POPULATION GENETICS OF GREATER CANE RAT (*THRYONOMYS SWINDERIUNUS*) ACROSS ITS RANGE AREAS IN AFRICA

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A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D) OF SOKOINE UNIVERSITY OF AGRICULTURE, MOROGORO, TANZANIA

2023

ABSTRACT

The African Greater Cane rat (AGC) (*Thryonomys Swinderiunus*) is a wild rodent species that belongs to the Family Thryonomydae and is historically endemic to Africa. The species is widely distributed in different parts of Africa and is considered a delicacy in some societies inhabiting the continent. Despite its food value, the rodent species is also a pest of crops and a potential host of bacteria and worms that can impair human health. There is a growing body of literature about cane rat species (*Thronomys spp*.) but most of the published work is patchy. Currently, the spatial population distribution across suitable habitats within its range areas in Africa, and the species' biology, particularly in the wild, are unknown. These gaps limit broader species use for example, for commercial game farming, for developing the potential strategies for controlling the species' destructive impacts on crops, and the measures to advocate for the species conservation in the wild. This Ph.D. study was conceived to fill in these gaps and to advance knowledge on the genetics of this species that is critical to the management and conservation of the cane rat population in the wild. The research aimed specifically to answer four objectives; first, to review the state of knowledge that is currently available about the cane rat species, second; to assess the genetic diversity and population demography of the cane rat populations inhabiting two mountains within the Eastern Arc Mountains range, third, to assess the morphometrics of the cane rat skull to improve evolutionally knowledge of the cane rat species and fourth, to assess the genetic relatedness of the cane rat populations inhabiting the East and West African regions.To understand the breadth of knowledge about the cane rat species, I conducted a systematic review of 56 years (1964 - 2020) of cane rat research published in various outlets including theses and peer-reviewed journal articles accessed from credible literature archives such as Web of Science, Scopus and Google scholar. I found that the available literature on the cane rat was mostly biased towards the West African region, and no single study published from East and Southern Africa was available. I also found that most of the published literature was based on captive cane rat individuals and that substantial knowledge gaps were remaining in various topics including genetics and species biogeography, food biology, and conservation. The knowledge generated in this systematic assessment, helped me to focus on the identified gaps in the next chapters. Further, to answer the question of genetic diversity and population demography of the cane rat species in the Eastern Arc Mountains, I conducted field surveys to collect cane rat samples from traps set in four localities in the Udzungwa south, Udzungwa north, Uluguru rural, and Uluguru urban areas all located in the Udzungwa and Uluguru mountains respectively. I used DNA sequencing of the D-loop region of MtDNA (515 bp) from 46 cane rat samples and various

molecular techniques to analyze these data. I found that there were high genetic differences between than within these four populations. I also found that these four AGC populations in Eastern Arc Mountains (EAMs) have experienced a recent population expansion, especially among the urban population due perhaps to the influence of the urbanization process that may have favored and/or assisted species movements across the rural-urban landscapes. To understand the evolutionary information on the greater cane rats in the Eastern and Southern African regions, I studied *T. swinderiunus* populations in Tanzania to link molecular and geometric-morphological evidence to characterize these populations' diversity using tissue samples and skulls. I found that both molecular and geomorphometric evidence were aligning *T. swinderiunus* into three clades based on the location where samples were collected. This suggested that genetic and morphometric methods could complement each other in understanding the evolutionary biology and within-species diversity of vertebrate species that do not exhibit strong intra-species differentiation.Further, to assess the maternal origin of the African Greater cane rats populations found in the spatially isolated localities in Africa, I compared mt-DNA D-loop sequences from samples collected from two Eastern Arc Mountains, Tanzania, and three agro-ecological zones in Ghana. I found a high genetic differentiation between AGC populations from Tanzania and Ghana with high variation coming from between while low variation was within the AGC populations. I also found that the populations from Tanzania had higher haplotype diversity indicating that they are healthier demographically than those of Ghana. I also found that the populations from Ghana do not share common maternal lineage with those of Tanzania suggesting that the species has evolved as distant populations that lacked connectivity.This study has important implications for the conservation, taxonomy, farming and ranching of African Greater Canerats (AGCs) in Tanzania. The genotype data can help to inform policy and decision-makers on conservation priorities and potential conservation measures including the need for the establishment of germ-plasm banks in vivo and in vitro for maintaining the genetic pool through selection processes. Also, the results can be useful in selecting parental stocks for establishing cane rat farming and ranching to provide farmers with viable stocks. The study will enable the resolution of taxonomic uncertainties which is mandatory for understanding species biology. The study has provided genetic sequences which have been deposited in the NCBI gene bank and are available for public use. The species is presently not endangered but if the needs arises these data can be accessed without going back to the field and used for further research.

Keywords: African Greater Canerat (*Thryonomys swinderiunus*), Eastern Arc Mountains, geometric-morphology, Genetic diversity, martenal lineage, conservation

DHAHANIA

Ndezi (*Thryonomys Swinderionus*) ni aina ya panya pori ambao wako kwenye Familia ya Thryonomydae na wameeenea barani Afrika. Ndezi wamesambaa sana katika sehemu tofauti za Afrika na wana nyama yenye ladha nzuri na wanaliwa na jamii tofauti katika bara la Africa. Licha ya thamani yake kama chakula, aina hii ya panya pia ni waharibifu wa mazao na wanabeba bakteria na minyoo ambayo inaweza kuathiri afya ya binadamu. Kuna mwongezeko wa fasihi za kisayansi juu ya aina za Ndezi (*Thryonomys* spp.) lakini kazi nyingi zilizochapishwa zimetawanyika. Hivi sasa, hakuna ufahamu kamili kuhusu maeneo yanayofaa kukaliwa na hawa panya katika nchi wanapopatikana barani Afrika na pia hakuna ufahamu kamili wa biolojia ya hawa panya, haswa maporini. Mapungufu haya hupunguza matumizi mapana ya hawa panya. Kwa mfano, ufugaji wa kibiashara, pamoja na kutafuta mikakati ya kudhibiti uharibifu wanaosababisha kwenye mazao, na kuchukua hatua za uhifadhi wa hawa panya porini. Utafiti huu wa shahada ya Uzamivu (Ph.D.) ulifanyika ili kujaza mapengo haya na kuendeleza maarifa juu ya vinasaba vya aina hii ya panya, elimu ambayo ni muhimu kwenye usimamizi na uhifadhi wa Ndezi. Utafiti huu ulikuwa na malengo manne; kwanza, kukagua hali ya maarifa ambayo yapo na yamechapisha kwa sasa juu ya Ndezi, pili; kutathmini utofauti wa vinasaba na demografia ya Ndezi wanaoishi katika milima miwili ndani ya Milima ya Tao la Mashariki (MTM), ya tatu, kutathmini maumbile (jiometriki-mofolojia) ya fuvu la Ndezi ili kuboresha maarifa ya mabadiliko ya kievolushenari ya spishi hii na nne, kutathmini uhusiano wa jenetikia ya Ndezi wanaoishi katika maeneo ya Afrika Mashariki, Afrika Magharibi na Afrika Kusini. Kuelewa upana wa maarifa juu ya Ndezi, nilifanya mapitio ya majarida yote yaliyochapishwa ndani ya miaka 56 (1964 - 2020) ya utafiti wa hawa panya na kuchapishwa katika majarida mbali mbali ikijumuisha maandiko tasnifu (thesis) na majarida ya kisayansi yanayotathiminiwa kitaalamu kabla ya chapisho, na yanayopatikana kutoka kwenye kumbukumbu za fasihi za kuaminika kama vile Wavuti ya Sayansi (Web of Science), Scopus na Google Wasomi (Google Scholar). Niligundua kuwa fasihi zilizopo kuhusu Ndezi zinapatikana kwa wingi zaidi kwenye nchi za Afrika Magharibi, na hakuna utafiti wa Ndezi wowote uliochapishwa kutoka Mashariki na Kusini mwa Afrika uliopatikana. Niligundua pia kuwa fasihi nyingi zilizochapishwa zilitokana na Ndezi wanaofugwa na kwamba kuna mapungufu makubwa ya maarifa hasa kwenye mada mbali mbali za Ndezi wanaopatikana porini ikijumuisha jenetikia na biojieografia yao , biolojia ya chakula anachokula, na uhifadhi. Ujuzi uliotokana na tathmini hii ya kimfumo, ulinisaidia kuzingatia mapungufu yaliyotambuliwa katika sura zifuatazo. Ili kuelewa zaidi na kujibu swali la utofauti wa jenetikia na jiometriki-mofolojia ya spishi hii ya Ndezi katika milima ya

Tao la Mashariki, nilifanya utafiti wa Ndezi waliopo porini na kukusanya sampuli kwa kutumia mitego iliyotegwa katika maeneo manne ya milima. Mitego ilitegwa Udzungwa kusini, Udzungwa kaskazini, Uluguru vijijini, na Uluguru mjini. Nilitumia mpangilio wavinasaba kutoka sampuli 46 za Ndezi na mbinu mbali mbali za Masi kuchambua data hizi. Niligundua kuwa kulikuwa na tofauti kubwa za vinasaba kati ya Ndezi kutoka maeneo manne yaliyotajwa hapo juu. Niligundua pia kuwa kumekuwa ongezeko la idadi ya Ndezi kutoka maeneo hayo manne ya MTM hasa yanayotokana na upanuzi wa miji ambao umewezesha aina hii ya panya hii kusambaa zaidi katika maeneo ya vijijini na mijini kwa sababu ya kuwepo maeneo yanayowafaa kwa makazi na upatikanaji wa chakula. Ili kuelewa zaidi kuhusu mabadiliko ya Kiivolutionary juu ya Ndezi katika maeneo ya Mashariki na Kusini mwa Afrika, nilitafiti Ndezi kutoka nchini Tanzania ili kuunganisha ushahidi wa Masi na jiometri-mofologia ili kuonyesha utofauti kwa kutumia sampuli za tishu na fuvu. Niligundua kuwa ushahidi wote wa Masi na jiometria-mofolojia ulikuwa unagawanyisha Ndezi kwenye makundi matatu kulingana na eneo ambalo sampuli zilikusanywa. Hii inapendekeza kwamba jenetikia na mofolojia zinaweza kuambatana katika kuelewa biolojia ya mabadiliko na utofauti wa spishi ambazo hazionyeshi utofauti wa maumbile ya nje kati yao. Nilitathmini uasilia wa mama wa Ndezi wanaopatikana katika maeneo tofauti yaliyotenganishwa kwa umbali barani Afrika kwa kulinganisha mlolongo wa vinasaba vinavyopatikana kwenye mitokondria kutoka sampuli zilizokusanywa kutoka Milima miwili ya Tao ya Mashariki, Tanzania, maeneo matatu tofauti ki-ikolojia nchini Ghana (Afrika ya Magharibi) na maeneo manne tofauti kusoka Afrika ya kusini. Niligundua utofauti mkubwa wa vinasaba kati ya Ndezi kutoka Tanzania,Ghana na Afika kusini. Niligundua pia kuwa Ndezi kutoka Tanzania walikuwa na utofauti wa hali ya juu unaonyesha kuwa wana ubora kijenetikia kuliko wale wa Ghana na Afrika kusini. Niligundua pia kuwa Ndezi kutoka Ghana na wale kutoka Tanzania na Afrika kusini hawakutokana na ukoo mmoja wa mama kiuasili hii ikimaanisha waligawanyika kuwa tofaouti muda mrefu.Utafiti huu una umuhimu kwa uhifadhi, uainishaji wa kisayansi (takisonomia), kilimo na ufugaji wa Ndezi nchini Tanzania. Takwimu za aina jeni zinaweza kusaidia kuwajulisha watunga sera na watoa maamuzi juu ya vipaumbele vya uhifadhi na hatua zinazowezekana za uhifadhi ikiwa ni pamoja na mahitaji ya uanzishwaji wa benki za chembe za vinasaba kwenye maabara na kwenye mazingira asilia kwa kudumisha hifadhi ya vinasaba kwa kuchagua mbegu bora. Pia, matokeo haya yanaweza kuwa muhimu katika kuchagua wazazi bora kwa kuanzisha kilimo cha Ndezi na ufugaji ili kuwapa wakulima panya wazazi anzilishi wanaofaa. Utafiti huu utaondoa utatanishi wa taksionomia, jambo ambalo ni muhimu kwenye kuelewa biolojia ya Ndezi. Utafiti huu umegundua na kutayarisha mlolongo wa vinasaba ambao umehifadhiwa katika benki ya

vinasaba ya NCBI na unapatikana kwa matumizi ya umma. Ndezi kwa sasa hawajahatarishwa lakini ikiwa mahitaji yanatokea data hizi zinaweza kupatikana bila kurudi porini kukusanya sampuli kwa upya na unaweza kutumika kwa utafiti zaidi.

Maneno muhimu: Ndezi (*Thryonomys swinderiunus*), Milima ya Tao la Mashariki, jiometriki-mofolojia, utofauti wa maumbile, ukoo wa mama asilia, uhifadhi

DECLARATION

I SHADIA IBRAHIM KILWANILA do hereby declare to the senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

Shadia Ibrahim Kilwanila **Signed……Date……..**

The above declaration is confirmed by:

Dr Alfan Rija **Signed…… Date…….**
Main Supervisor

Dr.Charles Lyimo **Signed……Date……..**
Co-Supervisor

LIST OF PUBLISHED PAPERS/MANUSCRIPTS

i) Kilwanila, S. I., Msalya, G. M., Lyimo, C. M., & Rija, A. A. (2021). Geographic biases in cane rat (*Thryonomyds*) research may impede broader wildlife utilization and conservation in Africa: A systematic review. *Scientific African*, *12*, e00785.

ii) Kilwanila, S. I., Lyimo, C. M., & Rija, A. A. (2022). Mitochondrial genetic diversity of the Greater Cane rat (*Thryonomys swinderianus*) populations from the Eastern Arc Mountains ecosystem, Tanzania. *Molecular Biology Reports*, 1-12.

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ACKNOWLEDGMENT

First of all, I thank almighty God (Allah S.W) for blessing me with the will, energy, strong mind, and ability to carry out this study.

I would like to express my sincere gratitude to my supervisors: Dr. Alfan A. Rija and Dr. Charles M. Lyimo for their thoughtful and creative advice, guidance and all forms of academic support extended during this study. I am also indebted to Prof.Rhodes Makundi for critical review of different chapters and manuscripts and Prof Shombe Hassan for his continual guidance and moral support.

I am grateful to the Africa Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development (ACEII-IRPM&BTD) (GRANT No 5799-TZ.) for the full PhD scholarship that enabled me to conduct these studies.

I wish to acknowledge the assistance provided by Mr. Ismail Rashidi during data collection in the field.

Last but not least, I would like to acknowledge the support and encouragement given by my brothers Dr. Kilwanila Hussein Kiiza and Mudathiru Ibrahim Kilwanila. I thank them for their prayers and for standing by me, especially during difficult times.

Permits to conduct the research were granted by the Tanzania Wildlife Research Institute (TAWIRI) and Tanzania Commission for Science and Technology (COSTECH) (research permit number 2020-247-NA-2020-158). Further, permits to work in the study sites were provided by Morogoro Rural District, Morogoro Urban District and Kilombero District Councils.

DEDICATION

This work is dedicated to my deceased parents, the late Mr. Ibrahim Kiiza Kilwanila and Mrs. Mariath Abdulmarik for their love during my upbringing, care and support throughout childhood and for providing me with education that enabled me to accomplish studies at college up to completion of my PhD. studies at Sokoine University of Agriculture. May Allah rest you in eternal peace.

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LIST OF ABBREVIATIONS AND ACRONYMS

AGC	–	African Greater Cane rats
EAM	–	Eastern Arc Mountains
MTM	–	Milima ya Tao la MAshariki
TAWA	–	Tanzania Wildlife Authority
MNRT	–	Ministry of Natural Resources and Tourism
FST	–	Fixation Index
CVA	–	Canonica Variate Analysis
mt-DNA	–	Mitochondrial DNA
AMOVA	–	Analysis of Molocular Variance
NCBI	–	National Center for Biotechnology Information
MEGA	–	Molecular Evolutionary Genetics Analysis
DNAsp	–	DNA Sequence Polymorphism

ORGANIZATION OF THE THESIS

This thesis is organized in seven chapters. The first chapter deals with background information, problem statement and justification. The second chapter is the review on available cane rat literature. The third chapter is on the genetic diversity of AGC in the spatially isolated Eastern Arc Mountains. The fourth chapter deals with studying the variation of AGC using both molecular and morphometric methods and the fifth chapter is on comparison of the martenal lineage of AGC population found in Tanzania and Ghana. The chapter six is the general discussion of all chapters. and lastly in the conclusions and recommendations.

Each data chapter is presented as a stand-alone manuscript that is ready for sending out to a journal for publication. For the objectives whose manuscripts have been published, a typical published article is included rather than a manuscript.

CHAPTER ONE

1.0 Introduction

Cane rats belong to the sub-order Hystricomorpha. The name '*cane rat'*, differs from location to location depending on the habitat and food they prefer (Adu *et al*., 2017). In West Africa, they are known as grasscutters because they prefer grasses while in Southern Africa they are called cane rats because they prefer cane fields (Adenyo *et al*., 2013). Cane rat is the second largest rodent species in Africa after porcupines, which belongs to the genus Thryonomys (Lopez-Antonanzas *et al*., 2004). This genus consists of two species; the greater cane rat *(Thryonomys swinderianus)* and smaller cane rat (*Thryonomys gregorianus)* (Sacramental *et al.,* 2012). These two species vary in body size, thus their names.

Rodent distribution like that of other mammals is influenced by various factors such as soil, vegetation type and altitude (Mulungu *et al.,* 2008). Cane rats are widely distributed in the sub-Sharan regions of Africa ranging as far from Senegal to South Africa (Jori *et al*., 1995; Opara, 2010; Olatidoye *et al*., 2019) and mostly occupy habitats with dense grasses (Delany and Happold 1979; Aluko *et al*., 2015). The greater cane rat is much more widespread in Africa than the lesser cane rat; it occupies almost all African countries west of the Sahara (Skinner and Chimimba, 2005). The lesser cane rat can be found in a narrow belt from northern Cameroon to East Africa, where they are common and widespread, and further south as far as Zimbabwe and parts of Mozambique (Aluko *et al*., 2015). Although there are overlaps in their distribution, they occupy different ecological niches (Aluko *et al*., 2015).

In Tanzania, these species are widely distributed in different areas including EAM (Stainley *et al*., 1998), although a detailed account of their population is lacking. Cane rats are found in cultivated areas that they have invaded especially in sugar cane plantations, rice fields, maize, groundnut farms; or tubers like cassava and sweet potatoes. These species are sometimes considered serious pests of crops (Owen *et al.,* 2012; Uloko *et al*., 2017). Further, cane rats inhabit the borders of humid zones and marshes with vegetation composed of savanna, sparse forests and of rocky areas (Adu *et al*., 2003). However, as Lopez-Antonanzas *et al.,* (2004) reported, cane rats can utilize both lowlands habitats during the dry season and upland habitats in the wet season. These areas are used dependent on the availability of habitats and cover. Despite care rats mostly preferring good grass cover, they also reside in rock crevices, under rocks, or in abandoned holes of springhare (Aluko *et al*., 2015). Further, cane rats maintain major trails connecting their

areas of abode and foraging areas when food and cover resources are scarce. This behavior makes them vulnerable to hunting by experienced hunters who know their trails (Mensah *et al.,* 2007; Akinola *et al*., 2015).

The Eastern Arc Mountains (EAMs) are habitats for several wild animal species (Stanley *et al*., 1998). Species inhabiting the EAMs are threatened by pressures from human population increase (Burgess *et al*., 2004). The threats include clearing of the forest for different human activities, wildfires and illegal hunting of animal species including rodents (Stanley *et al*., 1998). Habitat clearing over time has led to decline of forests and habitat fragmentation (Burges *et al*., 2004). A government report recently suggested that more than 70% of forest cover in EAMs has been lost to habitat clearing (MNRT, 2010). Further, habitat fragmentation has been shown to reduce genetic diversity in mammals (Frankham, 2003; Newmark *et al*.,2017; Newmark *et al*.,2018), increase inbreeding depression (Herbert *et al*., 2003; Shirk *et al*.,2010) and lead to accumulation of deleterious mutation (Keyghobadi, 2007; Sjölund *et al*., 2019). It also reduces species diversity and abundance in mammal species (Ofori *et al.,* 2013; Chen *et al.,* 2019) due to the shrinking of home range sizes, dispersal routes and movement rates (Gehring and Swihart, 2004; Newmark *et al*.,2018; Sjölund *et al*., 2019). Effective management of species faced with such threats may require more information on the species' biology, genetics, and or ecology (Seiler *et al*., 2014).

Cane rats are aggressively hunted illegally in different parts of Africa including Nigeria, Togo, Benin, Ghana and Cote d voire because it is a delicacy (Annor *et al*., 2011; Adenyo *et al*., 2017). There have been efforts over the years to domesticate this species to make it more easily available for the people in West Africa and subsequently in other parts of the continent because of existing hunting pressure to rescue the wild population (Asibey, 1981; Jori *et al*., 1995; Addo *et al*., 2002; Mensah and Okeyo, 2006; Annor *et al*., 2008). The method used in hunting cane rats involves the use of poisonous baits, dogs and fire to the peril of wildlife habitats, the environment and consumers (Adenyo *et al*., 2013). The use of fire most often gets out of hand leading to bushfires that destroy the habitats of many wildlife species, thereby raising serious biodiversity and environmental concerns (Adenyo *et al*., 2017). Even though there are concerns about the techniques used for hunting cane rats in the wild, the cane rats game meat trade continues to flourish, making significant contributions to the economies of African countries where this meat is consumed (Ntiamoa-Baidu, 1997). The meat is eaten by all classes of people with no religious prohibitions (Opara, 2010b), and is also exported to continental Europe and the

United States (Adu *et al*., 1999). Therefore, cane rat domestication is important so that it can contribute even more to those economies without jeopardizing wildlife diversity and the environment. In West African countries cane rat domestication started in the 1970s mainly aimed to reduce poaching but have since given little success (Adenyo *et al*., 2013). Rearing attempts suffered from high mortality due to the aggressive nature of the species, (Adu *et al*., 1999). In any domestication process, selection for desirable traits is of great importance to ensure ease of handling and for profitable production in the case of the cane rat, which is being developed as a mini-livestock in Sub-Saharan Africa to alleviate poverty and to cater for the protein needs of the people (Jori *et a.,* 1995). Various aspects of cane rat biology have been studied in order to better manage the species under domestic conditions. These include reproduction (Henry, 2011), nutrition (Karikari, and Nyameasem, 2009), parasites and diseases (Kankam *et al*., 2009) and genetics (Adenyo *et al*., 2013, Adenyo *et al*., 2017). However, in Tanzania, such studies are lacking.

The genetic structure, diversity and phylogenetic relationships of greater cane rats have been of focus for research in recent years. For example, Adenyo *et al*., (2012) developed microsatellite markers which acted as a baseline for individual identification in studying the genetic structure and diversity and phylogeny relationships of cane rats. Correspondingly, Adenyo *et al*., (2017) conducted the study to apply novel microsatellite markers they developed in determining the genetic structure and diversity of cane rats populations in four spatially isolated agro-ecological zones (guinea Savanna, forest, coastal Savanna and Volta region). They found that populations of cane rats in Ghana are genetically differentiated according to agro ecological zones and the Volta Lake could be serving as a barrier to gene flow. Furthermore, Adenyo *et al*., (2013) investigated the genetic diversity of greater cane rats from agro-ecological zones (Guinea Savanna, Forest and Coastal Savanna) in Ghana using mitochondrial D-loop. They found that the Ghanaian populations of cane rats are highly diverse but are less distinctive. Additionally, Lopez-Antonanzas *et al*., (2004) did a cladistics analysis by using skulls to review the systematics and phylogeny of the Thryonomyids species in different parts of the world including West Africa. They found that this genus consists of two species which are restricted in Africa there are some species in that genus spread out of Africa during the Miocene age eastward to southern Asia (Pakistan), making the family Thryonomidae have 20 species. Given this diversity, it is not clear whether cane rats inhibiting east African regions follow suit, yet no study has attempted to investigate this question in Tanzania.

The morphometric characteristics of cane rats have been studied extensively by various scientists in different parts of Africa. For example in Kenya, Winkler (2003) and Kingdon, (2012) described *T. gregorianus* as the smaller sized cane rats, weighing between 2.6 and 7.5 kg, with a short tail (6.5 -14 cm), bulbous nose, and very deeply grooved incisors. In contrast, *T. swinderianus* was described as relatively larger in size, weighing between 4.5 and 8.8 kg, with a longer tail (17 - 26 cm), less protuberant nose and restricted fine grooving of the incisors. In another study, Isabelle *et al*., (2012) used morphometric characteristics such as body length, tail length, ear length, hind leg length without claws, neck perimeter, head perimeter, chest perimeter and body weight to determine the age of cane rats in Benin. Whereas, in Nigeria Adu *et al*., (2003) reported the importance of using the ano-genital distance in sex determination of the cane rats. Additionally, Olude *et al*., (2014) were able to determine the sexual dimorphism that exists within the greater cane rats by using morphometric characteristics of the skull in Nigeria.

1.1 Problem Statement and Justification

The long-term survival of wild species in isolated or fragmented habitats depends on habitat connectivity which ensures gene flow between adjacent landscapes. Physical barriers such as water bodies, altitudinal variation, topography, fragmented habitats and anthropogenic features (e.g. roads) have been observed to limit animal dispersal, affecting genetic diversity/variation and structures of populations (Riley *et al*. 2006; Gauffre *et al.,* 2008; Shirk *et al*., 2010). Habitat fragmentation in particular confines populations in a smaller area and overtime may influence evolutionary processes i.e. changes in genetic traits (Keyghobadi, 2007; Kuussaari *et al*., 2009) and may make them vulnerable to extinction due to failure to cross large gaps (Burgess *et al*., 2004). The EAMs flora and fauna are threatened by habitat fragmentation due to the clearing of forests for different human activities (Burges *et al*., 2004). The impact of habitat fragmentation on species may further be compounded by increased animal exploitation due to activities such as poaching. (Burges *et al*., 2004; Newmark *et al*., 2017; Newmark *et al*., 2018). Collectively, the impacts of these threats (fragmentation and poaching) may be even more severe for little-known species such as cane rats. Most studies on cane rats have been conducted in West Africa, particularly the genetic structure and diversity, diet and reproduction; this knowledge has improved our understanding of these species (Adu *et al*., 2003; Adenyo *et al*., 2013; Adenyo *et al*., 2017a; Adenyo *et al*., 2017b). Despite this, it is not clear whether this knowledge may be representative of the cane rats found in a different geographic region, in this case, the East Africa region. The subtle differences between the West and East African regions in terms of the rainfall regimes, vegetation

types, existing threats on potential habitats and local culture may shape the landscapes used by *Thryonomys spp.*, potentially influencing species evolutionally divergence (Schluter, 2015). For example, the species names reflect the habitats they utilize between the West (grass cutter) and East (Cane rats), yet the biological and or ecological information about these species inhabiting the East African region is still not known and presumed to be different from their West African counterparts (Caumul and Polly, 2005; Cardini *et al*., 2010). In Tanzania there is no published information about any of the species of cane rats despite that habitats used by these species across the EAMs (Udzungwa, Uluguru and Eastern Usambara) are increasingly threatened by human activities. Further, the few studies available (Stanley *et al*. 1998; Kilwanila *et al*., 2021) suggest poaching of cane rats for bush meat is common, there is the danger that these species may experience drastic population decline and potentially face local extinction without our knowledge. Information on the genetic structure and phylogenetic relationship of the greater cane rat species, their diet ecology, population size, species morphometric differentiation and level of exploitation by humans for bush meat are necessary to improve our understanding of this species in Tanzania. This study intends to generate new knowledge on the greater cane rat in Tanzania by studying the population genetics and ecology of this species in two spatially isolated mountains faced with fragmentation and poaching pressure (Newmark, 2002; Burgess *et al*., 2007). The new knowledge generated from this study will provide baseline data on the greater r cane rats in Tanzania and East Africa region. The new information on the poaching levels of these species will be useful for improving conservation planning of this potential game species particularly in informing the game farming and ranching policy currently being implemented by the Tanzania Wildlife Authority (TAWA) in the Ministry of Natural Resources and Tourism (MNRT).

1.2 Objectives

This study was aimed at improving knowledge of the genetic structure and ecology of the greater cane rat, *T. swinderianus,* in two spatially isolated mountains within the Eastern Arc biodiversity hotspot area as well as other range areas across Africa.

1.2.1 Specific Objectives

i) To assess the state of knowledge of the greater cane rat based on information already published elsewhere.

ii) To estimate the genetic diversity and population demography of the greater cane rat population inhabiting two mountain blocks within the Eastern Arc mountain

iii) To examine morphometric traits of the greater cane rat skulls from the two mountains areas to improve evolutionary knowledge about this species

iv) To assess the genetic relatedness of the greater cane rat population inhabiting the east, west and south African regions.

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CHAPTER TWO[1]

2.0 Geographic biases in cane rat (Thryonomyds) research may impede broader wildlife utilization and conservation in Africa: A systematic review

Scientific African 12 (2021) e00785

Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

Geographic biases in cane rat (Thryonomyds) research may impede broader wildlife utilization and conservation in Africa: A systematic review

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ARTICLE INFO

Article history:
Received 20 January 2021
Revised 26 April 2021
Accepted 8 May 2021

Editor: Dr B. Gyampoh

Keywords:
Bushmeat hunting
Cane rat literature
Game ranching
Insitu conservation
Spatiotemporal trend
Wildlife farming

ABSTRACT

There is a growing body of literature about cane rat species but most of the published work is patchy and current spatial distribution is unknown which limits its wide application in the utilization of the species for the broader commercial game industry and for improving wildlife conservation across Africa. We conducted a systematic review of 56 years (1964 - 2020) of cane rat research to understand existing research gaps, to analyze the spatiotemporal and thematic patterns, and investigated factors that influence the publication of the cane rat research in widely recognized journal outlets. We found 308 publications on the cane rat species from 14 countries authored by 39 nationalities globally. The publications increased significantly over the study period, with 97.7% of these biased geographically and thematically towards the west and central African region. Further, the published research mostly covered one species, the greater cane rat, and none had covered the biogeography, food biology, and conservation of any of the two cane rat species in situ. Also, the author's nationality had the strongest influence on publishing the research in journals with or without impact factor. These results suggest that the financial limitation and quality of the research influenced most cane rat research published in local national or regional journals which mostly had limited accessibility for widespread research use to improve applied conservation programs. Expanding coverage of the cane rat research in other species-range countries in the east and southern African regions will be necessary to tap the species as a priority commercial game to reducing exploitation pressure on the wild mammal populations particularly in the African savannas where illegal hunting for bushmeat consumption is a growing problem.

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https://doi.org/10.1016/j.sciaf.2021.e00785

[1] This chapter was published as an article in the journal Scientific African

Introduction

Cane rats are wild rodent species that belong to the Thryonomydae family, historically endemic to Africa and widely distributed in eastern Cameroon, Central African Republic, Sudan, Kenya, Tanzania, Uganda, The Democratic Republic of Congo, Zambia, Malawi, Mozambique, and Zimbabwe [43]. Two recognized species, the smaller (*Thryonomys gregoriunus*) and greater cane rat (*Thryonomys swinderiunus*) occupy different habitats including swampy lowland along riverbanks and streams and higher altitudes in drier and rocky areas [13]. These small mammals are important both to man and the environment through maintaining food webs and chains [23]. They consume plant material and enhance the mineralization of organic matter making them an essential component of the ecosystem [26]. They are also agricultural pests, feeding on different plants ranging from leguminous fodder, tubers (e.g. cassava (*Manihot esculenta*) and sweet potatoes (*Ipomoea batatas*)), fruits (e.g. pawpaw (*Carica papaya*), pineapple (*Ananas comosus*), and mango (*Mangifera indica*)) to food crops such as rice (*Oryza sativa*) and maize (*Zea mays*) [1]. Also, they harbor parasites that transmit diseases to human beings and other animals, for example, salmonellosis, trypanosomosis, gastrointestinal parasites, and ticks [17,30].

Further, in some African countries, cane rats body parts such as pancrease and hairs are used in traditional medicine for healing wounds, restoring fertility in women, and diabetes treatment [14]. The species are increasingly regarded as important game animals of high-quality meat and delicacy across many countries in Africa and for this reason, they are hunted aggressively in their range areas [6]. Furthermore, meat from the cane rats is considered to be in high demand partly because its consumption has no religious, gender, age, and ethnic prohibitions at least in some countries [3,33], making cane rats a potentially suitable game species for commercial wild meat industries in Africa. As a result, there have been efforts and attempts to domesticate them to curb the potential over exploitation of wild populations especially in the west and central African countries [8]. Domestication provides an alternative source of income for farmers and increases farmers' access to and utilization of animal protein for dietary needs [31]. According to Ajayi et al. [5], cane rats have a great turn-over rate for meat production within a short period, thus making it a prospective good source of generating income. Coupled with this, their potential as a source of ecotourism to entertain interested viewers in zoological gardens has substantially increased efforts to domesticate cane rats in recent decades in these countries [32]. Accordingly, cane rat farming could be promoted in other African countries where the growing demand for wild meat (henceforth named as bushmeat) is fueling wildlife poaching crisis for bushmeat across the African savannas [48], causing wild mammal population decline in many targeted protected areas [38]. Domestication of cane rats in form of wildlife farms, zoos, or ranches would promote conservation of wild mammals in protected areas and livelihood of local communities, especially where cane rats are considered as commercial game animals for farming or ranching programs.

Although advocating for cane rat farming looks intuitive, however, its wider acceptance and implementation among many societies in east and southern African regions may be particularly challenging. This is because most cane rat information is based in the west and central African countries, and available knowledge is scattered and patchy, rendering wide use difficult. To date, only one study, by Mustapha et al. [27] has attempted to collate the available literature on cane rats. However, that study covered only one species, the greater cane rat, searched very few databases, ignored theses, and did not analyze important information such as the methods used in the published research, types of data collected, and where the research was published to facilitate the wider application of the research work in other regions.

Further, the approach used by Mustapha et al., [27] underreported the studies currently available and may limit the use of the research for advancing science and for guiding conservation and livelihood programs such as cane rats farming or ranching. A study that pulls together all the information available from all sources would be useful in informing future direction on the cane rat research and application. This study aimed to conduct a systematic assessment of the literature about cane rat species to understand the state of knowledge and to explore the future research options and the potential use of the cane rat species for wider conservation and livelihood programs in sub-Saharan Africa. Specifically, this study addressed four questions; what are the existing knowledge gaps both spatiotemporal and thematically, what are the research methodologies used, what outlets have been used by the current research to publish results, and what factors influence publication in the chosen journal outlets? This paper provides recommendations on the future research options and application of the cane rat knowledge in tackling conservation and development challenges in the African savanna regions.

Methodology

Performing search

A quantitative systematic review of available cane rat research was conducted following four steps that included planning (i.e. the formulation of the topic, review protocol and keywords), searching (i.e. selection of relevant data and assessment of publications), data extraction and database creation, and data synthesis [34,36]. All searches were conducted in the ISI Web of Science, Scopus, Google, and Google Scholar to access a comprehensive database of published information about cane rats from 19th March to 8th June 2020 and additional searches conducted from 20th October to 25th November 2020 (Table SM1). The search terms used were "cane rats" and "grasscutter". These search terms were used together with the established themes covering broader fields as ecology, conservation, genetics, production, reproduction, anatomy, nutrition, and diseases. The first search used a single search term together with any of the themes above. The second search was conducted using both search terms separated by either the OR operator or AND operator followed by the theme (see all

search terms in Table SM1). The same procedures were repeated until all themes were covered. All searches were done from Sokoine University of Agriculture (SUA) library, Tanzania to access journals which SUA library has subscribed to. Most journals where the retrieved papers were published were free access. For the journals that required paying, the papers were requested from individuals in other institutions that had access. We included all original articles, reviews, book chapters, published conference or symposium proceedings, and theses and excluded grey literature. No time frame limit was used in the search to allow for the retrieval of as much publication as possible [38]. If an article was available in non-English languages, the article was translated by a proficient speaker of that language based in the Language department at SUA. Search results were reported following the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) protocol [25].

Paper screening

Retrieved papers from online databases were screened using a two-stage process [38,48]. First, titles and abstracts of the papers were read to look for relevance, and secondly, for any relevant paper identified based on the selection criteria below, the whole paper was read in-depth to extract the needed information (see extracted data in the following section-literature database). Only papers based on empirical studies and reviews of cane rats were included. To check for the validity of the screening process, two data sets of an equal number of papers were worked on by two independent reviewers working against the set exclusion criteria i.e. search terms not in the title, abstract, or mentioned few times in the whole paper and not an empirical study [48]. Both reviewers found 96.6% of all papers were relevant for inclusion in the review.

Building published literature database

The database of the cane rat literature required to answer the research questions addressed above included author name, title of the paper, publication year, research theme and subtheme, author nationality, study country, research collaborating nationalities, geographic location of the study (only country names were recorded as most studies did not indicate exact coordinate points where data collection were conducted), study methodology used, research type, outlet journal and impact factor (based on the journal online page and Clarivate Web of Science), journal coverage (i.e. whether local, regional or international based on description given on online journal page), species studied (greater or smaller cane rats or both) and number of studies (see Supplementary raw data I). Because most publications did not report the exact coordinate location of their study sites, we used Q-GIS version 3.10.3 to spatially locate the number of studies conducted in each country mentioned in the publication. The four stages followed when conducting systematic review are presented in Fig SM1.

Data analysis

Prior to conducting formal analysis, data cleaning and manipulation were conducted with the 'dplyr' package in the statistical software R version 3.6.3. We checked for distribution shapes of continuous variables using the histogram and created a visual display of most variables as most of the research objectives required simple analyses. To understand the predominant research topics and sub-topics, the research themes and subthemes were grouped and plotted as barplots and word cloud using 'ggplot2' and 'wordcloud' packages respectively. The trend of publications was examined using the Cox and Stuart trend test [9] and, the geographic distribution of research, and the research methods used in the cane rat research were assessed using descriptive statistics as most data were count. Most of the cane rat research works were published in online journals with or without impact factor. To assess what factors influence publishing in a journal with or without impact factors, we used generalized linear mixed models (GLMMs) with a binomial error term and logit link function implemented in the R-package'lme4' [7]. Before running the model cells with NAs (i.e. Not Applicable which represented non-journal article publications e.g. conference paper and book chapters) from a response variable- Journal impact factor (JIF) i.e. whether a paper was published in a journal with (scored as 1) or without impact factor (scored as 0) were excluded. We also, excluded countries with very few data points ($n<8$) in the model analysis, i.e. Cameroon, Zimbabwe, USA, Japan, Germany, Gabon, Senegal, France, Benin, and Kenya to remove noise. To analyze these data, we fitted the mixed model with the 'glmer' function implemented in the 'lme4' package where four fixed factors: author nationality, number of co-authoring countries, geographical location, and number of authors were used. As different publications were covering similar research topics and research areas, we included a research theme and country of study as random effects in the model. We examined the relative contribution of each fixed effect by using step-wise deletion of non-significant variables using 'drop1' function and tested the model significance with the Chi-square test [7]. We obtained model confidence intervals around variables showing statistical significance in the minimum adequate model using the Wald-method [7]. To select the most parsimonious model, we compared two competing models using the Akaike information criterion (AIC), where the model with the smallest AIC value was selected. Furthermore, we built a prediction model of the significant fixed effect using a prediction package to understand how best the independent variable predicted the response variable in the model. The prediction was plotted using the 'ggplot2' function. Finally, we calculated the squared correlation between the response variable and the predicted values to understand the model variance explained by the fixed effect [16].

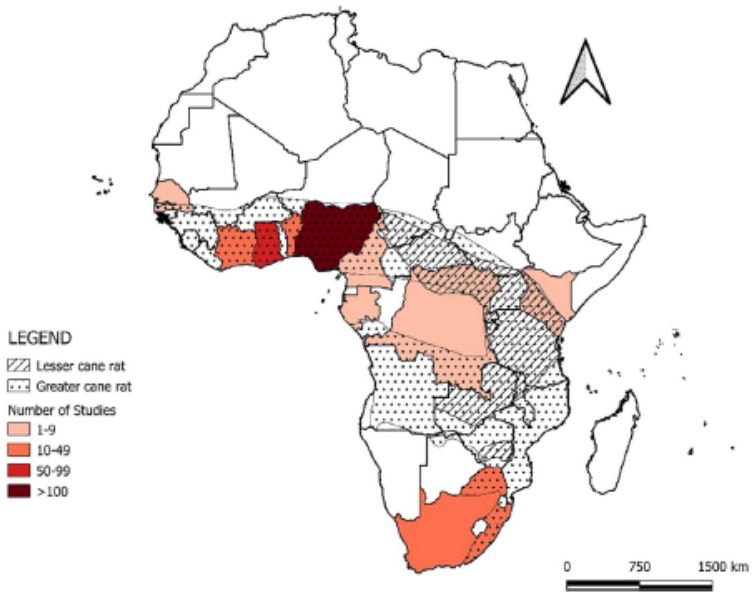

Fig. 1. Distribution of the published articles on cane rat in countries where samples were sourced and where first author of the publication was based. The cane rat species ranges presented here were constructed based on Skinner and Chimimba [43] and map used was sourced from Natural Earth, which is an open access map source.

Results

Spatial coverage, thematic pattern and research methods used

We analysed 308 papers over a period of 56 years mostly distributed in West and Central Africa (Fig 1). The cane rat studies were reported from 14 countries in four continents namely Africa, Europe, America, and Asia. Most published research studies were biased on study species, methods, and themes. Most papers (99.35%, n = 293) focused on a single species- the greater cane rat, mostly used captive individuals (87.99%, n = 271), and fewer were based on field surveys (17.21%, n = 53). Only twenty studies researched field-derived and captive animals (6.49%, n = 20) together (Fig SM2). The publications covered 29 themes and 36 subthemes with the popular theme and subtheme being ecology and reproduction respectively (Fig 2). Most studies (96.76%) had their main goal to improve the domestication of the cane rats in these regions with only five studies (3.24%) targeting to improve cane rat conservation in the wild.

Research trend, collaborations among countries and journal outlet

The publications on cane rats research have increased significantly over the study period ($z = 3.6056$, n = 39, $p = 0.0003$, Fig 3). The first topic to be published was on biodiversity followed by physiology in the same decade. About one-third of all the published papers were conducted in the first four decades (1964–2008) followed by a sharp publication spurt in the last decade (2009–2020) with almost two-thirds of the publications documented within this period. About 85.37% (n = 263) of the cane rat studies were conducted by researchers from the same country particularly from the west and central Africa and 14.61% (n = 45) was conducted by several collaborating countries from Africa and Europe, Asia and America (Fig. 4). About 2.59% (n = 8) of the papers was conducted by authors from non-African countries. The reviewed papers were published in 217 different outlets including 181 journals, 11 theses, 6 proceedings, 1 symposium, and 2 books. Most of the journals claimed to be international in coverage (68.06%) and few were regional or national (31.94%).

Factors influencing choice of a journal outlet for the cane rat research

Author nationality was found to have the strongest influence on the choice of the journal (Table SM2). Authors from South Africa had significantly higher probability of publishing in the journal with high impact factor (2.4079 ± 1.1441,

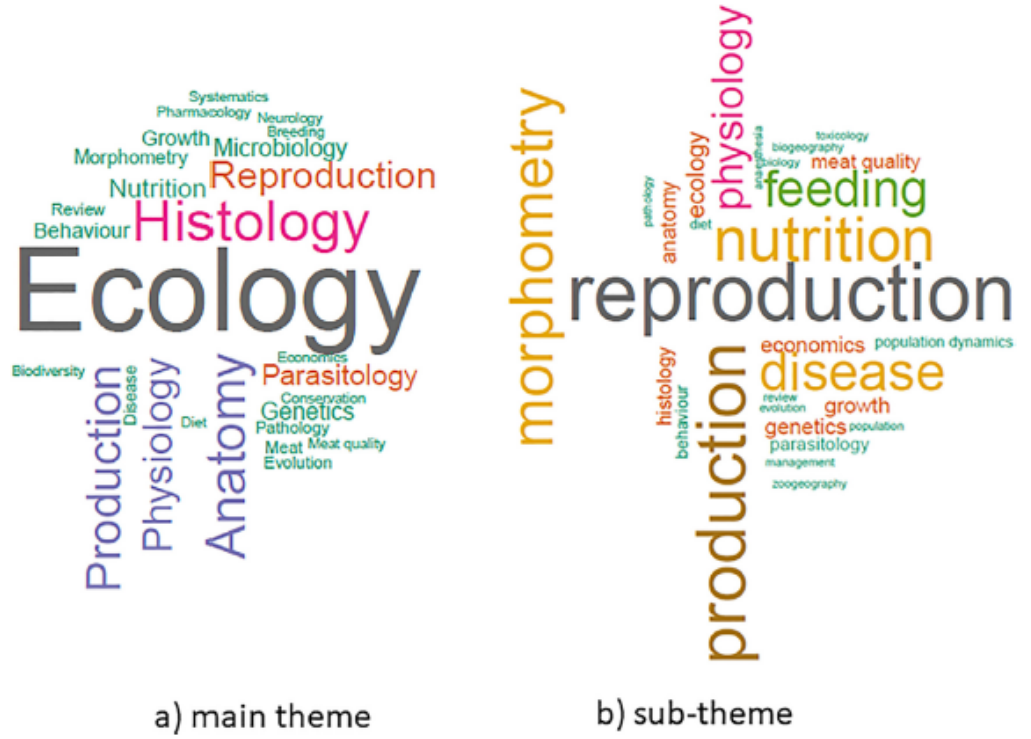

Fig. 2. Word clouds of the main theme (a) and sub theme (b) investigated in the cane rat literature. The size of each word increases with the increase in its frequency in the data.

$z = 2.105$, $p = 0.0353$) and while authors from Nigeria had lower probability (mean = -1.0016 ± 0.4943, z= -2.026, $p = 0.0428$, Fig. 5). There was no apparent effect of other factors included in the model such as the geographic location of research, research collaborator (co-country) and, number of authors in publishing in a journal with or without impact factor.

Discussion

This research aimed to understand the current breadth of cane rat research and to explore research gaps and examine factors that influence the publication of the cane rat research in the chosen journal. We found that publications on cane rats have increased significantly in the last 56 years, particularly in journals without impact factor. Most research focused on domesticating cane rats and were mostly in West and Central Africa. More studies focused on ecological themes particularly reproduction to improve cane rat domestication and no empirical studies conducted on the biogeography of the cane rats across its geographical range in Africa or on the species feeding ecology in the wild. Further, the available literature was also biased in favor of ex-situ rather than in situ conservation of the cane rat species. Furthermore, we found author nationality significantly influenced publication of the cane rat research in journals with an impact factor. Authors from South Africa were most likely to publish in high impact factor journals than authors from countries in west and central Africa where most of the cane rat research was conducted.

The geographical bias in cane rat research observed in this study is surprising given that both cane rat species have a much wider distribution in Africa [43]. The concentration of studies in west and central Africa could be attributed to increasing efforts to domesticate cane rats for bushmeat consumption cultures embedded among the residents and its impact on the wild population in these regions. Several studies on bushmeat harvesting across west and central Africa have reported severe declines in harvested wild species [8,12]. For example, 40% of primate species in West and Central Africa are now threatened with extinction due to hunting for bushmeat and habitat destruction through deforestation [11,19], several other non-primate wild mammals continue to experience severe range contraction and population declines in these regions due to hunting for commercial bushmeat trade and subsistence consumption [10,38]. Persistent harvesting of wild species

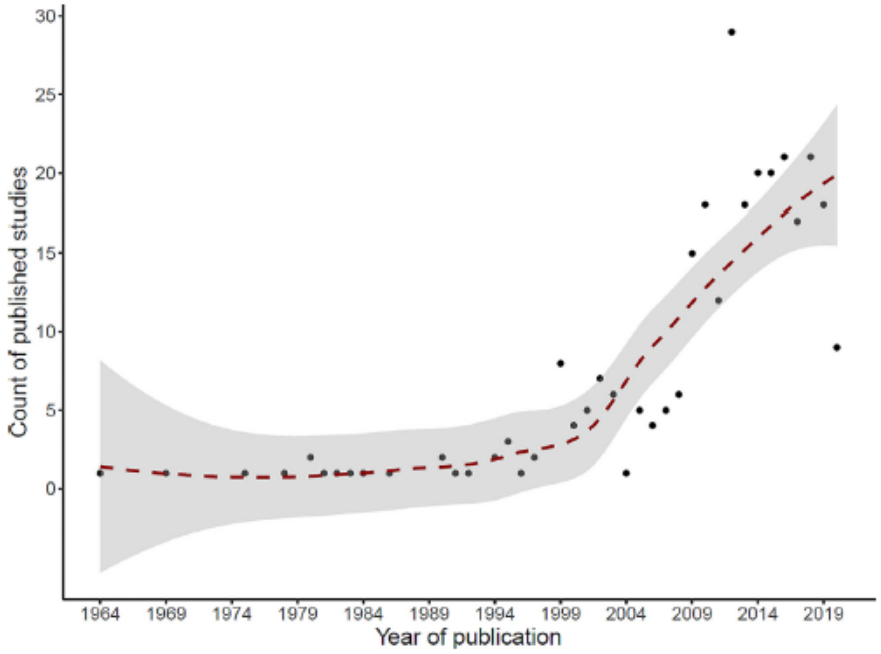

Fig. 3. Trend of publication on cane rat studies from 1964–2020. Shaded area indicates 95% confidence limit of the estimates.

is likely to disadvantage smaller and highly reproducing mammals such as cane rats in these regions [40]. This may have prompted more studies to improve alternative avenues for sourcing wild meat protein through domestication of wild cane rats [2,4,15].

Further, the quest to provide wild meat protein may have increased studies on reproduction, nutrition, production, and disease associated with animal husbandry observed in this study. Cane rat meat is considered a delicacy among several West African communities [2]. The species high reproductive potential together with the small area and relatively low financial capital needed to establish indoor husbandry [4] may have attracted wider domestication in the west and central Africa [15]. This is supported by the observed large number of publications on cane rat from Nigeria and Ghana which have many commercial cane rat farms [14,50]. The increased rate of publications on the cane rats, particularly in the last ten years (2009–2020) provides more evidence to the increasing interest in generating information to improving the cane rat farming programs in these west African countries.

The scant research on cane rat in east and southern Africa, on another hand, could probably be because the species has not fetched priority among the wildlife species normally consumed for bushmeat despite the species being a potential game animal for commercial wildlife zoos and ranches. Most illegal hunters in East Africa prefer larger mammals than smaller ones which provide them with bulky meat and high economic gains and there is a tendency for disregarding small mammals in poaching trips especially in communities where poaching for commercial gain is common [28,39]. Despite this, however, poaching of cane rats for subsistence is still common in several communities across the African savannah [29,38], suggesting the species importance on the dietary protein menu of the local communities in the continent. An alternative explanation could be that there is still low awareness among the local human population on the protein and economic potential of the cane rats which may have been due to limited knowledge about the species in East and southern Africa. We argue that the cane rat species could be utilized through game ranches and zoo farming in sub-Saharan savanna regions alike to alleviate the increasing wild meat demands especially in the rural communities where poaching for bushmeat is a growing conservation problem [37,48]. Notwithstanding this, however, there is a growing urge in countries in east and southern Africa such as Tanzania, Zimbabwe, and South Africa to improving the economic development of the local people through investing in wildlife businesses such as game farming and ranching. In Tanzania for example, this urge has been emphasized by his Excellence President Magufuli at several public addresses in 2019 and recently at the inauguration of the 12th Parliament held on 13th November 2020 (pg 47–49) in Dodoma that local Tanzania citizens should proactively engage in commercial wildlife ranching and farming to ensure the economic benefit from the wildlife resources [35]. Consequently, this has been mainstreamed within the government sector particularly through the Tanzania Wildlife Authority (TAWA) by

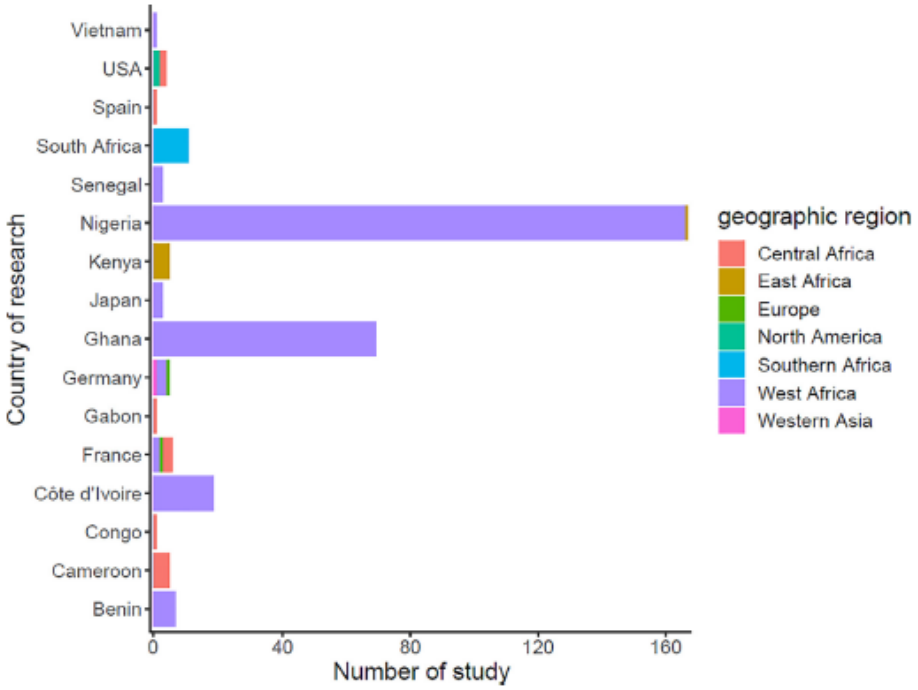

Fig. 4. Collaborating countries in conducting research on cane rat in four continents: Africa, Asia, Europe and America.

formulating game ranching/farming policy and enacting laws and regulations that will guide the implementation of wildlife farming and ranching in Tanzania [47]. It remains unclear, however, how the cane rat species will out-compete other wild species in such programs to remain sound and priority animal species among the local wildlife farmers and ranchers. This is particularly concerning given none of the two cane rat species has been studied in Tanzania or any dry savanna country to help guide the potential farming and ranching business.

There was a high number of cane rat research publications from countries in west and central Africa in local national, regional, and internationally-claimed journals. This may be attributed to three reasons. Firstly, the publication in local national journals could have been contributed by the financial limitations by the authors of the papers to publish in high-quality international journals that charge publication fees, a common impediment among many developing country researchers [18,21]. For instance, many papers from these regions were authored by the nationals of Nigeria and other West African countries both as single or multiple local authors and without collaboration with authors from outside these countries or regions. This has the potential of limiting wide readership and citations as most of such papers may only be accessed and utilized locally [42]. Secondly, the publishing in the local national journals could be because the results being communicated were less competitive in terms of their quality to be published in supposedly good international journals where publication space is often limited and that only good quality papers get through the usually stringent screening and review process of such journals [21,42]. Due to the lack of rigorous review, such local journals do not choose what they publish. For example, previous review research by [27] reported some duplicates in publications by authors from different countries repeatedly publishing similar research, a problem potentially caused by limited online accessibility by these local journals. Thirdly, although publishing in local journals does not necessarily imply low-quality research as some publish important and practical information that would be declined in the international journals [21], the perceived bias in some reviewers and editors in an international journal published in developed countries in judging articles from developing countries [22,45] could have increased the authors' propensity in publishing the cane rat research in the local national journals.

The strong influence of the author's nationality in publishing the cane rat research in high impact factor journals echoes the importance of collaboration with international authors from outside in furthering science. Although our mixed model analysis removed papers published from the developed countries such as the USA, Spain, and Japan due to low numbers of data points, however, the papers collaboratively authored by West African nationals and these developed countries were published in international journals with impact factors - a proxy of good quality [46]. Also, papers published by authors from South Africa had the highest probability of being published in high impact factor journals than papers authored by

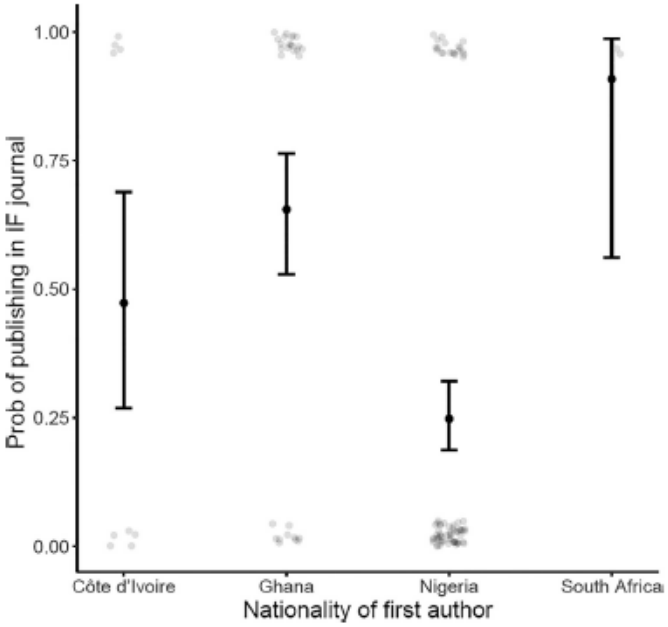

Fig. 5. Probability of publishing cane rat papers in impact factor journal. South Africa showed high probability of publishing in the journal with impact factor while Nigeria showed the lowest probability of publishing the paper in the journal with impact factor.

Nigeria and Cameroon nationals alone. The quality of research and journal attributes (i.e. strong editorial board and review process, impact factors, etc.) are currently acknowledged to influence the choice of a journal when publishing research [46], this study has added author nationality on the list of factors that influence the publication of the cane rat research in a journal with or without impact factors. The author nationality has nothing to do with race [41], but underscores the quality of the research and its output [44] that are strongly related to the wealth of the nations of the authors [18,49]. Furthermore, an alternative explanation to the authors from South Africa publishing in journal with impact factor could be caused by local institutional requirements and or regulations that compel them to do so. Such requirements could probably be non-existing in some institutions in West and Central Africa.

Most of the published articles were based on the greater cane rat in captivity but also focused on observational study or surveys. Further, many of the survey studies also relied on the direct questioning of the cane farmers to document the economic gain associated with animal husbandry. The biases on only one species and captive breeding programs may limit the wider potential utilization of both cane rat species in other regions of Africa. The observational studies on the captive cane rat provide limited information, particularly when considering in situ conservation and the implementation of the game ranching or farming in the savanna regions in east and southern Africa. This is because findings from such studies can hardly be generalized for all the cane rat species across their geographical range due to local variability in habitats and weather conditions that may have substantial impact on the reproduction and local population dynamics [20,24].

Conclusion and future directions

This study has revealed important insights into the available and accessible literature on the cane rat that is presently available. The study research indicates strong geographic, thematic, and species biases which most research outputs have been published in local national, or regional outlets where wider readership and thus utilization of the research findings are suggestively limited or low. There was generally an increasing trend in the publication of the cane rat research over the last 56 years and this trend looks set to increase as the species get widely accepted as a source of meat protein in many societies in the west and central Africa. However, to gain wide use as a commercial game species in other countries particularly of the dry savanna regions, there still a few issues to be addressed for this species to compete with the wild ungulates in the game/ranching programs across the southern African region. First, new research should focus to understanding the biological and ecological aspects of both cane rat species in the wild to inform any potential ranching/farming program in East and Southern Africa regions. Currently, there is a lack of understanding of the local species biogeography across its range states, the food spectrum of the lesser cane rat species [43], and the genetic information of isolated populations within a country

are yet unknown. Secondly, there appears to be a potential cultural barrier in meat consumption among tribes in the region (e.g. [28]). A study focusing to understand how the cultural norms among the local societies in the east and southern Africa shape the consumption patterns of the cane rat bushmeat will be a useful contribution to the development of the cane rat farming/ranching industry in these regions. Such research will also provide information on where the markets and demands for the cane rat bushmeat are. Strong scientific information generated in the region will improve the conservation of the cane rat species insitu and expedite the wide use for improving the food security and cash income among many societies in Africa. Also, a well-grounded cane rat ranching/farming industry will directly reduce the bushmeat poaching pressures on the protected areas in the savanna regions.

Declaration of Competing Interest

There is no competing interest.

CRediT authorship contribution statement

Shadia I. Kilwanila: Conceptualization, Data curtion, Formal analysis, Validation, Writing – original draft, Writing – review & editing. **George M. Msalya:** Writing – review & editing. **Charles M. Lyimo:** Writing – review & editing. **Alfan A. Rija:** Conceptualization, Formal analysis, Validation, Writing – review & editing.

Acknowledgements

The authors would like to thank anonymous reviewers for the comments on this manuscript. Shadia I. Kilwanila thanks African Centre of Excellence for Innovative Rodent Pest Management & Biosensor Technology Development (IRPM&BTD) for PhD scholarship at the Sokoine University of Agriculture.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sciaf.2021.e00785.

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CHAPTER THREE[2]

3.0 Mitochondrial genetic diversity of the Greater Cane rat (*Thryonomys swinderianus*) populations from the Eastern Arc Mountains ecosystem, Tanzania

Molecular Biology Reports
https://doi.org/10.1007/s11033-022-07823-1

ORIGINAL ARTICLE

Mitochondrial genetic diversity of the Greater Cane rat (*Thryonomys swinderianus*) populations from the Eastern Arc Mountains ecosystem, Tanzania

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Received: 14 February 2022 / Accepted: 26 July 2022

Abstract

Background Management of most herbivorous small mammal species considered to be pests in Africa is still challenging partly because of the paucity of information on their biological traits that would help to manage their destructive impacts. This gap also precludes the potential for tapping species with potential food-value to improving the economy of rural communities through, for example, sustainable game farming programs in Africa. This study investigates the genetic diversity and population demography of the African Greater Cane rat (AGC), a rodent pest of crops and game species inhabiting two isolated blocks of the Eastern Arc Mountains (EAMs), Tanzania to contribute to the species management and conservation.

Methodology and results We used non-invasive sampling techniques and DNA sequencing of the D-loop region of MtDNA (515bp) from 46 cane rats (*Thryonomys swinderianus)* samples to characterize the genetic diversity and structure of the species and potential population threats faced in natural habitats. We found 25 haplotypes:15 from Uluguru and 9 from Udzungwa mountains populations, containing 49 polymorphic regions (32 parsimoniously informative and 17 singleton sites). Haplotype diversity (range: 0.849–0.995) did not differ substantially across populations but the median haplotype diversity for Udzungwa South was overall lower than for other populations. Nucleotide diversity averaged 0.00641, 0.01528, 0.0111 and 0.01313, respectively for Udzungwa South, Udzungwa North, Uluguru Rural and Uluguru Urban, suggesting high genetic diversity within the four populations. Analysis of molecular variance (AMOVA) indicated significantly high genetic differences between the four populations (FST = 0.16, p = 0.00098) whereas neutrality test (FU's Fs) values were negative, indicating historical population expansion. Similarly, the Bayesian skyline analysis indicated a recent demographic expansion suggesting limited bottlenecks in the recent past in this population.

Conclusions Our results show the AGC population in EAMs consists of four distinct populations which have experienced a recent population expansion, especially among the urban population due perhaps to influence of urbanization process that may have favored assisted species movements across the rural-urban landscapes. Future research should focus on understanding impact of geographical isolation on the genetic structure and diversity of this species.

Keywords Conservation threats · Mt DNA D-loop region · Geographic isolation · Haplotype diversity · Phylogenetic pattern · Rodents

Part of this abstract has been published in a conference proceeding (link: https://www.sua.ac.tz/sites/default/files/documents/research/SUA-SCIENTIFIC-CONFERENCE-2021-BOOK-OF-ABSTRACTS-AND-CONFERENCE-PROGRAMME.pdf).

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Published online: 15 September 2022

[2] The contents of this chapter were published as an article in the journal Molecular Reports

Introduction

Control of rodent pests in the world remains challenging to date due to several issues associated with intrinsic population expansion of pest species and the climate change impacts that demand constant adaptation of pest management strategies [4, 9, and 22]. The challenge is also compounded by limited knowledge on important biological and ecological traits of some pest species. Despite this, rodent pests have remained a substantial threat to food security and pose significant health risks to humans across the globe [38, 39]. The African Greater cane rat-AGC (*Thryonomys swinderianus*) is considered to be an agricultural crop pest species across its range areas in Africa [17] but management of the pest impacts in most rural areas in Africa remains elusive and less well-documented partly because of the difficult in establishing species population size in the wild as well as other important biological information that would inform the potential control strategies. Most available knowledge on the biology and ecology of this species is strongly biased in the wet regions of West Africa and mostly from captive individuals [17]. However, similar information is currently lacking in most savanna and montane regions with heterogeneous landscape topography in eastern and southern Africa where the species also widely distributes [36] . Understanding the species genetic diversity and population trends may be useful for planning the management and conservation of the species in the wild.

The Eastern Arc Mountains (EAMs) in East Africa: one of the global biodiversity hotspots harbors African Greater Cane rat populations across various habitats ranging from lowland savanna to montane forests. Despite its pest nature, the habitats of this species across its range areas are fraught with pressure from various human activities such as land clearance for agriculture [1], logging [7] and wildfires [16]. Further, the AGC populations in these habitats are also under pressure of trapping for bushmeat consumption [17, 26]. Also, human development activities within biodiversity hotpots pose additional pressure on the cane rat population, mainly through potential population fragmentation and assisted species movements across urbanization landscapes. Such threats have the potential to influence on the species genetic traits and population dynamics. For example, vegetation structural simplification and exploitation by humans may lead to decreased small mammal diversity [27], genetic diversity [13, 14and 24] and change in population size and community structure of animals and plants [29, 30]. Despite this, our understanding of how these threats may have shaped the genetic diversity and structure of the AGC species in Eastern Africa remains low, limiting potential species management and utilization initiatives to improving the local economies through game farming or ranching practices [17].

The AGC populations within the EAM blocks are naturally isolated due to large spatial geographic distance (at least 100km apart) between them [7] potentially affecting species dispersal patterns and therefore, gene flow [11, 34, and 41]. Given greater spatial isolation, we expect the AGC populations from different blocks to show greater differentiation (i.e., spatial genetic divergence) due to drift and dispersal limitations. Further, selection by environment is expected to limit gene flow in the AGC populations within individual mountain block that inhabit similar or different environment (e.g., at different elevation gradients), a pattern observed on other mammals elsewhere [10, 20, 34]. Furthermore, selection by environment may happen due to anthropogenic activities (e.g., farming that fragment habitats) and urbanization (i.e., loss of or reduced habitat quality). Thus, urbanization is expected to cause greater genetic differentiation between the AGC populations inhabiting rural and relatively urbanized landscapes within the Uluguru mountain block. However, a different pattern of gene flow may occur in the Uluguru Mountains between the rural and urban population due to accidental introduction of animals caught from elsewhere brought in the urban for food. The combined effect of the urbanization and hunting pressure on the AGC population genetic structure is yet unknown but could be greater as has been observed in several other species elsewhere [3, 24, 33].

Here we use the mtDNA D-Loop to characterize the genetic structure of the AGC population inhabiting spatially isolated habitats (i.e., Udzungwa, Uluguru Rural and Uluguru Urban) that are also faced with local hunting and urbanization pressure.In this study we use Uluguru Rural and Uluguru Urban to refer to areas sorrounding Northern side of the Uluguru Mountains in Morogoro Rural and Urban Districts respectively. We aimed to answer three questions; (i) what is the distribution pattern of haplotypes in AGC populations in Udzungwa and Uluguru Mountains? (ii) Which AGC habitat shows a higher level of polymorphism? and (iii) How far are the sampled populations different genetically and is there evidence for population expansion or bottlenecks in the AGC populations due to the existing threats? These data provide insights into the biology of the AGC and the potential impacts of the existing threats on its population.

Materials and methods

Study area description

This study was conducted in two mountain blocks: Uluguru (37°65'E, 07°10'S) and Udzungwa (36°53'E, 07°50'S) within the Eastern Arc Mountains (Fig.1). The Uluguru Mountains cover approximately 256.2 km^2 with altitudinal range of 600–2,700m above sea level [25]. Rainfall and temperature in the mountains vary with altitude and aspect. The western side of the mountains receives rainfall ranging from 650 to 1200mm to 800–1700mm at altitudes of 600 and 1600m a.s.l., respectively. In contrast, the eastern side receives higher rainfall ranging from 1600 to 3000mm and 2700–2900mm at altitudes of 600 and 1600m a.s.l., respectively. The mean temperature is about 24.3°C with maximum of 34°C in December and a minimum of 21°C in July [25].

Udzungwa Mountains on the other hand cover approximately 10,000 km^2 in South central Tanzania between 200 and 2600m altitude and consist of three protected areas: Kilombero Nature Reserve, Udzungwa Mountains National Park and Udzungwa Scarp Nature Reserve. The Udzungwa Mountains have a unimodal rainfall pattern, with the wet season between November and May. Although cane rats are also widely distributed in tropical savannahs, there is no current evidence to suggest that they can move between the different blocks of the EAMs, unless they are assisted by humans through accidental introduction for bushmeat consumption. Cane rats prefer non-forested habitats of mostly grass distributed along rivers and lakes and also occupy moist savannas. Cane rats in the Udzungwa and Uluguru Mountains are more likely to be found in human disturbed habitats where forests have been cleared.

Data Collection

Data collection in the Udzungwa was conducted in localities lying at altitudes between 300 and 2600m.a.s.l. In the Uluguru Mountains, samples were collected at altitudes ranging from 300 to 1800m.a.s.l. Areas above 1800m.a.s.l. in the Uluguru Mountains are protected natural forests, which are uninhabitable for cane rats. We relied on local expert knowledge on the distribution of cane rats in the study sites to collect these data because there is no documented study on the AGC in Tanzania or Eastern and Southern Africa. We used four local hunters who use nets to catch the animals for food to get the samples for this analysis. For the purpose of this study, the hunters were first trained on how to handle the animals when caught in nets to ensure that the samples were collected in the most appropriate way without causing harm to the animals. Each captured animal was carefully removed from the net and placed in a separate cotton bag to defecate. The fecal samples were picked with forceps and placed in 1.5 ml falcon tubes containing 80% ethanol and were transported to the lab at Sokoine University of Agriculture for further analysis. In total, 50 fecal samples of individual cane rats were collected from Uluguru (n = 30) and Udzungwa Mountains (n = 20) between 2019 and 2021. In the Uluguru Mountains, the samples were collected within human-dominated landscapes (i.e., urban areas- ULU (n = 20)) and in the rural areas henceforth, Uluguru rural (10) while in Udzungwa samples were collected from Udzungwa South (n = 10) and Udzungwa North(n = 10). The caught animals were later released at the point of capture. There were no accidental fatal cases among the animals associated with the capture process.

DNA extraction from fecal samples, PCR amplification and sequencing

In the laboratory, the collected samples were stored at – 20°C. DNA from each sample was extracted from 5g of fecal samples using Zymo research kit following manufacturer's protocol and quantified using Nano Drop Spectrophotometer (Thermo Scientific, USA). Mitochondrial displacement loop (D-loop) was amplified in a Polymerase Chain Reaction (PCR) using both forward (5'-CCAACTCCCAAAGCTGATGT-3') and reverse (5'GGCACCAACATCATCACAAA-3') primers following procedures described in [2]. The PCR mixture contained 0.75 U of *LA-Taq* DNA polymerase (TaKaRa, Shiga, Japan), PCR buffer, 400 µM of each dNTP, 0.4 µM each of forward and reverse primers, and 20 ng of template DNA in a total volume of 15µl. PCR cycling conditions consisted of an initial denaturation at 95°C for 2min, followed by 35 cycles at 95°C for 30s, 55°C for 30s, 74°C for 1min and a final extension of 74°C for 10min. To check amplification efficiency aliquots of 5µl of the PCR products were electrophoresed on 1.5% agarose gel. DNA bands were visualized after Ethidium Bromide staining under UV light. The expected size was determined in relation to a DNA size standard. The PCR products were Sanger sequenced by Macrogen (Europe) using standard protocols involving chain termination method as described in [35].

Data analysis

Forward and reverse sequences were first edited manually and later aligned to get the consensus sequences using

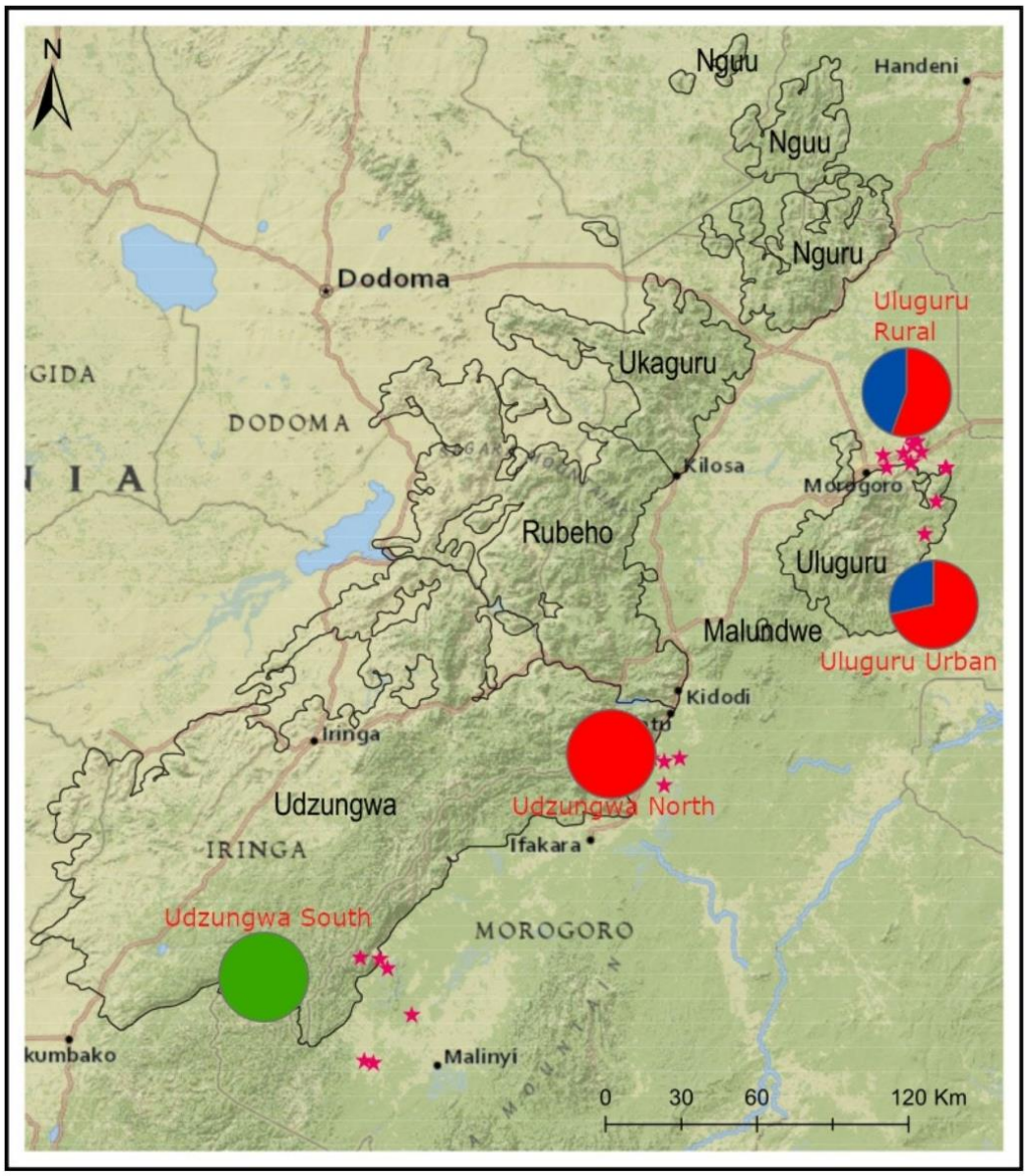

Fig. 1 Spatial distribution of haplotypes of African Greater Cane rat populations in the Eastern Arc Mountains in Tanzania and sites (red stars) where cane rat samples used in this study were collected. Pie charts indicate haplotype distribution across three clades: Clade A (green) representing unique haplotype found in Udzungwa South, Clade B (blue) indicating haplotypes shared between Uluguru rural and Urban and Clade C (red) showing haplotypes commonly found in Udzungwa North, Uluguru urban and rural Uluguru

BioEdit program version 7.0.9.0. After quality control procedures, 46 sequences of mt-DNA remained for analysis and were deposited in NCBI gene bank with accession number OM475549-OM475594. To establish the genetic diversity

Table 1 Genetic diversity indices of population neutrality across cane rat population in four sites

Population	Haplotype (h)	Haplotype Diversity (Hd)	Nucleotide diversity (Pi)	Tajima's D	Fu's Fs statistics
Udzungwa South	4	0.900 ± 0.051	0.00641	-0.99083 ± 0.10	-0.176 (0.343)
Udzungwa North	5	0.933 ± 0.015	0.01528	0.35109 ± 0.10	0.419 (0.396)
Uluguru Rural	7	0.933 ± 0.062	0.01118	0.74663 ± 0.10	-0.403 (0.234)
Uluguru Urban	9	0.937 ± 0.029	0.01313	-0.44699 ± 0.10	-2.867 (0.033)

of AGC, indices including position and number of polymorphic sites, number of haplotypes, haplotype diversity and nucleotide diversity were estimated using DnaSP program version 6.12.03 [31].

The evolutionary history of AGC was inferred by Neighbor-Joining (NJ) phylogenetic tree using MEGA program version 6.0. Neighbor-Joining (NJ) phylogenetic tree was constructed using T92 + G substitution model which was identified as the model with the lowest Bayesian Information Criterion (BIC) i.e., 3180.62 and gamma distribution (G) of 0.07 [18]. The G-value was used to compute the NJ.

We checked robustness of the nodes of the phylogenetic trees and the variation among sites using bootstrap modelling with 100,000 replicates. To produce haplotype Media Joining network to depict the phylogenetic and geographical relationships of haplotypes, we used outputs from DNA alignment version 1.3.3.2 that were imported in Network software version 4.6. The haplotype network was computed under haplotype pairwise differences, giving the number of mutation steps between haplotypes. Furthermore, to determine the population structure of the AGC, we used Arlequin program version 3.5.2 to calculate the Pairwise FST and Analysis of Molecular Variance (AMOVA) using haplotype frequencies to examine the differences among the studied sites. AMOVA was calculated to quantify the partitioning of genetic variation present within and between populations using 1000 permutations.

The level of genetic differentiation was determined using Weir and Cockerham's (1984) estimation of Wright's (1951) fixation index- pairwise FST. Computation of the genetic distance for each population was calculated at $P < 0.05$ with the algorithm suggested by [12], in Arlequin program vers. 3.5.2.

Based on the genetic structure found, demographic history of the African Greater Cane rat was examined as each site presents a single genetic population. The demographic history was inferred by Tajima's D and Fu's Fs indexes and their corresponding p-values to detect departures from neutrality using DNASP [31]. The demographic history of AGC was further examined by Bayesian Skyline Plot (BSP) implemented in BEAST2 [5] to check potential recent population expansion or bottlenecks. The BSP analyses were run using HKY + G model which is closest to T92 + G model in MEGA. We ran two independent runs of 10^9 iterations of the Marcov Chain Montel Carol (MCMC) process, sampling every 100,000 generations. All other settings were left as default. Two independent runs with effective sample size > 200 were combined using Log Combiner with a 10% burn in. The Bayesian skyline plots were generated in Tracer [28] to visualize pattern of population trajectory.

RESULTS

Diversity indices of cane rat populations

We found 515bp of the Mt-DNA D-loop from 46 individual greater cane rats from the sampled populations. Forty-nine polymorphic sites were identified translating to 25 haplotypes (Table S1). The most frequently shared haplotypes in Uluguru urban and rural populations were the haplotype 12 (24.4%), followed by haplotype 13 and haplotype 14 each constituting 20%. Two other haplotypes were shared by at least two populations from Uluguru Urban and Udzungwa North. Four haplotypes (16%) were unique to Udzungwa South population.

High mean haplotypes diversity (0.937 ± 0.029) of the cane rat population was observed in Uluguru urban while low mean haplotype diversity (0.900 ± 0.051) was observed in Udzungwa South (Table1). Nucleotide diversity was high (0.01313) in Udzungwa North and low (0.00641) in Udzungwa South population (Table1). There were substantial variations in the haplotype diversity across the studied populations where the Udzungwa South population had lower diversity than other populations (Table2; Fig.2). Further, results from AMOVA based on haplotype frequencies revealed 59.70% of the genetic variation occurred within populations whereas only 40.30% of the genetic variation occurred between populations (Table2). Fixation index (FST) suggested evidence of significant genetic divergence of the populations in the four localities: Udzungwa North, South, Uluguru Urban and Rural (FST = 0.40297, P < 0.00001; Table2).

Table 2 Molecular Variance (AMOVA) of African Greater Cane rat population from the three study sites indicating significant difference between samples sourced from various localities

Source of Variation	df	Sum of Squares	Variance Components	Percentage of Variations	FST	P Value
Between Populations	3	71.452	2.14201 Va	40.30	0.40297	0.00001
Within Population	42	133.287	3.17349 Vb	59.70		
Total	45	204.739	4.86248			

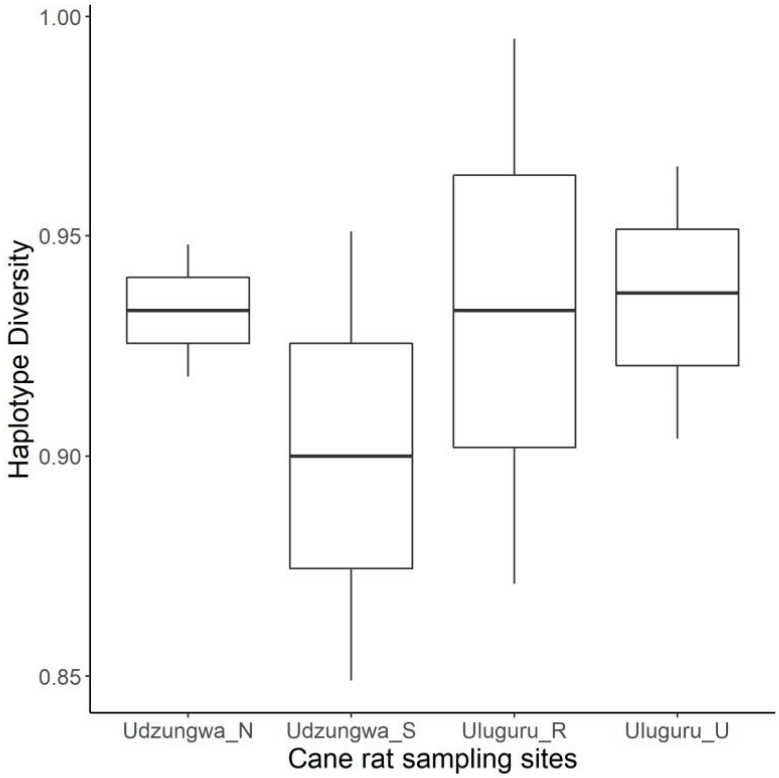

Fig. 2 Patterns of haplotype diversity of four cane rat populations based on field samples collected from the study area. Boxplot shows median diversity of each surveyed population from Eastern Marc Mountains, Tanzania

Phylogenetic structure, demography and evolutionary relationship of the AGC

The neighbor joining phylogenetic tree showed the evolutionary history of AGC had three clusters composing populations from four localities (Fig.3). The haplotypes clustered into three clades (or haplogroups) depending on the geographical location where the samples were sourced. Clade A (n = 4, 16%) constituted individuals from Udzungwa South alone. Clade B, the second largest haplogroup (n = 6, 24%) had individuals from both Uluguru Rural and Uluguru Urban. Clade C, the largest haplogroup (n = 15, 60%) had individuals pooled from Uluguru Urban, Uluguru Rural and Udzungwa North. The structuring of the AGC population into three clades also coincided with the Median joining network that revealed similar grouping of the populations (Fig.4). Further, examining the number of mutations across the population structure, we found Clade A was separated by 1–8 mutations, Clade B separated by 1–13 mutations whereas in Clade C the number of mutations between the haplotypes ranged from 1 to 21 contributing highest proportion of mutations (i.e.,1–12 mutations).

The estimated fixation index (FST) for each population showed significant genetic divergences between Udzungwa South and Uluguru Rural populations (FST = 0.72284, p = 0.0001), Udzungwa South and Udzungwa North (FST = 0.70524, p = 00238) and Udzungwa South and Uluguru Urban populations (FST = 0.67988, p = 0.0001, Table3).

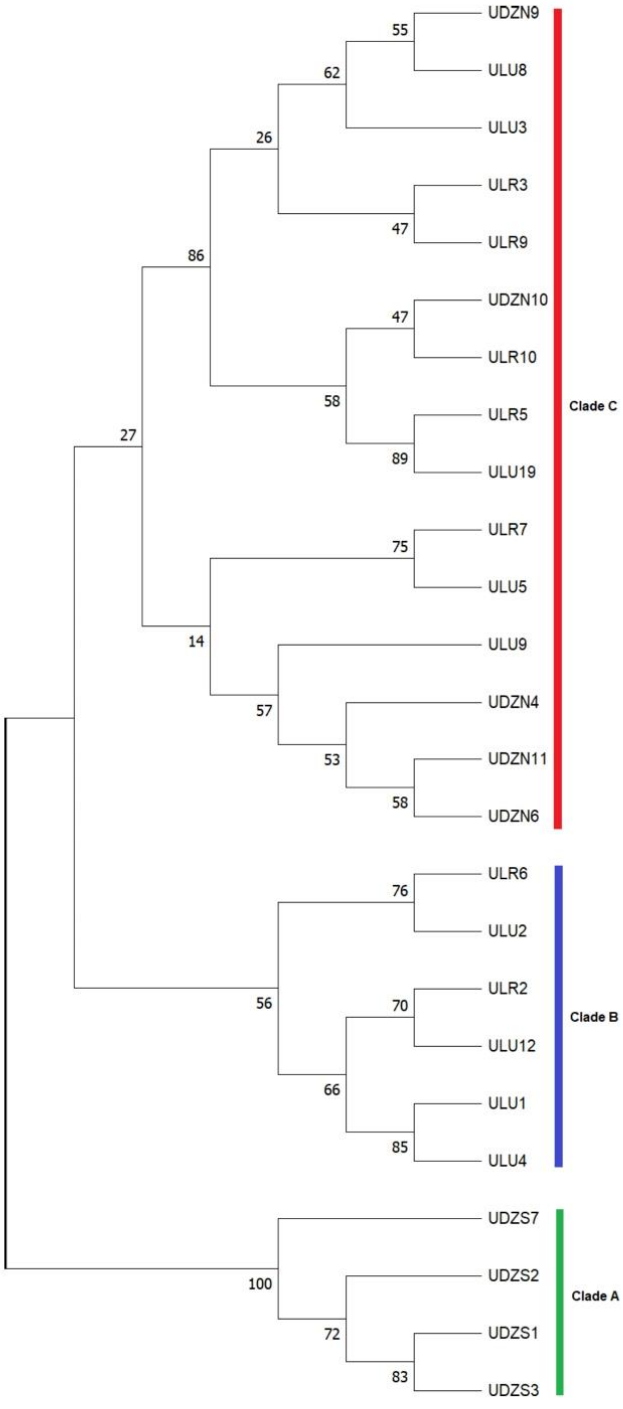

Fig. 3 Neighbour joining phylogenetic tree of mitochondrial D-loop nucleotide sequences based on 25 haplotypes indicating evolutionary history of the greater cane rat populations in three clusters. The numeral at each branch indicates the bootstrap value of replications

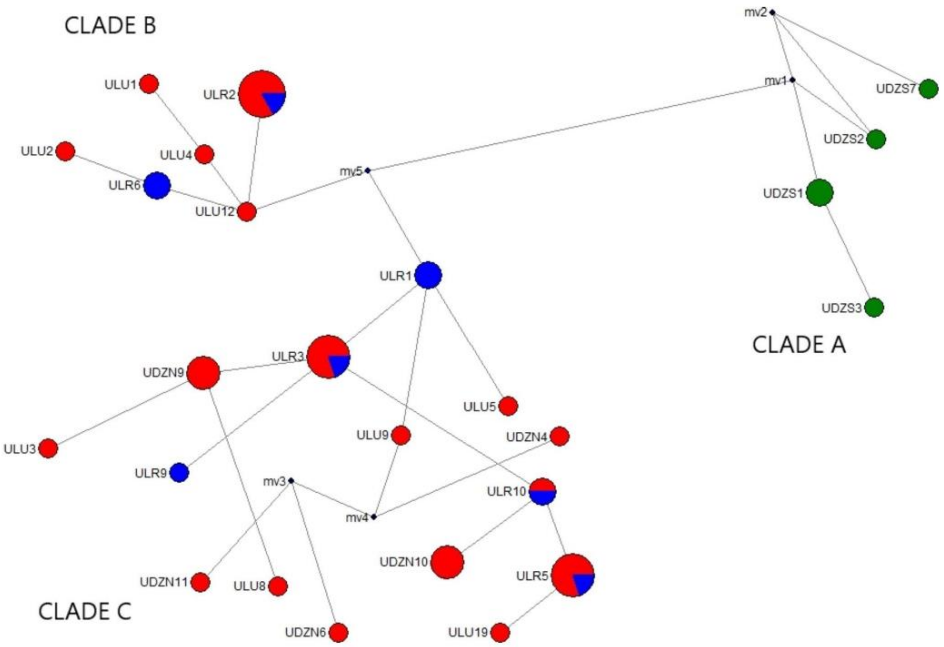

Fig. 4 Median-joining network of 25 mt-DNA D-loop haplotypes observed in AGC populations inhabiting Udzungwa and Uluguru urban and rural areas. The network is based on the polymorphic sites of the mitochondrial DNA D-loop region. Size of circle is proportional to the haplotype frequencies. Blue dot circles represent median vectors connecting indirectly-related haplotypes. The numbers on the line correspond to mutational positions connecting haplotypes. Different shades of the circles correspond to distinct populations

Table 3 FST values of four populations from Udzungwa and Uluguru Mountains. Two stars (**) indicate very strong and significant genetic divergence between populations while single star(*) shows strong and significant genetic divergence within populations

POPULATIONS	Uluguru Urban	Uluguru Rural	Udzungwa South	Udzungwa North
Uluguru_Urban	0	-0.018 (0.52094)	0.679 (0.0001)**	0.088 (0.08346)
Uluguru_Rural	-0.018 (0.52094)	0	0.723 (0.0001)**	0.101 (0.10009)
Udzungwa South	0.679 (0.0001)**	0.723 (0.0001)**	0	0.705 (0.00238)*
Udzungwa North	0.088 (0.08346	0.101- (0.10009)	0.705 (0.00238)*	0

Furthermore, the neutrality test revealed a significant Fu's Fs at $P < 0.05$ on the Uluguru Urban population alone. Also, examining population trend based on BSP analysis we found evidence of population expansion of the cane rat starting from mid 2019 (Fig. 5).

Discussion

Our study indicates variations in genetic diversity and structure of the cane rat populations inhabiting two spatially isolated Eastern Arc Mountains (Uluguru and Udzungwa), Tanzania. We found higher diversity of haplotypes in the Uluguru Urban but, higher nucleotide diversity in the Udzungwa North population. There was higher genetic variation within than between populations. Three clades were revealed by the median and neighbour joining trees, whereas individual population FST revealed high genetic distance between Udzungwa and Uluguru populations. Our findings also indicate recent expansion of the cane rat populations.

The high haplotype and nucleotide diversity observed in this study is comparable to the haplotype (0.853–0.978) and nucleotide diversity (0.007–0.012) reported for cane rat populations in Ghana [2]. The high haplotype and nucleotide diversity observed in the present study may be attributed to high mutation rates usually found in organisms with shorter

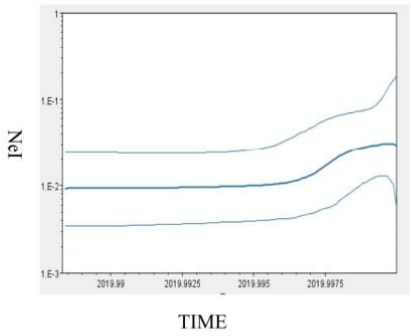

Fig. 5 Bayesian skyline plot showing population expansion of AGC that occurred in mid 2019

generation time [19] such as rodents [8] that are also influenced by demographic processes such as high reproduction rate and high mortality. Such demographic processes determine the strength of genetic drift [33] thereby shape genetic variation within and among populations [6].

Our results revealed high haplotype diversity in the Uluguru than the Udzungwa cane rat population. This could be due to relatively smaller and restricted grassland habitats used by the species available in montane forests within the Udzungwa ecosystem, than is available in the Uluguru urban ecosystem dominated by vast grasslands due to urban landscape modification created by urbanization activities. This is because habitat specificity, habitat fragmentation and historical population decline are known to greatly influence genetic diversity in rodent species (*Georychus capensis*) [40], a situation that may have influenced the cane population in our study area.

On another hand, the high haplotype and low nucleotide diversity observed in the Udzungwa South AGC population, could be reflective of a recent population expansion as supported by Tajima's D and FU's indicating excess recent mutations. This pattern may be linked to the conservation history in this site. Prior to being a national park in 1992, Udzungwa ecosystem was under threats of forest clearing, fragmentation and hunting, factors that may have reduced effective population size of AGC, thus affecting its genetic diversity as has been observed in other rodents species; the southern plains wood rat (*Neotoma micropus*) in Southern Texas, USA [23]. Further, our findings are comparable to the high haplotype and low nucleotide diversity of AGC population reported by [2] in Guinean forest and Coastal Savanna in West Africa where the authors reported genetic drift to have contributed to the splitting of the AGC ancestral lineage due to habitat fragmentation. Furthermore, Uluguru Urban AGC population was found to have high nucleotide diversity while Uluguru Rural had low nucleotide diversity. This could suggest that the Uluguru Urban AGC was more polymorphic than the Uluguru Rural population, indicative of a restricted gene flow among small isolated populations in rural than urban areas. The rural area population is more prone to several threats such as cultivation and hunting that altogether may have increased genetic loss while the urban population may have benefited from assisted migration from rural areas by cane rat hunters [32].

There was evidence of population divergence of Udzungwa South population from the rest suggesting high genetic differentiation within these populations. The AMOVA results confirmed great genetic variation in the spatially isolated AGC populations particularly within than between populations. Indeed, the Eastern Arc Mountains are over 100million years old with the different blocks biogeographically isolated thus long making species distribution on them more similar to true islands than to mainland regions [15]. Due to pressures of habitat fragmentation and loss on these isolated blocks, the level of differentiation within was higher than between meta-populations. We therefore suggest apparent restricted gene flow between the isolated small size populations within the meta-populations of Udzungwa and Uluguru Mountain blocks to have contributed to greater genetic divergence. These observations are consistent with studies on AGC in West Africa which also indicated a high genetic variation within than between populations from different agro-ecological zones [2].

Furthermore, a strong geographical pattern representing Udzungwa South, Udzungwa North, Uluguru Rural and Uluguru Urban AGC populations was also revealed by the MJN and neighbour joining phylogenetic tree. The gene flow was high between Uluguru urban and Uluguru rural as revealed by the maximum likelihood phylogenetic, MJN analyses and low FST values and suggests haplotypes were not equally distributed in the populations. In a random distribution we would expect the haplotypes to be equally distributed in study populations [21]. However, our results showed a large overlap between different haplotypes

occurring in the Uluguru Mountains. We also observed shared haplotypes between Uluguru Rural, Uluguru Urban and Udzungwa North populations which may be a result of past ancestral DNA signatures. Sharing of haplotypes between isolated populations would not have been expected if they had no ancestral relatedness. Comparison between unrelated families has however shown multiple shared haplotypes in other studies [21]. The shared haplotypes may also indicate the relatedness in the three populations possibly attributed to new introductions which could have occurred due to immigration (Uluguru Rural vs. Uluguru urban) or human mediated animal introduction as a result of hunting for food (Uluguru vs. Udzungwa North). The AGC population in Uluguru Urban could be a recent colonization from Uluguru Rural and therefore making the genetic distance between them to be closer than for the Udzungwa North populations.

Finally, the neutrality test revealed a negative but non-significant Fus Fs values for Uluguru rural and Udzungwa South populations while Uluguru urban had a negative and significant Fus Fs value, suggesting evidence of recent population expansion in these populations. This also is observed in Bayesian skyline plot which indicated AGC population expanded around the mid of 2019. The pattern of population expansion is similar to previous studies on AGC in Ghana [2]and in Kangaroo rat [37]. In the current study, population expansion of the three AGC populations may be explained by the creation of new habitats with potentially more food resources associated with agriculture such as opening new farms that encourage grass vegetation that potentially attract AGC population. Also, our results showed low levels of sequence divergence and high frequency of unique mutations particularly for Clades B (Uluguru Urban and Uluguru rural population) and C (individuals from Udzungwa North and Uluguru) which can also be interpreted as a signature of rapid population expansion [32].

Implications for conservation

This is the first study on the genetic diversity of *T. swinderianus* populations in Eastern and Southern African region, providing comparable literature to previous studies in West Africa. Our results provide important insights into the population trend and diversity in the spatially isolated mountain blocks as well as potential impacts of urbanization threats on the cane rat populations. These data may be useful in informing future management strategies of this pest species and on the conservation planning especially on how the species could be tapped locally to improving the local economies of rural human populations though the game farming practices. Future research into this species should focus on the genetic structure and dispersal patterns using different markers such as microsatellites and single nucleotide polymorphism (SNPs) to further define the genetic variations across its range areas in eastern and southern Africa. This will enable informed conservation of each genetic variant and to monitor the continued evolution of the species in the presently changing environment.

Table S1

Distribution of haplotypes in three sampled cane rat populations

Haplotypes	Uluguru urban	Uluguru rural	Udzungwa South	Udzungwa North	Total
1	1				1
2	4	1			5
3	4	2			6
4	1				1
5	1		2		3
6	2		1		3
7	4	1			5
8	1				1
9	1				1
10	1	1			2
11	1				1
12	1				1
13	1				1
14	1				1
15	1				1
16		2			2
17		2			2
18		1			1
19			1		1
20			1		1
21			1		2
22				1	1
23				1	1
24				1	1
25				1	1
Total	25	10	6	4	46

Acknowledgements We thank several field assistants who helped with logistics and fieldwork across the study sites. This study was funded by Eastern and Southern Africa Higher Education Centers of Excellence Project –ACEII with credit no.5799-TZ through a scholarship to the first author. Tanzania Wildlife Research Institute (TAWIRI) and Tanzania Commission for Science and Technology (COSTECH) provided a research permit number 2020-247-NA-2020-158 to conduct this study. Further research permits to work in the study sites were provided by Morogoro Rural District, Morogoro Urban District and Kilombero District Councils, for which we owe many thanks.

Author contribution All authors conceived the study design. Shadia I. Kilwanila collected data, performed laboratory and statistical analysis and wrote original draft and edited manuscripts. Charles M. Lyimo performed formal analysis, validation and reviewed the manuscript.

Alfan A. Rija, performed data collection, supervised laboratory analysis, conducted statistical analysed and validation and reviewed the manuscripts drafts. Alfan A. Rija and Charles M. Lyimo supervised the research. All authors read and approved the final manuscript for publication.

Funding The first author has received full scholarship from Eastern and Southern Africa Higher Education Centers of Excellence Project –ACEII with credit no.5799-TZ to pursue PhD studies at Sokoine University of Agriculture.

Data Availability The sequences generated and analyzed in this study have been deposited in NCBI GenBank with accession number OM475549-OM475594.

Declarations

Conflict of interest The authors have no relevant financial and nonfinancial interests to disclose.

Consent to participate Not applicable.

Consent for publication Not applicable.

Ethical clearance All applicable institutional guidelines for the care and use of animals were followed. The study was conducted under the ethical clearance from Directorate of Postgraduate Studies, Research, Technology Transfer and Consultancy of Sokoine University of Agriculture, Tanzania.

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CHAPTER FOUR

4.0 Isolating Greater Cane Rat Populations *(Thryonomys swinderianus)* from Eastern Arc Mountains, Tanzania: Linking Diversity to Morphometric and Molecular Characteristics

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Abstract

Evolutionary information on the greater cane rat (*Thryonomys swinderianus*) in the Eastern and Southern African regions is scarce, making population management and conservation of the species challenging. We studied *T. swinderianus* populations from two spatially isolated Eastern Arc Mountains in Tanzania to link molecular and geometric–morphological evidence to characterize these populations' diversity. Fecal samples (n = 50) and skulls (n = 99) of *T. swinderianus* were collected from Udzungwa (north and south) and Uluguru mountains (urban and rural sites) and analyzed using molecular and geomorphometry techniques. Molecular analysis grouped the population into three distinct clades based on the location where the samples were collected, while the morphometric method was not able to distinctively separate the populations. Both methods revealed that the population obeyed the isolation by distance model with higher genetic distance between the Udzungwa and Uluguru populations and lower distance between Uluguru urban and rural populations. Both Mahalanobis and Procrustes distances in skull

landmarks between the Udzungwa and Uluguru populations were significantly higher across the dorsal, ventral, and lateral views of the skulls, suggesting strongly that molecular and morphometric methods applied together can be useful in characterizing the population traits of the least known species. Our study suggests genetic and morphometric methods could complement each other in understanding the evolutionary biology and within-species diversity of vertebrate species that do not exhibit strong intra-species differentiation.

Keywords: Cane rats; diversity; Mahalanobis distance; molecular characteristics; morphometric; skull shape; Eastern Arc Mountains

4.1 Introduction

Variability in organisms is one of the features useful in delineating species in biology. Several trait characteristics of the species including morphological, physiological, developmental, behavioral, ecological and genetic parameters have been useful in studying various taxa. The pattern of these traits is shaped by the evolutionary history of the species and is useful in inferring the biodiversity of an area [1, 2]. Morphological characteristics such as cranial shape and size are widely used in diversity studies in many taxa [3, 4, 5]. In rodents, which comprise 42% of known mammalian species [6] for example, phenotypic variations such as the morphology of skulls have evolved to adapt to a wide range of ecological niches [7]. In such a mammal group, phenotypic traits have long been used in describing species diversity [8], although more recently, due to the phenotypic variability in rodents [9, 10], evidence from genetic analyses is increasingly being applied to complement the description of species more accurately [11]. For example, [12], studying different strains of mice (*Mus spp.*) showed that genetically closely related strains do not always possess morphologically similar crania. This suggests that linking evidence from both morphology and genetics can be a useful way to accurately describe species that would otherwise be confused due to phenotypic variability. The greater cane rat (*Thryonomys swinderianus*) is a rodent species distributed across Eastern and Southern Africa, whose knowledge of morphometric and genetic traits is still lacking. Such information, when available, could be useful in managing the species in fragmented habitats where survival is threatened due to illegal hunting and habitat disturbances [13].

Many factors influence craniometric variations in rodents, although in our study, we were more concerned with the cranio-morphometric diversity and genetic differentiation of geographically isolated populations of cane rats. Some studies on vertebrates have

highlighted the potential factors influencing such variations. For example, a study of the central African rodent, *Praomys misonnei*, indicated precipitation gradients influenced both genomic and craniometrics variations, which was most likely due to effects on vegetation structure [14]. Other studies have reported strong gene–environment associations in determining cranial morphology [7], a strong influence of geographical distances on skull shapes [15, 16], and roles of environmental characteristics such as altitudinal variations, vegetation types, etc. on the skull shape of rodents [17] and in non-rodent species of vertebrates [18] . Furthermore, a study on *Mastomys natalensis* Smith 1834, a widely distributed species in Sub-Saharan Africa, has reported a micro-evolutionary process within populations inhabiting different environments using a geometric–morphometric approach [19]. These studies show that geometric–morphometric and molecular analysis approaches are increasingly being used to establish variations in isolated populations of the same species. Furthermore, rodents occupy a diverse range of environments and habitats ranging from farmland, woodlands, forests, savanna grasslands, and mountainous landscapes at varying altitudes [20] and therefore, geographically isolated populations are likely to form groups that are morphologically different in size and genetics [21]. For example, among the Muroid rodents, a remarkable anatomical variety of the head skeleton even among closely related lineages has been reported [22].

Quantitative craniometrical traits incorporated into population genetic methods can provide insight into the cane rats' population structure in the Eastern Arc Mountains. Some studies on other vertebrate species have suggested that skull morphology has substantial potential to evolve and that craniometrical characters can provide consistent phylogenetic signals [23]. [24] suggested that the craniometry of rodents could provide a good systemic model to study the relationship between genetic variation and cranial shape evolution. Therefore, it is plausible that the morphological diversity of the cranium should reflect the phylogenetic and functional traits of a species inhabiting heterogeneous habitats where the populations are isolated from each other.

The greater cane rats show intra-species variability in body size, but the association between morphological variability and genetic differentiation is little known. The species has a wide geographical distribution across Africa [13], occupying diverse habitats. A molecular approach in addition to craniometrical measurements can confirm the variability between and within these populations. Many studies have been conducted on cane rats but more widely covering ecology and reproduction, with most of them concentrated in

West Africa [13]. Other studies conducted in West Africa have focused on sexual dimorphism [1], gross morphology and morphometry of the spinal cord [25], the brain [26] and characterization of the morphology of the brain across age groups [27]. [28] investigated the craniofacial and ocular morphometrics of male *T. swinderianus* in Nigeria aimed at early detection of the characteristic facial appearance of some syndromes. Although these studies are useful in providing information about this species morphometrically, they are more confined to West Africa and on domesticated cane rats, which implies that they cannot be representative of all other regions in Africa. Furthermore, the studied populations in West Africa inhabit wet equatorial regions with a strong ecological contrast with the savanna and montane biomes in Eastern and Southern Africa. Comparable information on the cane rat population distribution in the savanna and montane eco-regions is necessary for augmenting this species' biology across Africa.

The aim of this study was to investigate the molecular and cranial morphometric diversity in isolated populations of the greater cane rats in two mountain blocks of the Eastern Arc, which is located in Morogoro, Tanzania. We hypothesized that skull shape will be strongly associated with genetic variations of cane rat populations occupying geographically isolated habitats in the Eastern Arc Mountains. Specifically, we assessed how cranial shapes of three skull views—lateral, ventral and dorsal—are scaled with the age and species locality and how such insights are mirrored in the genetic diversity of the cane rat populations. These data add to the biological repertoire of this species and may be useful in devising potential population management strategies, conservation and game farming and ranching within its range of distribution in Eastern and Southern Africa.

4.2 Materials and methods

4.2.1 Sampling Sites and Sampling Procedure

Field data collection was conducted between April 2019 and December 2020 in Udzungwa and Uluguru mountains, which lie within the Eastern Arc Mountains in Tanzania (Figure 1). Uluguru populations were further divided into Uluguru urban and Uluguru rural depending on the location where samples were collected. Uluguru urban and Uluguru rural are distinct areas based on varying land use patterns and urbanization levels, which are known to greatly influence behavior of vertebrate species [29]. Cane rats were captured at altitudes ranging from 400 to 1200 m above sea level in fallow, grasslands or bushed grasslands in both Udzungwa and Uluguru Mountains. Samples from Udzungwa were collected from the northern and southern parts of the mountain block.

To collect data for morphometric analyses, we deployed experienced local hunters in each location to capture *Thryonomys swinderianus* using local methods, as there is no documented standardized method available for capturing greater cane rats in the wild. Cane rats are among the wild mammal species hunted for meat supply in rural communities surrounding the Eastern Arc Mountains [13, 30]. The local hunters were deployed to obtain the required samples allowing them to retain the rest of the carcasses. The locality where an animal was captured or hunted was marked and was later visited by a research assistant to record the GPS coordinates. Because the hunters use locally made nets to trap cane rats which may capture juvenile animals, only sub-adults and adults were used for this study. A live captured *Thryonomys swinderianus* was sacrificed, and the head was removed for craniometric measurements of the skull. The age of each animal was recorded and was further refined in the laboratory as explained below. Heads were cleaned of tissue remains and debris using boiled water, sodium hypochlorite, and hydrogen peroxide after the outer skin had been removed with surgical blades [31]. Fecal samples were collected for molecular analysis.

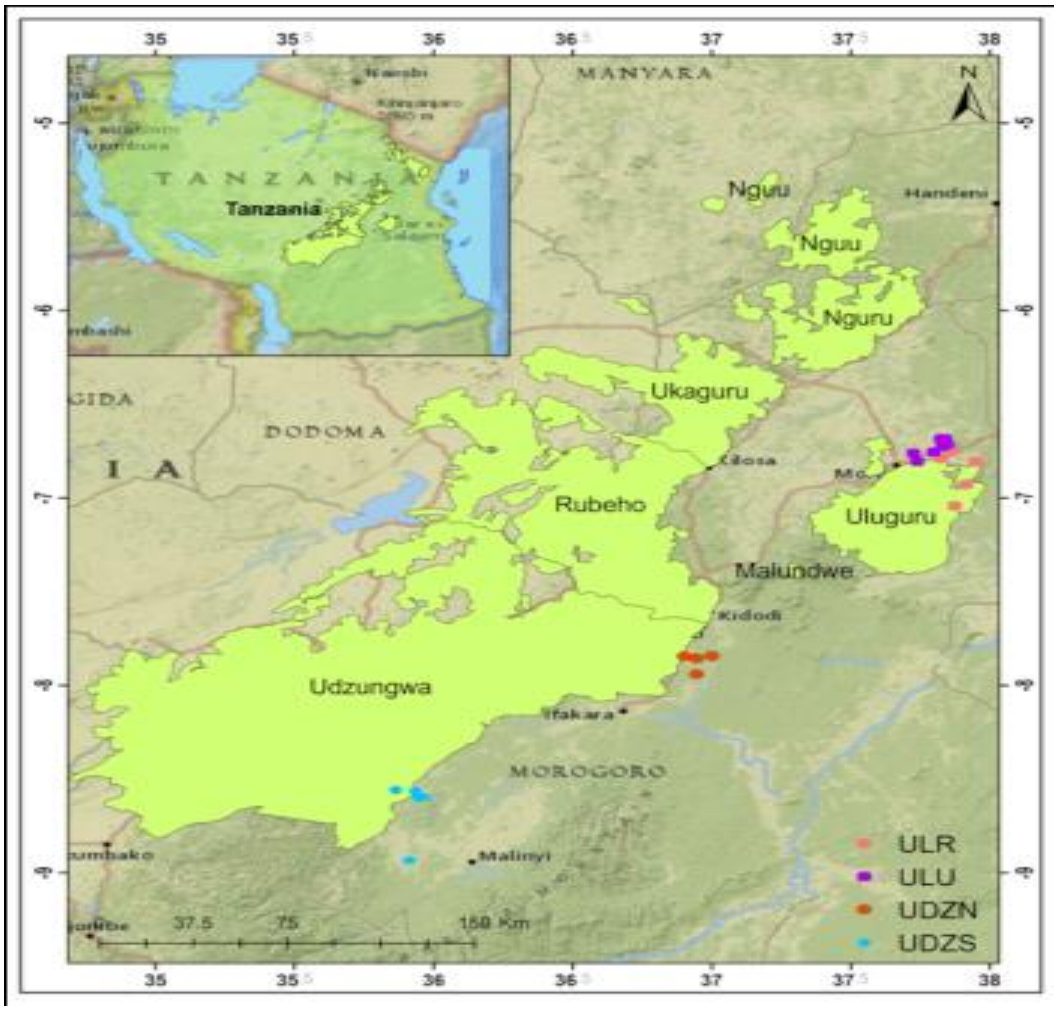


Figure 4.1: Map of study site, showing the Eastern Arc Mountains and locations (colored bullets) where data collection took place in the Udzungwa and Uluguru Mountains. The lower inset map is Tanzania with the Eastern Arc Mountains.

DNA extraction was completed in 50 fecal samples using a zymo-research kit for fecal samples following the manufacturer's protocol without modifications. Amplifications of the 515 bp region mt-DNA D-loop via Polymerase Chain Reaction (PCR) were performed using both forward and reverse primers following [32]. PCR was conducted under various conditions: initial denaturation at 95 °C for 2 min; 35 cycles of denaturation at 95 °C for 30 sec, annealing at 55 °C for 30 sec, extension at 74 °C for 1 min and a final extension at 74 °C for 7 min. The PCR products were Sanger sequenced by macrogen (Europe).

Refining the age classes of each skull was based on the eruption and wear of molar teeth and the degree of exposure [33, 6]. Adults had the third molar fully erupted, showing signs of wear with exposed dentin on all teeth and roots partially exposed, whereas the sub-adults had all molars erupted with cusps still enameled, little exposed dentin and roots completely in the alveoli [33]. Furthermore, to create landmarks for analysis, each skull was photographed from the dorsal, ventral and left lateral views. Damaged skulls were photographed only in the view(s) with the landmark regions. The photographs were taken using a Nikon D3100 camera with a resolution of 14.2 megapixels. The camera lens was positioned parallel to the photographic background. For photographs of the dorsal view, specimens were positioned with the molar surface facing the background. For the ventral view, the specimens were placed with parietal bones facing the background. For photographs of the lateral view, skulls were fixed on the background by the zygomatic arch. The landmark digitization was carried out using 286 high-quality images out of the 386 photographs taken.

4.2.2 Data Analysis

4.2.2.1 Geometric Morphometric Assessment

To investigate the craniometric features of *T. swinderianus*, we made a TPS file from photographs with .jpg format using TPSUtil software ver 1.82 [34]. Thirteen two-dimensional landmarks were digitized in the ventral view (Figure 2; Supplementary Table S1), nine were digitized in the left lateral view of the skull (Figure 3; Supplementary Table S2)) and twelve were digitized in the dorsal view (Figure 4; Supplementary Table S3), using the TPSDig software version 2.32 [34]. Depending on the quality of the skull (i.e., not broken), the number of digitized photographs of the skull varied in number with 80 ventral, 80 dorsal, and 96 lateral views. Damaged skulls were left out from analysis but were cataloged and archived in the Zoology laboratory at Sokoine University of Agriculture for future research. Landmarks were imported into MorphoJ software version 10.11 in TPS format, and coordinates were superimposed using the Generalized Procrustes Analysis (GPA) algorithm to extract shape information. GPA is a procedure that removes the effects of scale, orientation and position differences to avoid potential biases in the results [10] and leaves only shape variation. Procrustes analysis is a form of statistical shape analysis used to analyze the distribution of a set of shapes using landmarks. The size of each skull in each view was estimated from its centroid size, which is defined as the square root of the sum of squares of the distances of landmarks from the centroid [35]. Data distribution and the outliers were inspected graphically by plotting the cumulative distribution of the squared Mahalanobis distances against a multivariate normal

distribution fitted to the data as described in MorphoJ [36]. To assess the morphometric differences that exist between samples collected from different locations, we used the centroid size of each population group to perform a Kruskal–Wallis test, which was implemented in software R version 3.6.3. Furthermore, to assess the patterns of shape variation that may exist among population groups under study, we used Canonical Variate Analysis (CVA) [37]. CVA works by transforming the original measurements of the specimen, e.g., landmarks into a set of new variables called canonical variates, which are uncorrelated with each other but capture the most importance sources of variation between groups. The number of canonical variates is equal to the number of groups minus one, and each one represents a specific combination of original variables that maximizes the separation between groups. The p-value of the CVA was checked by calculating Procrustes distances with 1000 iterations per comparison.

During analysis, confidence ellipses were set at a probability of 0.9. Mahalanobis and Procrustes distances were calculated and used to draw neighbor-joining phylogram using SplitsTree software ver. 5.3.0 [38] (Figure 5) to show the relationship that exists between populations. An individual Procrustes distance matrix was generated using the “geomorph” package in the R environment [39, 40]. The Mantel test was then performed in R using the “vegan” package [40, 41] with Procrustes distance and genetic distance matrices. The genetic distance matrix was generated in MEGA software ver 6.0 [42] using the most appropriate substitution model as described in [43]. Furthermore, to assess the relative amount of shape variation (representing biological variation) among individuals, we ran a Procrustes analysis of variance (ANOVA) using location and age as classifiers.

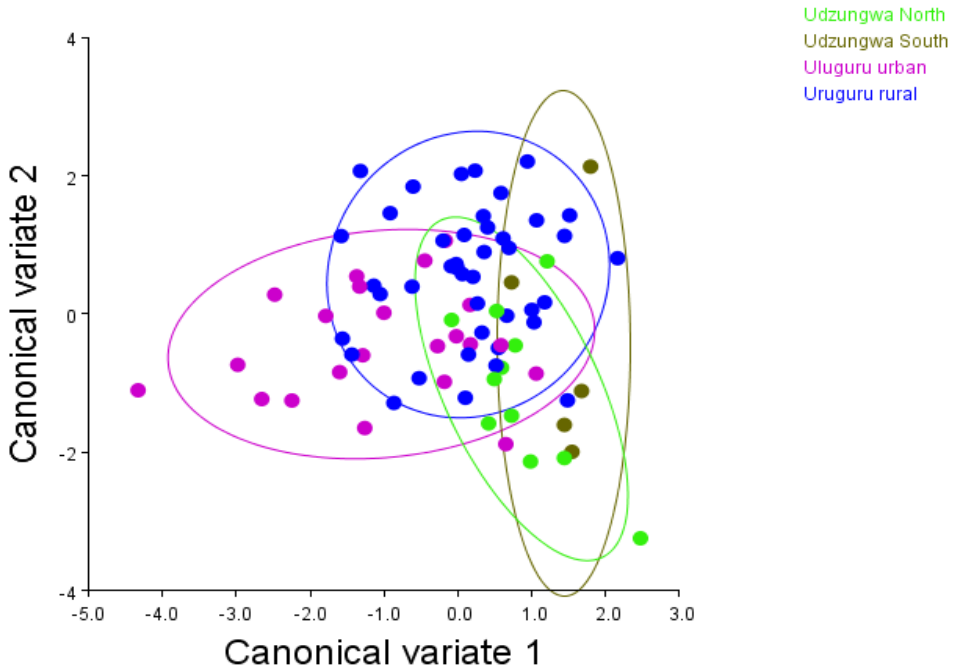
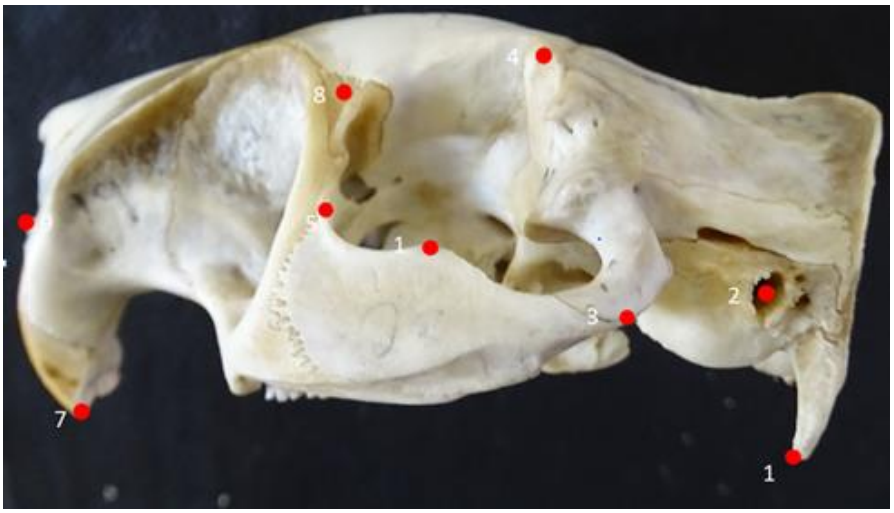


Figure 4.2: Landmarks locations with the name of each location mentioned (each landmark is represented by a number in the photo) in the Supplementary Material, Table S1, and scatter plots of the ventral view of the *T. swinderianus* from four populations. The points on the graph represent individuals in the morphospace.



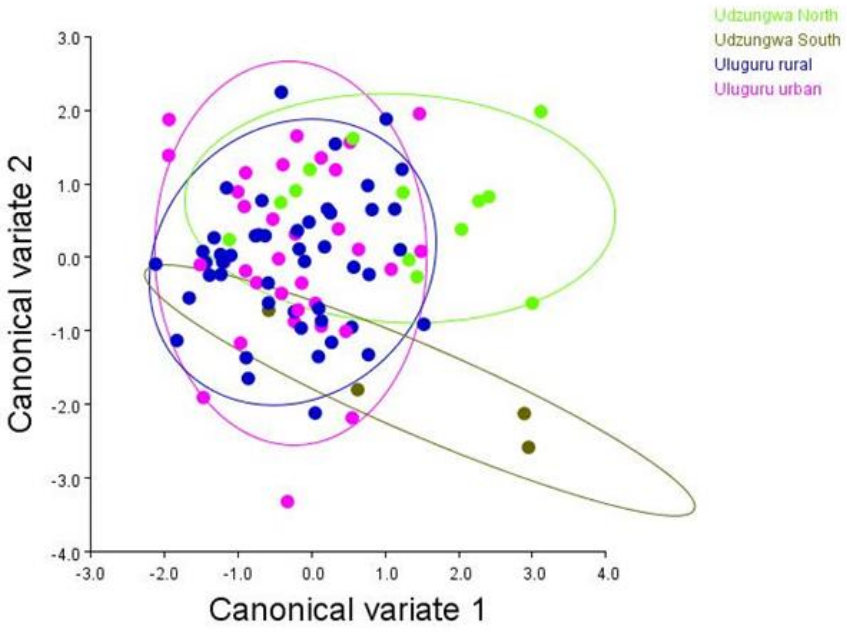
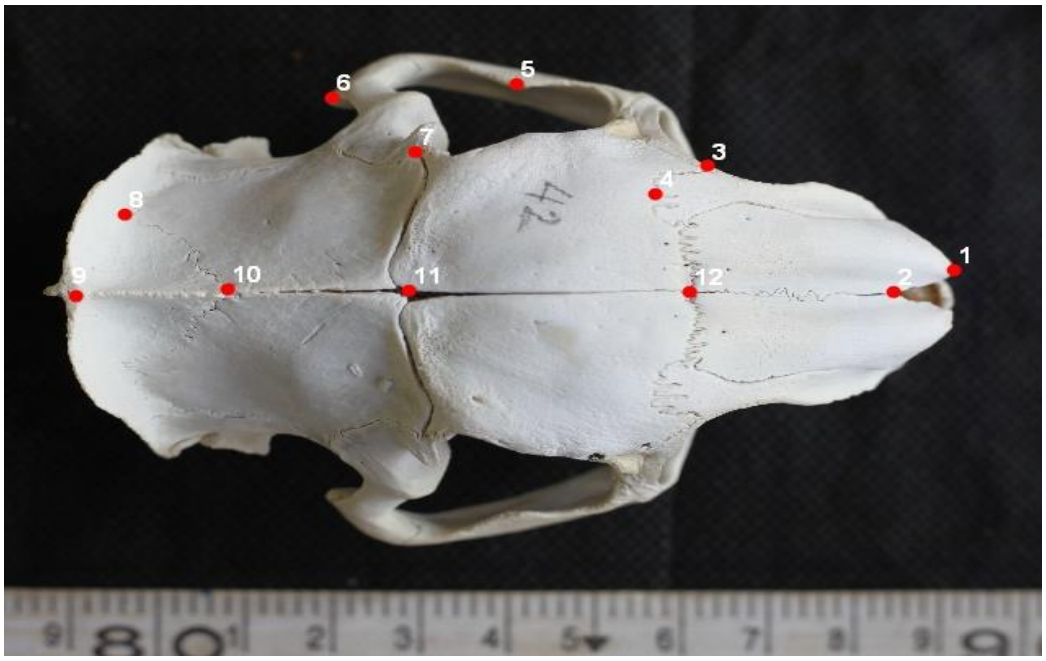


Figure 4.3: **Landmarks locations with the name of each location mentioned (each landmark is represented by a number in the photo) in the Supplementary Material, Table S2, and scatter plots of the lateral view of the *T. swinderianus* from four populations. The points on the graph represent individuals in the morphospace.**



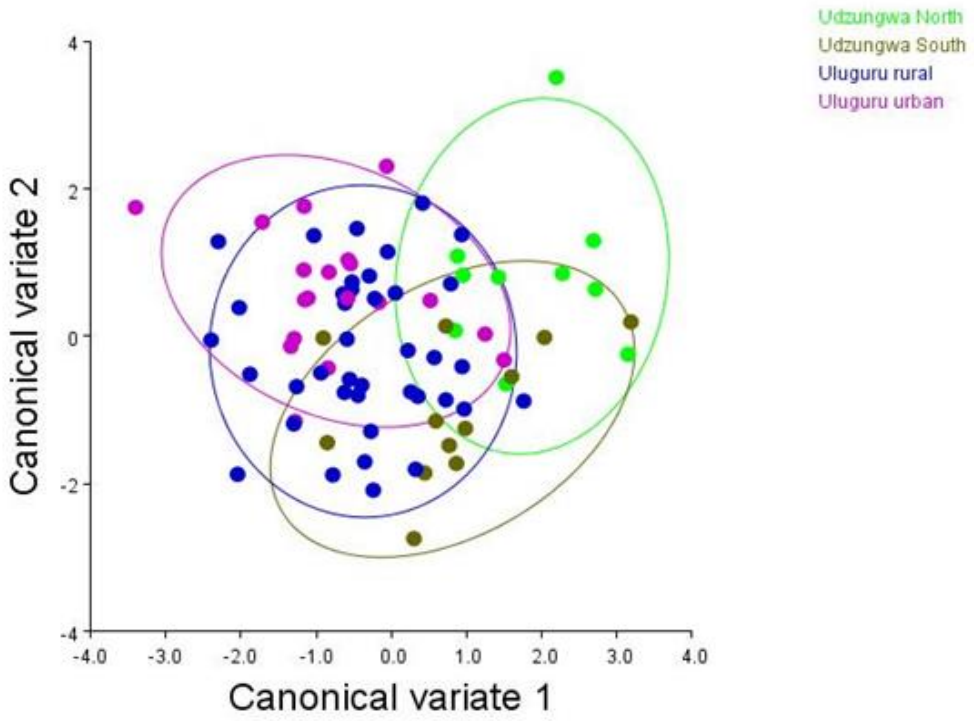


Figure 4. 4: Landmarks locations with the name of each location mentioned (each landmark is represented by a number in the photo) in the Supplementary Material, Table S3, and scatter plots of the dorsal view of the *T. swinderianus* from four populations. The points on the graph represent individuals in the morphospace.

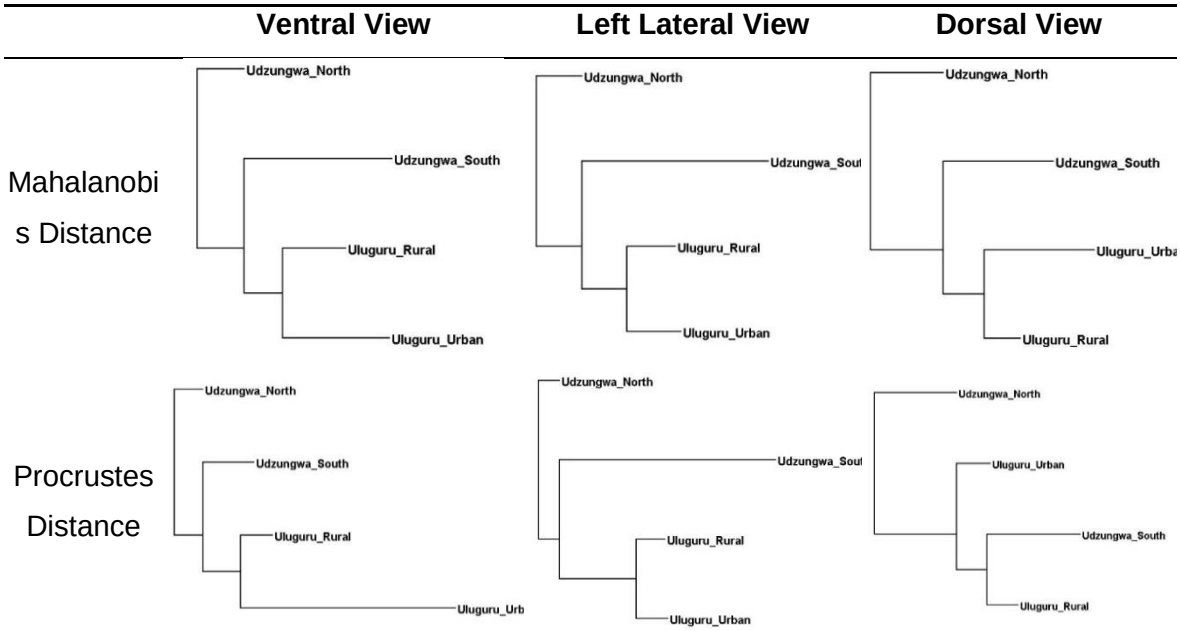


Figure 4.5: Phylogram showing the relationship between *T. swinderianus* populations using Procrustes and Mahalanobis distances.

4.2.2.2 Molecular Analysis

Nucleotide sequence editing and alignment were carried out using the BioEdit program version 7.0.9.0 [44]. Following quality control procedures, 46 sequences were retained for analysis and were deposited in the NCBI gene bank with accession numbers OM475549-OM475594. Using MEGA program version 6.0, aligned sequences were used to infer the evolutionary history of *T. swinderianus* by constructing a neighbor-oining phylogenetic tree as explained in [43]. The nodes' robustness was tested using 100,000 bootstraps. A median-joining network was built using 25 haplotypes implemented in Network software version 4.6 to observe haplotype phylogenetic and geographical relationships. The population structure of *T. swinderianus* was determined using Arlequin version 3.5.2.2 and Analysis of Molecular Variance (AMOVA). The sampling localities and significant clades discovered in the median-joining analysis were used to define hierarchical levels used in AMOVA. An Individual Pairwise FST was estimated using the algorithm proposed by [45] in the Arlequin program version 3.5.2.2.

A Mantel test was performed between geographic and genetic distances at 1,000,000 permutations in software R version 3.6.3 using "Geodist", "ape" and "vegan" packages to establish isolation by distance pattern [46].

4.3 Results

4.3.1 Matching Geometric and Genetic Variation Evidence in T. swinderianus Populations

4.3.1.1 Differences between Populations

We found no significant differences between the four populations based on the centroid size in the ventral, dorsal and left lateral views. In addition, CVA revealed that there was non-significant shape variation among four populations in the scatter plots in all three views (Figures 3–5).

In the ventral view, the first discriminant function explains 47.49% of the between-group variability, and the second discriminant function explains 17.512% of the between-group variability (Figure 3).

A higher differentiation in shape (Mahalanobis distance) was revealed for the Udzungwa south population when compared to the Uluguru urban (Mahalanobis distance = 2.7865, *p* = 0.1180) and Uuguru rural populations (Mahalanobis distance = 2.2598, *p* = 0.3564). Shape differentiation (Mahalanobis distance) between Uluguru rural and urban was low and non-significant (Mahalanobis distance = 1.5776, *p* = 0.0111). Furthermore, examining the absolute magnitude of shape deviation (Procrustes distance) of *T. swinderiunus*, we found the Udzungwa south population had higher but non-significant deviation from Uluguru urban (Procrustes distance = 0.0575, *p* = 0.4616) and Uluguru rural (Procrustes distance = 0.0218, *p* = 0.5781). In addition, the magnitude of shape deviation between Uluguru rural and urban was low and non-significant (Procrustes distance = 0.0462, *p* = 0.0895) (Table 1; Figure 3).

Table 4.1: Mahalanobis and Procrustes distances of the four populations generated from the Canonical Variate Analysis of the ventral view. The numbers outside the brackets are the distances and the numbers inside the brackets are the *p*-values generated from permutations.

Mahalanobis Distance			
Population	**Udzungwa North**	**Udzungwa South**	**Uluguru Urban**
Udzungwa south	2.2472 (0.7864)		
Uluguru urban	2.1609 (0.0548)	2.7865 (0.1180)	
Uruguru rural	1.8783 (0.0582)	2.2598 (0.3564)	1.5776 (0.0111)
Procrustes Distance			
Population	**Udzungwa North**	**Udzungwa South**	**Uluguru Urban**
Udzungwa south	0.0202 (0.7139)		
Uluguru urban	0.0572 (0.3530)	0.0575 (0.4616)	
Uruguru rural	0.0244 (0.2347)	0.0218 (0.5781)	0.0462 (0.0895)

On the other hand, the amount of the variation between groups explained by the first discriminant function was 48.146%, while the second discriminant function explains (24.167%) in the left lateral view (Figure 4).

The Udzungwa south population had higher but non-significant shape differentiation (Mahalanobis distance) when compared to Uluguru urban (Mahalanobis distance = 2.5772, p = 0.0473) and Uluguru rural (Mahalanobis distance = 2.5284, p = 0.0418). In addition, we found no significant shape differentiation between Uluguru rural and urban (Mahalanobis distance = 0.9281, p = 0.2344; see Table 2). The absolute magnitude of shape differentiation (Procrustes distance) revealed a large but not significant deviation of the Udzungwa south population when compared to Uluguru urban (Procrustes distance = 0.0630, p = 0.0124) and Uluguru rural (Procrustes distance = 0.0628, p = 0.03941). The magnitude of the shape deviation between Uluguru urban and rural was low and non-significant (Procrustes distance = 0.0117, p = 0.7840; Table 2; Figure 4).

Table 4.2: Mahalanobis and Procrustes distances of the four populations generated from the Canonical Variate Analysis of the left lateral view. The numbers outside the brackets are the distances, and the numbers inside the brackets are the *p*-values generated from permutations.

Mahalanobis Distance			
Populations	**Udzungwa North**	**Udzungwa South**	**Uluguru Rural**
Udzungwa south	2.5012 (0.2837)		
Uluguru rural	1.6627 (0.0109	2.5284 (0.0418)	
Uluguru urban	1.7111 (0.0142)	2.5772 (0.0473)	0.9281(0.2344)
Procrustes Distance			
Populations	**Udzungwa North**	**Udzungwa South**	**Uluguru Rural**
Udzungwa south	0.0501 (0.3014)		
Uluguru rural	0.0285(0.2059)	0.0628 (0.0394)	
Uluguru urban	0.0292 (0.1532)	0.0630 (0.0124)	0.0117 (0.7840

Analyzing the dorsal view (Figure 5), we found the first discriminant function explains 56.935% of the between group variation, while the second discriminant function explains only 16.574%. The shape differentiation (Mahalanobis distance) between Udzungwa north and Uluguru urban was high and non-significant (Mahalanobis = 2.7200, p = 0.0005) as was that between Uluguru rural (Mahalanobis distance = 2.4629, p = 0.0003). The Mahalanobis distance between Uluguru rural and Uluguru urban was low (Mahalanobis distance = 1.2913, p = 0.3434). There was also high but non-significant shape deviation (Procrustes distance) between Udzungwa north and Udzungwa south (Procrustes

distance = 0.0341, *p* = 0.0182) as well as Udzungwa north and Uluguru rural (Procrustes distance = 0.0279, *p* = 0.0154), but it was low between Uluguru rural and urban (Procrustes distance = 0.0109, *p* = 0.5324) (Table 3; Figure 5).

Table 4.3: Mahalanobis and Procrustes distances of the four populations generated from the Canonical Variate Analysis of the dorsal view. The numbers outside the brackets are the distances, and the numbers inside the brackets are the *p*-values generated from permutations.

Mahalanobis Distance			
Population	**Udzungwa North**	**Udzungwa South**	**Uluguru Rural**
Udzungwa South	2.2643 (0.1584)		
Uluguru rural	2.4629 (0.0003)	1.7541(0.0725)	
Uluguru urban	2.7200 (0.0005)	2.2279 (0.0040)	1.2913 (0.3434)
Procrustes Distance			
Population	**Udzungwa North**	**Udzungwa South**	**Uluguru Rural**
Udzungwa South	0.0341 (0.0182)		
Uluguru rural	0.0279 (0.0154)	0.0150 (0.3154)	
Uluguru urban	0.0239 (0.0828)	0.0196 (0.0927)	0.0109 (0.5324)

Pooling evidence from the genetic analysis, we found 25 haplotypes were explained by three main clades/haplogroups based on their evolutionary relationships and the location where samples were collected. Clade A consisted of individuals from Udzungwa south populations only, clade B consisted of individuals from Uluguru urban and rural, while clade C consisted of individuals from all three populations (Udzungwa north, Uluguru rural and urban) (Figures 6 and 7). We also found most of the differentiation (83.36%) of *T. swinderianus* was within the three clades, and only 16.64% was found between clades (Table 4). Examining the extent to which populations are similar or different from one another, a higher genetic distance for the Udzungwa south and Uluguru rural populations and low genetic distance between Uluguru rural and urban populations were reported in [43]. The haplotype and nucleotide diversity indices were also reported in [43]. The Mantel test revealed a positive correlation between geographic and genetic distance (r = 0.51, $p < 0.0001$) as well as between Procrustes and genetic distance (r = 0.7, $p < 0.0001$).

Table 4.4: Molecular Variance (AMOVA) of greater cane rats from the four study sites showing significant differences between samples sourced from different localities.

Source of Variation	df	Sum of Squares	Variance Components	Percentage of Variations	FST	*p*-Value
Between Populations	3	71.452	2.14201	40.30	0.402970.00001	
Within Population	42	133.287	3.17349	59.70		
Total	45	204.739	4.86248			

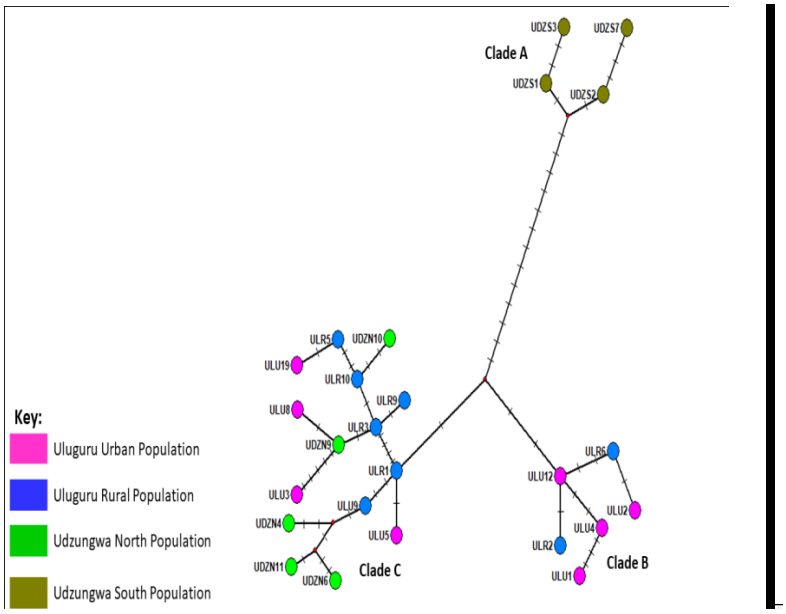


Figure 4.6: Median-joining network of 25 haplotypes of greater cane rats inhabiting Udzungwa and Uluguru urban and rural areas.

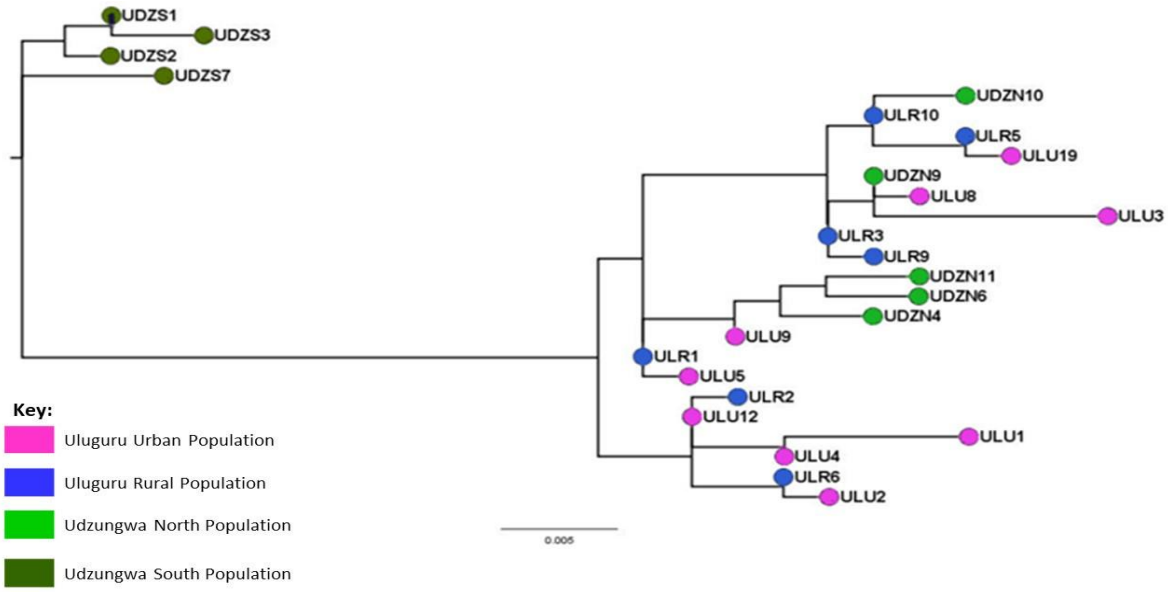


Figure 4.7: Neighbor-joining phylogenetic tree of mitochondrial D-loop nucleotide sequences based on 25 haplotypes of greater cane rats.

4.3.2. Variations in Skull Size

Procrustes ANOVA of the ventral view revealed no significant variations in the size of cane rat skulls between locations (F = 0.35, p = 0.7917) but significant between age classes (adult and sub-adult) (F = 24.16, p < 0.0001). Similarly, the left lateral view also depicted no significant differences in the skulls between locations (F = 1.05, p = 0.3087) and age (F = 0.12, p = 0.9503). For the dorsal view, non-significant variations in the size of skulls were observed between locations (F = 2.21, p = 0.0935), but a significant variation between age classes (adult and sub-adult) (F = 17.10, p < 0.0001) was evident (Supp1emantary Tables S4–S6).

4.3.3 Variations in Skull Shape

There were no significant skull shape differences in the ventral view between study sites (Pillai trace = 0.83, F = 1.47, p = 0.0091) and age classes (Pillai trace = 0.49, F = 1.48, p = 0.0708). However, the left lateral view showed no significant variations in the shape of the skulls between study sites (Pillai trace = 0.59, F = 1.58, p = 0.0109) and age classes (Pillai trace = 0.21, F = 0.63, p = 0.8417). On the other hand, the dorsal view revealed non-significant differences in the skull shapes between study sites (Pillai trace, 0.88, F = 1.79, p = 0.0002), while significant differences in skull shape between age classes were observed (Pillai trace = 0.36, F = 5.24, p < 0.0001) (Supplementary Material Tables S7–S9).

4.4 Discussion

The aim of this study was to link geometric and genetic evidence to the diversity of cane rat populations in the Eastern Arc Mountain blocks. The molecular analysis was able to differentiate four populations (Udzungwa north, Udzungwa south, Uluguru rural and Uluguru urban) collected from different localities. However, not all the morphometric measurements could substantiate the observed genetic distinction of four populations. Comparison using a phylogram derived from Procrustes and Mahalanobis distances also revealed four distinct populations. In contrast, the Procrustes ANOVA and Kruskal–Wallis test failed to differentiate between the four populations based on morphometric measurements.

The observed variations leading to four distinct populations could be due to the effect of isolation by distance; similar results were revealed by FST analysis. This was further supported by a positive correlation between Procrustes and genetic distances and between geographic and genetic distances using the Mantel test.

The Procrustes ANOVA results illustrated the importance of the dorsal view in both size and shape to demonstrate age differences within and between populations. The ventral view also showed size differences for individuals of different age classes within populations. This is consistent with the AMOVA results, which indicated high genetic variation within rather than between populations. In our study, we did not investigate sex-linked variations in skull morphometrics, but [1] in West Africa reported dimorphisms associated with the sex of individuals in *T. swinderianus*. Some studies have pointed out that genetics and food processing influence cranial size and shape [47]. Furthermore, variations in skull size and shape have also been attributed to local adaptations and morphological differentiations without genetic structuring [1]. Food availability also correlated to the multimammate rats' cranial characteristics [1]. In our study areas in the EAM, human activities including vegetation clearing for settlements, agricultural activities, and burning of bushes have resulted in habitat fragmentation [48], which may consequently have led to limited dispersal events between populations. *T. swinderianus* populations locked in different mountain blocks, therefore, experience geographic isolation and barriers to gene flow and could result in morphological differentiation as observed in the cranial morphometry in our study. Studies elsewhere indicated that a loss of functional connectivity between landscapes reduces gene flow between populations and may lead to genetic variations [49, 50, 51].

Our study shows that the skull shapes and size were different between age classes and locations. Although not all variables were significantly different, it is obvious that the longer the separation by distance, the more likely these populations differed in skull shape because animals may respond to ecological variations by genetic and morphological adaptations [52]. The Procrustes distance, Mahalanobis distance and FST were high in populations separated by long distance (Udzungwa south and Uluguru urban) and were low in neighboring populations (Uluguru rural and Uluguru urban). These findings are consistent with the isolation-by-distance model, which predicts that gene flow will decrease with an increase in geographic distance, thus promoting a genetic divergence between “subgroups” [46]. Gene flow between populations is an important biological process, which shapes and maintains biodiversity [53]. Inter-population variations in skull morphology correlated with geographic distance, which is consistent with previous studies on echimyids [17] and murid rodents [54].

In our study, we were also able to evaluate the geographical distances by the Mantel test and to compare the genetic divergence (genetic distances) of the cane rat populations found in the Uluguru and Udzungwa mountain blocks. We found a positive correlation which indicated a spatial genetic divergence between these populations. Calculations of the genetic distances between these populations indicated a positive correlation between Procrustes and genetic distance. Our study also revealed the FST and Procrustes distances increased with geographical isolation between populations, supporting our hypothesis of genetic variability in cane rat populations due to isolation by distance. It is obvious that the Uluguru urban and Uluguru rural populations were genetically more similar, as these populations were much closer to each other than the Udzungwa south and north populations. These findings are consistent with studies on other vertebrates. For example, it was reported that in Lake Tanganyika, Tanzania, the genetic distances between populations were strongly associated with geographic distances [55]. Geographic distances limit dispersal, and therefore, the rate of migration becomes higher between nearby populations than between distant populations [56]. This pattern is consistent with our data for the dorsal and lateral views of the *T. swinderianus* skulls in which those from nearby localities (Uluguru urban and Uluguru rural) had small morphological distances, suggesting the existence of dispersal and gene exchange in these populations [43].

The observed cranial morphological variations in cane rats are probably widely found in rodents occupying different landscapes. For example, [19] reported a population-level differentiation in *M. natalensis* with significant variation in skull shape attributed to different

ecological conditions (e.g., rainfall, habitat heterogeneity, and seasonal variations) occupied by these populations. In this study, it is evident that there are morphological and genetic variations between populations occurring in different mountain blocks of the Eastern Arc. These variations suggest that gene flow between these populations is limited, and they have adapted to the prevailing conditions within each mountain block. These, coupled with environmental conditions such as vegetation type, could increase the fitness of the species in the local environment, enabling a continued evolutionary divergence process. Therefore, the phenotypic and genetic traits demonstrated in our study could be a common phenomenon in *T. swinderianus* across African landscapes where the species is found.

Some unknown environmental effects can also account for the observed variations. In other similar studies, for example, it has been shown that skull morphological differentiation in murids, *Dipodillus* [57], *Mastomys* [19], *Taterillus* [58], *Ctenomys minutus* [53] and the Japanese shrew-mole, *Urotrichus talpoides* [59], were attributed, among other factors, to environmental heterogeneity including vegetation type, rainfall, habitat variability, and altitude. Other variables known to limit gene flow and potentially influencing cane rat populations in the EAM, such as habitat heterogeneity, rainfall, anthropogenic activities and isolation, are worth further investigation.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1. Ventral view landmarks; Table S2. Left lateral landmarks; Table S3. Dorsal view landmarks; Table S4. Centroid size variation of the ventral view; Table S5. Centroid size variation of the left lateral view; Table S6. Centroid size variation of the dorsal view; Table S7. Shape variation based of the ventral view; Table S8. Shape variation based of the left lateral view; Table S9. Shape variation of the dorsal view.

Author Contributions: S.I.K and A.A.R. designed the study, collected field data, and performed morphometric data analysis and part of molecular data analysis. C.M.L. performed analysis of the genetic aspects of the data. S.I.K. and R.H.M. prepared the first draft of the manuscript. A.A.R. read and provided comments and revisions of the final manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the Eastern and Southern Africa Higher Education Centers of Excellence Project—ACEII (Africa Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development—Sokoine University of Agriculture)—Credit no.5799-TZ.

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Author Contributions:

S.I.K and A.A.R. designed the study, collected field data, and performed morphometric data analysis and part of molecular data analysis. C.M.L. performed analysis of the genetic aspects of the data. S.I.K. and R.H.M. prepared the first draft of the manuscript. All authors read and provided comments and revisions of the final manuscript. All authors have read and agreed to the published version of the manuscript.

Funding:

This study was funded by the Eastern and Southern Africa Higher Education Centers of Excellence Project—ACEII (Africa Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development—Sokoine University of Agriculture)—Credit no.5799-TZ.

Institutional Review Board Statement:

All applicable institutional guidelines for the care and use of animals were followed. The study was conducted after issuance of an ethical clearance from Directorate of Postgraduate Studies, Research, Technology Transfer and Consultancy of Sokoine University of Agriculture, Tanzania on 10thMay, 2019 with an Institutional Review Ref. No. SUA/ DPRTC/186/17.

Data Availability Statement: The sequences generated and analyzed in this study have been deposited in NCBI GenBank with accession number OM475549-OM475594. The skulls used for this study are at the department and may be made available on special request to the corresponding author.

Acknowledgments: The Tanzania Wildlife Research Institute (TAWIRI) and Tanzania Commission for Science and Technology (COSTECH) provided a research permit number 2020-247-NA-2020-158 to conduct this study. We thank various field assistants for helping with data collection in the field.

Conflicts of Interest: The authors declare no conflict of interest

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CHAPTER FIVE

5.0 Phylogeographic patterns of Greater cane rat (*Thryonomys swinderianus*) populations from eastern, western and southern Africa and implications for wildlife conservation

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Abstract

The African Greater Cane rat (AGC) populations in eastern, western and southern Africa bear a single ancestral origin. However, to date, information is lacking on their genetic differentiation due to long-time geographical isolation and the environmental and anthropogenic pressures the populations face in each region. This gap limits our ability to understand potential speciation in these populations and how this species could widely be used to enhance the conservation of wildlife across the forest and savannah biomes in Africa. We analyzed the genetic sequences of cane rat samples from three geographic regions: eastern Africa, western Africaand southern Africa to characterize the phylogeographical patterns of the populations based on the mt-DNA.The D-loop sequences used comprised samples collected from two Eastern Arc Mountains in Tanzania, three agroecological zones in Ghana and four sites in South Africa. **Results:** Tanzania revealed higher haplotype diversity than populations from other countries. AMOVArevealed a considerably high genetic variation within than between populations in all geographic regions. Demographic history analysis revealed a negative and significant Tajima'D for the populations from southern Africa. The Fu'sFs was negative and significant for all populations across the three regions indicating population increase in

these regions.This is the first study to compare maternal lineages of AGC populations from eastern, western and southern Africa and provides a basis for future genetic studies. High genetic diversity and the negative and significant Fus Fs values in some of the population from all geographical locations indicate that greater cane rat populations are currently not threatened concurring with the current IUCN status of Least Concern. Also, the distinct haplotypes observed in each region suggests that the populations can be managed as metapopulations. Potential local game farming programs can greatly benefit from breeding individuals from distinct metapopulations.

Keywords: African Greater Canerats, Demographic history, conservation genomics, Evolutionary history, mt-DNA, maternal origin, , *Thryonomys swinderianus*

5.1 Introduction

The African Greater cane rats (AGC) *(Thryonomys swinderianus)* is widely distributed in Africa with subpopulations in eastern, western and southern African regions, which are isolated due to topographical and environmental barriers that limit natural dispersal (Collier, 2006). These barriers limit gene flow between these populations, making them appear different, at least genetically (Kilwanila et al., 2021). For example, habitat fragmentation and seasonality have been shown to correlate with genetic diversity in spatially isolated populations of Mexican spotted owls (Wan et al.,2018). Given the long-time separation of the southern, eastern and western African regions (about 543 million years ago, Suess, 1888; Sloss, 1963), exposure to different biotic and abiotic conditions may have influenced profound genetic differentiation in the cane rat populations. However, to date, there is a scarcity of information and a lack of knowledge on these populations' demographic trajectories and genetic variations. This knowledge gap limits a broader understanding of the potential speciation process that may exist and is an impediment to the development of management strategies and conservation efforts where the species is considered a pest or is threatened, respectively, across its distribution range in Africa (Kilwanila et al. 2022). Further, the species utilization either as bush-meat through illegal hunting or through ranching requires a deeper understanding of the genetic make-up of these populations in order to enhance its conservation across these regions (Kilwanila et al., 2021). An improved understanding of the cane rat ecology and biology could foster widespread farming and ranching practices of this species thereby relieving the pressure of exploitation on threatened slow-breeding wild large mammals across the savannah biomes in Africa (Rija et al. 2020).

Maternal lineages have traditionally been established using mitochondrial DNA (mt-DNA) because of the lineage markers that provide uni-parental genetic information which can be transmitted without homologous recombination, while its polymorphism is exclusively generated by mutation (Hutchison et al., 1974; Mauki and Adeola, 2021). Animal mt-DNA is deemed to strictly follow maternal inheritance and is highly variable. It evolves rapidly within a species, particularly the control region sequence (which includes the D-loop) compared with the nuclear DNA (Kolosov et al*.,* 2021). Mt-DNA has been used to assess haplogroup histories, maternal lineage and the genetic affinities of various vertebrate populations including mammals (Guo et al., 2005; Kamalakkannan et al., 2021), birds (Di Lorenzo et al., 2015), reptiles (Candan et al., 2021) and amphibians (Wang et al*.,* 2021). Mitochondrial DNA can also tell recent demographic processes acting on a population (Wanjala et al*.,* 2021), potentially informing the development of conservation strategies. For example, an analysis of mt-DNA can reveal whether a population has undergone a recent demographic expansion or has a more complex history (Bruford et al., 2003). Further, mt-DNA is valuable for characterizing populations genetically and comparing one population with others occurring in distant localities by considering sequences of the hypervariable D-loop region. For example, populations exhibiting low levels of genetic diversity and haplotype richness may likely reflect extreme isolation (Melosic et al*.,* 2017). Such studies are valuable to understanding species differentiation. A study of the common hamster (*Cricetus cricetus*) in Western Europe for example, has led to an understanding of its evolutionary relationships and possible migration flows (Neumann et al*.,* 2004, 2005). In another study, Aplin et al*.* (2011) surveying mitochondrial DNA of black rats collected across their global range found a strong phylogeographical pattern with well-differentiated lineages native to South Asia, the Himalayan region, southern Indochina, and northern Indochina to East Asia. These studies suggest strongly that long-time isolation and large geographic distances may act upon speciation leading to greater genetic differentiation among seemingly similar species.

The AGC occurs in almost all African countries west of the Sahara in grassland and wooded grassland habitats and prefers feeding on grasses and vegetation along waterways (Adu et al., 2017). The presence and predominance of dense thick cane-like grasses such as elephant grass (*Pennisetum purpureum*) and guinea grass (*Panicum maximum*) influence the geographical distribution of these rodent species (Kilwanila et al*.,* 2021). Within any particular range country, the AGC either has a wide or restricted distribution that corresponds to the habitat preferences, though information on population structure and distribution in the natural habitats across Africa is still lacking (Kilwanila et

al., 2021). Since mt-DNA is maternally inherited, uniparental gene flow could be reflected in populations with effective dispersal of females. For example, phylogenetic analysis of mt-DNA of Saxicolous Mice (*Phyllotis xanthopygus*) in Patagonia (Chile) provided evidence showing two geographically isolated groups due to lack of gene flow (Kim et al*.,* 1998) Among the AGC populations across isolated regions in Africa we lack genetic information to compare these populations. In our study, we investigate the maternal lineages of AGC populations in three countries in southern, eastern and western Africa. We analyzed mt-DNA D-loop sequence polymorphisms to assess the population history of AGC sampled in Tanzania, South Africa and Ghana, determined the haplotype clusters that exist between AGC sub-populations from the three countries and established the population genetic distances and genetic relatedness between AGC from the three geographic regions.

5.2 Materials and Methods

5.2.1 Source of data

The data analyzed in this study were obtained from three countries in eastern, southern and western Africa, namely Tanzania, Ghana, and South Africa respectively. In Tanzania, field-collected fecal samples from two blocks (Uluguru and Udzungwa) of the Eastern Arc mountains (Fig. 1) were analyzed. Details of the field sampling and DNA extraction have been provided elsewhere (Kilwanila et al., 2022). The data for South Africa and Ghana were retrieved from NCBI GenBank. The data consisted of previously published D-loop sequences from South Africa (accession nos. OP121209–OP121231) and Ghana (accession nos. AB675385 - AB675410). The data from Tanzania were deposited in the NCBI GeneBank with accession numbers OM475549 - OM475594. The locations of data collection in Ghana. Tanzania and South Africa are shown in Fig. 1.

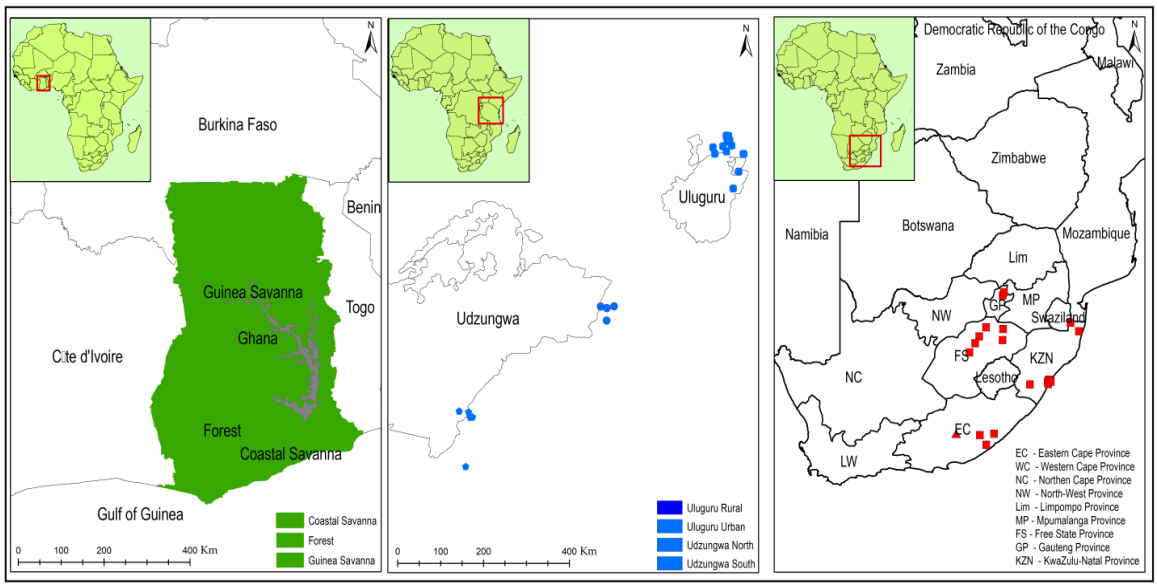

Figure 5.1: Location of samples collected in Tanzania, Ghana and South Africa

5.2.2 Data analysis

5.2.3 Establishing the genetic diversity indices between populations

We edited and aligned the sequences from all three countries using MEGA software (Kumar et al., 2016). The diversity indices (number of haplotypes - h, haplotype diversity - Hd and nucleotide diversity - π), were calculated using DNASP 5.10 (Ramos-Onsins and Rozas, 2002).

5.2.4 Examination of the phylogenetic relationships between Tanzania, Ghana and South Africa

The relationship between haplotypes was estimated by haplotype network using the minimum spanning method (Lwagami et al., 2010). The evolutionary relationships and probable ancestral connections among haplotypes from Tanzania, Ghana and South Africa were established by constructing a phylogenetic tree and median-joining network. The best nucleotide substitution model for haplotype alignments was determined using MEGA version 6.0 (Kumar et al., 2016) before phylogenetic tree construction. The model identified to have the lowest BIC was HKY+G model. The G value (0.48) was used in the construction of the phylogenetic tree. *Fukomys damarensis* and *Hystrix indica* sequences were used as outgroups. The node's confidence was tested using 1,000 replications. Median-joining network was constructed in NETWORK software version 4.6.1.0 (Bandelt et al., 1999). Genetic variations within and among AGC populations were separated using Analysis of Molecular Variance (AMOVA) (Excoffier et al., 1992) and computed conventional F-statistics from haplotypes with 1000 permutations using Arlequin software

(Excoffier et al., 2005). Genetic distances between populations were measured by MEGA software 6.0 (Kumar et al., 2016) to establish how the populations are distantly separated. The region genetic distance was calculated from pairwise FST (Fixation Index) coefficient.

5.3 Examination of the demographic history of the populations

To measure deviation from neutrality we used Tajima's D and Fu's FS implemented in the program Arlequin 3.5 (Fu,1997; Excoffier & Lischer, 2010) and p-values generated using 1000 simulations under a model of selective neutrality to test whether there was evidence for past population expansion. A mismatch distribution was estimated using Arlequin 3.5.2.1. The Harpending's raggedness pairwise distribution of observed and simulated frequencies was used to test the deviation the sudden expansion model (Harpending, 1994).

5.4 Results

5.4.1 Diversity indices of the Greater cane rat populations from three geographical regions

The East Africa population revealed high haplotype diversity (0.900-0.937) followed by the West Africapopulation (0.853-0.978) whereas the South African population had the lowest (0.250-0.970). There were slight differences in the nucleotide diversity ranging from 0.008-0.014 for West Africa, 0.006-0.011 for East and 0.002-0.011 for South Africa (Table 1).

Table 5.1: Diversity indices and neutrality tests of AGC (*Thryonomys swinderianus)* population sequences from eastern, western and southern Africa

Region	N	Haplotype number (h)	Haplotype diversity (Hd)	Nucleotide diversity (π)	Tajima's D	Fu's FS
Eastern African Populations (Tanzania)						
Udzungwa South	5	4	0.900 ± 0.051	0.00641	-0.991 ^{NS}	-0.176 (0.343)
Udzungwa North	6	5	0.933 ± 0.015	0.01528	0.351 ^{NS}	0.419 (0.396)
Uluguru Rural	10	7	0.933 ± 0.062	0.01118	0.747 ^{NS}	-0.403 (0.234)
Uluguru Urban	25	9	0.937 ± 0.029	0.01313	-0.447 ^{NS}	**-2.867 (0.033)**
Southern African population (South Africa)						
Eastern Cape	4	2	0.500 ± 0.265	0.002	-0.710 ^{NS}	1.099 (0.458)
Free State	8	2	0.250± 0.180	0.011	**-1.832****	**6.673 (0.016)**
KwaZulu-Natal	9	8	0.972 ± 0.064	0.006	-1.000 ^{NS}	**-4.479 (0.010)**
Gauteng	2	1	0 ± 0.000	0.0000	-	-
Western African Population (Ghana)						
Guinea Savannah	17	15	0.978 ± 0.031	0.012	0.189 ^{NS}	**−7.134 (0.002)**
Forest	22	7	0.853 ± 0.037	0.007	0.560 ^{NS}	0.989 (0.706)
Coastal Savannah	45	13	0.875 ± 0.024	0.012	1.442 ^{NS}	0.839 (0.673)

5.4.2 Variation within and between

The AMOVA results (Table 2) indicated significantly high variations within than between populations in the three geographical locations. The lowest genetic diversity between populations was observed between West African Canerat (0.1421). As expected, the distance between West and South Africa was much higher (0.869) than between East and South Africa (0.858) and was lowest East and South Africa (0.71022).

Table 5.2: Within and between population variations of AGC sequences from eastern, southern and western Africa

	Source of variation	DF	Sum of Squares	Variance Components	Percentage of Variations	FST	P Value
East Africa Populations	Between Populations	3	71.452	2.14	40.30	0.40297	0.00001
	Within Populations	42	133.287	3.17	59.70		
	Total	45	204.739	4.86	100		
South Africa Populations	Between Populations	3	63.420	3.23	43.90	0.43902	0.00001
	Within Populations	19	78.319	4.12	56.10		
	Total	22	141.739	7.35	100		
West Africa Populations	Between Populations	2	29.46	0.47	14.21	0.1421	0.0002
	Within Populations	81	229.57	2.83	85.79		
	Total	83	259.03	3.3	100		

5.5 Evolutionary relationship of *T. swinderianus* population collections from eastern, western and southern Africa

In order to determine the evolutionary history of the AGC populations from eastern, western and southern Africa, we established whether there was any haplotype clustering between AGC sub-populations from the three regions. Fig. 2 shows the phylogenetic tree of 60 haplotypes observed from AGC populations from Tanzania, South Africa and Ghana. Haplotypes from the same geographic region tended to cluster together.

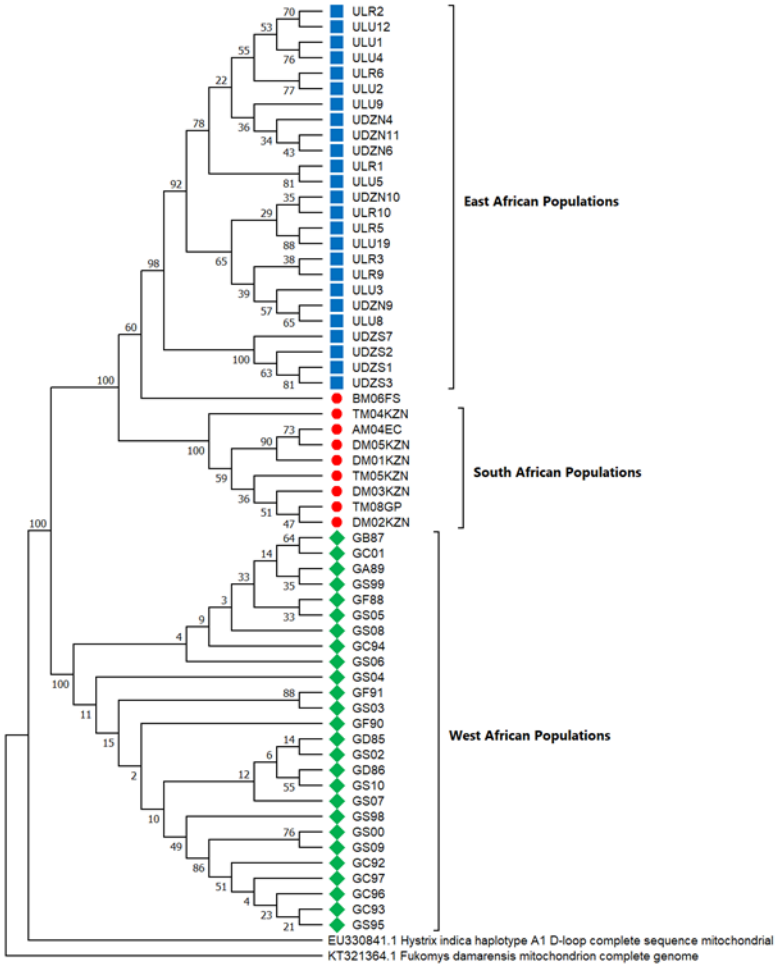

Figure 5.2: Maximum Likelihood phylogenetic tree of 60 haplotypes observed from AGC populations from Tanzania, South Africa and Ghana. The percent bootstrap value is represented by the numbers at the node after 1000 replication

5.5.1 Phylogenetic relationships of greater cane rat populations between western, southern and eastern Africa

To establish the phylogenetic relationships, we determined the population genetic distances and genetic relatedness between AGC from the three geographic regions. The haplotype network revealed that no haplotypes were shared between geographical regions (Fig. 3). The haplotypes could be divided into three main groups each one representing a geographical region (western southern and eastern Africa), but for Tanzania (East Africa) the group could further be divided into three main clades (Clade A, B and C) and two clades (D and E) for Ghana (West Africa), while the population from South Africa revealed no structuring (Fig 3).

The most dominant clade in all AGC populations from Tanzania was clade C which consisted of 12% haplotypes specific to Udzungwa North population, 20% haplotypes specific to Uluguru urban population and 8% haplotypes specific to Uluguru rural. Thirty-two percent (32%) of the haplotypes were shared between Udzungwa North and Uluguru urban and 40% of the haplotypes were shared between Uluguru rural and urban. Clade B was the second largest haplogroup consisting of majority (44%) of the haplotypes specific to Uluguru urban population. Twelve percent (12%) of the haplotypes were specific to Uluguru rural population and 16% were shared between Uluguru urban and rural. Clade A was the smallest haplogroup consisting of 16% haplotypes specific to Udzungwa South population. The most dominant clade in Ghanaian population was Clade D which is a complex consisting of haplotypes from all three zones. It consisted of guinea savannah specific haplotypes (30.76%), forest zone specific haplotypes (11.54%), coastal zone-specific haplotype (3.85%), haplotype shared between forest and coastal savannah zones (3.85%) and common haplotypes in all tree populations (15.39%). The second clade in the Ghana population was haplogroup E which is less complex consisting of coastal savannah zone-specific haplotypes (15.39%), guinea savannah zone-specific haplotypes (3.85%) and haplotypes shared between guinea savannah and coastal savannah (7.69%). The haplotype network of the South African populations revealed that KwaZulu-Natal had six unique haplotypes (66%) while the Free State had only one unique haplotype (11.1%). Also the network revealed that all South African populations shared one common haplotype (11.1%) while KwaZulu-Natal and Eastern Cape shared one common haplotype (11.1%).

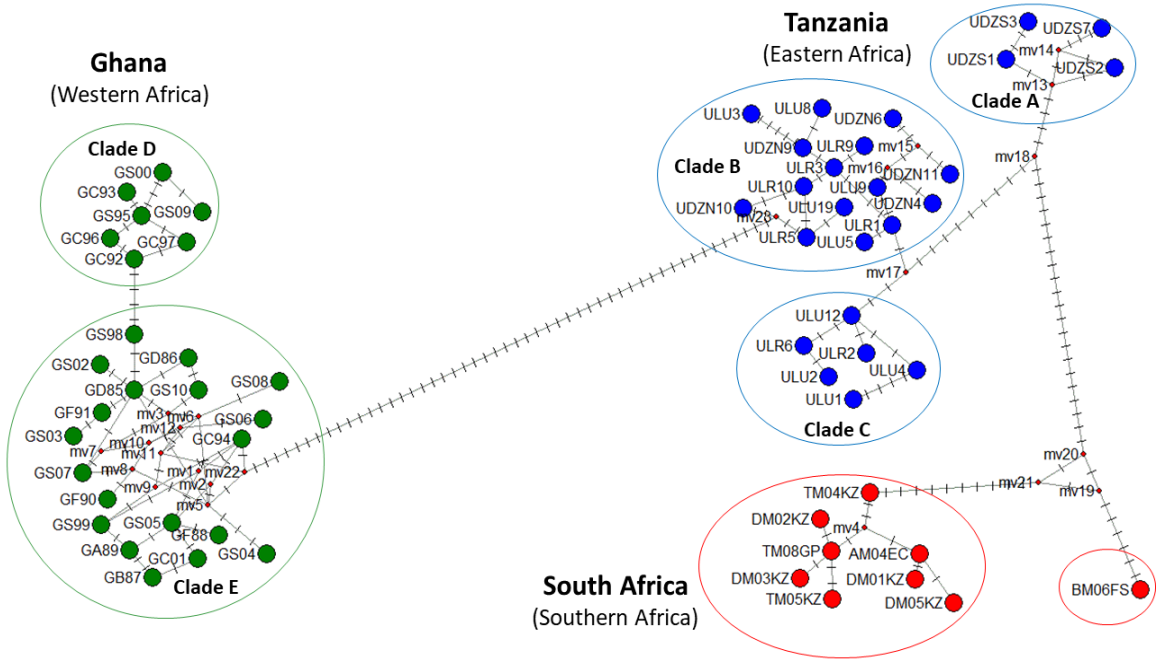

Figure 5.3: Median joining network of three AGC populations from western Africa (Ghana), eastern Africa (Tanzania) and southern Africa (South Africa) (A branch represents a single nucleotide change; red dots on branches represent inferred missing haplotypes (single nucleotide changes)).

5.5.2 Description of acronyms for haplotypes (Fig. 3)

Tanzania: ULU: Uluguru urban, ULR: Uluguru rural, UDZS: Udzungwa South, UDZN: Udzungwa North; Ghana: GS98, GC01 and GC94: coastal savannah zone, GD 86: shared between Guinea savannah and forest zone, GS06, GS05, GS08, GS04, GS02, GS03 and GS10: Guinea savannah specific, GF90, GF91, GF88: forest zone specific, GB87: shared between coastal and forest zone and GA89: common to all zones, GS99: shared between guinea and coastal savannah; Eastern Cape: AM04EC, Free State: BM06FS, KwaZulu-Natal: TM04KZ, TM05KZ, DM01KZ, DM02KZ, DM03KZ and DM05KZ; and Gauteng: TM08GP.

5.5.3 Demography and neutrality tests

Both Tajima'D values for estern and western Africa greater canerat populations were not significant while for Southern Africa, the Free State population revealed a negative and significant Tajima's D value (-1.832). In Tanzania, only the Uluguru urban population had a significant negative Fu's Fs value (-2.867 (0.033)). The Guinea savannah population from Ghana had a negative significant Fus FS values (−7.134 (0.002). The Free State population from South Africa had a positive and significant Fus FS value (6.673 (0.016))

while Kwazulu-Natal population had a negative and significant Fus Fs value (-4.479 (0.010) (Table. 1). A mismatch distribution analysis from eastern, western and south African AGC populations revealed a ragged mismatch plots (Figure S1), that indicating the populations are in equilibrium.

5.5 Discussion

We compared Mt-DNA d-loop haplotypes to establish the genetic diversity, population structure, neutrality and demographic history of cane rat populations that exist in three geographical regions of Africa (Tanzania, Ghana and South Africa). We found high haplotype diversity in AGC populations from Tanzania and Ghana while South African populations had relatively low haplotype diversity. Also, Ghana had slightly high nucleotide diversity compared to Tanzania AGC while South Africa populations had the lowest. The AMOVA results revealed that AGC populations from all the three geographic regions had significant FST values indicating high variations within than between populations. The phylogenetic tree and median joining network revealed strong population structuring. We also found a negative significant Fus Fs value from all populations. The Free State population from South Africa had a negative significant Tajima's D. Additionally, the neighbour joining network revealed three main clades in Tanzania and two main clades in Ghana.

Our analysis shows an earlier divergence of the West African population, and a later split of the other into southern and eastern African populations. Hence the Tanzania and South African populations presumably share a much more recent history than the western African populations. We also observed, a high haplotype diversity in eastern and western African AGC populations and relatively low diversity of the southern African populations, but was presumably due to small sample sizes. The high diversity and lack of shared haplotypes between populations may be caused by ancient common ancestry and independent colonisation events driving mutation rates in western, southern and eastern Africa (Kraatz et al., 2013; Coetzer, 2013).

By excluding the southern African AGC populations due to small sample size, the western African populations had low nucleotide diversity compared to those in eastern Africa. The low nucleotide diversity of AGC populations indicates small differences between haplotypes which suggests that the genetic diversity of the AGC in west Africa has declined. This may be attributed to a long domestication history in Ghana which is associated with artificial inbreeding probably as the result of small base populations that

are highly susceptible to inbreeding and genetic drift (Guerier et al., 2012). Domestication has been reported to reduce diversity in both flora and fauna (Liu et al., 2019). Further, inbreeding reduces fitness, increasing susceptibility to diseases and accelerating loss of genetic diversity (Smallbone et al., 2016). Furthermore, defaunation caused by overhunting for bushmeat trade in most west African countries including Ghana (Benítez-López et al., 2019) is known to have reduced species diversity and consequently the genetic diversity of mammals (Korner et al. 2017). Populations with little divergence could potentially be genetically less diverse and coupled with an inbreeding depression could affect many different fitness-related traits, including survival and reproductive success (Smallbone et al., 2016).

The network shape indicated a significant and strong geographical structure and very high level of sequence divergence or admixture, with every sampling locality presenting as a unique genetic entity since the results clearly show that no haplotypes were shared between western, eastern and southern Africa AGC populations. The indicated geographical structure among all populations was more visible when populations are compared across geographical locations. These results clearly mirror those from the diversity indices and AMOVA which show high within-population variance in all populations. The strong genetic differentiation points to a very minimal historical gene flow and no intermixing between the populations. Similar results were observed in Cape mole rats in South Africa (Visser et al.,2018). The strength of genetic structure relates to geographical distance and genetic distance (Foll & Gaggiotti, 2006).). The geographical distance between Ghana and Tanzania is approximately 3000 km and the distance between Ghana and South Africa is approximately 5000 km. Further, the genetic distance between the three groups was high which also conforms with the genetic differentiation index results. Wright (1951) reported that the degree of differentiation would be high if the Fst was >0.25. The AGC populations in this study met this criterion suggesting that the long history of spatial isolation between them has affected their genetic makeup. An alternative explanation for the observed genetic differences is that there was no female-mediated gene-flow between these populations (Tserenbataa et al., 2004). Also, the structure observed in this study may be caused by their territorial behaviour. This has been observed in other mammals including lions although Lyke et al. (2013) argued that not all social structure reflects breeding structure in some social mammals.

Further, the median joining network revealed that the populations from Ghana have little divergence and share the most common haplotypes between the three agro-ecological

zones. Less divergent haplotypes within animals and geographical locations suggest that the gene flow has occurred on a regional scale during some time in the recent past and the animals have not been subdivided by long-term biogeographic barriers (Simon et al., 2022). The AGC populations from Tanzania on the other hand were more structured with some haplotypes unique to a single population (Udzungwa South) while other haplotypes were shared between populations (Udzungwa North, Uluguru urban and Uluguru rural) indicating low genetic exchange. The South African population revealed non-significant clear geographic structuring indicating that there was gene flow between these populations and hence less divergence. The high gene flow between South African AGC populations may be due to the fact that AGC can cross different habitats including those which are terrestrial and aquatic (Kilwanila et al., 2021)

The analyses of demographic history revealed a recent population expansion for eastern, western and south African AGC populations. We found a negative and significant Tajima's D only for the Free State (South African population). This signifies an excess of low frequency polymorphisms, which was not expected and indicates population size expansion (e.g. after a bottleneck or a selective sweep). The population expansion in this area may be caused by the intense agricultural activities which open up suitable habitats for AGC.On the other hand, the mismatch revealed multimodal and ragged shape from all three geographical regions. The multimodal mimsmatch observed in the eastern and southern Africa population would be attributed to a stable and structured populations. Also, the bimodality of the mismatch distributions observed in our study could be interpreted as a result of the presence of different haplogroups (as seen in the haplotype network), rather than demographic stability.

Our study has revealed a strong genetic differentiation between eastern, western and southern African AGC populations and a unique maternal origin of each population. Future studies should look into the origin of these populations at finer scale and explore the potential speciation across the regions.

5.7 Implications for conservation of wild mammals across the savannah biomes

The high genetic diversity and the negative and significant Fus Fs values in some of the populations from the three geographical locations (East, West and South Africa), and the ragged mismatch distribution revealed that they were not threatened; this concurred with the current IUCN status of least concern. However, the phylogenetic tree and median joining network revealed that there were no haplotpyes shared between these three regions. This suggests that these populations need to be conserved since any potential

threat leading to the loss of haplotypes in any of the regions could result into disappearance of these haplotypes across Africa.

Also, the *T .swinderianus* populations revealed distinct phylogeographic patterns within the species indicating that the species has undergone different historical processes that have influenced its evolution. This information is important in informing the conservation strategies aimed at protecting the genetic diversity of the species across the regions. This will ensure the genetic diversity is preserved, and that local adaptations to specific environments are well preserved.

The AGC populations from Tanzania were richer in diversity than the two other groups from southern and western Africa. For *T. swinderianus* farming cross breeding of the eastern Africa stock with either Ghanaian or southern African stock is recommended to improve the genetic diversity of the species and enhance their economic value.

Acknowledgements

We wish to acknowledge the financial support from the Africa Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development (Grant no. 5799-TZ) for the PhD Scholarship offered to Shadia Ibrahim Kilwanila. Specimen collections in the field were assisted by Mr Ismail Issa and is cordially thanked.

Funding:

This study was funded by the Eastern and Southern Africa Higher Education Centers of Excellence Project—ACEII (Africa Centre of Excellence for Innovative Rodent Pest Management and Biosen-sor Technology Development—Sokoine University of Agriculture)—Credit no.5799-TZ.

Declaration of conflicts of Interest:

The authors declare no conflict of interest

Ethical clearance

All applicable institutional guidelines for the care and use of animals were followed. The study was conducted after issuance of an ethical clearance from Directorate of Postgraduate Studies, Research, Technology Transfer and Consultancy of Sokoine University of Agriculture, Tanzania on 10thMay, 2019 with an Institutional Review Ref. No. SUA/ DPRTC/186/17.

Data Availability Statement:

All sequences used in this study are in NCBI gene bank and are available for public use with accession numbers Tanzanian sequences OM475549 - OM475594, Ghanaian sequences AB675385 - AB675410 and South African sequences OP121209–OP121231

Author contribution

All authors contributed to the study conception and design. SIK collected data, performed laboratory and statistical analysis and with RHM prepared the first and final drafts of the manuscript. CML performed formal analysis, validation and reviewed the manuscript. AAR supervised data collection, and reviewed the manuscript drafts. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript for publication.

Permits

The Tanzania Wildlife Research Institute (TAWIRI) and Tanzania Commission for Science and Technology (COSTECH) provided a research permit number 2020-247-NA-2020-158 to conduct this study.

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CHAPTER SIX

6.0 GENERAL DISCUSSION

My study was undertaken to address the knowledge gap on cane rats in Africa particularly on the genetics and biology of the species in the wild, focusing more on isolated populations inhabiting the two Eastern Arc Mountain blocks in Tanzania. I further compared the maternal lineages of these populations with those occurring in West and South Africa to establish whether these geographically isolated populations could have common or different lineages, based on mitochondrial DNA. This missing knowledge is important for improving the management and conservation challenges as well as formulating intervention options where necessary. In the first chapter, I showed that cane rats, which could also apply to other wild species of mammals inhabiting EAMs, are distributed in isolated suitable habitats. These populations of AGCs are affected by anthropogenic activities including urbanization, illegal hunting and habitat fragmentation. These threats affect demographic processes such as migration and emigration, the rate of gene-flow, and survival of the species. One of the immediate impacts of illegal hunting is genetic loss through the removal of reproductively active individuals from the population. It affects gene flow between nearby demes whereas the fitness of the population is reduced not only by removing the genetically effective individuals, but also interfering with recruitment of new individuals. Additionally, bushmeat obtained through illegal hunting can lead to the transmission of zoonotic disease such as leptospirosis and leishmaniasis.

In chapter 3, I found AGC populations isolation which has led to two distinct main clades/haplo-groups to be linked to loss and fragmentation of habitats. Urbanization which requires transforming the landscape for settlements and infrastructure development is a major contributing factor in this process. As the human population increases, the areas under urbanization will expand into the suitable habitats for the AGCs thus compounding the ecological stress on these populations. This study clearly shows that the population from Udzungwa South was totally separated from the other three populations (Udzungwa North, Uluguru urban and Uluguru rural) which form two panmictic clades. The effect of fragmentation in the EAM is the limitation of gene flow between the isolated populations. Other pressures on these populations include increased consumption of cane rat meat obtained through hunting and shrinking of the population size which reduces population fitness. Previous studies in Tanzania (Rija, 2009) have demonstrated that hunting either illegally or legally is a threat to the sustainability of wildlife populaations throughout Africa. A shrinking population size of cane rats could increase the hunting pressure on other

species of mammals which are also facing the impact of habitat loss and urbanization. How this will impact on them is not currently known but it can be presumed that most of these other species including the porcupines and hares will be more seriously threatened. In conservation terms, in order for these populations to remain genetically viable, one of the strategies could be to re-establish protected vegetation or habitat corridors to encourage unhindered movements which are necessary to enable gene flow between isolated populations. In order for this to be successful, both policies and by-laws will need to be put in place to protect these movement corridors and stem out illegal hunting.

In chapter four, I found no strong genetic relatedness between AGC from Tanzania, Ghana and South Africa with every sampling locality presenting a unique genetic entity (i.e., no haplotypes were shared between populations). This suggests that AGC populations in Africa form separate major groups which have been isolated from each other by inhospitable biomes for the species. The study shows that the AGC populations of Tanzania were healthier with high genetic diversity than those from Ghana and South Africa. Ghanaian populations being less healthier might have been due to domestication and illegal hunting pressure which have effects on genetic diversity of the species.

The molecular biology aspects of the study have implications on game farming and ranching practices of cane rats as a source of protein for communities, as well as reducing the incidences of illegal hunting. An understanding of the genetics of the species in the wild can assist in the selection of parental stock for domestication n purposes to ensure that farmers are supplied with animals with quality features. Farming and ranching practices in Tanzania are guided by the management of wildlife in captive facilities regulation of 2020, the Wildlife Conservation Act of 2009, and the Wildlife Policy of 2007 which among other provisions includes one on wildlife ranching, farming, breeding, zoos, orphanage centers and game sanctuaries. Understanding of the genetic of the species will strengthen the implementation of these regulations, acts and policies based on solid scientific knowledge.

Also the subpopulations revealed in the study due to isolation have implications on conservation of the species since it show which sub-population is healthier and which population may need urgent conservation attention.

There are gaps in taxonomic understanding of the species within its distribution range. The study has provided new knowledge about the taxonomy of the species in Tanzania, and therefore will be the basis for eliminating taxonomic uncertainties in future, without going for new field collections. The genetic sequences have been deposited in the NCBI gene bank and are available for public use, if the needs arise these data can be accessed without going back to the field.

CHAPTER SEVEN

7.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

i. This study has shed light on the available AGC literature. It revealed that the available literature is biased both geographically and thematically. The available literature is published in local journals which limit wider utilization by scientists from different parts of the world.

ii. Both morphometric and genetic approaches revealed three morphotypes and haplo-groups/clades (Clades A, B and C) of AGCs from the EAM.

iii. The D-loop sequences comprising of samples collected from two EAM in Tanzania and three agro-ecological zones in Ghana showed a high nucleotide diversity in the AGC population from Tanzania and low nucleotide diversity in Ghana. Thus, there is significant genetic differentiation between AGC populations in Tanzania and Ghana. Further, the AGC populations in Tanzania and Ghana do not share common haplotypes indicating that these populations do not have the same maternal lineage.

iv. The study shows that genetic and morphometric methods could complement each other in understanding the evolutionary biology of vertebrate species that do not exhibit strong intra-species differentiation.

v. The findings of this study have broader applications, particularly in the farming and ranching industry of AGCs, conservation of the species, and eliminating taxonomic uncertainties of the species in Tanzania.

7.2 Recommendations for Future Research on Cane Rats

i) The results of this study were linked to urbanization impact on species, illegal hunting and habitat fragmentation. I suggest more studies into what and how environmental and habitat characteristics influence the genetic diversity

ii) Studies should focus on understanding how cultural norms among the local communities in the East and Southern Africa regions affect the consumption patterns of cane rat meat. Such studies will be a useful contribution to the development of the cane rat farming/ranching industry in these regions by informing decision-makers on markets and demands for the bush meat of cane rats.

iii) Future molecular studies on this species should focus on the genetic structure and dispersal patterns using different markers such as microsatellites and single nucleotide polymorphism (SNPs) to further define the genetic variations across its range areas in eastern and southern Africa for informed conservation of each genetic variant and to monitor the continued evolution of the species in a currently changing environment.

iv) More studies should be conducted on the geometric-morphometrics of AGC correlating morphometric features with other environmental aspects such as temperature and rainfall.

v) Future studies should focus on the feeding biology and diet of AGC as there is no published information in their natural range in the wild.

vi) There is no published information on the home range and movement patterns of AGC in the wild. I suggest future studies using technologies like telemetry and electronic loggers establish movements and dispersal pattherns.

vii) Future studies should also focus on the smaller cane rats as this study focused only on one species, the Greater Cane rat (*T. swinderianus)*.

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