainage system of Tanzania is complex due to general declination from the western highlands towards the Indian Ocean (Bwathondi & Mwamsojo 1993). Most of the country's rivers and streams drain into the Indian Ocean while few drain into lakes (Bwathondi & Mwamsojo 1993). Major water courses are from north to south and include the Umba, Lukuledi, Pangani, Msangasi, Mligasi, Mandera, Ruvu, Matandu, Mavuji and Mambi rivers (Mbwana 1986). These rivers drain into the eastern margin and slopes of the interior plateaus to the Indian Ocean. Rufiji River has sources in the Kipengere Mountains at the northern tip of Lake Malawi and drains into a huge basin in central southern Tanzania while Ruvuma River drains into Lake Malawi, Rukwa basin and the Indian Ocean (Temple & Sundbrog 1972).

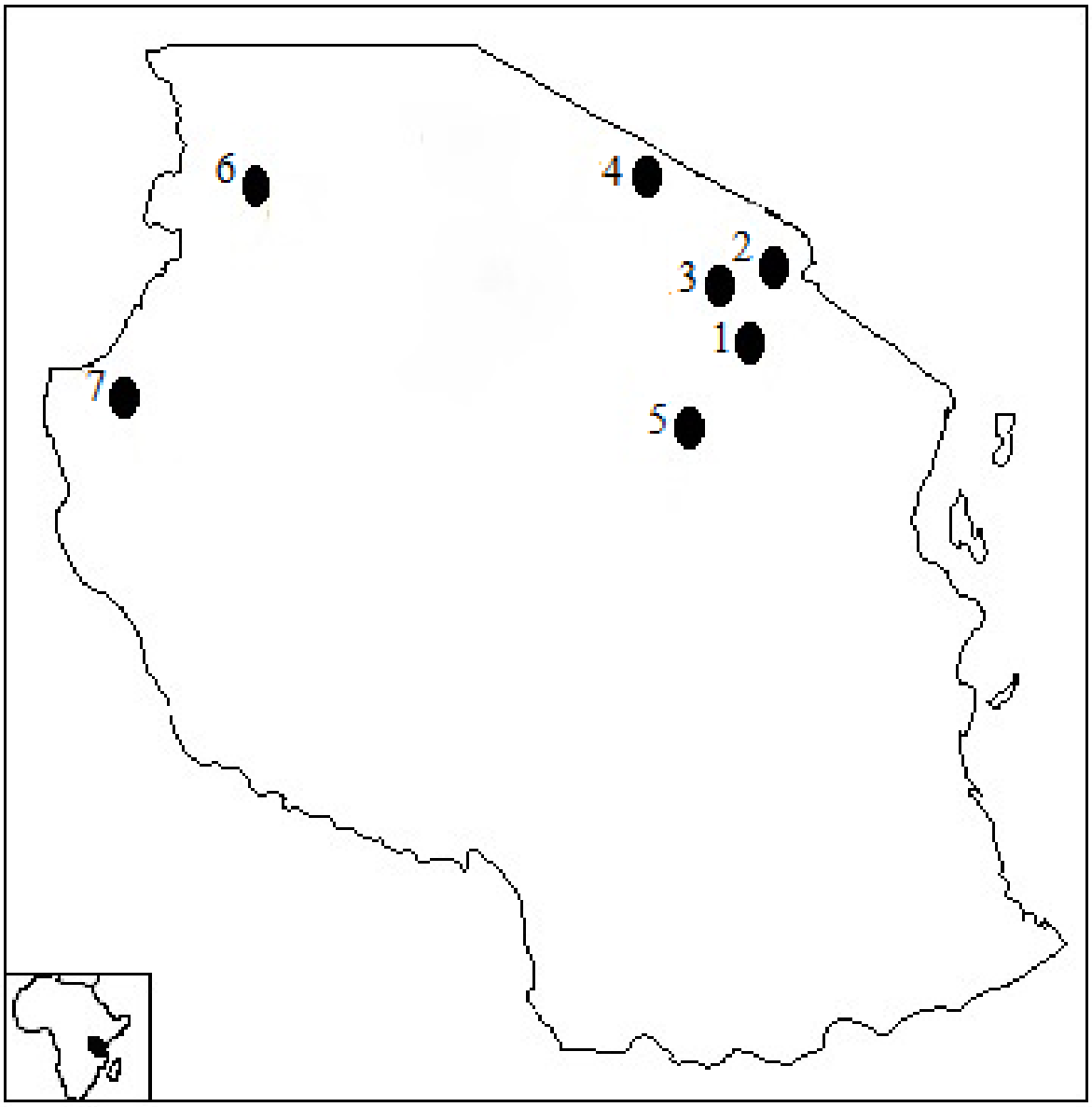

Figure 1: Map of Tanzania showing study areas (1-3 = Kilimanjaro region; 4 = Arusha region; 5 = Manyara region; 6 = Kagera region; 7 Kigoma region) and study sites for various biological aspects: 1 = Uru-Shimbwe; 2 = Keni-Aleni; 3 = Marangu; 4 = Tengeru; 5 = Endugulda; 6 = Katanga; 7 = Nyaruboza.

1.7 Animal sampling and processing

East Africa root rats were sampled from an agro-ecological zone. Animals were sampled using both Hickman live traps (Hickman 1979) and hand-capturing during burrow excavations. After sampling and during transportation to the laboratory, animals were kept in 20 litre plastic buckets. In the laboratory, animals were subsequently euthanased using chloroform (Merck, Johannesburg, South Africa) inhalation except live animals maintained in the laboratory for behavioural studies. Prior to anaesthesia, the animals were sexed and standard external measurements were recorded including body mass, head and body length, tail length, and length of the hind foot. Euthanased animals were dissected and blood drawn from the heart by heparinized syringe. The heart, liver, kidney, leg muscles, gastrointestinal tract, skull and reproductive organs were removed, fixed and stored for subsequent analyses. Storage and preservation of specimens followed the reagents manufacturers' protocols. Live animals were maintained under laboratory conditions in the University of Dar es Salaam (UDSM: Department of Zoology & Wildlife Conservation), Tanzania for two weeks before they were exported to the University of Pretoria, South Africa. Voucher specimens were prepared using standard procedures for the preparation of natural history specimens and are deposited in the mammal reference collection of the University of Dar Es salaam in the Department of Zoology and Wildlife Conservation, Tanzania and all nucleic acid sequences were placed in Genbank.

1.8 Ethical note

Animals were maintained under the guidelines of the American Society of Mammalogists (Animal Care and Use Committee 1998; http://www. mammalogy.org/committees/index/asp) and as approved by the Animal Ethics Committee of the University of Pretoria (ethics clearance

number ECO47-10), Pretoria, South Africa. Research permit (2011-44-NA-2010-204) and export permits (permit No. 65054 and 63816) were granted by the Commission of Science and Technology (COSTECH), Ministry of Natural Resources & Tourism and Ministry of Livestock & Fisheries, Dar es Salaam, Tanzania. The import permits (13/1/1/30/2/9/6-265 & CPC5 No. 01600 and 13/1/1/30/2/9/6-241 & CPB6 No. 003784) were granted by the Gauteng Department of Nature Conservation and the Department of Agriculture, Forestry and Fisheries, Johannesburg, South Africa.

1.9 Thesis overview

Apart from the general introduction (Chapter 1) and the general discussion (Chapter 7), this thesis consists of five stand alone research chapters, some of which have been submitted as manuscripts. Chapter 2 assessed the burrow architecture of *T. splendens* between sexes and seasons with an emphasis on fractal dimensions as a measure of habitat exploration efficiency. Chapter 3 investigated circadian rhythm of locomotory activity in *T. splendens* under various light regimes. Chapter 4 assessed the reproductive status of specimens collected over a calendar year in order to determine whether *T. splendens* is a seasonal or an aseasonal breeder and what environmental variables may influence its reproduction. This included an analysis of the histology of gonads, steroids profiles and field assessment from post-mortem dissections of the reproductive tracks of females, and also with reference to visible teats, perforated vagina and presence of lactating females, infants, juveniles as well as sub-adults. Chapter 5 investigated the nature and extent of craniometric sexual dimorphism and ontogenetic variation in *T. splendens* from Tanzania. Chapter 6 investigated phylogenetic relationships of *T. splendens* from Tanzania

based on molecular data that included an assessment of the D-Loop (control region) and cyt-*b* (cytochrome b) of the mitochondrial genome.

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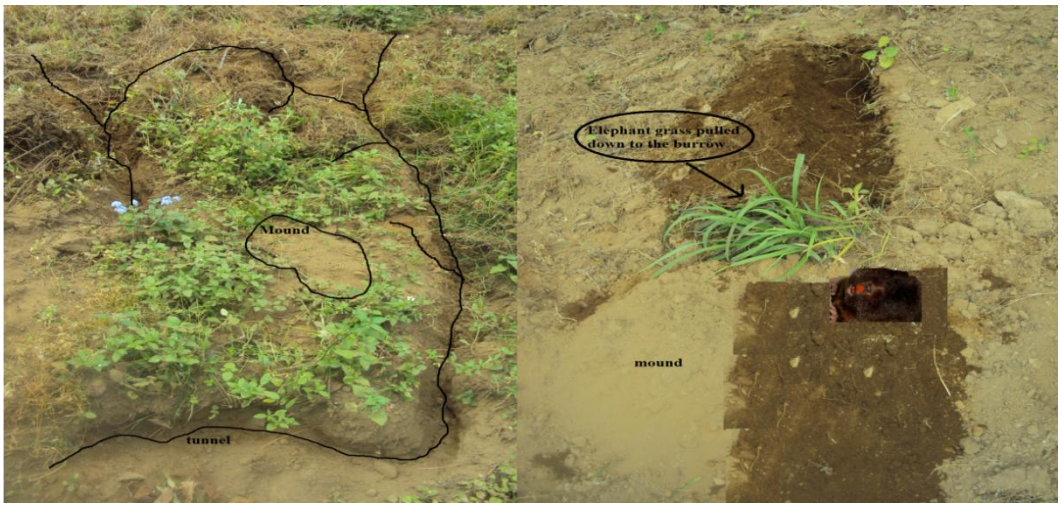
Chapter 2: Burrow architecture



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Title: Sweeping the house clean: burrow architecture and seasonal digging activity in the East African root rat, *Tachyoryctes splendens* from Tanzania

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Abstract

We investigated changes in burrow architecture and fractal dimension across seasons and between the sexes in the solitary East African root rats, *Tachyoryctes splendens* over an entire calendar year. The basic burrow system comprised a main tunnel reticulating into foraging tunnels, a nest consisting of food store chamber, latrine and sleeping area, and a bolt hole. Main tunnel length was strongly affected by sex, and contrary to expectations was higher for females and for males (during both the dry and wet seasons). The number and length of foraging tunnels was affected by both sex and seasons, with females' burrows having more foraging tunnels than males in both the dry and wet seasons. Females also had burrows with higher fractal dimension than males, while fractal dimension increased with burrow length for both sexes. We suggest that unlike the solitary bathyergid mole-rats, males *T. splendens* do not construct larger burrows than females in the search for mates, but rather females have larger burrows with more foraging tunnels resulting from the increased need for provisioning to overcome energy costs for burrow maintenance and caring of pup(s).

Keywords: *Tachyoryctes splendens*, burrow architecture, subterranean rodent, mole-rat, fractal dimension, root rat

Introduction

The East Africa root rat, *Tachyoryctes splendens* (Family: Spalacidae) is a solitary dwelling fossorial rodent, widely distributes across East Africa. The Spalacidae are classified within the suborder Myomorph of rodentia and are divergent from better-known African mole-rats of

Family Bathyergidae, suborder Hystricomorpha. While overlapping geographical ranges with several bathyergid mole-rat species, *Tachyoryctes* has convergently adopted subterranean life style and investigation of comparative distributions and habitat use are of broad interest. As with other rodent moles, *T. splendens* feeds on underground plant storage organs and roots (which influence burrow structure and dynamics), but is also known to occasionally forage above-ground (Jarvis & Sale, 1971). Where they are associated with farmland, *T. splendens* is a major pest species (kokiso & Bekele, 2008). Previous excavation of small numbers of adult burrows in Kenya have show that the typical architecture comprises of a main tunnel with a number of superficial foraging tunnels branching from this, a deeper bolt hole and a centrally-positioned multi-purpose nest chamber which comprises food store, latrine and sleeping area (Jarvis & Sale, 1971). Following a study of two adult and two juvenile burrows during flooding, Hickman (1983) also suggest the bolt hole may also act as a drainage sump during heavy rains. Although a common subterranean species in areas where present, no comprehensive study of *T. splendens* has been carried out to investigate the detailed structure and geometry of burrows with respect to the sex of the occupant, and seasonally-induced changes (e.g. over wet and dry seasons).

Most burrowing activity in subterranean species occurs following rainfall where there is increased soil moisture that makes the soil more friable and workable, and it is also easier to extrude the soil workings onto the surface as mounds (Miller, 1957). Other factors that may also be related to rainfall and which can seasonally influence burrowing patterns are mate acquisition and foraging (Miller & Bond, 1960; Hickman & Brown, 1973). Thus, in species occurring in regions of marked wet and dry seasons, as is the case in many African mole-rats (Family: Bathyergidae), some aspects of the architecture of the burrow system change seasonally and are

influenced by both biotic and abiotic factors. Burrows may be extended for: a) the primary purpose of food acquisition, especially in the case where food resources are geophytic and occur below ground; or b) tunneling may be triggered for the purpose of mate searching, or during dispersal, for example, when a juvenile leaves the maternal burrow system as is the case in solitary species or emigration occurs from a parent colony for social species (Bennett & Faulkes, 2000).

Among solitary bathyergid mole-rats, males seek out mates and as a consequence they construct longer tunnel systems, communicating with and attracting females in the subterranean niche via seismic signals (Bennett & Jarvis, 1988; Herbst & Bennett, 2006; Le Comber *et al.*, 2006; Thomas *et al.*, 2012). We therefore predicted that the burrow systems of male and female *Tachyoryctes* would show sex differences reflecting sex-specific mate seeking behaviour. We further predicted that the burrow systems of both sexes of *T. splendens* would be longer and more reticulated during the wet season as they actively search for food items.

The complexity of a burrow system and the exploration efficiency of the surrounding environment, or reticulation of the system, can be examined through fractal dimensions (Kenkel & Walker, 1996). Fractal dimension provides a measure of burrow shape since it provides a measure of the extent to which a one-dimensional structure fills a plane, with a low fractal dimension (i.e. close to 1.0) describing a burrow that explores relatively little of the surrounding area whereas a high fractal dimension (i.e. close to 2.0) designates a burrow which permeates the surrounding area more thoroughly (Romañach & Le Comber, 2004). Fractal dimension is thus a valuable measure of burrow shape when the burrow is used for foraging (Le Comber *et al.*, 2006). While there are no differences in the general burrow architecture between seasons in the

solitary, silvery mole-rat, *Heliophobius argenteocinereus*, the burrows had higher fractal dimension at the peak of the dry season which suggests the burrow occupant increases its search for food (Šumbera *et al.*, 2003). Similarly, in the social giant Zambian mole-rat, *Fukomys mechowii*, total burrow length did not vary with season, but the fractal dimension was greater during the rainy season than the dry season indicating greater exploratory tunneling without an overall increase in total tunnel length, which increases the chances of finding food resources (Sichilima *et al.*, 2008).

Thus in subterranean rodents, while the overall home range remains relatively constant, burrow systems are usually in a state of dynamic flux in response to environmental cues and mate acquisition. This is the first study to undertake an extensive survey of *T. splendens* in Tanzania, excavating burrow systems on a monthly basis over an entire calendar year. The objectives were (i) to test the prediction that burrow size and fractal dimension were larger in males, and (ii) to quantify seasonal differences with the prediction that increases in burrow length, the number of foraging burrows and fractal dimension occur in the wet season.

Materials and Methods

Study site

This study was conducted over 12 consecutive months from January to December 2011, in farmlands at Mamba Komakundi village, located on the slopes of Mount Kilimanjaro in the Moshi Rural District, north-east Tanzania (03˚16.54' S, 037˚32.49' E; 1495 m above sea level). The site is characterized as an agro-ecological zone with very fertile volcanic soils supporting a variety of food and cash crops (Kilimanjaro Regional Profile (KRP), 1998). The area has two

rainy seasons with a mean annual rainfall of 1250 mm. The long rainy season is between March and June, while the short rainy season is between October and December. The dry season spans from January to early March and from July to early October. Annual average temperature ranges from 15-30° C with high humidity during October, March, April and May (KRP, 1998).

Excavation of burrow systems

Burrow systems were excavated manually with hoes to expose the tunnels along their entire length. Occupants were captured, weighed and sexed. A total of 60 burrows were excavated (i.e. five burrow systems per month). During each month we captured *T. splendens*, the animals were weighed and sexed prior to excavating the burrow system. Equal numbers of each sex were collected (15 males and 15 females) from wet and dry seasons respectively. Animals caught and removed from burrows were used as samples for other on-going parallel studies.

The lengths of the tunnels and their dimensions and shape were recorded *sensu* Thomas *et al.*, (2009; 2012) and measured to the nearest 0.1 cm using a tape measure; the depth from the ground surface to the top of the tunnel (recorded approximately every 2 m), height and width of the tunnel, the length of the main tunnel and length of foraging tunnels. The number of foraging tunnels was also noted, and burrow fractal dimension calculated as detailed below. Tunnels were defined as either: deep, semi-permanent (> 20 cm) or shallow, foraging (< 20 cm) tunnels (determined by the depth of the bulbs and roots of the plants in the sites reached). The location and dimensions of any nests, food stores, bolt holes and latrines were recorded. Nests were defined as chambers with only a single entrance and filled with nesting material. Food stores were blind-ended tunnels filled with either bulbs or roots and the contents of these caches were

recorded as percentages of food type. Bolt holes were steep-angled tunnels (almost vertical) that were > 30 cm in length used as either anti-predatory function, thermoregulation or as drainage sumps (Hickman, 1983, 1990; Nevo, 1999). Latrines were blind-ended tunnels packed with soil and faeces. The study was approved by the Animal Ethics Committee of the University of Pretoria (Ethics clearance number ECO47-10), the Tanzania Commission for Science and Technology (COSTECH) (Ref. 2011-44-NA-2010-204) and the University of Dar es Salaam, Tanzania.

Data analyses

To quantify the geometric configuration of the burrow in a way that was independent of burrow size we calculated fractal dimension. This provides a measure of the extent to which the burrow explores the surrounding area, particularly in subterranean mammals where the burrow is primarily used for foraging (Le Comber *et al.*, 2002, Šumbera *et al.*, 2003; Romañach & Le Comber, 2004). All previous studies on mole-rat tunnel systems have assessed 2-D rather than 3-D structures, since the vertical variation is very slight (perhaps around 1 m). Since this study was primarily concerned with the burrow and its relation to foraging, we also only quantified how the burrows are distributed in 2-D. The geometry of the burrow systems was mapped to a scale of 1:100 on graph paper for estimation of fractal dimension (Le Comber *et al.*, 2002; Sichilima *et al.*, 2008; Thomas *et al.*, 2009, 2012). Each drawing was then digitized separately and subjected to image-processing software GIMP version 2.6.12 (http://sd-cf.en.softonic.com) and Inkscape version 0.48.3.1(http://www.flvplayerpro.net), for re-draw and to convert the burrow system

image into a readable format for 2-D fractal dimension calculation using Fractal Dimension Calculator version 1.2.0 (Thomas *et al.*, 2012).

Data from the short and long rainy periods and the two intervening dry seasons respectively were combined to produce "wet" and "dry" season comparisons, to simplify the analysis and increase sample size. Other environmental factors such as temperature do not differ between the short and long rainy/dry periods. All data were then analysed using R (R Core Team, 2012). The effects of sex and season on burrow length (i.e. length of the main tunnel), number of foraging tunnels and foraging tunnel length were analysed using a two-way analysis of variance, including a sex*season interaction. A similar approach was taken to study factors affecting burrow fractal dimension, but in this case burrow length, number of foraging tunnels and foraging tunnel length were included as additional predictors in a general linear model. Data were summarized in boxplots generated in R, showing 'hinges' around the median (versions of the first and third quartiles), and notches showing 1.58 times the interquartile range/$\sqrt{n}$ which approximately correspond to 95% confidence limits (R Core Team, 2012).

Results

A single animal was sampled in each burrow system excavated, except for five adult lactating females that had pups with them in their burrows. The burrow systems comprised a bolt hole, multi-functional nests and side branches ending at mole hills diverging from a main tunnel (Figure 1). The food store chamber was located on the side of the nest and connected to the nest by one exit point. In the food store, the composition of the cache was rich in roots, tubers, bulbs

and rhizomes, as well as grasses and shoots of forbs that may either have been pulled into the burrow tunnel or harvested during foraging above-ground.

Several nest chambers were excavated within individual burrow systems of *T. splendens* but only one was active at any particular time, with other nest chambers back-filled with soil and no longer in use. An active nest was connected to the main tunnel by a single entry/exit point. Within the nest chamber, dry plant materials were found proximal to the entrance, while the latrine was found in the distal section. Nesting comprised of 90.4% non-cultivated vegetation such as grasses and forbs, 5.5% crop materials, 4% faecal pellets and 0.09% invertebrates. Invertebrates observed in the nest chamber included spiders (54.4%), termites (20%), earthworms (7.1%), flies (6.5%), ants (6.2%) and beetles (5.8%). In the same section of the tunnel, disused nests contained dry plant material and old faecal pellets mixed with soil.

Foraging tunnels originated from the main tunnel (Figure 1), and in some burrow systems they branched into several peripheral tunnels and terminated at mole mounds. These mole mounds were characterized by both old and freshly excavated piles of soil, the latter indicating recent burrowing activity. The diameter of the foraging tunnels was large enough to allow easy movement of the occupant without excessive digging. The burrow system of *T. splendens* also contained a single bolt hole which was located at the end of the main tunnel, and at a greater depth than the nest chamber. During intense rains and dry months, the bolt holes were used as nest chambers.

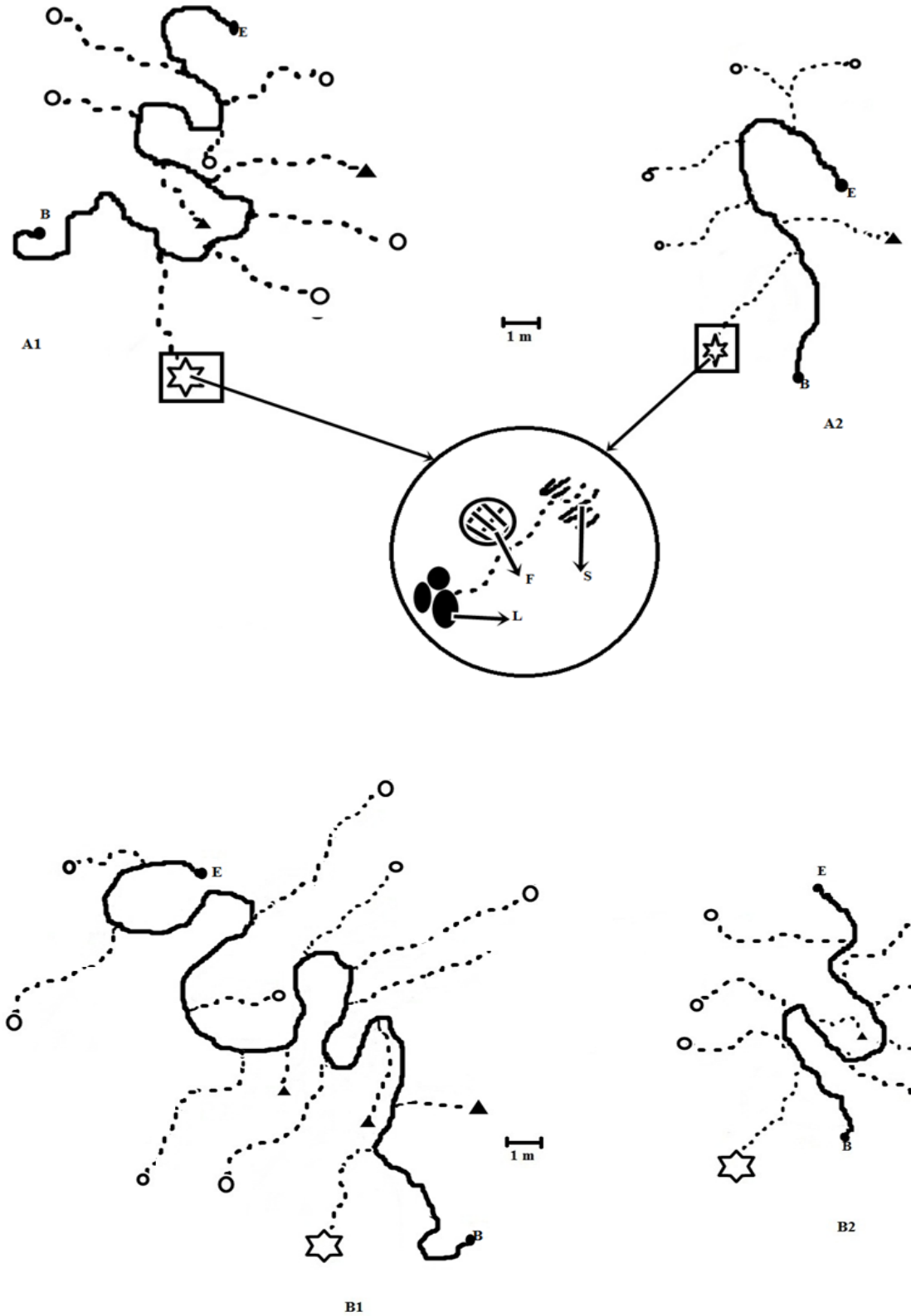

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Figure 1: The burrow system of *T. splendens* studied over entire calendar year 2011 at Mamba Komakundi, Kilimanjaro Region, Tanzania. A1 = typical female burrow system; A2 = typical male burrow system; B1 = female burrow system during the wet season; B2 = male burrow system during the wet season; C1 = female burrow system during the dry season; C2 = male burrow system during the dry season; Continuous thick line (~~) = main tunnel; E = entrance; broken line (- - -) = foraging tunnel; O = mole mound; ✡ = active nest; ▲ = old/disused nest; L = latrine; S = sleeping area, F = food store chamber and B = bolt hole.

The effects of sex and season on burrow structure

Burrow length (total length of the main tunnel) was strongly affected by sex but not season (ANOVA: sex: $F_{(1,56)} = 14.80$, $p = 0.0003$; season: $F_{(1,56)} = 0.65$, $p = 0.424$; sex*season: $F_{(1,56)} = 0.15$, $p = 0.70$, and was higher for females than for males. Tukey post-hoc tests showed that this difference was significant during both the dry and wet seasons (Figure 2). Mean ± SD (range): male wet: 6.64 ± 1.12 m, (5.00–8.50 m), male dry: 6.16 ± 1.06 m, (4.51–8.50 m); Female wet: 8.17 ± 2.17 m, (5.85–13.51 m), female dry: 7.99 ± 2.16 m, (5.85–11.50 m).

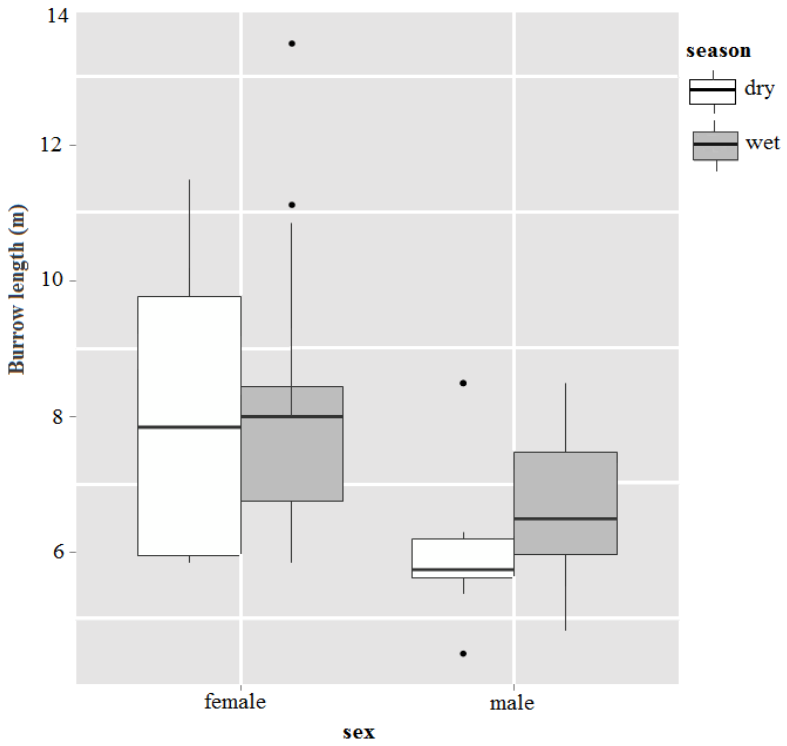

Figure 2: Box plot of burrow length (length of main tunnel as illustrated in Figure 1) versus sex of burrow occupant for wet and dry seasons. Each boxplot shows "hinges" (boxes) which are version of the first and third quartiles around the median (bold horizontal lines), and "notches" (vertical lines) showing 1.58 times the interquartile range/$\sqrt{\boldsymbol{n}}$, which approximately correspond to 95% confidence limits. Outliers are represented by circular plot sysmbol (R Core Team, 2012).

The number of foraging tunnels was affected by both sex and season (ANOVA: sex: $F_{(1,56)} = 20.46$, $p < 0.0001$; season: $F_{(1,56)} = 6.50$, $p = 0.014$; sex*season: $F_{(1,56)} = 0.17$, $p = 0.68$). Tukey post-hoc tests showed that females' burrows had more foraging tunnels than males' burrows in both the dry and wet seasons, while overall burrows had more foraging tunnels in the wet season than the dry season; this latter difference was driven largely by differences between females/wet and male/dry (Figure 3). Mean ± SD (range): male wet: 5.13 ± 0.92, (4.00–6.00), male dry: 3.93 ± 0.96, (3.00–5.00); Female wet: 6.80 ± 2.08, (4.00–10.00), female dry: 5.93 ± 1.94, (4.00–8.00).

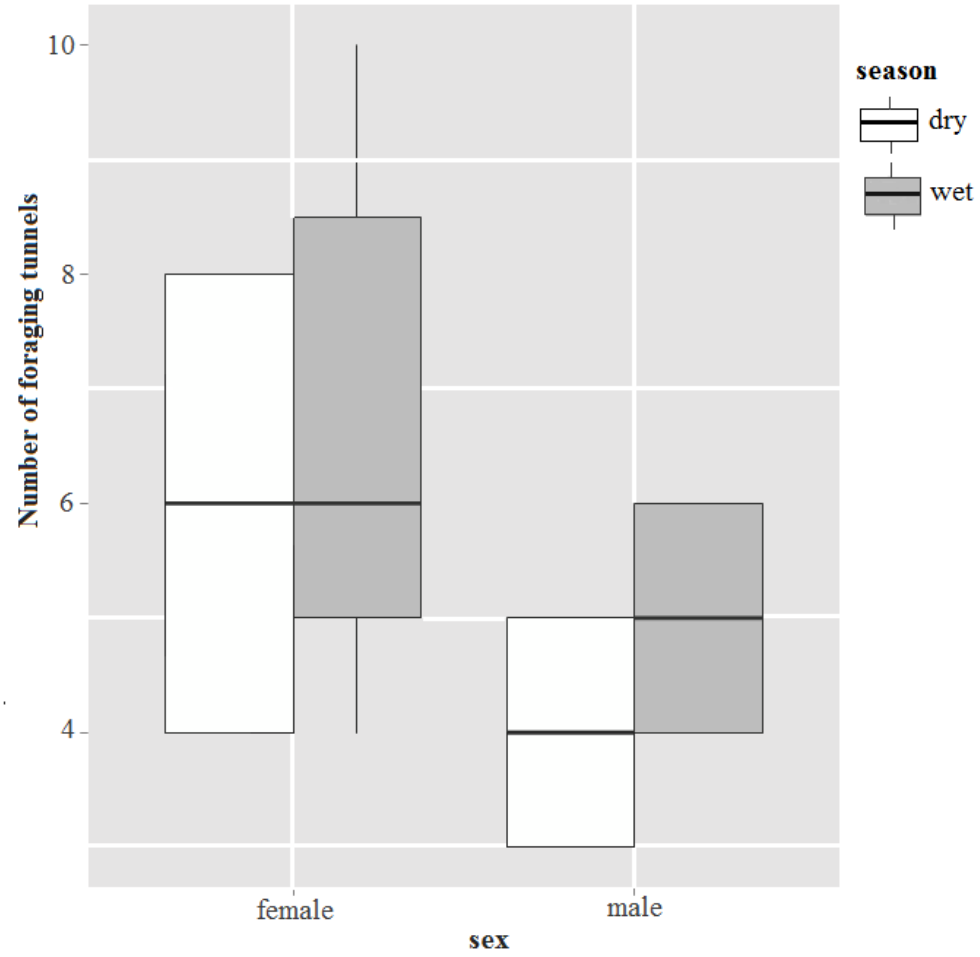

Figure 3: Box plot of foraging tunnels versus sex of burrow occupant for wet and dry seasons.

Foraging tunnel length showed a slight different pattern, with significant differences due to sex and sex*season (ANOVA: sex: $F_{(1,56)} = 38.15$, $p < 0.0001$; season: $F_{(1,56)} = 0.79$, $p = 0.378$; sex*season: $F_{(1,56)} = 5.83$, $p = 0.019$). Tukey post-hoc tests showed that overall females had burrows with longer foraging tunnels than males, in both the wet and dry seasons (Figure 4). Mean ± SD (range): male wet: 4.96 ± 2.36 m, (2.52–7.63 m), male dry: 3.35 ± 1.46 m, (2.52–6.19 m); Female wet: 6.79 ± 1.45 m, (5.21–8.63 m), female dry: 7.53 ± 2.10 m, (5.18–9.82 m).

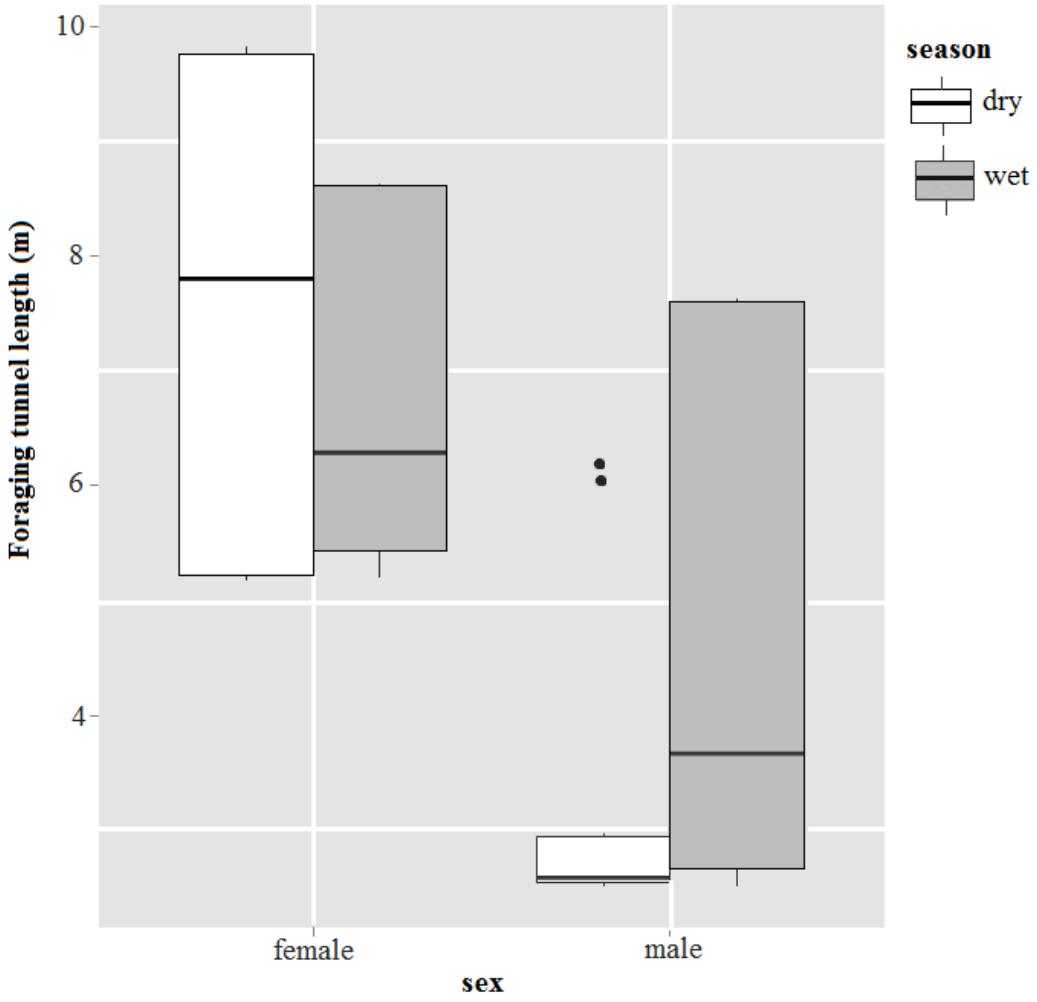

Figure 4: Box plot of foraging tunnel length versus sex of burrow occupant for wet and dry season.

Fractal dimension was strongly affected by both sex and burrow length. Females had burrows with higher fractal dimension than males (Figure 5), while fractal dimension increased with burrow length (Figure 6; results from GLM: sex: $F_{(1,53)} = 59.49$, $p < 0.0001$; season: $F_{(1,53)} = 0.35$, $p = 0.56$; sex*season: $F_{(1,53)} = 2.43$, $p = 0.13$; burrow length: $F_{(1,53)} = 77.29$, $p < 0.0001$; number of foraging tunnels: $F_{(1,53)} = 0.03$, $p < 0.87$; foraging tunnel length: $F_{(1,53)} = 1.56$, $p < 0.22$). Mean ± SD (range): male wet: 1.19 ± 0.04, (1.15–1.25), male dry: 1.17 ± 0.04, (1.14–1.24); Female wet: 1.23 ± 0.04, (1.18–1.29), female dry: 1.24 ± 0.04, (1.16–1.27).

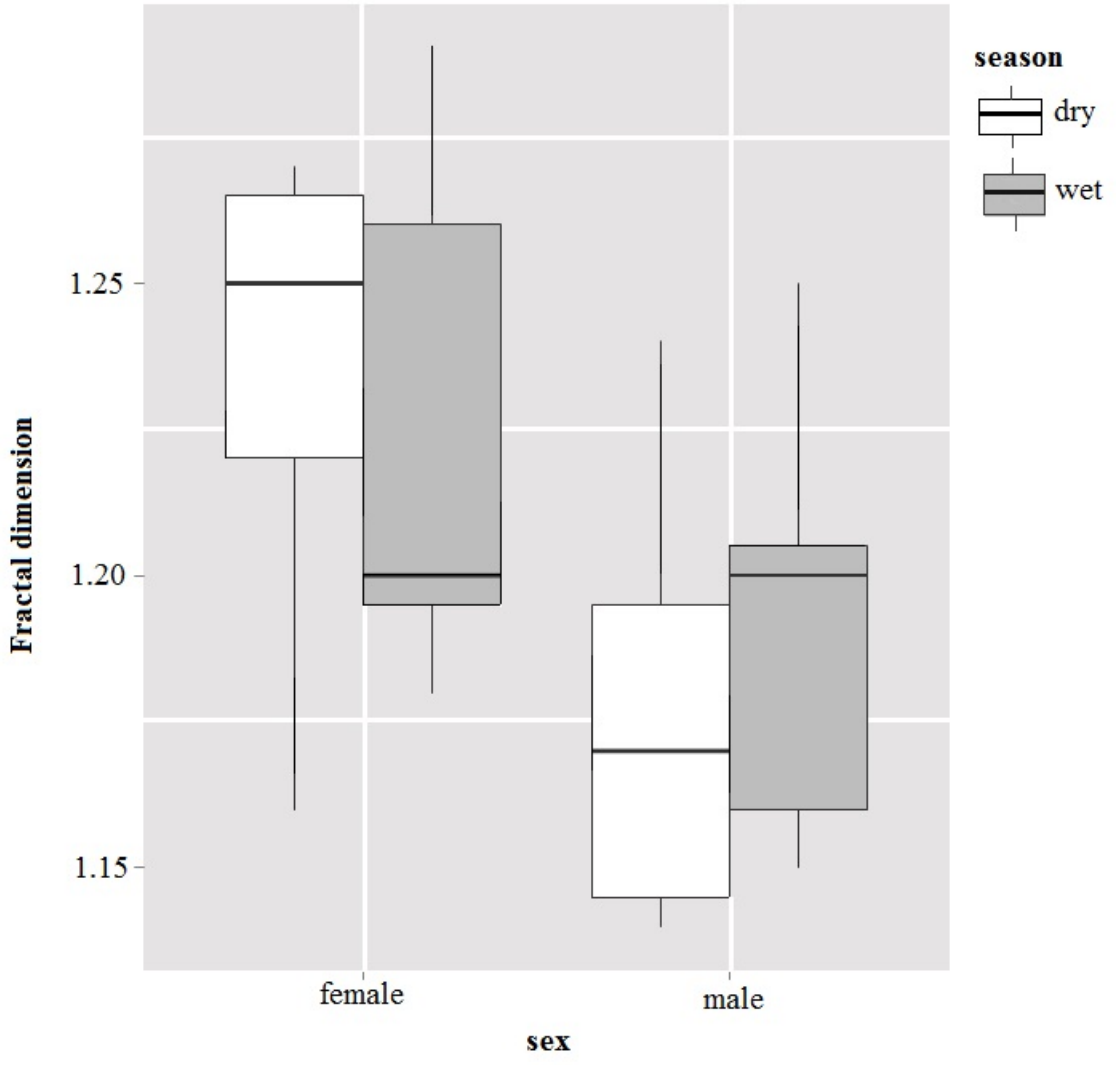

Figure 5: Box plot of fractal dimension versus sex of burrow occupant for wet and dry seasons.

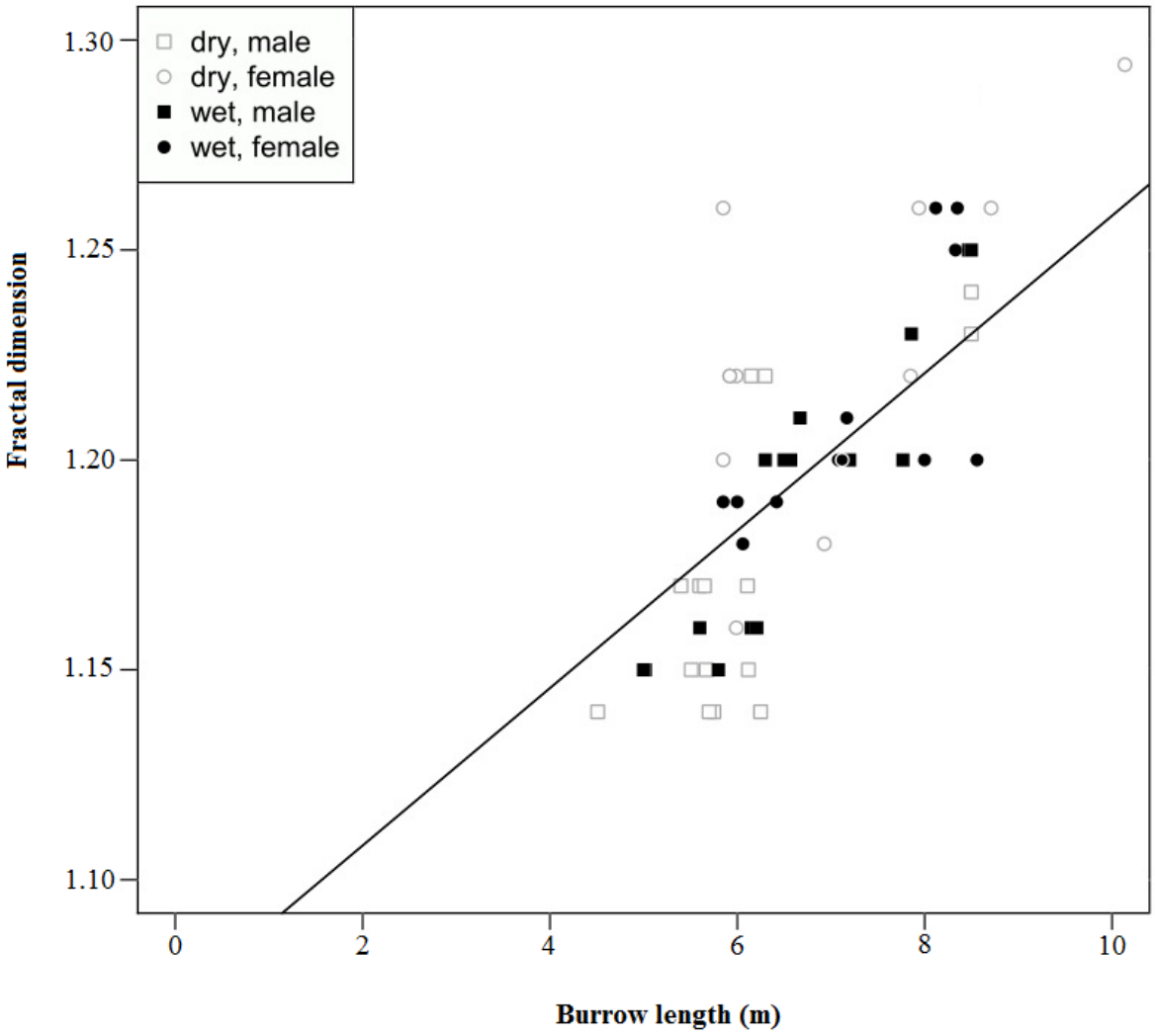

Figure 6: The relationship between fractal dimension and burrow length (main tunnel length) for sexes and seasons (male wet: ■, dry □; female wet: ● dry: ○).

A comparison of overall burrow fractal dimensions (FD) between *T. splendens* (mean ± SD = 1.21 ± 0.08) and other previously studied solitary bathyergids including the silvery mole-rat (*Heliophobius argenteocinereus*) = 1.32 ± 0.05, the Cape mole-rat (*Georychus capensis*) = 1.27 ± 0.08, and the Cape dune mole-rat (*Bathyergus suillus*) = 1.35 ± 0.12 (Le Comber *et al.*, 2002) showed fractal dimension to be similar.

Discussion

Our prediction that burrow length and associated burrow architecture would differ between the sexes was supported, but interestingly, and in marked contrast to the bathyergids, we found that females rather than males, had longer burrow systems (length of the main tunnel), together with more foraging tunnels of greater lengths and higher fractal dimension (Herbst & Bennett, 2006; Thomas *et al.*, 2009, 2012). We speculated that sexual dimorphism in burrow geometry of *T. splendens* is most likely attributed to maintenance of burrow for maternal care of pups and the associated increase food provisioning (and therefore digging) that is required when the female has juveniles young co-habiting the burrow. The greater fractal dimension of the burrows of female *T. splendens* is noteworthy since in other solitary mole rats such as *Thomomys bottae* (Reichman *et al.*, 1982), *Ctenomys minutus* (Gastal, 1994), *Bathyergus janetta* (Herbst *et al.*, 2006) and *Bathyergus suillus* (Thomas *et al.*, 2009), it is the male burrows that have greater complexity which may reflect mate searching strategies.

A recent study has shown that in *T. splendens*, seismic signaling using head raps against the tunnel roof may be used to communicate for purpose such as attraction (Hrouzkova *et al.*, 2013). Such a mechanism, coupled with close proximity of male and female burrows may mitigate against mate searching by burrowing alone. Seismic signaling has also been suggested as a mate attraction mechanism in the solitary bathyergid, *Georychus capensis* which uses drumming of the hind feet to create seismic vibrations (Bennett & Jarvis, 1988). While the overall burrow geometry of *T. splendens* is similar to the solitary bathyergids in terms of range of values recorded for fractal dimension, reflecting aspects of their similar life styles, interesting

differences (e.g. more complex nest and the aforementioned sex differences) undoubtedly reflect their convergent exploitation of the subterranean niche.

The effect of sex on burrow architecture that we observed tended to override the predicted seasonal differences but the general trend was as expected, in that digging activity was greater during the rainy seasons. Such differences between wet and dry periods were clearer for male burrows, possible because increased provisioning by females was also necessary during the dry seasons (Figure 2-5). Pregnancies were observed to peak during both rainy seasons and were occasionally in the dry period in June and July (Katandukila *et al.*, 2013), thus female may be found either lactating or provisioning young over much of the year with the accompanying need for increased foraging. Burrow length was longer in the wet season especially for females, and both males and females dug more foraging tunnels in the wet periods, although this was only reflected in a greater mean foraging tunnel length for females in the wet versus the dry periods. Greater burrowing activity during the wet season resulting in increasing number and length of foraging tunnels has been noted previously for subterranean rodents (Andersen, 1987; Šumbera *et al.*, 2003; Šklíba *et al.*, 2009) as the soil is more workable ((Jarvis & Sale, 1971; Vleck, 1981; Reichman & Smith, 1990; Antinuchi & Luna, 2006; Šklíba *et al.*, 2011) and the food quality and quantity increases from sprouting and regeneration following the rains (Bennie, 1991; Barber, 1995; Pregitzer & King, 2005). It has been found that during foraging, straight paths are more efficient for finding food resources (Zollner & Lima, 1999). However, once resources are found, increased reticulation may result as a function of the vegetative propagation of tubers, bulbs and corms (Bennett & Faulkes, 2000). Increased fractal dimension of the burrow system has been linked to the period of rainfall in the solitary mole-rats, *B. suillus* and *B. janetta*, (Herbst *et al*.,

2006; Thomas *et al*., 2009, 2012) as well as in the social giant Zambian mole-rat, *Fukomys mechowii* (Sichilima *et al*., 2008).

Extensive survey of *T. splendens* burrows undertaken for this study confirms earlier reports with respect to their general structure, which was consistent across all samples (multi-function nest, main tunnel, foraging tunnels and bolt hole: Jarvis & Sale, 1971; Hickman, 1983; Kokiso & Bekele, 2008). The burrow systems of *T. splendens* are inhabited by a single individual, with plural occupancy either during breeding or when females have young. In this study, the burrow systems of five females were found to include young individuals. Although adopting a similar lifestyle and found across similar ranges to bathyergid mole-rats, the burrow system of *T. splendens* shows some marked differences, particularly with respect to the multi-functional nest incorporating a sleeping area, food store chamber and latrine area. The food store chamber in the burrow system of *T. splendens* is located within the sides of the active nest chamber and is comprised mainly of grasses, forbs and underground plant organs including tubers, roots, rhizomes, and bulbs. Similar observations were recorded for *T. splendens* in Kenya and Ethiopia (see Jarvis & Sale, 1971; Hickman, 1983; Kokiso & Bekele, 2008). Interestingly, in the abandoned food stores some bulbs and tubers were found sprouting. The storage of geophytes and crop materials suggests that *T. splendens* have similar dietary needs to other herbivorous subterranean rodents such as bathyergids, geomyids, ctenomyids and Asian spalacids (Keith *et al*., 1959; Jarvis & Sale, 1971; Huntly & Reichman, 1994; Bennett & Faulkes, 2000; Rosi *et al*., 2000; Spinks *et al*., 2000; Šumbera *et al*., 2004; Romañach, 2005; Herbst & Bennett, 2006; Kokiso & Bekele, 2008; Šklíba *et al*., 2009; Šklíba *et al*., 2011).

A series of shallow disused nests were observed in the tunnel before the active nest chamber. In fossorial animals, the presence of multiple nests along a tunnel is common and has been reported in the burrow systems of silvery mole-rats, *Heliophobius argenteocinereus* and Cape dune mole rats, *B. suillus* (Hickman, 1977; Šumbera *et al.*, 2003; Šklíba *et al.*, 2009, 2011; Thomas *et al*., 2009). Multiple nests are reported to be a mechanism for reducing ecto-parasite infestation within burrows. The active nest chamber is identified by the presence of dry grasses and husks of geophytes at the proximal part of the nest entrance. Within the nest chamber, all adjacent above-ground geophytes had been consumed. This indicates that feeding also occurs within the nest chambers of *T. splendens* as reported for *Geomys* sp. and bathyergids (Jarvis & Sale, 1971; Hickman, 1983; Andersen, 1987; Bennett *et al*., 1988; Bennett & Faulkes, 2000).

Apart from the food store and sleeping site in the nest chamber, accumulations of faecal pellets were recorded at the distal part of this chamber, forming a latrine. The fermenting faeces and decomposition of nesting material in the burrows of *T. splendens* raises the temperature in their underground chambers (Jarvis & Sale, 1971; Flynn, 1990; Nevo, 1999; Nowak, 1999) and concurrently, these decomposed faecal pellets, food remains and nesting add to soil fertility. Thus the nest chamber in *T. splendens* functions as a sleeping site, a latrine and also as a feeding chamber, as has also been reported in the blind mole rat, *Spalax ehrenbergi* (Nevo, 1961; Jarvis & Sale, 1971; Hickman, 1983; Kokiso & Bekele, 2008). Other subterranean rodents such as bathyergids, geomyids and ctenomyids differ in that they have a distinct toilet chamber (Jarvis & Sale, 1971; Hickman, 1977; Šumbera *et al.*, 2003, 2004; Cutrera *et al*., 2006; Šklíba *et al.*, 2009, 2011; Thomas *et al*., 2012).

In conclusion, our findings for *Tachyoryctes* show common features with other rodent moles in terms of increased burrowing following rains, but interesting differences in sexual dimorphism of burrow architecture. Species-specific differences in burrow structure were also evident, particularly with respect to their multi-function nest chamber. Across its range, *Tachyoryctes* overlaps with species of the solitary bathyergid mole-rats *Heliophobius* and the social *Fukomys*, especially in Tanzania (Beolchini & Corti, 2004; Faulkes *et al.*, 2010, 2011). Despite this, so far there are no confirmed published reports of any of these three genera co-habiting the same area, and neither did this study find such evidence. Whether this is a result of stochastic or historic process, or differences in habitat use remains to be determined.

Acknowledgements

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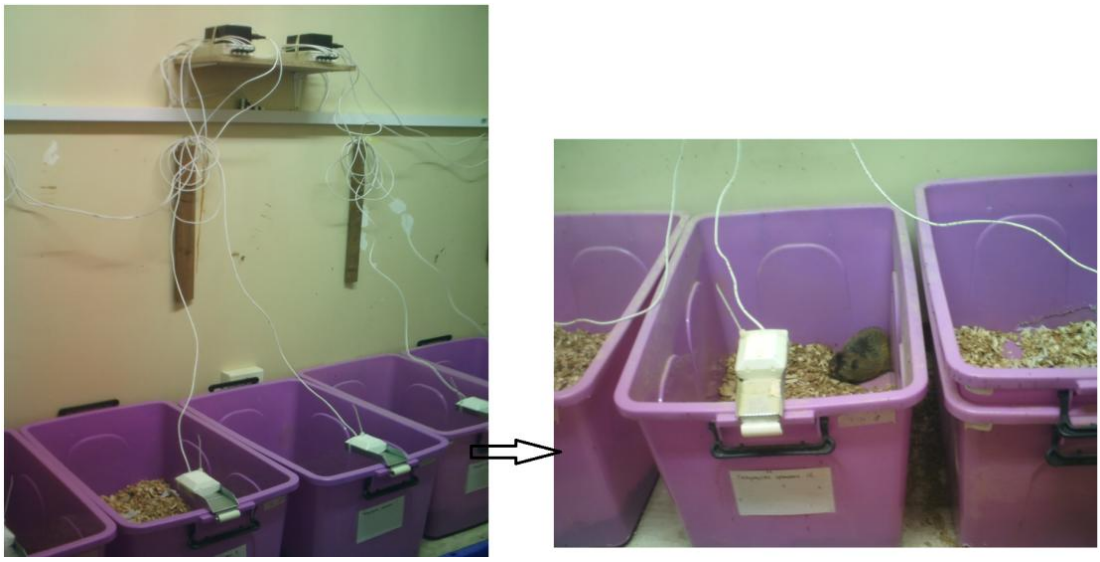
Chapter 3: Laboratory Activity



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Title: Locomotor activity patterns of captive East African root rats, *Tachyoryctes splendens* (Rodentia: Spalacidae) from Tanzania, East Africa

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Abstract

The East African root rat, *Tachyoryctes splendens* (Rüppell 1835) is a solitary fossorial rodent occurring in the eastern parts of central Africa. Unlike bathyergid mole-rats, *T. splendens* occasionally goes above-ground to feed and consequently it is periodically exposed to the natural light-dark cycle. The locomotor activity of *T. splendens* was assessed under various light regimes. *T. splendens* entrains its activity to light cycles and displays distinct nocturnal activity with the total percentage of activity during the dark phase at 87.78 ± 11.01%. In constant darkness (DD), *T. splendens* shows free-running rhythms of slightly shorter than 24 h (23h40 ± 0h13), while still exhibiting most of its activity during subjective night. Upon inversion of the light cycle, time of re-entrainment was exceptionally long. Nocturnal activity time (α) was shortened in response to a shorter night length from 11h29 to 7h46; however, when the dark phase was lengthened, α remained roughly similar to that of the 12L:12D at 11h24. A difference in circadian activity is apparent from the laboratory and field findings, thus in a natural situation, other environmental factors may influence activity patterns.

Key words: Circadian rhythm, entrainment, light, subterranean, *Tachyoryctes splendens*, Tanzania, East/central Africa.

Introduction

Many organisms exhibit daily rhythmicity of various biological functions. Circadian rhythms are biological rhythms that persist with a period of around 24 h in the absence of external cues (Aschoff 1960). Circadian rhythms are reset or entrained daily by external stimuli in order to remain synchronized with the environment. Undoubtedly, the most prominent entraining cues are

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light and temperature (Edery 2000). The mammalian circadian system is regulated by a central pacemaker contained in the suprachiasmatic nucleus (SCN) in the basal hypothalamus (Reppert & Weaver 2002). The basic mechanism of the circadian clock relies on interacting positive and negative transcriptional feedback loops that drive recurrent rhythms in gene expression of the relevant clock genes (Reppert & Weaver 2002). The SCN controls multiple peripheral circadian oscillators throughout the body. These circadian oscillators regulate output rhythms in physiology and behaviour, one of which is locomotor activity (Chong et al. 2003; Ko & Takahashi 2006).

Subterranean mammals spend a large proportion of their time underground and as a result have evolved specialized morphological and physiological adaptations to their underground habitat that include microphthalmic eyes and enhancement of other senses such as olfaction and tactile stimuli (Nevo 1999). In their burrows, they are subjected to a unique micro-environment with buffered thermal regimes and limited exposure to light (Bennett et al. 1988; Bennett & Faulkes 2000). As a consequence of their subterranean lifestyle, they are not regularly exposed to the natural day-night cycle. However, previous studies indicate that the majority of subterranean mammals do perceive light (Rado et al. 1992; Wegner et al. 2006; Kott et al. 2010) and are able to entrain locomotor activity to light (Bennedix 1994; Tobler et al. 1998; Riccio & Goldman 2000; Begall et al. 2002; Oosthuizen et al. 2003; Hart et al. 2004; Vasicek et al. 2005; Schöttner et al. 2006; Valentinuzzi et al. 2009; Tomotani et al. 2012).

The genus *Tachyoryctes* comprises several cryptic species, and is widely distributed in East Africa and the eastern parts of Central Africa (Musser & Carleton 2005). The East African root rat, the *Tachyoryctes splendens* is a solitary, fossorial rodent that occurs in central Africa in

a wide variety of habitat types including agricultural areas where it is regarded as a pest (Kokiso & Bekele 2008). *Tachyoryctes splendens* does not possess as many morphological modifications for a subterranean lifestyle as do mole-rats (both African and Asian) having an appearance more like a rat or a vole (Jarvis & Sale 1971). Unlike mole-rats, they do feed above-ground, providing an opportunity for contact with the external environment (Jarvis & Sale 1971). Little is known about the circadian system of the Spalacidae of Africa including the East African root rat. A single study was conducted on activity patterns of *T. splendens* (Kenyan population) in their natural environment using radio-active tracing (Jarvis 1973). In this study, predominantly diurnal activity was reported. To date, locomotor activity rhythms of *T. splendens* have not been investigated under controlled laboratory conditions.

We therefore undertook experiments under controlled lighting conditions to assess whether *T. splendens* sampled from Tanzania in East Africa exhibits rhythmic locomotor activity over the 24 hour day, as well as the response to a drastic phase shift (inversion) of the light cycle. We anticipated that *T. splendens* would display nocturnal activity and hence change its activity in response to an inverted light cycle. We also investigated whether an endogenous circadian rhythm was present under constant conditions (DD) and determined the free run period. We expected free-running periods of slightly shorter than 24 hours as is the case with most other nocturnal mammals. Furthermore, we assessed the effect of increasing and decreasing day length on the daily locomotor activity rhythm. Occurring near the equator, this is not an environmental phenomenon that this species would ever encounter in their natural habitat and we predicted that these animals may have some difficulty in adjusting the lengths of active time.

Materials and Methods

Experimental animals – Experimental animals (6 males {mean body mass 250.11 ± 13.09 g}, 5 females {mean body mass 218.4±12.8 g}) were sampled from the Kilimanjaro region, Tanzania (03˚16.466' S, 037˚32.887' E; 1495 m above sea level), East Africa and shipped (export permit No. 65054 granted by The Ministry of Natural Resources and Tourism Tanzania; and the veterinary permit No. 0000875 from the Ministry of Livestock Development and Fisheries, Tanzania) to the Department of Zoology and Entomology, University of Pretoria, Pretoria in South Africa (import permit No. 13/1/1/30/2/9/6-241from the Department of Agriculture, Forestry and Fisheries, and CPB6 No. 003784 granted by Gauteng Nature Concervation).

Before commencement of experiments, *T. splendens* was acclimatized to a light-controlled laboratory 12L:12D at room temperature (25±1° C) for 30 days. Animals were housed singly in plastic cages (50 × 40 × 40 cm) lined with wood shavings and paper tissue provided as nesting material, and fed *ad libitum* on chopped sweet potatoes and carrots. During the experiments, ambient temperature was maintained at 25^{o} C and cages were cleaned during cycle changes (Oosthuizen et al. 2003; Hart et al. 2004). Human disturbance in the laboratory was contorolled during experiment; one person was feeding animals at non-specified time to avoid conditional cease of activity during time of food supply throughtout the experiment. Red dim light was used by food supplier for visualization to avoid unnecceasry hiting of cages which may consequently affect activity of experimental animals. Research on live animals followed the guidelines of the American Society of Mammalogists (ASM; Sikes et al. 2011) and was approved by the Animal Ethics Committee of the University of Pretoria (Ethics clearance number ECO47-10).

Activity Recording – 11 cages were fitted with infrared motion detectors (Quest PIR internal passive infrared detector; Elite Security Products (ESP), Electronic Lines, London, UK) (Oosthuizen et al. 2003; De Vries et al. 2008) in such a way that activity could be detected over the entire floor space of the cage. Infrared captors were connected to receivers that were connected to a Minimitter recording system (Vital View™; Minimitter Co. Inc., Sunriver, OR, USA; http://www.minimitter.com) installed on a computer outside the temperature and light controlled animal room. The activity was collectively recorded per minute (Oosthuizen et al. 2003; De Vries et al. 2008; Alagaili et al. 2012).

Animals were placed on a 12L:12D light cycle (LD1; L:07h00-19h00) for 22 days to ascertain whether they were able to entrain to light. Subsequently, the animals were subjected to a DD cycle (constant darkness) for 46 days. Afterwards, the animals were re-entrained to a 12L:12D (LD2; L:07h00-19h00) for 22 days, where after the light cycle was inverted (DL; 12D:12L; L:19h00-07h00) for 25 days. Animals were re-entrained to a 12L:12D light cycle prior to successive light cycles. In order to assess the effect of different day length on activity of the East African root rats, animals were maintained on a long day cycle (LLD; L:05h00-21h00) for 22 days, followed by a short cycle (SLD;L:09h00-17h00) for 31 days.

Data analyses

Activity patterns were visualized with double plotted actograms using Actiview Biological Rhythm Analyses 1.2 software (Minimitter™ Co., Inc., Sunriver, OR, USA; www.minimitter.com). The phase angle (difference in time between the beginning of the dark cycle and onset of activity) and percentage of nocturnal and diurnal activity was determined for the light cycles of each animal using Clock Lab (ClockLab ™, Actimetrics Evanston, II U.S.A.:

www.actimertics.com). The circadian period (*tau* = **τ**) was determined during DD, also the percentage of activity during subjective night and day. Mean ± 1 Standard Deviation (SD) of percentage of nocturnal and diurnal activity was evaluated using descriptive statistical analysis (Begall et al. 2002; Hart et al. 2004; Alagaili et al. 2012). Multivariate comparison of percentage of nocturnal and diurnal activity between LD1, LLD and SLD was determined using ANOVA; whereas percentage of nocturnal and diurnal activity between light cycles was compared using *t*-test.

Results

LD1 (12L:12D) – All animals entrained their activity to the light-dark cycle, with the majority of their activity during the dark phase of the light cycle (*n* =11, mean ± SD = 87.78 ± 11.01%). Activity commenced after the offset of the light at 19h18 ± 0h 02 and ceased before the onset of light at 06h43 ± 0h 03. The mean ± SD active time (α) of the animals was 11h20 ± 0h04 (Figure 1).

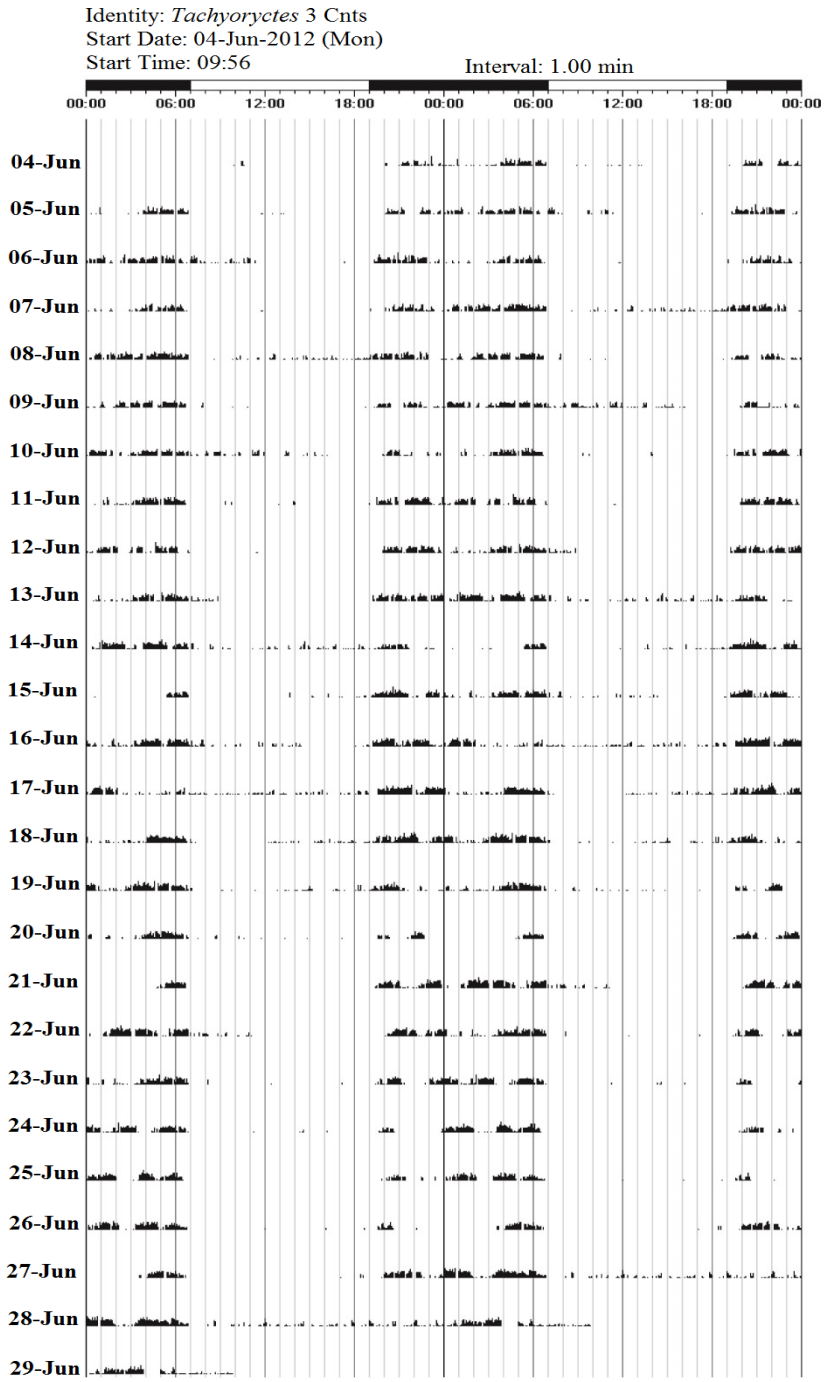

Figure 1: A double plotted *Tachyoryctes splendens* actogram of an animal displaying predominantly nocturnal activity and a small amount of diurnal activity during a 12L:12D light cycle: Black bars on the top illustrate hours of darkness and white bars for hours of lightness; Y-axis is days and x-axis is hours.

DD (constant darkness) – All animals showed free-running rhythms with a mean ± SD period (τ) of 23h40 ± 0h13 (range = 23h31–24h08). The majority of activity was concentrated during the subjective night (n = 11, mean ± SD = 86.76 ± 12.05%). 3 of 11 animals displayed activity during both the subjective day and subjective night however; animals were still more active during the subjective night. No difference was observed in the amount of nocturnal activity of LD1 and activity during the subjective night of DD ($t_{(10)}$ = 0.84, P = 0.42; n_1 = 11, n_2 = 11)(Figure 2).

LD2 (12L:12D) and DL (12D:12L) – The activity of *T. splendens* was re-entrained to the LD cycle after constant darkness. Upon inversion of the light cycle, most animals ceased activity for a few days before starting to re-entrain activity to the new light cycle. Full re-entrainment from the time the light cycle was inversed, was exceptionally long, and ranged between 5 and 18 days. While re-entraining, activity was masked during the light phase, once entrained, *T. splendens* displayed a mean ± SD of 92.17 ± 7.82 % (n = 11) of their activity during the dark phase.

Activity commenced slightly before the offset of the lights at 6h36 ± 0h16 and ceased activity right after the onset of the light at 19h05 ± 0h16 whereas the mean active time (α) was 11h36 ± 0h05. Comparison of percentage of activity of the dark phase between LD1 and DL was not significantly different ($t_{(10)}$ = 0.15, P = 0.883, n_1 = 11, n_2 = 11) (Figure 3).

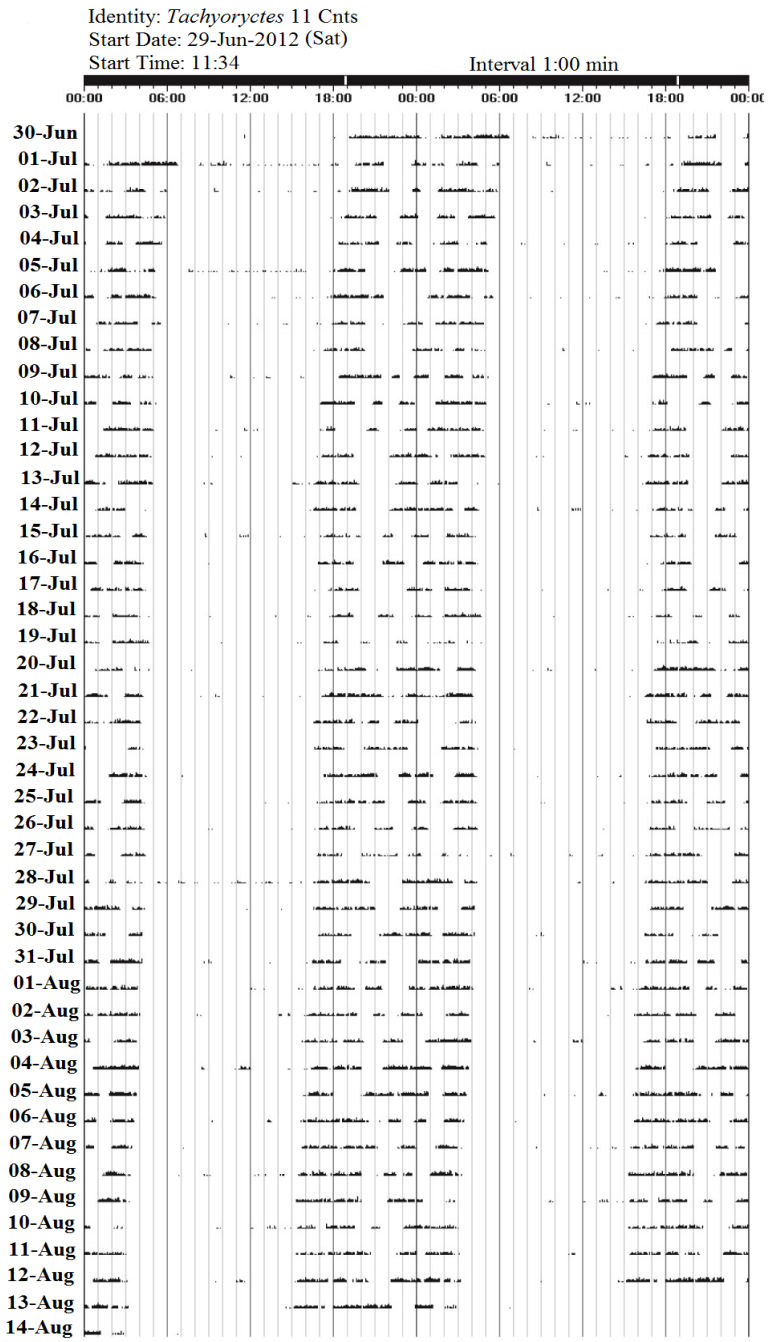

Figure 2: A double plotted *Tachyoryctes splendens* actogram demonstrating an endogenous circadian rhythm with a period slightly shorter than 24h during DD: Black bars on the top illustrate hours for subjective night and white bars for subjective day; Y-axis is days and x-axis is hours.

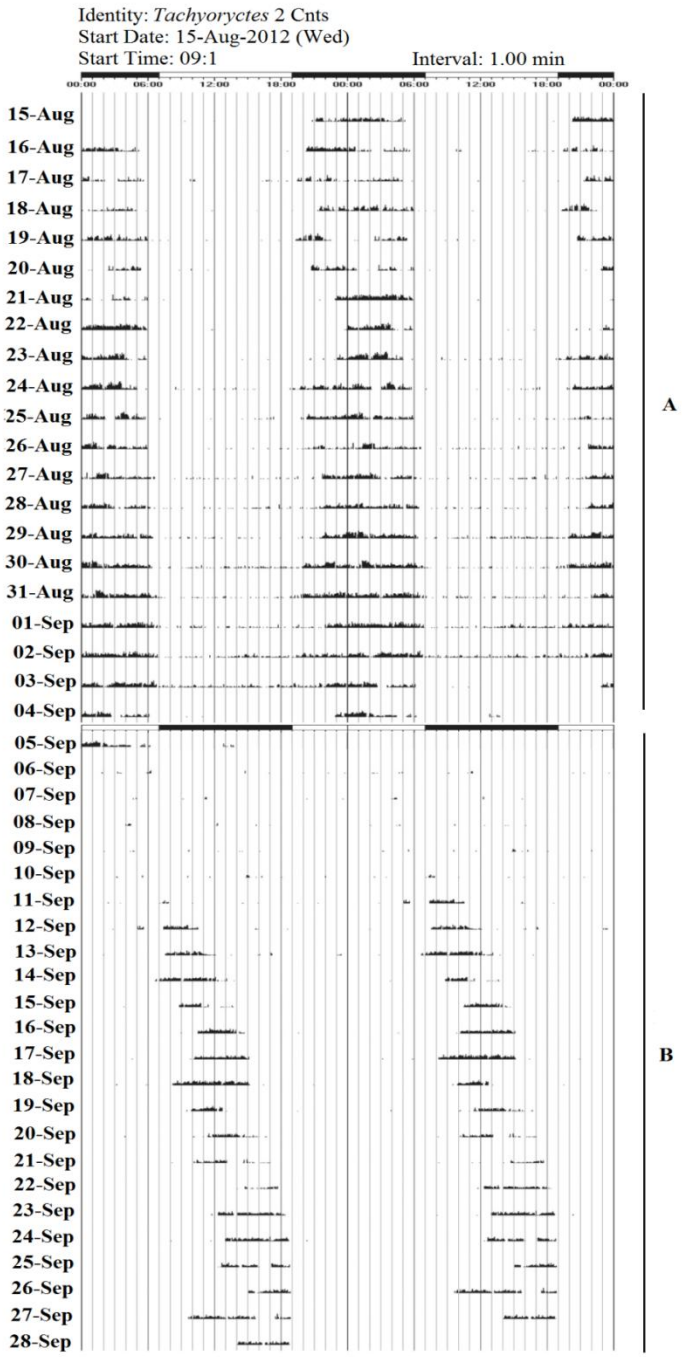

Figure 3: A *Tachyoryctes splendens* actogram illustrating the re-entrainment of activity when the light cycle was inversed from a 12L:12D cycle (A) to a 12D:12L cycle (B): Black bars on the top illustrate hours of darkness and white bars for hours of lightness; Y-axis is days and x-axis is hours.

LLD (16L:8D) and SLD (8L:16D) – When subjected to a long day (LLD), 3 of 11 animals exhibited activity during both the day and night activity although more activity peaks were observed during the dark phase. All animals shortened their activity time to be only during the dark phase of the light cycle. Animals were active for a mean ± SD of 94.43 ± 6.33 % (n = 11) during the dark phase with onset of activity before onset of darkness at 20h43 ± 0h35 and ceased activity before onset of light at 04h46 ± 0h35 The mean ± SD active time (α) was 7h46 ± 0h15 (Figure 4).

During the SLD cycle, 2 out of 11 animals were active during dark and light phases of the cycle with few peaks during the dark phase. Animals were active for a mean ± SD 92.68 ± 5.34 % (n = 11) during the dark phase of this cycle with the onset of activity almost two hours after the transition from light to dark at 18h58 ± 0h43. Activity was terminated more than 2 hours before onset of the light at 06h27 ± 0h52 and the active time was 11h24 ± 0h16. Comparison of percentage of dark activity between LLD and SLD was not significantly different ($t_{(10)}$ = 0.78, P = 0.45; n_1 = 11, n_2 = 11,). Similarly the comparison of the percentage of nocturnal activity between LD_1, LLD and SLD was also not significantly different (n = 11, K = 3, $F_{(2, 30)}$ = 2.07, P = 0.14) (Figure 5).

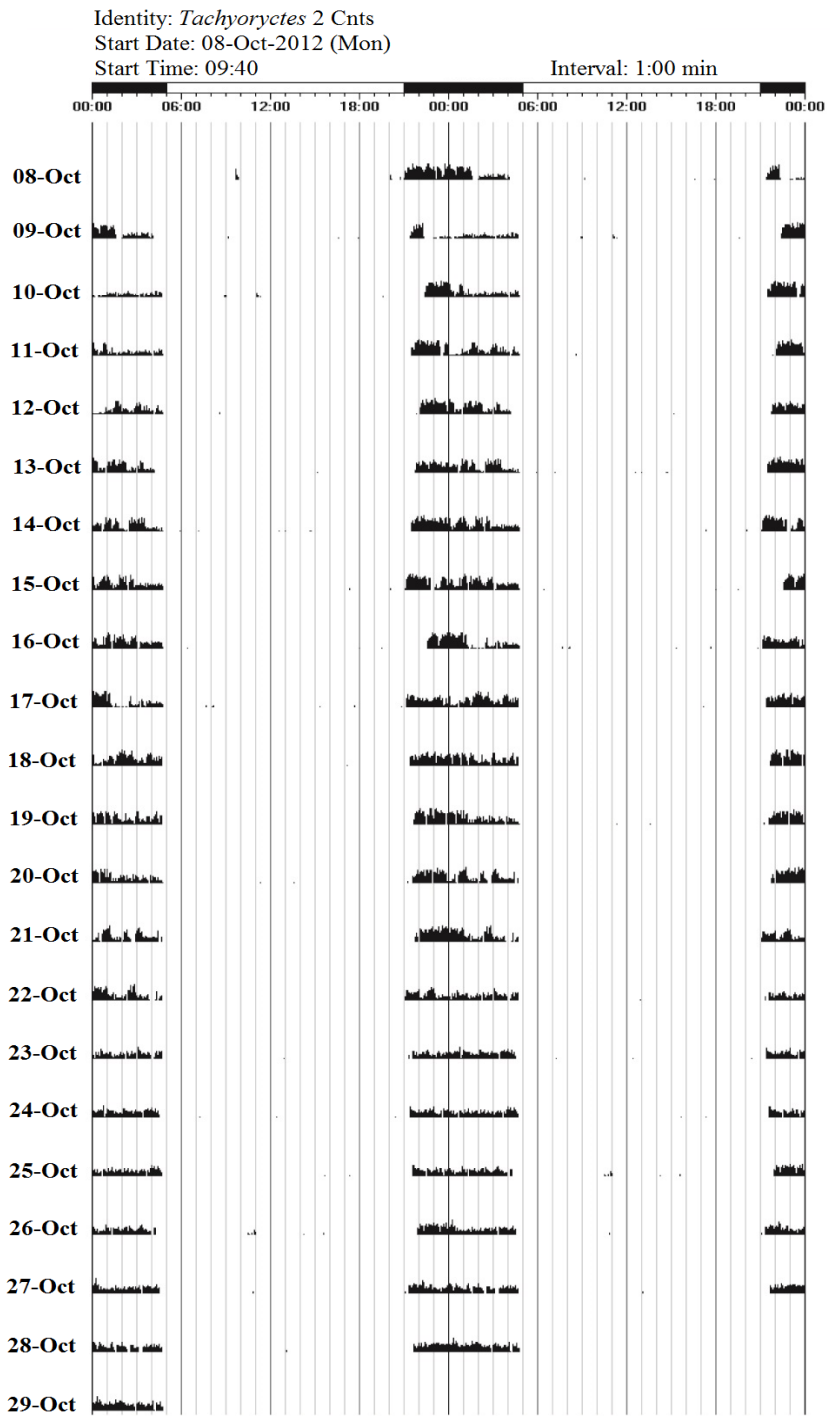

Figure 4: A *Tachyoryctes splendens* actogram exemplifying an animal that shortened its active time (α) to express activity only during darkness: Black bars on the top illustrate hours of darkness and white bars for hours of lightness; Y-axis is days and x-axis is hours.

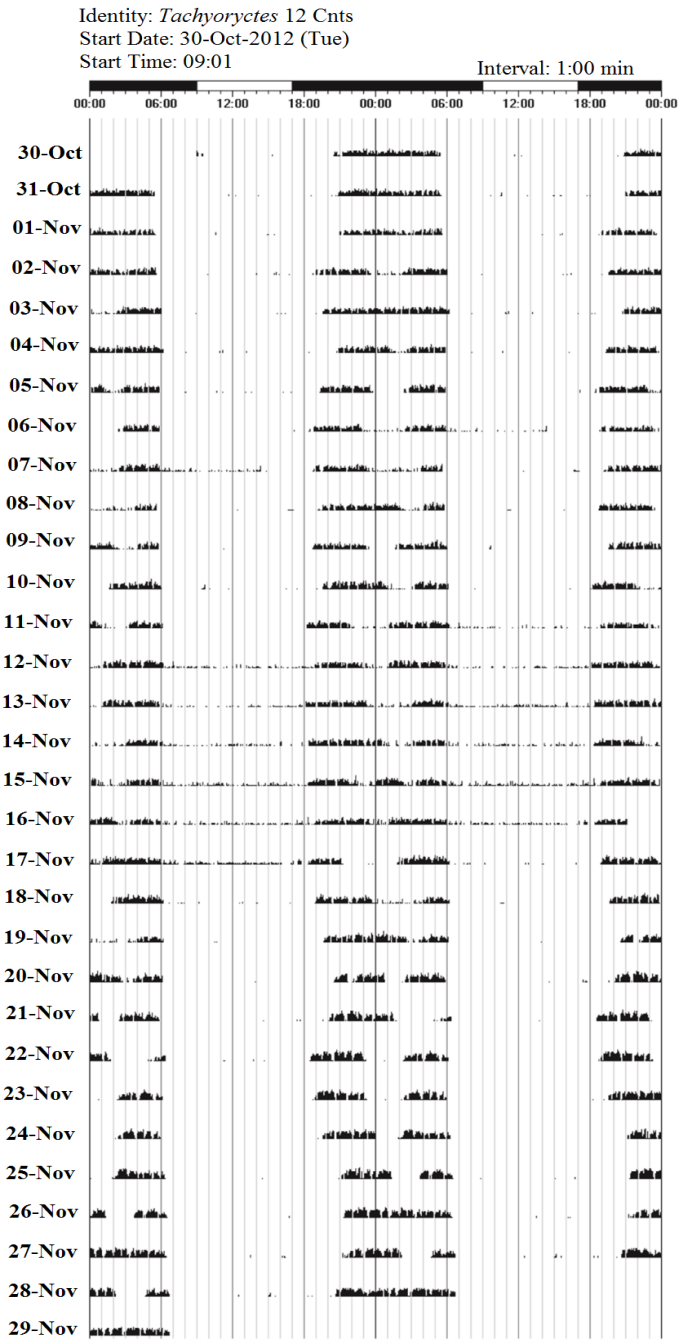

Figure 5: An example of a *Tachyoryctes splendens* actogram that shows that activity is not expanded to cover the whole period of darkness but rather remains relatively equal to that of 12L:12D: Black bars on the top illustrate hours of darkness and white bars for hours of lightness; Y-axis is days and x-axis is hours.

Discussion

A number of species worldwide exploit the subterranean niche, but these species differ in the degree of fossoriality (Nevo 1999). Some species, such as African mole-rats (Bathyergidae) are strictly subterranean, while others such as coruros (Octodontidae), tuco-tucos (Ctenomyidae) and root rats (Spalacidae) emerge from their burrows periodically to feed above-ground (Jarvis 1973; Bennett & Faulkes 2000; Begall et al. 2002; Tomotani et al. 2012). Along with the large degree of variation in fossoriality there is variation in circadian rhythmicity among these animals. Although circadian rhythmicity may be of little apparent use for strictly subterranean species, it may be of greater importance for species that do occasionally come above-ground.

Fossorial mammals differ in the degree to which they remain in their underground habitats. In controlled laboratory conditions, *T. splendens* showed a clear activity rhythm and displayed distinct preference for activity during the dark period. It is common for subterranean mammals to exhibit nocturnal activity under controlled conditions (Riccio & Goldman 2000; Begall et al. 2002; Schöttner et al. 2006; Tomotani et al. 2012), although some mole-rat species show less stable preferences for nocturnal or diurnal activity, such that some individuals of a species may display nocturnal activity while others display diurnal activity (Hart et al. 2004; Vasicek et al. 2005; De Vries et al. 2008). Other species such as the blind mole-rat (*Spalax ehrenbergi*) may even switch between nocturnality and diurnality (Tobler et al. 1998; Oster et al 2002). The silvery mole-rat, *Heliophobius argenteocinereus* is reported to be completely arrhythmic (Jarvis 1973).

The finding that *T. splendens* displays nocturnal activity is in contrast to a previous study conducted under natural conditions, where it was found to be foraging outside the burrow during the day (Jarvis 1973). However, several subterranean species have been reported to display

different activity patterns under controlled *versus* natural conditions. Both curoros (*Spalacopus cyanus*) and tuco-tucos (*Ctenomys knightii*) exhibit distinct nocturnal activity under controlled laboratory conditions (Begall et al. 2002; Valentinuzzi et al. 2009) whereas under natural conditions, animals are reported to be active during the day (Reig 1970; Tomotani et al. 2012). Furthermore, pocket gophers (*Geomys bursarius*) show a predictable activity pattern in the wild (Benedix 1994) whereas captive animals display no distinct activity patterns (Hickman 1984).

Since some of these species do feed above-ground, timing may be of more importance than for those that are more strictly subterranean. Predation risk may affect foraging behaviour in terms of timing and efficiency, as may food availability itself (Halle & Stenseth 2000). Predation is absent and food supply is non- limiting from the laboratory setting and may contribute significantly to differences in field and laboratory activity. Also, it has been suggested that soil temperature may act as a zeitgeber for behavioural activity rhythms in field studies (Šklíba et al. 2007; Lövy et al. 2013), however this is likely more pertinent to strictly subterranean mammals.

Under constant conditions, *T. splendens* displayed endogenous rhythms slightly shorter than 24 h, as is typical for nocturnal animals (Aschoff 1981). This is in congruence with the blind mole-rat and most of the African mole-rats, although a large amount of variability is present in the length of the circadian period (Tobler et al. 1998; Oosthuizen et al. 2003; Hart et al. 2004; Vasicek et al. 2005; Schöttner et al. 2006; De Vries et al. 2008). Tuco-tucos on the other hand display endogenous rhythmicity with a period much longer than 24 h (Valentinuzzi et al. 2009), while pocket gophers are arrhythmic with activity distributed throughout the 24 h period (Hickman 1984).

Upon inversion of the light cycle, the responding phase shift in *T. splendens* was exceptionally long. Similar results have been obtained for coruros and several species of African mole-rats, in response to a phase shift in the light cycle, activity was masked during the light hours and re-entrainment was rather slow (Begall et al. 2002; Oosthuizen et al. 2003; De Vries et al. 2008). The time of re-entrainment may be dependent on whether animals display nocturnal or diurnal activity, in diurnal chronotypes of the blind mole-rat *Spalax ehrenbergi* activity shifted immediately after a phase shift in the light cycle, whereas re-entrainment in nocturnal chronotypes spanned over almost two weeks (Tobler et al. 1998). Re-entrainment to an inversed light cycle confirms that *T. splendens* may have a preference for nocturnal activity under controlled laboratory conditions.

Another indication of their preference for nocturnal activity is apparent in the restricted active time when the length of the night is shortened. Onset of activity is after dark and offset again before the transition of dark to light, limiting activity completely to the dark hours of the light cycle. Activity was however, not expanded to spread throughout the time of darkness when the night length was expanded. In its natural habitat near the equator, the day length varies about one hour over the annual cycle, thus *T. splendens* would never encounter such drastic changes as has been tested. Normally, photoperiodic events related to day length would be of importance in seasonal breeding, and although *T. splendens* appears to be breeding seasonally, this is more related to rainfall than day length. Breeding coincides with peaks in the rain cycles, which has implications for food availability in terms of breeding success.

Tachyoryctes splendens shows a preference for nocturnal activity that is maintained through drastic phase shifts and shortening of the dark phase. Our study reiterates that circadian

investigations of subterranean species under controlled laboratory conditions may not reflect the true nature of circadian activity in the natural habitat since other environmental factors may influence active time in combination with the light dark cycle, which may have relevance for the survival of the animals.

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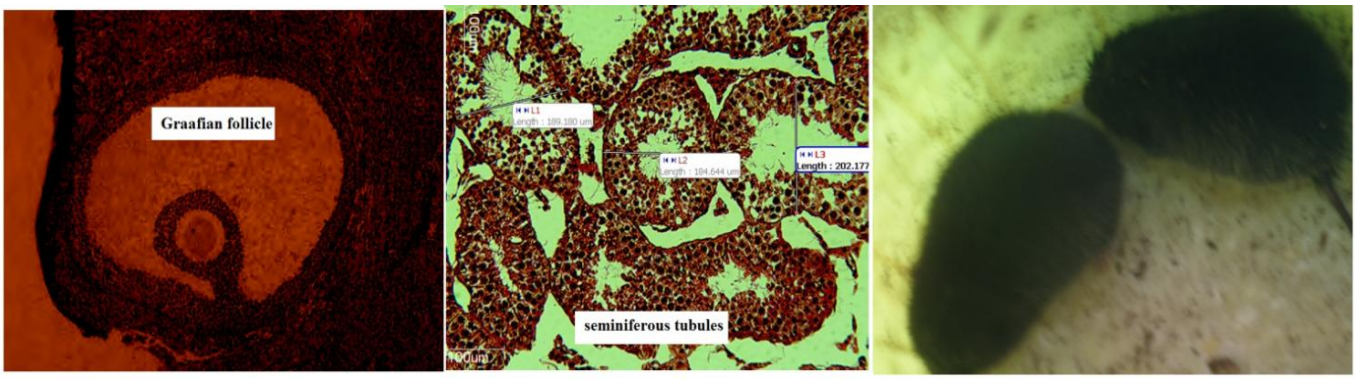
Chapter 4: Reproductive status



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Title: Reproduction in the East African root rat, *Tachyoryctes splendens* (Rodentia: Spalacidae) from Tanzania: the importance of rainfall

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Abstract

The East African root rat, *Tachyoryctes splendens* (Rüppell, 1835) is a solitary subterranean rodent mole. The present study investigated breeding patterns in both sexes of *T. splendens* from data collected at monthly intervals over an entire calendar year. The study focused on the analyses from post mortem examination of males and females to assess the presence of foetuses, gonadal histology, reproductive tract morphometrics, measurement of gonadal steroids (plasma progesterone and oestradiol-17β in females and testosterone in males) and field observations (i.e., the presence of infants, juveniles, sub-adults and lactating females). The objective of this study was to assess if the reproductive biology of the root rat reflected the bimodal pattern of rainfall that is characteristic of East Africa. Rainfall has been suggested to trigger breeding in many subterranean rodents and as a consequence, this study aimed to assess the relationship between rainfall and reproductive characteristics of *T. splendens*. Peaks in mean gonadal mass, increases in concentration of reproductive hormones and the presence of Graafian follicles and corpora lutea in the ovaries of females, and testes mass, seminiferous tubule diameter and testosterone titre mirrored the annual peaks of precipitation at the study area. Together with field observations of the temporal occurrence of pregnancies, infants, juveniles and sub-adults, the data show that *T. spendens* cues its breeding with the patterns of rainfall, such that offspring are born in the latter half of each rainy season, from April to July and November to December.

Keywords: *Tachyoryctes splendens*, solitary, hormones, histology, rainfall, radioimmunoassay, Tanzania, East Africa

Introduction

Reproduction in subterranean mammals is constrained by both the prevailing ecological conditions and the burrow environment (Bennett & Faulkes, 2000). The subterranean niche precludes the use of many common cues that are normally used to maximise reproductive success, for example, photoperiod is unlikely to be an important proximate cue, whereas rainfall in the form of changing soil moisture content is used to trigger breeding (Bennett & Faulkes, 2000; Herbst *et al*., 2004; Hart *et al*., 2006). Temperature is a potentially important environmental cue that mole-rats may utilise for their daily and seasonal activity patterns. The temperatures within mole-rat burrow systems are much less variable than those above ground; diurnal and seasonal fluctuations in temperature do occur but they are muted (Bennett *et al*., 1988; Roper *et al*., 2001).

Solitary subterranean rodents occurring at higher altitudes (particularly geomyids) exhibit marked breeding patterns and this may in part be due to the seasonal changes in temperature of their burrow environment (Kennerly, 1964). Rainfall is an important variable that can be detected underground since it softens the soil (if it reaches sufficient depths) and brings about subsequent plant growth and flushes of vegetation (Dennis & Marsh, 1997). Solitary subterranean bathyergid mole-rats time their breeding events with rainfall. These occur in winter rainfall regions and as a consequence mating occurs in the winter months, with young being born in the spring when food is abundant and the soil easily workable. This also facilitates dispersal and construction of independent burrow systems (Bennett & Jarvis, 1988; Herbst *et al*., 2004; Hart *et al*., 2006). Interestingly, the silvery mole-rat, *Heliophobius argenteocinereus* from the tropics differs in that it breeds seasonally, but not synchronously with rainfall patterns (Šumbera *et al*., 2003).

Many subterranean rodents are strictly solitary, highly xenophobic and vigorously defend the burrow system in which they reside (Bennett & Jarvis, 1988; Gazit & Terkel, 1998; Šumbera *et al.*, 2003). In solitary African mole-rats (Family Bathyergidae), courtship and subsequent copulation is brief and requires that the highly aggressive and xenophobic behaviours that are characteristic of these species are relaxed during these periods (Bennett & Jarvis, 1988; Narins *et al.*, 1992). Seismic signalling occurs with the different sexes announcing their presence and sexual status displayed by a particular frequency of foot drumming (Bennett & Jarvis, 1988; Narins *et al.*, 1992; Bennett *et al.*, 1999). Thus plural occupancy of the female's burrow system only occurs when courtship and mating is taking place and also when the female has young (Bennett & Jarvis, 1988; Bennett *et al.*, 1991).

All solitary southern African species of bathyergid mole-rats are seasonal breeders with their reproduction acutely tied to rainfall (Bennett & Jarvis, 1988; Herbst *et al*., 2004; Sandwyk & Bennett, 2005; Hart *et al*., 2006). In marked contrast, the majority of social bathyergids reproduce throughout the year (Bennett *et al.*, 1988, Sichilima *et al.*, 2008; 2011). Two social species, one from a winter rainfall region, the common mole-rat, *Cryptomys hottentotus hottentotus* (Spinks *et al.*, 1997; 1999) and one from a summer rainfall region, the highveld mole-rat, *Cryptomys hottentotus pretoriae* (van Rensburg *et al.*, 2002) are unusual in exhibiting seasonal reproduction, with the former also occurring sympatrically with solitary species the Cape dune mole-rat, *Bathyergus suillus* (Bennett & Faulkes, 2000).

A number of studies of reproduction in solitary subterranean rodents have been conducted, but many of these have focussed on laboratory housed animals (Altuna *et al.*, 1991; Bennett *et al.*, 1991; Gazit *et al.*, 1996) given the difficulties of studying subterranean rodents in the field (Rado & Terkel, 1989; Rado *et al.*, 1998). The reproductive biology of a number of

families of rodent moles have been studied and include *Geomys* (Wood, 1949; Vaughan, 1962), *Cannomys* (Eisenberg & Maliniak, 1973), *Thomomys* (Andersen, 1978), *Georychus* and *Bathyergus* (Bennett *et al.*, 1991), *Spalax* (Shanas *et al.*, 1995; Gazit *et al.*, 1998), *Ctenomys* (Weir, 1974; Camin, 2010) and *Heliophobius* (Šumbera *et al.*, 2003). Despite these, very little has been published on the East African root rat, *Tachyoryctes splendens* (Family Spalacidae). Jarvis (1973) investigated a Kenyan population of *Tachyoryctes* using field observations as well as the examination of gonadal histology from adult females. She reported that *T. splendens* has two breeding opportunities approximately 173 days apart; the gestation period was estimated to be 38.5 days with the time interval between birth and weaning of around 43 days, and the annual recruitment rate to be 2.73 young with an average of 2.1 litters produced annually from a single female.

To date, there has been no in-depth study on the reproductive biology of both male and female *Tachyoryctes* and this study addresses this deficiency on a population from Tanzania, East Africa where the species that is considered to be an agricultural pest. In this study we aimed to assess the reproductive biology of *T. splendens* using post-mortem data and investigation of plasma steroids from animals sampled on a monthly basis for an entire calendar year, as has been done for other subterranean mammals (e.g., Wood, 1949; Herbst *et al.*, 2004; Schoeman *et al.*, 2004; Hart *et al.*, 2006; de Bruin *et al.*, 2012). We predicted that peaks in reproductive activity and pregnancy would coincide with the bimodal rainfall patterns characteristic in East Africa, namely the long wet season from February to early June and the shorter wet season in September, October and November.

Materials and Methods

Tachyoryctes splendens were sampled from the Mamba Komakundi village which is located on the foot slopes of Mount Kilimanjaro in Moshi Rural District, north-east Tanzania, East Africa (03˚16.54' S, 037˚32.49' E; 1495 m above sea level). The study was conducted over 12 consecutive months, from January to December 2011. The study site is classified as agri-ecological zone with highly fertile volcanic soils supporting a variety of food and cash crops (Kilimanjaro Regional Profile [KRP], 1998). The study area has two rainy seasons (Figure 1) with a mean annual rainfall of 1250 mm; a longer period of rainfall (i.e., heavy rainfall) occurs in the latter half of February, March, April, and May while a shorter period of rainfall occurs in late September, October and November. The dry months are December, January, June, July, and August (KRP, 1998). June and July are the transition months between the heavy rains and dry season. Annual average temperature ranges from 15°- 40° C with high humidity during March, April, May and October (KRP, 1998).

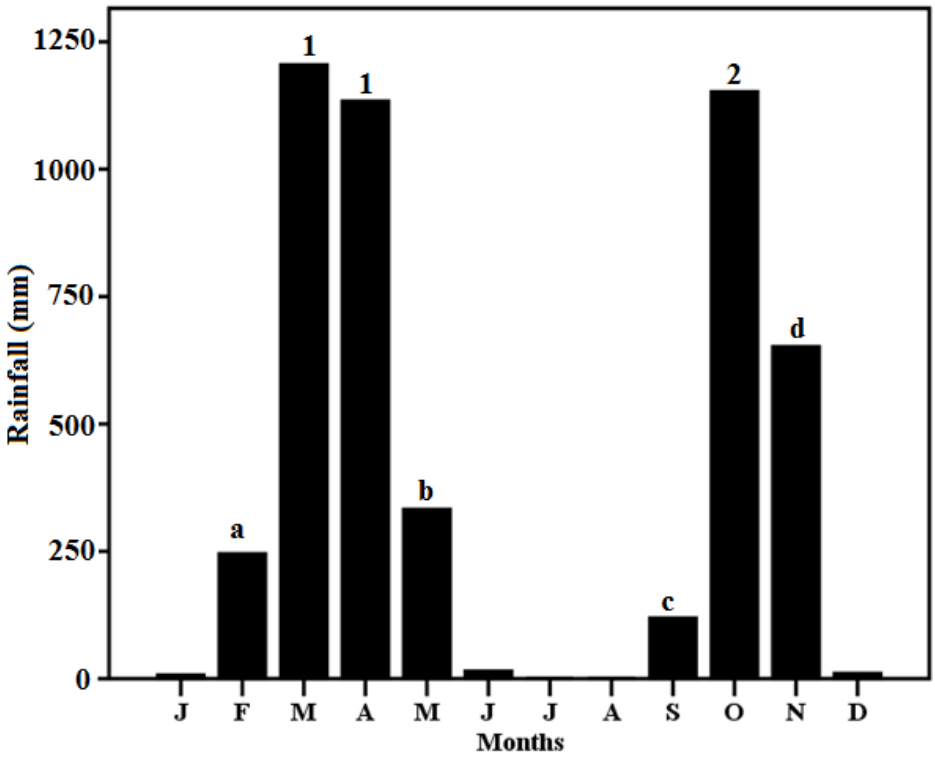

Figure 1: Mean monthly rainfall (mm) of Marangu ward, Kilimanjaro region Tanzania from January to December 2011. Data were obtained from the Tanzania Meteorology Agency (TMA): a = onset of heavy rainfall (wet season 1), b = offset of heavy rainfall, c = onset of short rains (wet season 2), d = offset of short rains, 1 = heavy rains, 2 = short rains. July, August and January are driest months.

Animals were sampled from areas subject to widespread crop destruction by *T. splendens*, and were sampled from their burrows by either using Hickman live traps or by manual excavation of burrow systems (Hart *et al*., 2006; Sichilima *et al.*, 2008). Ten adult female and ten adult male *T. splendens* were sampled each month for one calendar year resulting in a total of 240 animals (i.e., 120 adult males and 120 adult females). Burrows for sampling animals were selected randomly (Sichilima *et al.*, 2008; 2011). Animals were euthanized with an overdose of chloroform (Merck, Johannesburg, South Africa). Following expiration, standard

body measurements were recorded including body mass, head-body length, tail length and the length of hind foot (Hart *et al*., 2006).

The external reproductive characteristics such as presence of teats and a perforate vagina were recorded in females. Blood was drawn from the heart of the sacrificed animal using heparinized syringes and centrifuged at 500 g for 10 minutes. Plasma was separated using a pipette and subsequently stored at -20° C. Gonads (testes/ovaries) were removed and fixed in Bouin's fixative for 18 hrs before storing in 70% ethanol. The reproductive status of females was further assessed by recording either the presence or absence of embryos/foetuses after post-mortem dissection. Lactating females were identified by assessing the presence of prominent teats and occupancy of either juvenile(s) or infant(s) in their burrows.

Juveniles and sub-adults of both sexes were recorded on sampling to assess recruitment to the population of *T. splendens* from reproductive animals sampled, but their blood and gonads were not included in the hormonal and histological analyses of this study because their reproductive systems were not fully developed. Categorisation of individuals into either juveniles or sub-adults was based on tooth eruption, based on the right maxillary molar tooth-row using the method described by Taylor *et al.*, (1985), with modification appropriate to the specific dental formula of *T. splendens* (1/1, 0/0, 0/0, 3/3; Katandukila *et al.*, submitted). Infants were classed by having either two cheek teeth or less, with no eruption of the third tooth, and had less fur present on their body than juveniles. Juveniles were classed as having two fully-developed cheek teeth and an erupting third molar, and still inhabited the maternal burrow. Sub-adults had three fully-developed teeth and occupied an independent burrow adjacent to maternal burrow systems with immature reproductive organs (i.e., small testes and ovaries).

Histology: Mass (g) of fixed gonads was recorded using a Sartorius scale (Zeiss, Germany), while length (mm) and width (mm) were measured using a pair of digital calipers (Mitutoyo American Corporation Aurora, Illinois). Testicular and ovarian volumes were evaluated using the formula for the volume of an ellipsoid: $V = 4/3\pi ab^2$ where a = half the maximum length and b = half the maximum width as detailed by Woodall & Skinner (1989). All measured gonads were sequentially dehydrated in increasing concentrations of ethanol baths and embedded in a cube of paraffin wax before being sectioned at a thickness of 7 µm using a rotary microtome (820 Spencer, American Optical, Scientific Instrument Division, Buffalo, New York, U.S.A.). Gonad sections were mounted on microscopic slides after being dipped in warm water mixed with gelatine as an adhesive. Mounted sections were dried in an oven at 36° C for about 72 hrs and subsequently stained using Ehrlich's haematoxylin and counter-stained with eosin (Drury & Wallington, 1967). Stained sections were covered by a slide cover-slip and glued with resin solution (Microscopy Entellan glue, Germany) as adhesive and then dried in an oven at 36° C for about 48 hrs.

Testicular histology: 30 randomly selected sections from the mid region of the testes were chosen in order to measure the diameter of seminiferous tubules with a light microscope (Diaplan, Ernst Leitz Wetzlar GmbH, Germany). Seminiferous tubules were then photographed at 10× magnification with a digital camera (Moticam 1000 1.3 M Pixel USB 2.0, Motic China Group, LTD., Xiamen, China) attached to a microscope. The diameters (µm) of 3600 seminiferous tubules were measured using Motic Images Plus 2.0ML (Motic China Group, Ltd., Xiamen, China). It is assumed that greater diameter of the seminiferous tubule indicate active testes with higher production of spermatozoa.

Ovarian histology: Ovarian sections were examined under a light microscope at 100×, 200× and 400×magnification (van Rensburg *et al.,* 2002; Hart *et al.,* 2006). Each ovary was sectioned in its entirety and sections were examined in consecutive order using a light microscope and checked for the presence of primordial, primary, secondary, tertiary and Graafian follicles as well as corpora lutea following Bloom & Fawcett (1962), van Rensburg *et al.* (2002) and Hart *et al.* (2006). Follicles of each developmental stage per section were counted, avoiding double counts by matching follicles split between consecutive sections using the method of Borgeest *et al.* (2004). Sections were photographed using a digital camera (Moticam 1000 1.3 M Pixel USB 2.0, Motic China Group, LTD., Xiamen, China) attached to a light microscope.

Radioimmunoassay: In the laboratory, plasma from female animals was assayed for progesterone and oestradiol-17β while plasma from males was assayed for testosterone using Coat-A-Count kits following the manufacturer's specifications. All hormone assays were validated for use in mole-rats as described in Bennett *et al.* (1994) and the total hormonal concentrations were determined using a gamma counter.

Progesterone: A volume of 100µL of plasma was dispensed in duplicate into polypropylene tubes following the Coat-A-Count progesterone kit (Progesterone Diagnostics Products Corporation, U.S.A.) procedures. The Coat-A-Count progesterone antiserum is highly specific for progesterone with a particularly low cross-reactivity (< 1%) to other naturally occurring steroids except in 5α-Pregnan-3, 20-dione (9%), 17α-Hydroxyprogesterone (3.4%), β-pregnan-3, 20-dione (3.2%) and 11-Deoxycorticosterone (2.2%). A blood plasma sample with a high concentration of progesterone was double-diluted using the assay buffer as a matrix 1:1 to 1:8 then assayed. The slopes of serial double dilution and standard curve were compared to check for

parallelism (Analysis of covariance (ANCOVA): $F = 2.54$; $n = 4$; $P > 0.05$) following a log-logit transformation of the data (Chard, 1987). The intra-assay coefficient of variation for the plasma pool was 2% whereas the inter-assay coefficient was 9.9% and sensitivity of the assay was 1.35 nmol/L.

Oestradiol -17β: Oestradiol -17β assays were performed as described by Herbst *et al*. (2004) using a Coat-a-Count oestradiol kit (Diagnostic Products Corporation). Cross-reactivity of the antibody to all naturally occurring steroids was 10% with oestrone, <5% with oestriol, oestrone-β-D-glucoronide, oestone-3-sulphate, d-equilenin, 17β-oestradiol-3-monosulphate, testosterone and androsterone. The assay was validated for plasma of *T. splendens* by testing the slope of the curve produced using serial dilutions of un-extracted mole-rat plasma obtained from a female with high oestradiol concentrations (over the range 1:1 to 1:32) against the standard curve. After logit-log transformation of the data (Chard, 1987), slopes of the lines were compared and found not to differ significantly from the reference preparation (ANCOVA: $F = 0.72$; $n = 6$; $P > 0.05$). The intra-assay coefficient of variation (CV) for repeated determinations of a quality control was 8.3% and sensitivity of the assay was 10pg/ml.

Testosterone: A volume of 50 µl of plasma was dispensed in duplicate into polypropylene tubes following Coat-A-Count Total Testosterone kit (Testosterone Diagnostics Products Corporation, U.S.A.) procedures. The Coat-A-Count Total Testosterone antiserum is highly specific for testosterone with cross-reactivity for 19-Nortestosterone (22%), 4-Estren-17-ol-3-one (20%) and 11-Ketotestosterone (16%) whereas 5α-Dihydrotestosterone, 19-Hydroxyandrostenedione and other steroids has low cross-reactivity of < 3.5%. A blood plasma sample with highest testosterone concentration was double diluted from 1:1 to 1:32 then assayed. The slopes of serial double-dilution and that of standard curve was compared to the double-dilutions to check for

parallelism (ANCOVA: F = 2.87; n = 6; P > 0.05) following a log-logit transformation of the data (Chard, 1987). The intra-assay coefficient of variation for the plasma pool was 3.2% whereas the inter-assay coefficient was 4.7% and sensitivity of the assay was 1.39 nmol/L.

Data analyses

The number of young produced from adult females per annum was determined using the following population equation from Jarvis (1973) with modification:

$$\text{L}= Y/T \text{ where } T = \frac{G+W}{1-(\frac{n}{N})}$$

Where L= litter(s) born to one adult female per annum
Y= days in the year (≈ 365 days)
T= duration of one breeding cycle
G= Gestation period (≈ 38.5 days)
W= time between birth and weaning (≈ 43 day)
$\frac{n}{N}$= Proportional of number of adult females in the sample neither pregnant nor lactating to the total number of females in the sample

Descriptive and statistical analyses were performed using Statistical Package for the Social Sciences (*SPSS*; Schneider, 1988) version 20 (*IBM® SPSS®* Statistics *20)* statistical packages and Excel 2007. The relationship between variables such as gonadal mass, volumes and rainfall were evaluated using Pearson's correlation analysis. Normality of data within each month was initially tested using Kolmogorov-Smirnov (KS) test (Lilliefors, 1967) and parametric tests were used in subsequent analyses after the normality of the data were confirmed. Monthly variation in hormone concentrations and gonadal metrics was investigated using analysis of variance (ANOVA) with Student Newman Keuls (S-N-K: testosterone, progesterone and gonadal metrics) and Tukey's honest significant difference (HSD: oestradiol-17β) to evaluate

significant differences *post hoc*. All descriptive statistics in the illustrations are presented as mean ± one standard deviation (SD) except for rainfall.

Results

The plasma and gonads of 120 adult males and 120 adult females were sampled, while 5 pups and 121 juveniles were sampled in maternal burrows. Among juveniles, 60 were females and 61 were males. Juvenile and infant samples increased from April to May, thereafter decreasing from June to July, none in February, August, and September, but increasing again from October to December. Samples of juveniles and infants were thus restricted to the mid and latter end of peaks of rains (Figure 1). 83 sub-adults were sampled of which 43 were females and 40 were males, they were sampled in their burrow systems which were adjacent to their maternal burrows. Of the 120 adult females, 32.97% (n = 40) were lactating whereas 16.20% (n = 19) were pregnant. Pregnancies increased from February to March and decreased from April, June and July then increased again from September to early November (Figure 2). Pregnancies in June and July were considered as late pregnancies as they occurred when many other females were lactating rather than pregnant. Post-mortem dissection of pregnant females revealed that three females were carrying 2 foetuses, while the remaining 16 were pregnant with a singleton (giving a mean litter size of 1.1 pups). With two conception periods separated by 166 days, fitting the parameters into the equation of Jarvis (1973), the mean reproductive output was calculated as 2.2 pups per female per year.

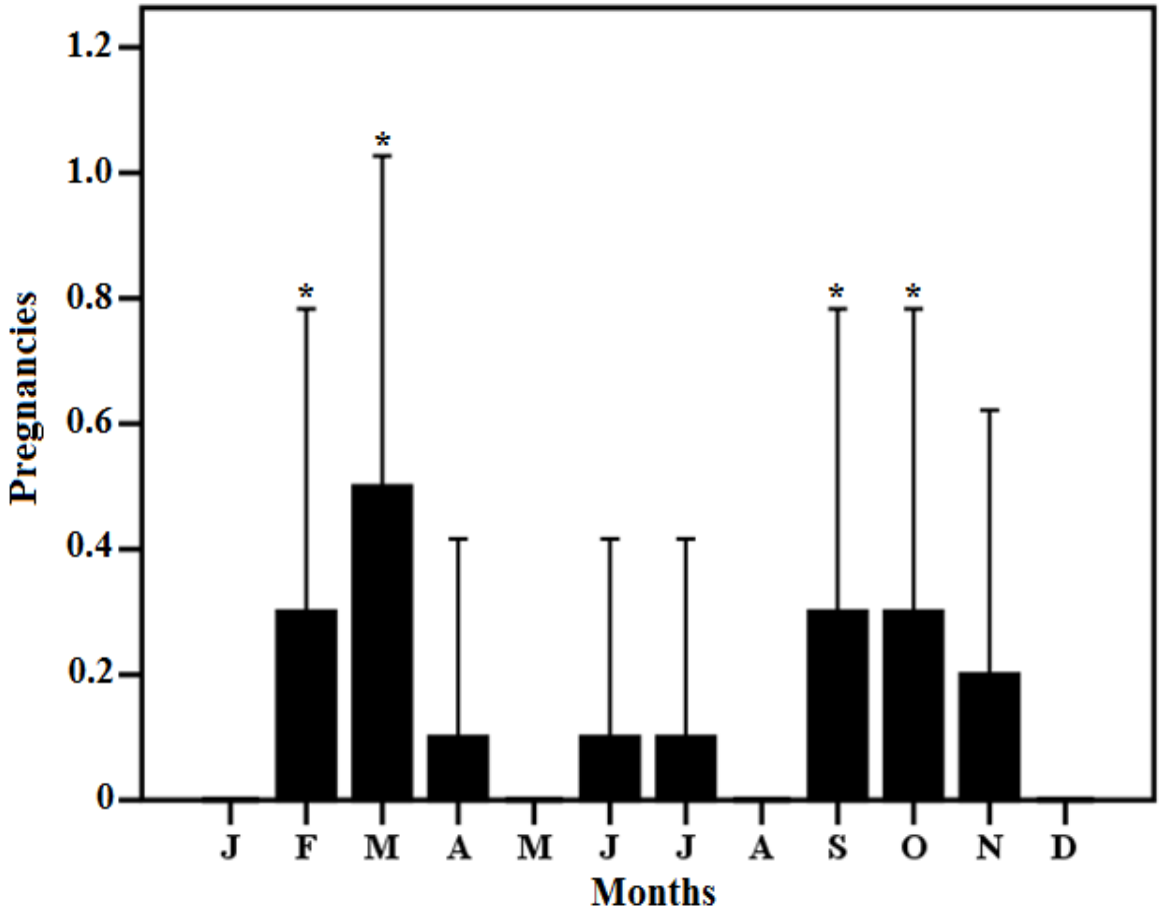

Figure 2: The mean ± SD number of pregnancies observed from post mortem examination in female *Tachyoryctes splendens* (*n* = 120) sampled from Tanzania from January to December 2011: * = Months having the highest number of pregnancies.

Histology

Testicular metrics: Testicular mass increased from February to March and decreased from April to August then increased again from September to October with the lightest testes mass observed in August (Figure 3). The mass of testes was significantly different between months ($F_{(11,108)}$ = 7.38; *n* = 120; *P* = 0.0001) with months of wet seasons having heavier testes than dry months

($F_{(1,118)}$ = 47.08; n = 120; P = 0.0001 = 120). The volumes of testes (n = 120) were highest in January but decreased from February to April and increased again in August and then decreased from September to November; however, volumes increased again in December (Figure 3). Testicular volumes were significantly higher during non-breeding than breeding months ($F_{(1,118)}$ = 12.34; n = 120; P = 0.010) inversely to testicular mass. Testicular mass increased in parallel with rainfall (Pearson's correlation: r = 0.78; n = 12; P = 0.001) in contrast to testicular volume (Pearson's correlation: r = -0.69; n = 12; P = 0.004).

The mean diameter of seminiferous tubules (n = 3600) was greatest in September, October and November; whereas the lowest mean diameter was in January and August (Figure 3). Variation in the diameter of seminiferous tubules between months was significant ($F_{(11,3588)}$ = 118.72; n = 3600; P = 0.0001) with breeding months (i.e., wet seasons) showing widened diameter of seminiferous tubules than non-breeding months (i.e., dry season: $F_{(1,3598)}$ = 185.77; n = 3600; P = 0.0001). The width of seminiferous tubules was positively correlated with peaks of rainfall (Pearson's correlation: r = 0.68; n = 12; P = 0.005).

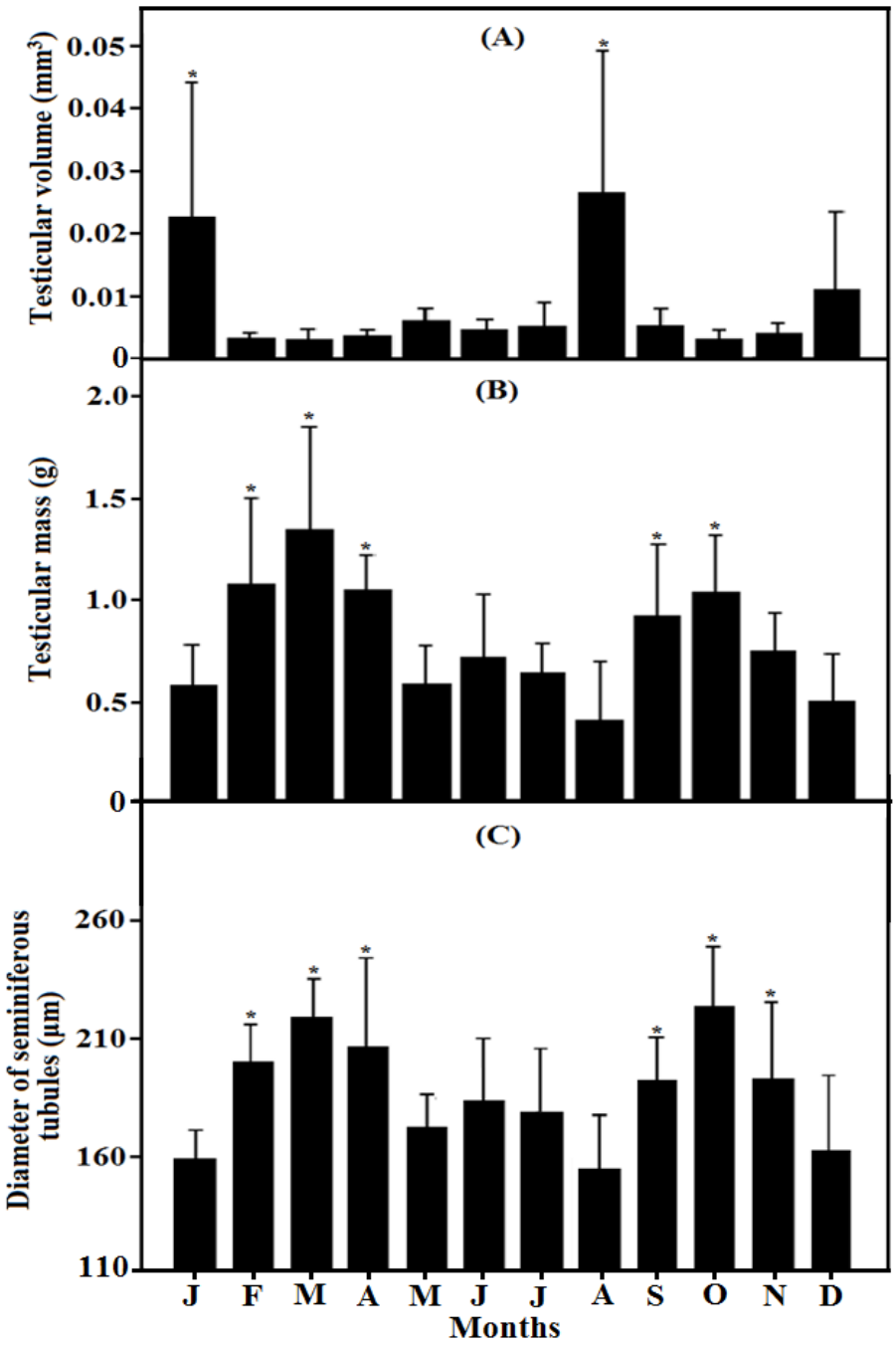

Figure 3: Monthly measurements (mean ± SD) of testicular volume (A), mass (B) and diameter of seminiferous tubules (C) in male *Tachyoryctes splendens* (*n* = 120) sampled in Tanzania from January to December 2011). * = Months of the greatest measurements

Ovarian metrics: The mass of the ovaries was greatest in February, March and April; and again in September, October and November with the lowest mean ovarian mass occurring in August (Figure 4). Ovarian mass varied between breeding and non-breeding months ($F_{(1,118)}$ = 9.88; n = 120; P = 0.002). Ovarian volumes were higher in January, March, August, November and December (Figure 4). Ovarian volumes varied significantly between months ($F_{(11,108)}$ = 3.76; n = 120; P = 0.0001) whereas non-breeding months (dry season) had highest volumes than breeding months. The mass of ovaries increased with increasing rainfall (Pearson's Correlation: r = 0.61; n = 12; P = 0.021) and was inversely proportional to ovarian volume (Pearson's correlation: r = -0.68; n = 12; P = 0.005).

Follicle development varied over the year, and as expected, primordial follicles were recorded in all ovaries (n = 120), with no significant variation in number over the year ($F_{(11,108)}$ = 0.94; n = 120; P = 0.36). The number of primary follicles was highest for *T. splendens* sampled in August and lowest in December. Secondary follicles showed the highest numbers in the animals sampled in January, March and April and lowest in May and August (Table 1); however, the number of tertiary follicles was highest in January, February and June with none recorded in August and in December. February and September had the greatest numbers of Graafian follicles while January, May and August had fewer Graafian follicles with none in November and in December (Figure 4). The number of corpora lutea were highest in March than in February, April, June and July with none observed in January, May, August and December. The maturation of Graafian follicles to corpora lutea matched the peaks of rainfall (Figures 1 and 4).

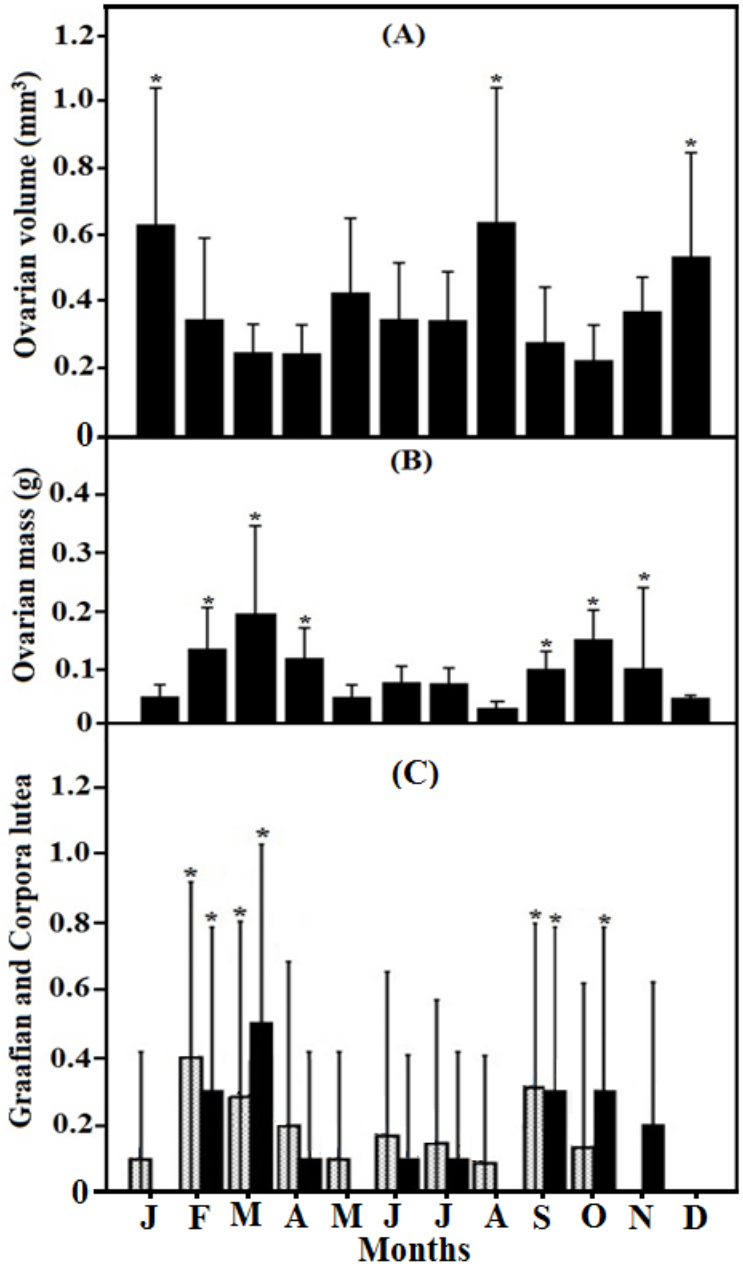

Figure 4: Monthly fluctuations (Mean ± SD) in ovarian volume (A), mass (B) and number of Graafian follicles and Corpora lutea (C) in female *Tachyoryctes splendens* (*n* = 120) sampled in Tanzania from January to December 2011; (C), hatched bars = Graafian follicles and solid black bars = Corpora lutea: * = Months of the greatest measurements.

Radioimmunoassay

Testosterone: The mean concentration of testosterone was highest in November and lowest in January and August (Figure 5). Concentration of testosterone varied significantly between months ($F_{(11,108)}$ = 8.15; n = 120; P = 0.001) whereas higher concentrations were observed in breeding than in non-breeding months. The concentration of testosterone in male *T. splendens* increased during the wet season and was lowest during the dry season with maxima coinciding with peaks of precipitation (see Figures 1 & 5). There was a positive correlation between rainfall and testosterone concentration (r= 0.58; n = 12; P = 0.03).

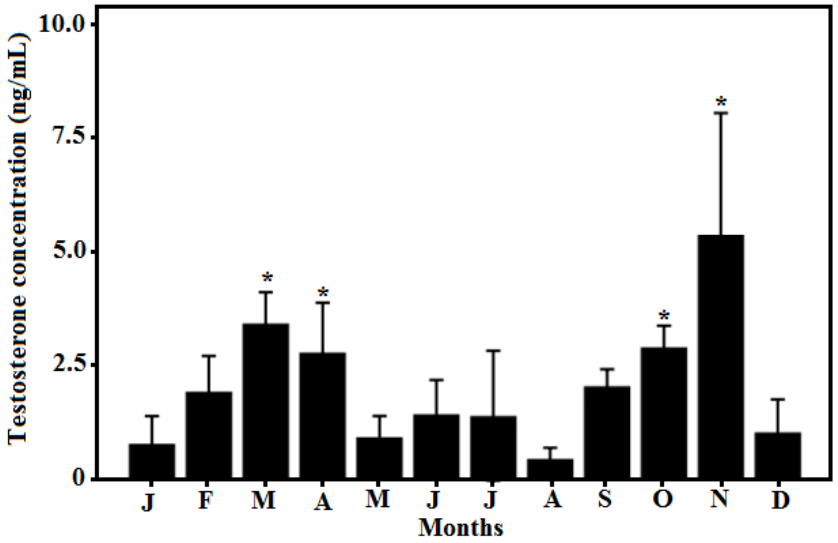

Figure 5: The concentration (mean ± SD) of testosterone (ng/mL) in male *Tachyoryctes splendens* (n = 120) sampled in Tanzania from January to December 2011: * = Months having the highest concentrations of testosterone.

Progesterone: The concentration of progesterone increased from February, March and April while the lowest occurred during the dry season in January, May and August (Figure 6). Progesterone concentration differed significantly between months ($F_{(11,108)}$ = 23.15; n = 120; P =

0.0001). There was a significant positive correlation between progesterone concentration and rainfall (r = 0.69; n = 12; P = 0.004) mirroring the periods with the highest number of pregnancies (Figure 2).

Oestradiol-17β: Oestradiol-17β concentration was significantly higher during the rains in February and March as well as in September and October with the lowest concentration observed in the dry month of May (Kruskal Wallis test: $H_{(11)}$ = 23.51; n = 10; K = 12; P = 0.02). Although these elevations in oestradiol-17β mirrored rainfall peaks, the correlation was not significantly different (r = 0.06; n = 12; P = 0.67; Fig. 6).

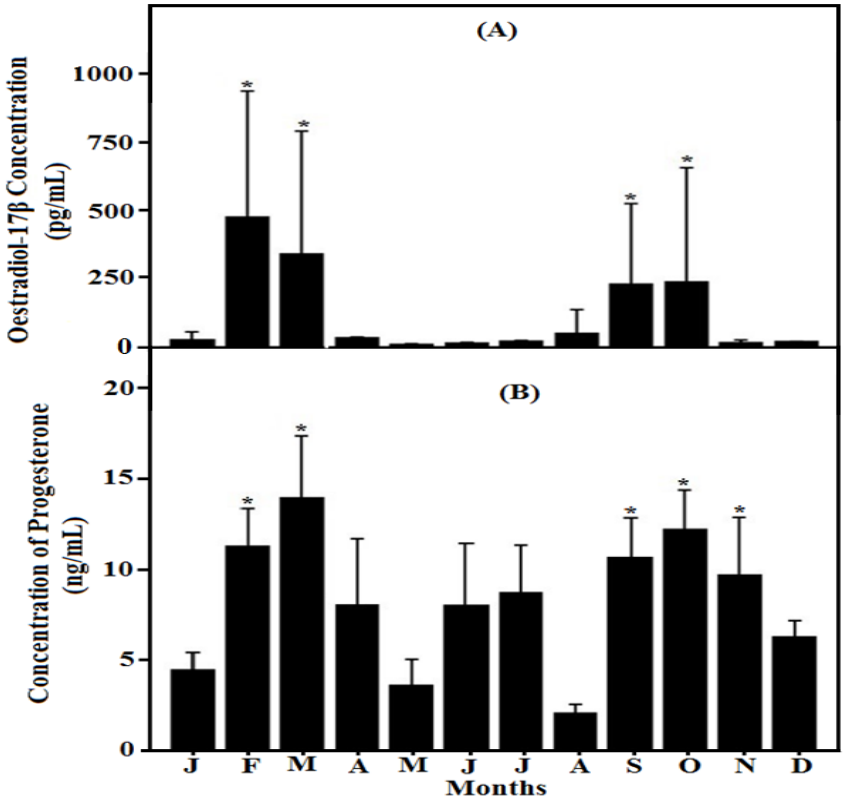

Figure 6: Monthly concentrations (mean ± SD) of Oestradiol-17β (A) and progesterone (B) in female *Tachyoryctes splendens* (n = 120) sampled in Tanzania from January to December 2011: * = Months having significant the highest concentrations.

Discussion

Post-mortem examination of a variety of reproductive parameters in *T. splendens* from Tanzania shows that it clearly synchronises its breeding with periods of precipitation. The reproductive tract morphometrics, gonadal histology and measurement of gonadal steroids revealed two distinct peaks of breeding that mirrored rainfall peaks at the study area. Plural occupancy of burrows generally only occurs either when adult male and female come together to mate or when females are caring for their young (Jarvis, 1973; Flynn 1990; Nowak, 1999). Coincident peaks of rainfall, oestradiol-17β and the presence of Graafian follicles in February, March, September and October indicate that female *T. splendens* ovulate during the onset of the wet months. Likewise, male *T. splendens* also show peaks in concentration of testosterone, have larger seminiferous tubule diameters and heavier testicular mass during the wet months. This suggests that it is during wet months that adult males and females prepare for mating, the raised testosterone levels coincident with the onset of the rains.

The mating period may therefore reflect increased territoriality and active mate searching as has been reported in other solitary subterranean mammals during breeding (see Bennett & Jarvis, 1988; Bennett *et al.*, 1991; Narins *et al.*, 1992). Furthermore, intra-male competition may be enhanced during this period as males actively seek female mates, as has been reported for the African mole-rat *Bathyergus suillus*, which may fight to death to acquire mates (Bennett & Faulkes, 2000; Hart *et al*., 2006). As with other subterranean rodents, adult *T. splendens* of both sexes synchronise the maturation of their reproductive cells to increase the chances of conception before the end of wet months so that when young are born food is abundant. This has also been reported for gophers, *Geomys* sp. (Wood, 1949; Villa-Cornejo & Engeman, 1995), the Middle Eastern mole-rat, *Spalax ehrenbergi* (Heth *et al.*, 1987; Rado *et al.*, 1987), African mole-rats,

Bathyergus suillus and *Georychus capensis* (Bennett & Jarvis, 1988; Sandwyk & Bennett, 2005; Hart *et al.,* 2006), *Bathyergus janetta* (Herbst *et al.,* 2004) and the tuco-tuco, *Ctenomys talarum* (Fanjul *et al.,* 2006). In such instances, rainfall results in soils becoming softer so that digging can occur and the caching and harvesting of both subterranean (geophytes) and sprouting above-ground vegetation can be utilised by the animals during these periods of resource abundance.

The presence of corpora lutea, higher circulating levels of progesterone and greater ovarian mass through the period when rainfall is high reflects the observations of pregnancies during this time (February-March and September-October). Although the majority of conceptions occurred at the onset and subsequent pregnancies were through the period of heavy rains, interestingly, some pregnancies were also recorded at the end of heavy rains (June and July months). These pregnancies possibly represent a period when an opportunistic chance to mate occurs as a consequence of lower constraints in mate acquisition, as suggested in other studies of solitary mammals (Emlen & Oring, 1977; Schulte-Hostedde *et al*., 2001; Isaac & Johnson, 2003). The late season pregnancies were validated by observations of a few new born pups at the end of June, early July and January. Although food resources might be more limiting during these months of the dry season (June, July and January) the adult females may cache food within their burrow and female *T. splendens* extend their burrows to increase foraging efficiency particularly when they have pups.

The estimation of 2 litters born per annum per adult female *T. splendens* corresponds well with the findings of Jarvis (1973). Jarvis (1973) documented two conception cycles of 173 days apart and the successful births of 2.1 litters per adult female, whereas in this study there is a similar cycle of 166 days with the birth of an average 2.2 litters per adult female a year. However, post-mortem observations revealed that three pregnant *T. splendens* had twin foetuses,

although pregnancy with a single foetus was by far the most common condition in pregnant females. Birth of pups during periods of good rainfall ensures the acquisition of quality food resources for lactating females and their young as the soil is softened and burrowing is made more efficient. Since precipitation is a major influence for the re-sprouting, regeneration and an overall increase in plants succession and subsequently increased vegetation cover (Bennie, 1991; Barber, 1995; Pregitzer & King, 2005), the numbers of pup births in *T. Splendens* over the periods of rainfall might be an ultimate factor for timing of their births. This observation is parallel to reports documented for bathyergids, geomyids, ctenomyids, Middle East spalacids and other arboreal small mammals (Vaughan, 1962; Andersen, 1978; Bronson, 1985; Lovegrove & Jarvis, 1986; Bennett & Jarvis, 1988; Ims, 1990; Bennett *et al.*, 1991; Malizia & Busch, 1997; Bennett & Faulkes, 2000; Herbst *et al.* 2004; Camin, 2010; Tassino & Passos, 2010).

Juvenile and/or sub-adult *T. splendens* were recorded throughout the year in this study demonstrating that although litters are most often a single pup, *T. splendens* has a steady recruitment rate, and that predation pressure may be low and competition for key resources such as food and space also low (Jarvis & Sale, 1971; Hickman, 1983; Kokiso & Bekele, 2008). To ensure young are adequately provisioned, female *T. splendens* construct a more complex burrow system (length range = 5.85–13.51 m; fractal dimension range = 1.16–1.29) compared to male burrows (length range = 4.51–8.50 m long; fractal dimension range = 1.14–1.24) (Katandukila *et al.*, submitted). The increased burrow length and fractal dimension ensures efficient foraging, and is similar to observations in females of other solitary subterranean rodents (Bennett & Faulkes, 2000; Herbst *et al.*, 2004; Sandwyk & Bennett, 2005; Hart *et al*., 2006).

In this study, the dispersal of sub-adult *T. spendens* peaked during rainy season months. This observation implies that the timing of dispersal of sub-adult *T. splendens* from their natal

burrow systems occurs at the onset of next rains to ensure that the offspring have maximal vegetation and food resources available for independence when setting up their own tunnel system. This breeding strategy and the associated dispersal from natal burrows have been well-documented for other solitary subterranean rodents (e.g., *Georychus capensis*: Jarvis & Bennett, 1991; *Bathyergus* species: Bennett & Faulkes, 2000; Herbst *et al.*, 2004). The dispersal of sub-adults during periods of rain when food is plentiful (Rado *et al.*, 1992; Le Galliard *et al.*, 2012) and the soil is workable (Williams & Cameron, 1984) enables sub-adult *T. splendens* to successfully establish their own independent burrow system (Bennett *et al.*, 1991; Rado *et al*., 1992; Selås 1997; Herbst *et al.*, 2004; Maher & Burger, 2011).

Breeding seasonally enables animals to time their reproductive events such that young are born when environmental conditions are favourable and they can maximise their reproductive success (Ims, 1990). Photoperiod plays a very important role as a proximate factor in triggering reproductive events in above-ground organisms with developed visual systems, living at higher latitudes (Lofts, 1970, Karsch *et al*., 1984; Nelson *et al*., 1998), but its role in the seasonality of breeding in visually regressed subterranean rodents is probably either limited or not important at all. Spalacid mole-rats are known to respond to photoperiod, such as effecting thermoregulation in *S. ehrenbergi* (Haim *et al*. 1983). African mole-rats also entrain their locomotory activity rhythms to different lighting schedules (Oosthuizen *et al.*, 2003; Hart *et al*., 2004; de Vries *et al*., 2008), but it is unlikely that it is important in reproduction. Rainfall is probably the most important environmental factor acting on subterranean mammals in that it can be detected underground since it softens the soil (if a sufficient amount falls), and brings about subsequent plant growth and flushes of vegetation (Dennis & Marsh, 1997). All solitary subterranean southern African bathyergid mole-rats studied to date time their breeding events with rainfall.

These occur in winter rainfall regions and as a consequence mating occurs in the winter months, with young being born in the spring when food is abundant and the soil easily workable. This also facilitates dispersal and construction of independent burrow systems (Bennett & Jarvis, 1988; Herbst *et al*., 2004; Hart *et al*., 2006). Interestingly, the silvery mole-rat, *Heliophobius argenteocinereus* from the tropics differs in that it breeds seasonally, but not synchronously with rainfall patterns (Šumbera *et al*., 2003). Likewise, *S. ehrenbergi* exhibits seasonal reproduction with breeding occurring in the winter months where they likely cue into rainfall (Heth *et al*., 1995).

Our findings from the reproductive hormone profiles, histological assessment of gonadal characteristics and field observations have all clearly revealed that *T. splendens* is a seasonal breeder having two periods of heightened reproductive activity within a year, which is consistent with the findings of studies undertaken in Kenyan by Jarvis (1973). *Tachyoryctes splendens* from Tanzania shows peaks of reproductive hormone concentrations, pregnancies, births and sub-adult dispersals concomitant with peaks of precipitation. This suggests that precipitation is the key factor which triggers their seasonal reproduction. The overall results in the present study confirm our earlier prediction that peaks in reproductive activity and pregnancy would coincide with the bimodal rainfall patterns characteristic in East Africa, namely the long wet season from February to early June and the shorter dry season in September, October and November.

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Chapter 5: Craniometrics

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Title: Craniometric analysis of ontogenetic variation and sexual dimorphism in the East African root rat, *Tachyoryctes splendens* (Rodentia: Spalacidae) from Tanzania

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Abstract

Ontogenetic variation and sexual dimorphism in the East African root rat, *Tachyoryctes splendens* (Rüppell 1835) from Tanzania, East Africa were examined using craniometric and body mass data. Ontogenetic variation was based on tooth eruption and wear in five relative age classes and revealed significant craniometric variation between them. Sexual dimorphism was evident in all three populations examined and revealed that males are larger in body size than females. The results of craniometric analysis of ontogenetic variation and sexual dimorphism in *T. splendens* are also reflected in the analysis of body mass data. These results suggest that male *T. splendens* invest energy into growth to facilitate male-male competition and sexual selection during the breeding season. Females however, show conservative growth with most energy being utilized for the excavation of complex burrow systems to increase foraging efficiency and allow for maternal care. Individuals of relative age classes 4 and 5 are reproductively mature and are thus capable of procreation.

Keywords: *Tachyoryctes splendens*, ageing, craniometrics, ontogeny, sexual dimorphism

Introduction

Ontogenetic refers to growth and development of organism from embryo to adulthood (Alberch et al. 1979; Badyaev 2000). As organism grows its body parts changes both on size and shape, and consequently results into ontogenetic variation from lower to higher age classes (Badyaev 2000). The ontogenetic variation has been assessed between age classes as all individuals within age class assumed to have equal ontogenetic variation (Zelditch 1988). Since it is difficult to

examine growth rate from embryonic stage, most of ontogenetic studies were conducted from birth to adult-hood whereas growth ceases (Zelditch 1988; Schultle-Hostedde et al. 2001; Schultle-Hostedde 2007; Chimimba et al. 2010).

Ontogenetic variation between age groups has been however reported to be concealed by sexual dimorphism (Badyaev 2002). The evidence of concealed ontogenetic variation as a result of sexual dimorphism was indicated by divergence of measurements between sexes within particular age class (Schultle-Hostedde et al. 2001). The nature and extent of sexual dimorphism reported be a result of different growth rate between sexes as a consequence of adaptation to reproductive or ecological roles (Badyaev 2000; Schultle-Hostedde et al. 2001). The sex-specific adaptation to attain certain size or shape for mate selection and/or dominance over other individuals of the same sex has been attributed to sexual dimorphism however some genetic traits are shared between sexes within population (Andersson 1994; Schutle-Hostedde et al. 2001; Badyaev 2002). Example: In polygynous mating system, males are larger than females as consequences of male-male competition to acquire more adult female mates which is reproductive potential with females utilize their energy to overcome reproduction costs related to pup caring (Trives 1972; Andersson 1994; Schutle-Hostedde *et al.* 2001). Ontogenetic characteristics and sexual dimorphism have been studied in number of animals including rodents such as Giant mole-rats (*Fukomys mechowii*: Chimimba et al. 2010), Cape dune mole-rats (*Bathyergus suillus*: Hart et al. 2007), social Highveld mole-rats (*Cryptomys hottentotus pretoriae*: van Rensburg et al., 2004), Tete veld rats (*Aethomys ineptus*: Abdel-Rahman *et al.*, 2009) and African Nile rat (*Arvicanthis niloticus* (Abdel-Rahman *et al.* 2009), Tuco-tuco (*Ctenomys* sp: Mora et al. 2003).

Studies of these rodents were based on analyses of craniometric data and body mass and reported the significant ontogenetic variation from sub-adult to adult-hood with presence of sexual dimorphism except but with absence of sexual dimorphism in *Cryptomys hottentotus pretoriae*. Despite ontogenetic variation and sexual dimorphism have been revealed on several species including subterranean rodent, none has been reported on spalacids including *Tachyoryctes splendens*. Although craniometrics have been analyses in *T. splendens* (Missone 1974; Corbert & Hill 1991; Beolchini & Corti 2004), all studies were focused on investigation of taxonomy of the species rather than assessment of ontogenetic and sexual dimorphism. As a consequence of missing information on literature, the present study assessed ontogenetic variation, nature and extent of sexual dimorphism. This study analysed craniometrics and body mass data based on age classes.

Since chronological age classes were unknown in a population structure of *T. splendens*, the present study also estimated age classes within population based on degree of maxillary molar eruption and wear as the method was used successful on estimation of relative age classes on small mammals given the difficult to determine absolute age class (van Rensburg et al. 2004; Hart et al. 2007). The determination of chronological age classes is a key towards evaluation of ontogenetic variation and sexual dimorphism. The prediction for the present study was presence of ontogenetic variation between age classes from young age classes to adulthood. The study also hypothesized the presence of sexual dimorphism being prominent from sub-adult individuals (i.e. transitional age class to adulthood) as a consequence of sex-specific adaptation to behavioural roles.

Materials and Methods

A total of 215 *T. splendens* (103 males and 112 females) were sampled from three localities in the Kilimanjaro region, Tanzania, East Africa (Figure 1). These include: Marangu (03˚16.54' S, 037˚32.49' E; 1450 m above sea level (a.s.l.); *n* = 75), Keni-Aleni (03˚13.53' S, 037˚35.37' E; 1236 m a.s.l.; *n* = 70) and Uru-Shimbwe (03˚25.32' S, 037˚16.21' E; 1415 m a.s.l.; *n* = 70). All sampled sites are classified as agri-ecological zones with highly fertile volcanic soils supporting a variety of food and cash crops (Kilimanjaro Regional Profile (KRP), 1998). Animal capture and care procedures followed the guidelines of the Animal Ethics Committee of the University of Pretoria (Ethics clearance number ECO47-10) and sampling permits from the Commission of Sciences and Technology Tanzania (COSTECH; Permit number 2011-44-NA-2010-204). Voucher specimen preparation followed standard protocols as described by van Rensburg et al., (2004) and deposited in the reference collection of the Department of Zoology and Wildlife Conservation, University of Dar es Salaam, Tanzania.

Figure 1: Map of the study site in Tanzania (with an insert of Africa: 1 = Uru-Shimbwe; 2 = Keni-Aleni; 3 = Marangu.

Tachyoryctes splendens has highly trophied hypsodont dentition and the dental formula of adult individuals is 1/1, 0/0, 0/0, 3/3 = 16. Based on this dentition, estimation of relative age of classes was determined by the degree of maxillary molar eruption and wear based on one side of the maxillary row as described by van Rensburg et al. (2004) and Hart et al. (2007). Consequently, all individuals were allocated into the following five relative age classes (Figure 2): 1) Age class 1: two erupted anterior maxillary molars; 2) Age class 2: two erupted anterior maxillary molars with the 3rd molar beginning to erupt; 3) Age class 3: two erupted maxillary molars, with the 3rd molar half-grown; 4) Age class 4: three fully-grown maxillary molars, with

worn crowns; and 5) Age class 5: three fully-grown maxillary molars, with extensively worn crowns.

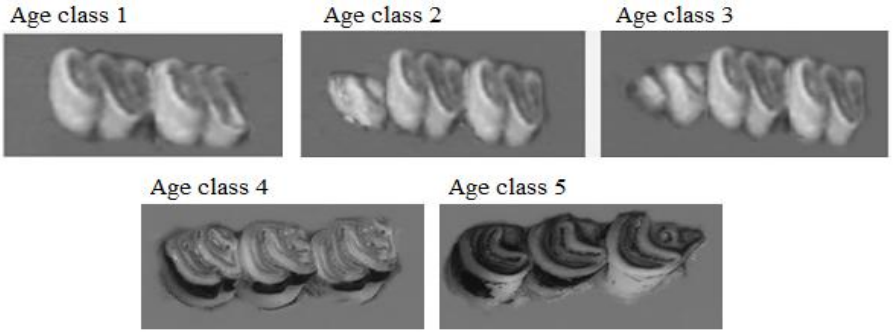


Figure 2: Relative age classes based on maxillary molar eruption and wear in *Tachyoryctes splendens* from Tanzania.

Ontogenetic variation and sexual dimorphism were assessed using 34 linear cranial measurements (Figure 3) following van Rensburg et al. (2004), Hart et al. (2007) and Chimimba et al. (2010) with slight modification. All linear cranial measurements were recorded to the nearest 0.05 mm using a pair of Mitutoyo digital calipers (Mitutoyo American Corporation, Aurora, Illinois, U.S.A.), while body mass was recorded to the nearest 0.05 g using a Pesola spring balance (Pesola dynamometer-5 kg/10 lbs, Switzerland).

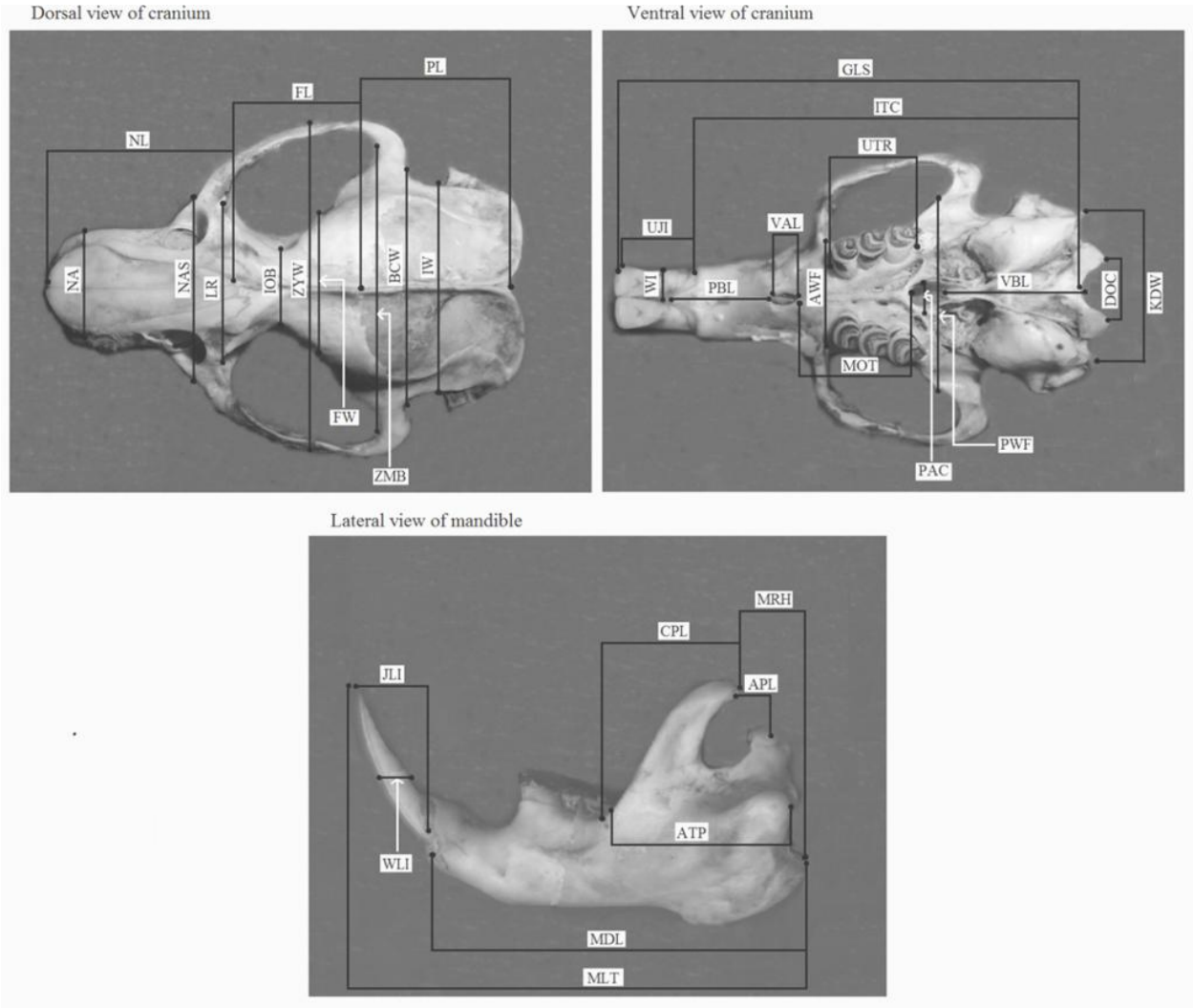


Figure 3: Linear cranial measurements used for craniometric analysis. Measurement abbreviations: 1) APL = Length of foramen articular facet; 2) ATP = Length of mandibular foramen-articular facet; 3) AWF = Ventral width from left to right of anterior frontal bone (at the anterior part of the 1st molar); 4) BCW = Brain case breadth; 5) CPL = Length of articular facet to edge of the 3rd molar; 6) DOC = Distance between right and left occipital condyle; 7) FL = Frontal bone length (sagittal border length of frontal bone); 8) FW = Frontal bone width; 9) GLS = Length from incisor to condyle (including incisor); 10) IOB = Least breadth of interorbital

constriction; 11) ITC = Length from incisor to condyle (excluding incisor); 12 IW = Interparietal bone width; 13) JLI = Lower incisor length; 14) KDW = Distance between left and right paroccipital process; 15 LR = Distance between left and right anterolateral corner of frontal bone; 16) MDL = Length of mandible from anterior to posterior part (excluding incisor); 17) MLT = Length of mandible from anterior to posterior part (including incisor); 18) MOT = Length of hard palate from posterior of incisive fossa to posterior nasal spine; 19) MRH = Height of mandible-ramus; 20) NA = Nasal width; 21) NAS = Nasal width at the middle; 22) NL = Nasal bone length; 23) PAC = Width of hard palate at point of constriction; 24) PBL = Length of premaxillar bone; 25) PL = Parietal bone length (sagittal border length of parietal bone); 26) PWF = Ventral width of posterior part of frontal bone (after 3rd molar); 27) UJI = Upper incisor length: 28) UTR = Crown length of maxillary teeth row; 29) VAL = Length of incisive fossa; 30) VBL = Length between vomer and condyle before foramen magnum; 31) WI = Width of upper incisor; 32) WLI = Width of lower incisor; 33) ZMB = Breadth between zygomatic processes of squamosals; and 34) ZYW = Width between outer margins of zygomatic arches.

Data analyses

Craniometric and body mass data from all populations of *T. splendens* were initially tested for normality and homogeneity of variances and subsequently subjected to a two-way analysis of variance (ANOVA; Zar, 1999) to test for ontogenetic variation, sexual dimorphism and the interaction between them. Where significant differences were detected, Student-Newman-Keuls

(SNK) *post hoc* test of ranked means following ANOVA assumptions (Sokal and Rohlf, 1981) was used to partition non-significant subsets of relative age classes ($P > 0.05$). The sum of squares (SSQ) from the derived two-way ANOVA table were used to partition potential sources of variation (Wonnacort & Wonnacort, 1972) with reference to relative age, sex, the interaction between them and error (= residual). The %SSQ was computed by dividing the SSQ associated with each source of variation by the total SSQ. All univariate analyses were based on algorithms in the *Statistical Package for the Social Sciences* (*SPSS*; Schneider, 1988) version 20 (IBM® SPSS® Statistics 20; Chicago, Illinois, USA).

Given the equivocal results of *post hoc* analysis of the univariate analyses between age classes, multivariate analyses were used to further evaluate the source of equivocal results of ontogenetic variation since analysis of sexes does not fit assumptions of post hoc. Consequently, the craniometric data of all three populations of *T. splendens* were also subjected to multivariate analyses using principal components analysis (PCA) of standardized variables and Unweighted Pair-Group Method of Arithmetic Averages (UPGMA) cluster analysis. The PCA was based on correlation coefficients among variables and UPGMA cluster analysis was based on Euclidean distances and correlation coefficients among groups (Sneath and Sokal, 1973. The distinct groups of sexes within age classes and overlap of sexes between age classes revealed on multivariate analyses subsequently resulted into analyses of data with sexes separately. All multivariate analyses were based on algorithms in *STATISTICA* version 10 (http://www.statsoft.com).

Results

Univariate analyses

As exemplified by the Marangu population which had the largest representative sample size (n = 75) and all the five relative age classes, both sexes showed an orderly increase in cranial dimensions with increasing age and males were larger in body size than females (Table 1).

Table 1: Standard descriptive statistics of cranial measurements (mm) of *Tachyoryctes splendens* from Marangu, Tanzania. SD = standard deviation; CV = coefficient of variation; f = female, m = male; sample size is indicated in parentheses. Cranial variables are defined and illustrated in Figure 3.

		Cranial measurements										
Age class	Statistics	APL	ATP	AWF	BCW	CPL	DOC	FL	FW	GLS	IOB	ITC
1f (2)	Mean	5.57	2.14	7.77	13.78	6.99	5.21	6.98	8.94	30.62	4.99	28.44
	SD	0.45	0.52	0.42	0.54	0.08	0.07	0.19	0.08	0.01	0.80	0.72
	CV	0.21	0.27	0.18	0.29	0.01	0.01	0.04	0.01	0.15	0.64	0.52
2f (8)	Mean	7.61	3.87	9.19	17.15	8.22	6.51	8.40	11.46	37.61	5.70	34.53
	SD	0.53	0.49	0.48	0.57	0.40	0.66	0.79	0.94	0.66	0.57	0.90
	CV	0.28	0.24	0.23	0.33	0.16	0.43	0.63	0.87	0.44	0.32	0.82
3f (8)	Mean	8.77	5.19	11.37	19.92	10.65	7.08	10.95	13.98	42.79	6.94	38.85
	SD	0.98	1.09	0.46	0.90	0.40	0.98	0.40	1.65	0.14	0.72	1.46
	CV	0.97	1.19	0.22	0.81	0.16	0.97	0.16	2.72	0.02	0.52	2.12
4f (18)	Mean	10.26	7.93	14.05	24.56	12.37	8.66	12.80	15.99	46.87	11.12	42.77
	SD	0.67	0.31	0.64	0.71	0.74	0.72	0.83	1.03	0.37	0.31	0.75
	CV	0.45	0.10	0.41	0.51	0.55	0.52	0.70	1.06	0.14	0.10	0.57
5f (2)	Mean	11.50	9.60	15.43	26.74	13.15	9.99	14.74	18.01	49.41	12.77	47.76
	SD	0.53	0.84	1.05	0.60	0.99	0.93	0.73	1.02	0.61	0.42	0.71
	CV	0.03	0.01	5.75	0.53	0.88	0.11	0.18	1.92	0.28	0.03	0.61

Females (continued)

		Cranial measurements										
Age class	Statistics	IW	JLI	KDW	LR	MDL	MLT	MOT	MRH	NA	NAS	NL
1f (2)	Mean	10.64	10.09	7.84	7.91	20.19	30.35	5.99	9.98	5.69	7.32	5.74
	SD	0.06	0.17	0.14	0.06	0.04	0.11	0.28	0.23	0.59	0.07	1.01
	CV	0.03	0.03	0.02	0.04	0.01	0.01	0.08	0.05	0.35	0.01	1.02
2f (8)	Mean	15.43	11.56	10.76	9.43	23.72	33.35	8.57	13.06	7.63	8.71	8.78
	SD	0.64	0.48	0.93	0.60	0.95	0.68	0.60	0.60	0.63	0.58	0.98
	CV	0.40	0.23	0.86	0.36	0.89	0.46	0.35	0.36	0.40	0.34	0.97
3f (8)	Mean	23.20	14.22	14.94	11.68	27.72	37.23	10.19	14.42	9.63	11.01	12.91
	SD	0.94	0.58	0.65	1.01	1.37	1.40	1.18	0.76	1.16	0.93	0.80
	CV	0.88	0.34	0.42	1.02	1.87	1.95	1.39	0.58	1.34	0.87	0.64
4f (18)	Mean	25.59	16.91	18.10	14.57	30.64	40.79	12.86	16.00	11.94	12.52	16.03
	SD	0.57	0.86	0.33	0.55	1.36	1.10	0.97	0.83	0.68	0.78	0.79
	CV	0.32	0.73	0.11	0.30	1.86	1.20	0.94	0.69	0.47	0.61	0.63
5f (2)	Mean	27.52	18.76	19.04	16.12	38.37	51.94	13.98	17.25	13.11	14.79	18.92
	SD	0.68	0.77	0.15	1.06	0.99	1.47	0.91	1.23	0.70	0.45	0.48
	CV	1.28	1.11	0.30	0.25	1.22	3.56	0.20	0.30	0.57	0.01	0.91

Females (continued)

Age class	Statistics	Cranial measurements											
		PAC	PBL	PL	PWF	UJI	UTR	VAL	VBL	WI	WLI	ZMB	ZYW
1f (2)	Mean	1.64	5.56	8.54	8.92	5.84	5.08	2.03	6.66	1.97	1.61	19.04	24.07
	SD	0.07	0.04	0.56	0.20	0.11	0.07	0.01	0.25	0.03	0.32	1.29	0.72
	CV	0.01	0.20	0.31	0.50	0.01	0.01	0.40	0.07	0.30	0.10	1.66	0.52
2f (8)	Mean	2.27	8.18	11.15	12.17	6.75	7.25	2.58	9.80	2.28	2.12	22.26	28.06
	SD	0.47	0.84	0.60	0.51	0.54	0.68	0.28	1.35	0.11	0.09	0.94	1.27
	CV	0.22	0.70	0.36	0.26	0.29	0.46	0.08	1.83	0.01	0.01	0.88	1.61
3f (8)	Mean	3.33	11.15	15.10	14.72	10.58	9.08	3.17	13.43	3.08	2.34	26.23	32.64
	SD	0.42	0.70	0.59	0.51	0.84	0.41	0.49	0.86	0.15	0.17	0.49	0.86
	CV	0.17	0.49	0.35	0.26	0.70	0.16	0.24	0.75	0.02	0.03	0.24	0.73
4f (18)	Mean	4.73	13.22	17.55	16.45	12.08	10.09	4.23	16.06	4.04	3.07	29.32	36.76
	SD	0.26	0.64	1.28	0.93	0.41	0.57	0.64	0.79	0.15	0.21	0.64	1.27
	CV	0.07	0.41	1.63	0.86	0.17	0.33	0.42	0.62	0.02	0.04	0.41	1.62
5f (2)	Mean	4.95	14.18	19.09	17.62	13.93	11.04	5.05	18.67	4.13	3.23	31.98	39.12
	SD	0.58	1.18	0.84	0.80	0.98	0.84	0.61	1.36	0.10	0.23	1.44	0.61
	CV	0.01	2.08	0.11	0.14	0.11	0.25	0.05	4.96	0.30	0.04	1.14	0.86

Age class	Statistics	Cranial measurements										
		APL	ATP	AWF	BCW	CPL	DOC	FL	FW	GLS	IOB	ITC
1m (3)	Mean	6.25	2.86	7.83	13.38	6.81	5.48	6.98	9.66	30.96	5.15	28.43
	SD	0.31	0.23	0.57	0.55	0.83	0.50	0.27	0.70	0.11	0.14	1.21
	CV	0.09	0.05	0.33	0.30	0.70	0.25	0.07	0.48	0.01	0.02	1.47
2m (4)	Mean	9.55	6.44	12.02	20.80	10.51	7.43	9.93	15.22	44.02	7.31	40.74
	SD	0.12	1.02	0.57	0.43	0.22	1.23	1.13	0.47	0.30	0.14	0.40
	CV	0.01	1.04	0.32	0.19	0.05	1.50	1.27	0.22	0.09	0.02	0.16
3m (15)	Mean	12.18	8.38	15.10	23.14	13.75	8.94	13.79	17.10	49.40	10.25	46.09
	SD	0.64	0.62	0.71	0.52	0.91	1.40	0.20	0.99	0.70	0.29	0.36
	CV	0.40	0.39	0.51	0.27	0.82	1.97	0.04	0.98	0.49	0.09	0.13
4m (14)	Mean	13.72	10.44	16.07	27.27	14.59	10.83	15.86	18.75	56.48	12.78	52.05
	SD	0.17	0.11	2.40	0.73	0.94	0.33	0.42	1.39	0.53	0.17	0.78
	CV	0.28	0.71	1.11	0.36	0.99	0.86	0.53	1.05	0.38	0.18	0.51
5m (1)	Mean	13.98	12.13	17.93	28.75	15.33	12.44	16.94	20.38	58.26	14.68	53.48

Males (continued)

Age class	Statistics	Cranial measurements										
		IW	JLI	KDW	LR	MDL	MLT	MOT	MRH	NA	NAS	NL
1m (3)	Mean	10.33	10.19	8.28	8.23	20.26	30.50	6.88	10.58	6.78	7.83	6.09
	SD	0.08	0.79	0.98	0.78	0.55	0.21	0.64	0.23	0.56	0.82	0.52
	CV	0.01	0.62	0.96	0.61	0.30	0.04	0.40	0.05	0.31	0.67	0.27
2m (4)	Mean	23.79	13.06	13.67	11.36	32.07	42.52	10.76	15.04	9.95	10.30	10.80
	SD	0.22	0.11	0.93	1.56	0.25	0.22	1.48	0.50	1.03	0.97	0.38
	CV	0.05	0.01	0.87	2.44	0.06	0.05	2.20	0.25	1.06	0.94	0.14
3m (15)	Mean	26.79	16.78	17.99	14.23	37.63	46.38	12.67	16.89	12.37	13.18	15.76
	SD	0.61	0.57	0.45	1.12	1.41	3.46	1.22	0.33	0.30	0.28	0.60
	CV	0.38	0.32	0.21	1.25	2.89	6.13	1.50	0.37	0.09	0.08	0.37
4m (14)	Mean	29.47	20.23	20.04	17.24	40.68	49.77	14.69	18.14	13.90	15.49	19.70
	SD	1.13	1.05	0.02	0.50	1.10	1.89	0.06	0.55	0.76	0.12	0.95
	CV	0.46	0.59	0.02	1.13	0.99	2.17	0.83	1.52	0.49	0.21	0.23
5m (1)	Mean	31.30	21.16	21.33	18.84	44.10	55.28	15.65	19.29	16.18	16.96	21.55

Males (continued)

Age class	Statistics	Cranial measurements											
		PAC	PBL	PL	PWF	UJI	UTR	VAL	VBL	WI	WLI	ZMB	ZYW
1m (3)	Mean	1.68	5.44	9.05	9.48	6.03	5.09	2.13	6.59	2.18	1.63	20.02	25.84
	SD	0.19	0.15	0.31	0.45	0.06	0.19	0.08	0.36	0.29	0.07	0.73	0.56
	CV	0.04	0.02	0.10	0.20	0.20	0.04	0.01	0.13	0.09	0.01	0.53	0.32
2m (4)	Mean	2.32	10.41	13.75	13.84	10.68	8.43	3.40	14.09	3.07	2.91	23.99	32.31
	SD	0.20	1.82	0.35	0.74	0.22	0.39	0.12	1.23	0.08	0.13	1.85	1.59
	CV	0.04	3.32	0.13	0.55	0.05	0.16	0.02	1.51	0.01	0.02	3.42	2.53
3m (15)	Mean	4.92	13.77	17.87	17.24	13.19	11.42	5.52	16.79	4.59	3.99	30.93	36.14
	SD	0.46	0.80	0.48	1.14	0.41	0.61	0.49	1.29	0.15	0.21	0.93	0.57
	CV	0.21	0.65	0.23	1.31	0.17	0.37	0.24	1.67	0.02	0.04	0.87	0.33
4m (14)	Mean	6.19	15.21	20.98	18.85	15.64	12.29	6.63	19.94	5.76	4.83	34.49	39.63
	SD	0.09	1.44	0.33	0.37	0.33	0.50	0.22	2.23	0.04	0.20	1.07	0.93
	CV	0.34	1.39	0.70	0.65	0.97	0.70	0.38	1.85	0.01	0.05	2.07	0.37
5m (1)	Mean	6.63	16.77	21.17	20.20	16.31	13.20	7.61	20.97	5.94	4.86	37.09	42.68

As exemplified by the Marangu population, the ANOVA results of the three populations showed that although most measurements varied significantly with relative age classes, sex and the interaction between the two, the largest *F*-values were associated with age, followed by sex, the residual component and the interaction between relative age and sex (Table 2). Variance partitioning in the three populations using %SSQ showed that ontogenetic variation contributed more to the potential source of variation followed by sex, the residual component and the interaction between age and sex [Age: Marangu – Mean %SSQ = 53.22% (range = 33.96–73.97%); Keni-Aleni – Mean %SSQ = 53.23% (range = 34.04–74.04%); Uru-Shimbwe – Mean %SSQ = 53.19% (range = 33.98–73.99); Sex: Marangu – Mean %SSQ = 18.34% (range = 2.84–38.41%); Keni-Aleni – Mean %SSQ = 18.37% (range = 2.87–38.39%); Uru-Shimbwe = 18.37% (range = 2.85–38.38); Sex/Age interaction: Marangu – Mean %SSQ = 11.27% (range = 1.77–29.89%; Keni-Aleni – Mean %SSQ = 11.28% (range = 1.79–29.89%); Uru-Shimbwe = 11.30% (range = 1.80–29.92)%]; and Error component (= residual): Marangu – Mean %SSQ = 17.17% (range = 3.94–36.01%; Keni-Aleni – Mean %SSQ = 17.12% (range = 3.98–36.01%); Uru-Shimbwe = 17.13% (range = 3.95–35.99%). The %SSQ of the residual component in all three populations suggests that, apart from age variation, sexual dimorphism and the interaction between the two, there are also other factors that may be influencing the nature and extent of ontogenetic variation and sexual dimorphism in *T. splendens* from Tanzania.

Table 2: Two-way analysis of variance (ANOVA) and percent sum of squares (%SSQ) of relative age classes (1-5) based on the degree of maxillary molar eruption and wear in male and female *Tachyoryctes splendens* from Marangu, Tanzania. * = $P < 0.05$; ** = $P < 0.01$; ***= $P < 0.001$. Cranial measurements are defined and illustrated in Fig. 3.

Cranial measurements	*F*-values			%SSQ			
	Age (A)	Sexes (S)	A x S	A	S	A x S	Error
APL	37.90***	16.06***	1.99	52.91	28.44	3.35	15.31
ATP	46.11***	9.41**	15.26***	50.97	15.94	20.59	12.50
AWF	75.01***	36.04***	6.09**	59.89	16.45	8.79	14.87
BCW	118.78***	60.13***	37.69***	52.45	20.03	15.74	11.79
CPL	39.97***	11.89**	15.95***	44.62	15.41	24.03	15.94
DOC	17.96***	7.29**	2.10	43.04	22.29	1.77	32.90
FL	91.14***	2.27	5.58**	60.56	7.99	19.85	11.60
FW	33.94***	11.34**	3.36*	58.52	10.55	5.92	25.01
GLS	130.85***	37.45***	19.14***	65.18	24.50	6.26	4.06
IOB	82.17***	22.99***	55.03***	33.96	21.51	29.89	14.64
ITC	171.95***	69.96***	11.90***	50.83	38.38	3.27	7.52
IW	121.02***	38.12***	22.17***	69.96	15.24	8.68	6.13
JLI	54.19***	19.89***	2.29	53.08	19.06	3.58	24.28
KDW	149.19***	48.06***	8.95***	73.97	14.28	5.23	6.52
LR	53.71***	10.06**	8.06***	49.11	12.60	10.50	27.80
MDL	97.85***	35.93***	11.48***	54.98	26.85	7.27	10.90
MLT	70.81***	26.24***	3.81*	51.58	34.89	9.51	4.02
MOT	42.58***	19.54***	0.97	48.94	13.81	7.31	29.94
MRH	21.85***	9.16**	5.06**	49.34	13.84	10.97	25.85
NA	86.13***	16.01***	6.31***	55.83	25.13	7.81	11.24
NAS	84.03***	42.96***	1.31	65.97	17.71	3.15	13.17
NL	90.95***	64.30***	10.27***	42.67	37.28	16.12	3.94
PAC	38.05***	14.25***	1.38	41.91	23.36	10.82	23.91
PBL	58.90***	13.02***	6.98***	61.12	10.91	8.34	19.63
PL	87.41***	10.93**	3.93*	71.69	4.35	2.44	21.52
PWF	139.16***	31.53***	4.13**	67.51	13.11	5.84	13.55
UJI	84.83***	7.37**	7.03***	66.26	9.50	8.56	15.69
UTR	49.11***	1.85	15.37***	47.03	6.86	26.40	19.71
VAL	42.94***	2.60	18.97***	42.99	2.86	27.79	26.37
VBL	56.59***	36.11***	5.01**	41.62	28.06	9.58	20.75
WI	20.13***	2.12	15.04***	39.99	3.12	20.88	36.01
WLI	87.14***	4.56*	5.13**	47.38	9.34	19.12	24.16
ZMB	118.04***	23.96***	12.04***	49.10	36.71	6.35	7.84
ZYW	88.12***	19.86***	6.07**	44.51	23.34	7.44	24.71
Mean				53.22	18.34	11.27	17.17

As exemplified by the Marangu population, the SNK tests are equivocal. These tests showed five major patterns (Table 3). The first pattern involved 33 out of 34 measurements that showed the separation of relative age class 1 from other relative age classes except for one measurement (IOB) which showed an overlap of relative age classes 1 and 2. The second pattern involved 16 out of the 34 measurements that grouped relative age classes 2 and 3 in the same non-significant subset. The third pattern involved 25 out of 34 measurements that grouped relative age classes 3 and 4 into the same non-significant subset. The fourth pattern involved 27 out of 34 measurements that showed overlaps of relative age classes 4 and 5. The fifth pattern involved seven out of 34 measurements that showed all relative age classes to be significantly different from each other.

Table 3: Student-Newman-Keuls (SNK) *post hoc* tests for cranial measurements (mm) of age classes 1-5 of *Tachyoryctes splendens* from Marangu, Tanzania. Results are presented as mean ± 1 standard deviation. S = all means are significantly different; underlined = non-significant subsets of relative age classes ($P > 0.05$).The sample size is shown in parentheses. Cranial measurements are defined and illustrated in Figure 3.

Cranial measurements	Age classes				
	1 (5)	2 (12)	3 (23)	4 (32)	5 (3)
APL	4.63 ± 0.32	8.72 ± 1.37	10.60 ± 2.41	12.03 ± 2.44	12.74 ± 1.68
ATP	1.97 ± 0.27	5.30 ± 1.82	6.91 ± 2.26	9.28 ± 1.77	10.88 ± 1.78
AWF	6.76 ± 0.38	10.75 ± 2.00	13.36 ± 2.64	15.06 ± 1.43	16.69 ± 1.76
BCW	12.68 ± 0.28	19.12 ± 2.58	21.66 ± 2.28	25.94 ± 1.91	27.76 ± 1.42
			S		
CPL	4.74 ± 0.38	9.51 ± 1.62	12.33 ± 2.19	13.53 ± 1.57	14.26 ± 1.54
DOC	4.30 ± 0.26	7.11 ± 0.65	8.14 ± 1.32	9.88 ± 1.53	11.23 ± 1.73
FL	6.73 ± 0.35	9.31 ± 1.08	12.50 ± 2.01	14.46 ± 2.16	15.85 ± 1.55
FW	8.60 ± 0.08	13.48 ± 2.66	15.63 ± 2.20	17.43 ± 1.95	19.21 ± 1.67
GLS	30.29 ± 0.24	40.84 ± 4.53	45.15 ± 4.68	49.15 ± 4.59	52.16 ± 4.23
			S		

Table 3 (continued)

Cranial measurements	Age classes				
	1 (5)	2 (12)	3 (23)	4 (32)	5 (3)
IOB	4.02 ± 0.19	6.65 ± 1.14	8.73 ± 2.34	11.96 ± 1.17	13.73 ± 1.35
ITC	26.83 ± 0.44	37.68 ± 4.39	42.51 ± 4.12 S	45.48 ± 5.56	49.95 ± 4.04
IW	10.89 ± 0.35	19.78 ± 3.86	25.13 ± 2.54	26.58 ± 2.74	29.41 ± 2.67
JLI	9.98 ± 0.44	12.48 ± 1.06	15.64 ± 1.81	18.67 ± 2.35	19.96 ± 1.69
KDW	7.41 ± 0.39	12.37 ± 2.06	16.60 ± 2.16	19.10 ± 1.37	20.19 ± 1.62
LR	7.27 ± 0.22	10.56 ± 1.36	13.06 ± 1.81	16.06 ± 1.89	17.48 ± 1.92
MDL	19.48 ± 0.40	27.94 ± 5.90	32.71 ± 6.01 S	35.73 ± 6.10	41.56 ± 4.15
MLT	30.28 ± 0.32	37.98 ± 6.48	41.85 ± 6.47 S	45.28 ± 5.35	49.91 ± 2.36
MOT	6.02 ± 0.37	9.83 ± 1.55	11.47 ± 1.76	13.82 ± 1.29	14.82 ± 1.18
MRH	9.89 ± 0.41	14.22 ± 1.40	15.79 ± 1.75	17.16 ± 1.51	18.28 ± 1.44
NA	4.99 ± 0.42	8.96 ± 1.64	11.14 ± 1.94	13.00 ± 1.38	14.65 ± 2.17
NAS	7.28 ± 0.36	9.66 ± 1.12	12.10 ± 1.54	14.02 ± 2.10	15.87 ± 1.53
NL	4.97 ± 0.32	9.96 ± 1.43	14.47 ± 2.02	17.96 ± 2.59	20.25 ± 1.86
PAC	1.36 ± 0.31	2.59 ± 0.39	3.17 ± 1.13	4.43 ± 1.03	5.01 ± 1.19
PBL	5.20 ± 0.34	9.46 ± 1.58	12.59 ± 1.85	14.20 ± 1.40	15.51 ± 1.83
PL	8.68 ± 0.47	12.59 ± 1.84	16.61 ± 1.96	19.42 ± 2.42	20.16 ± 1.47
PWF	8.72 ± 0.42	13.15 ± 1.18	16.11 ± 1.78	17.66 ± 1.70	18.94 ± 1.82
UJI	5.68 ± 0.47	8.86 ± 2.78	12.01 ± 1.85	14.00 ± 2.52	15.15 ± 1.68
UTR	5.21 ± 0.11	7.83 ± 0.83	9.27 ± 1.66	10.24 ± 1.55	10.49 ± 1.51
VAL	1.96 ± 0.25	2.99 ± 0.58	3.36 ± 1.66	4.41 ± 1.69	4.63 ± 1.81
VBL	5.93 ± 0.37	12.11 ± 3.03	15.24 ± 2.38	18.15 ± 2.74	19.85 ± 1.62
WI	2.08 ± 0.15	2.88 ± 0.77	3.34 ± 1.40	3.91 ± 1.21	4.15 ± 1.28
WLI	1.50 ± 0.13	2.57 ± 0.56	3.13 ± 1.17	3.44 ± 1.24	3.80 ± 1.15
ZMB	17.76 ± 0.37	23.27 ± 1.22	28.71 ± 3.33 S	32.03 ± 3.65	35.07 ± 3.61
ZYW	23.52 ± 0.32	30.33 ± 3.01	34.52 ± 2.48 S	38.40 ± 2.03	41.43 ± 2.51

As exemplified by the Marangu population (Figure 4), a plot of the first two principal components of the PCA confirmed the unequivocal separation of the sexes within relative age classes and the partial separation of relative age classes. Similar to the SNK tests, the PCA revealed the separation of relative age class 1 from all other age classes with overlaps of age classes 2 and 3, 3 and 4 and 4 and 5. This suggests that although there are age class differences within all three populations of *T. splendens*, the relative age groupings are confounded by sexual dimorphism.

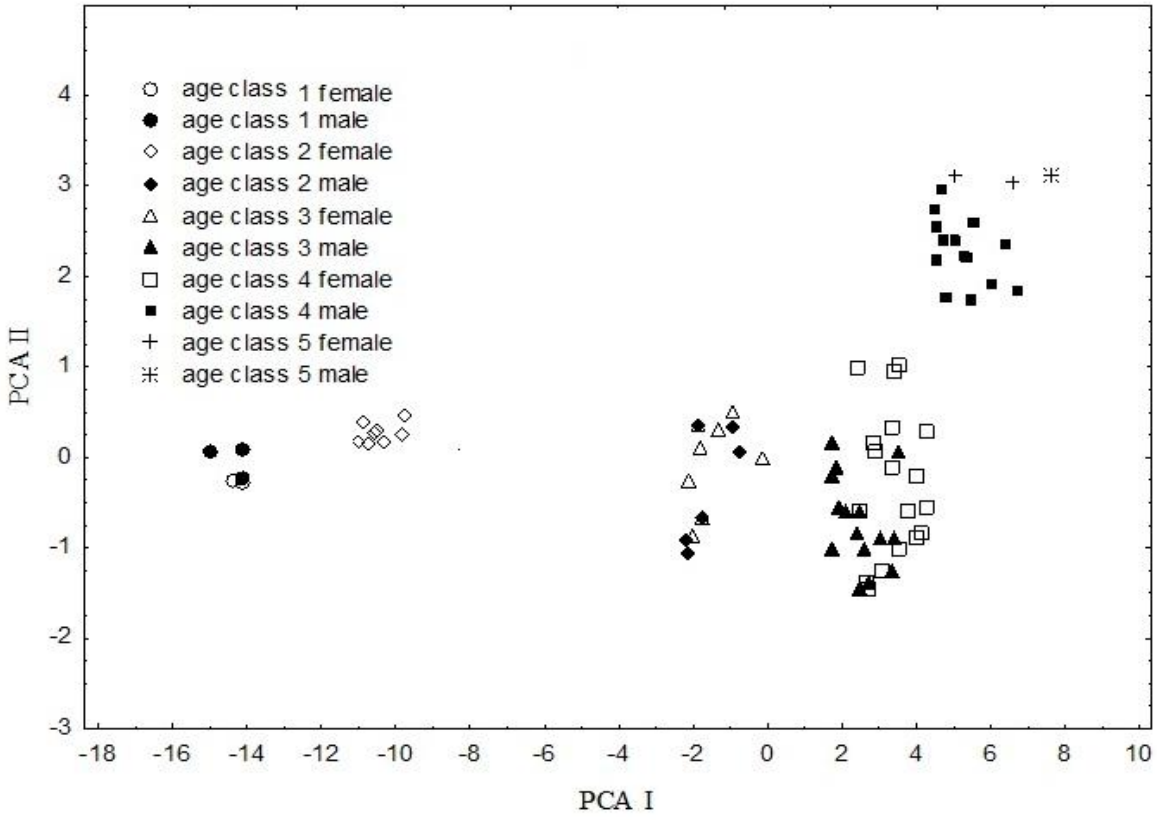
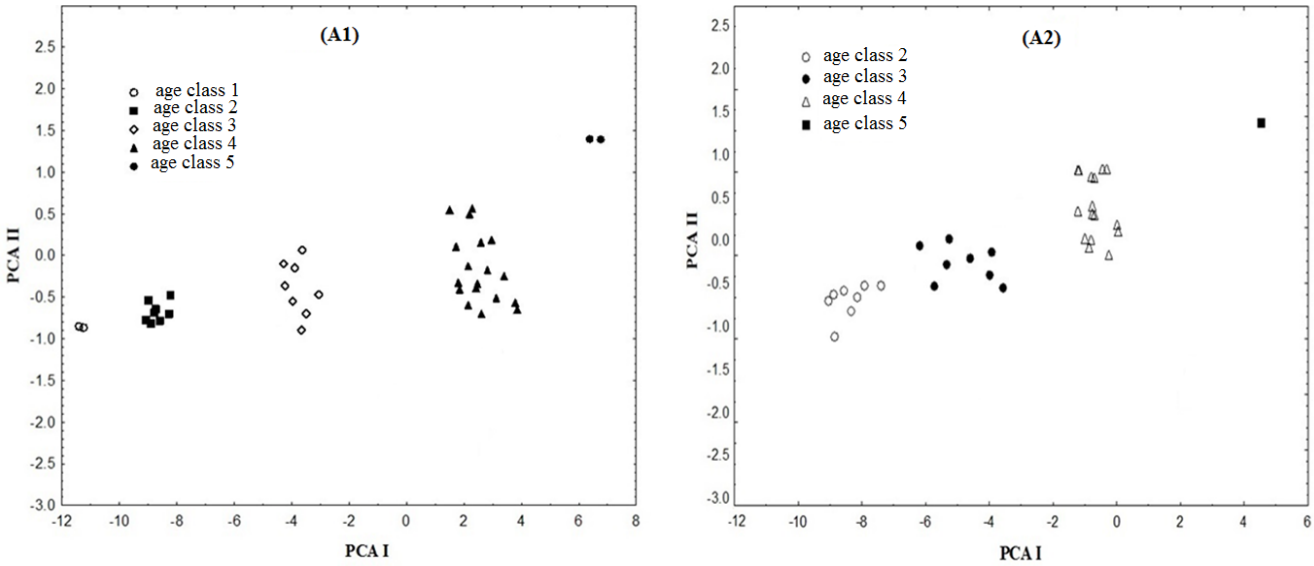


Figure 4: The first two principal components from a principal components analysis (PCA) of cranial measurements from male and female *Tachyoryctes splendens* of age classes 1-5 from Marangu, Tanzania.

The equivocal placement of relative age class groupings in the PCA and UPGMA cluster analysis necessitated separate PCA and cluster analyses of the sexes from the three populations of *T. splendens*. Both the PCA (Figure 5) and cluster analysis (Figure 6) of the three populations of females (A1, A2 & A3) and males (B1, B2 & B3) revealed a separation of all the relative age classes 1–5 that reflect increasing body size from relative age classes 1–5. All these results are also reflected in subsequent separate ANOVAs, SNKs and %SSQs of the sexes (not illustrated).



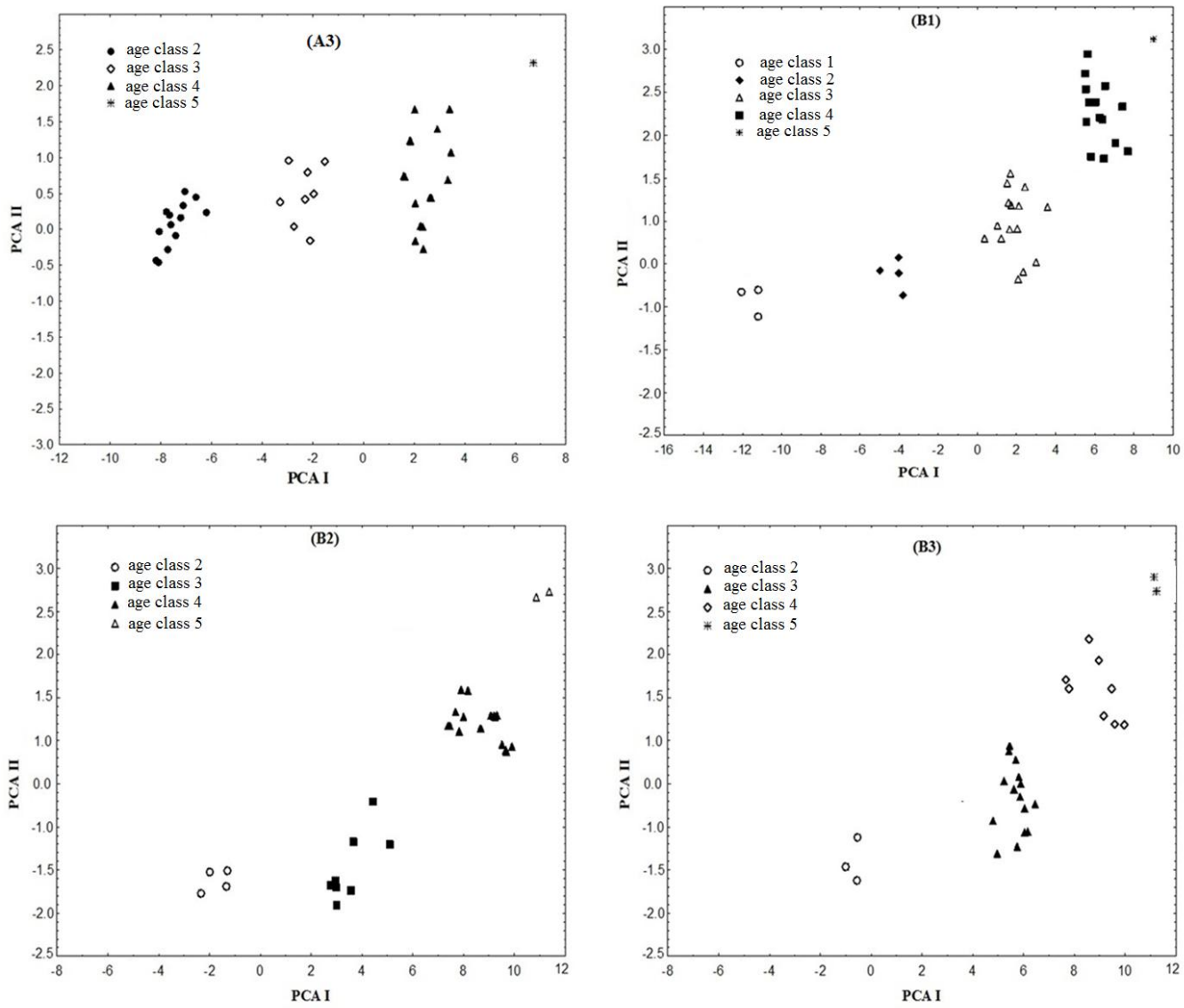
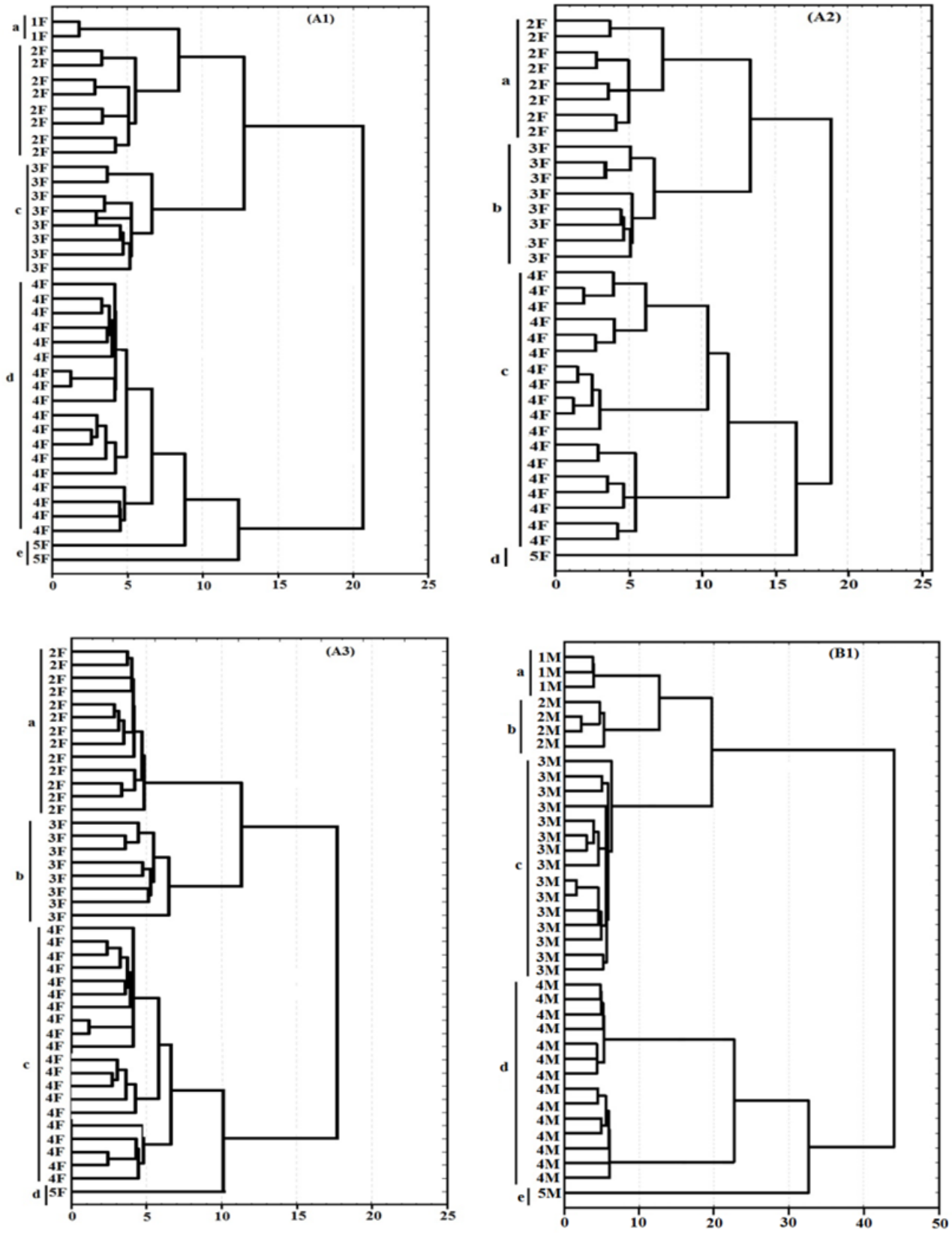


Figure 5: The first two principal components from a principal components analysis (PCA) of cranial measurements of female (A1-3) and male (B1-3) *Tachyoryctes splendens* of age classes 1-5 from Marangu (A1: males & B1: females), Keni-Aleni (A2: males & B2: females) and Uru-Shimbwe (A3: males & B3: females), Tanzania.



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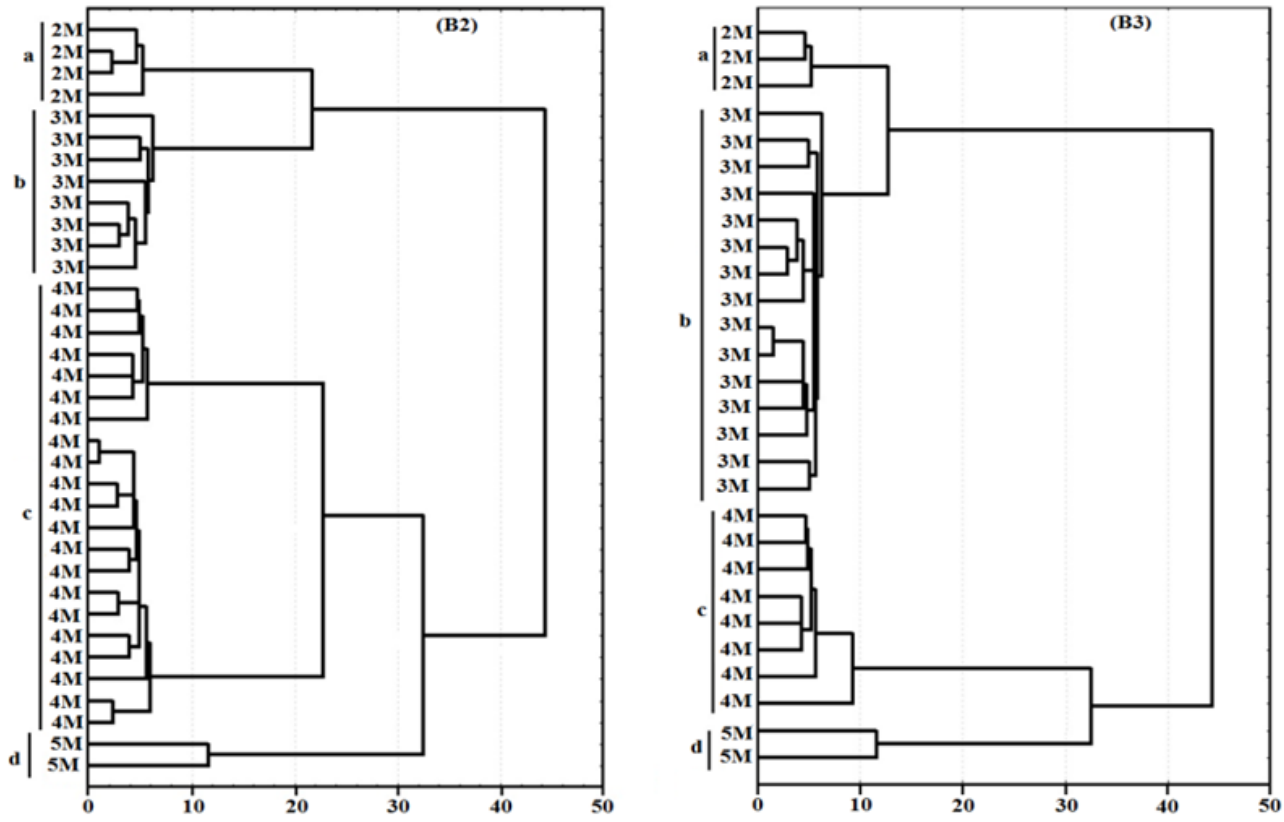


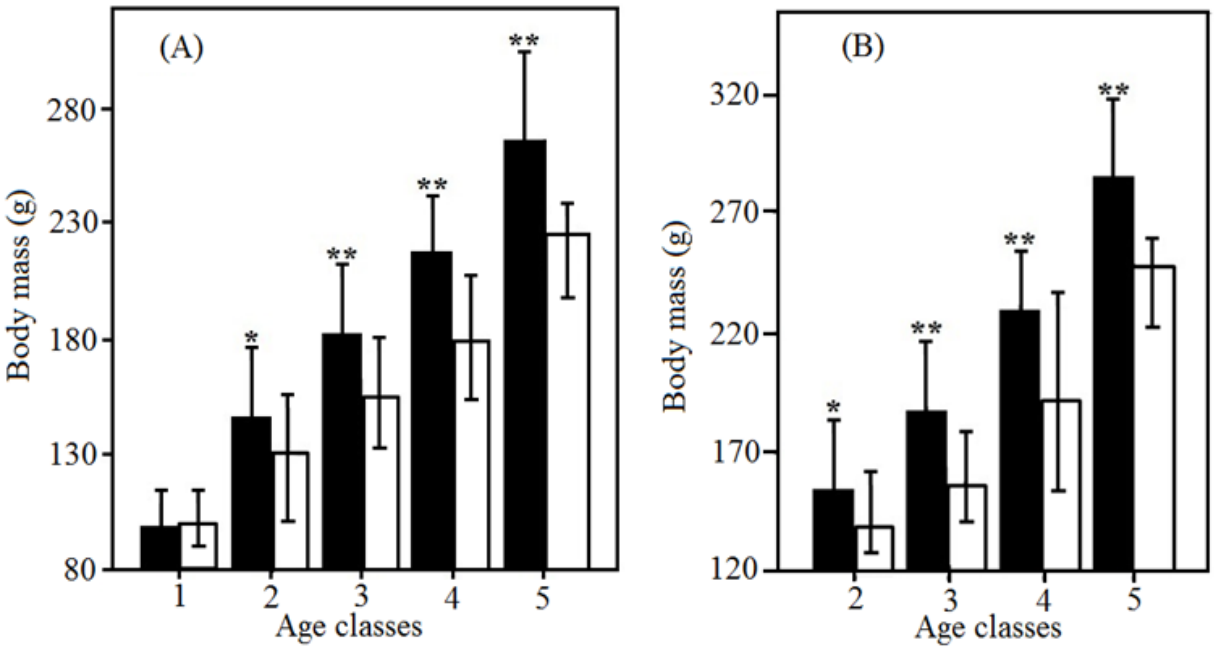
Figure 6: A Euclidean distance phenogram from an Un-weighted Pair-group Method using Arithmetic Averages (UPGMA) cluster analysis of cranial measurements of male (M) and female (F) *Tachyoryctes splendens* of age classes 1-5 (Sub- clusters a–e) from Marangu (A1: females & B1: males), Keni-Aleni (A2: females & B2: males) and Uru-Shimbwe (A3: females & B3: males), Tanzania.

The first principal components axis (PCA I) from the PCA explained > 88% of the total variance while PCA II explained < 5% of the total variance in all males and females of the three populations (Table 4). Most cranial measurements on PCA I in all males and females of the three populations loaded relatively highly than those on PCA II which collectively only has seven cranial measurements (APL, CPL, DOC, PAC, UTR, VAL & WI) loading relatively highly in all the analysis of males and females from the three populations. These relatively high loadings on PCA I suggest that cranial size rather than shape is an important component in age variation in *T. splendens* from Tanzania.

Table 4: Relative loadings of cranial measurements of the first two principal components axes (I and II) from a principal components analysis (PCA) of *Tachyoryctes splendens* from Marangu (age classes 1–5), Keni-Aleni and Uru-Shimbwe (age classes 2-5), Tanzania. Marangu: A1 = males & B1 = females; Keni-Aleni: A2 = males & B2 = females; and Uru-Shimbwe: A3 = males & B3 = females. Cranial measurements are defined and illustrated in Figure 3.

	Marangu				Keni-Aleni				Uru-Shimbwe			
Cranial measurements	PCA I (A1)	PCA II (A1)	PCA I (B1)	PCA II (B1)	PCA I (A2)	PCA II (A2)	PCA I (B2)	PCA II (B2)	PCA I (A3)	PCA II (A3)	PCA I (B3)	PCA II (B3)
APL	0.86	0.12	0.86	0.29	0.87	0.12	0.92	0.26	0.86	-0.19	0.96	0.25
ATP	0.93	-0.20	0.84	0.03	0.94	-0.20	0.92	0.15	0.96	0.14	0.97	0.10
AWF	0.96	0.14	0.92	0.02	0.97	0.14	0.95	-0.06	0.90	0.09	0.93	-0.06
BCW	0.92	-0.13	0.96	-0.07	0.93	-0.13	0.86	-0.05	0.99	-0.02	0.97	-0.13
CPL	0.95	-0.06	0.81	0.32	0.96	-0.06	0.87	0.31	0.86	0.02	0.94	-0.34
DOC	0.77	0.28	0.78	0.11	0.78	0.28	0.96	-0.05	0.87	-0.29	0.96	0.11
FL	0.93	-0.05	0.94	-0.07	0.94	-0.05	0.97	-0.13	0.92	0.02	0.77	-0.04
FW	0.89	0.08	0.88	-0.09	0.90	0.08	0.94	0.12	0.97	0.04	0.96	0.07
GLS	0.98	-0.02	0.96	0.12	0.99	-0.02	0.86	-0.01	0.96	0.02	0.93	-0.02
IOB	0.91	-0.13	0.77	-0.13	0.92	-0.13	0.78	0.09	0.93	-0.15	0.96	0.01
ITC	0.97	-0.03	0.98	-0.10	0.98	0.03	0.92	-0.02	0.96	0.01	0.91	0.12
IW	0.97	0.06	0.96	0.09	0.98	0.06	0.84	-0.06	0.93	0.18	0.96	0.06
JLI	0.89	0.13	0.86	0.12	0.90	0.10	0.88	0.03	0.86	0.10	0.94	-0.02
KDW	0.97	0.08	0.97	0.06	0.98	0.08	0.96	0.16	0.87	0.03	0.96	-0.06
LR	0.95	0.08	0.82	-0.02	0.96	0.08	0.86	-0.07	0.90	0.01	0.96	0.14
MDL	0.90	0.10	0.95	-0.14	0.91	-0.13	0.86	0.12	0.85	0.01	0.87	0.03
MLT	0.84	0.14	0.94	-0.10	0.85	0.11	0.92	0.14	0.78	0.12	0.97	-0.06
MOT	0.90	-0.06	0.85	0.17	0.91	-0.05	0.92	-0.02	0.93	0.16	0.88	0.12
MRH	0.92	0.14	0.83	0.08	0.93	0.14	0.85	0.16	0.93	-0.06	0.91	0.18
NA	0.95	-0.01	0.90	0.06	0.96	-0.01	0.86	0.14	0.94	-0.02	0.95	-0.06
NAS	0.92	0.04	0.91	-0.24	0.93	0.04	0.75	0.06	0.91	0.05	0.93	-0.01
NL	0.96	-0.04	0.96	0.06	0.97	0.00	0.87	-0.01	0.95	0.03	0.82	0.08
PAC	0.85	0.19	0.77	-0.33	0.86	0.02	0.96	-0.35	0.86	0.18	0.99	0.33
PBL	0.96	-0.02	0.92	0.07	0.97	-0.02	0.92	0.07	0.93	-0.02	0.92	-0.10
PL	0.91	-0.06	0.97	-0.10	0.92	-0.05	0.86	-0.09	0.90	0.01	0.88	-0.05
PWF	0.93	0.06	0.87	0.10	0.94	0.06	0.93	0.03	0.88	0.12	0.96	-0.03
UJI	0.93	0.18	0.88	0.04	0.94	0.18	0.91	0.04	0.92	0.02	0.91	0.18
UTR	0.80	0.30	0.75	-0.13	0.81	0.35	0.96	0.03	0.91	0.30	0.96	-0.13
VAL	0.86	0.16	0.78	0.31	0.87	0.16	0.96	0.31	0.97	0.02	0.95	-0.29
VBL	0.95	0.03	0.92	0.15	0.96	0.03	0.88	0.16	0.98	0.06	0.95	0.01
WI	0.87	-0.34	0.82	0.01	0.88	-0.33	0.89	0.01	0.90	-0.34	0.95	0.18
WLI	0.90	0.23	0.78	0.15	0.91	0.06	0.86	0.10	0.92	0.14	0.88	0.16
ZMB	0.96	-0.02	0.93	0.06	0.97	-0.02	0.83	-0.04	0.98	0.06	0.82	-0.02
ZYW	0.92	0.17	0.95	0.14	0.93	0.17	0.95	-0.03	0.93	-0.13	0.88	0.17
% of variance explained	91.43	4.52	88.23	3.08	92.42	3.19	89.52	4.44	91.47	2.15	92.57	2.63

The general trend of age variation and sexual dimorphism in populations of *T. splendens* shown by the preceding analyses was also evident in the analysis of body mass (Figure 7). The ANOVA of body mass showed significant variation in all three populations of *T. splendens* [Age: Marangu – $F_{(4,65)} = 31.21$; $n = 75$; $P < 0.001$; Keni-Aleni – $F_{(3,62)} = 26.44$; $n = 70$; $P < 0.001$; Uru-Shimbwe – $F_{(3,62)} = 25.91$; $n = 70$; $P < 0.001$; Sex: Marangu – $F_{(1,65)} = 15.14$; $n = 75$; $P < 0.001$; Keni-Aleni – $F_{(1,62)} = 15.08$; $n = 70$; $P < 0.001$; Uru-Shimbwe – $F_{(1,62)} = 14.91$; $n = 70$; $P < 0.001$. The SNK tests of the pooled data and sexes separately of the relative age classes with reference to body mass revealed the same results reflected by the analysis of craniometric ontogenetic variation and sexual dimorphism.



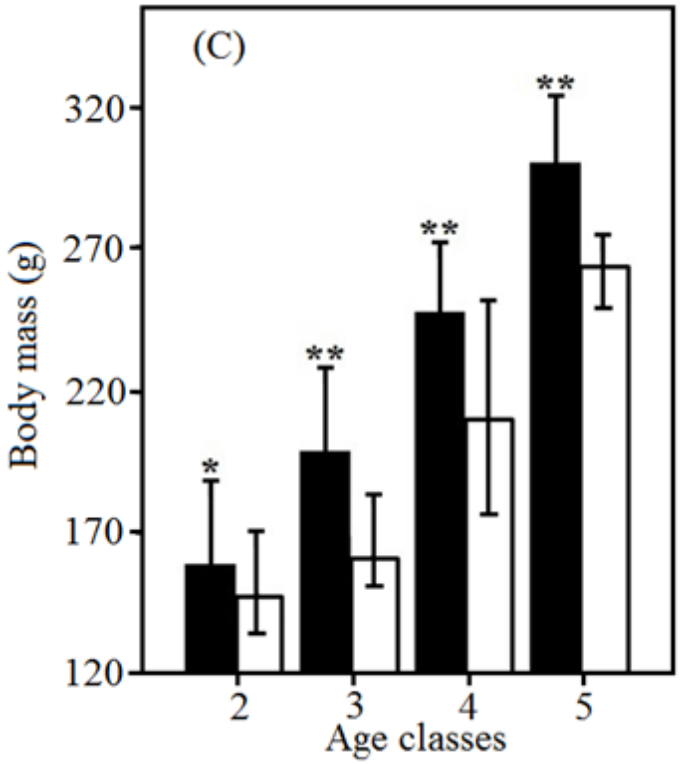


Figure 7: Body mass (mean ± SD) between males (black bars) and females (white bars) of *Tachyoryctes splendens* of age classes 1-5 from Marangu (A), Keni-Aleni (B) and Uru-Shimbwe (C), Tanzania. Statistical significance: * = $P < 0.05$; ** = $P < 0.01$.

Discussion

Based on the dental formula of adult *T. splendens*, the present study revealed five relative age classes in populations from Tanzania. Individuals of relative age classes 1 and 2 were sampled from maternal burrows suggesting that they are juveniles since the species is solitary except during pup caring, whereas of relative age class 3 were sampled from simple independent burrows adjacent to maternal burrows indicating that they are sub-adults. Individuals of relative age classes 4 and 5 were sampled from complex independent burrow systems suggesting that

they are adults as they possess fully-developed reproductive organs (Katandukila et al., 2013). Univariate analyses of craniometric data in the present study revealed craniometrics ontogenetic variation with age classes however it was difficult to determine trend of variation across age classes as a result of overlap between age classes except age class 1.

The multivariate analyses were however shown that sexual dimorphism was a source of concealed ontogenetic variation. Upon analyses of sexes separately, the clear ontogenetic variation between age classes was noted with measurements increasing on orderly pattern from lower to higher age classes indicate that growth increases with increasing age. The increased ontogenetic variation from age class 2 in *T. splendens* indicate that growth pattern which favours adult-hood ecological selection and survival is prominent form young age before dispersal with age class 2 being the preparatory to dispersal. The *T. splendens* disperse at age class 3 when individual starts to carry on adult-hood tasks including burrow construction, independent foraging and defense (Katandukila et al. submitted). The significant ontogenetic variation at age class 3 and 4 than age class 5 imply that mean growth variation is more obvious on mid age classes than the oldest and youngest age classes as reported on other rodents (Zelditch 1988; Hart et al. 2007; Chimimba et al. 2010).

The craniometric ontogenetic variation was also reported in Damaraland mole rats (*Cryptomys damarensis*; Bennett et al., 1990), Namaqua dune mole rats (*Bathyergus jannetta*; Jarvis and Bennett, 1991), Pocket gophers (*Geomys* sp.; Mauk et al., 1999), Talarum tuco tucos (*Ctenomys talarum*; Zenuto, 1999), Highveld mole-rats (*Cryptomys hottentotus pretoriae*; van Rensburg et al., 2004), Cape dune mole rats (*Bathyergus suillus*; Hart et al., 2007) and giant mole-rats (*Fukomys mechowii*; Chimimba et al., 2010). Similar to the present study, ontogenetic

variation the aforementioned subterranean rodents was reported between age classes with higher age classes possess the greatest measurements.

The cranial size of male *T. splendens* implies more utilization of energy for growth to attain large skeletal to support more muscles as adaptation to reproductive and ecological roles. As the species is solitary, males have to fight for adult female mates to foster reproductive success. The successful adaptation of large body size in males *T. splendens* intimate that the species has polygynandry mating strategy as this mating system has been reported to be characterized by larger males and smaller females as a consequence of both sexual selection and intense male-male competition (Bennett and Faulkes, 2000; Schutle-Hostedde et al., 2001; Schutle-Hostedde, 2007). *Tachyoryctes splendens* is aggressive and xenophobic to conspecifics of both sexes but the intense fights between adult males was observed prior to reproduction season. The increased male-male fights before onset of breeding seasons has been also reported in bathyergids whereas more aggressive interactions noted to occur before and during the reproductive phase (Bennett and Faulkes, 2000). Male-male aggression in *T. splendens* was evident in the wild where some males were found with wounds inflicted around their necks prior to becoming reproductively active. The larger-sized males rather than small body-sized males were observed to have their burrows in close proximity to those of adult females. Sampling of fewer small-sized males in the present study suggests that smaller-sized males were forced to live far from adult females and large-sized males to avoid injury from male-male aggressive interactions. Male *T. splendens* possesses more fatty deposits around the neck region than females, suggesting that these fatty deposits may act as a cushion to avoid extensive injuries during male-male aggressive interactions as suggested by studies of Cape dune mole rats

(*Bathyergus suillus*; Hart et al., 2007). Although all mature individuals of solitary species have an equal opportunity to mate, the body size of adult male *T. splendens* may have a reproductive advantage such that males strive to attain a larger body size in order to be competent in searching for and guarding female mates.

The smaller body size of female *T. splendens* imply limited growth as adaptation to conserve energy to overcome reproduction related costs including construction of burrow system which explores the earth thorough for nutrients acquisition for maintenance of burrow to accommodate lactating female and pups for space and foraging; burrow maintenance is also energy demanding activity. The investment of energy for reproduction was evidenced on burrow architecture of *T. splendens* whereas female constructs more complex and reticulated burrow systems with more long foraging tunnels than males (Katandukila et al., submitted). The foraging efficiency of females *T. splendens* would expect to have large sizes than males but in contrast they are smaller-sized than males intimate that reproduction selectivity among females is less based on large size rather other criteria which is still unknown. The lower female-female fight than male-male was noted prior to reproduction seasons indicate the absence of female-female combat to attain more male mates rather normal fight as *T. splendens* is generally aggressive and xenophobic.

As revealed on craniometric results, males are heavier than females except perhaps for individuals of age class 1 where there was no significant difference in the body mass in both sexes, although this may not be conclusive given the few individuals of this age class available for study and only from the Marangu population. The presence of sexual dimorphism in body mass and craniometric data in all populations studied suggests that body mass may also be an

appropriate indicator of sexual dimorphism in *T. splendens* from Tanzania. The suitability of body mass as an indicator of sexual dimorphism has also been demonstrated in Talarum tuco tucos (*Ctenomys talarum*; Zenuto, 1999), Damaraland mole-rats (*Fukomys damarensis*; Bennett and Faulkes, 2000), deer mice (*Peromyscus maniculatus*; Shuttle-Hostedde et al., 2001), bushy-tailed wood rats (*Neotoma cinerea*; Shuttle-Hostedde et al., 2001), southern red-backed voles (*Myodes gapperi*; Shuttle-Hostedde et al., 2001), Cape dune mole rats (*B. suillus*; Hart et al., 2007) and giant mole-rats (*F. mechowii*; Chimimba et al., 2010). Analyses of craniometrics and body mass is therefore signify that size attributed to nature and extent of sexual dimorphism rather than shape implying that sexual-size dimorphism is apparent in *T. splendens* as adaptation to ecological role. Sexual-size dimorphism was also documented on blind mole-rats (*Spalax*: Corti et al., 1996) reported to have sexual-size dimorphism on mandibular metrics.

Analyses of craniometrics and body mass within and between relative age classes (1-5) support our prediction of presence of ontogenetic variation between age classes. The sexual dimorphism was apparent as predicted with nature of dimorphism be attributed by size rather than shape. In contrast to other subterranean rodent showing sexual dimorphism at sub-adult (transitional age to adulthood), *T. splendens* shows sexual dimorphism from young age class (relative age class 2).

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Chapter 6: Population genetics

Molecular genetics of East African root rats from Tanzania

Abstract

The East African root rat, *Tachyoryctes splendens* is native to eastern and central Africa. The taxonomy of the genus at the specific level is uncertain due to various opinions on nomenclature based on type locality, morphometrics and morphological attributes. The present study set out to investigate the phylogenetics and phylogeographical characteristics of East African root rats using sequence analyses of mitochondrial markers including cytochrome *b* (cyt *b*) and D-loop (control region). The study used samples from across Tanzania where root rats are common in the rich soils where there is diversity of plants, mainly agricultural areas. Cyt *b* analysis revealed a 1.68% genetic distance between populations of East African root rats across the sampling distribution, suggesting that all populations belong to single species. The high similarity of East African root rat populations from Tanzania and Kenya implies the taxonomy of *Tachyoryctes splendens* (Rüppell 1835) similar to the findings from geometric morphometrics and craniometric studies. The big-headed East African root rat, *Tachyoryctes macrocephalus* which is endemic to Ethiopia was not included in the present study; therefore the taxonomy of *T. macrocephalus* is retained as suggested in previous studies (i.e. morphology, geometric morphometrics, craniometrics and type locality). Although East African root rats showed high genetic similarity at cyt *b*, differences were revealed at D-loop and a more extensive phylogenetic study was carried out using data from this locus. The greatest differences at D-loop were observed within and among geographically neighbouring populations, while the same haplotypes were shared across the sampling range. The most likely explanation for these patterns is historical processes rather than recent gene flow, although stochastic and sampling effects

cannot be ruled out. The retention of more ancestral haplotypes across the sampling range indicates a recent radiation within the species and adds support to taxonomic studies which suggest *Tachyoryctes splendens* should be considered the nominate and only true species over its currently recognized geographical range.

Key words: Cytochrome *b*, D-loop, East African root rats, haplotype, PCR, *Tachyoryctes splendens*, Tanzania

Introduction

The systematics of rodent taxa at both the generic and specific level has been debated for several decades since previous taxonomy has been based on plesiomorphic traits and confused by homoplasies (Carleton & Musser 1989) most of which are poorly supported as some of the shared characters are influenced by biotic and abiotic factors such as predation, food specialization and competition of space rather than shared ancestry (Delany 1975; Carleton & Musser, 1984; Thaler 1986; Lawrence, 1991; DeWalt *et al.* 1993; Nowak, 1999).

With the development of molecular techniques we have been fortunate to critically review the taxonomy of many rodent taxa using molecular techniques employing mitochondrial genes to assess the phylogenetic relationship between species and indeed to reconstruct phylogeographic structure of some families (Honeycutt & Williams 1982; Lee & Baker 1987; D'Erchia *et al.* 1996; Conroy & Cook 2000; Adkins *et al.* 2001, 2003). Apart from its importance for species-specific relationships, mitochondrial analyses have also revealed genetic diversity within individual species (Mora *et al.* 2007). The taxonomy and genetic diversity based on analyses of mitochondrial genes of several rodent taxa have been used successfully for several

taxonomic groups including Pocket gophers (*Cratogeomys* and *Pappogeomys*: DeWalt *et al.* 1993; *Thomomys*: Smith 1998), African grass rats (*Arvicanthis*: Ducroz *et al.* 1998;), Tuco-tucos (*Ctenomys*: D'Elia *et al.* 1999; Wlasiuk *et al.* 2003; Mora *et al.* 2007, 2013;), blind mole rats (*Spalax*: Nevo *et al.* 1999; Reyes *et al.* 2003), African mole rats (*Heliophobius* and *Heterocephalus*: Faulkes *et al.* 1997, 2004, 2011; *Fukomys*: Van Daele *et al.* 2007; Faulkes *et al.* 2010), Coruros (*Spalacopus cyanus*: Opazo *et al.* 2008), and Zokors (*Myospalax* and *Eospalax*: Su *et al*. 2013). Despite the broad analysis of phylogenetics and population genetics within rodent taxa in subterranean rodents, little has been done to investigate the population genetics of root rats.

The East African root rats are solitary subterranean rodent moles belonging to the genus *Tachyoryctes* (Bekele 1986; Baskevich *et al.* 1993; Musser & Carleton 1993). Root rats occur in rich soils that support a diversity of food resources which they feed upon including underground storage organs and aerial parts of a variety of plants (Jarvis & Sale 1971; Kokiso & Bekele 2008). The genus *Tachyoryctes* has been previously classified variously into 14 species (Allen 1939; Ellerman 1941) and 11 species *(*Musser & Carleton 1993) with both taxonomies based mainly on morphological characteristics. A subsequent study based on geometric morphometrics suggested the presence of just two species, with the other species identified earlier synonymized into *T. splendens* with the exception of *T. macrocephalus* (Beolchini & Cotri 2004). The recognition of two species within the genus was supported by craniometric analyses (Misonne 1974; Corbert & Hill 1991).

A recent taxonomy of the genus *Tachyoryctes* based on geography and ecological characteristics across the distributional range recognised 13 species (Musser & Carleton 2005). These species include *T. ankoliae, T. annectens, T. audax, T. daemon, T. ibeanus, T.*

macrocephalus T. naivashae, T. rex, T. ruandae, T. ruddi, T. spalacinus, T. splendens and T. storey. Musser & Carleton (2005) however suggested that *T. naivashae* and *T. ruddi* may be synonymous with *T. daemon* while *T. splendens, T. ibeanus* and *T. ruandae* were also thought to be synonymous because of their similarities in morphology and habitat characteristics. Given the inconclusive taxonomy of the genus, the present study employed molecular techniques for the first time to investigate the status of the species within a genus *Tachyoryctes* with more emphasize on phylogenetic and phylogeographic history within Tanzania. The analyses included data from mitochondrial gene loci such as Cytochrome *b* (cyt *b*) and control region (D-Loop). The cyt *b* has been successfully used to reveal systematic relationships both within and between populations that are not possible using other systematic techniques such as morphology and morphometrics (Patton & Smith 1994; Lessa & Cook 1998; Smith 1998; Faulkes *et al.* 2011; Su *et al.* 2013). The D-Loop has been widely shown to be useful on identifying phylogeographic history and population genetics (Larriza *et al.* 2002; Reyes *et al.* 2003; Mora *et al.* 2013).

The study was conducted in Tanzania since all previously 'species' described earlier by Musser & Carleton (2005) are reported to occur in this country with the exception of *T. macrocephalus* which is endemic to Ethiopia (Musser & Carleton 1993, 2005; Beolchin & Corti 2004). We predict that samples from areas across the distributional range for this study should represent the various species occurring in Tanzania. The null hypothesis was that 1) more than one species of *Tachyoryctes* would occur in Tanzania and 2) there would be phylogenetic diversity across the geographical distribution of the genus. The present study should add knowledge to the existing information on the taxonomy of the genus particularly for previously synonymized. The present study also investigated the status of potentially cryptic species (Bekele 1986; Baskevich *et al.* 1993; Beolchin & Corti 2004).

Materials and Methods

Tachyoryctes were sampled from various localities in Tanzania. The study sites included Arusha (03˚18 ' 44-45" S, 036˚46' 46-47"E; 1461-1530 metres above sea level (m a.s.l.)), Kagera (02˚20'08-09" S, 031˚25'34-35" E; 1264-1291 m a.s.l.), Kigoma (04˚35'21-22" S, 030˚05'35-36" E; 1649-1665 m a.s.l.), Kilimanjaro (03˚13-25'32-55" S, 037˚16-35'21-50" E; 1235-1451 m a.s.l.) and Manyara (04˚14'43-44" S, 036˚54'06-07" E; 1237-1242 m a.s.l.).

In the field, heart tissues were obtained from the specimens and preserved in alcohol (> 96 % ethanol) and then subsequently stored at -20 ° C. In the laboratory (School of Biological and Chemical Sciences, The Queen mary University of London, London-UK) , the DNA was extracted from the tissue digested by 30ml 10x TNE; 30ml 1M Tris pH 8; 270ml dH_2O; 8ml 25%SDS; proteinase K 10mg/ml (NBL) incubated overnight in a hot-block at 55° C. Procedures for DNA extraction followed protocols described by Müllenbach *et al.* (1989). Some DNA was also extracted using column extraction method using QIAamp Tissue DNA extraction kit (Qiagen, Germany) following the manufacturer's guidelines. The extracted DNA was subsequently eluted by 100µl double distilled H_2O (ddH_2O) and then stored in a freezer (-20 ° C) until needed.

The Polymerization Chain Reactions (PCRs) used to amplify cytochrome b (*cyt b*) were H15915 and L14724, and a species-specific primer MRL1TS (5'-GCCTCTTCCTCCACATTGGTCGA-3'), for D-loop, universal primers PheH, DLL and ThrL were used, as described for mole-rats in Faulkes *et al.* (1997). The PCRs used NEB taq (New England Biolabs) with thermopol Buffer with 2 mM Mg^{++} (when diluted) and KAPA taq (Kapa Biosystems, UK) following manufacturer's protocols. High quality PCR products were purified

using the QIAquick DNA purification kit (Qiagen) following the manufacturer's guidelines. Standard procedures for Sanger sequencing were carried out by by Eurofins MWG Operon (Anzinger str. 7 85560 Ebersberg, Germany).

Data analysis

Haplotypes found within East African root rat populations were identified following manual alignment of sequences from mitochondrial cyt *b* and the D-loop loci using MEGA version 5 (Tamura *et al.* 2004, 2011). Gaps within sequences were treated as insertion/deletion mutations (Faulkes *et al.* 2011). The estimate of evolutionary divergence between haplotypes was evaluated using Maximum Composite Likelihood (ML) models (Tamura & Kumar 2002; Tamura *et al.* 2004, 2011). Cyt *b* genetic differences were far fewer than at the D-Loop, and consequently the latter was used for phylogenetic analysis. These analyses included comparison of molecular diversity within and between regions as well as construction of phylogeographical relationships. All analyses of D-Loop locus involved the 23 distinct haplotypes identified. The phylogeographic relationships of haplotypes were inferred by the Neighbour-joining method (Tamura *et al.* 2004) and median spanning network (Bandelt *et al.* 1999) with genetic distances evaluated using uncorrected P-distance (Tamura & Nei 1993).

Results

Analysis of Cyt *b* sequences (n = 26) revealed 17-19 haplotypes with 3-9 haplotypes from each location sampled. Genetic distances between populations within Tanzania were low, with uncorrected P distances = 0.09-4.62% (mean = 1.68%). Between Tanzanian and the single

published Kenyan *Tachyoryctes* sequence (Jansa *et al.* 1999) values were similar to within the Tanzanian comparisons, with uncorrected P = 0.35-4.04% (mean = 1.89%).

The D-loop (n = 59) sequences revealed 23 haplotypes with 8-14 from each location sampled (Table 1). Genetic distances at the D-loop among populations of *T. splendens* in Tanzania were greater than at cyt-*b*: uncorrected P distance = 0.11-9.86% (mean = 7.41%; Table 2). Thus there were approximately 3x more mutations. Therefore the D-loop sequences were used for phylogenetic analysis as there was more phylogenetic information within the data. The neighbor-joining phylogram (NJ) and median spanning network (MSN) showed 3 distinct groups supported by bootstrap analysis on the neighbor-joining tree (Figure 1). Both phylogenetic relationship analyses (i.e. NJ and MSN) showed that some haplotypes were restricted to a particular region whereas some were shared between geographical locations (Figure 2). The highest sequence differences among haplotypes were found within and between neighbouring regions. For example, Arusha, and Kilimanjaro are the closest regions but share only one haplotype while these regions are far apart from Kagera and Kigoma regions but share more than one haplotype.

Table 1: Number of haplotypes and individuals bearing such haplotype in various regions of Tanzania; *h* = haplotype number, *n* = total individuals having such haplotype, *nr* = number of individuals having such haplotype within a region; AR = Arusha, KA = Kagera, KG = Kigoma, KL = Kilimanjaro, MA = Manyara.

h	*n*	*nr*
1	9	2 KG
		4 MA
		3 KL
2	8	1 KG

Table 1 (Continued)

		1 MA
		4 KL
		2 KA
		3 MA
3	5	2 KL
4	4	4 AR
		1 MA
		1 KL
		1 AR
5	4	1 KA
6	4	4 KG
		1 MA
7	3	2 KA
		1 KG
		1 MA
8	3	1 AR
9	2	2 KL
10	2	2 AR
11	2	2 KG
12	2	2 KA
13	1	1 KG
14	1	1 MA
15	1	1 AR
16	1	1 KL
17	1	1 AR
18	1	1 AR
19	1	1 AR
20	1	1 AR
21	1	1 AR
22	1	1 KA
23	1	1 KG

Table 2: Genetic distances among 23 haplotypes (uncorrected P-distances) at the mitochondrial D-Loop region within *T. splendens* populations from Tanzania.

	haplotype number (n)																					
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	0.010																					
2	0.016	0.015																				
3	0.004	0.008	0.016																			
4	0.086	0.089	0.082	0.089																		
5	0.077	0.080	0.077	0.080	0.054																	
6	0.007	0.010	0.014	0.009	0.087	0.080																
7	0.088	0.091	0.086	0.091	0.050	0.022	0.089															
8	0.083	0.086	0.079	0.086	0.002	0.052	0.084	0.050														
9	0.009	0.001	0.014	0.007	0.088	0.078	0.009	0.090	0.085													
10	0.088	0.091	0.089	0.091	0.062	0.025	0.092	0.024	0.060	0.090												
11	0.087	0.090	0.087	0.090	0.051	0.022	0.088	0.004	0.050	0.089	0.022											
12	0.027	0.028	0.034	0.027	0.092	0.087	0.032	0.099	0.089	0.027	0.097	0.097										
13	0.014	0.003	0.018	0.011	0.091	0.081	0.014	0.090	0.088	0.004	0.093	0.089	0.032									
14	0.011	0.001	0.016	0.009	0.091	0.081	0.011	0.090	0.088	0.002	0.093	0.089	0.029	0.002								
15	0.003	0.011	0.017	0.003	0.088	0.079	0.008	0.090	0.085	0.010	0.090	0.089	0.028	0.015	0.012							
16	0.006	0.009	0.012	0.008	0.088	0.082	0.001	0.090	0.085	0.008	0.090	0.089	0.030	0.012	0.010	0.007						
17	0.006	0.009	0.012	0.008	0.085	0.078	0.001	0.087	0.082	0.008	0.090	0.086	0.030	0.012	0.010	0.007	0.002					
18	0.082	0.085	0.077	0.085	0.003	0.053	0.082	0.052	0.001	0.083	0.061	0.052	0.087	0.086	0.086	0.083	0.084	0.080				
19	0.082	0.085	0.077	0.085	0.003	0.050	0.082	0.049	0.001	0.083	0.058	0.049	0.087	0.086	0.086	0.083	0.084	0.080	0.002			
20	0.092	0.095	0.089	0.095	0.037	0.032	0.092	0.011	0.037	0.094	0.034	0.016	0.102	0.094	0.094	0.094	0.094	0.091	0.038	0.036		
21	0.087	0.090	0.087	0.090	0.051	0.022	0.088	0.004	0.050	0.089	0.022	0.000	0.097	0.089	0.089	0.089	0.089	0.086	0.052	0.049	0.016	
22	0.080	0.083	0.080	0.083	0.057	0.002	0.083	0.024	0.054	0.081	0.025	0.024	0.090	0.084	0.084	0.082	0.085	0.081	0.056	0.053	0.034	0.024

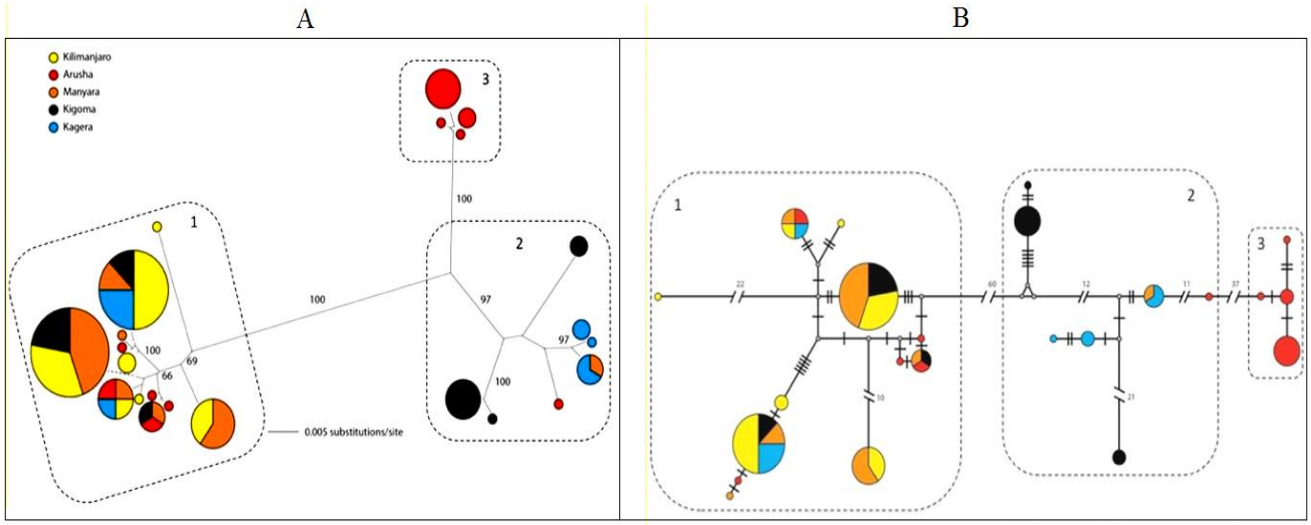

Figure 1: Phylogram showing 23 haplotypes (n = 59 samples) from mitochondrial D-Loop region within a populations of *T. splendens* from Tanzania; A = Neighbour-Joining tree, B = Median spanning network: 1-3 = clades: numbers on joining lines = bootstrap values.

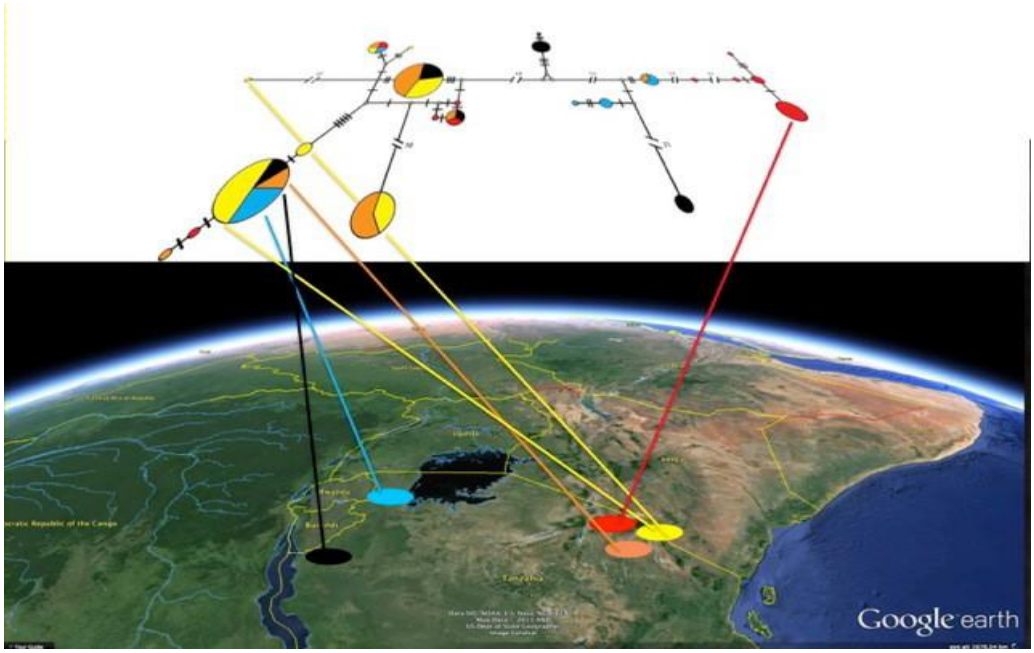

Figure 2: Phylogram showing occurrence of various haplotypes from the Mitochondrial D-Loop region across geographical distribution range of *T. splendens* in Tanzania.

Discussion

The populations of Tanzanian East African root rats revealed low sequence differences using Cyt *b* sequence analysis, although haplotype diversity was relatively high. Although geographically separate (Figure 2) all populations studied to date have revealed small genetic distances suggesting that there is a single species across its geographical range within Tanzania. Given that Tanzania possesses all 12 previously identified species (Musser & Carleton 2005), this molecular study provides strong support for synonymising the 12 *Tachyoryctes* species into a single species namely *Tachyoryctes splendens*. The suggestion of synonymizing species of East African root rats into a single species is also supported by the low percentage of genetic distance between the Kenyan and Tanzanian populations as the Kenyan root rats reported to be the *Tachyoryctes splendens* (Jansa *et al.* 1999). My study did not include any data from the big-headed root rat, *T. macrocephalus* so the degree of genetic differentiation between this species and *T. splendens* remains unknown. My findings lend support to studies involving geometric morphometrics and craniometrics (Missone1974; Corbert & Hill 1991; Beolchini & Corti 2004) but contrast with the systematic diversity of the genus based on classical morphology (Allen 1939; Ellerman 1941) and morphological and geographical characteristics (Musser & Carleton 2005).

The validity of allocating populations into monophyletic group as a result of the level of genetic distance of cyt *b* has been also reported on Tuco tucos (D'Elía *et al.* 1999) African mole-rats (Van Daele *et al.* 2007; Faulkes *et al.* 2004, 2011), Pocket gophers (DeWalt *et al.* 1993; Sudman *et al.* 2006) and Zokors (Su *et al.* 2013). These studies reported that high genetic distances at cyt *b* sequences reveal cryptic clade/species (Van Daele *et al.* 2007; Faulkes *et al.* 2011). The analyses of cyt *b* sequences and low genetic distances in this study do not support the

contention that the specimens in the populations of *Tachyoryctes* represent more than one species, despite the presence of genetic diversity.

The D-loop sequence analysis revealed some distinct genetic structure among populations (some haplotypes were restricted to particular locations; Figure 1 & 2), but there were also haplotypes shared among the populations across the sampling range with absence of species haplotype within distributional range. Moreover, some of the greatest genetic differences occurred both within and among closely neighbouring populations. The most likely explanation for these patterns is historical processes rather than recent gene flow, although stochastic and sampling effects cannot be ruled out. Although there was haplotype sharing between populations there was no haplotype shared by all populations.

These patterns differ markedly from studies on a member of the family Bathyergidae, that of Silvery mole-rat, *Heliophobius* (also found in Tanzania) which exhibits high variation of cyt *b* haplotypes among populations with deep and ancient divergences between populations (Faulkes *et al.* 2011). In this case study, *Heliophobius* was previously thought to have been represented by a single species, *Heliophobous argenteocinereus*, but recent molecular genetic studies have revealed a number of cryptic species occurring in East Africa (Faulkes *et al.* 2004, 2011). Conversely, the shallow genetic branches on the phylogenetic tree for *Tachyoryctes* suggest a recent divergence but argue against hidden species diversity. Although both genera (i.e. *Tachyoryctes* and *Heliophobius*) are solitary and occupy agricultural areas in Tanzania, their niches do not overlap, even though they occur in one geographical region (Beolchini & Corti 2004; Faulkes *et al.* 2011). For example, *Heliophobius* and *Tachyoryctes* occur in the Kigoma region but the former is restricted at Ujiji district whereas the latter occurs at Kasulu district. The

reason for niche separation between root rats and mole-rats is still not apparent despite them sharing similar ecological characteristics.

The results found from the molecular characteristics of cyt *b* locus within and among populations of *Tachyoryctes* in the present study suggest that *Tachyoryctes* is represented by a single species within Tanzania. The sharing of haplotypes between regions including those that are geographically very distant may suggest the recent genetic radiation within the genus and the retention of ancestral haplotypes across the current species range.

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Chapter 7: Synthesis

The East African root rat, *Tachyorytes splendens* from Tanzania is a solitary, subterranean rodent that occurs in a reticulated burrow system consisting of foraging tunnels, bolt holes and a multi-functional nest which comprises a sleeping area, food store and latrine, similar to reports in previous studies of *T. splendens* in Kenya and Ethiopia (Jarvis & Sale, 1971; Hickman, 1983; Kokiso & Bekele, 2008). In addition to the common burrow plan, this study revealed variation of fractal dimension with seasons and sexes of the occupants, whereas wet season showed greater fractal dimension and females' possess greater fractal dimension in both the wet and dry seasons. The preference to construct closed burrows system and place food resources in a food store in *T. splendens* is similar to bathyergids (Bennett & Faulkes, 2000) suggesting that the species has convergent habitat and dietary preferences with other herbivorous subterranean rodents. Although the root rat inhabits a closed burrow system of a similar common plan to that of Asian spalacids, *T. splendens* has a different burrow architecture from that of bathyergids, ctenomyids and geomyids (Šumbera *et al.*, 2003, 2004; Cutrera *et al*., 2006; Thomas *et al*., 2012) in that *T. splendens* has a multi-functional nest chamber encompassing a sleeping area, food store and latrine whereas bathyergids and geomyids possess distinct latrine chamber.

Tachyoryctes splendens excavates more burrows during the wet season than the dry season due to friable soils and abundant food sources. The female *T. splendens* exhibit higher burrow excavation with greater fractal dimension across all seasons. In most other solitary subterranean rodents, males are reported to have burrow systems with greater fractal dimension as a result of a mate searching strategy prior to onset of reproductive activity. The presence of invertebrates and abandoned nests has also reported in the burrows of other rodent moles, including other spalacids and bathyergids (Šumbera *et al.*, 2003; Thomas *et al*., 2009) suggesting

evolutionary convergence. Despite the fact that, *T. splendens*, *Fukomys* sp. and *Heliophobius* sp. occupy similar niches in Tanzania, there is no niche overlap reported.

Sexual dimorphism is found in *T. splendens* with males having a larger body size than females. The sexual dimorphism was revealed from analyses of measurements of the cranium and body mass of distinct age classes as determined by tooth wear and eruption patterns (relative age class 1-5). The analyses, however, revealed that nature of sexual dimorphism was attributed by size rather than shape divergence. Sexual dimorphism was prominent from age class 2 indicating that growth rates vary between the sexes from a very young stage (relative age class 2) and becomes more announced in adults. The larger size of males suggests they invest their energy in growth to attain large body size which has potential reproductive and ecological roles. The smaller size of females despite their greater foraging effort reflects that larger-body size is less important for reproductive competition among females. The ontogenetic variation and size-sexual dimorphism of *T. splendens* suggests that the species is polygynous and thus similar to other solitary subterranean rodents, as this mating system is characterized by larger-sized males that reflect reproductive competence for ecological potential (Schutle-Hostedde *et al.*, 2001; Schutle-Hostedde, 2007). Unlike other subterranean rodents, *Tachyoryctes splendens* showed sexual dimorphism at a young age class, whereas most subterranean rodent species reported to be sexual dimorphic from sub-adult stage which is a transitional age group to adulthood (Chimimba *et al.*, 2010). Further study on growth traits with relation to absolute age classes in a controlled environment is recommended to establish sex-specific growth curves which may provide further explanation of observed ontogenetic variation and sexual dimorphism.

Tachyoryctes splendens is a seasonal breeder having two breeding cycles per year that co-localise with the rainfall peaks. The greater number of pregnancies and higher concentration

of steroid hormones, maturation of gonadal cells indicates that high reproductive activity commences near the onset of wet seasons. The increased births at the latter end of wet seasons suggest that young are born when food is abundant following vegetative growth. The increased reproductive activity during the wet season supports the previous reports (Rensburg *et al.*, 2002) that reproductive activity is more pronounced when there is abundant food resources that enable enough energy to be available to overcome the reproductive costs such as courtship display, gestation and care of pup(s). However, in most solitary male subterranean rodents there is increased a courtship display, with males *T. splendens* using cues such as seismic signaling to communicate to females. The onset of reproduction during the wet season can be corralted with the role of increased burrow extension of *T. splendens* during wet seasons. However, some pregnancies and births were observed during onset of the dry season, possibly as a result of constraint of mate acquisition in subterranean niche. The extended reproduction activities (i.e. pregnancies and births) during the dry seasons may explain the higher fractal dimension observed in females burrows in both the dry and wet seasons to ensure energy acquisition for maintenance of burrow which can accommodate reproducing females and pup(s) for space and provisioning.

The reproductive seasonality is reported in most solitary subterranean rodents with onset of reproductive activity synchronized with food abundance (Bennett & Jarvis, 1988). The majority of social species, however, are aseasonal breeders given the co-operative care of pups and group foraging which ensures provisioning for breeding and non-breeding individuals with exception of the common mole-rat, (*Cryptomys hottentotus hottentotus*: Spinks *et al.*, 1997) and the highveld mole-rats (*Cryptomys hottentotus Pretoriae*: Rensburg *et al.*, 2002) which show a distinctly seasonal pattern of reproduction. Days between breeding cycles suggested in the

present study (i.e. 166 days apart) are supporting study by Jarvis (1973). An investigation of the pattern of ovulation is recommended to assess if it is a spontaneous of induced ovulator. As this study reported bimodal breeding patterns mirrored by rainfall patterns at the study area, it is highly recommended to investigate if reproduction is one per annum in regions that are characterized by unimodal rainfall pattern such as Kigoma and Kagera.

Activity patterns of captive *T. splendens* demonstrated nocturnal activity which was entrained by light indicating that the species is capable of perceiving light and using it as a zeitgeber to entrain the endogeneous clock. The species continued to express a nocturnal activity pattern during constant darkness of a similar duration and time period as that under the light dark cycle suggesting that the species is adapted to a subterranean habitat where light is limited, although it forage above-ground on some occasions. The locomotory activity of *T. splendens* under various light regimes is similar to reports of other subterranean rodents (Oosthuizen *et al.*, 2003). Further study on captive animals to unravel the activity patterns with respect to sex and ontogenetic variation is recommended to enlighten locomotory pattern preference with sex and age.

Molecular data undertaken on populations of *Tachyoryctes* in the present study suggests that a single species is found across the sampling range. Although the study covered wide range of occurrence of root rats, the small genetic distance of the cyt *b* (cytochrome *b* gene data) marker between the Tanzanian and Kenyan sequences (Jansa *et al.*, 1999) implies that a single species of root rat occurs in Tanzania and Kenya. These results support synominising the proposed 12 species into a single nominal species, *T. splendens* as suggested by previous study of the genus based on traditional (Missone, 1974; Corbert & Hill, 1991) and geometric morphometrics (Beolchini & Corti, 2004). There is, however, a need for further molecular

studies that broaden the sampling area to include a wide range of localities of root rats that may reveal cryptic species. Molecular analyses of the present study also revealed the absence of genetic structure in some, but not all mitochondrial d-loop (control region) haplotypes; several haplotypes were shared among populations across the sampling range. In addition, some of the most divergent haplotypes were found within rather than between geographically isolated populations. These data are best explained by ancestral processes (e.g., the retention of ancestral lineages among more derived lineages), rather than by contemporary patterns of dispersal and gene flow, although stochastic effects due to sampling and other factors cannot be fully ruled out at this stage. Thus the conservation of the species across its distributional range is recommended.

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