Faunal turnover between east and southern African terrestrial vertebrates: is Malawi the geographical break?

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DEDICATION

I dedicate this dissertation to my wife Lucy for being such an understanding companion, it was a painful time to allow me to be away while we were expecting the beautiful twins Bethany and Berachah, without her this work would never have been accomplished. To my beloved daughters Esther and Crystal, their flexibility and adaptability afforded the completion of this thesis.

DECLARATION

I hereby declare that the work presented in this dissertation is my own, unless otherwise stated. Apart from the guidance received from my supervisors, assistance from all institutions and individuals in this dissertation is acknowledged. This dissertation has not been previously submitted for the degree at this or any other university and I therefore present it for examination for the degree of Ph.D.

Signed by candidate

Signature Removed

17TH FEBRUARY 2014

Potiphar Menaheim Kaliba Date

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ABSTRACT

The study centred on the investigation of phylogeographic structure within three forest associated bird species and two small mammal species, as well as two woodland associated bird species distributed across the Malawi Rift of Africa. The key objective was to investigate the extent to which geographically structured lineages exist within several bird (Stripe-cheeked Greenbul *Andropadus milanjensis*, Malawi Batis *Batis dimorpha*, Bar-throated Apalis *Apalis thoracica*, Southern Puffback *Dryoscopus cubla* and White-browed Robin-chat *Cossypha heuglini*) and small mammal species (Delectable Soft-furred Mouse *Praomys delectorum* and Dark-coloured Brush-furred Rat *Lophuromys aquilus*) distributed across the Malawi Rift. Analyses of a combination of mtDNA (1041 bp ND2 in birds, and 1130-1143 bp Cytochrome-b and 461-466 bp control region in mammals) and nDNA (463-481 bp CHDZ, 569-572 MUSK and 594 bp TGFb2 in birds, and Beta-Fibrinogen intron-7 in small mammals) revealed significant population structure in each of the five forest associated species studied. In contrast, woodland associated birds exhibited reduced spatial genetic structure across the Malawi Rift. Collectively the result suggest that phylogeographic breaks for forest associated species occur in the southern highlands separating Mount Namuli in Mozambique and Mount Mulanje as well as between Mount Mulanje and Mount Zomba in Malawi; in the central highlands that split Malawi into two halves, and within the northern highlands separating the Misuku Hills and Nyika Plateau. The Misuku Hills are also separated from the Udzungwa Mountains of the Eastern Arc and volcanic Mount Rungwe in Tanzania. Genetic differences exhibited by the taxa investigated across the phylogeographic breaks and degree of lineage turnover revealed in the small mammals support observations by

Vrba (1985) based on fossil mammal assemblages that Malawi may be geographically key to understanding faunal turnover between southern and east African animal taxa. The detected phylogeographic breaks primarily occur in forest reserves that are not adequately protected with the exception of Nyika National Park. Illegal logging and uncontrolled fires are threatening the montane ‘sky islands’ population, thus compromising the conservation of the fauna and important evolutionary distinct units.

Chapter 1

Review of eastern and southern Africa phylogeography, the Malawi Rift and of the taxa investigated in this thesis

1.0 INTRODUCTION

1.1 Setting the Scene

Biogeography and phylogeography involves the investigation of geographical distributions and relationships in terms of the genealogy and demographic history of individual species (Avise 2000), or a group of related taxa (Sgariglia & Burns 2003). The relationships that exist among lineages and their distributions today likely arose as a result of dynamic processes involving both temporal and spatial scales (e.g. Bowie et al. 2004), which makes biogeographic and phylogeographic approaches necessary for adequate interpretation of pattern and process (Templeton 1998). The phylogenetic analysis of DNA sequences for a given taxon, when superimposed over a geographical area may help to explain whether the history of the species has been one of isolation, panmixia or a combination of both (Pavlova et al. 2003).

The historical geographical processes that can shape the genetic structure among species include population division, range expansion, as well as long distance colonisation, which are expected under a neutral model of evolution to produce distinct patterns and relationships among haplotypes/alleles (Templeton et al. 1995). Mitochondrial DNA (mtDNA) is most often used to infer the patterns of genetic variation because it is maternally inherited and does not undergo recombination and has a

relatively fast mutation rate (Avise 2000). Therefore, it enables the construction of matrilineal genealogies that often show clear relationships among individuals from different populations (Irwin 2002; Zink & Barrowclough 2008).

However, when inferring process, the use of a single molecular marker such as mtDNA can be misleading. For example, a phylogeny based on nuclear DNA (nDNA) in Hawaiian crickets (*Luapala* spp) strongly contradicted the mtDNA phylogeny (Shaw 2002). Comparison between these two molecular markers suggested the repeated hybridisation and interspecific mtDNA gene transfer in the recent history of the species. Therefore, although the phylogeographical pattern inferred from mtDNA will often be correct, caution should be used when inferring process (Irwin 2000; Barrowclough 2008).

1.2 The study

This study examines the biogeography, phylogeography and genetic diversity among several avian and mammalian taxa distributed throughout the Malawi Rift. Specifically, the resulting molecular data generated are used in combination with morphological evidence to determine species boundaries and relationships across the geographical region from southern Tanzania (Mount Rungwe, Fig. 1.1) through Malawi (Misuku Hills, Nyika Plateau, Ntchisi Highlands, Mount Zomba and Mount Mulanje) to northern Mozambique (Mount Namuli). Bowie et al. (2005) demonstrated that the Eastern Arc Mountains and northern Malawi Rift thrush species *Turdus abyssinicus nyikae* is closely related to the Albertine Rift taxa *T. a. baraka* and *T. a. bambusicola* and the Kenyan highland *T. a. abyssinicus*. These taxa are both genetically and morphologically distinguishable from the Northern Eastern Arc endemics *T. helleri* and

T. roehli, as well as the southern African clade comprising *T. olivaceus* and *T smithi*. The sister relationship of these clades suggests that southwestern East Africa, central Africa and the Kenyan highlands are biogeographically linked. A similar scenario of faunal turnover has also been demonstrated to occur in the large mammal fauna across the Malawi Rift (Vrba1985; Partridge et al. 1995). The above are but a few of the examples that illustrate that studies of phylogeography and genetic diversity can help delimit species boundaries and determine where phylogeographical breaks occur specifically, as well as determine when these breaks occurred, thereby providing a temporal perspective. A better understanding of the Malawi Rift system is critical if we are to understand the linkages and relative roles of ecology and history as processes separating the east African avifauna from that of southern Africa (Table 1.1). Determining how general this pattern of faunal turnover is in Malawi forms the central theme of this study.

1.3 Eastern and southern Africa phylogeography

Species turnover between eastern Africa and southern Africa is an important phenomenon, which has occurred as a consequence of the rapid changes in the environment between these regions. The African continent has undergone major climatic changes since the Plio-Pleistocene (deMenocal 1995), which are likely to have caused shifts in environmental pattern (Smith et al. 1997). Changes in regional topography caused by the 'African super swell' (Partridge et al. 1995) before 2 Myrs BP (million years before present) is thought to have resulted in the isolation of the eastern lowland forests (coastal) from the main Guinea-Congolian rainforest block (Lovett & Wassre 1993; Coppes 1994), and generally is thought to have had a profound effect on the

paleoclimate of Africa. Another significant geological change that affected the paleoclimate and hence the biota of eastern and southern Africa during the Tertiary period was the general drying of northern Africa over the past 5 Myrs BP, that resulted in considerable contraction of forest cover, after the continent collided with Asia in the early Miocene (Axelrod & Raven 1978; Voelker et al. 2010). The development of rift valley systems in eastern and southern Africa has also been postulated to have had a major vicariance impact on intraspecific divergence in many taxa, as well as on divergence among allopatrically distributed members of closely-related species complexes (Tolley et al. 2011; Lorenzen et al. 2012).

Plio-Pleistocene climate changes in Africa are probably one of the main forces driving diversification of African species. Several models have been proposed and tested to reveal the effect of vegetation shifts and landscape modifications during paleoclimatic changes on faunal assemblages through space and time. Many researchers have emphasized allopatric speciation, either in refugia (Haffer 1969), in isolated montane forests (Fjeldså & Lovett 1997; Roy et al. 2001; Bowie et al. 2004; Fjeldså & Bowie 2008; Fjeldså et al. 2013), in scrap and lowland forests (Marks 2010), or across major rivers and other topographic barriers (Haffer 1992; Moritz et al. 2000; Anthony et al. 2007; Voelker et al. 2013) as likely possibilities.

It has also been observed that of extant bird species that occur in the Malawi Rift, 67 species (Table 1.1) exhibit turnover (beta-diversity) across the rift (Dowsett-Lemaire & Dowsett 2006; del Hoyo, Elliot & Christie 2006). 88% (59) of these birds pattern of the turnover is latitudinal breaking the Malawi Rift into north-south lineages. Therefore, Malawi Rift is an important area to investigate if we are to understand faunal turnover

between eastern and southern Africa. This study seeks to detect, and if present, attempt to unravel the processes that have let to the formation of genetic structure and turnover in Malawian birds and small mammals. Further, coupled with morphological information available from the literature, this study seeks to re-evaluate the species status of putative endemic taxa (currently considered subspecies, e.g. *Apalis thoracica flavigularis*). This is critical from both a scientific as well as an economic perspective, as Malawi currently does not have an endemic bird species and the recognition of species with a firm scientific foundation could be of considerable importance for bird ecotourism initiatives, as well as for the promotion of sustainable community-based conservation initiatives. This research would provide a fundamental foundation for future ornithological and small mammal research in Malawi.

1.4 Malawi Rift

Malawi lies at the southern end of the Great African Rift Valley, between latitudes 9° 22' and 17° 07'S, and between longitudes 32° 40' and 35° 55'E. Its north-south length is about 900 km and width varies from 80 km to 160 km. Total surface area is 118,464 sq. km of which 24,208 sq. km is water. The rift depression is occupied by Lake Malawi, its narrow shore plains and the valley of the Shire River which drains the Lake to the lower course of the Zambezi River in Mozambique. Malawi has several isolated hills and high plateaus which from north to south include the Misuku Hills, Nyika Plateau, Viphya Plateau, Mount Zomba and Mount Mulanje, which is the highest point in south-central Africa at 3002 m a.s.l. (Survey Dept. 1983; Clarke 1983).

Malawi's climate is tropical with maritime influences from the Mozambique Channel. It is also influenced by Lake Malawi. Malawi has three main seasons: the cool and dry period from May to August, the warm and dry period from September to December, and the warm and wet period from December to April. Annual rainfall ranges from about 600 mm to 3000 mm. High rainfall is experienced in areas of higher elevation as a consequence of orographic rain (Clarke 1983).

Malawi falls within the Zambezian Regional Centre of endemism (Dowsett-Lemaire et al. 2001) and has a wide diversity of biotic communities ranging from the low lying rift valley woodlands to montane forest and grassland. It is likely that over the last two millennia man-induced fire has played a major role in determining the plant community structure, with a shift from open to closed canopy woodland having taken place (Clarke 1983).

1.5 Taxon selection

In the study, I examined three forest specific bird species or species complexes: the Stripe-cheeked Greenbul **(***Andropadus milanjensis***,** Pycnonotidae), Malawi Batis (*Batis dimorpha*, Platysteiridae) and Bar-throated Apalis (*Apalis thoracica*, Cisticolidae) in order to test whether the perceived low individual dispersal among forest species (Dowsett 1985; Bowie et al. 2005; 2006) is reflected in their phylogeographic structure. The White-browed Robin-chat (*Cossypha heuglini*, Turdidae) and Southern Puffback (*Dryoscopus cubla*, Malaconotidae), which are both woodland bird species, were also investigated in order to compare and contrast the extent of phylogeographic structure between forest and woodland bird taxa, with the expectation that the sky island nature of

forest patches will result in greater phylogeographic structure of forest associated taxa. The bird species investigated encompass a number of subspecies that are distributed across the Malawi Rift (Table 1.1) which will facilitate the study of phylogeographic structure; genetic variability and gene flow (Bowie et al. 2005). Phylogeographic structure was also investigated in two forest specific small mammals, the Delectable Soft-furred Mouse (*Praomys delectorum*; Muridae) and the Dark-coloured Brush-furred Rat (*Lophuromys aquilus,* Muridae) to enable comparisons to be made between bird and small mammal taxa. Theoretically, the small mammals should show greater genetic structure than forest birds due to perceived lower dispersal potential. By critically testing expected levels of genetic structure with observed levels of genetic structure in birds and small mammals I expect to meet the objectives of this study, as well as identify sampling gaps that could be filled in future studies of these and other taxa across the Malawi Rift.

Further, the genus *Praomys* has been demonstrated to encompass a rapid radiation (Lecompte et al. 2005), which has also been suggested for *Lophuromys* as this group too has undergone extensive genetic diversification (Lavrenchenko et al. 2004). Therefore, by using rodents it will help to test if the depth of the genetic breaks observed with different animal species can be related to historical events (Taberlet et al. 1998).

1.5.1 Forest Species

1.5.1.1. *Stripe-cheeked Greenbul*

The Stripe-cheeked Greenbul (*Andropadus milanjensis*, Shelley 1894) has been recorded from southern Kenya in Ol Doinyo Oroko, as well as from the Chyulu and Taita Hills. In Tanzania, the species occurs in Monduli, Mbulu, Usambara, Udzungwa,

Rungwe, and Isoko Mountains. In Malawi, it inhabits the Misuku Hills, Nyika Plateau, central highlands, and Mounts Zomba and Mulanje. In Mozambique, it is found in the Unangu, Namuli and Chiperone Mountains, and in Zimbabwe on Manica Plateau, Inyanga Hills and Selinda Mountain (Fig. 1.2). The species is commonly found in evergreen forests between 1000 m and 2000 m above sea level (Dowsett-Lemaire & Stjernstedt 1987; Keith 1992).

There are three putative subspecies of *Andropadus milanjensis*: *Andropadus milanjensis milanjensis* (Shelley 1894), *Andropadus m. olivaceiceps* (Shelley 1896) and *Andropadus m. striifacies* (Reichenow and Neumann 1895). *Andropadus m. milanjensis* has a body that is darker above and below with darker eyes and less clearly defined dark cheeks with a grey head, pale eye-ring and faint white streaks on the cheeks. It is confined to eastern Zimbabwe, northern Mozambique and Mount Mulanje in southern Malawi (Keith 1992; Sinclair & Ryan 2003; Dowsett-Lemaire & Dowsett 2006). *Andropadus m. olivaceiceps* differs from *A. m. milanjensis* by having paler eyes and a paler plumage, a body which is darker above with greener belly, a robust bill, an olive crown and less conspicuous eye-ring. This subspecies occurs in southern Tanzania, northern Mozambique and Malawi, but the ranges do not overlap with that of *A. m. milanjensis* (Keith 1992; Sinclair & Ryan 2003; Dowsett-Lemaire & Dowsett 2006). In contrast, *A. m. striifacies* has brighter yellow underparts with a paler throat, and eyes that are pale with an eye-ring that is not prominent, and an olive crown. It is confined to southern Kenya and Tanzania (Keith 1992; Sinclair & Ryan 2003).

1.5.1.2 *Malawi Batis*

The Malawi Batis (*Batis dimorpha*) occurs in Malawi and northern Mozambique is often treated as a subspecies of the Cape Batis (*Batis capensis*, Linnaeus 1766), a more widespread species in the Zimbabwean Mountains and forests of South Africa (Fig. 1.3). Male birds have a white wing bar with a broader breast band. Females have scapulars, which are blackish olive brown whereas the Cape Batis has rich rufous wing bars and chestnut flanks in both sexes, with males having a black breast band and golden eyes whereas females have a chestnut breast band. *Batis dimorpha* inhabits montane forests and secondary growth ranging between 1150 m to 2450 m a.s.l (Erard & Fry 1997; Sinclair & Ryan 2003).

The species has two subspecies *Batis d. dimorpha* (Shelley 1893) and *Batis d. sola* (Lawson 1964). *Batis d. dimorpha* is found in the southern part of Malawi and adjacent Mozambique. Males have white flanks and females have scapulars, which are blackish olive brown. *Batis d. sola* is restricted in northern Malawi, male birds are like male *dimorpha*, whereas females have a grayer mantle than female *dimorpha* and scapulars that are black on the lateral side, as well as black lesser coverts with tertials fringed white (Erard & Fry 1997).

1.5.1.3 *Bar-throated Apalis*

The Bar-throated Apalis (*Apalis thoracica*, Shaw 1811) is a small forest associated bird and contains 19 recognised subspecies (Ryan et al. 2006). The species has five subspecies which are distributed across the Malawi Rift (Fig. 1.1 & 1.4): the *Apalis thoracica murina* (Reichenow 1904), *Apalis thoracica youngi* (Kinnear 1936), *Apalis*

thoracica whitei (Grant & Mackworth-Praed 1937), *Apalis thoracica flavigularis* (Shelley, 1893), and *Apalis thoracica lynesi* (Vincent 1933). Two of the recognised subspecies (*A. t. flavigularis* and *A. t. lynesi*) are of conservation concern and are regarded as Endangered and Near-threatened, respectively (BirdLife International 2010). The striking geographic variation in plumage makes its species complex an interesting clade for phylogeographic studies across the Malawi Rift, as it will help to relate plumage variation and the phylogeographic breaks identified across the Malawi Rift.

1.5.1.4 *Delectable Soft-furred Mouse*

The Delectable Soft-furred Mouse (*Praomys delectorum*, Thomas 1910) belongs to the *Praomys* group which is considered one of the most diverse and successful groups of Old World rodents. The *Praomys* group complex contains a minimum of 40 species. Traditionally the taxonomy of the *P. delectorum* group has a single species *P. delectorum* that encompasses three junior synonyms: *melonotus* (Allen & Loveridge 1933), *taitae* (Heller, 1912) and *actomastis* (Hatt 1912). Recent extensive revision of this complex provides convincing evidence to elevate the taxa *P. melonotus* as well *P. taitae* to full species, together with *P. delectorum*, (Carleton & Stanley 2012). The group has successfully colonised various biomes in Africa as well as the Arabian Peninsula, ranging from the equatorial rain forest to the Sahelian savannas (Lecompte et al. 2002b; 2005). *Praomys delectorum* group has been recorded to inhabit high plateaus and isolated mountains within its distribution range (see Fig. 1.5) with *P. taitae* extending from southeast Kenya to southern Tanzania (Udzungwa). *Praomys melonotus* from Mt. Rungwe in Tanzania to northern Malawi (Misuku and Nyika Plateau), and *P. delectorum* from central Malawi to Mozambique and northeast Zambia (Ansell 1978; Ansell &

Dowsett 1988; Carleton & Stanley 2012). In the *Praomys delectorum* group the individuals have soft brown fur, a long snout, and tail without any tuft of hairs on the tip, with a dirty white underbelly. The species lives on the ground and is rarely observed in trees, bushes, or in other arboreal habitats, (Stanley et al. 2005). It is listed as a Near-threatened species (IUCN Red List 2010).

1.5.1.5 *Dark-coloured Brush-furred Rat*

The *Lophuromys* group contains two subgenera, *Kivumys* (3 species) and the *Lophuromys* (18 species), to which *L. aquilus* belongs (Nowak 1999). Allopatric speciation is considered to have played a major role in shaping the evolution of this genus (Lavrenchenko et al. 2004). Research that has been conducted on the group reveals that isolated species exist a relatively short distance away from each other (Dierterlin 1976; Stanley et al. 2005).

The Dark-coloured Brush-furred Rat (*Lophuromys aquilus*, True 1892) occurs in lowland and montane habitats. Its distribution range (Fig. 1.6) extends from north-eastern Angola through the eastern Democratic Republic of Congo, Burundi, Uganda, Kenya, and south through Tanzania, Malawi, northern Zambia and northern Mozambique (Delany 1975; Smithers & Lobao Tello 1976; Ansell 1978; Ansell & Dowsett 1988; Corti et al. 2000). *Lophuromys aquilus*, derives its name from the unique stiff hairs that make up its speckled pelage, providing a texture of a soft brush. The skin is delicate and the animal appears to use this as a predator avoidance mechanism. The tail breaks easily and could be lost so that the animal can escape. However, once lost it does not regenerate.

The species appears to feed to a greater extent on animal matter than what most muroids normally do (Dierterlin 1976; Nowak 1999).

1.5.2. Woodland Species

1.5.2.1 *Southern Puffback*

The Southern Puffback (*Dryoscopus cubla* Latham 1801) is a resident of East African and Southern African mesic woodlands. It is widespread and common from sea level to about 2200 m a.s.l. It occurs in Somalia, Kenya, Tanzania, Democratic Republic of Congo, Rwanda, Burundi, Zambia, Zimbabwe, Malawi, Mozambique, Angola, Namibia, Botswana, South Africa and Swaziland (Fig. 1.7). Males are strikingly pied, with a black crown and back, pure white rump and underparts, with wings patterned black and white, and it has red eyes. Females are duller and less pure white, and have a grey rump (Fry 1997).

The species has one subspecies which occurs across the study area, *Dryoscopus cubla hamatus* (Hartlaub 1863). This taxa, *D. c. hamatus*, is found in Kenya west of the Rift Valley to the Kavirondo Gulf in Tanzania, extending to the northeast Democratic Republic of Congo, northern Angola, northern and western Zambia, eastern Zimbabwe, Malawi, north eastern South Africa. Adult males have a narrow line under the eye the crown, nape and sides of its neck side are glossy bluish black. The underparts are white. In adult females the lores are whitish as opposed to pale as in male birds (Fry 1997).

1.5.2.2 *White-browed Robin-chat*

The White-browed Robin-chat (*Cossypha heuglini* Hartlaub 1866) is resident in northeastern Nigeria, northern Cameroon, southwestern Chad, southwest and northeastern Central African Republic, southern Ethiopia, southern Somalia through most of central Africa to southern Angola, northeastern Namibia, northwestern Botswana, and north eastern South Africa (Fig. 1.8). This species has black head, a long white supercilium, grey wings and red-orange underparts (Oatley, Fry & Keith 1992).

The species has one subspecies which occurs within the study area, *Cossypha heuglini heuglini* (Hartlaub 1866). Its distribution ranges from southern Chad, west and southern Sudan, north and southwest Central African Republic east to Ethiopia, south to eastern Angola, northern Botswana, Zimbabwe and northern South Africa (Oatley, Fry & Keith 1992).

1.6 Study objectives

The key objective of this thesis was to investigate whether phylogeographically there are genetic differences among and within several birds and small mammal species distributed across the Malawi Rift. This was in order to determine species biogeographic relationships across the geographical region from southern Tanzania (Rungwe Mountains), through Malawi to northern Mozambique (Mount Namuli).

1.7 Hypotheses

Given the rapid changes in the environment and species induced by Africa's changing climate the extent of turnover exhibited in bird and mammal taxa between

eastern and southern Africa (Vrba 1985; Partridge et al. 1995; Bowie et al. 2005; Table 1.1), I hypothesise that: phylogeographically there are genetic differences among some (if not many) forest associated species in Malawi, with potential breaks occurring: 1) across the lowland gap between Mt. Zomba and Mt. Mulanje in southern Malawi, 2) in the central highlands, splitting Malawi into two, and 3) across the lowland gap that separates Nyika from the Misuku Hills in northern Malawi (Fig. 1.1). I expect the forest associated species to show greater phylogeographic structure than the woodland associated species.

1.8 Research questions

To test these hypotheses, the research should be able to answer the following questions.

1. Can genetic turnover in animal species distributed between east and southern Africa be detected and does it occur across the Malawi Rift?
2. Are geographically restricted taxa endemic to Malawi, and what are their distributions in Malawi?
3. Where are the common phylogeographic breaks in Malawi and how do they relate to the current placement of national parks?

Fig. 1.1 Malawi Rift depicting the montane highland regions sampled.

Figure 1.2 Distribution of *Andropadus milanjensis*.

Figure 1.3 Distribution of *Batis dimorpha*.

Figure 1.4 Distribution of *Apalis thoracica*.

Figure 1.5 Distribution of *Praomys delectorum.*

Figure 1.6 Distribution of *Lophuromys aquilus*.

Figure 1.7 Distribution of *Cossypha heuglini.*

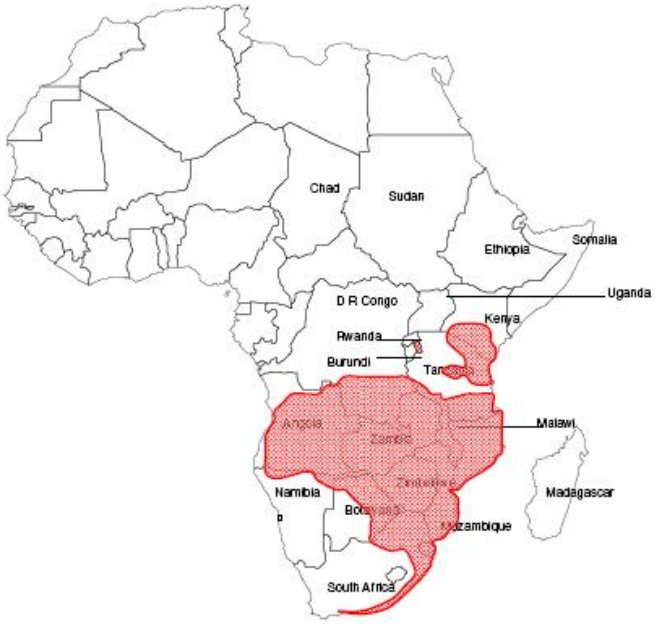


Figure 1.8 Distribution of *Dryoscopus cubla*.

Table 1.1 Taxa with a southern range limit (turnover point) in southern Malawi = 20, with a southern range limit in north of Malawi = 21, with a northern range limit in northern Malawi = 2, with northern range limit in central Malawi = 3, with southern range limit in central Malawi = 3, with northern range limit in southern Malawi = 3, with eastern range limit in Malawi = 7, with western range limit in Malawi = 1, with southeastern range limit in Malawi = 6, and with a southwestern range limit in Malawi = 1, (adapted from Dowsett-Lemaire & Dowsett 2006; del Hoyo, Elliot & Christie 2006).

SPECIES NAME	**SPECIES TURNOVER POINT IN MALAWI**
Hildebrandt' Francolin- *Francolinus hildebrandti*	S Malawi southern range limit
Scaly Francolin - *Francolinus squamata doni*	N Malawi southern range limit
Swainson Francolin-*Francolinus swainsonii lundazi*	Northeastern range limit
Double-banded Sandgrouse - *Pterocles bicinctus multicolor*	Eastern range limit
Pink-breasted Turtle Dove- *Streptopelia lugens lugens*	N Malawi southern range limit
Mountain Nightjar - *Caprimulgus poliocephalus guttifer*	N Malawi southern range limit
Bar-tailed Trogon - *Apaloderma vittatum*	S Malawi southern range limit
Böhm's Bee-eater - *Merops boehmi*	Eastern range limit
Pale-billed Hornbill - *Tockus pallidirostris neumanni*	S Malawi southern range limit
Green Barbet – *Stactolaema olivacea rungweensis*	N Malawi northern range limit
Green Barbet – *Stactolaema olivacea belcheri*	S Malawi southern range limit
Moustached Green Tinkerbird – *Pogoniulus leucomystax*	S Malawi southern range limit
Miombo Pied Barbet – *Tricholaema frontata*	Eastern range limit
Black-backed Barbet - *Lybius minor macclounii*	Southeastern range limit
Stierling's Woodpecker – *Dendropicos stierlingi*	Western range limit
Fischer's Sparrow-Lark – *Eremopterix leucopareia*	C Malawi southern range limit
White-headed Saw-wing – *Psalidoprocne albiceps albiceps*	C Malawi southern range limit
Rufous-chested Swallow – *Cecropis semirufa semirufa*	Eastern range limit
Pearl-breasted Swallow – *Hirundo dimidiata marwitzi*	Eastern range limit
Shelley's Greenbul – *Andropadus masukuensis masukuensis*	N Malawi southern range limit
Eastern Mountain Greenbul – *Andropadus nigriceps fusciceps*	S Malawi southern range limit
Little Greenbul – *Andropadus virens zombensis*	S Malawi southern range limit
Grey-olive Bulbul – *Phyllastrephus cerviniventris*	S Malawi southern range limit
Cabanis's Greenbul – *Phyllastrephus cabanisi placidus*	S Malawi southern range limit
Thyolo Alethe - *Pseudalethe choloensis choloensis*	S Malawi northern range limit
Sharpe's Akalat – *Sheppardia sharpei sharpie*	N Malawi southern range limit
Cinnamon Bracken Warbler – *Bradypterus cinnamomeus nyassae*	S Malawi southern range limit
Evergreen Forest Warbler – *Bradypterus lopezi granti*	S Malawi southern range limit
Mountain Yellow Warbler – *Chloropeta similis*	N Malawi southern range limit
Southern Hyliota – *Hyliota australis inornata*	C Malawi northern range limit
Brown Warbler – *Sylvia lugens jacksoni*	S Malawi southern range limit
Tinkling Cisticola - *Cisticola rufilatus ansorgei*	Eastern range limit
Churring Cisticola – *Cisticola njombe mariae*	N Malawi southern range limit
Trilling Cisticola – *Cisticola woosnami lufira*	N Malawi southern range limit
Black-lored Cisticola – *Cisticola nigriloris*	N Malawi southern range limit
Rudd's Apalis – *Apalis ruddi caniviridis*	S Malawi northern range limit
Grey Apalis – *Apalis cinerea alticola*	Southeastern range limit
Chapin's Apalis – *Apalis chapini strausae*	C Malawi southern range limit
Böhm's Flycatcher – *Muscicapa boehmi*	Southeastern range limit
White-eyed Slaty-flycatcher – *Melaenornis fischeri nyikensis*	S Malawi southern range limit
Dark Batis - *Batis crypta*	Southwestern range limit
Malawi Batis - *Batis dimorpha sola*	N Malawi northern range limit
Malawi Batis - *Batis dimorpha dimorpha*	C Malawi northern range limit

White-tailed Blue Flycatcher – *Trochocercus albicauda*	S Malawi southern range limit
Spot-throat – *Modulatrix stictigula*	N Malawi southern range limit
Mountain Illadopsis- *Illadopsis pyrrhoptera nyasae*	N Malawi southern range limit
African Hill Babbler – *Pseudoalcippe abyssinica stictigula*	S Malawi southern range limit
White-winged Black Tit – *Parus leucomelas insignis*	S Malawi southern range limit
Anchieta's Sunbird - *Anthreptes anchietae*	Southeastern range limit
Green-headed Sunbird – *Nectarinia verticalis viridisplendens*	Southeastern range limit
Eastern Double-collared Sunbird – *Nectarinia mediocris bensoni*	S Malawi southern range limit
Scarlet-tufted Malachite Sunbird – *Nectarinia johnstoni nyikensis*	N Malawi southern range limit
Sousa's Shrike – *Lanius souzae tacitus*	Southeastern range limit
Fülleborn's Black Boubou – *Laniarius fuelleborni fuelleborni*	N Malawi southern range limit
Olive Bush Shrike – *Malaconotus olivaceus makawa*	S Malawi northern range limit
Olive Bush Shrike – *Malaconotus olivaceus bertrandi*	S Malawi northern range limit
Waller's Red-winged Starling – *Onychognathus walleri walleri*	N Malawi southern range limit
Slender-billed Starling – *Onychognathus tenuirostris theresae*	N Malawi southern range limit
Chestnut-mantled Sparrow-weaver – *Plocepasser rufoscapulatus*	Eastern range limit
Baglafecht Weaver – *Ploceus baglafecht nyikae*	N Malawi southern range limit
Bertram's Weaver – *Ploceus bertrandi*	S Malawi southern range limit
Mountain Marsh Widowbird – *Euplectes psammocromius*	N Malawi southern range limit
Crimson-rumped Waxibill – *Etrilda rhodopyga centralis*	N Malawi southern range limit
African Citril – *Serinus citrinelloides hypostictus*	S Malawi southern range limit
Lemon-breasted Canary – *Serinus citrinipectus*	S Malawi northern range limit
Streaky Seedeater – *Serinus striolatus whytii*	N Malawi southern range limit
Oriole Finch – *Linurgus olivaceus kilimensis*	N Malawi southern range limit

CHAPTER 2

The phylogeography of the Delectable Soft-furred Mouse and Dark-coloured Brush-furred Rat across the Malawi Rift

2.1 ABSTRACT

I investigated phylogeographic structure among montane highland populations of the Delectable Soft-furred Mouse (*Praomys delectorum*) and the Dark-coloured Brush-furred Rat (*Lophuromys aquilus*) across the Malawi Rift. Analyses of a combination of mtDNA (Cytochrome-b and control region) and nDNA (Fib7) from populations sampled throughout the Malawi Rift, as well as from the Eastern Arc Mountains to the north, and from central Mozambique in the south, revealed significant population structure. Results suggest that phylogeographic breaks occur in the southern highlands separating Mount Namuli in Mozambique and Mount Mulanje, as well as between Mount Mulanje and Mount Zomba in Malawi. Additional breaks occur in the central highlands splitting Malawi into two halves, and in the northern highlands separating the Misuku Hills and Nyika Plateau, as well as the Misuku Hills from Mount Rungwe. Genetic differences exhibited by the rodent taxa investigated, highlight the transition from southern to eastern African lineages in central Malawi, in support of the observations by Vrba (1985) derived from the analysis of fossil mammal assemblages. Thus, Malawi may be geographically key to understanding where turnover between southern and east African lineages occurs. The results of this study and ongoing research suggest that molecular DNA data have an important role to play in helping to manage and conserve divergent populations of montane animal taxa in Malawi.

2.2 INTRODUCTION

The strength of comparative phylogeography is that it enables one to test whether co-distributed taxa exhibit similar phylogeographic patterns, which may reveal similar ecological requirements, shared geological or climatically altered histories (Moritz & Faith 1998). Such congruent phylogenetic patterns may predict similar partitioning of genetic variation for other co-distributed taxa not yet investigated (Zink et al. 2002). Phylogeographic patterns are most often formed when formerly contiguous populations become isolated for extended periods of time resulting in a reduction of gene flow among them (Avise 2000). This vicariant fragmentation into allopatric lineages can result from a variety of geographical events including mountain-formation, changes in river courses, and shifts in the distribution of suitable habitat due to climatic change (Knowles 2000; 2001; Pereira & Baker 2004; DeChaine & Martin 2005; Epps et al. 2006; Anthony et al. 2007; Shepard & Burbrink 2009). During climatic fluctuations, populations of species that closely track environmental conditions likely experienced alternating periods of isolation and connectivity as habitats scale and descend along elevational or aridity gradients in response to environmental change (Hewitt 2004; Wiens & Graham 2005; Kozak & Wiens 2006; Lorenzen et al. 2012). As vegetation shifts across the landscape, the extent of gene flow between for instance discrete mountain or lowland tracts of forest may be heavily influenced by the degree of isolation of suitable blocks of habitat, i.e. refugia (Hewitt 1996; 2004; Wiens 2004).

Phylogeographic structure due to environmental change has influenced patterns of genetic diversity in the large mammal fauna of Africa, which is thought to have contributed to the high species diversity that exceeds that of Eurasia or North America

(Vrba 1995; Lorenzen et al. 2012). It has been demonstrated that the Impala *Aepyceros melampus*, Greater Kudu *Tragelaphus strepsicelos*, Wildebeest *Connocatus taurinus* and Sable Antelope *Hippotragus niger* exhibit considerable genetic diversity among southern and eastern African populations. As a whole, the southern African antelope populations have higher nucleotide diversity than those from East Africa, suggesting that the southern African populations may be older than the East African populations. Hence, several species of antelope could have spread from southern Africa to eastern Africa during periods of suitable habitat connectivity, possibly via the hypothesised arid corridor (Vrba 1985), which linked south-western Africa with north-eastern Africa (Nersting & Arctander 2001; Lorenzen et al. 2012). Paleontological studies of African bovid evolution during the late Miocene, suggests that migration had an important effect on the composition of lineages in the Malawi Rift, thereby influencing lineage turnover between taxa predominantly distributed in the north and taxa predominantly distributed in the south (Vrba 1985). From a palaeontological perspective, major shifts in the composition of the antelope fauna, with simultaneous changes in hominid populations across the Malawi Rift signified broader faunal turnover in this region (Partridge et al. 1995). Thus, it seems likely that the distribution of mid to large sized mammals has been significantly modified by anthropogenic activities, and their ranges have become fragmented and reduced. In contrast, small mammals, which are not usually subject to hunting pressure are perhaps more suited to determining the relative roles of ecological change, the importance of river and geological barriers, and habitat patchiness in structuring diversity patterns. To date studies conducted on small mammals distributed in the montane

highlands of Africa are relatively few (Que´rouil et al. 2001; Herron et al. 2005; Stanley & Olson 2005; Nicolas et al. 2008; Bryja et al. 2014).

This study seeks to detect, and if present, to unravel the processes that have led to the formation of genetic structure and turnover that occurs in two small mammal species, the Delectable Soft-furred Mouse (*Praomys delectorum* Thomas 1910) and the Dark-coloured Brush-furred Rat (*Lophuromys aquilus* True 1892), distributed across the Malawi Rift.

Praomys delectorum is an indigenous sub-Saharan rodent; a member of a genus that has rapidly radiated and presently comprises c. 22 species (Lecompte et al. 2002b; Musser & Carleton 2005; Bryja et al. 2014). The taxonomic delineation of *P. delectorum* and associated taxa has been clouded since its description, because several names are synonymous with other small African murines (Musser & Carleton 2005). A recent review of the East African montane forms of the *Praomys delectorum* group recognises three species: *P. delectorum* (Thomas 1910), *P. melonotus* (Allen & Loveridge 1933), *P. taitae* (Heller, 1912) and *actomastis* (Hatt 1912) as a synonym (Carleton & Stanley 2012, see also Bryja et al. 2014). The *L. aquilus* species complex comprises six taxa: *cinereus* (Dieterlen & Gelmroth 1974), *laticeps* (Thomas & Wroughton 1907), *major* (Thomas & Wroughton 1907), *margarettae* (Heller 1912), *rita* (Dollman 1910), *rubecula* (Dollman 1909), with *aquilus* occurring across the Malawi Rift (Ansell & Dowsett 1988). It has been observed that *L. aquilus* shares similar habitat characteristics with *P. delectorum* (Musser & Carleton 2005) and it would be interesting to understand the phylogeographic patterns in these species.

This study examines the phylogeography and genetic diversity in *P. delectorum* group and *L. aquilus* throughout the Malawi Rift (Fig. 1.1). Specifically, the resulting molecular data generated are used to determine species relationships across the geographical region from southern Tanzania (Mount Rungwe) through Malawi (Misuku Hills, Nyika Plateau, Mount Ntchisi, Mount Zomba and Mount Mulanje) to northern Mozambique (Mount Namuli).

For the *P. delectorum* and *L. aquilus*, I obtained DNA sequence data from two mitochondrial DNA (mtDNA) markers, Cytochrome-b and the noncoding control region, as well as a nuclear marker, Beta-Fibrinogen intron 7, in order to examine the genetic relationships and population structure in these species. Given the rapid evolutionary changes in the environment and lineage turnover (beta-diversity) that is exhibited in the mammalian and bird fauna between eastern and southern Africa (Vrba 1985; Partridge et al. 1995; Bowie et al. 2005), I hypothesised that there would be genetic differences among small mammal population distributed across the Malawi Rift, with potential breaks occurring: 1) across the lowland gap between Zomba and Mulanje in southern Malawi, 2) in the central highlands, splitting Malawi into two, and 3) across the lowland gap that separates Nyika Plateau from the Misuku Hills in northern Malawi. To test these hypotheses, I sought to answer the following questions: (i) Can genetic turnover between east and southern Africa be detected and does it occur in Malawi? (ii) Are these taxa endemic to Malawi, and what are their distributions in Malawi? (iii) Where are the common phylogeographic breaks in Malawi and how do they relate to the current placement of national parks?

2.3 MATERIALS AND METHODS

2.3.1 Population Sampling

Tissue samples were obtained from 91 *P. delectorum* and 92 *L. aquilus* individuals collected during research expeditions in Malawi from 2001 to 2009 (see appendix E & F). In order to understand whether the turnover of east to southern African lineages occurs across the Malawi Rift, populations of *P. delectorum* and *L. aquilus*, were also sampled from southern Tanzania (Rungwe) and northern Mozambique (Namuli). For the sites I could not visit I obtained preserved tissue from The Field Museum of Natural History. *Hylomyscus* was used as an outgroup to *P. delectorum* (Lecompte et al. 2002) and *Deomys ferrugineus* as an outgroup to *L. aquilus* (Lecompte et al. 2008; Schenk et al. 2013).

2.3.2 Laboratory procedures

Total genomic DNA was extracted from 0.25 g or less muscle tissue using a DNeasy Kit (QIAGEN, Germantown, MD, USA) following the manufacturer's animal tissue protocol with an overnight Proteinase K digestion at 55^{o}C. In total 1130 base pairs (bp) for *L. aquilus* and 1143 bp for *P. delectorum* of the mitochondrial Cytochrome-b genome was amplified by polymerase chain reaction (PCR) with primers L14723 (5'-ACCAATGACATGAAAAATCATCGTT-3') and H15915 (5'-TCT CCATTTCTGGTTTACAAGAC-3'), as described in Lecompte et al. (2002b). A 466 bp fragment for *L. aquilus* and a 461 bp fragment for *P. delectorum* of the mitochondrial control region was amplified with primers N777 (5'-TACACTGGTCTTGTAAAACC-3') and DLH-1 (5'- TTGAAGTAGGAACCAGAT-3'). The primers BFibR1 and BFibR2

(Seddon et al. 2001) were used to amplify a 376 bp fragment of *L. aquilus* and a 713 bp fragment of *P. delectorum* of Beta-Fibrinogen intron 7. Double-stranded PCR-amplifications were carried out in 25 µl reaction volumes containing: 2.5 µl 10 x buffer, 0.5 µl of 10 mM dNTP's, 0.5 µl of 10 mg/ml of bovine serum albumin, 0.75 µl of 50 mM $MgCl_2$, 1.25 µl of 10 µM of the forward and reverse primer in the presence of 0.25 µl of Taq polymerase (Perkin-Elmer) and genomic DNA.

The Cytochrome-b gene was PCR-amplified with an initial denaturation step at 94°C for 3 minutes, followed by 38 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 45 seconds, and a final extension at 72°C for 7 minutes. The control region was PCR-amplified with an initial denaturation step at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 48°C for 30 seconds and extension at 72°C for 30 seconds, with a final extension at 72°C for 7 minutes. Fib7 was amplified with an initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 30 seconds, with a final extension at 72°C for 7 minutes. Standard precautionary measures included negative controls (template or DNA free PCR reactions) that were used to test for any contamination.

I electrophoresed 5µl of the PCR product on a 1% agarose gel mixed with ethidium bromide and observed it under ultra-violet light to check for the correct fragment size and to control for specificity of the PCR-amplification. The amplified DNA was purified by using ExoSAP-IT (USB; Cleveland, OH, USA) or with GELase (Epicentre; Madison, WI, USA) and cycle sequenced using the ABI PRISM BigDye

Terminator Kit v.3.1 (Perkin-Elmer). Sephadex spin columns were utilised to clean-up the cycle sequencing reactions (CureHunter, Oregon, USA). For each locus both the reverse and forward directions were sequenced on an ABI 3730 automated DNA Analyser (Applied Biosystems) using the same primers as used in the PCR-amplification.

2.3.3 Determining the phase of alleles

In order to infer genealogies and to estimate demographic parameters from molecular DNA sequence data it is important to resolve the phase of the bi-allelic sequence data. Some introns had more than one polymorphic site (single nucleotide polymorphism [SNP]). A Bayesian method implemented in the programme PHASE v.2.1.1 (Stephens et al. 2001; Stephens & Donnelly 2003) was used to resolve the phase of all linked polymorphisms. A threshold of 0.75 (Harrigan et al. 2008) was used to consider a SNP correctly phased; all SNPs that did not satisfy this threshold were removed from further analyses.

2.3.4 Data analysis

Sequence alignment

Sequences were obtained from both strands of DNA for each individual and some individuals were sequenced several times in the event of any base ambiguity encountered. Sequence alignment was performed by computation using MAFFT (Katoh et al. 2009) and checked by eye. For Cytochrome-b, sequences were checked for insertions or deletions and that stop-codons were not present.

Phylogenetic analyses

Parsimony and Maximum Likelihood analyses were performed on the mitochondrial DNA (Ctychrome-b and Control region). Parsimony analyses were conducted in PAUP*10b (Swofford 2002) using a heuristic search with TBR branch-swapping and 1000 random addition replicates. Parsimony bootstrap values were obtained from 1000 pseudoreplicates with five random addition replicates being performed for each bootstrap pseudoreplicate.

Maximum Likelihood analyses were conducted using RAXML (Stamatakis 2006), partitioned by codon position for Cytochrome-b, under a general-time-reversible model of nucleotide substitution and a gamma model of rate heterogeneity via the CIPRES portal (Stamatakis et al. 2008; Miller et al. 2009). One-thousand bootstrap pseudoreplicates were performed to evaluate support at specific nodes.

Haplotype network construction

Due to the problems that arise in the construction of intraspecific phylogenies (e.g. Posada & Crandall 2001) TCS v.1.01 was used to construct a statistical parsimony network of haplotypes (Clement et al. 2000) for each locus. The connection limit was set at 95%. The above analysis included only the in-group haplotypes.

Analysis of molecular variance (*AMOVA*)

In order to determine molecular variation within and among populations as well as among larger geographical regions, several hierarchical analyses of molecular variance (AMOVA) were conducted. This enabled the determination of how genetic variability was partitioned within and among major lineages by using $\Phi_{ST,}$ which is an analogue of F_{ST} that incorporates both haplotype frequencies and the difference in number of

nucleotides between each pair of haplotypes (Excoffier et al. 1992). The levels of significance for AMOVA were obtained by using a non-parametric permutation with 10000 iterations and was carried out using ARLEQUIN v. 3.0 (Excoffier et al. 2005).

Mismatch distributions and test of selective neutrality

The pairwise mismatch distributions and two tests of selective neutrality, Tajima's D (Tajima 1989) and Fu's Fs test (Fu 1997) were calculated to test if populations were in mutation drift equilibrium under an infinite sites model. These indices were used as indicators of recent demographic change that could result from population expansion or contraction (Fu 1997; Rodgers & Harpending 1992). Both tests were conducted using ARLEQUIN v.3.0 (Excoffier et al. 2005).

Divergence times

I used the program BEAST v.1.7.1 (Drummond et al. 2006; Drummond & Rambaut 2007) to estimate divergence times for Cytochrome-b using the mean rate of divergence and associated standard deviation reported by Nicholas et al. 2011 (see also Demos et al. 2014). This rate is based on two African Murid fossil dates. In BEAST, a Yule process speciation prior and an uncorrelated lognormal model of rate variation (relaxed clock) were implemented, with individual codon positions having separate substitution models determined using MRMODELTEST. The MCMC analyses were run for 1 x 10^7 generations with parameters sampled every 5000 steps, and a 10% burn-in. TRACER v.1.5 (Rambaut & Drummond 2007) was used to determine the effective sample size of each parameter and calculate the mean, and upper and lower bounds of the 95% highest posterior density interval (95% HPD) for divergence times. Tree topologies

were assessed using TreeANNOTATOR v.1.5.3 (Drummond & Rambaut 2007) and FIGTREE v.1.3.1 (Rambaut 2008).

2.4 RESULTS

2.4.1 Sequence variation

Mitochondrial DNA

A final alignment of 1130 bp of the Cytochrome-b gene was obtained from 92 individuals of *L. aquilus*. Removal of identical sequences recovered 31 unique haplotypes (Table 2.1a). Of the 1130 characters, 919 (81.33%) were constant, 172 (15.22%) were variable but parsimony uninformative, and 39 (3.45%) were parsimony informative. A final alignment of the entire 466 bp of the control region was obtained from 85 individuals of *L. aquilus*. Removal of identical sequences recovered 32 unique haplotypes (Table 2.1b). Of the 466 characters, 335 (71.89%) were constant, 97 (20.81%) were variable but parsimony uninformative, and 34 (7.30%) were parsimony informative.

A final alignment of the entire 1143 bp of the Cytochrome-b gene was obtained from 88 individuals of *P. delectorum*. Removal of identical sequences recovered 29 unique haplotypes (Table 2.1b). Of the 1143 characters, 926 (81.01%) were constant, 164 (14.35%) were variable but parsimony uninformative, and 53 (4.64%) were parsimony informative. From 91 individuals of *P. delectorum* a final alignment of the 461 bp of the control region recovered 30 unique haplotypes (Table 2.1b). Of the 461 characters, 376 (81.56%) were constant, 55 (11.93%) were variable but parsimony uninformative, and 30 (6.51%) were parsimony informative.

Nuclear DNA

A final alignment of the entire 729 bp of the nuclear intron Fib7 gene was obtained from 82 individuals of *L. aquilus*. Removal of identical sequences recovered seven unique alleles (Table 2.1b). Of the 376 characters, 370 (98.4%) were constant, two (0.5%) were variable but parsimony uninformative, and four (1.1%) were parsimony informative.

A final alignment of the 713 bp of the intron Fib7 gene was obtained from 83 individuals of *P. delectorum*. Removal of identical sequences recovered seven unique alleles (Table 2.1b). Of the 713 characters, 707 (99.1%) were constant, two (0.30%) were variable but parsimony uninformative, and four (0.56%) were parsimony informative.

2.4.2 Phylogenetic analysis

Mitochondrial DNA

For the *L. aquilus*, Maximum Parsimony and Maximum Likelihood combined analyses of Cytochrome-b and control region recovered trees that were nearly identical (Fig 2.2). The topologies for each individual mtDNA marker were also nearly identical to those recovered during the combined analyses (see appendices A & B). Six broadly defined groups of haplotypes were recovered and represent from north to south: Mount Rungwe, Misuku Hills, Nyika Plateau, Mount Zomba, Mount Mulanje and Mount Namuli. The Misuku Hills haplotype from individual FMNH196245 is closely allied to the Rungwe haplotypes, and the Mount Rungwe haplotype from individual FMNH163604 is closely allied to Misuku haplotypes. The Nyika Plateau haplotype from individual FMNH192249 is closely allied to Mulanje haplotypes (Fig 2.2).

The *P. delectorum* Maximum Parsimony and Maximum Likelihood combined analyses of Cytochrome-b and control region recovered trees that were nearly identical (Fig 2.3), and were congruent with those of each individual locus (see appendices C & D). Five groups of haplotypes were recovered and represent from north to south: Mount Rungwe, Misuku Hills, Nyika Plateau, Mount Mulanje and Mount Namuli. The Mount Rungwe haplotype from individual FMNH183725 is closely allied to individuals from the Misuku Hills, and the Mount Namuli individual FMNH196838 is closely allied to individuals sampled on Mount Mulanje (Fig. 2.3).

Nuclear DNA

The Maximum Parsimony and Maximum Likelihood analyses for the intron Beta-Fibrinogen intron 7 for both *L. aquilus* and *P. delectorum* formed a large polytomy (not presented).

2.4.3. Haplotype network construction

Mitochondrial DNA

Construction of a haplotype network using 1130 bp of the Cytochrome-b gene of *L. aquilus* recovered one subnetwork (Fig. 2.4) with Mt. Rungwe, Misuku Hills, Nyika Plateau, Mt. Zomba, Mt. Mulanje and Mt. Namuli grouped together. The northern populations are separated from the southern populations by 11 mutational steps. The populations on Mt. Rungwe, Misuku Hills and Nyika Plateau are separated by one mutational step, whereas Mt. Zomba haplotypes were separated from Mt. Mulanje by four mutational steps, and from Mt. Namuli by eight mutational steps. Individuals from

Mt. Mulanje were separated from Mt. Namuli by five mutational steps indicating substantial geographical structuring among the southern populations (Fig. 2.4). All individuals sampled from Mt. Zomba shared the same haplotype (Table 2.1a).

Construction of a haplotype network using 463 bp of the control region of *L. aquilus* recovered two subnetworks (Fig. 2.5) that represented the northern clade (I) Rungwe, Misuku and Nyika, and the southern clade (II) Zomba, Mulanje and Namuli.

Haplotype network construction using 1143 bp of the Cytochrome-b gene of *P. delectorum* recovered two subnetworks (Fig. 2.8) that matched those which were recovered in the phylogenetic analyses (Fig. 2.3). The two subnetworks represented the northern clade (I) Rungwe, Misuku and Nyika, and the southern clade (II) Mulanje and Namuli. The two clades are distinct and there is no haplotype sharing between northern and southern populations across the Malawi Rift.

Construction of haplotype network using 641 bp of the control region of *P. delectorum* also recovered two subnetworks (Fig. 2.9). The two subnetworks represent the northern clade (A) Rungwe, Misuku and Nyika, and the southern clade (B) Mulanje and Namuli.

Nuclear DNA

For Beta-Fibrinogen intron 7 (Fib7) for *L. aquilus* TCS suggested that subnetworks connecting alleles by eight steps or fewer had a cumulative probability of greater than 95% of being correct. The same subnetworks and the degree of geographical structuring as that displayed by the mitochondrial Cytochrome-b and the control region (Fig. 2.6) were not observed. Most alleles were connected to the common central allele

that occurred in several populations: Mt. Rungwe, Misuku Hills, Nyika Plateau, Mt. Zomba, Mt. Mulanje and Mt. Namuli (allele 1, Fig. 2.6). Within *L. aquilus*, Misuku (= 4), Nyika (= 2), Mulanje (= 4), and Namuli (= 2) had higher allelic variation than Rungwe (= 1) and Zomba (= 1) (Table 2.1b).

For *P. delectorum* Fib7, TCS suggested that subnetworks connecting alleles by 11 steps or fewer had a cumulative probability of greater than 95% of being correct. The same subnetworks and the degree of geographical structuring as that displayed by the mitochondrial Cytochrome-b gene and the control region (Fig. 2.7 & 2.8) were not observed. Most alleles were connected to the central allele which occurred in several populations: Nyika, Mulanje and Namuli (allele 1, Fig. 2.7). Within *P. delectorum*, Rungwe (= 2), Misuku (= 3), Nyika (= 3), Mulanje (= 4), and Namuli (= 2) had high allelic variation (Table 2.1b).

2.4.4 Analysis of molecular variance

Mitochondrial DNA

An AMOVA for *L. aquilus* Cytochrome-b was conducted among the six sampled populations (Table 2.1a). The genetic variation within groups was 6.82%, variation within populations 19.87%, and variation among groups 73.31%. There was considerable population substructure as indicated by the high value of Φ_{ST} (0.801, $P < 0.0001$), and this was supported by high pairwise Φ_{ST}-values that were significant (Table 2.2). Using only the five sampled populations with large sample sizes (Rungwe, Misuku, Nyika, Mulanje and Namuli, Table 2.1a) the genetic variation within groups was 6.74%,

variation within population 20.5%, and variation among groups 72.77% (Φ_{ST} = 0.795, P < 0.0001; Table 2.2). For the control region, AMOVA was conducted among the six sampled populations (Table 2.1b). The genetic variation within groups was 66.65%, variation within populations 26.05%, and variation among groups 7.3%. There was considerable population structuring as indicated by the high value of Φ_{ST} (0.740, P < 0.0001), and this was supported by the pairwise Φ_{ST}-values, which were significant (Table 2.3). Using the five sampled populations with larger sample sizes (Rungwe, Misuku Hills, Nyika, Mulanje and Namuli; Table 2.1b) the genetic variation within groups was 66.93%, variation within population 25.34%, and variation among groups 7.73% (Φ_{ST} = 0.747, P < 0.0001; Table 2.3).

The *P. delectorum* Cytochrome-b AMOVA was conducted among the five sampled populations (Table 2.1a). The genetic variation within groups was 1.99%, variation within population 13.39%, and variation among groups 84.62%. There was significant population structuring as indicated by the high value of Φ_{ST} (0.866, P < 0.0001), and this was supported by the significant pairwise Φ_{ST}-values (Table 2.4). For the control region, AMOVA was conducted among the five sampled populations (Table 2.1b). The genetic variation within groups was 1.66%, variation within population 27.87%, and variation among groups 70.47%. There is considerable population substructure as indicated by the high value of Φ_{ST} (0.721, P < 0.0001), and this is supported by the significant pairwise Φ_{ST}-values (Table 2.5).

Nuclear DNA

AMOVA for Fib7 for *L. aquilus* was conducted among the six sampled populations (Table 2.1b). The genetic variation among groups was 12.58%, within groups

was 0% (-3.38), and within populations was 90.8%. Although population substructure was not as pronounced it remained significant (Φ_{ST} = 0.092, P < 0.001). Using the five sampled populations with larger sample sizes (Rungwe Mounts, Misuku Hills, Nyika Plateau, Mount Mulanje and Mount Namuli, Table 2.1b) the genetic variation among groups was 11.55%, within groups was 0% (-1.49) and, within populations was 89.94%; Φ_{ST} remained significant (0.101, P < 0.004, Table 2.2).

AMOVA for Fib7 for *P. delectorum* was conducted on all five sampled populations (Table 2.1b). The genetic variation among groups was 61.21%, variation within groups 0.4%, and variation within population 38.39%. Population structuring was also observed among the populations (Φ_{ST} = 0.616, P < 0.0001). Using the four sampled populations with larger sample sizes (Misuku Hills, Nyika Plateau, Mount Mulanje and Mount Namuli, Table 2.1b) the genetic variation among groups was 63.67%, variation within groups 0.85%, and variation within population 35.48%; Φ_{ST} remained significant (0.645, P < 0.0001, Table 2.3).

2.4.5 Mismatch distributions and test of selective neutrality

Mismatch profiles that follow a modified Poisson distribution (a unimodal bell shaped curve when population expansion is recent) are thought to be associated with past events of population growth, for instance range expansion (Rogers & Harpending 1992; Harpending et al. 1993). For the five *L. aquilus* Cytochrome-b mismatch distribution profiles (Fig. 2.9) constructed for populations with adequate samples and sufficient variation (Zomba had one haplotype), all (Rungwe, Misuku, Nyika, Mulanje and Namuli) followed a Poisson distribution. The Tajima's D and Fu's Fs statistics for Rungwe,

Misuku, Nyika and Mulanje were not significant indicating that if these populations have undergone a range expansion, it occurred a long time ago. The Namuli population also followed a Poisson distribution (Fig. 2.10) and both Tajima's D and Fu's Fs values were significant (Table 2.6); these results are consistent with recent population expansion.

The mismatch distribution profiles of *L. aquilus* populations (Rungwe, Misuku, Nyika, Mulanje and Namuli) using the control region data, all followed a Poisson distribution. The Tajima's D and Fu's Fs statistics for all populations were not significant indicating that if these populations have undergone a range expansion, it occurred a long time ago.

The mismatch distribution profiles of *P. delectorum* (Rungwe, Misuku, Nyika, Mulanje and Namuli) using the Cytochrome-b data (Fig. 2.11), all followed a Poisson distribution. The Tajima's D and Fu's Fs statistics for Rungwe, Misuku, Nyika and Mulanje were not significant, indicating that if these populations have undergone a range expansion, it occurred a long time ago.

The mismatch distribution profiles of *P. delectorum* using control region data (Fig. 2.12) indicated that all populations (Rungwe, Misuku, Nyika, Mulanje and Namuli) follow a Poisson distribution. The Tajima's D and Fu's Fs statistics for all the populations were not significant, indicating that if these populations have undergone a range expansion, it occurred a long time ago.

2.4.6 Divergence times

From the dating results (Fig. 2.14) the northern *L. aquilus* population comprising Mount Rungwe, Misuku Hills, and Nyika Plateau diverged from the southern population comprising Mount Zomba, Mount Mulanje and Mount Namuli at about 0.39 Myrs BP.

From the dating results (Fig. 2:15) the southern *P. delectorum* population of Mount Mulanje and Namuli became isolated from the northern Malawi Rift population of Nyika, Misuku and Rungwe at about 0.72 Myrs BP. The population on Nyika Plateau diverged from the northern population at about 0.10 Myrs BP.

2.5. DISCUSSION

Lophuromys aquilus

The combined mtDNA phylogenetic analyses (Cytochrome-b and control region) for *L. aquilus* recovered six groups of haplotypes that represent from north to south: Mount Rungwe, Misuku Hills, Nyika Plateau, Mount Zomba, Mount Mulanje and Mount Namuli (Fig.2.2). There is sharing of haplotypes between the northern and southern populations; however, there is strong geographical structuring indicating that the lack of reciprocal monophyly may not be due to recurrent gene flow, but is more likely a consequence of ancestral polymorphism due to these lineages having recently diverged.

The mtDNA Cytochrome-b network analyses of the *L. aquilus* populations resulted in one network. There is substantial phylogeographic structuring among the sampled populations (Fig. 2.4). The southern populations of Mount Zomba, Mount Mulanje and Mount Namuli show fine-scale geographic structuring (4-8 mutational steps - see also Bryja et al. 2014), a pattern that has also been observed in other less dispersive

taxa in the southern Malawi Rift montane forests (Lawson 2013). The northern populations of Mount Rungwe, Misuku Hills and Nyika Plateau are more closely clustered and are separated by one mutational step (Fig. 2.4).

The control region dataset recovered two distinct subnetworks that comprise populations from Rungwe-Misuku-Nyika and Zomba-Mulanje-Namuli (Fig. 2.5), a result consistent with the north-south division of populations as suggested by the Cytochrome-b data. The use of nuclear marker (Fib7) recovered some phylogeographic structuring, as Φ_{ST} was significant, supporting the mtDNA data (Table 2.2 & 2.3). However, this structuring was not well displayed by the network (Fig. 2.6) as most of the alleles were connected to the central and most common allele, suggesting that differences in the frequency distribution of the alleles is influencing the Φ_{ST}-value, as opposed to population specific alleles (i.e. private) being present.

The mismatch distribution for *L. aquilus* for Namuli (Fig. 2.10), Tajima's D and Fu's Fs tests were significant indicating, suggesting the population is expanding. The direction of expansion is towards lower nucleotide diversity (Sgariglia & Burns 2003), thus this is likely southwards towards Mount Namuli as the Mount Mulanje population has higher nucleotide diversity (Table 2.6).

Praomys delectorum

Praomys delectorum mtDNA combined phylogenetic analyses (Cytochrome-b and control region), recovered five groups of haplotypes and represent from north to south: Mount Rungwe, Misuku Hills, Nyika Plateau, Mount Mulanje and Mount Namuli (Fig. 2.3). There is no sharing of haplotypes between the northern and southern populations. This suggests that the northern populations and southern populations of *P.*

delectorum are reciprocally monophyletic. These results support the morphological analyses of Carleton and Stanley (2012) and morphological and molecular analyses of Bryja et al. (2014) conducted on the greater *P. delectorum* complex.

The mtDNA Cytochrome-b network analyses of the sampled *P. delectorum* populations resulted in two discrete subnetworks that comprise northern Rungwe-Misuku-Nyika and southern Mulanje-Namuli lineages (Fig. 2.8). Within both the northern and southern clades of *P. delectorum,* individuals from Rungwe and Nyika in the northern clade, and Mulanje and Namuli in the southern clade, share haplotypes (Fig. 2.3, Table 2.1a). This could be an indication of gene flow between populations, but considering the strong geographical structuring among the populations (Table 2.4), the shared haplotypes could also be a consequence of incomplete lineage sorting due to recent divergence.

Similarly, analyses of the control region dataset recovered two subnetworks that comprise northern (Rungwe-Misuku-Nyika) and southern (Mulanje-Namuli) populations (Fig. 2.9). There is phylogeographic structuring among the five sampled populations. The populations of Mount Mulanje and Mount Namuli did not share haplotypes as it was observed with Cytochrome-b (Fig. 2.3, 2.8 & 2.9) indicating complete differentiation of the lineages in the two populations reinforcing the view that there is strong phylogeographic structuring in southern Malawi Rift montane forests (Lawson 2013; see also Bryja et al. 2014).

The use of nuclear marker (Fib7) in *P. delectorum* revealed some geographical structuring, as the Φ_{ST} was significant, thereby supporting the mtDNA dataset (Table 2.4 & 2.5). However, this structuring was not well illustrated by the network (Fig. 2.7) as

most of the alleles were connected to the central and most common allele suggesting that differences in the frequency distribution of alleles, rather than the presence of private alleles is driving the significant Φ_{ST}-value.

The mismatch distributions (Fig. 2.12 & 2.13) and tests for selective neutrality (Tajima D & Fu's Fs) were not significant (Table 2.7) suggesting that the populations are at equilibrium and likely stable (Rodgers & Harpending 1992; Harpending et al. 1993), thereby contributing to the observed levels of phylogeographic structure among the five sampled populations of *P. delectorum*.

Faunal turnover and comparative phylogeography in L. aquilus and P. delectorum across the Malawi Rift

Several sampled populations of *L. aquilus* and *P. delectorum* distributed across the Malawi Rift montane forests are distinct. Both species exhibit phylogeographic structure in the Malawi Rift suggesting that the historical processes that occurred during the Plio-Pleistocene may have acted similarly on these two species. Biogeographic barriers in the Malawi Rift, comprise valleys (gaps between montane forests) and forest fragmentation (Misuku and Nyika), both of which have played a role in shaping the genetic structure in the two rodent taxa and thereby influencing the distribution of lineages across the eastern Rift Valley of Africa (Vrba 1985). The geographical structuring observed is at fine spatial scales, especially among the southern populations, supporting the view that these isolated montane massifs are important refuges for diversification, and that these massifs require further sampling for other vertebrate

species if we are to understand the environmental changes that underlie the transition of southern African to east African lineages across the Malawi Rift.

The populations for both taxa form two distinct clades comprising northern and southern lineages. Thus, a major geographical break for both species occurs in the central highlands of Malawi (Fig. 2.16). This central break is also reflected in the deepest divergence time for both taxa (Fig. 2.14 & 2.15), whereby the initial split is the isolation of northern and southern populations. However, the exact location of the phylogeographic break in central Malawi is hard to precisely determine, as during surveys in 2005 and 2007, no samples of either *L. aquilus* or *P. delectorum* were obtained from Ntchisi and Dedza, the only forest highlands in central Malawi, despite similar trapping effort as performed at other localities: Misuku = 5000 trap hours, Nyika = 10000, Ntchisi/ Dedza = 5000 and Mulanje = 5000 trap hours (Museums of Malawi, Stanley unpub. data). Historically the species are thought to have occurred throughout the central highlands (Happold & Happold 1987), but the lack of observations of these species during sampling in 2005 and 2007, as well as 2004 (Chitaukali 2004), and by Bryja et al. (2014), suggests that *L. aquilus* and *P. delectorum* may no longer occur in the central highlands of Malawi. Both *L. aquilus* and *P. delectorum* are forest specific species (Stanley et al. 2005) and the fragmentation of much of the central highlands of Malawi (Van der Straeten & Agwanda 2004; Nicolas et al. 2008) may explain their apparent disappearance from the central highlands. If this does indeed prove to be correct, then it would further accentuate the north-south break in these species, and strengthen the arguments based on fossil data from other mammalian species that faunal turnover between eastern and southern Africa taxa occurs within the Malawi Rift (Vrba 1985).

The concordant structuring that has been observed among populations of both *L. aquilus* (Table 2.2 & 2.3) and *P. delectorum* (Table 2.4 & 2.5) from Rungwe, Misuku and Nyika delineates two important phylogeographic breaks, one between Rungwe and Misuku, and another between Misuku and Nyika (Fig. 2.16). The presence of a geographical barrier (lowland gap) that separates Rungwe on the Tanzanian side and Misuku on the Malawian side could prevent gene flow, as could the lowland gap separating Nyika Plateau from the Misuku Hills.

In the south, the sampled populations on Mount Zomba and Mount Mulanje form distinct clades revealing the presence of a phylogeographic break between Mount Zomba and Mount Mulanje (Fig. 2.16). Further, the population sampled on Mount Namuli is also distinct from individuals sampled on Mount Mulanje, thereby revealing an additional phylogeographic break. These populations could be isolated either by a barrier or distance, because animals that are likely poor dispersers are more likely to show phylogeographic breaks than animals with greater dispersal abilities. Strong structuring in the southern populations is perhaps not surprising because the southern highlands of Malawi have been heavily impacted by anthropogenic activities due to urbanisation and high human population density, that have resulted in the fragmentation of forests (Van der Straeten & Agwanda 2004; Mzumara et al. 2012, Bryja et al. 2014). In contrast, in northern Malawi the forests are still in a near-pristine state and are less fragmented. All the sampled population exhibited haplotype variation for mitochondrial and nuclear DNA markers, a result consistent with these populations now being stable.

Most national parks in Malawi are located on each side of the identified geographic breaks. Nyika National Park in the north is strategically located to encompass

most of the evolutionary processes north of the central break. This park comprises varied habitats that cover both montane as well as woodland biomes (Cater et al. 1993). The majority of parks in the south and central Malawi Rift are located in the lowlands and because these areas are generally dry and wooded, they do not encompass other important ecosystems such as the montane forests that are known to promote the accumulation of recently diverged species (Roy 1997; Fjeldså et al. 2012; this study).

The location and proclamation of most national parks in Malawi has largely been the consequence of an ad hoc approach that has not typically followed modern conservation planning strategies (Margules & Pressey 2000). Therefore, the existing protected areas fail to include all species which effective conservation planning seeks to achieve (Pressey 1994). Most of the places where the phylogeographic breaks occur are forest reserves that are not adequately protected with the exception of Nyika National Park where both taxa occur. Illegal logging and uncontrolled fires are threatening the montane 'sky island' populations, thus compromising the conservation of their fauna and flora, and as suggested by this study, important evolutionary lineages of more widespread species.

The results of this study suggest that molecular DNA data have an important role to play in helping identify the phylogeographic breaks of animals and plants across the Malawi Rift. Looking at the distribution of taxa within the Malawi Rift, important habitats for the conservation of evolutionary units are identified, which will contribute to modern conservation planning in Malawi. The northern and southern lineages of *L. aquilus*, and *P. delectorum* should be managed separately because they comprise distinct evolutionary lineages and are likely distinct species.

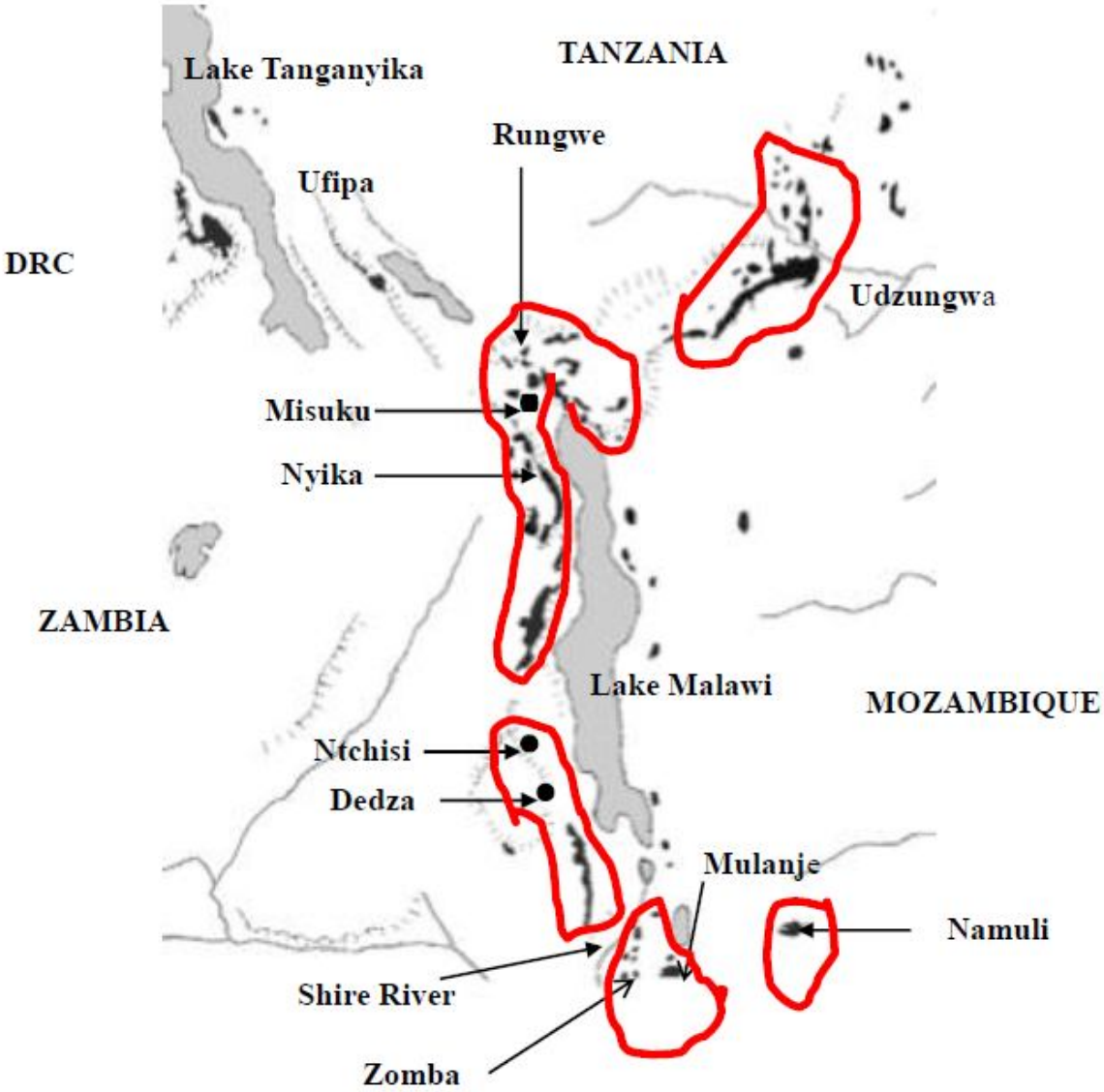


Figure 2.1 Malawi Rift depicting the distribution of two co-occurring rodent taxa *Lophuromys aquilus* and *Praomys delectorum* and sampling sites.

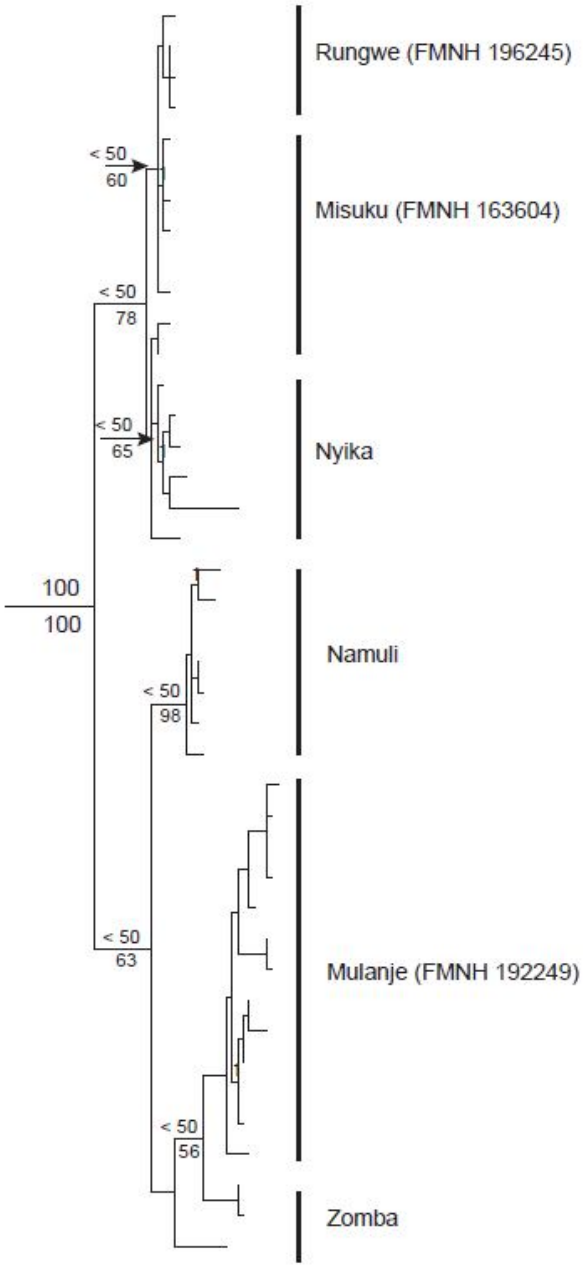


Figure 2.2 A Maximum Parsimony topology depicting phylogenetic relationships using concatenated data for both Cytochrome-b and control region among *Lophuromys aquilus* populations sampled across the Malawi Rift, as well as representative populations to the north and south. Values above the nodes are ML bootstrap support values and those below are MP bootstrap support values. Individuals' from a different haplotype clade are indicated by their museum voucher number – see Appendices E and F.

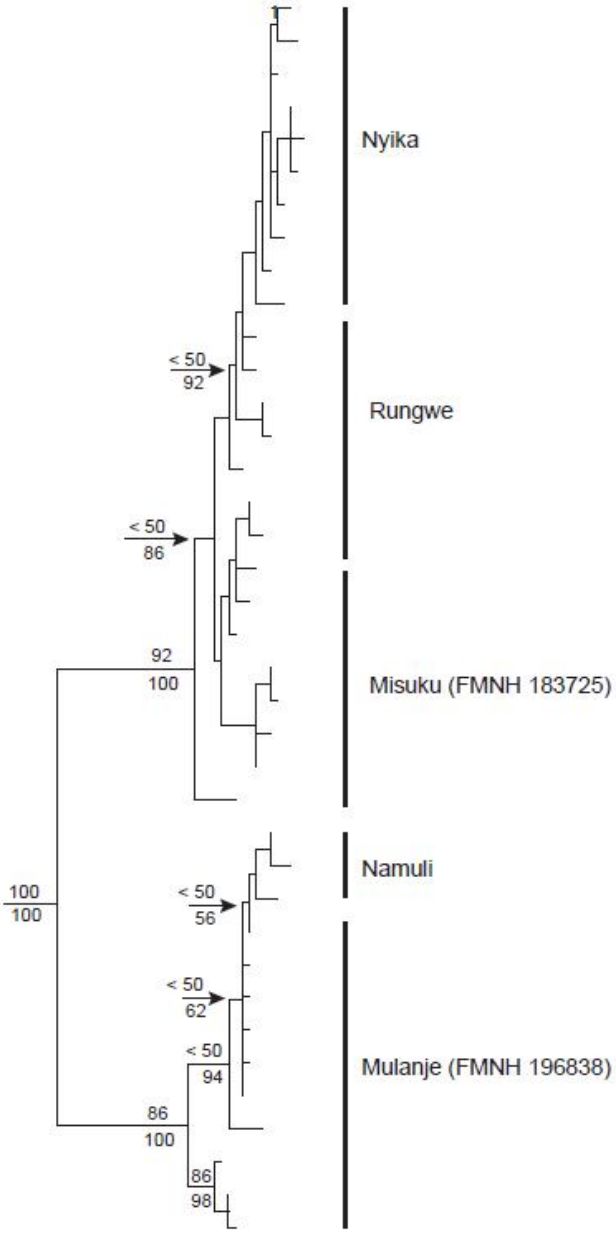


Figure 2.3 A Maximum Parsimony topology depicting phylogenetic relationships using concatenated data for both Cytochrome-b and control region among *Praomys delectorum* populations sampled across the Malawi Rift, as well as representative populations to the north and south. Values above the nodes are ML bootstrap support values and those below are MP bootstrap support values. Individuals from a different haplotype clade are indicated by their museum voucher number – see Appendices E and F.

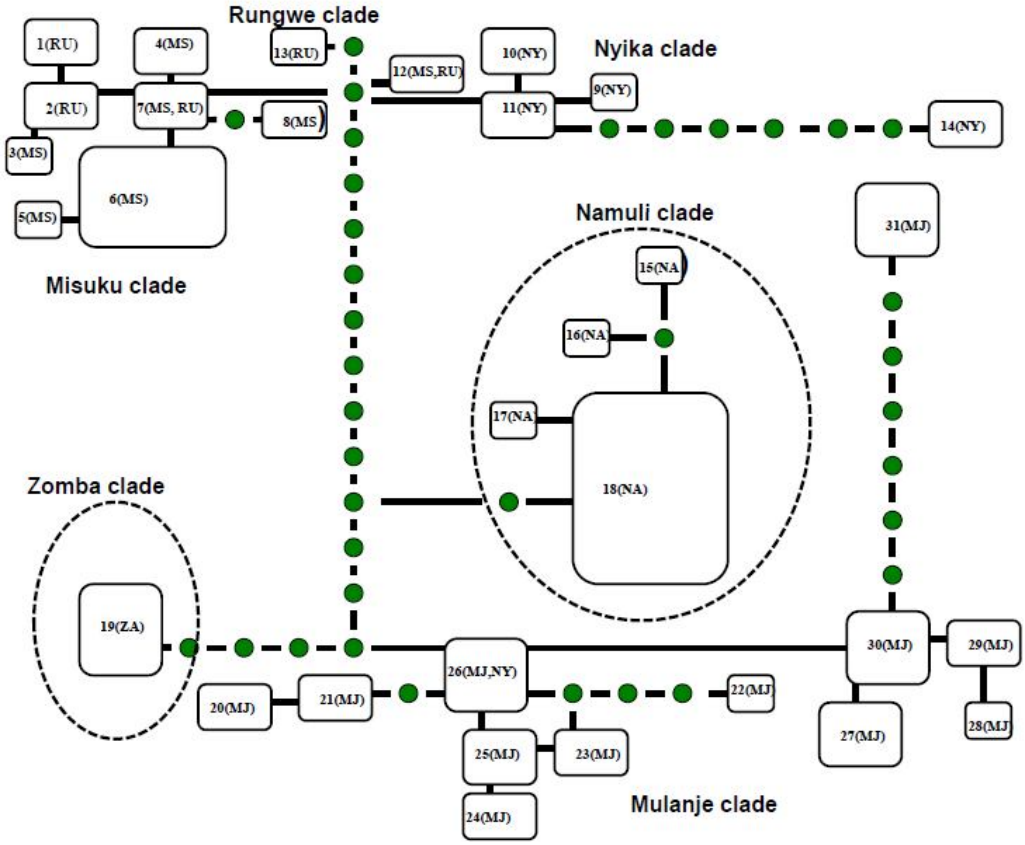


Figure 2.4 Statistical parsimony network obtained with Cytochrome-b for the 31 haplotypes of *Lophuromys aquilus* (Table 2.1a). The subnetworks that are connected to each other do not exceed the 95% confidence limit of nine steps. Dots indicate unsampled or extinct haplotypes. The size of each box is proportional to the frequency of the haplotype. Haplotype codes correspond to the population of origin: RU = Rungwe, MS = Misuku, NY = Nyika, ZA = Zomba, NA = Namuli and MJ = Mulanje.

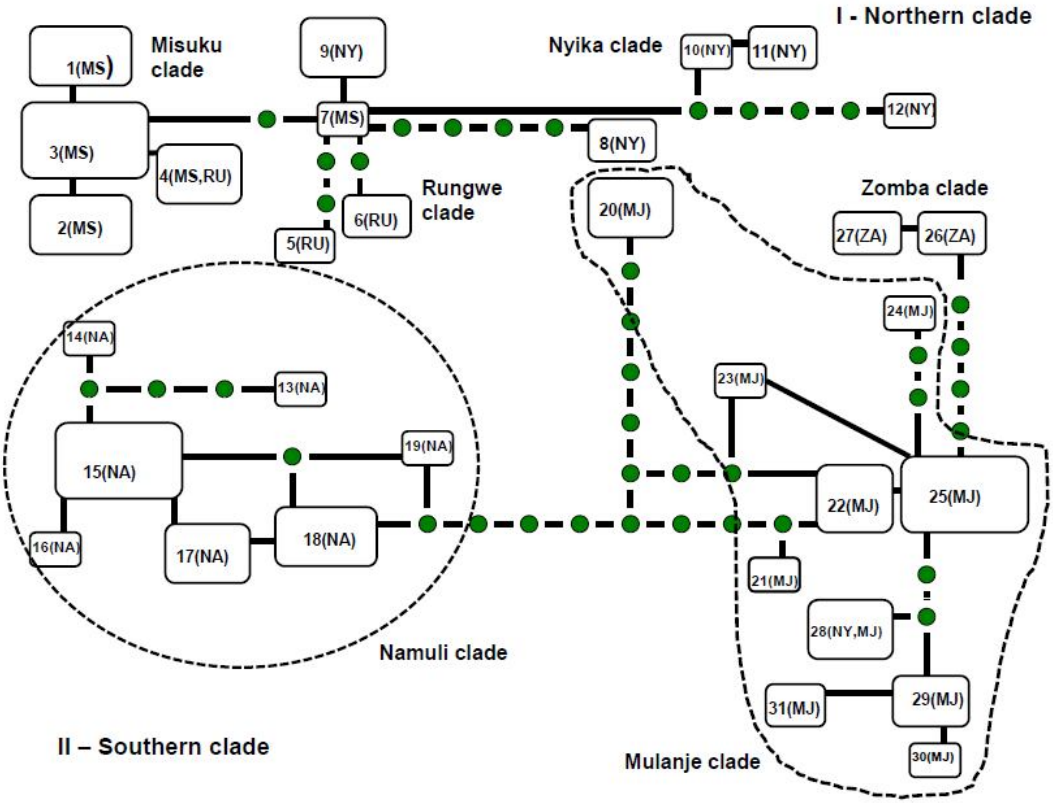


Figure 2.5 Statistical parsimony network obtained with control region for the 32 haplotypes of *Lophuromys aquilus* (Table 2.1b). The subnetworks that are connected to each other do not exceed the 95% confidence limit of 14 steps. Dots indicate unsampled or extinct haplotypes. The size of each box is proportional to the frequency of the haplotype.

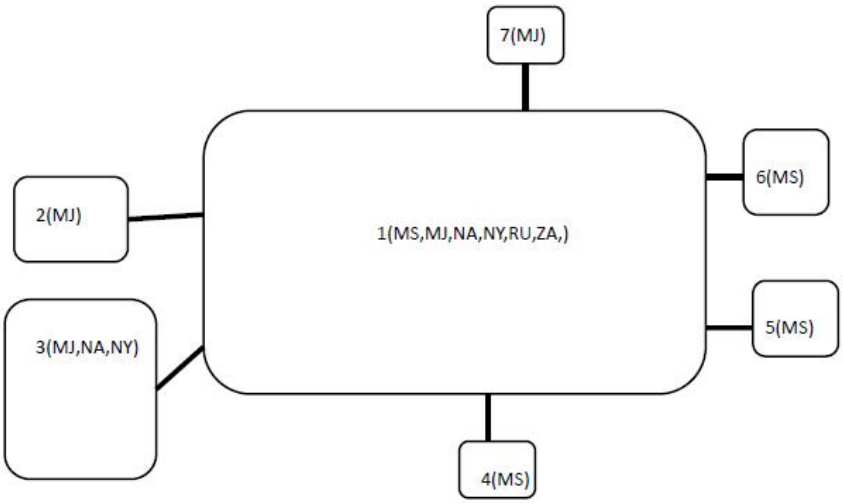


Figure 2.6 Statistical parsimony network obtained with Beta-Fibrinogen intron 7 for seven alleles of *Lophuromys aquilus* are connected to each other and satisfy the 95% confidence limit of 8 steps. The size of each box is proportional to the frequency of the allele.

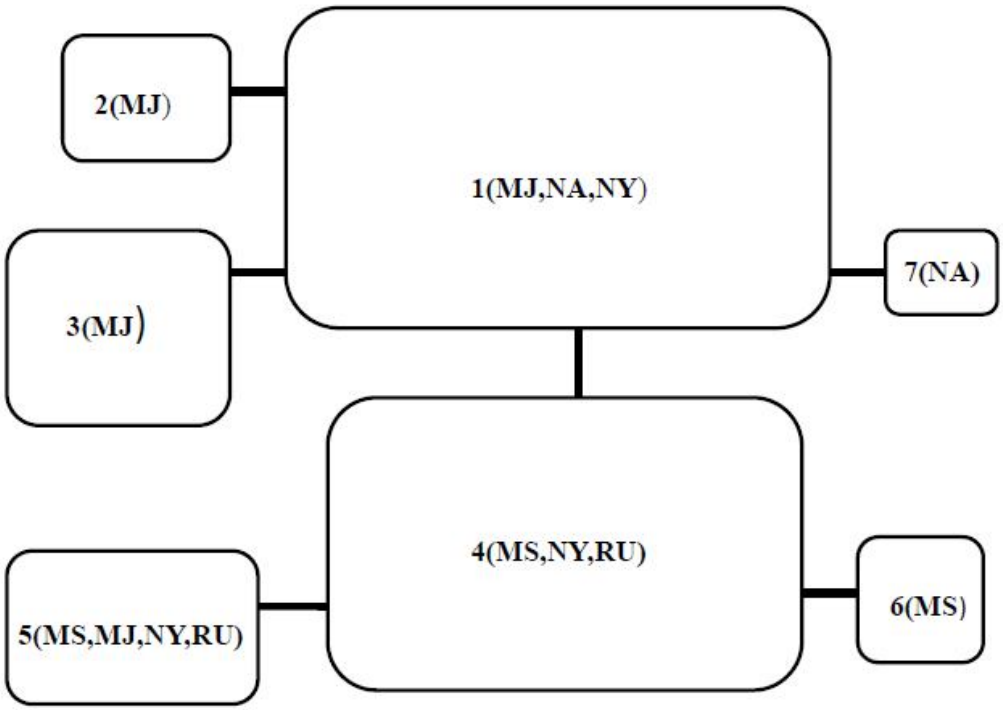


Figure 2.7 Statistical parsimony network obtained with Beta-Fibrinogen intron 7 for seven alleles of *Praomys delectorum* are connected to each other and satisfy the 95% confidence limit of 11 steps. The size of the boxes is proportional to the frequency of the allele.

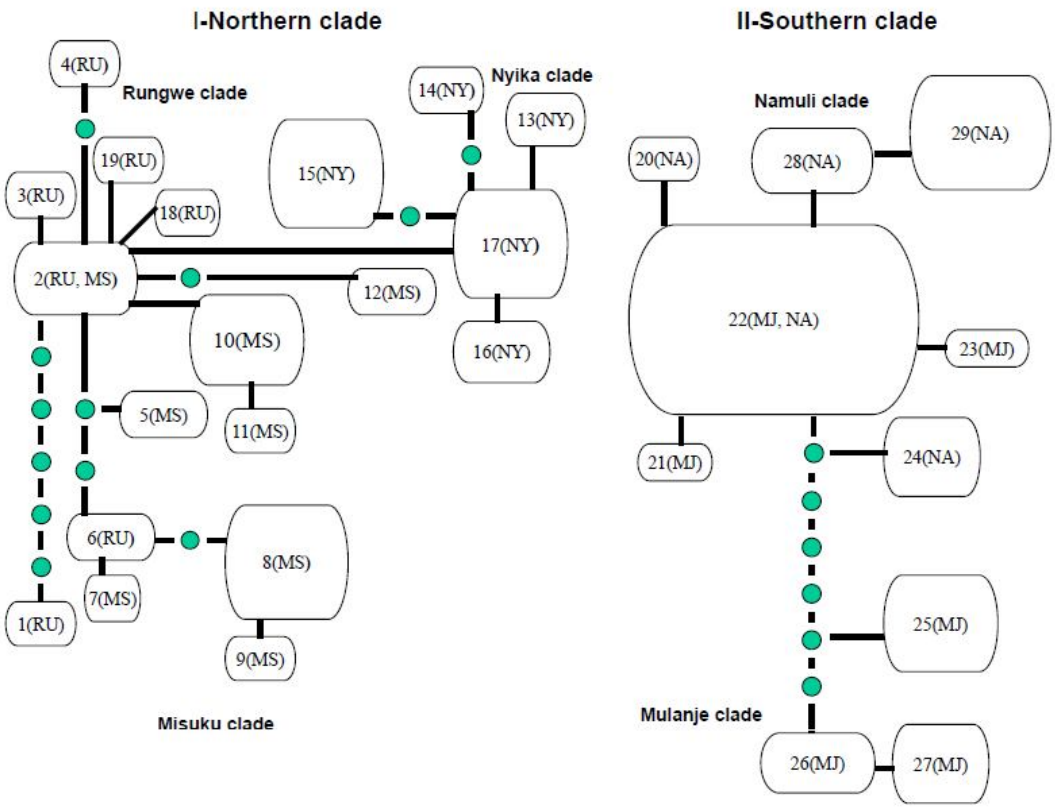


Figure 2.8 Statistical parsimony network obtained with Cytochrome-b for the 31 haplotypes of *Praomys delectorum* (Table 2.1a). The subnetworks that are connected to each other do not exceed the 95% confidence limit of 14 steps. Dots indicate unsampled or extinct haplotypes. The size of each box is proportional to the frequency of the haplotype.

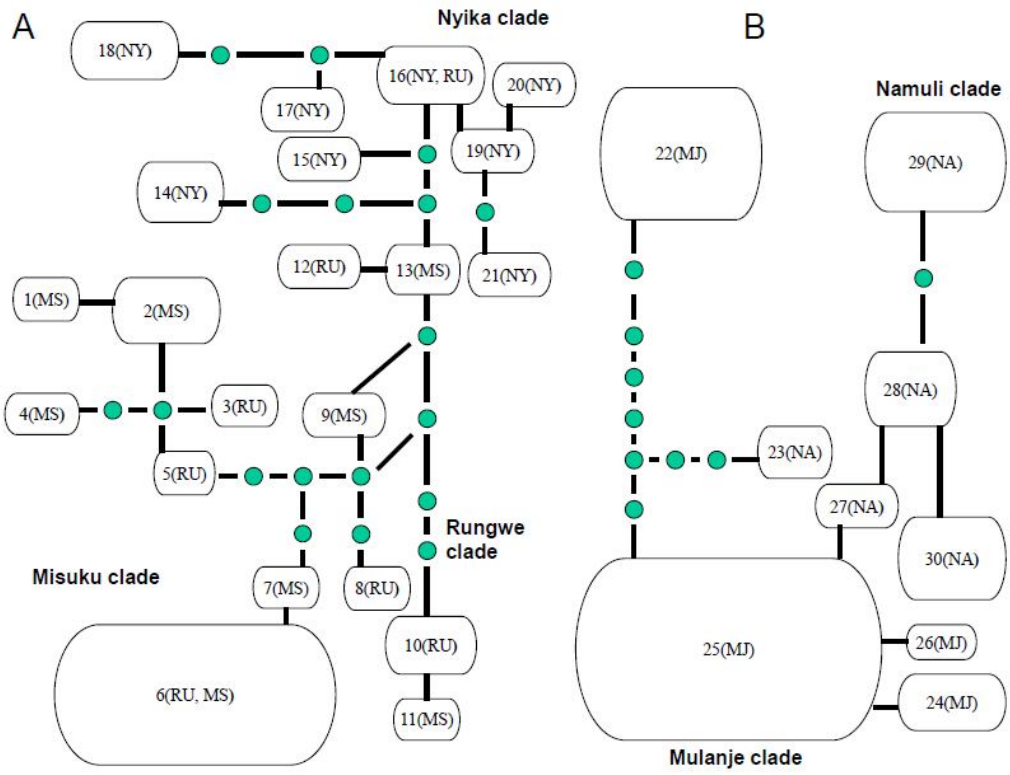
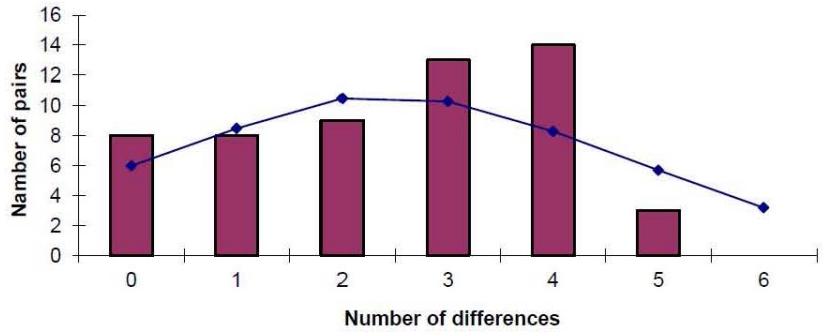
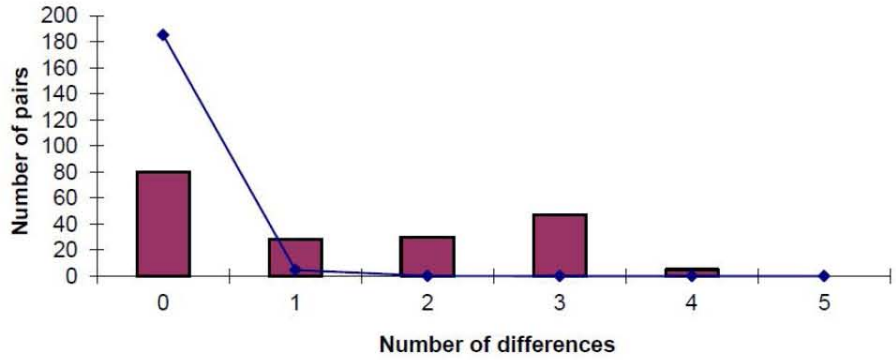


Figure 2.9 Statistical parsimony network obtained with control region for the 30 haplotypes of *Praomys delectorum* (Table 2.1b). The subnetworks that are connected to each other do not exceed the 95% confidence limit of nine steps. Dots indicate unsampled or extinct haplotypes. The size of the boxes is proportional to the frequency of the haplotype.

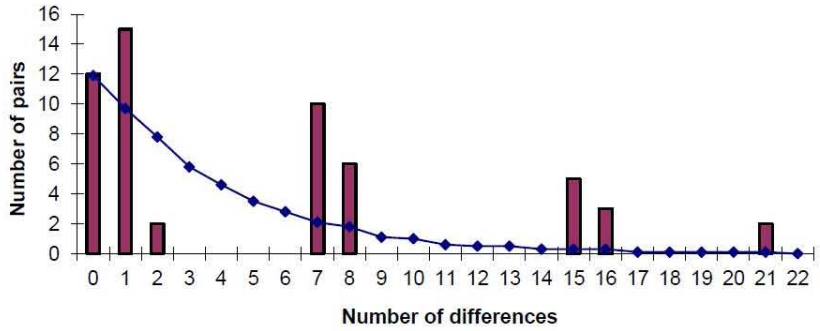
Rungwe



Misuku



Nyika



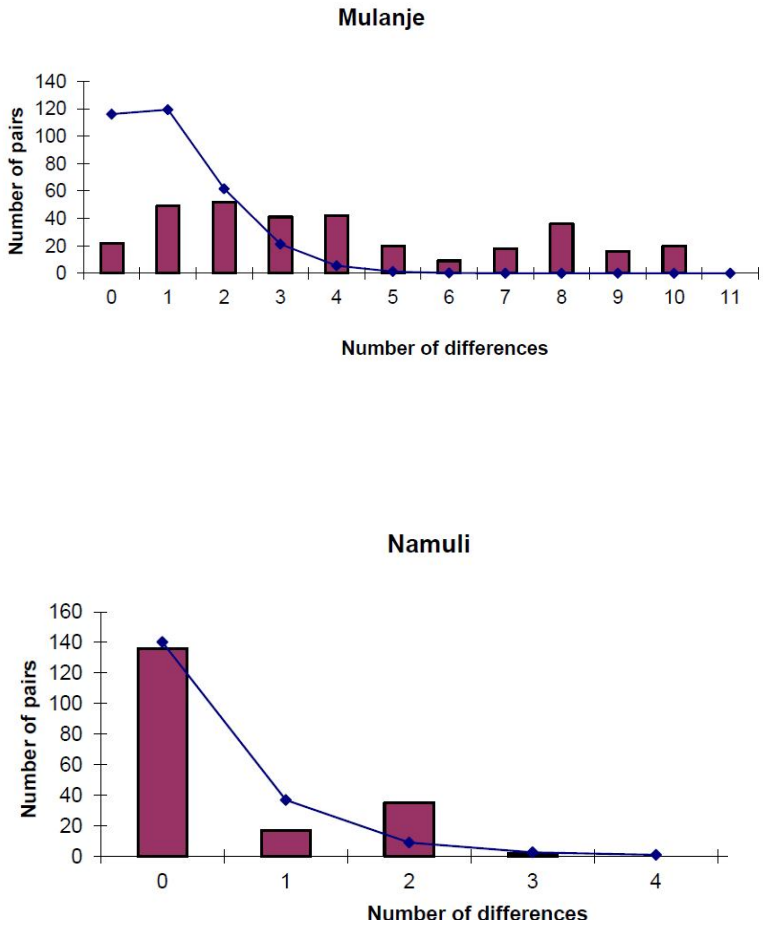
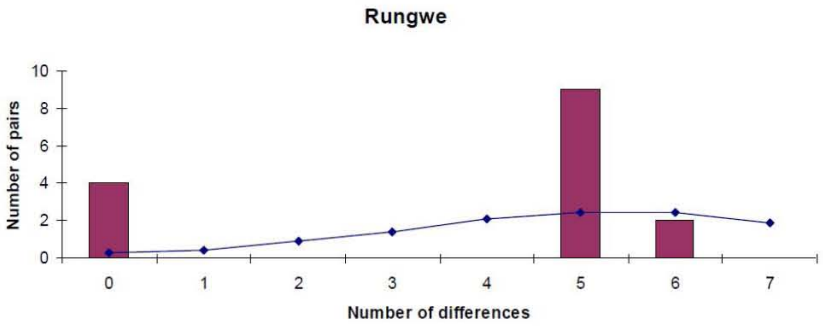
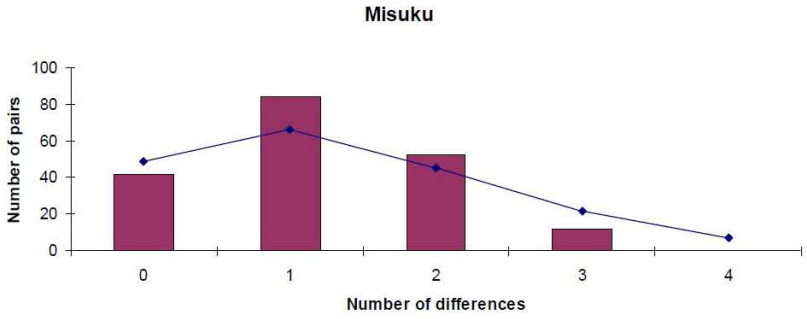
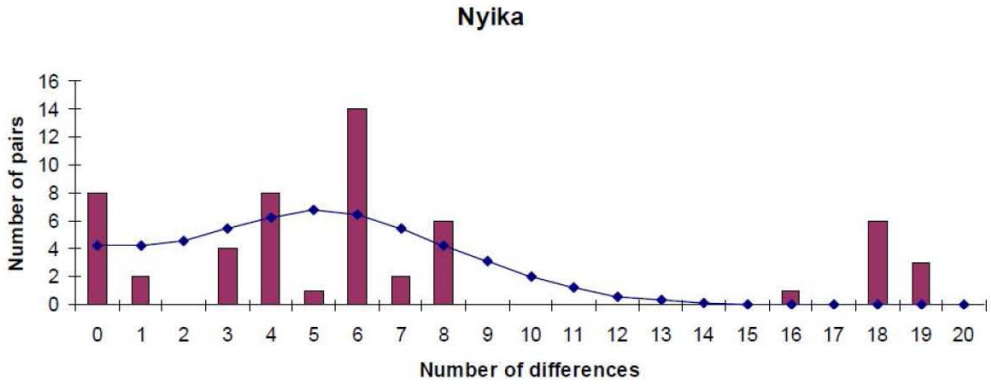


Figure 2.10 Mismatch distributions for Cytochrome-b for selected *Lophuromys aquilus* populations. Histograms represent the observed distributions and the line the expected distribution for a growing population under the same mean.


Rungwe
Number of pairs
10
8
6
4
2
0
0
1
2
3
4
5
6
7
Number of differences


Misuku
Number of pairs
100
80
60
40
20
0
0
1
2
3
4
Number of differences


Nyika
Number of pairs
16
14
12
10
8
6
4
2
0
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
Number of differences

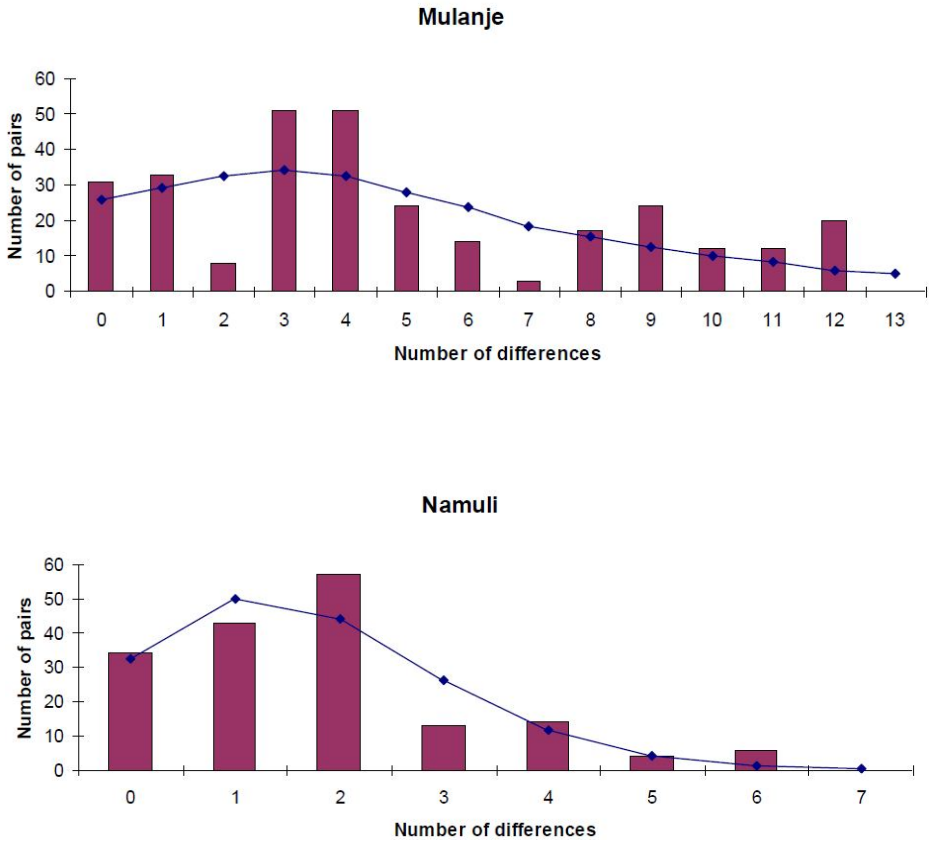
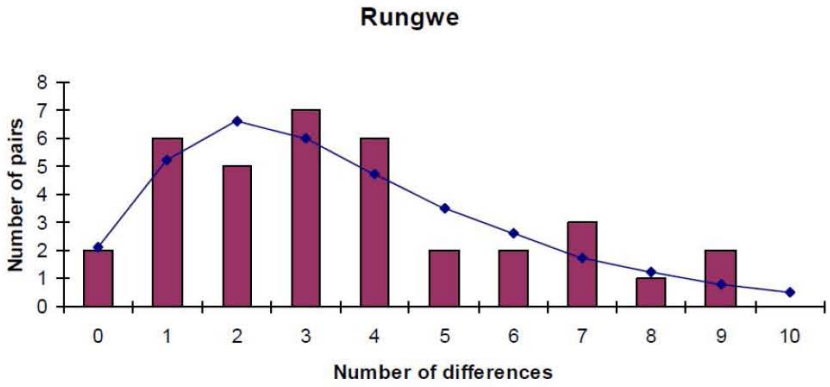
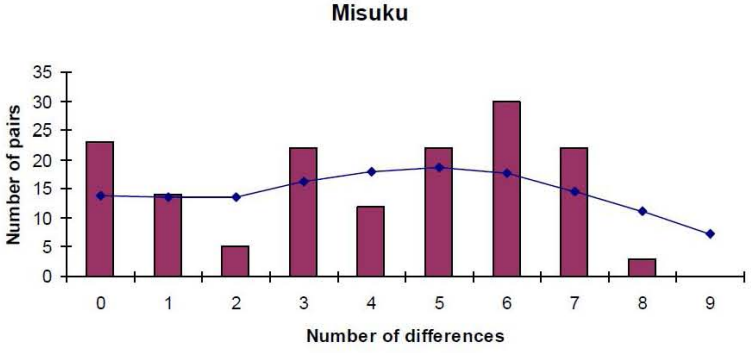
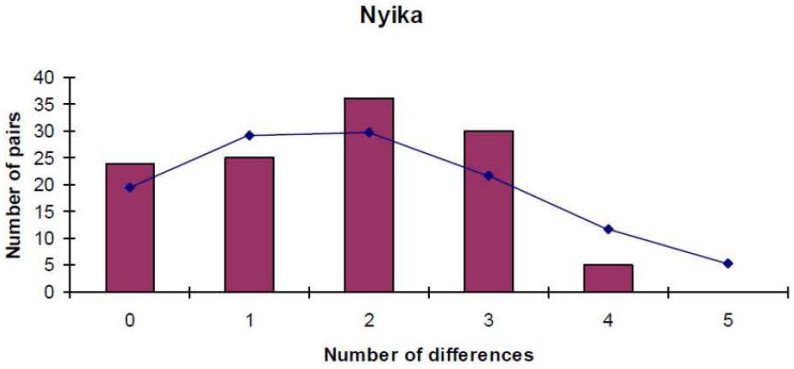


Figure 2.11 Mismatch distributions for control region for selected *Lophuromys aquilus* populations. Histograms represent the observed distributions and the line the expected distribution for a growing population under the same mean.


Rungwe
Number of pairs
0
1
2
3
4
5
6
7
8
Number of differences
0
1
2
3
4
5
6
7
8
9
10


Misuku
Number of pairs
0
5
10
15
20
25
30
35
Number of differences
0
1
2
3
4
5
6
7
8
9


Nyika
Number of pairs
0
5
10
15
20
25
30
35
40
Number of differences
0
1
2
3
4
5

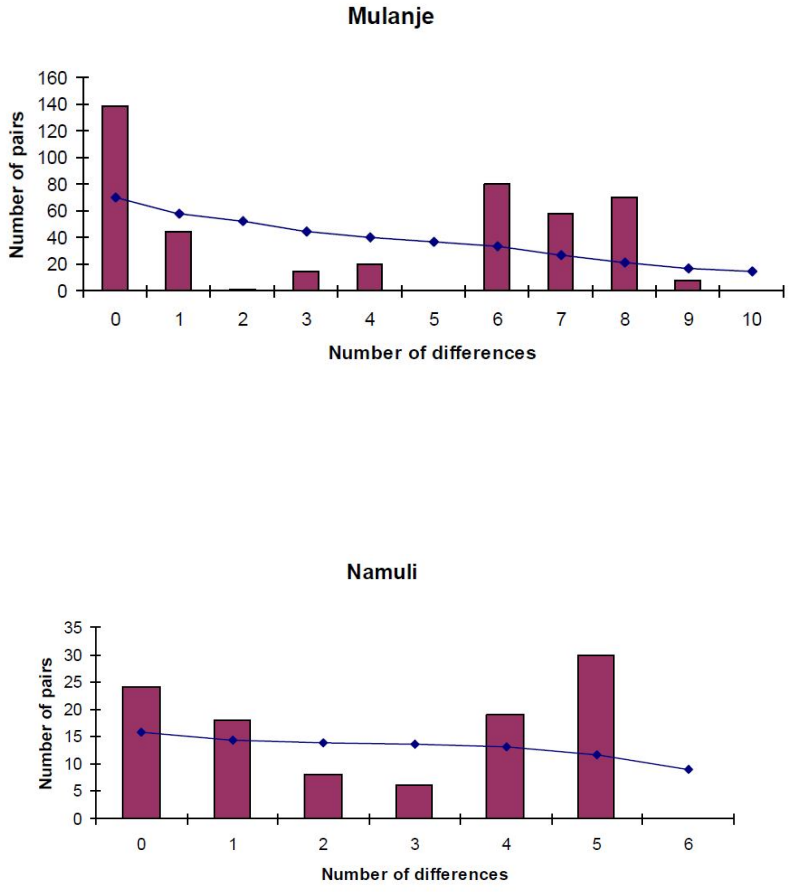
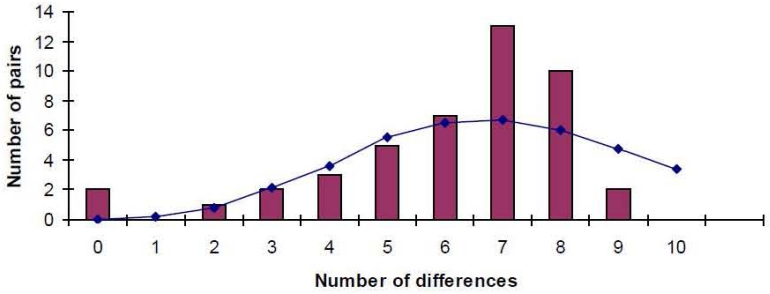
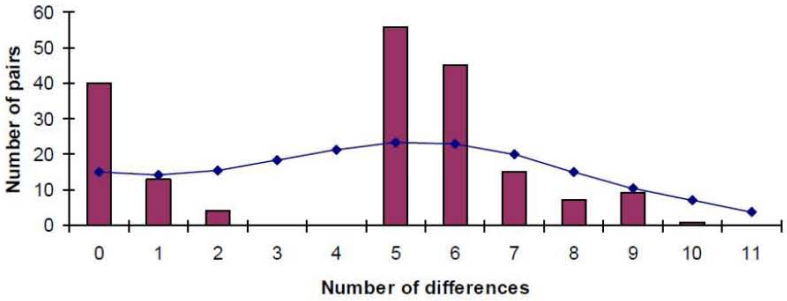


Figure 2.12 Mismatch distributions for Cytochrome-b for *Praomys delectorum* populations. Histograms represent the observed distributions and the line the expected distribution for a growing population under the same mean.

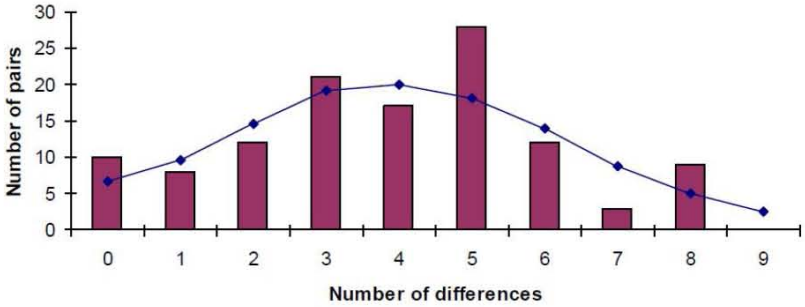
Rungwe



Misuku



Nyika



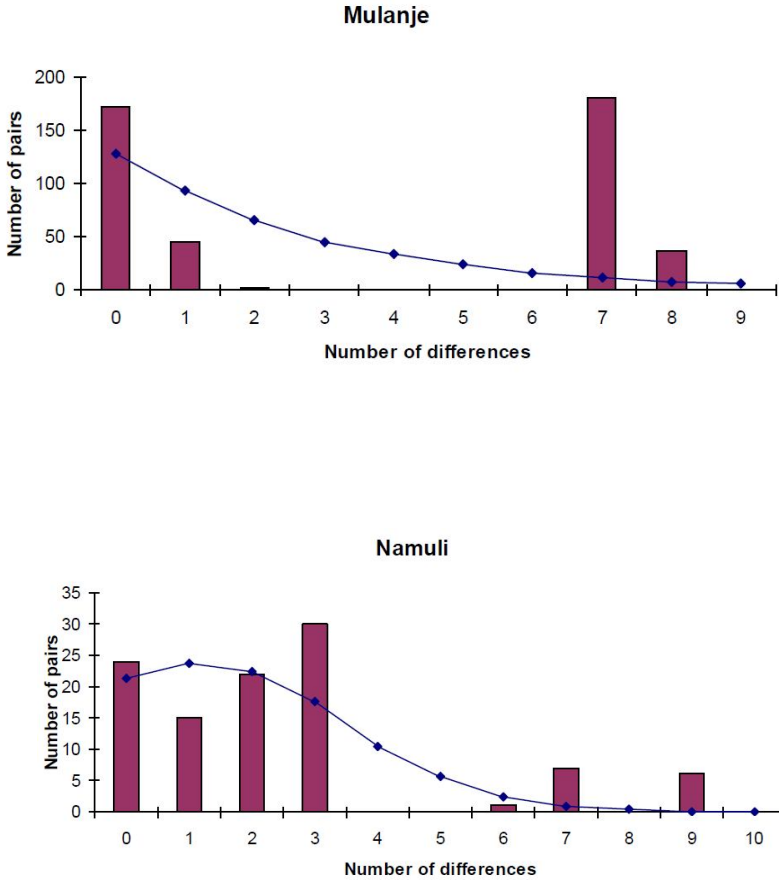


Figure 2.13 Mismatch distributions for control region for *Praomys delectorum* populations. Histograms represent the observed distributions and the line the expected distribution for a growing population under the same mean.

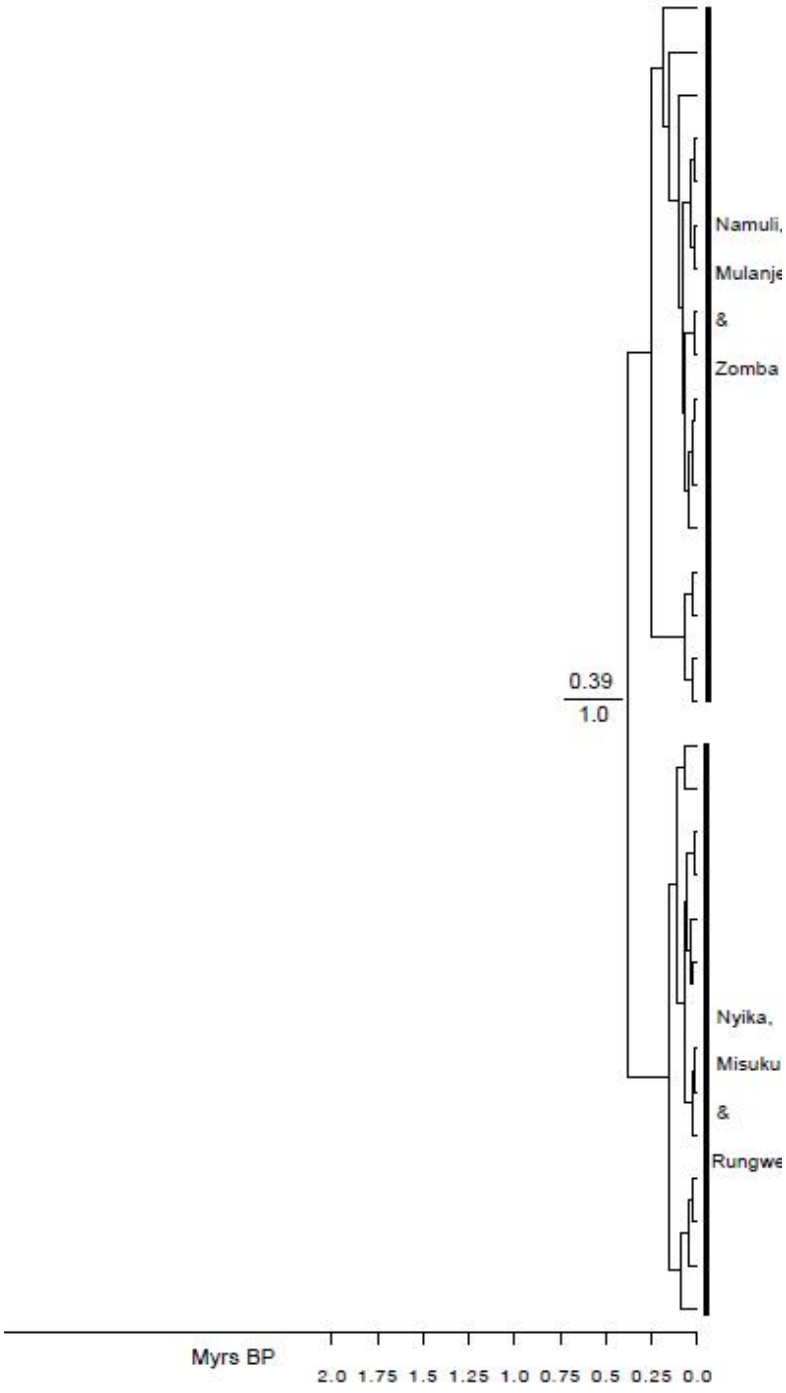


Figure 2.14 Divergence times among populations of *Lophuromys aquilus* in millions of years before present (Myrs BP) above the nodes and below the nodes are BEAST posterior probabilities.

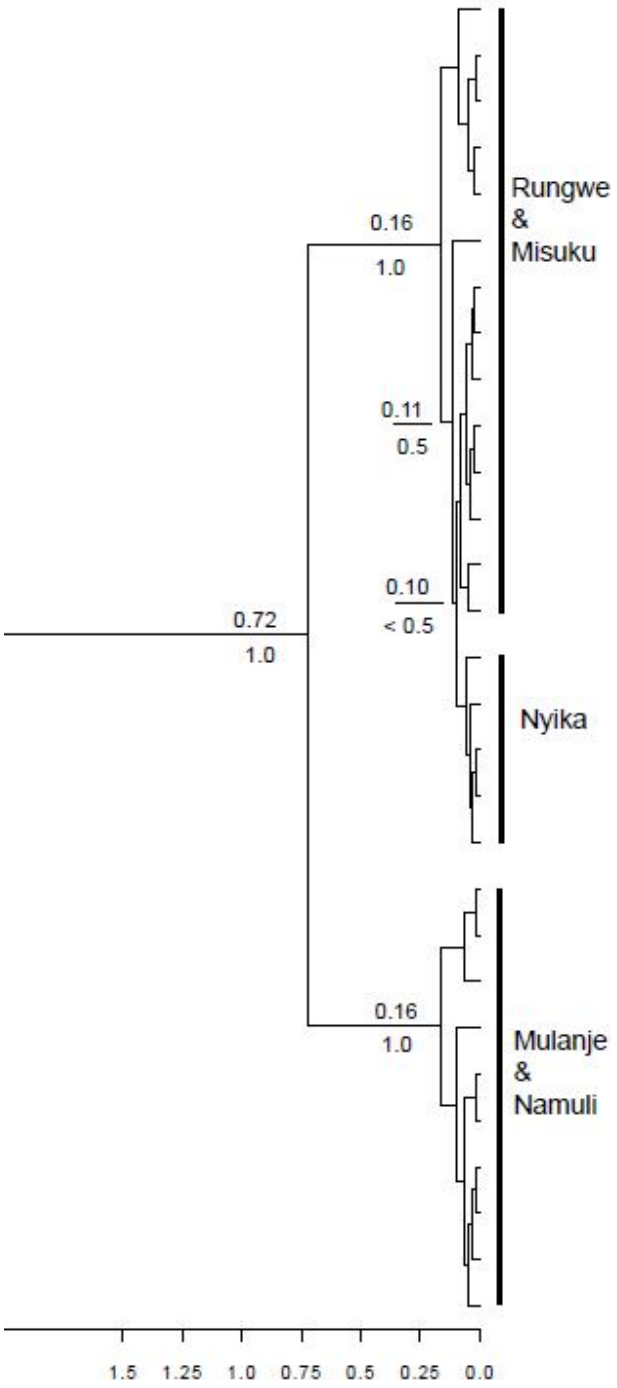


Figure 2.15 Divergence times among populations of *Praomys delectorum* in millions of years before present (Myrs BP) above the nodes and below the nodes are BEAST posterior probabilities.

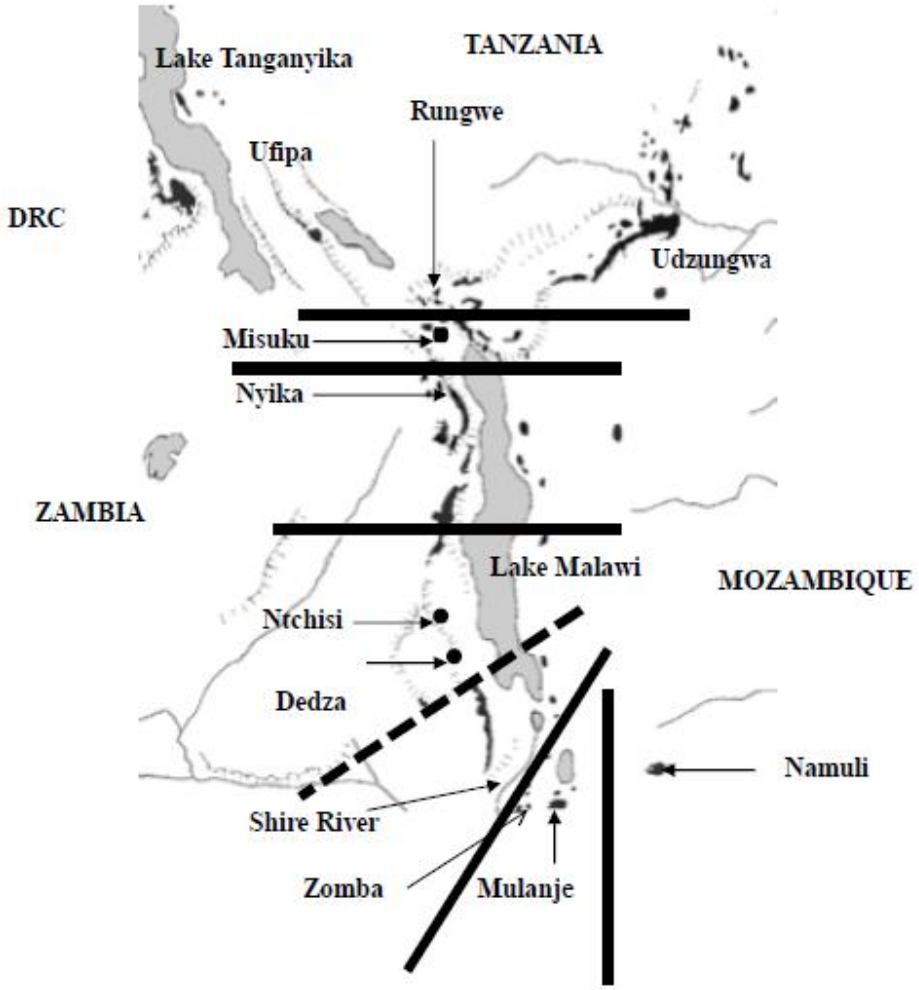


Figure 2.16 The Malawi Rift with the geographical breaks (solid lines) detected among sampled populations of *Lophuromys aquilus* and *Praomys delectorum*. The dashed line indicates potential or weaker breaks.

Table 2.1a Taxa analysed in this study for Cytochrome-b, sampling localities, geographical coordinates and frequencies of haplotypes. [ ] = haplotypes restricted to a specific population, ( ) = occurrence in more than one population.

Species	Locality	Country	Sample size	Latitude	Longitude	Haplotypes mtDNA (Cytochrome b)
Lophuromys aquilus	Rungwe	Tanzania	11	07.80S	36.41E	1[2] 2[2] 3[1] 7(1) 12(4) 13[1]
	Misuku	Malawi	20	09.68S	33.50E	4[2] 5[1] 6[13] 7(1) 8[2] 12(1)
	Nyika	Malawi	11	10.57S	33.70E	9[1] 10[2] 11[5] 14[2] 26(1)
	Zomba	Malawi	4	15.40S	35.30E	19[4]
	Mulanje	Malawi	26	15.55S	35.38E	20[1] 21[1] 22[1] 23[2] 24[2] 25[2] 26(4) 27[3] 28[1] 29[2] 30[4] 31[4]
	Namuli	Mozambique	20	15.39S	37.05E	15[1] 16[1] 17[1] 18[17]
Praomys delectorum	Rungwe	Tanzania	9	07.80S	36.41E	1[1] 2(2) 3[1] 4[1] 6[2]18[1] 19[1]
	Misuku	Malawi	18	09.68S	33.50E	2(1) 5[2] 7[1] 8[6] 9[1] 10[4] 11[1] 12[2]
	Nyika	Malawi	16	10.57S	33.70E	13[2] 14[1] 15[5] 16[3] 17[5]
	Mulanje	Malawi	30	15.55S	35.38E	21[1] 22(16) 23[1] 25[5] 26[3] 27[4]
	Namuli	Mozambique	15	15.39S	37.05E	20[1] 22(1) 24[4] 28[3] 29[6]

Table 2.1b Taxa analysed in this study for control region and Fib7, sampling localities, geographical coordinates and frequencies of haplotypes/alleles. [ ] = haplotypes/alleles restricted to a specific population, ( ) = occurrence in more than one population.

Species	Locality	Country	Sample size	Latitude	Longitude	Haplotypes mtDNA control region	Alleles nDNA Fib7
Lophuromys aquilus	Rungwe	Tanzania	6	07.80S	36.41E	4(2) 5[1] 6[3]	1(12)
	Misuku	Malawi	20	09.68S	33.50E	1[5] 2[5] 3[7] 4(2) 7[1]	1(35) 4[1] 5[2] 6[2]
	Nyika	Malawi	11	10.57S	33.70E	8[2] 9[4] 10[1] 11[2] 12[1] 28(1)	1(21) 3(1)
	Zomba	Malawi	4	15.40S	35.30E	26[2] 27[2]	1(8)
	Mulanje	Malawi	25	15.55S	35.38E	20[4] 21[1] 22[3] 23[1] 24[1] 25[6] 28(3) 29[3] 30[1] 31[2]	1(45) 2[4] 3(3) 7[1]
	Namuli	Mozambique	19	15.39S	37.05E	13[1] 14[1] 15[7] 16[1] 17[3] 18[5] 19[1]	1(29) 3(11)
Praomys delectorum	Rungwe	Tanzania	10	07.80S	36.41E	3[1] 4[1] 5[1] 6(2) 8[1] 10[2] 12[1] 16(1)	4(4) 5(2)
	Misuku	Malawi	20	09.68S	33.50E	1[1]2[5] 6(8) 7[1] 9[2] 11[1] 13[2]	4(34) 5(5) 6[1]
	Nyika	Malawi	16	10.57S	33.70E	14[3] 15[1] 16(2) 17[1] 18[3] 19[2] 20[2] 21[2]	1(4) 4(24) 5(2)
	Mulanje	Malawi	30	15.55S	35.38E	22[12] 24[2] 25[15] 26[1]	1(35) 2[7] 3[17] 5(1)
	Namuli	Mozambique	15	15.39S	37.05E	23[1]27[1] 28[3] 29[6] 30[4]	1(29) 7[1]

Table 2.2 Pairwise Φ_{ST}-values of six sampled populations of *Lophuromys aquilus* mtDNA (Cytochrome-b) below diagonal and nDNA (Fib7) above diagonal.

	Rungwe	Misuku	Nyika	Mulanje	Namuli
Rungwe	-	-0.31	-0.33	-0.29	-0.08
Misuku	0.35***	-	0.01	0.03	0.18**
Nyika	0.28**	0.46***	-	-0.01	0.12
Mulanje	0.79***	0.82***	0.72***	-	0.12**
Namuli	0.91***	0.93***	0.83***	0.72***	-

***P < 0.0001
**P < 0.001
*P < 0.01

Table 2.3 Pairwise Φ_{ST}-values of five sampled populations of *Lophuromys aquilus* mtDNA (control region) below diagonal and nDNA (Fib7) above diagonal.

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	Rungwe	Misuku	Nyika	Mulanje	Namuli
Rungwe	-	-0.31	-0.33	-0.29	-0.08
Misuku	0.42**	-	0.01	0.03	0.18**
Nyika	0.14*	0.43***	-	-0.01	0.12
Mulanje	0.71***	0.78***	0.63***	-	0.12**
Namuli	0.85***	0.90***	0.74***	0.71***	-

***P < 0.0001
**P < 0.001
*P < 0.01

Table 2.4 Pairwise Φ_{ST}-values of five sampled populations of *Praomys delectorum* mtDNA (Cytochrome-b) below diagonal and nDNA (Fib7) above diagonal.

	Rungwe	Misuku	Nyika	Mulanje	Namuli
Rungwe	-				
Misuku	0.10	-	0.04	0.67***	0.84***
Nyika	0.36***	0.43***	-	0.57***	0.78***
Mulanje	0.89***	0.89***	0.90***	-	0.15**
Namuli *	0.91***	0.90***	0.93***	0.29***	-

***P < 0.0001
**P < 0.001
*P < 0.01

Table 2.5 Pairwise Φ_{ST}-values of five sampled populations of *Praomys delectorum* mtDNA (control region) below diagonal and nDNA (Fib7) above diagonal.

	Rungwe	Misuku	Nyika	Mulanje	Namuli
Rungwe	-				
Misuku	0.03	-	0.04	0.67***	0.84***
Nyika	0.36***	0.48***	-	0.57***	0.78***
Mulanje	0.73***	0.76***	0.75***	-	0.15**
Namuli	0.80***	0.83***	0.82***	0.47***	-

**P < 0.0001
**P < 0.001
*P < 0.01

Table 2.6 Measures of haplotype diversity, nucleotide diversity and for tests of selective neutrality (Tajima's D and Fu's Fs) based on Cytochrome-b and control region for *Lophuromys aquilus*.

	Number of individuals	**Haplotype diversity**	**Nucleotide diversity**	**Tajima D**		**Fu's Fs**	
				D	P	Fs	P
Cytochrome-b							
Rungwe	11	0.8545 ± 0.0852	0.002188 ± 0.001442	0.141	0.591	-1.013	0.231
Misuku	20	0.5789 ± 0.1242	0.001160 ± 0.000843	-1.104	0.153	-1.458	0.140
Nyika	11	0.7818 ± 0.1073	0.004859 ± 0.002857	-1.375	0.086	2.165	0.850
Zomba	4	0	0	0	1.000		
Mulanje	26	0.9323 ± 0.0231	0.003692 ± 0.002112	-0.410	0.389	-2.225	0.162
Namuli	20	0.2842 ± 0.1284	0.000433 ± 0.000433	-1.638	0.031**	-1.613	0.046**
Control region							
Rungwe	6	0.7333 ± 0. 0.1552	0.008207 ± 0.005543	0.499	0.675	2.321	0.879
Misuku	20	0.7789 ± 0.0496	0.002546 ± 0.001905	-0.500	0.347	-0.695	0.317
Nyika	11	0.8545 ± 0.0852	0.014844 ± 0.008539	-0.739	0.239	1.556	0.772
Zomba	4	0.6667 ± 0.2041	0.001440 ± 0.001616	1.633	0.963	0.540	0.478
Mulanje	25	0.8967 ± 0.033	0.010619 ± 0.005963	0.112	0.600	-0.173	0.503
Namuli	29	0.8012 ± 0.0631	0.003890 ± 0.002623	-1.048	0.162	-1.637	0.140

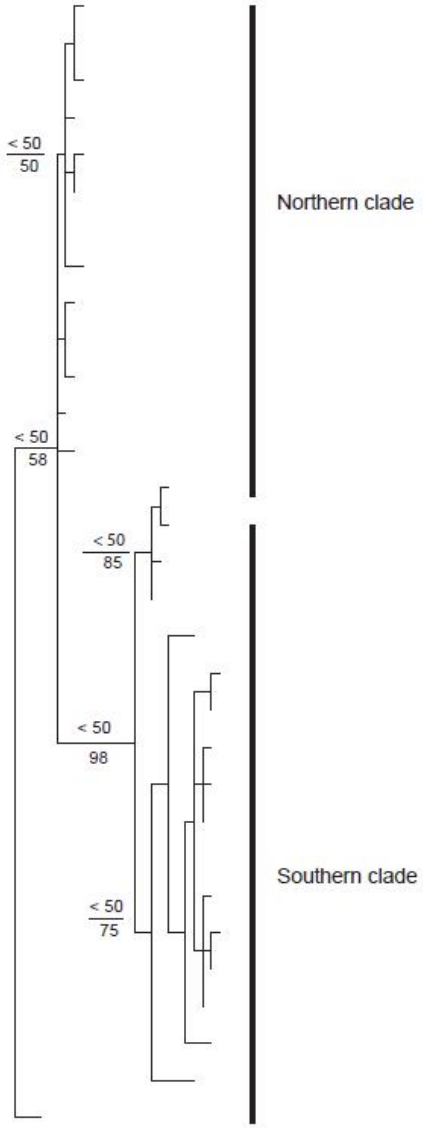
**Significant values based on 10000 permutations, indicate that the population is out of equilibrium, suggestive of recent demographic change.

Table 2.7 Measures of haplotype diversity, nucleotide diversity and for tests of selective neutrality (Tajima's D and Fu's Fs) based on Cytochrome-b and control region for *Praomys delectorum*.

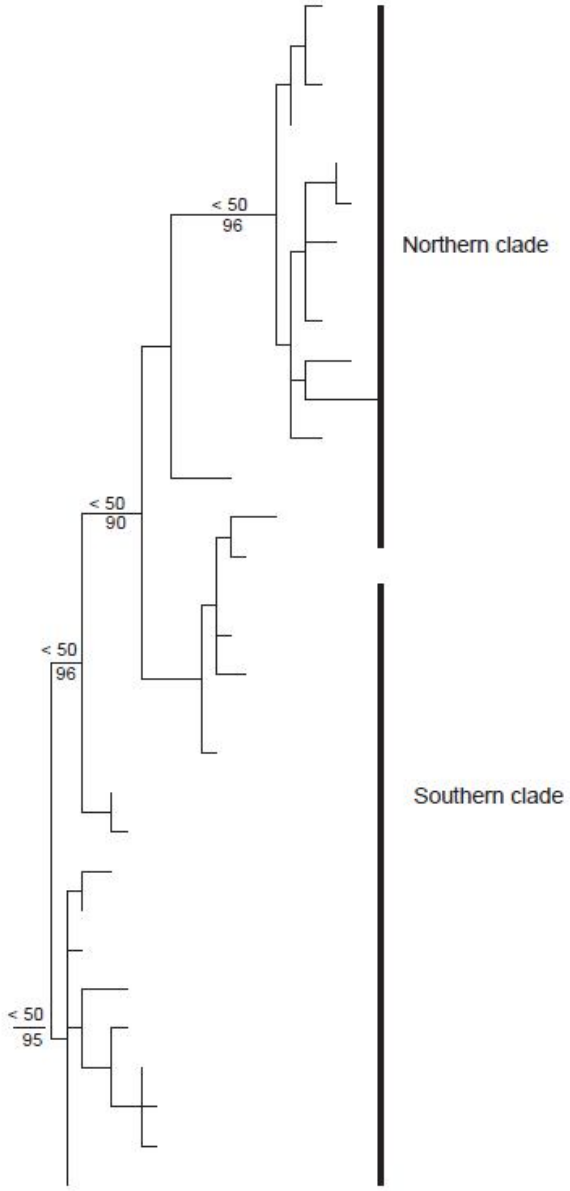
	Number of individuals	**Haplotype diversity**	**Nucleotide diversity**	**Tajima D**		**Fu's Fs**	
				D	P	Fs	P
Cytochrome-b							
Rungwe	9	0.9444 ± 0. 0.0702	0.003159 ± 0.002004	-1.4435	0.076	-2.040	0.083
Misuku	18	0.8497 ± 0.0603	0.003465 ± 0.002034	0.4 00	0.731	-0.282	0.455
Nyika	16	0.8000 ± 0.0572	0.001509 ± 0.001039	-0.157	0.469	-0.063	0.485
Mulanje	30	0.6805 ± 0.0775	0.003397 ± 0.001952	1.282	0.914	2.810	0.890
Namuli	15	0.7714 ± 0.0720	0.002316 ± 0.001467	0.828	0.810	0.934	0.709
Control region							
Rungwe	10	0.9556 ± 0.0594	0.013304 ± 0.007820	0.960	0.582	-1.452	0.187
Misuku	20	0.7895 ± 0.0684	0.009396 ± 0.005417	0.358	0.677	1.158	0.729
Nyika	16	0.9167 ± 0.0375	0.008460 ± 0.005016	-0.017	0.537	-0.686	0.364
Mulanje	30	0.6046 ± 0.0520	0.007964 ± 0.004612	1.914	0.975	5.240	0.972
Namuli	15	0.7714 ± 0.0720	0.005330 ± 0.003422	-0.421	0.364	0.733	0.671

**Significant values based on 10000 permutations, indicate that the population is out of equilibrium, suggestive of recent demographic change.

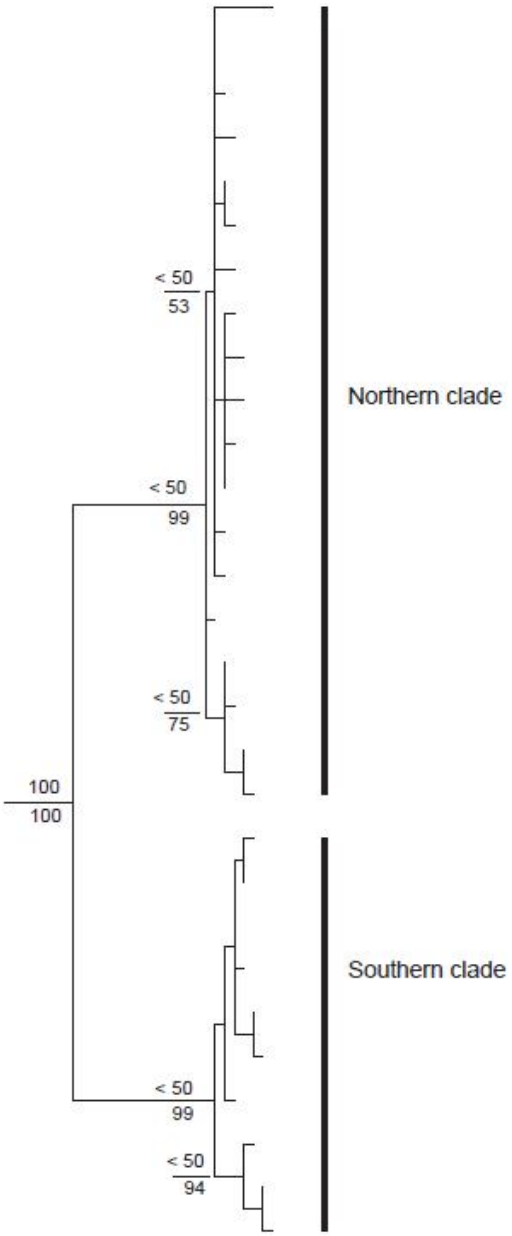
APPENDICES



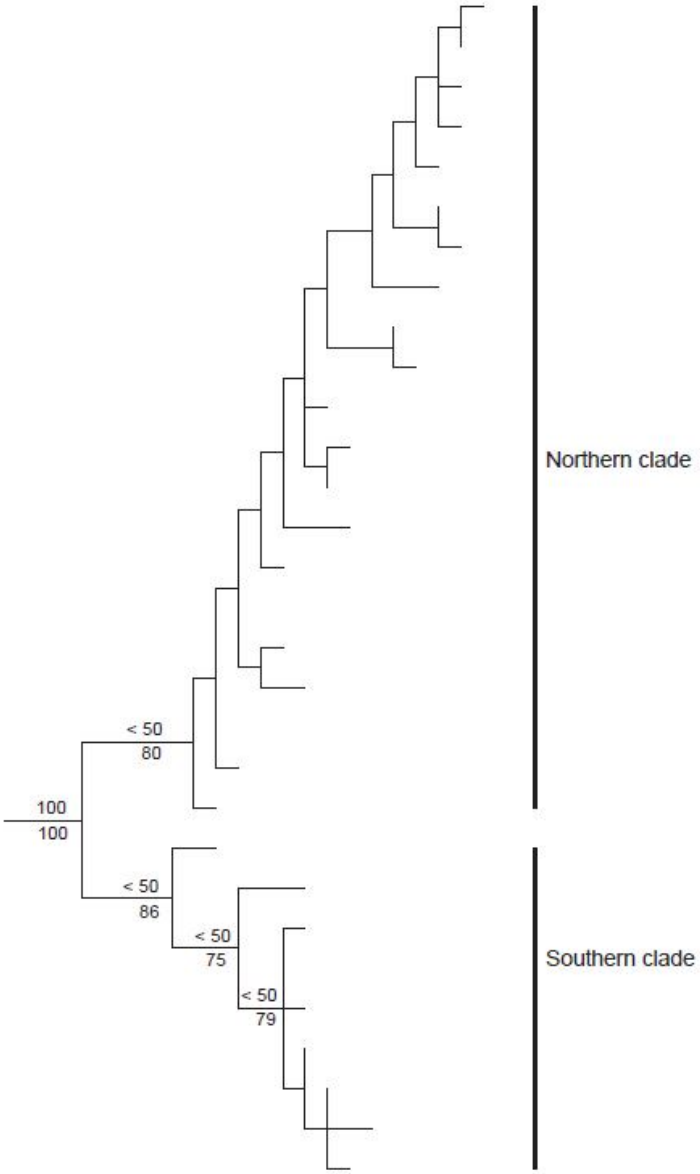
Appendix A. A Maximum Parsimony topology depicting phylogenetic relationships among *Lophuromys aquilus* (Cytochrome-b) populations sampled across the Malawi Rift, as well as representative populations to the north and south. Values above the nodes are ML bootstrap support values and those below are MP bootstrap support values.



Appendix B. A Maximum Parsimony topology depicting phylogenetic relationships among *Lophuromys aquilus* (control region) populations sampled across the Malawi Rift, as well as representative populations to the north and south. Values above the nodes are ML bootstrap support values and those below are MP bootstrap support values.



Appendix C. A Maximum Parsimony topology depicting phylogenetic relationships among *Praomys delectorum* (Cytochrome-b) populations sampled across the Malawi Rift, as well as representative populations to the north and south. Values above the nodes are ML bootstrap support values and those below are MP bootstrap support values.



Appendix D. A Maximum Parsimony topology depicting phylogenetic relationships among *Praomys delectorum* (control region) populations sampled across the Malawi Rift, as well as representative populations to the north and south. Values above the nodes are ML bootstrap support values and those below are MP bootstrap support values.

Appendix E: *Praomys delectorum* specimens examined in this study

Genus/Species	Locality	Coordinates	Museum number
Praomys delectorum	Rungwe, Tanzania	07.80S 36.41E	FMNH163627
Praomys delectorum	Rungwe, Tanzania	07.80S 36.41E	FMNH163628
Praomys delectorum	Rungwe, Tanzania	07.80S 36.41E	FMNH163630
Praomys delectorum	Rungwe, Tanzania	07.80S 36.41E	FMNH163632
Praomys delectorum	Rungwe, Tanzania	07.80S 36.41E	FMNH163634
Praomys delectorum	Rungwe, Tanzania	07.80S 36.41E	FMNH163635
Praomys delectorum	Rungwe, Tanzania	07.80S 36.41E	FMNH163636
Praomys delectorum	Rungwe, Tanzania	07.80S 36.41E	FMNH163637
Praomys delectorum	Rungwe, Tanzania	07.80S 36.41E	FMNH163641
Praomys delectorum	Rungwe, Tanzania	07.80S 36.41E	FMNH163642
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181195
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181196
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181197
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181198
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181199
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181200
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181201
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181202
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181203
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181204
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181218
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181219
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181220
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181221
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181222
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181223
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181224
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181225
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181226
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181227
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181231
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181232
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181235
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181237
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181238
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181239
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181240
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181243
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181245
Praomys delectorum	Mulanje, Malawi	15.55S 35.38E	FMNH181246
Praomys delectorum	Nyika, Malawi	10.57S 33.70E	MLWM136
Praomys delectorum	Nyika, Malawi	10.57S 33.70E	MLWM213
Praomys delectorum	Nyika, Malawi	10.57S 33.70E	MLWM214
Praomys delectorum	Nyika, Malawi	10.57S 33.70E	MLWM220
Praomys delectorum	Nyika, Malawi	10.57S 33.70E	MLWM224

Praomys delectorum	Nyika, Malawi	10.57S 33.70E	MLWM225
Praomys delectorum	Nyika, Malawi	10.57S 33.70E	MLWM229
Praomys delectorum	Nyika, Malawi	10.57S 33.70E	MLWM235
Praomys delectorum	Nyika, Malawi	10.57S 33.70E	MLWM236
Praomys delectorum	Nyika, Malawi	10.57S 33.70E	MLWM238
Praomys delectorum	Nyika, Malawi	10.57S 33.70E	MLWM249
Praomys delectorum	Nyika, Malawi	10.57S 33.70E	MLWM252
Praomys delectorum	Nyika, Malawi	10.57S 33.70E	MLWM254
Praomys delectorum	Nyika, Malawi	10.57S 33.70E	MLWM264
Praomys delectorum	Nyika, Malawi	10.57S 33.70E	MLWM265
Praomys delectorum	Nyika, Malawi	10.57S 33.70E	MLWM267
Praomys delectorum	Namuli,Mozambique	15.39S 37.05E	FMNH183705
Praomys delectorum	Namuli,Mozambique	15.39S 37.05E	FMNH183706
Praomys delectorum	Namuli,Mozambique	15.39S 37.05E	FMNH183707
Praomys delectorum	Namuli,Mozambique	15.39S 37.05E	FMNH183708
Praomys delectorum	Namuli,Mozambique	15.39S 37.05E	FMNH183709
Praomys delectorum	Namuli,Mozambique	15.39S 37.05E	FMNH183710
Praomys delectorum	Namuli,Mozambique	15.39S 37.05E	FMNH183711
Praomys delectorum	Namuli,Mozambique	15.39S 37.05E	FMNH183712
Praomys delectorum	Namuli,Mozambique	15.39S 37.05E	FMNH183724
Praomys delectorum	Namuli,Mozambique	15.39S 37.05E	FMNH183725
Praomys delectorum	Namuli,Mozambique	15.39S 37.05E	FMNH183726
Praomys delectorum	Namuli,Mozambique	15.39S 37.05E	FMNH183727
Praomys delectorum	Namuli,Mozambique	15.39S 37.05E	FMNH183728
Praomys delectorum	Namuli,Mozambique	15.39S 37.05E	FMNH183729
Praomys delectorum	Namuli,Mozambique	15.39S 37.05E	FMNH183730
Praomys delectorum	Misuku Hills, Malawi	09.68S 33.50E	FMNH196357
Praomys delectorum	Misuku Hills, Malawi	09.68S 33.50E	FMNH196358
Praomys delectorum	Misuku Hills, Malawi	09.68S 33.50E	FMNH196361
Praomys delectorum	Misuku Hills, Malawi	09.68S 33.50E	FMNH196362
Praomys delectorum	Misuku Hills, Malawi	09.68S 33.50E	FMNH196363
Praomys delectorum	Misuku Hills, Malawi	09.68S 33.50E	FMNH196364
Praomys delectorum	Misuku Hills, Malawi	09.68S 33.50E	FMNH196365
Praomys delectorum	Misuku Hills, Malawi	09.68S 33.50E	FMNH196837
Praomys delectorum	Misuku Hills, Malawi	09.68S 33.50E	FMNH196838
Praomys delectorum	Misuku Hills, Malawi	09.68S 33.50E	FMNH196839
Praomys delectorum	Misuku Hills, Malawi	09.68S 33.50E	FMNH196840
Praomys delectorum	Misuku Hills, Malawi	09.68S 33.50E	FMNH196841
Praomys delectorum	Misuku Hills, Malawi	09.68S 33.50E	FMNH196842
Praomys delectorum	Misuku Hills, Malawi	09.68S 33.50E	FMNH196843
Praomys delectorum	Misuku Hills, Malawi	09.68S 33.50E	FMNH196887
Praomys delectorum	Misuku Hills, Malawi	09.68S 33.50E	FMNH196888
Praomys delectorum	Misuku Hills, Malawi	09.68S 33.50E	FMNH196889
Praomys delectorum	Misuku Hills, Malawi	09.68S 33.50E	FMNH196890
Praomys delectorum	Misuku Hills, Malawi	09.68S 33.50E	FMNH196892
Praomys delectorum	Misuku Hills, Malawi	09.68S 33.50E	FMNH196893

Appendix F: *Lophuromys aquilus* specimens examined in this study

Genus/Species	Locality	Coordinates	Museum number
Lophuromys aquilus	Nyika, Malawi	10.57S 33.70E	FMNH191611
Lophuromys aquilus	Nyika, Malawi	10.57S 33.70E	FMNH191610
Lophuromys aquilus	Nyika, Malawi	10.57S 33.70E	FMNH192249
Lophuromys aquilus	Nyika, Malawi	10.57S 33.70E	FMNH191612
Lophuromys aquilus	Nyika, Malawi	10.57S 33.70E	FMNH191613
Lophuromys aquilus	Nyika, Malawi	10.57S 33.70E	FMNH191614
Lophuromys aquilus	Nyika, Malawi	10.57S 33.70E	FMNH191615
Lophuromys aquilus	Nyika, Malawi	10.57S 33.70E	FMNH191616
Lophuromys aquilus	Namuli Mozambique	15.39S 37.05E	FMNH183662
Lophuromys aquilus	Namuli Mozambique	15.39S 37.05E	FMNH183663
Lophuromys aquilus	Namuli Mozambique	15.39S 37.05E	FMNH183664
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH192250
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH192251
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH192252
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH192253
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH192254
Lophuromys aquilus	Nyika, Malawi	10.57S 33.70E	FMNH191607
Lophuromys aquilus	Nyika, Malawi	10.57S 33.70E	FMNH191608
Lophuromys aquilus	Nyika, Malawi	10.57S 33.70E	FMNH191609
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH192255
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH180980
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH181182
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH181183
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH181184
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH181185
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH181186
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH181187
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH192246
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH192247
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH192248
Lophuromys aquilus	Rungwe Tanzania	07.80S 36.41E	FMNH163620
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH180998
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH180999
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH181000
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH181016
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH181188
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH181189
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH181190
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH181191
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	FMNH180975
Lophuromys aquilus	Rungwe, Tanzania	07.80S 36.41E	FMNH163601
Lophuromys aquilus	Rungwe, Tanzania	07.80S 36.41E	FMNH163602
Lophuromys aquilus	Rungwe, Tanzania	07.80S 36.41E	FMNH163603
Lophuromys aquilus	Rungwe, Tanzania	07.80S 36.41E	FMNH163604
Lophuromys aquilus	Rungwe, Tanzania	07.80S 36.41E	FMNH163605

Lophuromys aquilus	Rungwe, Tanzania	07.80S 36.41E	FMNH163611
Lophuromys aquilus	Rungwe, Tanzania	07.80S 36.41E	FMNH163612
Lophuromys aquilus	Rungwe, Tanzania	07.80S 36.41E	FMNH163613
Lophuromys aquilus	Rungwe, Tanzania	07.80S 36.41E	FMNH163618
Lophuromys aquilus	Rungwe, Tanzania	07.80S 36.41E	FMNH163619
Lophuromys aquilus	Misuku, Malawi	09.68S 33.50E	FMNH196268
Lophuromys aquilus	Misuku Hills, Malawi	09.68S 33.50E	FMNH196269
Lophuromys aquilus	Misuku Hills, Malawi	09.68S 33.50E	FMNH196270
Lophuromys aquilus	Misuku Hills, Malawi	09.68S 33.50E	FMNH196271
Lophuromys aquilus	Misuku Hills, Malawi	09.68S 33.50E	FMNH196272
Lophuromys aquilus	Misuku Hills, Malawi	09.68S 33.50E	FMNH196701
Lophuromys aquilus	Misuku Hills, Malawi	09.68S 33.50E	FMNH196702
Lophuromys aquilus	Misuku Hills, Malawi	09.68S 33.50E	FMNH196704
Lophuromys aquilus	Misuku Hills, Malawi	09.68S 33.50E	FMNH196700
Lophuromys aquilus	Misuku Hills, Malawi	09.68S 33.50E	FMNH196705
Lophuromys aquilus	Misuku Hills, Malawi	09.68S 33.50E	FMNH196235
Lophuromys aquilus	Misuku Hills, Malawi	09.68S 33.50E	FMNH196236
Lophuromys aquilus	Misuku Hills, Malawi	09.68S 33.50E	FMNH196237
Lophuromys aquilus	Misuku Hills, Malawi	09.68S 33.50E	FMNH196238
Lophuromys aquilus	Misuku Hills, Malawi	09.68S 33.50E	FMNH196239
Lophuromys aquilus	Misuku Hills, Malawi	09.68S 33.50E	FMNH196240
Lophuromys aquilus	Misuku Hills, Malawi	09.68S 33.50E	FMNH196241
Lophuromys aquilus	Misuku Hills, Malawi	09.68S 33.50E	FMNH196243
Lophuromys aquilus	Misuku Hills, Malawi	09.68S 33.50E	FMNH196244
Lophuromys aquilus	Misuku Hills, Malawi	09.68S 33.50E	FMNH196245
Lophuromys aquilus	Namuli Mozambique	15.39S 37.05E	FMNH183675
Lophuromys aquilus	Namuli Mozambique	15.39S 37.05E	FMNH183774
Lophuromys aquilus	Namuli Mozambique	15.39S 37.05E	FMNH183665
Lophuromys aquilus	Namuli Mozambique	15.39S 37.05E	FMNH183670
Lophuromys aquilus	Namuli Mozambique	15.39S 37.05E	FMNH183666
Lophuromys aquilus	Namuli Mozambique	15.39S 37.05E	FMNH183671
Lophuromys aquilus	Namuli Mozambique	15.39S 37.05E	FMNH183676
Lophuromys aquilus	Namuli Mozambique	15.39S 37.05E	FMNH183775
Lophuromys aquilus	Namuli Mozambique	15.39S 37.05E	FMNH183667
Lophuromys aquilus	Namuli Mozambique	15.39S 37.05E	FMNH183672
Lophuromys aquilus	Namuli Mozambique	15.39S 37.05E	FMNH183771
Lophuromys aquilus	Namuli Mozambique	15.39S 37.05E	FMNH183773
Lophuromys aquilus	Namuli Mozambique	15.39S 37.05E	FMNH183668
Lophuromys aquilus	Namuli Mozambique	15.39S 37.05E	FMNH183673
Lophuromys aquilus	Namuli Mozambique	15.39S 37.05E	FMNH183772
Lophuromys aquilus	Namuli Mozambique	15.39S 37.05E	FMNH183669
Lophuromys aquilus	Namuli Mozambique	15.39S 37.05E	FMNH183674
Lophuromys aquilus	Mulanje, Malawi	15.55S 35.38E	MLW1215
Lophuromys aquilus	Zomba, Malawi	15.40S 35.30E	JLP23926
Lophuromys aquilus	Zomba, Malawi	15.40S 35.30E	JLP23927
Lophuromys aquilus	Zomba, Malawi	15.40S 35.30E	JLP23944
Lophuromys aquilus	Zomba, Malawi	15.40S 35.30E	JLP23945

CHAPTER 3

The phylogeography of the Stripe-cheeked Greenbul and Malawi Batis across the Malawi Rift

3.1 ABSTRACT

This study investigated phylogeographic structure of the Stripe-cheeked Greenbul (*Andropadus milanjensis*) and Malawi Batis (*Batis dimorpha*) complex across the Malawi Rift of eastern Africa. Analyses of a combination of mtDNA (1041 bp ND2) and nDNA (463 - 481 bp CHDZ and 569 - 572 bp MUSK) from populations sampled throughout the Malawi Rift, as well as from the Eastern Arc Mountains to the north, and from South Africa to the south, revealed significant population structure for both species complexes. Results suggest that phylogeographic breaks occur in the southern highlands separating Mount Namuli from Mount Mulanje; between Mount Mulanje and Mount Zomba; in the central highlands splitting Malawi into two halves; in the northern highlands separating the Misuku Hills from Nyika Plateau, as well as between the Misuku Hills and the Udzungwa Mountains, the southern terminus of the Eastern Arc Mountains. These breaks agree closely with taxon boundaries, and suggest that each complex may comprise multiple species. The results of this study and ongoing research suggest that molecular DNA data have an important role to play in helping to identify, manage and conserve divergent populations of montane birds in Malawi.

3.2 INTRODUCTION

The Pleistocene was characterized by intense fluctuations in average global temperature accompanied by the repeated advance and retreat of glacial ice sheets in both the Northern and Southern Hemisphere (Dawson 1992). This glacial activity is thought to have had a profound impact on species distributions by causing range contractions during glacial advance and range expansion during periods of climatic amelioration (Hewitt 1996; 2000). In Africa, there were no large glacial ice sheets which could 'wipe the landscape clean', indicating that patterns of lineage diversification through time are likely to be both well preserved and complex (Voelker et al. 2010). Therefore, historical processes are likely to be important determinants of local patterns of diversity because they influence the characteristics of the species pools from which local assemblages are drawn (Caley 1997). It has also been proposed that extensive endemism in the African tropics is partly due to climatic stability through the Pleistocene, whereby some higher elevation sites were shielded from the large climatic fluctuations that caused repeated local and regional extinctions at higher latitudes (Fjeldså & Lovett 1997; Hewitt 2000; Fjeldså et al. 2012). This climatic buffering may have permitted speciation through genetic drift in allopatry or via ecological diversification in areas that remained stable during periods when global and regional climate change were severe enough elsewhere to force major shifts in species distributions. As forests retracted, they became isolated to a greater degree leading to reduced gene flow and hence enhanced opportunities for divergence (Fjeldså & Lovett 1997; Fjeldså et al. 1997). This has contributed to the exceptionally high phylogeographic structure recovered for vertebrates (e.g. Fjeldså & Bowie 2008; Lawson 2010; Bryja et al. 2014) along the eastern arm of the African Rift

system that includes the Malawi Rift (deMenocal 1995; 2004; Vincens et al. 2003). While the impact of the Pleistocene climate fluctuations on the phylogeographic history of northern hemisphere species has been amply studied (Taberlet et al. 1998; Hewitt 2000) few studies have been conducted on this topic in Africa (Bowie et al. 2006).

Using published DNA-based phylogenies of several African bird groups (Johansson et al. 2007; Fuchs et al. 2012), it has been demonstrated that lineages of recent origin are often clustered in montane regions, whereas the faunas of lowland rainforests tend towards being older (Fjeldså et al. 2007; Fjeldså & Bowie 2008; Fjeldså et al. 2012). A more detailed phylogenetic analysis of the African greenbuls (*Andropadus tephrolaemus, A. muasukuensis* and *A. milanjensis*), demonstrated that the montane species form a monophyletic group of Plio-Pleistocene age, while the lowland species of African greenbuls predominately represent branches of Miocene age (Johansson et al. 2007; Fjeldså et al. 2007). This analysis infers a complex relationship between early vicariant events and dispersal events between the Albertine Rift Mountains and the mountains comprising the eastern African and the Malawi Rift, leading to local faunal diversification (Roy 1997; Roy et al. 1998; Fjeldså et al. 2007; Johansson et al. 2007). To further explore the impact of climatic cycling during the Pleistocene on montane birds in Africa, I here conduct a multilocus phylogeographic study of two characteristic montane species complexes: (1) the Stripe-cheeked Greenbul complex (*Andropadus milanjensis*: Pycnonotidae) and (2) the Cape Batis complex (*Batis capensis*: Platysteiridae). The Stripe-cheeked Greenbul (*A. milanjensis*, Shelley 1894) is traditionally subdivided into three subspecies: *A. milanjensis milanjensis* (Shelley 1894), *A. m. olivaceiceps* (Shelley 1896) and *A. m. striifacies* (Reichenow and Neumann 1895) (Fig. 3.1).

Batises are small African insectivores that exhibit sexual dimorphism in plumage (Erard & Fry 1997; Sinclair & Ryan 2003). As for many montane forest species, they exhibit regional variation among isolated mountains. The complex of *Batis* taxa that occur across the Malawi Rift was previously divided into two species, *B. mixta* that occurs predominantly in the Eastern Arc Mountains and *B. capensis* that extends from South Africa to encompass populations in central and southern Malawi (Dowsett & Dowsett-Lemaire 1993; Erard & Fry 1997; Sinclair & Ryan 2003). *Batis mixta* has recently been split into two species (Fjeldså et al. 2006), with *B. mixta* occupying the northern Eastern Arc Mountains and *B. crypta* occupying the central and southern Eastern Arc Mountains of Tanzania and the Misuku Hills in northern Malawi (Fjeldså et al. 2006). The remaining Malawi population was also split from *B. capensis* to *B. dimorpha,* which comprises two subspecies: *B. dimorpha sola* restricted to central Malawi and *B. dimorpha dimorpha* restricted to southern Malawi and central and northern Mozambique (Newman et al. 1992) (Fig. 3.2).

For *A. milanjensis* and *B. dimorpha*, I obtained DNA sequence data from one mitochondrial (mtDNA) marker (NADH dehydrogenase subunit 2 [ND2]) and two nuclear markers (MUSK and CHDZ) to examine the genetic relationships and population structure in each species complex. Considering the temporal changes in the environment and the extent of phylogeographic structure that has been uncovered in some avifauna lineages distributed between eastern and southern Africa (Bowie et al. 2004; 2005), I hypothesised that there are genetic differences among members of the *A. milanjensis* complex and within *B. dimorpha* distributed across the Malawi Rift, with potential breaks occurring: 1) on the lowland gap between Zomba and Mulanje in southern Malawi , 2) in

the central highlands, splitting Malawi into two, and 3) across the lowland gap that separates Nyika Plateau from the Misuku Hills in northern Malawi (Figs. 3.1 and 3.2). In order to determine whether these phylogeographic breaks are present, I address the following questions: (i) Does genetic turnover between east and southern Africa lineages occur, and is it centred geographically in Malawi? (ii) If so are lineages endemic to Malawi, and what are their distributional limits within Malawi? (iii) Where are the common phylogeographic breaks in Malawi and how do they relate to the current placement of national parks?

3.3 MATERIALS AND METHODS

3.3.1 Population Sampling

Tissue samples were obtained from 112 *A. milanjensis* and 68 *B. dimorpha* individuals collected during research expeditions conducted in the northern, central and southern highlands of Malawi from 2001 to 2009 (see appendix G & H). In order to understand whether the faunal turnover of southern to east Africa lineages occurs within the Malawi Rift, populations of *A. milanjensis* from the Udzungwa Mountains in Tanzania and *B. capensis* from South Africa were also sampled. For *A. milanjensis* the Udzungwa Mountains correspond to *A. m. striifacies*; the Misuku Hills, Nyika Plateau, Viphya Plateau, Mount Ntchisi and Mount Zomba to *A. m. olivaceiceps*; and Mount Mulanje and Mount Namuli to *A. m. milanjensis* (Fig. 3.1). For *B. dimorpha*, the Nyika Plateau and Mount Ntchisi population corresponds to *B. d. sola*, and populations from Mount Zomba, Mount Mulanje and Mount Namuli correspond to *B. d. dimorpha*. For the sites I could not visit, I obtained preserved tissue from the Museum of Vertebrate Zoology, The Field Museum of Natural History, and the National Museum of Natural

History, Denmark. For the greenbuls *A. nigriceps* and *A. muasukuensis* were used as outgroups (Johansson et al. 2007). *Batis soror* and *B. molitor* was used as outgroups for the *B. capensis* species complex (includes *B. mixta*, *B. crypta* and *B. dimorpha*; Fjeldså et al. 2006).

3.3.2 Laboratory procedures

Total genomic DNA was extracted from 0.25 g or less of muscle tissue or blood using a DNeasy Kit (QIAGEN, Germantown, MD, USA) following the manufacturer's animal tissue protocol with an overnight proteinase K digestion at 55^{o}C. The entire 1041 base pair (bp) ND2 gene of the mitochondrial genome was amplified by polymerase chain reaction (PCR) with primers L5204 (5'-GCTAACAAAGCTATCGGGCCCAT-3') and H6312 (5'-CTTATTTAAGGCTTTGAAGGCC-3') (Cicero & Johnson 2001). Intron 15 of the gene CHDZ was amplified for *A. milanjensis* (481 bp) and for *B. dimorpha* (463 bp) using primers 15E (5' -TAGAGAGATTGAGAACTACAGT-3') and 16E (5'-GACATCCTGGCAGAGTATCT- 3') (Griffiths & Korn 1997). A 569 bp fragment for *A. milanjensis* and 572 bp fragment for *B. dimorpha* of the MUSK intron 13 was amplified using primers 13F (5'-CTTCCATGCACTACAATGGGAAA-3') and 13R (5'-CTCTGAACATTGTGGATCCTCAA-3') (Kimball et al. 2009). Double-stranded PCR-amplification was carried out in 25 µl reaction volumes containing: 2.5 µl 10 x buffer, 0.5 µl of 10 mM dNTPs, 0.5 µl of 10 mg/ml of bovine serum albumin, 0.75 µl of 50 mM $MgCl_2$, 1.25 µl of 10 µM of the forward and reverse primer in the presence of 0.25 µl of Taq polymerase (Perkin-Elmer) and genomic DNA.

ND2 was PCR-amplified with an initial denaturation step at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 7 minutes. For CHDZ initial denaturation was at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 7 minutes. For MUSK initial denaturation was at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 7 minutes. Standard precautionary measures included negative controls (template or DNA free PCR reactions) that were used to test for the presence of any contamination.

I electrophoresed 5 µl of the PCR product on a 1% agarose gel stained with ethidium bromide and observed it under ultraviolet light to check for the correct fragment size and to control for specificity of the PCR-amplification. The amplified DNA was purified by using either ExoSAP-IT (USB; Cleveland, OH, USA) or with GELase (Epicentre; Madison, WI), and cycle-sequenced using the ABI PRISM BigDye Terminator kit v.3.1 (Perkin-Elmer). Sephadex spin columns were utilised to clean-up the cycle-sequencing reactions (CureHunter, Oregon, USA). For each locus both the reverse and forward directions were sequenced, using the same primers as used in the PCR-amplification, on an ABI 3730 automated DNA Analyser (Applied Biosystems).

3.3.3 Determining the phase of alleles

In order to infer genealogies and to estimate demographic parameters from molecular DNA sequence data it is important to resolve the phase of the bi-allelic sequence data. Some introns had more than one polymorphic site (single nucleotide polymorphism [SNP]). A Bayesian method implemented in the programme PHASE v.2.1.1 (Stephens et al. 2001; Stephens & Donnelly 2003) was used to resolve the phase of all linked polymorphisms. A threshold of 0.75 (Harrigan et al. 2008) was used to satisfactory consider a SNP as phased; all SNPs that did not satisfy this threshold were removed from further analyses.

3.3.4 Data analysis

Sequence alignment

Sequences were obtained from both strands of DNA for each individual and some individuals were sequenced several times in the event of any base ambiguity encountered. Sequence alignment was performed by computation using MAFFT (Katoh et al. 2009) and checked by eye. For ND2, sequences were checked for insertions or deletions, as well as to ensure that stop-codons were not present.

Phylogenetic analyses

Parsimony and Maximum Likelihood analyses were performed. Parsimony analyses were conducted in PAUP*10b (Swofford 2002) using a heuristic search with TBR branch-swapping and 1000 random addition replicates. Parsimony bootstrap values were obtained from 1000 pseudoreplicates, with five random addition replicates being performed for each bootstrap pseudoreplicate.

Maximum Likelihood analyses were conducted using RAXML (Stamatakis 2006) on the ND2 dataset, partitioned by codon position, under a general-time-reversible model of nucleotide substitution and a gamma model of rate heterogeneity via the CIPRES portal (Stamatakis et al. 2008; Miller et al. 2009). One-thousand bootstrap pseudoreplicates were performed to evaluate support at specific nodes.

Haplotype network construction

Due to the problems that arise in the construction of intraspecific phylogenies (e.g. Posada & Crandall 2001) TCS v.1.01 was used to construct a statistical parsimony network of haplotypes (Clement et al. 2000) for each locus. The connection limit was set at 95%. The above analysis included only the in-group samples.

Analysis of molecular variance (*AMOVA*)

In order to determine molecular variation within and among populations as well as among larger geographical regions, several hierarchical analyses of molecular variance (AMOVA) were conducted. This enabled the determination of how genetic variability was partitioned within and among major lineages by using $\Phi_{ST,}$ which is an analogue of F_{ST} that incorporates both haplotype frequencies and the difference in number of nucleotides between each pair of haplotypes (Excoffier et al. 1992). The levels of significance for AMOVA were obtained by using a non-parametric permutation with 10000 iterations, and was carried out using ARLEQUIN v.3.0 (Excoffier et al. 2005).

Mismatch distributions and tests of selective neutrality

Pairwise mismatch distributions and two tests of selective neutrality, Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997) were calculated to determine if populations were in mutation drift equilibrium under an infinite sites model. These indices were used as

indicators of recent demographic change that could result from population expansion or contraction (Fu 1997; Rodgers & Harpending 1992). Both tests were conducted using ARLEQUIN v.3.0 (Excoffier et al. 2005).

Divergence times

I used the program BEAST v.1.7.1 (Drummond et al. 2006; Drummond & Rambaut 2007) to estimate divergence times for ND2 using the mean rate of divergence and associated standard deviation reported by Lerner et al. (2011) for ND2 ($2.9*10^{-2}$ [$2.3-3.3*10^{-2}$] Myrs BP). This rate is derived from the sequence of lineage splits in a passerine clade (Hawaiian Honeycreepers: Fringillidae), and calibrated using the well-established dates of sequential uplift of the Hawaiian Archipelago. In BEAST, a Yule process speciation prior and a clock-like model of rate variation were implemented, under an HKY-model of nucleotide substitution and a gamma model of rate heterogeneity. The MCMC analyses were conducted twice for 1×10^7 generations with parameters sampled every 5000 steps, and a 10% burn-in. TRACER v.1.5 (Rambaut & Drummond 2007) was used to determine the effective sample size of each parameter and to calculate the mean, and upper and lower bounds of the 95% highest posterior density interval (95% HPD) for divergence times. The two independent runs were combined using LOGCOMBINER and tree topologies were assessed using TreeANNOTATOR v.1.5.3 (Drummond & Rambaut 2007) and FIGTREE v.1.3.1 (Rambaut 2008).

3.4 RESULTS

3.4.1 Sequence variation

Mitochondrial DNA

The entire 1041 bp of the ND2 gene was obtained from 112 individuals of *Andropadus milanjensis*. Removal of identical sequences recovered 22 unique haplotypes (Table 3.1a). Of the 1041 characters, 690 (66.28%) were constant, 190 (18.25%) were variable but parsimony uninformative, and 161 (15.47%) were parsimony informative.

The entire 1041 bp of the ND2 gene was obtained from 68 individuals of *Batis dimorpha*. Removal of identical sequences recovered 22 unique haplotypes (Table 3.1a). Of the 1041 characters, 844 (81.08%) were constant, 40 (3.84%) were variable but parsimony uninformative, and 157 (15.08%) were parsimony informative.

Nuclear DNA

A final alignment of the entire 481 bp of the CHDZ intron 15 was obtained from 112 individuals of *Andropadus milanjensis*. Removal of identical sequences recovered 16 alleles (Table 3.1b). Of the 481 characters, 462 (96.05%) were constant, three (0.62%) were variable but parsimony uninformative, and 16 (3.33%) were parsimony informative. Final alignment of the entire 569 bp fragment of the MUSK intron 13 was obtained from 112 individuals of *Andropadus milanjensis*. Removal of identical sequences recovered 23 unique alleles. Of the 569 characters, 536 (94.2%) were constant, 11 (1.93%) were variable but parsimony uninformative, and 22 (3.87%) were parsimony informative.

A final alignment of the entire 463 bp fragment of the CHDZ intron 15 was obtained from 68 individuals of *Batis dimorpha*. Removal of identical sequences recovered 11 unique alleles. Of the 463 characters, 453 (97.84%) were constant and all 10 (2.16%) variable characters were parsimony informative. Final alignment of the entire 572 bp fragment of MUSK intron 13 was obtained from 68 individuals of *Batis dimorpha*. Removal of identical sequences recovered six unique alleles. Of the 572

characters, 567 (99.13%) were constant, one (0.17%), was variable but parsimony uninformative, and four (0.70%) were parsimony informative.

3.4.2 Phylogenetic analysis

Mitochondrial DNA

The Maximum Parsimony and Maximum Likelihood mitochondrial DNA analyses recovered trees that were nearly identical for both *A. milanjensis* and *B. dimorpha* (Figs. 3.3 & 3.4). For *A. milanjensis* six groups were recovered and represent from north to south: (1) the Udzungwa Mountains, (2) Misuku Hills, (3) Nyika and Viphya Plateaus, (4) Mount Ntchisi, (5) Mount Zomba, (6) Mount Mulanje and Mount Namuli. Individuals sampled from the Udzungwa Mountains correspond to *A. m. striifacies*. Birds from the Misuku Hills, Nyika and Viphya Plateaus, Mount Ntchisi and Mount Zomba correspond to *A. m. olivaceiceps.* Birds from Mount Mulanje and Mount Namuli correspond to *A. m. milanjensis*.

With respect to the *Batis capensis* complex, the South African samples were recovered as sister to the Malawi Rift taxa with the geographically more proximate Misuku population (*B. crypta*) sister to this clade. For *B. dimorpha* five clades were recovered and represent from north to south: (1) Nyika Plateau, (2) Mount Ntchisi, (3) Mount Zomba, (4) Mount Mulanje, and (5) Mount Namuli (Fig. 3.4). There was no sharing of haplotypes among the sampled populations (Table 3.1a). Nyika Plateau and Mount Ntchisi correspond to *B. d. sola*. Birds from Mount Zomba, Mount Mulanje and Mount Namuli correspond to *B. d. dimorpha*. All sampled taxa for *A. milanjensis* and *B. capensis*/*B. dimorpha* are reciprocally monophyletic for mitochondrial DNA.

Nuclear DNA

The Maximum Parsimony and Maximum Likelihood analyses of the CHDZ and MUSK intron alleles, for both *A. milanjensis* and *B. dimorpha*, formed a polytomy and hence are not presented here.

3.4.3 Haplotype network construction

Mitochondrial DNA

Construction of a haplotype network using 1041 bp of the ND2 gene of *A. milanjensis* indicated that subnetworks connected by 14 steps or fewer had a cumulative probability of greater than 95% of being correct. The TCS analysis recovered four subnetworks (Fig. 3.5) with individuals from Nyika, Viphya, Ntchisi, and Zomba grouping together; individuals from Mulanje and Namuli grouped together, but individuals from Udzungwa and Misuku formed subnetworks of their own. Although Mount Zomba comprised part of the same subnetwork as individuals sampled from Nyika-Viphya, these individuals were separated by two mutational steps. Nyika and Viphya shared haplotypes, whereas all samples from Ntchisi had the same haplotype, separated from Nyika-Viphya by one mutational step. Mount Mulanje and Mount Namuli also shared haplotypes. On average there was considerably haplotype diversity with sampled populations of *A. milanjensis*: Udzungwa (= 6), Misuku (= 5), Viphya (= 2), Zomba (= 3), Mulanje (= 4) and Namuli (= 3). In contrast birds from Ntchisi (n = 36) and Nyika (n = 3) all shared the same haplotype (Table 3.1a).

Construction of the haplotype network using 1041 bp of the ND2 gene for *B. dimorpha* indicated that subnetworks connected by 14 steps or fewer had a cumulative

probability of greater than 95% of being correct. The TCS analysis recovered three subnetworks (Fig. 3.6) with individuals recovered from Nyika, Ntchisi, Mulanje, Zomba and Namuli grouping together, whereas those from Misuku (*B. crypta*) and South Africa (*B. capensis*) formed subnetworks of their own. Individuals from Nyika grouped together and were separated from Ntchisi haplotypes by one mutational step. Nyika haplotypes were separated from Mulanje individuals by 13 mutational steps. Individuals from Mulanje grouped together and were separated from Zomba by six mutational steps, and from Namuli by four mutational steps (Fig. 3.6). There was generally high haplotype diversity in *B. dimorpha*: Nyika (= 6), Zomba (= 5), Mulanje (= 7), and Namuli (= 3), with the exception of birds from Ntchisi (n = 13), all of which shared the same haplotype (Table 3.1a).

Nuclear DNA

A TCS network for the CHDZ alleles sampled for *A. milanjensis* suggested that subnetworks connecting alleles by nine steps or fewer had a cumulative probability of greater than 95% of being correct. The degree of geographical structuring was reduced relative to that recovered by the ND2 dataset. Most alleles were connected to the common central allele that occurred in several populations: Misuku, Nyika, Viphya, Ntchisi and Zomba (allele 1, Fig. 3.7). Within *A. milanjensis,* Udzungwa (= 6), Misuku (= 7), Viphya (= 2), Ntchisi (= 2) and Namuli (= 3) had allelic variation, whereas Nyika, Zomba, and Mulanje were invariable (Table 3.1b).

A TCS network for the MUSK alleles sampled for *A. milanjensis* suggested that subnetworks connecting alleles by 10 steps or fewer had a cumulative probability of greater than 95% of being correct. Most alleles were connected to the common central

allele that was recovered in several populations: Misuku, Nyika, Viphya, Ntchisi and Mount Zomba (allele 1, Fig. 3.8). Within *A. milanjensis,* Udzungwa (= 10), Misuku (= 4), Nyika (= 2) Viphya (= 2), Ntchisi (= 3), Zomba (= 2), Mulanje (= 5) and Namuli (= 3) all had allelic variation (Table 3.1b).

A TCS network for the CHDZ alleles sampled for *B. dimorpha* suggested that subnetworks connecting alleles by nine steps or fewer had a cumulative probability of greater than 95% of being correct. The degree of geographical structuring was reduced relative to that recovered by the ND2 dataset. Most alleles were connected to the common central allele which occurred in several populations: Nyika, Ntchisi and Zomba (allele 1, Fig. 3.9). Within *B. dimorpha*: Nyika (= 3), Ntchisi (= 2), Zomba (= 3), Mulanje (= 4) and Namuli (= 3) all had allelic variation (Table 3.1b).

A TCS network for the MUSK alleles sampled for *Batis dimorpha* suggested that subnetworks connecting alleles by nine steps or fewer had a cumulative probability of greater than 95% of being correct. Most alleles were connected to the central allele that was recovered from the South African *B. capensis* (allele 1, Fig. 3.10). Within *B. dimorpha*: Nyika (= 2) and Namuli (= 2) had allelic variation, whereas for Ntchisi (= 1), Zomba (= 1) and Mulanje (= 1) only a single allele was recovered (Table 3.1b).

3.4.4 Analysis of molecular variance

Mitochondrial DNA

An AMOVA for *A. milanjensis* was conducted among the eight sampled populations (Table 3.1a). The genetic variation within groups was 0.39%, variation

within populations was 1.86%, and variation among groups was 97.75%. There is considerable population substructure as indicated by the high value of Φ_{ST} (0.981, $P < 0.0001$). For an AMOVA conducted with the seven populations with large sample sizes (Nyika and Viphya Plateaus were combined). Genetic variation within groups was 0.20%, variation within populations was 1.88%, and variation among groups was 97.92%. The same high Φ_{ST}-value (0.981, $P < 0.0001$) was recovered, which is reflected by the high pairwise Φ_{ST}-values, which are nearly all significant, with the exception of Zomba versus Namuli (Table 3.2a).

An AMOVA for *Batis dimorpha* was conducted among the five sampled populations (Table 3.1a). The genetic variation within groups was 14%, variation within populations was 6.7%, and variation among groups was 79.3%. There is considerable population geographical structure as indicated by the high value of Φ_{ST} (0.933, $P < 0.0001$). For an AMOVA conducted on four populations with large sample sizes (Namuli was removed, Table 3.1b) the genetic variation among groups was 79.82%, variation within groups was 13.69%, and variation within populations was 6.49%. The high Φ_{ST}-value (0.935, $P < 0.0001$) was reflected in all pairwise comparisons being significant (Table 3.3a).

Nuclear DNA

An AMOVA for CHDZ for *A. milanjensis* was conducted on all eight sampled populations (Table 3.1b). The genetic variation among groups was 49.38%, variation within groups was 24.89%, and variation within populations was 25.73%. Although population substructure was not as pronounced it remained highly significant (Φ_{ST} = 0.743, $P < 0.0001$). An AMOVA conducted on seven populations with sufficient samples

(Nyika and Viphya Plateaus combined) the genetic variation among groups was 49.38%, variation within groups was 24.87%, and variation within populations was 25.75%, with a Φ_{ST}-value that was significant ($\Phi_{ST} = 0.742$, $P < 0.0001$).

An AMOVA for MUSK for *Andropadus milanjensis* was conducted on all eight sampled populations (Table 3.1b). The genetic variation among groups was 70.71%, variation within groups was 6.44%, and variation within populations was 22.85%. Although population substructure was not as pronounced it remained highly significant ($\Phi_{ST} = 0.771$, $P < 0.0001$). An AMOVA conducted on seven populations with sufficient samples (Nyika Plateau and Viphya Plateau combined, Table 3.1b) the genetic variation among groups was 69.76%, variation within groups was 6.24% and variation within populations was 24%, with a Φ_{ST}-value that was significant ($\Phi_{ST} = 0.760$, $P < 0.0001$).

An AMOVA for CHDZ for *Batis dimorpha* was conducted on the five sampled populations (Table 3.1b). The genetic variation among groups was 0% (-40.11), variation within groups was 93.44% and variation within populations was 46.67%. Although population substructure was not as pronounced it remained highly significant ($\Phi_{ST} = 0.533$, $P < 0.0001$). An AMOVA conducted on three populations with sufficient samples (Mount Zomba and Mount Namuli removed) the genetic variation among groups was 24.70%, variation within groups 28.74% and variation within populations was 46.56%, with a Φ_{ST}-value that was significant ($\Phi_{ST} = 0.534$, $P < 0.0001$).

An AMOVA for MUSK for *B. dimorpha* was conducted on the five sampled populations (Table 3.1b). The genetic variation among groups was 80.07%, variation within groups was 16.94%, and variation within populations was 2.99%. Although

population substructure was not as pronounced it remained highly significant (Φ_{ST} = 0.970, P < 0.0001).

3.4.5 Mismatch distributions and tests of selective neutrality

Mismatch profiles that follow a modified Poisson distribution (a unimodal bell shaped curve when population expansion is recent), are thought to be associated with past events of population growth, for instance range expansion (Rogers & Harpending 1992; Harpending et al. 1993). In the four *A. milanjensis* mismatch distribution profiles (Fig. 3.11) constructed for populations with adequate sample sizes (Udzungwa, Misuku, Zomba, Mulanje and Namuli) and sufficient variation (Ntchisi and Nyika had one haplotype, and Viphya had three individuals) all followed a Poisson distribution, with Misuku and Zomba being significantly skewed to the left, a result indicative of more recent population expansion. Tajima's D and Fu's Fs statistics for Udzungwa, Mulanje and Namuli were not significant, indicating that if these populations have undergone a range expansion, it occurred a long time ago. The populations of Misuku and Zomba both had significant negative Tajima's D and Fu's Fs values (Table 3.4), consistent with a more recent change in population size.

In the three *B. dimorpha* mismatch distribution profiles (Fig. 3.12) constructed for populations with adequate sample sizes (Nyika, Zomba and Mulanje) and sufficient variation (Ntchisi had one haplotype and Namuli had four individuals), all three populations followed a Poisson distribution. Tajima's D and Fu's Fs statistics for Nyika, Zomba and Mulanje were not significant, indicating that if these populations have undergone a range expansion, it occurred a long time ago (Table 3.4).

3.4.6 Divergence times

From the dating results (Fig. 3.13), I can infer that the Mulanje and Namuli populations of *A. milanjensis* diverged from the remaining East African taxa at about 1.2 Myrs BP. The Eastern Arc Mountain population sampled from the Udzungwa Mountains split from the remaining Malawi Rift populations at about 0.89 Myrs BP, followed by the Misuku Hills becoming isolated from the central Malawi Rift at about 0.48 Myrs BP. Mount Zomba split from the central and northern highland populations at about 0.10 Myrs BP.

From the dating results (Fig. 3.14), it can be inferred that the Misuku Hills population comprising *B. crypta* diverged from its sister and more southerly distributed taxa (*B. capensis* and *B. dimorpha*) at about 4.14 Myrs BP. The South African *B. capensis* split from the Malawi Rift *B. dimorpha* at about 0.53 Myrs BP, followed by the three southern Malawi Rift populations becoming isolated from the northern and central populations at about 0.34 Myrs BP. The population on Mount Zomba split from the southern Malawi Rift populations at about 0.19 Myrs BP, with the Mount Mulanje population becoming isolated from nearby Mount Namuli at about 0.14 Myrs BP.

3.5 DISCUSSION

Andropadus milanjensis

The mtDNA phylogenetic analyses recovered six well supported clades comprising from north to south: the Udzungwa Mountains, Misuku Hills, Nyika and Viphya Plateaus, Mount Ntchisi, Mount Zomba, Mount Mulanje and Mount Namuli (Fig. 3.3). The Udzungwa Mountains corresponds to *A. m. striifacies*; the Misuku Hills, Nyika

Plateau, Viphya Plateau, Mount Ntchisi and Mount Zomba to *A. m. olivaceiceps*; and Mount Mulanje and Mount Namuli to *A. m. milanjensis*.

The mtDNA ND2 network analyses resulted in four distinct subnetworks that comprised birds from the Udzungwa Mountains, Misuku Hills, Nyika-Viphya-Misuku-Zomba, and Mulanje-Namuli, respectively (Fig. 3.5). The sharing of haplotypes between Nyika and Viphya Plateaus, as well as Mount Mulanje and Mount Namuli, may not be due to recurrent gene flow but incomplete lineage sorting. All 36 individuals sampled from Mount Ntchisi had the same haplotype, suggestive of a bottleneck that likely occurred in the population's recent past, or the consequence of a recent founder event (see e.g. Clegg et al. 2002).

The use of nuclear markers (CHDZ and MUSK) recovered some phylogeographical structuring, as the Φ_{ST}-values were significant and in general agreement with the mtDNA dataset (Table 3.2a and 3.2b). However, this phylogeographic structuring was not well displayed by the network (Figs. 3.7 & 3.8), as most of the alleles were connected to the central and most common allele revealing that the differences in the frequency distribution of alleles is driving the high Φ_{ST}-value.

The mismatch distribution for *A. milanjensis* from the Misuku Hills and Mount Zomba (Fig. 3.11), and Tajima's D and Fu's Fs tests suggest that these populations have recently experienced population growth. Zomba has higher diversity than Ntchisi therefore the direction of expansion is towards the central Malawi Rift where Ntchisi is located. Misuku is made up of three hills: Matipa, Mughese and Wilindi. It is possible that populations of forest birds in the Misuku Hills are expanding, but further

investigation is warranted because Mughese it is prone to anthropogenic disturbance and could be acting as a sink.

Batis dimorpha

The mtDNA phylogenetic analyses recovered two well-supported clades one comprising the predominantly Eastern Arc distributed *Batis crypta* and the other comprising the southern African clade *Batis capensis*, which is the sister-clade to the Malawi Rift *Batis dimorpha* (Fig.3.2). Five well-supported clades were recovered across the Malawi Rift and represent from north to south: Nyika Plateau, Mount Ntchisi, Mount Zomba, Mount Mulanje and Mount Namuli.

The Nyika Plateau and Mount Ntchisi populations correspond to *B. d. sola*, whereas the Mount Zomba, Mount Mulanje and Mount Namuli populations correspond to *B. d. dimorpha*. Thus, the central highlands of Malawi are inhabited by *B. d. sola*, which has a very restricted distribution. Mount Zomba is closer to Mount Mulanje and Mount Namuli suggesting that Mount Zomba is the most northern extent for *B. d. dimorpha,* a result contrary to earlier surveys that indicated that *B. d. dimorpha* extended onto the central highlands of Malawi (Newman et al. 1992; Erard & Fry 1997). As indicated above the recovered topology suggests that these taxa may be reciprocally monophyletic.

The mtDNA network analyses of the *B. dimorpha* populations recovered one subnetwork that comprises from north to south: Nyika-Ntchisi-Zomba-Mulanje-Namuli (Fig.3.6). There was substantial geographical structuring among all the sampled populations, as revealed by lack of sharing of haplotypes suggesting that there is no gene flow (Table 3.1a). Interestingly, in *A. milanjensis*, Ntchisi was also genetically deporpriate, suggesting that Ntchisi could be a sink population that exposes the birds to

various bottlenecks in response to varying environmental conditions (Zink & Dittmann 1993), or that the forest has recently become established and only recently been colonised (i.e. founder events).

Further, clear links in the subnetworks between the two subspecies (*B. d. dimorpha* and *B. d. sola*) suggests that they have diverged in allopatry from one common ancestor. This is further supported in the phylogenetic analysis (Fig. 3.4) with each subspecies of the Malawi Batis being reciprocally monophyletic. The congruence between morphologically defined subspecies and phylogeographic structure in the molecular data contrast with many avian species in which subspecies classification does not reflect evolutionary history (Zink 2004).

The use of nuclear marker (CHDZ and MUSK) in *Batis dimorpha* revealed some geographical structuring, as the Φ_{ST}-value was significant, thereby supporting the mtDNA results (Tables 3.3a & 3.3b). However, this structuring was not clearly depicted by the networks (Figs. 3.9 & 3.10) as most of the alleles were connected to the central allele.

The mismatch distribution for *B. dimorpha* for all the sampled populations (Fig. 3.12), Tajima's D and Fu's Fs tests were not significant indicating that demographic changes in the population occurred a long time ago, and that these populations (with the possible exception of Ntchisi that may be acting as a sink - see above) are probably stable supporting the phylogenetic results that most populations comprise distinct evolutionary units.

Faunal turnover and phylogeography of Andropadus milanjensis and Batis dimorpha across the Malawi Rift

The mtDNA analyses clearly suggest that *A. m. striifacies*, *A. m. olivaceiceps* and *A. m. milanjensis* are reciprocally monophyletic, and likely warrant recognition as distinct species, as suggested in recent field guides (e.g. Sinclair & Ryan 2003). Within *A. olivaceiceps* there is sharing of haplotypes between Viphya and Nyika, both northern highland plateaus. This may not be a consequence of gene flow but ancestral polymorphism, considering that the divergence of both populations is recent (Fig. 3.13). Larger sample sizes are required to test the phylogeographic structure between these populations. Although the populations of *A. milanjensis* on Mount Mulanje and Mount Namuli share haplotypes recurrent gene flow may not be taking place because there is substantial geographical structure as revealed by the high pairwise Φ_{ST}-value. The mismatch distributions for both Mulanje and Namuli, and the Tajima's D and Fu's Fs were not significant indicating the populations are in equilibrium, hence the sharing of haplotypes is possibly due to ancestral polymorphism.

Both *A. striifacies* and *A. olivaceiceps* are reciprocally monophyletic for mtDNA revealing a phylogeographical break between the Udzungwa Mountains and Misuku Hills (Fig. 3.15). This break is not surprising because this is a unique region, located at the boundary of two major hydrobasins. A similar positioned break has also been recovered in other highland species (e.g. *Otomys dentilacustris*, Taylor et al. 2009; *Praomys delectorum*, Carleton & Stanley 2012; Bryja et al. 2014; *Hyperolius substriatus*, Lawson 2013). Within *A. olivaceiceps*, the Misuku Hills, Nyika-Viphya Plateaus, Mount Ntchisi, and Mount Zomba comprise distinct populations (Figs. 3.3 & 3.5), thereby revealing a

weaker geographical break to gene flow between the Misuku Hills and Nyika-Viphya-Ntchisi-Zomba, as the southern most extent. *Andropadus milanjensis* is distinct and Mount Mulanje is the northern most extent of this taxon, thereby revealing a break between Mount Zomba and Mount Mulanje, The significant Φ_{ST}-values suggest a more recent and weaker break between Mount Mulanje and Mount Namuli (Fig. 3.15).

In *Batis dimorpha* the Misuku Hills were clearly separated and inhabited by the Eastern Arc species *B. crypta*. The Nyika Plateau and Mount Ntchisi populations comprise birds of the same subspecies, but there was no sharing of haplotypes, suggesting that gene flow may be limited between the two populations of *B. d. sola*. However, this distribution range needs further investigation as this subspecies was not previously thought to occur in Ntchisi, but rather to be restricted to 'northern' Malawi (Newman et al. 1992; Erard & Fry 1997). Birds from Mount Zomba, Mount Mulanje and Mount Namuli did not share haplotypes; despite the distances being relatively small among these highlands (~ 75 km between Zomba and Mulanje) suggesting that the southern Malawi Rift montane forests exhibit high levels of population structure (see also Lawson 2013; Bryja et al. 2014).

The Mount Zomba, Mount Mulanje and Mount Namuli populations (*B. d. dimorpha*) are distinct revealing a geographical break between Mount Zomba and Mount Mulanje, and between Mount Mulanje and Mount Namuli (Fig. 3.16). There is a potential break between Nyika and Ntchisi as mismatch distribution (Fig. 3.12) and Tajima D and Fu's Fs (Table 3.4) are not significant for Nyika, suggesting that this population is stable, and given the complete lack of genetic diversity in Ntchisi (Tables 3.1a & 3.4), Nyika may well have acted as a source population at some point in the recent past.

In summary, the distribution of members of the *A. milanjensis* species complex exhibit phylogeographic breaks within the Malawi Rift, namely along the lowland gap between the Udzungwa Mountains and Misuku Hills; between the Misuku Hills and Nyika Plateau; between Mount Zomba and Mount Mulanje.(Fig. 3.15). *Batis dimorpha* phylogeographic breaks occur along the lowland gap between the Misuku Hills and Nyika Plateau; between Nyika Plateau and Mount Ntchisi; between Mount Ntchisi and Mount Zomba; and weaker breaks between Mount Zomba and Mount Mulanje and between Mount Mulanje and Mount Namuli (Fig. 3.16). This is in accordance with the expectation of Malawi being a transition zone (faunal turnover) between east and southern Africa lineages.

The gazetted national parks across the Malawi Rift are located on each side of the identified geographical breaks. Nyika National Park, which is the largest park, situated in northern Malawi is strategically located to encompass important evolutionary processes north of the central phylogeographic break. This park comprises varied habitats that encompass both montane as well as woodland biomes (Cater et al. 1993). Most of the parks in southern and central Malawi are established in the lowlands and because these areas are generally dry *Brachystegia* woodland, they do not encompass other important biomes such as the montane forests that are known to promote the accumulation of recently diverged species (Roy 1997; Fjeldså et al. 2012; this study).

The proclamation of most national parks in Malawi has largely been the consequence of an ad hoc approach that has not typically followed modern conservation planning strategies (Margules & Pressey 2000). Therefore, the existing protected areas fail to include all species or evolutionary lineages which effective conservation planning

seeks to achieve (Pressey 1994). Most of the places where the phylogeographic breaks occur are forest reserves that are not adequately protected with the exception of Nyika National Park where *A. olivaceiceps* and *B. d. sola* occur. Deforestation and uncontrolled fires are threatening the montane populations, for example, *A. milanjensis* on Mount Mulanje (Mzumara et al. 2012; Bryja et al. 2014), thereby compromising the conservation of the birds and important evolutionary units in general.

The results of this study suggest that molecular DNA data has an important role to play in helping identify phylogeographic breaks within *Andropadus milanjensis* and *Batis dimorpha*. Looking at the distribution of taxa within the Malawi Rift, important habitats for the conservation of evolutionary units are identified which will contribute to modern conservation planning in Malawi. Therefore, *A. olivaceiceps* (Misuku Hills, Nyika Plateau, Mount Ntchisi and Mount Zomba), *A. milanjensis* (Mount Mulanje and Mount Namuli), *B. d. sola* (Nyika Plateau and Mount Ntchisi) and *B. d. dimorpha* (Mount Zomba, Mount Mulanje and Mount Namuli) should be managed separately as they comprise discrete evolutionary lineages that with further investigation may warrant recognition as distinct species.

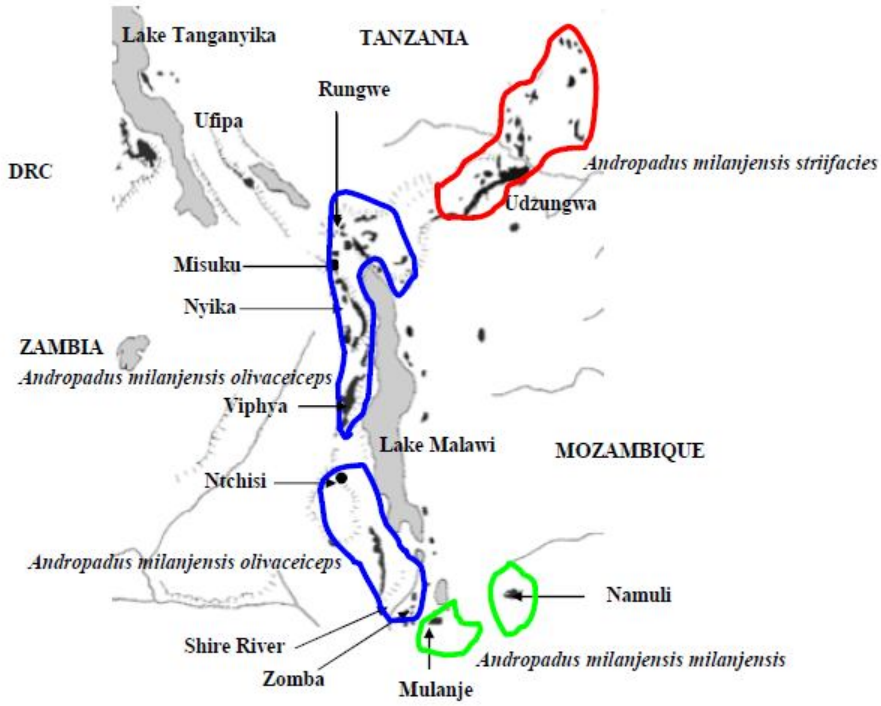


Figure 3.1 The Malawi Rift depicting the distribution of *Andropadus milanjensis* subspecies and sampling sites.

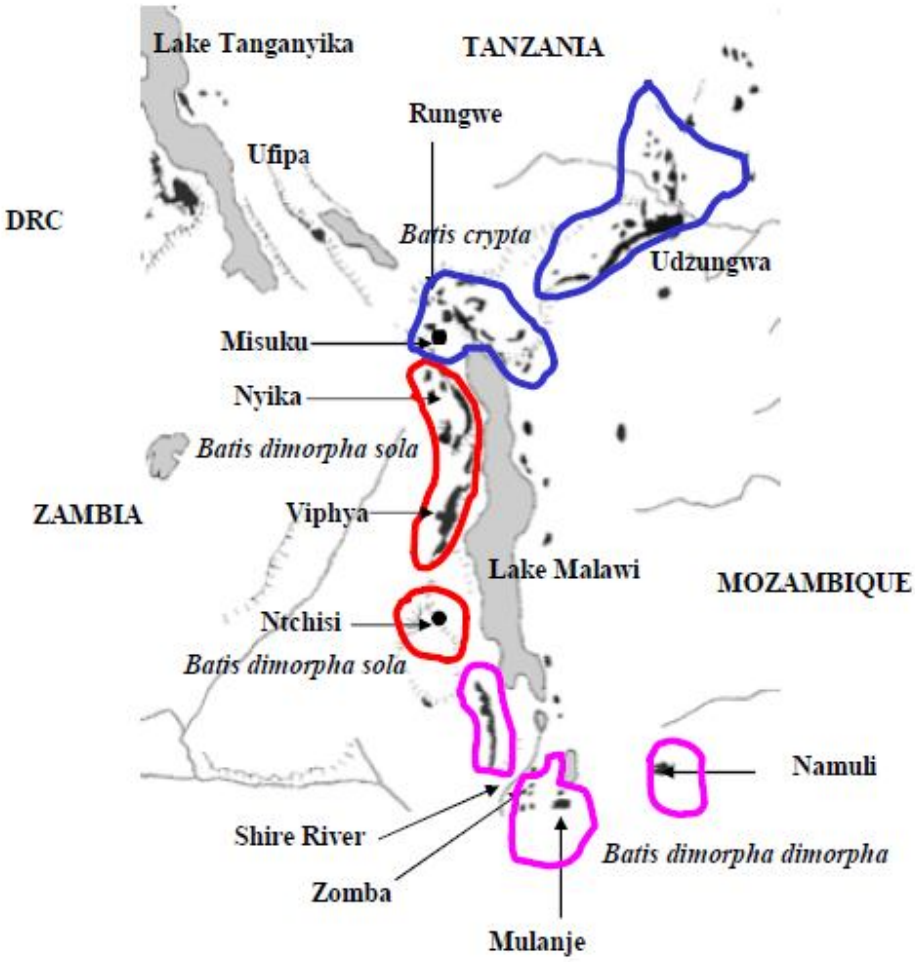


Figure 3.2 The Malawi Rift depicting the distribution of *Batis dimorpha* subspecies and sampling sites.

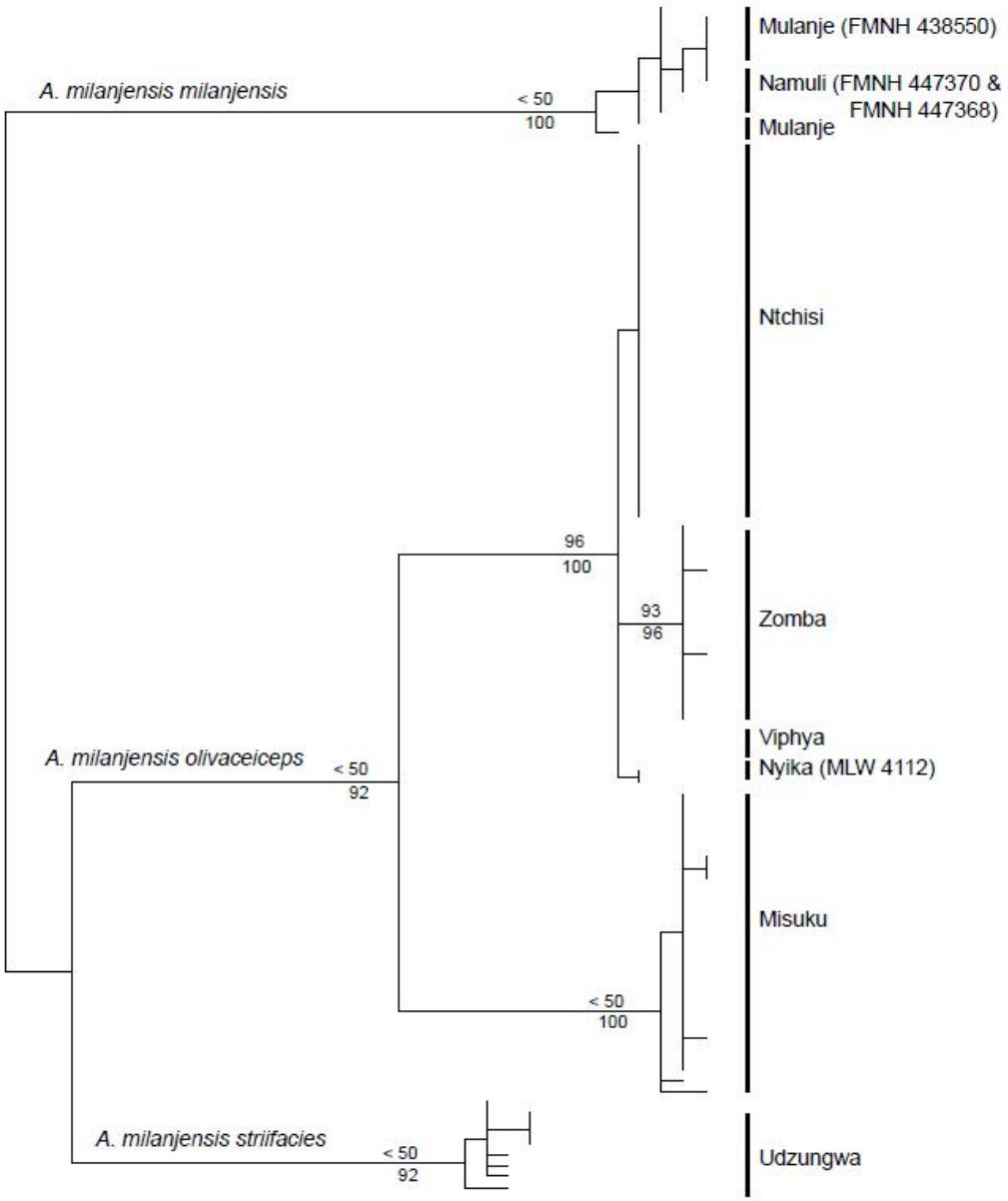


Figure 3.3 A Maximum Parsimony topology depicting phylogenetic relationships among taxa in the *Andropadus milanjensis* species complex occurring across the Malawi Rift, as well as representative taxa distributed to the north and south. Values above the nodes are ML bootstrap support values and those below are MP bootstrap support values. Individuals from a different haplotype clade are indicated by their museum voucher number – see Appendices G and H.

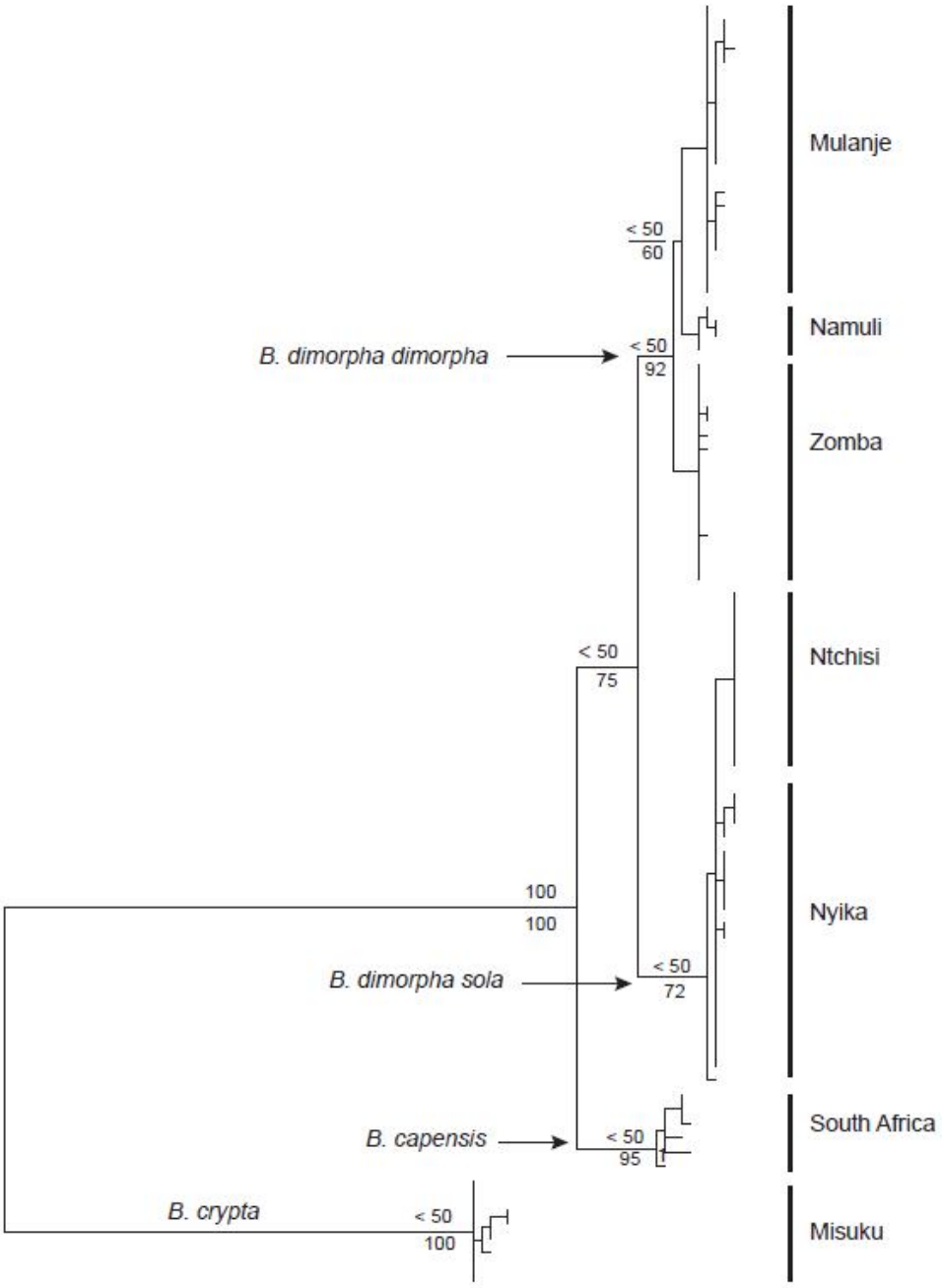


Figure 3.4 A Maximum Parsimony topology depicting phylogenetic relationships among taxa in the *Batis capensis* species complex occurring across the Malawi Rift, as well as representative taxa distributed to the north and south. Values above the nodes are ML bootstrap support values and those below are MP bootstrap support values.

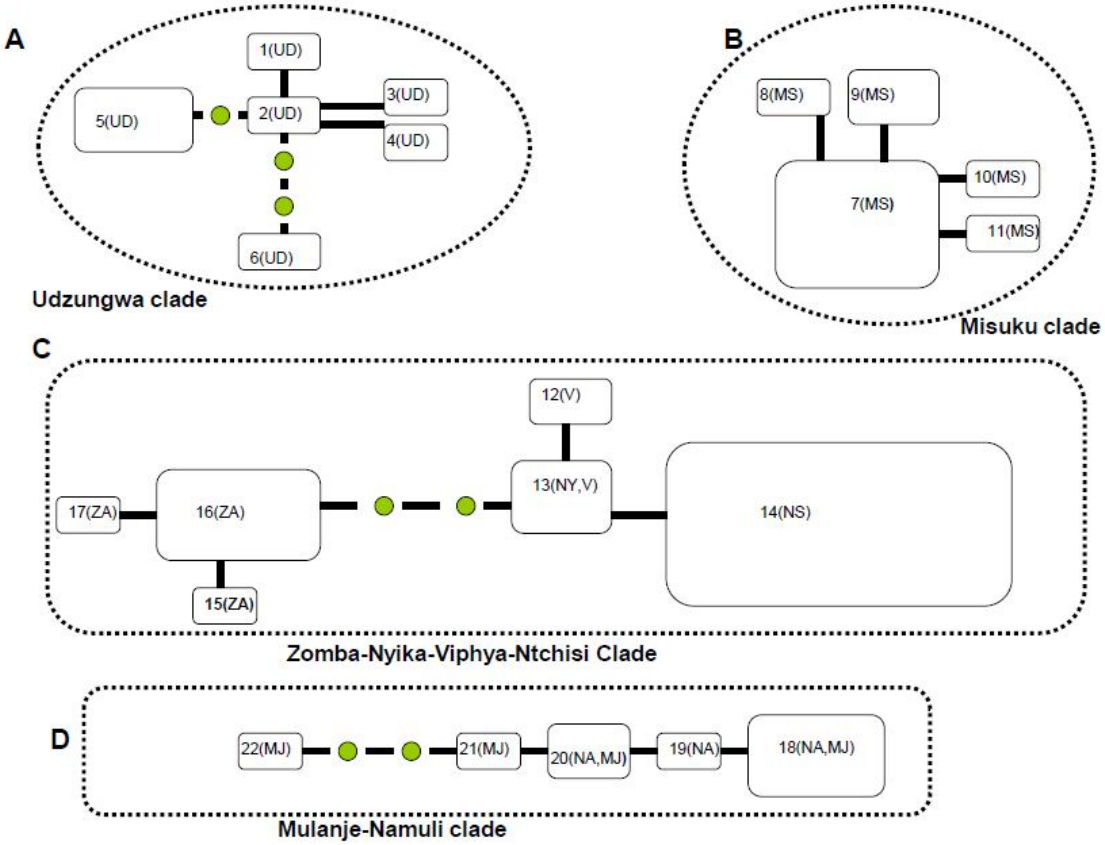


Figure 3.5 Statistical parsimony network obtained with ND2 for the 22 haplotypes of *Andropadus milanjensis* (Table 3.1a). Unconnected networks exceed the 95% confidence limit of 14 steps. Dots indicate unsampled or extinct haplotypes. The size of each box is proportional to the frequency of the haplotype. Haplotype codes correspond to the population of origin: NS = Ntchisi, MS = Misuku, ZA = Zomba, NA = Namuli, MJ = Mulanje, NY = Nyika, V = Viphya and UD = Udzungwa.

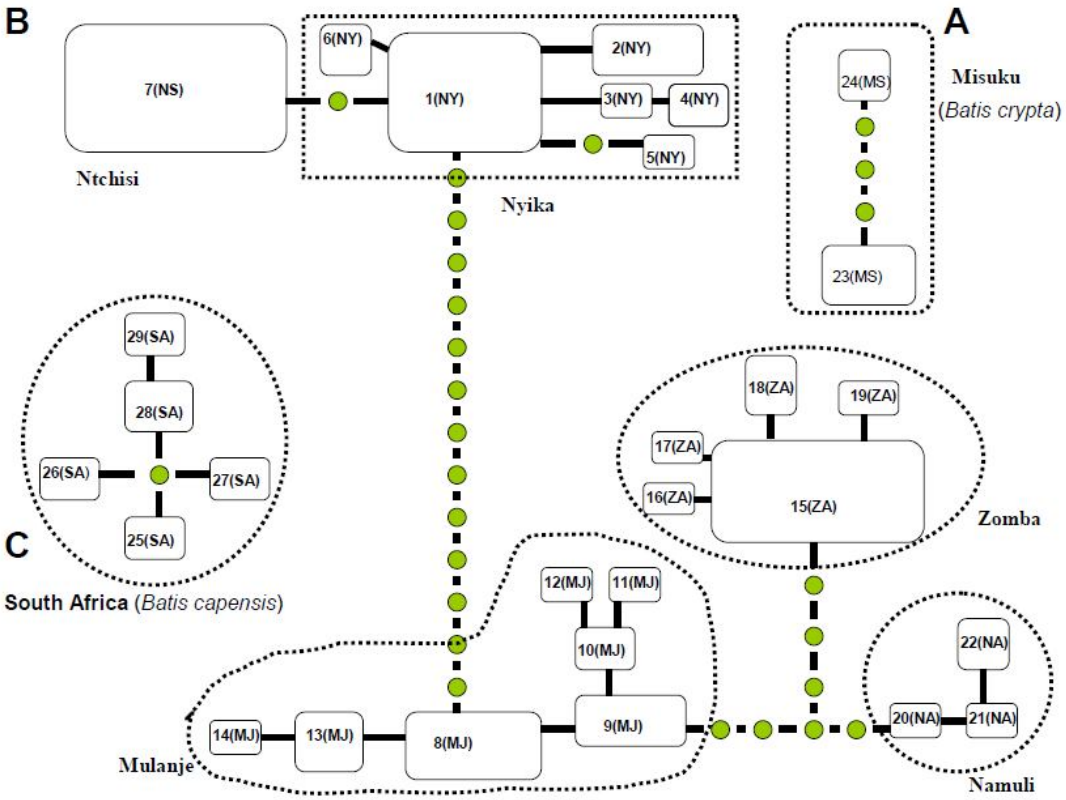


Figure 3.6 Statistical parsimony network obtained with ND2 for the 22 haplotypes of *Batis* (Table 3.1a). Unconnected networks exceed the 95% confidence limit of 14 steps. Dots indicate unsampled or extinct haplotypes. The size of each box is proportional to the frequency of the haplotype. Haplotype codes correspond to the population of origin: NY = Nyika, NS = Ntchisi, ZA = Zomba, MJ = Mulanje and NA = Namuli.

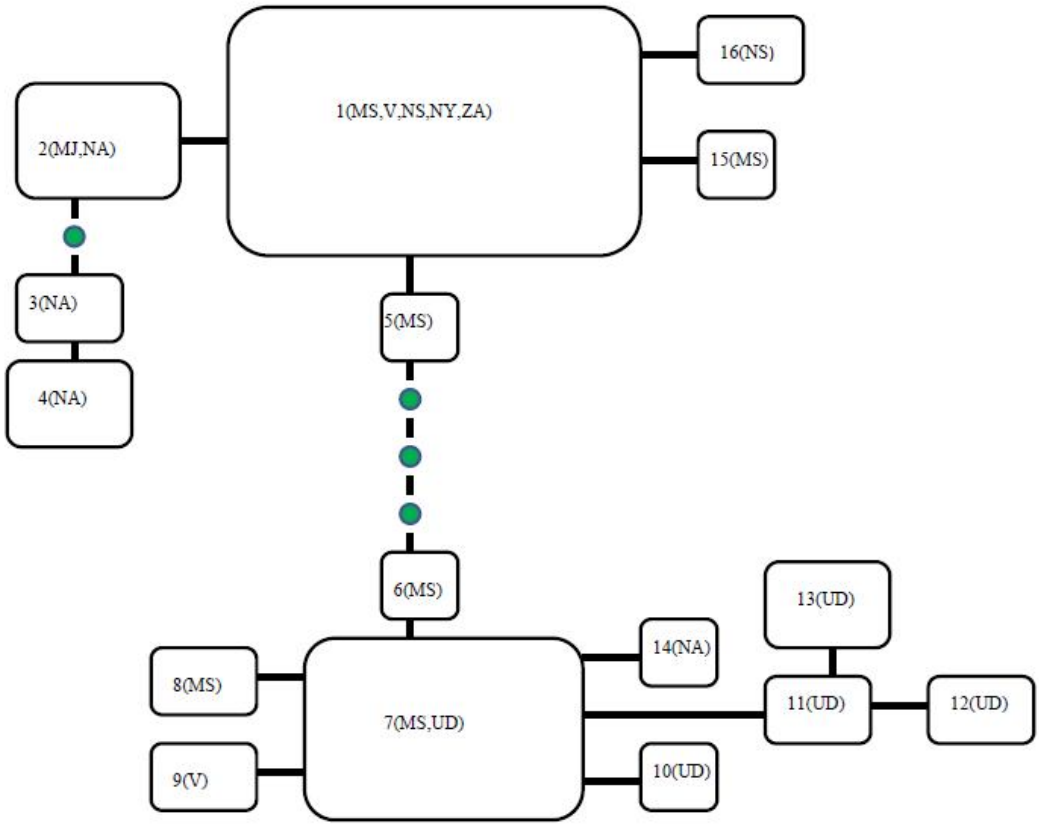


Figure 3.7 Statistical parsimony network obtained with CHDZ for the 16 alleles of *Andropadus milanjensis* (Table 3.1b). The subnetworks are connected to each other having not exceeded the 95% confidence limit of nine steps. Dots indicate unsampled or extinct alleles. The size of each box is proportional to the frequency of the allele. Allele codes correspond to the population of origin: UD = Udzungwa, MS = Misuku, NY = Nyika, V = Viphya, NS = Ntchisi, ZA = Zomba, MJ = Mulanje and NA = Namuli.

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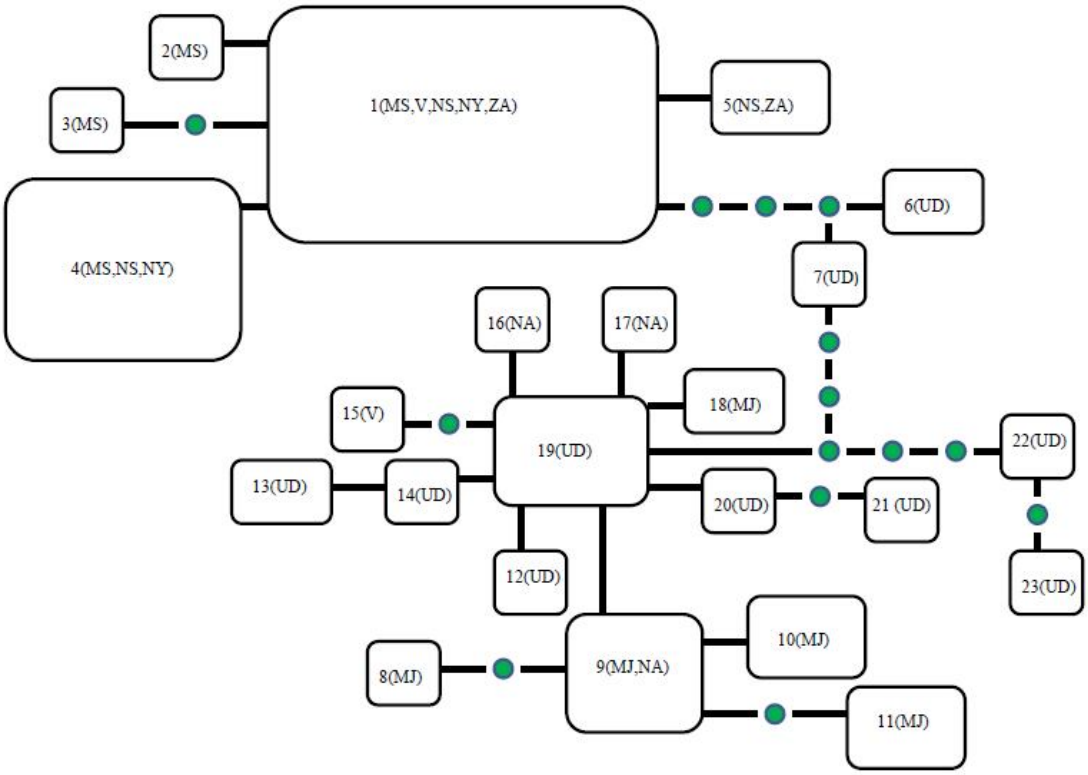


Figure 3.8 Statistical parsimony network obtained with MUSK for the 23 alleles of *Andropadus milanjensis* (Table 3.1b). The subnetworks are connected to each other having not exceeded the 95% confidence limit of 10 steps. Dots indicate unsampled or extinct alleles. The size of each box is proportional to the frequency of the allele. Allele codes correspond to the population of origin: UD = Udzungwa, MS = Misuku, NY = Nyika, V = Viphya, NS = Ntchisi, ZA = Zomba, MJ = Mulanje and NA = Namuli.

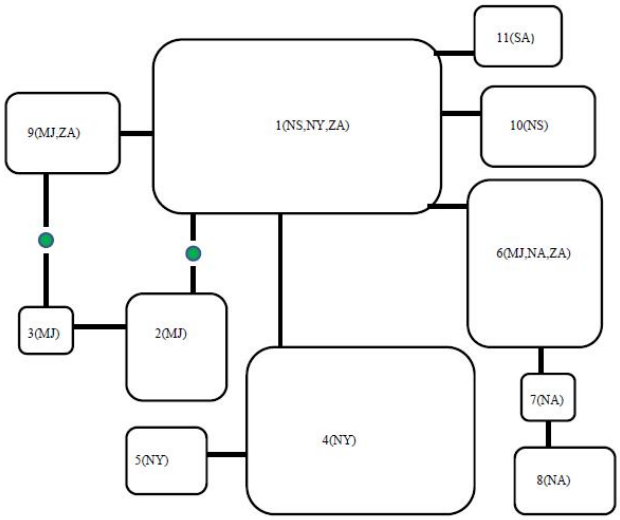


Figure 3.9 Statistical parsimony network obtained with CHDZ for the 11 alleles of *Batis dimorpha* (Table 3.1b). The subnetworks are connected to each other after satisfying the 95% confidence limit of nine steps. Dots indicate unsampled or extinct alleles. The size of each box is proportional to the frequency of the allele. Allele codes correspond to the population of origin: NY = Nyika, NS = Ntchisi, ZA = Zomba, MJ = Mulanje, NA = Namuli, and SA = South Africa.

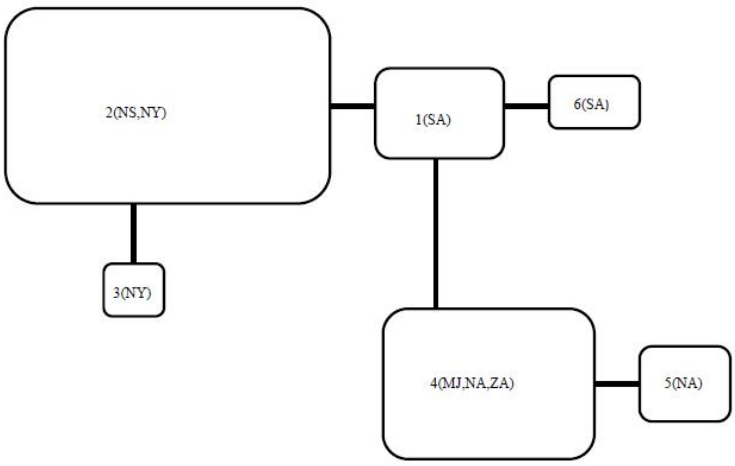
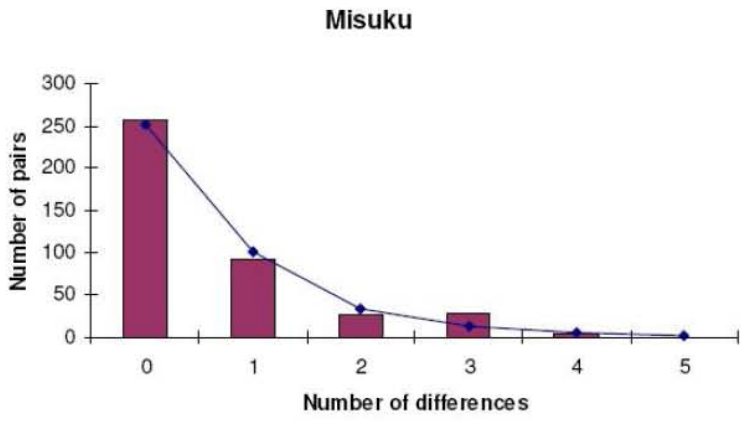
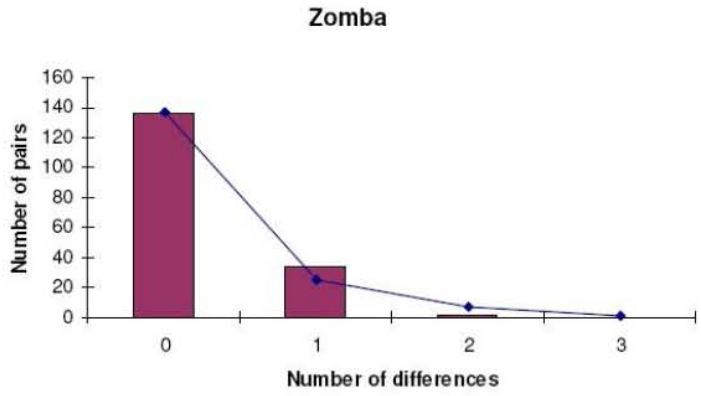
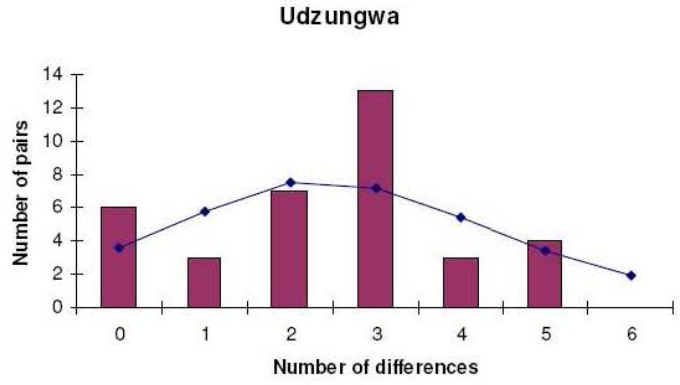


Figure 3.10 Statistical parsimony network obtained with MUSK for the six alleles of *Batis dimorpha* (Table 3.1b). The subnetworks are connected to each other after satisfying the 95% confidence limit of nine steps. Dots indicate unsampled or extinct alleles. The size of each box is proportional to the frequency of the allele. Allele codes correspond to the population of origin: NY = Nyika, NS = Ntchisi, ZA = Zomba, MJ = Mulanje, NA = Namuli, and SA = South Africa


Misuku
Number of pairs
300
250
200
150
100
50
0
0
1
2
3
4
5
Number of differences


Zomba
Number of pairs
160
140
120
100
80
60
40
20
0
0
1
2
3
Number of differences


Udzungwa
Number of pairs
14
12
10
8
6
4
2
0
0
1
2
3
4
5
6
Number of differences

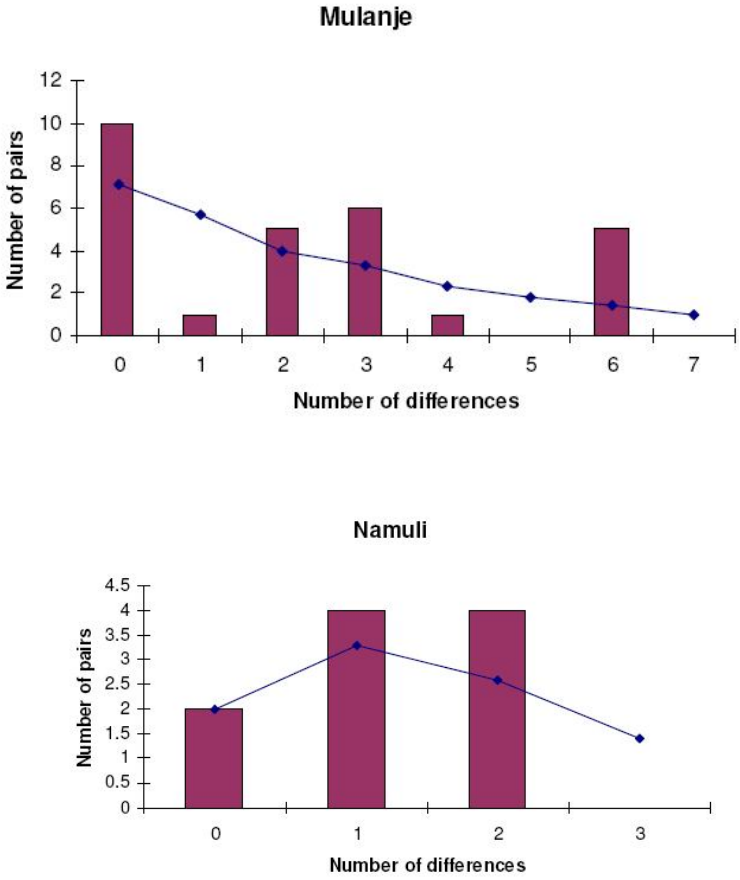


Figure 3.11 Mismatch distributions for selected *Andropadus milanjensis* populations. Histograms represent the observed distribution and the line the expected distribution for a growing population under the same mean.

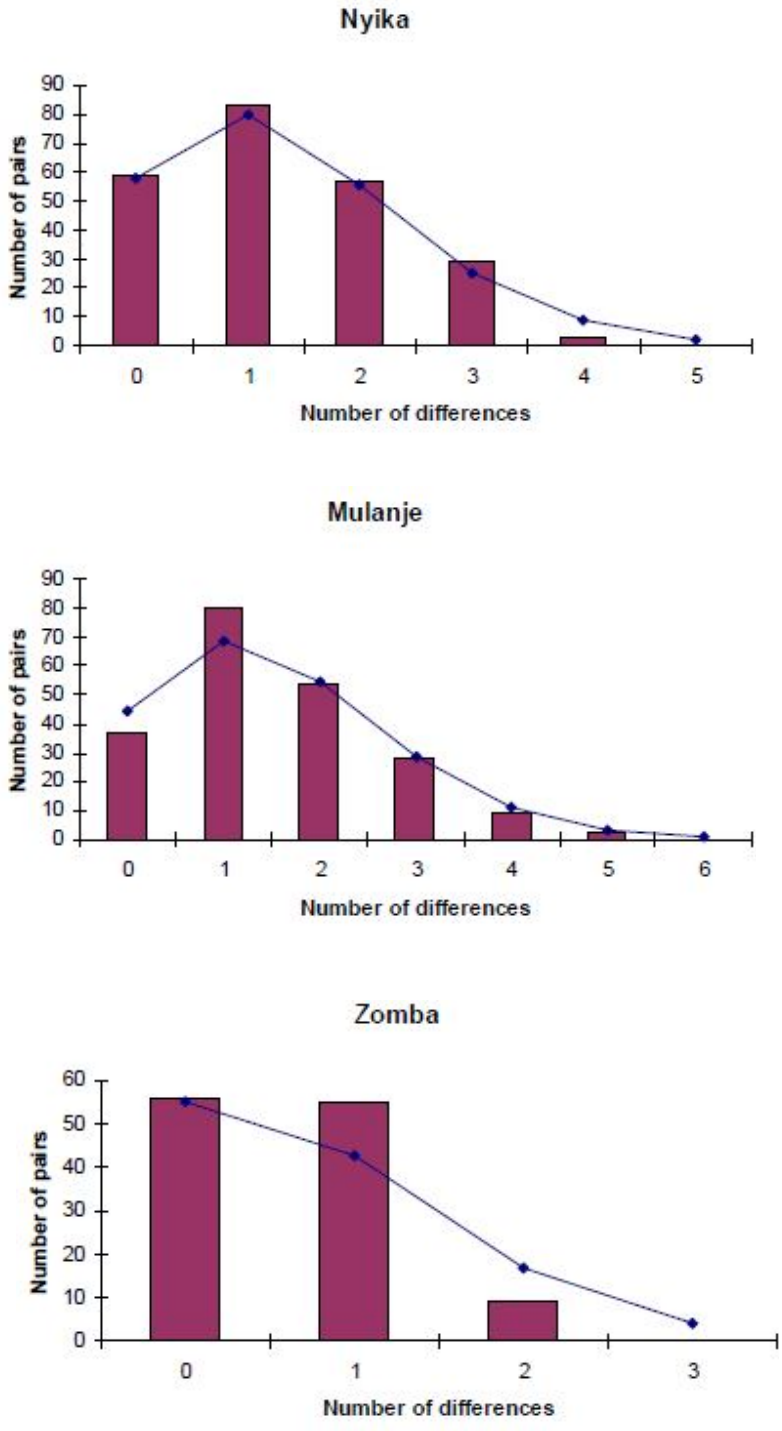


Figure 3.12 Mismatch distributions for selected *Batis dimorpha* populations. Histograms represent the observed distribution and the line the expected distribution for a growing population under the same mean.

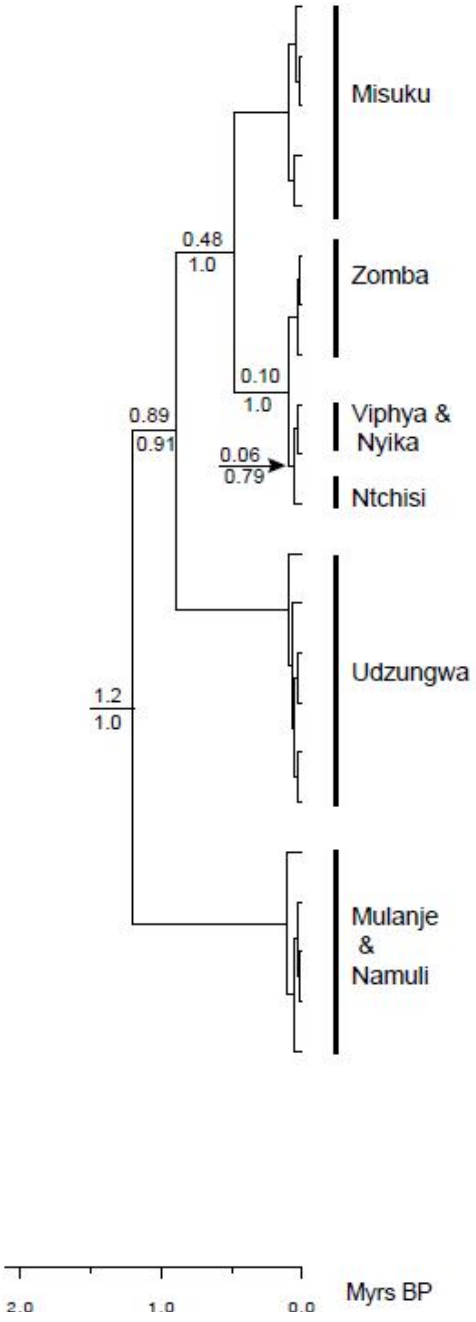


Figure 3.13 Divergence times among populations of *Andropadus milanjensis* in millions of years before present (Myrs BP). Values above the nodes are divergence times and below are BEAST posterior probability support values.

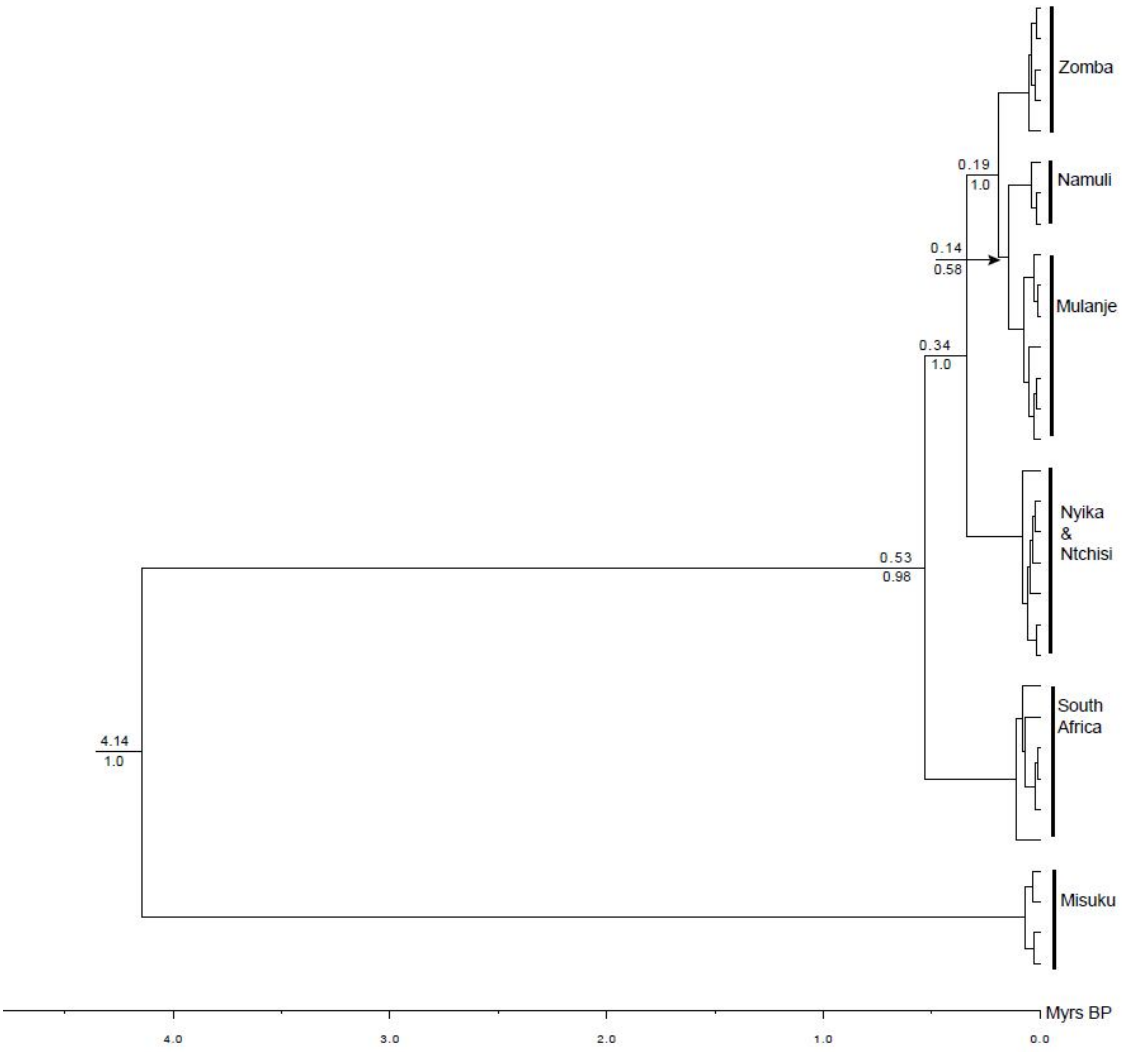


Figure 3.14 Divergence times among populations of *Batis dimorpha* in millions of years before present (Myrs BP). Values above the nodes are divergence times and below are BEAST posterior probability support values.

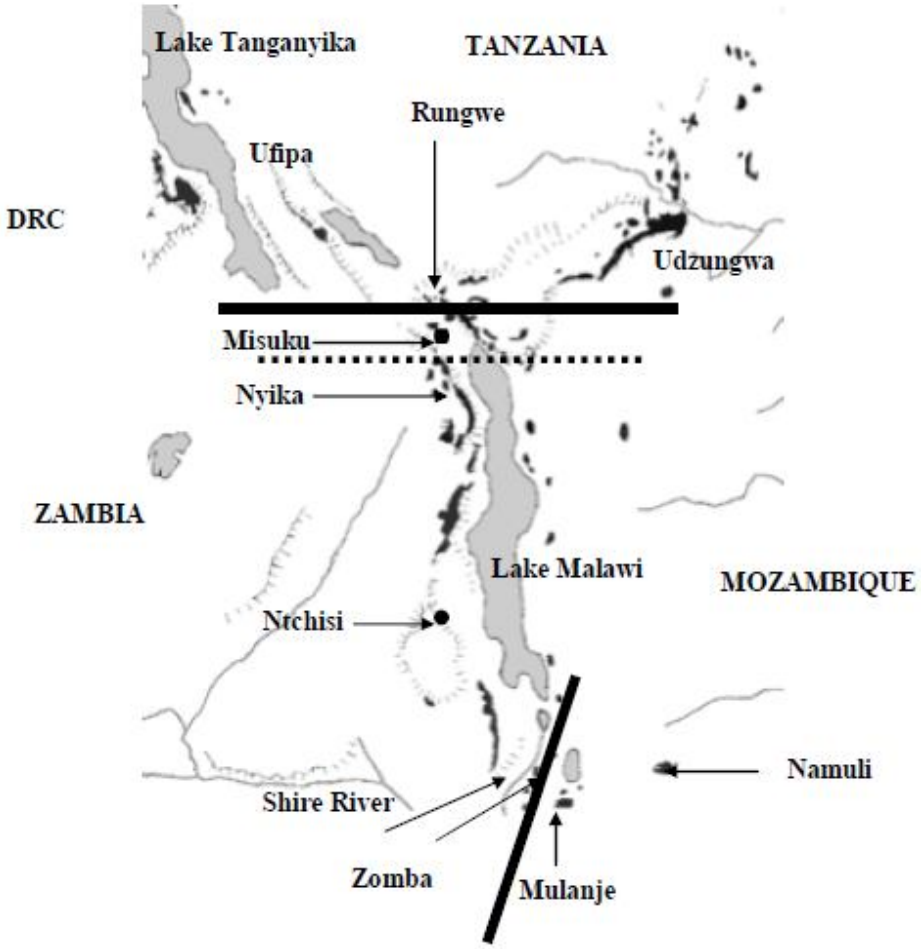


Figure 3.15 The Malawi Rift showing geographical breaks detected among sampled populations of *Andropadus milanjensis*. Solid lines indicate deep breaks and dashed lines weaker breaks.

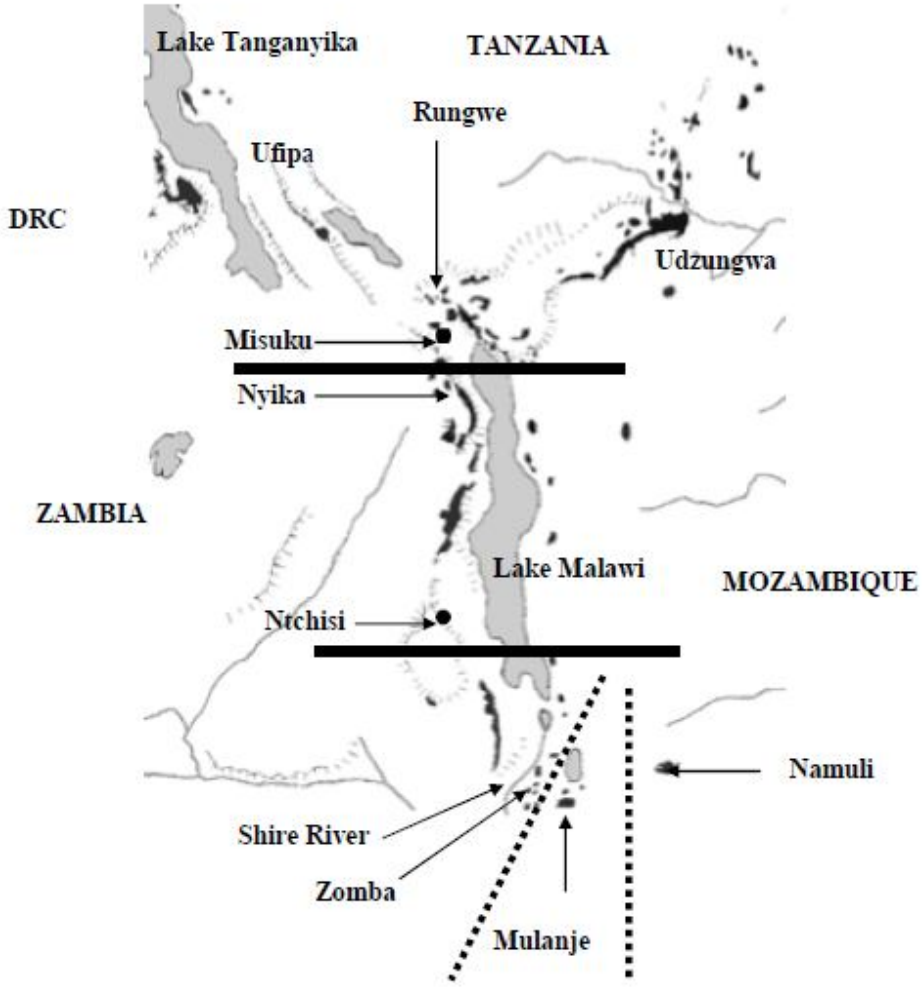


Figure 3.16 The Malawi Rift showing geographical breaks detected among sampled populations of *Batis dimorpha*. Solid lines indicate deep breaks and dashed lines weaker breaks.

Table 3.1a Taxa analysed in this study for ND2, sampling localities, geographical coordinates and frequencies of haplotypes. [ ] = haplotypes restricted to a specific population, ( ) = occurrence in more than one population.

Species	Locality	Country	Sample size	Latitude	Longitude	Haplotypes mtDNA (ND2)
Batis dimorpha	Nyika	Malawi	22	10.57S	33.70E	1[10] 2[5] 3[1] 4[3] 5[1] 6[2]
	Ntchisi	Malawi	13	13.38S	34.00E	7[13]
	Zomba	Malawi	16	15.40S	35.30E	15[11] 16[1] 17[1] 18[2] 19[1]
	Mulanje	Malawi	21	15.55S	35.38E	8[7] 9[5] 10[3] 11[1] 12[1] 13[3] 14[1]
	Namuli	Mozambique	4	15.39S	37.05E	20[1] 21[1] 22[2]
Andropadus milanjensis	Udzungwa	Tanzania	9	7.48S	36.41E	1[1] 2[1] 3[1] 4[1] 5[4] 6[1]
	Misuku	Malawi	29	09.68S	33.50E	7[23] 8[1] 9[3] 10[1] 11[1]
	Nyika	Malawi	3	10.57S	33.70E	13(3)
	Viphya	Malawi	3	11.26S	33.55E	12[2] 13(1)
	Ntchisi	Malawi	36	13.38S	34.00E	14[36]
	Zomba	Malawi	19	15.40S	35.30E	15[1] 16[17] 17[1]
	Mulanje	Malawi	8	15.55S	35.38E	18(5) 20(1) 21[1] 22[1]
	Namuli	Mozambique	5	15.39S	37.05E	18(2) 19[1] 20(2)

Table 3.1b Taxa analysed in this study for CHDZ and MUSK, sampling localities, geographical coordinates and frequencies of alleles. [ ] = alleles restricted to a specific population, ( ) = occurrence in more than one population.

Species	Locality	Country	Sample size	Latitude	Longitude	Alleles nDNA	
						CHDZ	MUSK
Batis dimorpha	Nyika	Malawi	23	10.57S	33.70E	1(7) 4[34] 5[5]	2(45) 3[1]
	Ntchisi	Malawi	19	13.38S	34.00E	1(26) 10[10]	2(38)
	Zomba	Malawi	4	15.40S	35.30E	1(2) 6(4) 9(2)	4(8)
	Mulanje	Malawi	21	15.55S	35.38E	2[12] 3[1] 6(21) 9(8)	4(42)
	Namuli	Mozambique	4	15.39S	37.05E	6(1) 7[1] 8[6]	4(4) 5[4]
Andropadus milanjensis							
	Udzungwa	Tanzania	8	7.48S	36.41E	7(7) 10[1] 11[2] 12[2] 13[1] 14[3]	6[2] 7[1] 12[1] 13[2] 14[1] 19[5] 20[1] 21[1] 22[1] 23[1]
	Misuku	Malawi	29	09.68S	33.50E	1(16) 8[3] 9(2) 15[1] 7(34) 5[1] 6[1]	1(55) 2[1] 3[1] 4(1)
	Nyika	Malawi	3	10.57S	33.70E	1(6)	1(5) 4(1)
	Viphya	Malawi	3	11.26S	33.55E	1(5) 9(1)	1(5) 15[1]
	Ntchisi	Malawi	62	13.38S	34.00E	1(120) 16[2]	1(50) 4(72) 5(2)
	Zomba	Malawi	21	15.40S	35.30E	1(42)	1(26) 5(16)
	Mulanje	Malawi	8	15.55S	35.38E	2(16)	8[1] 9(4) 10[6] 11[3] 18[2]
	Namuli	Mozambique	5	15.39S	37.05E	2(4) 3[3] 4[3]	9(8) 16[1] 17[1]

Table 3.2a Pairwise Φ_{ST}-values of seven sampled populations of *Andropadus milanjensis* mtDNA (ND2) below diagonal and nDNA (CHDZ) above the diagonal

	Udzungwa	Misuku	Nyika	Ntchisi	Zomba	Mulanje	Namuli
Udzungwa	-	0.21*	0.81***	0.98***	0.95***	0.93***	0.86***
Misuku	0.98***	-	0.45***	0.76***	0.63***	0.63***	0.63***
Nyika	0.96***	0.97***		0.25	0.13	0.67***	0.57***
Ntchisi	0.99***	0.99***	0.94***	-	0	0.97***	0.93***
Zomba	0.96***	0.98***	0.92***	0.99***	-	1.00***	0.87***
Mulanje	0.98***	0.98***	0.97**	0.99***	0.99***	-	0.57*
Namuli	0.96**	0.99***	0.99*	1.00***	-0.08	0.99***	-

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***P < 0.0001
**P < 0.001
*P < 0.01

Table 3.2b Pairwise Φ_{ST}-values of seven sampled populations of *Andropadus milanjensis* mtDNA (ND2) below diagonal and nDNA (MUSK) above the diagonal

	Udzungwa	Misuku	Nyika	Ntchisi	Zomba	Mulanje	Namuli
Udzungwa	-	0.82***	0.54***	0.84***	0.76***	0.29***	0.21**
Misuku	0.98***	-	0.10	0.44***	0.33***	0.94***	0.97***
Nyika	0.96***	0.97***	-	0.31***	0.20*	0.78***	0.82***
Ntchisi	0.99***	0.99***	0.94***	-	0.47***	0.92***	0.93***
Zomba	0.96***	0.98***	0.92***	0.99***	-	0.90***	0.93***
Mulanje	0.98***	0.98***	0.97**	0.99***	0.99***	-	0.10
Namuli	0.96**	0.99***	0.99*	1.00***	-0.08	0.99***	-

.
***P < 0.0001
**P < 0.001
*P < 0.01

Table 3.3a Pairwise Φ_{ST}-values of four sampled populations of *Batis dimorpha* mtDNA (ND2) below diagonal and nDNA (CHDZ) above the diagonal.

	Nyika	Ntchisi	Mulanje	Zomba
Nyika	-	0.64***	0.53***	
Ntchisi	0.74***	-	0.32***	
Mulanje	0.91***	0.95***	-	
Zomba	0.93**	1.00***	0.84**	-

***P < 0.0001
**P < 0.001
*P < 0.01

Table 3.3b Pairwise Φ_{ST}-values of four sampled populations of *Batis dimorpha* mtDNA (ND2) below diagonal and nDNA (MUSK) above the diagonal.

	Nyika	Ntchisi	Mulanje	Zomba
Nyika	-	0	0.99***	
Ntchisi	0.74***	-	1.00***	
Mulanje	0.91***	0.95***	-	
Zomba	0.93**	1.00***	0.84**	-

***P < 0.0001
**P < 0.001
*P < 0.01

Table 3.4 Measures of haplotype diversity, nucleotide diversity and results for tests of selective neutrality (Tajima's D and Fu's Fs) based on ND2 for *Batis dimorpha* and *Andropadus milanjensis.*

	Number of individuals	**Haplotype diversity**	**Nucleotide diversity**	**Tajima D**		**Fu's Fs**	
				D	P	Fs	P
Batis dimorpha							
Nyika	22	0.7446 ± 0.0728	0.001231 ± 0.000896	-0.686	0.285	-1.367	0.166
Ntchisi	17	0	0	0	1.00	-	-
Mulanje	21	0.8238 ± 0.0516	0.001455 ± 0.001016	-0.289	0.411	-1.980	0.087
Zomba	4	0.5000 ± 0.2652	0.000480 ± 0.000595	-0.612	0.364	0.172	0.347
Namuli	4	0.8333 ± 0.2224	0.001121 ± 0.001064	0.592	0.835	-0.658	0.132
Andropadus milanjensis							
Udzungwa	8	0.8333 ± 0.1265	0.002348 ± 0.001588	-0.770	0.251	-1.660	0.099
Misuku	29	0.3695 ± 0.1097	0.000577 ± 0.000527	-1.746	0.014**	-2.022	0.051**
Nyika/Viphya	6	0.7333 ± 0.1552	0.000512 ± 0.000563	0.851	0.883	-1.225	0.018
Ntchisi	62	0	0	0	1	-	-
Mulanje	8	0.6429 ± 0.1841	0.002161 ± 0.001510	-0.129	0.450	0.395	0.560
Zomba	21	0.2047 ± 0.1191	0.000202 ± 0.000287	-1.511	0.048**	-1.804	0.012**
Namuli	5	0.8000 ± 0.1640	0.001153 ± 0.001020	1.459	0.957	-0.186	0.265

**Significant values based on 10000 permutations, indicate that the population is out of equilibrium, suggestive of recent demographic change.

Appendix G: *Andropadus milanjensis* specimens examined in this study

Genus/Species	Locality	Coordinates	Museum number
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444130
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444136
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444137
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444140
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444129
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444131
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444124
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444139
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444122
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444123
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444126
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444133
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444135
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444121
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444144
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444125
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444142
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444118
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444132
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444141
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444127
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444138
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444128
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444120
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444134
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444143
Andropadus milanjensis	Ntchisi , Malawi	13.38S 34.00E	FMNH444119
Andropadus milanjensis	Mulanje, Malawi	15.55S 35.38E	FMNH447363
Andropadus milanjensis	Mulanje, Malawi	15.55S 35.38E	FMNH447364
Andropadus milanjensis	Mulanje, Malawi	15.55S 35.38E	FMNH447365
Andropadus milanjensis	Mulanje, Malawi	15.55S 35.38E	FMNH447366
Andropadus milanjensis	Mulanje, Malawi	15.55S 35.38E	FMNH447367
Andropadus milanjensis	Mulanje, Malawi	15.55S 35.38E	FMNH447368
Andropadus milanjensis	Mulanje, Malawi	15.55S 35.38E	FMNH447370
Andropadus milanjensis	Mulanje, Malawi	15.55S 35.38E	FMNH447369
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MOM2005.1.102
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MOM2005.1.127
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MLW985
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MLW1029
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MLW1028
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MLW1004
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MOM2005.1.111
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MOM2005.1.71
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MOM2005.1.115
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MOM2005.1.113

Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MLW1002
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MLW934
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MOM2005.1.141
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MLW942
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MLW986
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MOM2005.1.72
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MLW973
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MLW944
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MOM2005.1.53
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MOM2005.1.103
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MLW1027
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MLW1007
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MOM2005.1.70
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MOM2005.1.86
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MLW920
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MLW930
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MLW981
Andropadus milanjensis	Misuku Hills,Malawi	09.68S 33.50E	FMNH439188
Andropadus milanjensis	Namuli, Mozambique	15.39S 37.05E	FMNH438550
Andropadus milanjensis	Namuli, Mozambique	15.39S 37.05E	FMNH438545
Andropadus milanjensis	Misuku Hills,Malawi	09.68S 33.50E	FMNH439196
Andropadus milanjensis	Namuli, Mozambique	15.39S 37.05E	FMNH438546
Andropadus milanjensis	Misukun Hills,Malawi	09.68S 33.50E	MOM2003.2.149
Andropadus milanjensis	Misukun Hills,Malawi	09.68S 33.50E	MOM2003.2.154
Andropadus milanjensis	Misukun Hills,Malawi	09.68S 33.50E	MOM2003.2.141
Andropadus milanjensis	Misukun Hills,Malawi	09.68S 33.50E	MOM2003.2.139
Andropadus milanjensis	Misukun Hills,Malawi	09.68S 33.50E	FMNH439200
Andropadus milanjensis	Misukun Hills,Malawi	09.68S 33.50E	FMNH439199
Andropadus milanjensis	Misukun Hills,Malawi	09.68S 33.50E	FMNH439190
Andropadus milanjensis	Misukun Hills,Malawi	09.68S 33.50E	FMNH439205
Andropadus milanjensis	Misukun Hills,Malawi	09.68S 33.50E	FMNH439194
Andropadus milanjensis	Misukun Hills,Malawi	09.68S 33.50E	FMNH439189
Andropadus milanjensis	Misukun Hills,Malawi	09.68S 33.50E	FMNH439187
Andropadus milanjensis	Misukun Hills,Malawi	09.68S 33.50E	FMNH439198
Andropadus milanjensis	Namuli, Mozambique	15.39S 37.05E	FMNH438549
Andropadus milanjensis	Misuku Hills,Malawi	09.68S 33.50E	FMNH439186
Andropadus milanjensis	Misuku Hills,Malawi	09.68S 33.50E	FMNH439201
Andropadus milanjensis	Misuku Hills,Malawi	09.68S 33.50E	FMNH439193
Andropadus milanjensis	Misuku Hills,Malawi	09.68S 33.50E	FMNH439185
Andropadus milanjensis	Misuku Hills,Malawi	09.68S 33.50E	FMNH439192
Andropadus milanjensis	Misuku Hills,Malawi	09.68S 33.50E	FMNH439203
Andropadus milanjensis	Misuku Hills,Malawi	09.68S 33.50E	FMNH439191
Andropadus milanjensis	Misuku Hills,Malawi	09.68S 33.50E	FMNH439197
Andropadus milanjensis	Namuli, Mozambique	15.39S 37.05E	FMNH438544
Andropadus milanjensis	Misuku Hills, Malawi	09.68S 33.50E	FMNH439195
Andropadus milanjensis	Misuku Hills, Malawi	09.68S 33.50E	FMNH439206
Andropadus milanjensis	Misuku Hills, Malawi	09.68S 33.50E	FMNH439204
Andropadus milanjensis	Misuku Hills, Malawi	09.68S 33.50E	FMNH439202

Andropadus milanjensis	Zomba, Malawi	15.40S 35.30E	GAV2520
Andropadus milanjensis	Zomba, Malawi	15.40S 35.30E	DHB4868
Andropadus milanjensis	Zomba, Malawi	15.40S 35.30E	GAV2512
Andropadus milanjensis	Zomba, Malawi	15.40S 35.30E	DBH4869
Andropadus milanjensis	Zomba, Malawi	15.40S 35.30E	JK02-406
Andropadus milanjensis	Zomba, Malawi	15.40S 35.30E	JK02-408
Andropadus milanjensis	Zomba, Malawi	15.40S 35.30E	JK02-407
Andropadus milanjensis	Misuku Hills, Malawi	09.68S 33.50E	MOM2002.1.17
Andropadus milanjensis	Misuku Hills, Malawi	09.68S 33.50E	MOM2002.1.69
Andropadus milanjensis	Misuku Hills, Malawi	09.68S 33.50E	MOM2002.1.36
Andropadus milanjensis	Mulanje, Malawi	15.55S 35.38E	MOM2001.3.126
Andropadus milanjensis	Mulanje, Malawi	15.55S 35.38E	MOM2001.3.142
Andropadus milanjensis	Mulanje, Malawi	15.55S 35.38E	MOM2001.3.150
Andropadus milanjensis	Zomba, Malawi	15.40S 35.30E	MOM2001.3.34
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MLW B3
Andropadus milanjensis	Zomba, Malawi	15.40S 35.30E	MLW B139
Andropadus milanjensis	Zomba, Malawi	15.40S 35.30E	MLW B131
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	RB1099
Andropadus milanjensis	Zomba, Malawi	15.40S 35.30E	RCKB1466
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	RB1093
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	RB1105
Andropadus milanjensis	Zomba, Malawi	15.40S 35.30E	RCKB1443
Andropadus milanjensis	Zomba, Malawi	15.40S 35.30E	RCKB1428
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MoM2007.2.178
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MoM2007.2.194
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MoM2007.2.208
Andropadus milanjensis	Zomba, Malawi	15.40S 35.30E	MoM2007.2.389
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MoM2007.2.223
Andropadus milanjensis	Ntchisi, Malawi	13.38S 34.00E	MoM2007.2.185
Andropadus milanjensis	Zomba, Malawi	15.40S 35.30E	MoM2007.2.384
Andropadus milanjensis	Zomba, Malawi	15.40S 35.30E	MoM2007.2.303
Andropadus milanjensis	Udzungwa, Tanzania	7.48S 36.41E	RB3572
Andropadus milanjensis	Udzungwa, Tanzania	7.48S 36.41E	RB3577
Andropadus milanjensis	Udzungwa, Tanzania	7.48S 36.41E	RB3579
Andropadus milanjensis	Udzungwa, Tanzania	7.48S 36.41E	RB3582
Andropadus milanjensis	Udzungwa, Tanzania	7.48S 36.41E	RB3586
Andropadus milanjensis	Udzungwa, Tanzania	7.48S 36.41E	RB3593
Andropadus milanjensis	Udzungwa, Tanzania	7.48S 36.41E	RB3595
Andropadus milanjensis	Udzungwa, Tanzania	7.48S 36.41E	RB3603
Andropadus milanjensis	Udzungwa, Tanzania	7.48S 36.41E	RB3606
Andropadus milanjensis	Udzungwa, Tanzania	7.48S 36.41E	RB3618

Appendix H: *Batis dimorpha* and *Batis capensis* specimens examined in this study

Genus/Species	Locality	Coordinates	Museum number
Batis dimorpha	Ntchisi, Malawi	13.38S 34.00E	FMNH444392
Batis dimorpha	Ntchisi, Malawi	13.38S 34.00E	FMNH444394
Batis dimorpha	Ntchisi, Malawi	13.38S 34.00E	FMNH444393
Batis dimorpha	Ntchisi, Malawi	13.38S 34.00E	FMNH444390
Batis dimorpha	Ntchisi, Malawi	13.38S 34.00E	FMNH444395
Batis dimorpha	Ntchisi, Malawi	13.38S 34.00E	FMNH444391
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	FMNH447709
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	FMNH447710
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	FMNH447711
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	FMNH447712
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	FMNH447713
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	FMNH447714
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	FMNH447715
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	FMNH447716
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	FMNH447717
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	FMNH447718
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	FMNH447720
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	FMNH447721
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	FMNH447722
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	FMNH447723
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	FMNH447724
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	FMNH447725
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	FMNH447726
Batis dimorpha	Ntchisi, Malawi	13.38S 34.00E	MOM2005.1.57
Batis dimorpha	Ntchisi, Malawi	13.38S 34.00E	MLW1019
Batis dimorpha	Ntchisi, Malawi	13.38S 34.00E	MOM2005.1.96
Batis dimorpha	Ntchisi, Malawi	13.38S 34.00E	MLW913
Batis dimorpha	Ntchisi, Malawi	13.38S 34.00E	MLW998
Batis dimorpha	Ntchisi, Malawi	13.38S 34.00E	MLW974
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440843
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440862
Batis dimorpha	Namuli, Mozambique	15.39S 37.05E	FMNH438608
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440842
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440848
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440850
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440861
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440864
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440847
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440845
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440855
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440851
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440859
Batis dimorpha	Namuli, Mozambique	15.39S 37.05E	FMNH438610
Batis dimorpha	Namuli, Mozambique	15.39S 37.05E	FMNH438607
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440854

Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440860
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440856
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440849
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440857
Batis dimorpha	Namuli, Mozambique	15.39S 37.05E	FMNH438611
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440844
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440863
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440858
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440853
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440852
Batis dimorpha	Nyika, Malawi	10.57S 33.70E	FMNH440846
Batis capensis	South Africa		JK00-105
Batis capensis	South Africa		JK00-106
Batis capensis	South Africa		JK00-101
Batis capensis	South Africa		GAV1621
Batis dimorpha	Zomba, Malawi	15.40S 35.30E	GAV2535
Batis dimorpha	Zomba, Malawi	15.40S 35.30E	GAV2536
Batis dimorpha	Zomba, Malawi	15.40S 35.30E	JK02-445
Batis dimorpha	Ntchisi, Malawi	13.38S 34.00E	RB1137
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	RCKB1292
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	RCKB1281
Batis dimorpha	Ntchisi, Malawi	13.38S 34.00E	RB1138
Batis dimorpha	Ntchisi, Malawi	13.38S 34.00E	MLW B31
Batis dimorpha	Mulanje, Malawi	13.38S 34.00E	MLW B57
Batis dimorpha	Ntchisi, Malawi	13.38S 34.00E	MoM2007.2.192
Batis dimorpha	Ntchisi, Malawi	13.38S 34.00E	MoM2007.2.195
Batis dimorpha	Ntchisi, Malawi	13.38S 34.00E	MoM2007.2.195B
Batis dimorpha	Ntchisi, Malawi	13.38S 34.00E	MoM2007.2.210
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	MoM2007.2.282
Batis dimorpha	Mulanje, Malawi	15.55S 35.38E	MoM2007.2.288
Batis dimorpha	Zomba, Malawi	15.40S 35.30E	MLW B138
Batis dimorpha	Zomba, Malawi	15.40S 35.30E	MoM2009.2.66
Batis dimorpha	Zomba, Malawi	15.40S 35.30E	MoM2009.2.84
Batis dimorpha	Zomba, Malawi	15.40S 35.30E	MoM2009.2.65
Batis dimorpha	Zomba, Malawi	15.40S 35.30E	MoM2009.2.138
Batis dimorpha	Zomba, Malawi	15.40S 35.30E	MoM2009.2.200
Batis dimorpha	Zomba, Malawi	15.40S 35.30E	MoM2009.2.169
Batis dimorpha	Zomba, Malawi	15.40S 35.30E	MoM2009.2.168
Batis dimorpha	Zomba, Malawi	15.40S 35.30E	MoM2009.2.68
Batis dimorpha	Zomba, Malawi	15.40S 35.30E	MoM2016.2.121
Batis dimorpha	Zomba, Malawi	15.40S 35.30E	MoM2009.2.64
Batis dimorpha	Zomba, Malawi	15.40S 35.30E	MoM2009.2.63
Batis dimorpha	Zomba, Malawi	15.40S 35.30E	MoM2009.2.67

Chapter 4

The phylogeography of the Bar-throated Apalis complex with a focus on populations distributed across the Malawi Rift

4.1 ABSTRACT

This study investigated phylogeographic structure of the Bar-throated Apalis (*Apalis thoracica*) complex across the Malawi Rift. Analyses of a combination of mtDNA (1041 bp ND2) and nDNA (594 bp TGFb2) from populations sampled through the Malawi Rift, as well as from the Eastern Arc Mountains to the north, and from South Africa in the south revealed significant population structure. Results suggest that phylogeographic breaks occur in the southern highlands separating Mount Namuli in Mozambique and Mount Mulanje as well as between Mount Mulanje and Mount Zomba in Malawi. In the central highlands splitting Malawi into two halves, in the northern highlands separating Misuku Hills and Nyika Plateau, as well as separating the Misuku Hills from Udzungwa Mts. The West Usambara Mts, Udzungwa Mts and the Misuku Hills population corresponds to *Apalis thoracica murina*, Nyika Plateau corresponds to *Apalis thoracica youngi*, Mount Dedza corresponds to *Apalis thoracica whitei*, Mount Zomba and Mount Mulanje corresponding to *Apalis thoracica flavigularis*, and Mount Namuli corresponding to *Apalis thoracica lynesi*. The results of this study and ongoing research suggest that molecular DNA data has an important role to play in helping to manage and conserve divergent populations of montane birds in Malawi.

4.2 INTRODUCTION

Our understanding of the evolutionary history of species has been significantly enhanced by the acquisition of molecular data that has revealed the distribution of genetic variation within and among populations: the field of phylogeography (Avise 2000; Zink & Barrowclough 2008). In recent years phylogeography has been used as a tool with which to interpret the historical processes that are responsible for shaping the genetic diversity within a species, and has over the past two decades been applied to several groups of African vertebrates (e.g. Fjeldså & Bowie 2008; Lorenzen et al. 2012 for reviews).

From paleontological records and marine sediments it has been inferred that major ecological changes occurred in tropical Africa over the past few million years, with climatic oscillations between wet and dry conditions occurring in response to glacial cycles at higher latitudes (de Menocal 1995; 2004). However, long-term paleoclimatic data for Africa are scarce, with the most comprehensive dataset of wind-blown (eolian) dust in deep-sea drill cores extending back c. 5 million years (Myrs) (deMenocal 1995). These data, together with pollen and sedimentation data reveal that sub-Saharan Africa became much more arid during Pleistocene glacial periods. The increased aridity is thought to have facilitated the expansion of the savanna and semi-arid biomes and contraction of the forest biomes (Fjeldså & Bowie 2008; Voelker et al. 2010). It has been postulated that isolation in forest refugia is the primary mechanism of speciation of African forest birds (Crowe & Crowe 1982; Mayr & O'Hara 1986; Fjeldså & Bowie 2008; Voelker et al. 2010). Dating of forest bird divergence times (e.g. Bowie et al. 2006) are consistent with results from eolian deposition that suggest that the pendulum swung

towards savanna and away from forest during the Plio-Pleistocene at 2.8, 1.7 and 1.0 Myrs BP (de Menocal 1995; Kennett 1995), which coincided with peaks of aridity in Africa.

African rainfall patterns are known to be unstable and have been documented to change from decade to decade over a great extent of the continent (Nicholson 1994). However, the Afrotropical montane highlands, many of which receive considerable orographic rain, are thought to have maintained a more consistent precipitation regime. This consistency of rainfall is thought to have helped maintain persistent forest cover on high elevation mountains and plateaus throughout glacial-interglacial cycles (Fjeldså & Lovett 1997; Servat et al. 1998), and thereby provided a refuge for many species.

While phylogeographic studies using DNA markers have been carried out on African montane birds (e.g. Bowie et al. 2004; 2005; 2006; Fjeldså et al. 2006; Voelker et al. 2010; Fuchs et al. 2011), very few studies have included samples from the Malawi Rift. Thus, phylogeographical studies of Malawi Rift species are required if we are to better understand the biogeographical history of this important highland area.

The Bar-throated Apalis (*Apalis thoracica*, Shaw 1811) is restricted to forests and thickets with a distribution range (Fig. 1.4) extending from southeastern Kenya, through the highlands of central and eastern Tanzania, eastern Zambia, Malawi, interior Mozambique, eastern and southwestern Zimbabwe, eastern Botswana to the coastal forest mosaic of South Africa (Erard 1997). The plumage of the species is highly variable with different combinations of brown, grey, or black on the head, a green back and white or yellow underparts. All the subspecies share a black band separating the throat from the breast (Erard 1997).

The *Apalis thoracica* complex has five subspecies whose distribution range is within the study area (from southern Tanzania through Malawi to northern Mozambique, Fig. 1.4, 4.1 & Table 4.1): *Apalis thoracica murina* (Reichenow 1904), *Apalis thoracica youngi* (Kinnear 1936), *Apalis thoracica whitei* (Grant & Mackworth-Praed 1937), *Apalis thoracica flavigularis* (Shelley, 1893) and *Apalis thoracica lynesi* (Vincent 1933).

A previously study conducted on the Bar-throated Apalis complex (Solms 2003), examined the complex throughout its distribution range using mtDNA. Solms (2003) expected to recover limited genetic structure among subspecies which were contiguously distributed in southern Africa (*Apalis t. thoracica*, *A. t. griseopyga, A. t. venusta, A. t. drakensbergensis, A. t. spelonkensis, A. t. lebomboensis, A. t. capensis, A. t. claudei, A. t. darglensis* and *A. t. flaviventris*) as opposed to among the montane restricted and hence isolated subspecies in eastern Africa (*A. t. rhodesiae*, *A. t. arnoldi, A. t. whitei, A. t. lynesi, A. t. flavigularis, A. t. youngi*, *A. t. murina*, *A. t. fuscigularis, A. t. uluguru* and *A. t. griseiceps*), which formed a complex separate from the South Africa taxa. However, deep phylogeographic breaks were also observed between South African subspecies. Here I focus only on taxa distributed across the Malawi Rift in order to resolve their taxonomic status particularly in light of considerable plumage polymorphism among these taxa, and thereby help to determine the appropriate conservation strategies to implement given the range restricted nature of their distribution.

For *Apalis thoracica* I obtained DNA sequence data from one mitochondrial (mtDNA) marker (NADH dehydrogenase subunit 2 [ND2]) and two nuclear markers (Beta-Fibrinogen intron 5 and TGFb2 intron 5) in order to examine the genetic relationships and population structure in the East African species complex. Considering

the temporal changes to the environment and extent of phylogeographic structure that has been uncovered in some avian lineages distributed between eastern and southern Africa (Bowie et al. 2004; 2005), I hypothesise that there are genetic differences among members of the *Apalis thoracica* complex within the Malawi Rift, with potential breaks occurring: 1) on the lowland gap between Zomba and Mulanje in southern Malawi, 2) in the central highlands, splitting Malawi into two, and 3) across the lowland gap that separates Nyika from the Misuku Hills in northern Malawi (Fig. 4.1).

These predicted phylogeographic breaks correspond with known plumage variation as delineated by subspecies boundaries of range restricted taxa within the study area. For instance, the southern most extent of *A. t. murina* is the Misuku Hills, with *A. t. youngi* inhabiting Nyika Plateau and the Viphya Plateau as its most northern and southern extent, respectively, *A. t. whitei* occupies the central highlands of Malawi, *A. t. flavigularis* inhabits the mountains southeast of the Shire River which include Mount Zomba, Mount Malosa and Mount Mulanje, and *A. t. lynesi* is restricted to Mount Namuli in northern Mozambique (Fig 1.4 & 4.1). Because these subspecies are range restricted, the loss of habitat due to human activities (Mzumara et al. 2012) is affecting them to the extent that *A. t. flavigularis* is classified as Globally Endangered whereas *A. t. lynesi* is Near-threatened (BirdLife International 2010).

In order to determine whether phylogeographic breaks (lack of gene flow) are present among the divergent plumage forms, I address the following questions: (i) Does genetic turnover between east and southern African lineages occur, and is it centered geographically in Malawi? (ii) If so, are lineages endemic to Malawi, and what are their

distribution limits within Malawi? (iii) Where are the common phylogeographical breaks in Malawi and how do they relate to the current placement of national parks?

4.3 MATERIALS AND METHODS

4.3.1 Population sampling

Tissue samples were obtained from 82 *Apalis thoracica* individuals collected during research expeditions conducted in the northern, central and southern highlands of Malawi from 2001 to 2009 (see appendix I). In order to understand whether the faunal turnover of lineages occurred in the Malawi Rift, populations of *Apalis thoracica* from South Africa, Tanzania (Udzungwa and West Usambara) and northern Mozambique (Namuli) were also sampled. For the sites I could not visit, I obtained preserved tissue from the Museum of Vertebrate Zoology, The Field Museum of Natural History and the National Museum of Natural History, Denmark. The taxa *Oreolais ruwenzorii*, *Oreolais pulcher*, *Phragmacia substriata* and *Apalis flavida* were used as outgroups to the *Apalis thoracica* species complex (Olsson et al. 2013).

4.3.2 Laboratory procedures

Total genomic DNA was extracted from 0.25 g or less of muscle tissue or blood using a DNeasy Kit (QIAGEN, Germantown, MD, USA) following the manufacturer's animal tissue protocol with an overnight proteinase K digestion at 55^{o}C. The entire 1041 base pair (bp) ND2 gene of the mitochondrial genome was amplified by polymerase chain reaction (PCR) with primers L5204 (5'-GCTAACAAAGCTATCGGGCCCAT-3') and H6312 (5'-CTTATTTAAGGCTTTGAAGGCC-3') (Cicero & Johnson 2001). The primers Fib5 (5'-CGCCATACAGAGTATACTGTGACAT-3') and Fib6 (5'-

GCCATCCTGGCGATTCTGAA -3') (Fuchs et al. 2004, Kimball et al. 2009) were used to amplify 574 bp of Beta-Fibrinogen intron 5. A 594 bp fragment from TGFb2 intron 5 was amplified using primers 5F (5'GAAGCGTGCTCTAGATGCTG-3') and 6R (5'-AGGCAGCAATTATCCTGCAC-3') (Primmer et al. 2002). Double-stranded PCR amplifications were carried out in 25 µl reaction volumes containing: 2.5 µl 10 x buffer, 0.5 µl of 10 mM dNTPs, 0.5 µl of 10 mg/ml of bovine serum albumin, 0.75 µl of 50 mM $MgCl_2$, 1.25 µl of 10 µM of the forward and reverse primer in the presence of 0.25 µl of Taq polymerase (Perkin-Elmer) and genomic DNA.

ND2 was PCR-amplified with an initial denaturation step at 94^{o}C for 3 minutes, followed by 35 cycles of denaturation at 94^{o}C for 45 seconds, annealing at 56^{o}C for 30 seconds, and extension at 72^{o}C for 30 seconds, with a final extension at 72^{o}C for 7 minutes. Fib5 was PCR-amplified by initial denaturation at 94^{o}C for 3 minutes, 35 cycles of denaturation at 94^{o}C for 45 seconds, annealing at 54^{o}C for 30 seconds and extension at 72^{o}C for 30 seconds, with a final extension at 72^{o}C for 7 minutes. For TGFb2 initial denaturation was at 94^{o}C for 3 minutes, followed by 35 cycles of denaturation at 94^{o}C for 30 seconds, annealing at 60^{o}C for 30 seconds, and extension at 72^{o}C for 30 seconds, with a final extension at 72^{o}C for 7 minutes. Standard precautionary measures included negative controls (template or DNA free PCR reactions), that were used to test for the presence any contamination.

I electrophoresed 5 µl of the PCR product on a 1% agarose gel mixed with ethidium bromide and observed it under ultraviolet light to check for the correct fragment size and to control for specificity of the PCR-amplification. The amplified DNA was purified by using either ExoSAP-IT (USB; Cleveland, OH, USA) or with GELase

(Epicentre; Madison, WI), and cycle-sequenced using the ABI PRISM BigDye Terminator kit v.3.1 (Perkin-Elmer). Sephadex spin columns were utilised to clean-up the cycle-sequencing reactions (CureHunter, Oregon, USA). For each locus both the reverse and forward directions were sequenced, using the same primers as used in the PCR-amplification, on an ABI 3730 automated DNA Analyser (Applied Biosystems).

4.3.3 Determining the phase of alleles

In order to infer genealogies and to estimate demographic parameters from molecular DNA sequence data it is important to resolve the phase of the bi-allelic sequence data. Some introns had more than one polymorphic site (single nucleotide polymorphism [SNP]). A Bayesian method implemented in the programme PHASE v.2.1.1 (Stephens et al. 2001; Stephens & Donnelly 2003) was used to resolve the phase of all linked polymorphisms. A threshold of 0.75 (Harrigan et al. 2008) was used to satisfactory consider a SNP as phased; all SNPs that did not satisfy this threshold were removed from further analyses.

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4.3.4 Data analysis

Sequence alignment

Sequences were obtained from both strands of DNA for each individual and some individuals were sequenced several times in the event of any base ambiguity encountered. Sequence alignment was performed by computation using MAFFT (Katoh et al. 2009) and checked by eye. For ND2, sequences were checked for insertions or deletions, as well as to ensure that stop-codons were not present.

Phylogenetic analyses

Parsimony, Bayesian and Maximum Likelihood analyses were performed on the mitochondrial DNA (ND2). Parsimony analyses were conducted in PAUP*10b (Swofford 2002) using a heuristic search with TBR branch-swapping and 1000 random addition replicates. Parsimony bootstrap values were obtained from 1000 pseudoreplicates with 5 random addition replicates being performed for each bootstrap pseudoreplicate.

Maximum Likelihood analyses were conducted using RAXML (Stamatakis 2006) on the ND2 dataset, partitioned by codon position, under a general-time-reversible model of nucleotide substitution and a gamma model of rate heterogeneity via the CIPRES portal (Stamatakis et al. 2008; Miller et al. 2009). One-thousand bootstrap pseudoreplicates were performed to evaluate support at specific nodes.

The best-fitting model for the Bayesian analysis under the Akaike Information Criterion was identified in MRMODELTEST v.2.2 (Nylander 2004). Bayesian analyses were performed using MRBAYES v.3.1.2 (Huelsenbeck & Ronquist 2001) on the ND2 dataset, partitioned by codon position. Two runs were conducted with each analysis starting from a random tree, using four chains, and run for 5×10^6 generations, sampling every 1000 generations. Stationarity was assessed using TRACER v.1.5 (Rambaut & Drummond 2007), by plotting $-\ln L$ values, and ensuring that the sampling of the posterior probabilities had reached a sufficient effective sample size (ESS) for meaningful parameter estimation (ESS > 200). In addition, posterior probabilities were plotted against generation number in AWTY (Nylander et al. 2004). Runs stabilized after the first 1×10^6 generations, thus the burn-in was set at 10%. Convergence among runs

was evaluated by comparing consensus topologies from each of the two runs, and by checking that the potential scale reduction factor (PRSF) approached 1 for all parameters, and that the average standard deviation scale factor (ASDSF) approached 0.005. The two runs, with the burn-in trees removed, were combined to calculate a majority-rule consensus tree.

Haplotype network construction

Due to the problems that arise in the construction of intraspecific phylogenies (e.g. Posada & Crandall 2001) TCS v.1.01 was used to construct a statistical parsimony network of haplotypes (Clement et al. 2000) for each locus. The connection limit was set at 95%. The above analysis included only the in-group samples.

Analysis of molecular variance (*AMOVA*)

In order to determine molecular variation within and among populations as well as among larger geographical regions, several hierarchical analyses of molecular variance (AMOVA) were conducted. This enabled the determination of how genetic variability was partitioned within and among major lineages by using $\Phi_{ST,}$ which is an analogue of F_{ST} that incorporates both haplotype frequencies and the difference in number of nucleotides between each pair of haplotypes (Excoffier et al. 1992). The levels of significance for AMOVA were obtained by using a non-parametric permutation with 10000 iterations, and was carried out using ARLEQUIN v.3.0 (Excoffier et al. 2005).

Mismatch distributions and tests of selective neutrality

Pairwise mismatch distributions and two tests of selective neutrality, Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997) were calculated to test if populations were in mutation drift equilibrium under an infinite sites model. These indices were used as

indicators of recent demographic change that could result from population expansion or contraction (Fu 1997; Rodgers & Harpending 1992). Both tests were conducted using ARLEQUIN v.3.0 (Excoffier et al. 2005).

Divergence times

I used the program BEAST v.1.7.1 (Drummond et al. 2006; Drummond & Rambaut 2007) to estimate divergence times for ND2 using the mean rate of divergence and associated standard deviation reported by Lerner et al. (2011) for ND2 ($2.9*10^{-2}$ [$2.3-3.3*10^{-2}$] Myrs BP). This rate is derived from the sequence of lineage splits in a passerine clade (Hawaiian Honeycreepers: Fringillidae), and calibrated using the well-established dates of sequential uplift of the Hawaiian Archipelago. In BEAST, a Yule process speciation prior and an uncorrelated lognormal model of rate variation (relaxed clock) were implemented, with individual codon positions having separate substitution models determined using MRMODELTEST. The MCMC analyses were conducted for 1×10^7 generations with parameters sampled every 5000 steps, and a 10% burn-in. TRACER v.1.5 (Rambaut & Drummond 2007) was used to determine the effective sample size of each parameter and to calculate the mean, and upper and lower bounds of the 95% highest posterior density interval (95% HPD) for divergence times. Tree topologies were assessed using TreeANNOTATOR v.1.5.3 (Drummond & Rambaut 2007) and FIGTREE v.1.3.1 (Rambaut 2008).

4.4 RESULTS

4.4.1 Sequence variation

Mitochondrial DNA

A final alignment of the entire 1041 bp of the ND2 gene was obtained from all 82 individuals of *Apalis thoracica* included in this study. Removal of identical sequences recovered 26 unique haplotypes (Table 4.2). Of the 1041 characters, 645 (61.96%) characters were constant, 124 (11.91%) were variable but parsimony uninformative, and 272 (26.13%) characters were parsimony informative.

Nuclear DNA

A final alignment of 594 bp fragment of the TGFb2 intron 5 was obtained from 71 individuals of *Apalis thoracica*; combining identical sequences recovered nine unique alleles. Of the 594 characters, 584 (98.3%) characters were constant, two (0.3%) characters were variable but parsimony uninformative, and eight (1.4%) characters were parsimony informative. Beta-Fibrinogen intron 5 sequenced poorly due to considerable length-polymorphism and hence, was not further analysed.

4.4.2 Phylogenetic analysis

Mitochondrial DNA

The Maximum Parsimony, Bayesian and Maximum Likelihood mitochondrial DNA analysis recovered trees that were nearly identical (Fig 4.2). South African samples are sister to the Malawi Rift and Eastern Arc populations. Nine clades were recovered and represent from north to south: West Usambara Mts, Udzungwa Mts, Misuku Hills, Nyika Plateau, Mount Dedza, Mount Zomba, Mount Mulanje, Mount Namuli and South Africa. However, two haplotypes from Dedza (Fig 4.2, FMNH 444337 & FMNH 444336) appear closer to the Nyika clade while the other haplotype (FMNH 444339) is closer to individuals from Mount Zomba. The Udzungwa Mts, West Usambara Mts and

Misuku Hills correspond to *Apalis thoracica murina* which based on the recovered topology is not monophyletic since individuals from Misuku are closer to Nyika than Eastern Arc populations. Birds from Nyika Plateau correspond to *Apalis thoracica youngi*, and birds from Mount Dedza corresponding to *Apalis thoracica whitei*. Individuals from Mount Zomba and Mount Mulanje correspond to *Apalis thoracica flavigularis* which based on the topology recovered also appear to not be monophyletic since birds from Mount Zomba are more closely related to individuals from the central highlands of Malawi (Dedza and Nyika) than to Mount Mulanje. Birds from Mount Namuli corresponding to *Apalis thoracica lynesi*, and were recovered as a divergent lineage.

Nuclear DNA

The Bayesian and Maximum Likelihood analysis for the intron TGFb2 alleles formed a large polytomy (not presented).

4.4.3 Haplotype network construction

Mitochondrial DNA

Construction of a haplotype network using the 1041 bp fragment of the ND2 gene of *Apalis thoracica* indicated that subnetworks connected by 14 steps or fewer had a cumulative probability of greater than 95% of being correct. The TCS analysis recovered six subnetworks (Fig. 4.3) with Udzungwa, Misuku, Nyika, Dedza and Zomba grouping together whereas individuals from West Usambara, Mulanje, Namuli and South Africa, formed subnetworks of their own (Fig. 4.3 I, II, III, IV, V and VI). The individuals from

the Udzungwa Mts grouped together and were separated from Mount Dedza and Mount Zomba haplotypes by 12 mutational steps; individuals from the Misuku Hills were grouped together and were separated by four mutational steps from Nyika Plateau and Mount Dedza haplotypes. Mount Zomba haplotypes were separated from Mount Dedza by three mutational steps and from Nyika Plateau by six mutational steps.

There was generally high haplotype diversity in *Apalis thoracica*: Nyika (= 6), Udzungwa (= 3) and Misuku (= 3), Mulanje (= 3) and Dedza (= 2), and Namuli comprised only one sampled individual. In contrast individuals from Mount Zomba (n = 20) all had the same haplotype despite adequate sampling (Table 4.2). The South African individuals sampled formed two distinct subnetworks indicating geographical structuring, as was also reported by Solms (2003). From the phylogenetic analysis (Fig. 4.2), it has been observed that haplotypes from different localities in the north and south are not monophylectic because of the position of the Dedza haplotypes (FMNH 444337 & FMNH 444336), which are closely allied to Nyika haplotypes in the northern clade, or the Zomba haplotypes in the southern clade (FMNH 444339).

Nuclear DNA

For TGFb2 intron 5 TCS suggested that subnetworks connecting alleles by 10 steps or fewer had a cumulative probability of greater than 95% of being correct. The degree of geographical structuring was reduced relative to that recovered by the ND2 dataset (Fig. 4.4). Most alleles were connected to the common central allele which occurred in several populations: Udzungwa Mts, Nyika Plateau, Mount Dedza, Mount Mulanje, Mount Zomba, Mount Namuli, and South Africa (allele 1, Fig. 4.4). Within *Apalis thoracica,* Nyika Plateau (= 2), Udzungwa Mts (= 2) and Misuku Hills (= 2) had

higher allelic variation than Mount Mulanje (= 1), Mount Dedza (= 1) and Mount Zomba (= 1), and Mount Namuli had only one sample (Table 4.2). Only the Misuku Hills, West Usambara Mts and the South Africa population sampled had private alleles restricted to each highland.

4.4.4 Analysis of molecular variance

Mitochondrial DNA

An AMOVA for *Apalis thoracica* was conducted among the seven sampled populations (Table 4.2). The genetic variation within groups was 8.88%, variation within populations was 4.2%, and variation among groups was 86.92%. There is considerable population substructure as indicated by the high value of Φ_{ST} (0.958, $P < 0.0001$), and this is supported by the pairwise Φ_{ST} values, which are mostly significant (Table 4.3). Using four sampled populations with sufficient sample sizes (Misuku Hills, Nyika Plateau, Mount Zomba and Mount Mulanje, Table 4.3) the genetic variation within groups was 35.42%, variation within population was 15.09%, and variation among groups was 49.49%. The Φ_{ST} (0.849, $P < 0.0001$) also showed structuring among the populations (Table 4.3).

Nuclear DNA

AMOVA for TGFb2 intron 5 for *Apalis thoracica* was conducted on all the seven populations (Table 4.2). The genetic variation among groups was 38.13%, variation within groups was 0% (-1.01), and variation within populations was 62.88%. Although population substructure was not as pronounced, it remained highly significant (Φ_{ST} = 0.371, $P < 0.0001$). For the AMOVA conducted on the four populations with sufficient

sample sizes (Table 4.2), the genetic variation among groups was 49.64%, variation within groups was 0% (-1.32), and variation within populations was 51.68%, (Φ_{ST} = 0.483, P < 0.0001).

4.4.5 Mismatch distributions and test of selective neutrality

Mismatch profiles which follow a modified Poisson distribution (a unimodal, bell shaped curve when population expansion is recent), are thought to be associated with past events of population growth, for instance range expansion (Rogers & Harpending 1992; Harpending et al. 1993). In the three *Apalis thoracica* mismatch distribution profiles (Fig. 4.5) conducted for populations with adequate sample sizes and sufficient genetic variation (Zomba had one haplotype), all these populations (Misuku, Nyika and Mulanje) followed a Poisson distribution with Mulanje being significantly skewed to the left, indicative of the most recent expansion. Tajima's D and Fu's Fs statistics for Misuku were not significant, indicating that if these populations have undergone a range expansion, it occurred a long time ago. The population on Nyika Plateau has both Tajima's D and Fu's Fs values which are negative, although with varying levels of significance (Table 4.3).

4.4.6 Divergence times

From the dating results (Fig. 4.6), I can infer that the South African *Apalis thoracica* taxa diverged from the East African taxa about 1.1 Myrs BP. The Mount Mulanje population split from the remaining East African populations about 0.62 Myrs

BP, followed by the Namuli population becoming isolated about 0.5 Myrs BP. The Eastern Arc Mountain populations split from the Malawi Rift populations about 0.35 Myrs BP, with the Usambara (North Eastern Arc) diverging from the Udzungwa (South Eastern Arc) about 0.30 Myrs BP. Divergence times among taxa distributed across the centre of the Malawi Rift appears to have occurred within the past 0.25 Myrs BP. It is also important to note that the Dedza haplotypes (FMNH 444337 & FMNH 444336) are closely allied to Nyika haplotypes in the northern clade, and Dedza haplotype (FMNH 444339) is closely allied to Zomba haplotypes (Fig. 4.2 & 4.3), as also recovered in the earlier phylogenetic analyses.

4.5 DISCUSSION

The mtDNA phylogenetic analysis recovered two well supported clades one comprising the South African *Apalis thoracica* taxa and the other the Malawi Rift and Eastern Arc taxa (Fig 4.2). The Malawi Rift and Eastern Arc clades were well supported and represent from north to south the: West Usambara Mts, Udzungwa Mts, Misuku Hills, Nyika Plateau, Mount Dedza, Mount Zomba, Mount Mulanje and Mount Namuli. The Misuku clade appears to be closer to Nyika than Udzungwa as would be expected based on subspecies boundaries (Fig. 4.1). The Dedza haplotypes do not cluster together which could be due to either recurrent gene flow in *A. t. whitei* or recent vicariance among the sampled localities such that lineage sorting is not complete. All 20 individuals sampled from Zomba had the same haplotype that is not sister to birds from Mulanje as would be expected based on plumage similarity (Fig 4.1, *A. t. flavigularis*).

The West Usambara Mts, Udzungwa Mts and the Misuku Hills population corresponds to *Apalis thoracica murina*, Nyika Plateau correspond to *Apalis thoracica youngi*, Mount Dedza corresponds to *Apalis thoracica whitei*, Mount Zomba and Mount Mulanje corresponding to *Apalis thoracica flavigularis*, and Mount Namuli corresponding to *Apalis thoracica lynesi*. As indicated above the recovered topology suggests that some of these taxa may not be monophyletic.

The mtDNA network analyses of the *Apalis thoracica* populations resulted into six distinct subnetworks that comprise birds from the West Usambara Mts, Udzungwa-Misuku-Nyika-Dedza-Zomba, Mulanje, Namuli, and South Africa respectively (Fig 4.3). This suggests considerable geographical structuring among all the sampled populations, a result further re-enforced by the lack of shared haplotypes among the seven populations sampled in the Malawi Rift study area indicating that there probably is at present no gene flow (Table 4.2).

Mount Dedza haplotype samples from individuals FMNH 444337 and FMNH 444336 are closely allied to Nyika Plateau haplotypes in the northern clade, and the Mount Dedza haplotype from individual FMNH 444339 is closely allied to Zomba haplotypes (Fig 4.2 & 4.3). Interestingly the divergence time for Nyika Plateau, Mount Dedza and Mount Zomba populations is roughly concurrent (Fig. 4.6), therefore it could be inferred that the Mount Dedza population might have been colonised from both Nyika Plateau and Mount Zomba or that the sharing of haplotypes may be reflective of ancestral polymorphism. This suggests that the northern and southern populations presently circumscribed as subspecies are not monophyletic, rather they originally came from different ancestors (Nicholls & Austin 2005). Greater sample sizes from Mount Dedza

are required to fully understand the dynamics of observed patterns of structure in *A. t. whitei* in the Malawi Rift.

The use of nuclear marker (TGFb2) recovered some geographical structuring, as the Φ_{ST} was significant, and in general agreement with the mtDNA dataset (Table 4.3). However, this geographical structuring was not well displayed by the network (Fig 4.4) as most of the alleles were connected to the central and most common allele suggesting that differences in frequency distribution of alleles is driving the Φ_{ST} value. The lower level of geographical structuring displayed in the nDNA relative to the mtDNA is as a result of nuclear DNA having a four times slower coalescent time (Hare 2001). It is likely that incomplete lineage rather than recurrent gene flow accounts for the sharing of alleles in recently diverged taxa (Palumbi & Baker 1996), especially because in birds, females tend to be the more dispersed sex (Greenwood 1980; Ribeiro et al. 2012).

The mismatch distribution for Nyika (Fig 4.5) and Fu's Fs test was significant indicating that the population is expanding. The distribution of the subspecies *A. t. youngi* extends from Nyika Plateau to the southern part of the Viphya Plateau in central Malawi (Table 4.1). The direction of expansion is towards an area of lower nucleotide diversity (Sgariglia & Burns 2003) and this could be true for the subspecies as it has also been observed in *Andropadus milanjensis olivaceiceps,* where the direction of expansion is from Nyika towards the Ntchisi highlands in central Malawi (Kaliba, MSc Thesis 2006). For Mulanje the mismatch distribution indicated population expansion but both Tajima's D and Fu's Fs were not significant, suggesting that despite the population undergoing a recent demographic change it has remained in equilibrium (Rodgers & Harpending 1992; Harpending et al. 1993).

The mtDNA reveals *A. t. murina* (to the exclusion of the Misuku population), *A. t. youngi*, *A. t. lynesi* and possibly *A. t. whitei* as well as Mulanje population of *A. t. flavigularis* as being distinct. It is interesting to note that despite Mount Zomba and Mount Mulanje being inhabited by *A. t. flavigularis* (Erard 1997; Ryan et al. 2006; Dowsett-Lemaire & Dowsett 2006) the two populations are distinct with no detected gene flow across the two populations despite, the aerial distance being less than 100 km. This could be attributed to the low dispersal of forest birds (Dowsett 1985). Also inferred from the evolutionary history of the subspecies in the two localities (Fig 4.6) the Mulanje population diverged earlier than Mount Zomba from other isolated populations in the Malawi Rift. It is surprising that the Mount Zomba and Mount Mulanje populations are not closer relatives given the striking plumage similarity. It is difficult to explain this pattern, especially as each population was well sampled (Table 4.2). It is plausible that the two populations did not originate from one ancestor but through convergent evolution. In the Mount Zomba population there is no haplotype diversity in the mtDNA and no allelic diversity, this could be a result of recent colonization that might have taken place and has the potential of lowering the diversity of the new population (Clegg et al. 2002). It might also be that the population went through a severe bottleneck thus leading to an increase in inbreeding depression (Heber & Briskie 2010). *Apalis t. flavigularis* is restricted to Mount Mulanje, Mount Zomba and Mount Malosa. The population of Mount Mulanje is stable (Mzumara et al. 2012), and Mount Malosa is less than 10 km from Mount Zomba (Potiphar Kaliba per. obs.), which is likely inhabited by the same population as on Mount Zomba. Sampling Mount Malosa is required in order to fully

understand the underlying phylogeographic pattern, as well as if *A. t. flavigularis* is to be effectively managed and conserved.

The population of the subspecies *A. t. murina* in southern Tanzania (Udzungwa Mts) and in the Misuku Hills of northern Malawi form distinct clades implying the presence of a geographical break between the Udzungwa Mts and Misuku Hills (Fig 4.7). The distribution of *A. t. murina* does not extend beyond the Misuku Hills onto Nyika Plateau, despite the Misuku Hills population being closer to Nyika Plateau, with the Karonga-Chitipa Valley forming a geographical break. The distribution of *A. t. youngi* has the Nyika Plateau at its northern most extent, thereby being restricted to the central highlands of Malawi thereby creating a geographical between Nyika Plateau and the central highlands. The distribution of *A. t. whitei* extends from central Malawi encompassing Mount Dedza, to south-west of the Shire River. The population of Mount Zomba and Mount Mulanje (*A. t. flavigularis*) are distinct (Fig 4.2) revealing a geographical break between Mount Zomba and Mount Mulanje, with the Mount Zomba population not being sister to the birds from Mount Mulanje despite the birds having similar plumage (Fig 4.7). There is another break to the southwest of Mount Zomba for *A. t. whitei* that also acts as the northern most extent of *A. t. flavigularis*. *Apalis t. lynesi* restricted to the Namuli highlands is distinct indicating the presence of a phylogeographic break between Mount Namuli and Mount Mulanje, in agreement with the distinct plumage characters of birds on Mount Mulanje and Mount Namuli. The identification of these geographical breaks (Fig. 4.7) is expected considering also the high value of $\Phi_{ST,}$ and the pairwise Φ_{ST}-values which were mostly significant (Table 4.3) among the members of the *Apalis thoracica* I sampled (Fig. 4.1).

In summary, *Apalis thoracica* shows phylogeographical breaks within the Malawi Rift along the lowland gap between the Udzungwa Mts and Misuku Hills; between the Misuku Hills and Nyika Plateau; within the central highlands of Malawi; south-west of Mount Zomba; between Mount Zomba and Mount Mulanje, and between Mount Mulanje and Mount Namuli (Fig. 4.7). This is accordance with the expectation of Malawi being a transition zone (faunal turnover) between east and southern Africa taxa.

Most national parks in Malawi are located on each side of the identified phylogeographic breaks. Nyika National Park is a large park in northern Malawi making it strategically located to encompass most of the evolutionary processes north of the central break. This park comprises varied habitats that encompass both montane as well as woodland habitats (Cater et al. 1993). The majority of parks in the south and central Malawi are located in the lowlands and because these areas are generally dry and *Brachystegia* woodland, they do not encompass other important ecosystems such as the montane forests that are known to promote the accumulation of recently diverged species (Roy 1997; Fjeldså et al. 2012; this study).

The location and proclamation of most national parks in Malawi has largely been the consequence of an ad hoc approach that has not followed modern conservation planning strategies (Margules & Pressey 2000). Therefore, the existing protected areas fail to include all species which effective conservation planning seeks to achieve (Pressey 1994). Most of the places where the phylogeographic breaks occur are forest reserves that are not adequately protected with the exception of Nyika National Park where *A. t. youngi* occurs. Illegal logging and uncontrolled fires are threatening the montane 'sky islands' population, for example, *A. t. flavigularis* on Mount Mulanje (Mzumara et al.

2012), thus compromising the conservation of the birds and important evolutionary units in general.

The results of this study suggest that molecular DNA data has an important role to play in helping identify the phylogeographic breaks within the *Apalis thoracica* complex. Looking at the distribution of taxa within the Malawi Rift, important habitats for the conservation of evolutionary units are identified which will contribute to modern conservation planning (Margules & Pressey 2000) in Malawi. Therefore, *A. t. murina* (Misuku Hills), *A. t. youngi* (Nyika Plateau), *A. t. flavigularis* (Mount Zomba) and *A. t. flavigularis* (Mount Mulanje) should be managed separately because they are different populations. Further research is required to determine the status of birds sampled from Mount Dedza (*A. t. whitei*)

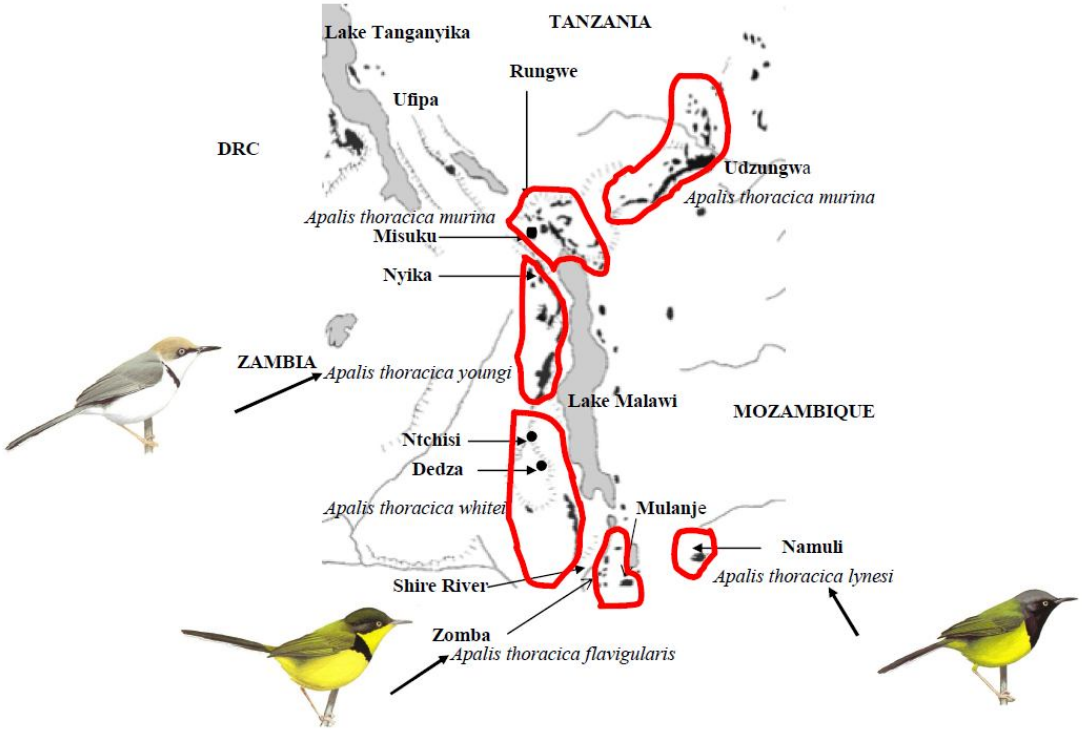


Figure 4.1 Malawi Rift depicting the distribution of *Apalis thoracica* subspecies and sampling sites. Bird images are from del Hoyo et al. (2011).

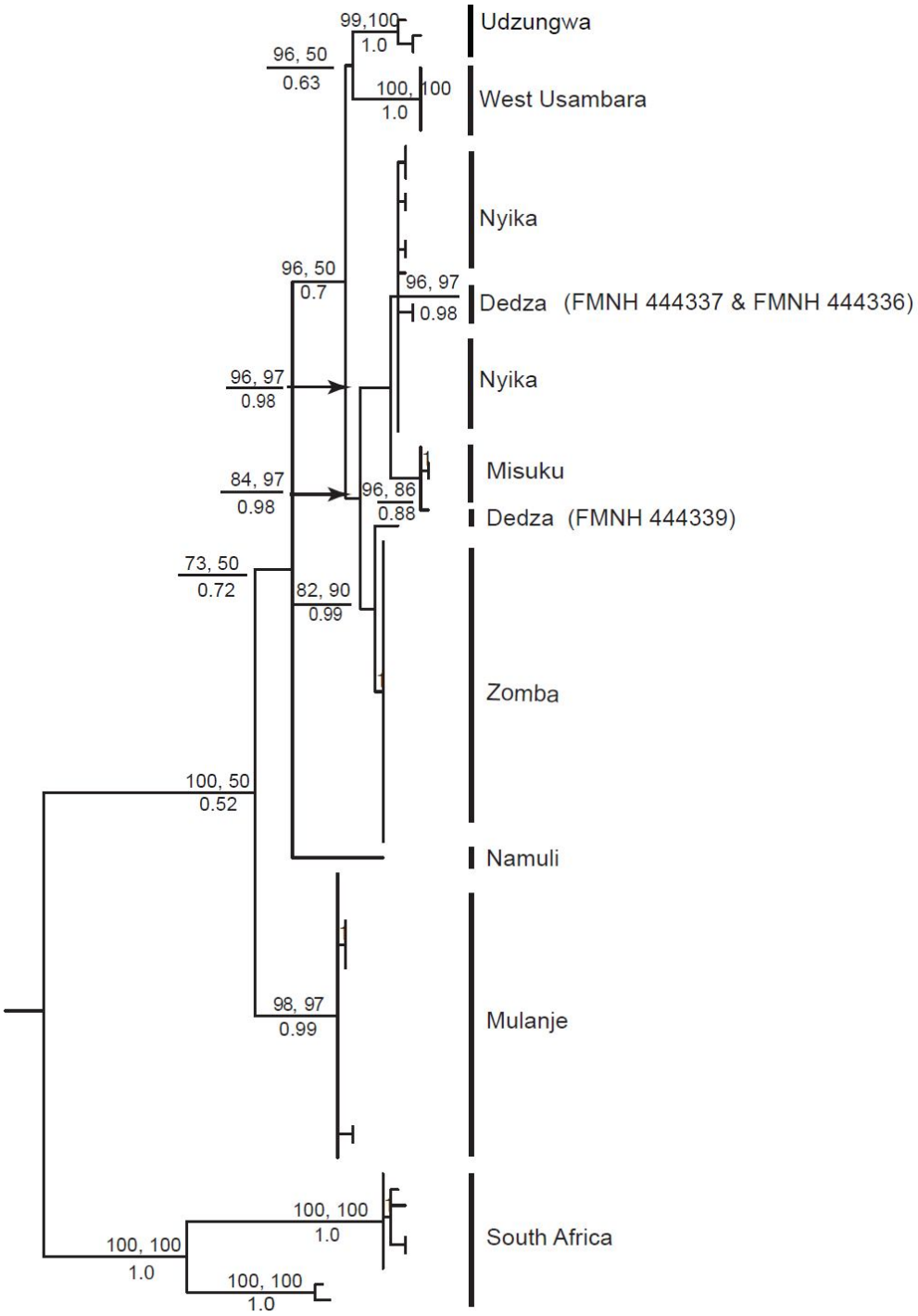


Figure 4.2 Maximum Parsimony topology depicting phylogenetic relationships among taxa in the *Apalis thoracica* species complex occurring across the Malawi Rift, as well as representative taxa to the north and south. Values above the nodes are MP and ML bootstrap support values whereas those below are posterior probabilities. Individuals from a different haplotype clade are indicated by their museum voucher number – see Appendix I.

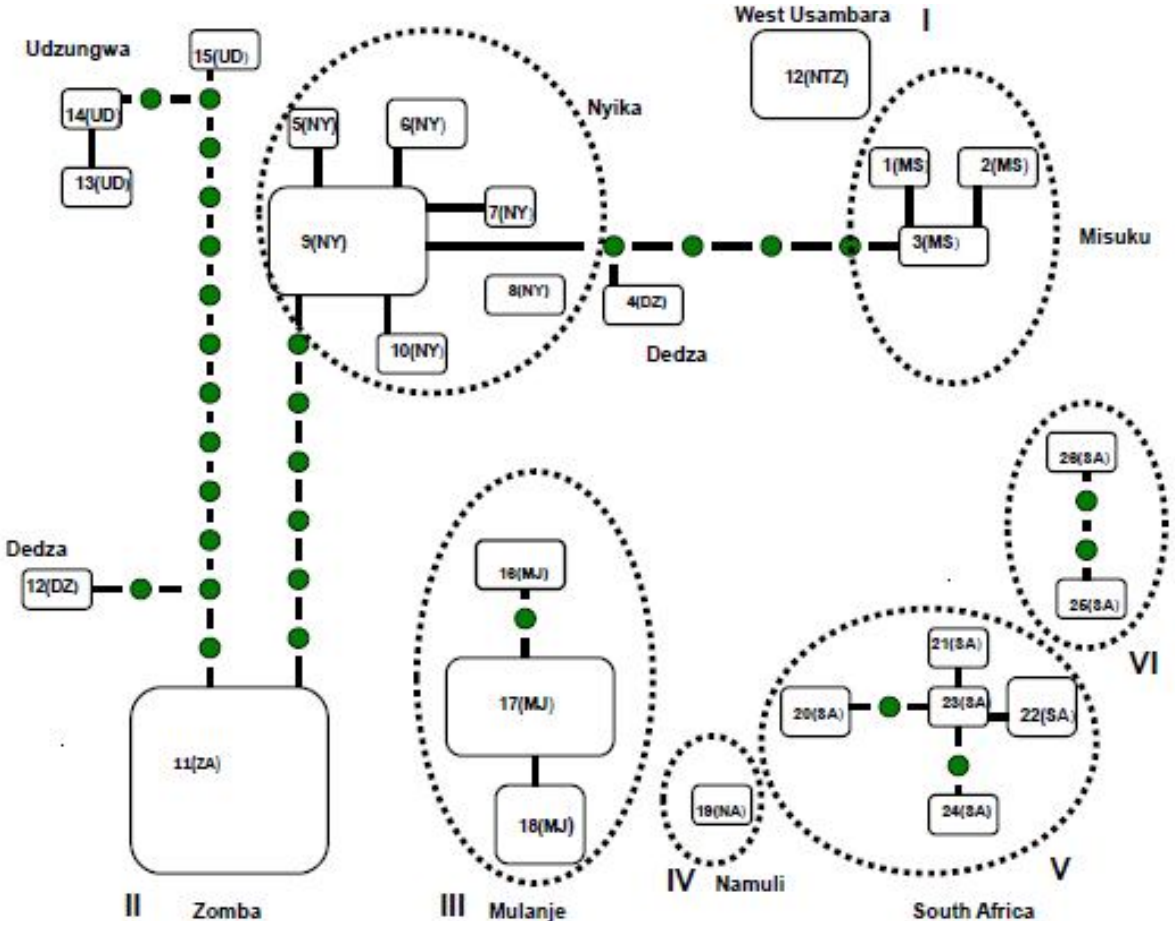


Figure 4.3 Statistical parsimony network obtained with ND2 for the 26 haplotypes of *Apalis thoracica* (Table 4.2). The subnetworks that are not connected to each other exceeded the 95% confidence limit of 14 steps. Dots indicate unsampled or extinct haplotypes. The size of each box is proportional to the frequency of the haplotype. Haplotype codes correspond to the population of origin: UD = Udzungwa, MS = Misuku, NY = Nyika, NTZ = West Usambara, DZ = Dedza, ZA = Zomba, MJ = Mulanje, NA = Namuli, and SA = South Africa.

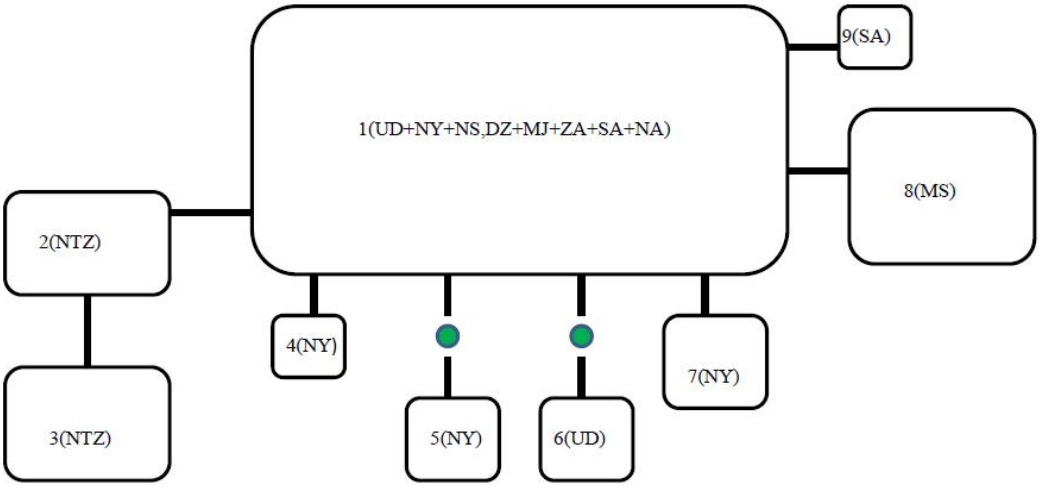


Figure 4.4 Statistical parsimony network obtained for the nine alleles of TGFb2 (Table 4. 2). The subnetworks are connected to each other after satisfying the 95% confidence limit of 10 steps. Dots indicate unsampled or extinct alleles. The size of each box is proportional to the frequency of the alleles. Allele codes correspond to the population of origin: UD = Udzungwa, MS = Misuku, NY = Nyika, NTZ = West Usambara, DZ = Dedza, ZA = Zomba, MJ = Mulanje, NA = Namuli, and SA = South Africa.

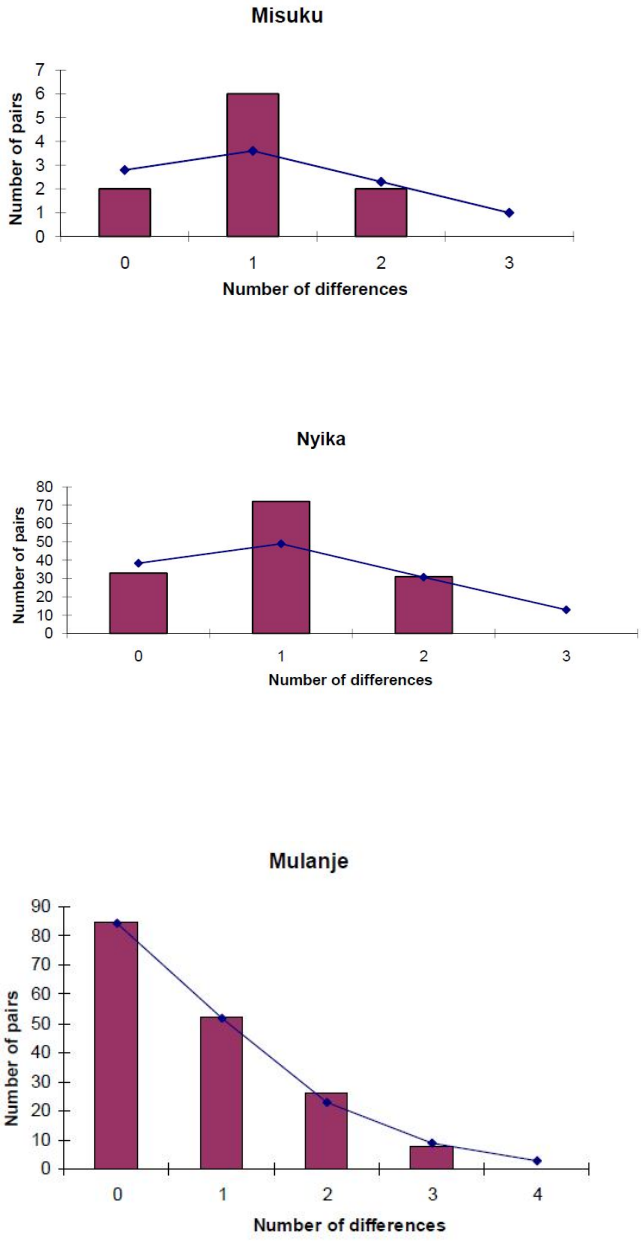


Figure 4.5 Mismatch distributions for selected *Apalis thoracica* populations. Histograms represent the observed distribution and the line the expected distribution for a growing population under the same mean.

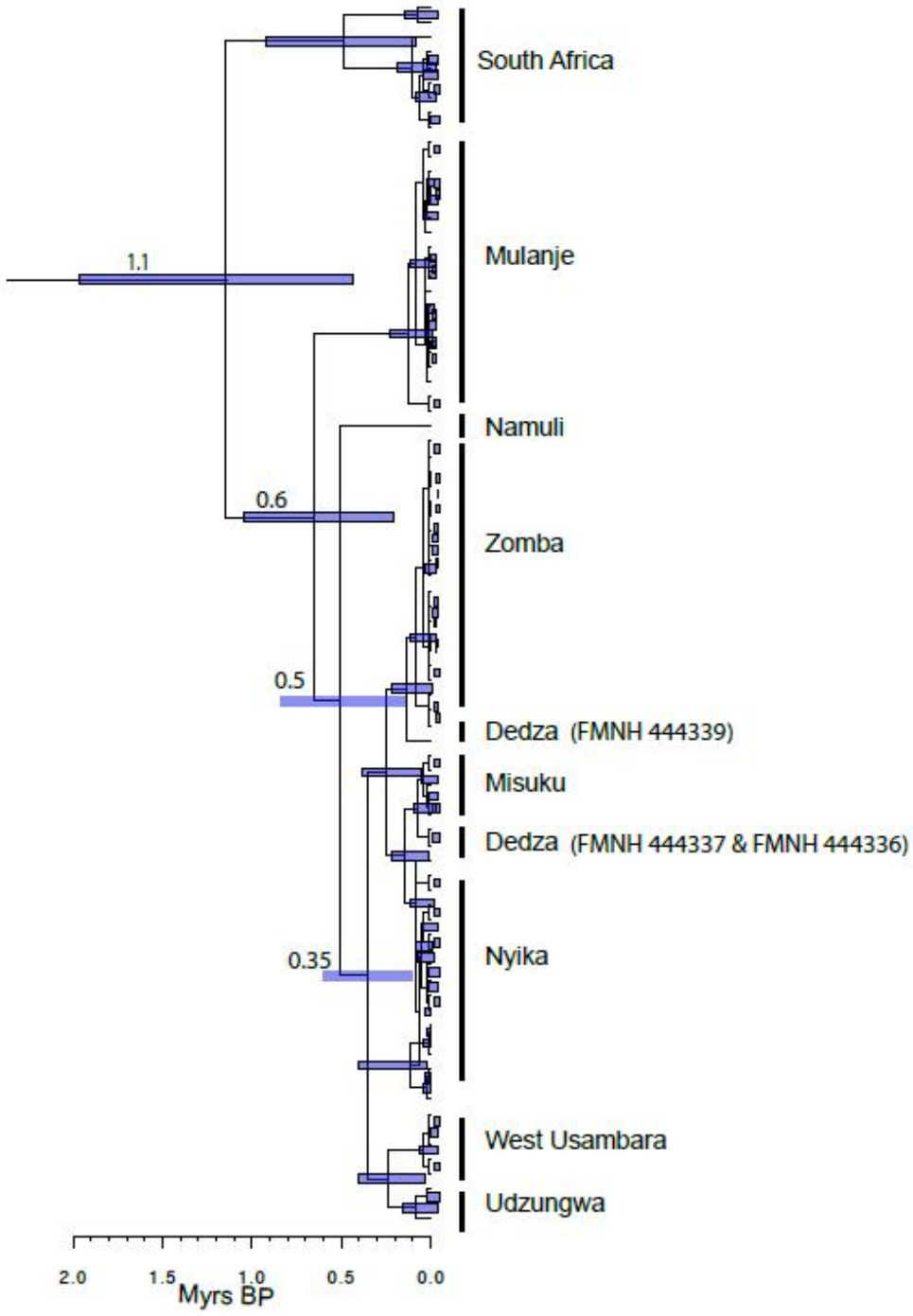


Figure 4.6 Divergence times among populations of *Apalis thoracica* in millions of years before present (Myrs BP). The blue lines represent the 95% HPD interval of divergence times as estimated using the software BEAST.

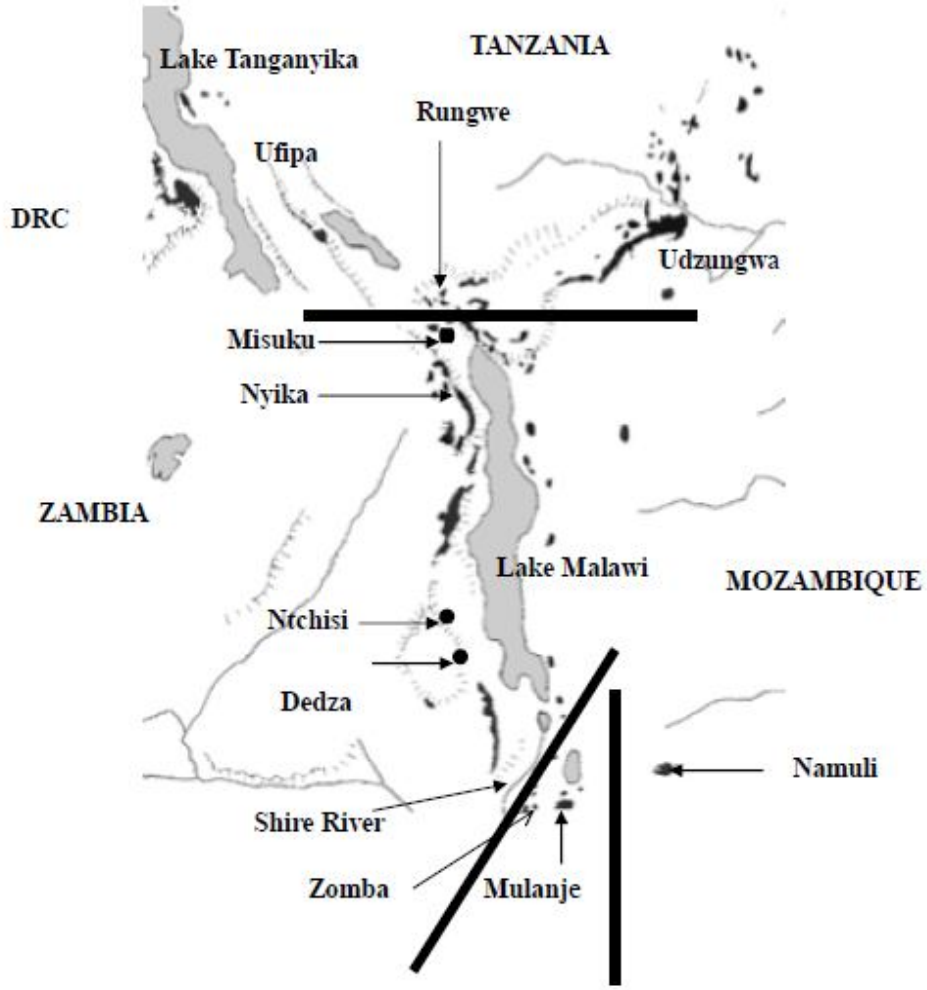


Figure 4.7 Malawi Rift showing geographical breaks (solid lines) detected among sampled populations of *Apalis thoracica*.

Table 4.1 Distribution and morphology of taxa within the East African clade of *Apalis thoracica* (modified from Erard 1997; Ryan et. al. 2006; Dowsett-Lemaire & Dowsett 2006).

Subspecies name	**Geographical distribution**	**Morphology**
Apalis thoracica murina	Northeastern Tanzania to northern Malawi (Mafinga, Wilindi and Matipa) and adjacent northeastern Zambia	Brown crown, olive-washed grey back, white under-parts with yellow belly and olive flanks.
Apalis thoracica youngi	Southwest Tanzania (Ufipa Plateau at Sumbawamba), along the northeast Malawian littoral zone (Livingstonia, Nyankhowa and Uzumara), Nyika Plateau, and South Viphya Mountains.	Brown crown, the back to rump and uppertail-coverts are clear slate grey or olive washed. The belly to undertail-coverts and flanks are clear white.
Apalis thoracica whitei	Eastern Zambia, through central Malawi (Dzalanyama to Kirk Range Mts and Ntchisi) to Mozambique (Zobue).	Birds have a brown crown, green back and the belly is washed yellow.
Apalis thoracica flavigularis	Southeast Malawi-Shire Rift (Mulanje, Zomba and Malosa).	Males have forehead, crown and sides of head black with greenish wash. The ear-coverts are dark grey, the mantle and back bright green, a black tail with the outer webs of feathers margined green. The wings are green, underwing-corverts yellowish, underparts bright yellow except for the black breast-band, and the flanks are washed olive. The females have a sooty brown crown and blackish loral streak.
Apalis thoracica lynesi	Mount Namuli in Mozambique.	Grey head and cheeks; black chin to breast which extends beyond the normal *thoracica* breast-line area. This taxon has a stronger greenish back and yellow lower underparts. Top of the head and ear-coverts are grey.

Table 4.2 Taxa analysed in this study for ND2 and TGFb2, sampling localities, geographical coordinates and frequencies of haplotypes/alleles. [ ] = haplotypes/alleles restricted to a specific population, ( ) = occurrence in more than one population.

Taxon	**Locality**	**Country**	**Sample size**	**Latitude**	**Longitude**	**Haplotypes mtDNA (ND2)**	**Alleles TGFb2**
A. t. Murina	Udzungwa	Tanzania	3	07.80S	36.41E	13[1] 14[1] 15[1]	1(4) 6[2]
A.t. Murina	Misuku	Malawi	5	09.68S	33.50E	1[1] 2[2] 3[2]	1(3) 8[7]
A.t. Youngi	Nyika	Malawi	17	10.57S	33.70E	5[1] 6[3] 7[1] 8[2] 9[8] 10[2]	1(28) 4[1] 5[2] 7[3]
A.t. Whitei	Dedza	Malawi	3	11.26S	33.55E	4[2] 12[1]	1(6)
A.t. Flavigularis	Zomba	Malawi	20	15.40S	35.30E	11[20]	1(40)
A.t. Flavigularis	Mulanje	Malawi	19	15.55S	35.38E	16[2] 17[13] 18[4]	1(38)
A.t. Lynesi	Namuli	Mozambique	1	15.39S	37.05E	19[1]	1(2)

Table 4.3 Pairwise Φ_{ST}-values of four sampled populations of *Apalis thoracica* for mtDNA (ND2) below diagonal and nDNA (TGFb2) above diagonal.

	Misuku	Nyika	Zomba	Mulanje
Misuku	-	0.51***	0.85***	0.84***
Nyika	0.81***	-	0.05**	0.04**
Zomba	0.98***	0.93***	-	0.00
Mulanje	0.78***	0.80***	0.82***	-

***P < 0.0001
**P < 0.001
*P < 0.01

Table 4.4 Measures of haplotype diversity, nucleotide diversity, and results for tests of selective neutrality (Tajima's D and Fu's Fs) based on ND2 for *Apalis thoracica*.

	Number of individuals	**Haplotype diversity**	**Nucleotide diversity**	**Tajima D**		**Fu's Fs**	
				D	**P**	**Fs**	**P**
Apalis thoracica							
Udzungwa	3	1.0000 ± 0.2722	0.002562 ± 0.002299	0	0.829	-0.341	0.186
Misuku	5	0.8000 ± 0.1640	0.000961 ± 0.000894	0.243	0.723	-0.475	0.192
Nyika	17	0.7574 ± 0.0912	0.000946 ± 0.000753	-1.077	0.159	-2.603	0.011**
Dedza	3	0.6667 ± 0.3143	0.007685 ± 0.006143	0.000	0.709	3.784	0.917
Zomba	20	0.000	0.000	0.000	1.000	-	-
Mulanje	19	0.5029 ± 0.1128	0.000719 ± 0.000620	-0.348	0.392	0.539	0.584
Namuli	1	1. 000	0.000	0.000	1.000		

**Significant values based on 10000 permutations, indicate that the population is out of equilibrium, suggestive of recent demographic change.

Appendix I: *Apalis thoracica* specimens examined in this study

Genus/Species	Locality	Coordinates	Museum number
Apalis thoracica	Nyika, Malawi	10.57S 33.70E	FMNH440762
Apalis thoracica	Nyika, Malawi	10.57S 33.70E	FMNH440767
Apalis thoracica	Nyika, Malawi	10.57S 33.70E	FMNH440771
Apalis thoracica	Nyika, Malawi	10.57S 33.70E	FMNH440774
Apalis thoracica	Nyika, Malawi	10.57S 33.70E	FMNH440776
Apalis thoracica	Nyika, Malawi	10.57S 33.70E	FMNH440768
Apalis thoracica	Nyika, Malawi	10.57S 33.70E	FMNH440775
Apalis thoracica	Nyika, Malawi	10.57S 33.70E	FMNH440778
Apalis thoracica	Misuku Hills, Malawi	09.68S 33.50E	FMNH439334
Apalis thoracica	Misuku Hills, Malawi	09.68S 33.50E	FMNH439333
Apalis thoracica	Dedza, Malawi	11.26S 33.55E	FMNH444337
Apalis thoracica	Nyika, Malawi	10.57S 33.70E	FMNH440777
Apalis thoracica	Nyika, Malawi	10.57S 33.70E	FMNH440763
Apalis thoracica	Nyika, Malawi	10.57S 33.70E	FMNH440772
Apalis thoracica	Nyika, Malawi	10.57S 33.70E	FMNH440773
Apalis thoracica	Nyika, Malawi	10.57S 33.70E	FMNH440765
Apalis thoracica	Nyika, Malawi	10.57S 33.70E	FMNH440770
Apalis thoracica	Nyika, Malawi	10.57S 33.70E	FMNH440764
Apalis thoracica	Nyika, Malawi	10.57S 33.70E	FMNH440769
Apalis thoracica	Nyika, Malawi	10.57S 33.70E	FMNH440766
Apalis thoracica	Mulanje, Malawi	15.55S 35.38E	FMNH447606
Apalis thoracica	Mulanje, Malawi	15.55S 35.38E	FMNH447605
Apalis thoracica	Mulanje, Malawi	15.55S 35.38E	FMNH447604
Apalis thoracica	Mulanje, Malawi	15.55S 35.38E	FMNH447603
Apalis thoracica	Mulanje, Malawi	15.55S 35.38E	FMNH447602
Apalis thoracica	Mulanje, Malawi	15.55S 35.38E	FMNH447601
Apalis thoracica	Mulanje, Malawi	15.55S 35.38E	FMNH447600
Apalis thoracica	Mulanje, Malawi	15.55S 35.38E	FMNH447599
Apalis thoracica	Dedza, Malawi	11.26S 33.55E	FMNH444339
Apalis thoracica	Dedza, Malawi	11.26S 33.55E	FMNH444336
Apalis thoracica	Misuku Hills, Malawi	09.68S 33.50E	FMNH452790
Apalis thoracica	Misuku Hills, Malawi	09.68S 33.50E	FMNH452791
Apalis thoracica	Misuku Hills, Malawi	09.68S 33.50E	FMNH452789
Apalis thoracica	Mulanje, Malawi	15.55S 35.38E	FMNH447613
Apalis thoracica	Mulanje, Malawi	15.55S 35.38E	FMNH447612
Apalis thoracica	Mulanje, Malawi	15.55S 35.38E	FMNH447611
Apalis thoracica	Mulanje, Malawi	15.55S 35.38E	FMNH447610
Apalis thoracica	Mulanje, Malawi	15.55S 35.38E	FMNH447609
Apalis thoracica	Mulanje, Malawi	15.55S 35.38E	FMNH447608
Apalis thoracica	Mulanje, Malawi	15.55S 35.38E	FMNH447607
Apalis thoracica	Zomba, Malawi	15.40S 35.30E	JK02-380
Apalis thoracica	Zomba, Malawi	15.40S 35.30E	GAV2510
Apalis thoracica	Zomba, Malawi	15.40S 35.30E	JK02-382
Apalis thoracica	Zomba, Malawi	15.40S 35.30E	GAV2578
Apalis thoracica	Zomba, Malawi	15.40S 35.30E	MoM2009.2.165

Apalis thoracica	Zomba, Malawi	15.40S 35.30E	MoM2009.2.125
Apalis thoracica	Zomba, Malawi	15.40S 35.30E	MoM2009.2.195
Apalis thoracica	Zomba, Malawi	15.40S 35.30E	MoM2009.2.203
Apalis thoracica	Zomba, Malawi	15.40S 35.30E	MoM2009.2.150
Apalis thoracica	Zomba, Malawi	15.40S 35.30E	MoM2009.2.19
Apalis thoracica	Zomba, Malawi	15.40S 35.30E	MoM2009.2.149
Apalis thoracica	Zomba, Malawi	15.40S 35.30E	MoM2009.2.18
Apalis thoracica	Zomba, Malawi	15.40S 35.30E	MoM2009.2.164
Apalis thoracica	Zomba, Malawi	15.40S 35.30E	MoM2009.2.93
Apalis thoracica	Zomba, Malawi	15.40S 35.30E	MoM2009.2.197
Apalis thoracica	Zomba, Malawi	15.40S 35.30E	MoM2009.2.94
Apalis thoracica	Mulanje, Malawi	15.40S 35.30E	RB1298
Apalis thoracica	Zomba, Malawi	15.40S 35.30E	RCKB1423
Apalis thoracica	Zomba, Malawi	15.40S 35.30E	RCKB1435
Apalis thoracica	Mulanje, Malawi	15.55S 35.38E	RCKB1271
Apalis thoracica	Mulanje, Malawi	15.55S 35.38E	RCKB1289
Apalis thoracica	Mulanje, Malawi	15.55S 35.38E	RCKB1304
Apalis thoracica	South Africa		JF580
Apalis thoracica	South Africa		JF587
Apalis thoracica	South Africa		JF588
Apalis thoracica	South Africa		JF609
Apalis thoracica	South Africa		JF626
Apalis thoracica	South Africa		JF651
Apalis thoracica	South Africa		JF699
Apalis thoracica	South Africa		JF662
Apalis thoracica	South Africa		JF778

CHAPTER 5

The phylogeography of the Southern Puffback and White-browed Robin-chat with a focus on populations distributed across the Malawi Rift

5.1 ABSTRACT

The study investigated phylogeographic structure of the Southern Puffback (*Dryoscopus cubla*) and White-browed Robin-chat (*Cossypha heuglini*) across the Malawi Rift. Analyses of mtDNA (1041 bp) from populations sampled throughout the Malawi Rift as well as from South Africa to the south, revealed reduced population structure for both species. Results did not identify phylogeographic breaks as revealed for forest-associated taxa in earlier chapters (Chap. 2-4). Interestingly, even for woodland-associated species geographical structuring was observed among highland populations as opposed to lowland populations. The results of this study and ongoing research suggests that molecular DNA data have an important role to play in helping to identify, manage, and conserve bird populations across the Malawi Rift.

5.2 INTRODUCTION

Plio-Pleistocene climatic fluctuations led to extensive modification of the African flora, which in turn altered the distribution patterns of diverse taxa (Axelrod & Raven 1978; deMenocal 1995; Voelker et al. 2010). For example, during periods of reduced rainfall, once continuous blocks of forest were fragmented into smaller patches that became refuges, thereby promoting divergence in allopatry of diverse vertebrate lineages.

With the return of more mesic conditions these forest "islands" expanded, simultaneously with the shrinking of savanna and broad-leaved woodland, leading to the formation of contact zones among lowland forest associated taxa as a consequence of secondary contact (Nicolas et al. 2008). Similarly, there have been repeated glacial and interglacial cycles in which species are forced to retreat into refugia by advancing ice and tundra but are able to expand from refugia during interglacial warming (Hewitt 1996). The genetic consequences of these forced movements have been modeled theoretically (Nicols & Hewitt 1994; Ibrahim et al. 1996) thereby demonstrating how different dispersal conditions may influence the underlying pattern of genetic diversity. Reduction of a once contiguous distribution range can lead to bottlenecks and the loss of genetic diversity.

The Malawi Rift extends over 900 km from the Rungwe volcanic province in southern Tanzania to the Uremba graben in Mozambique. It belongs to the western branch of the East African Rift system. The Malawi Rift is divided into five segments, from north to south are the Karonga, Nkata, Nkhotakota, Monkey Bay and Shire segments (Ebinger et al. 1993). The main feature of the Malawi Rift are several isolated mountains and high plateaus (1500-2000 meters above sea level) lakes (Lake Malawi), the narrow shore plains, and the valley of the Shire River that drains the lake to the lower course of the Zambezi River in Mozambique. The ecoregion consists of montane forests, woodlands and grassland (Clarke 1983). The rift has high phylogeographic structure of montane lineages (Chapters 2-4, Lawson 2013, Bryja et al. 2014) and high genetic diversity, features that are often attributed to the long-term (30 Myrs BP) climate stability of these highland refugia, with repeated climate fluctuations thought to have

predominantly affected intervening lowland areas (deMenocal 1995; 2004; Vincens et al. 2003; Osmaston & Harrison 2005; Ashley 2007; Mumbi et al. 2008; Lawson 2013).

This study examines the phylogeography and genetic diversity of *Dryoscopus cubla* and *Cossypha heuglini* populations distributed throughout the Malawi Rift (Fig. 1.1). Considering that these taxa are woodland species and hence are more broadly distributed than montane taxa, I expect that there will be reduced genetic structuring among the Malawi Rift populations, but that genetic diversity will likely be high due to the larger effective population size of woodland over forest-associated species. However, Fuchs et al. (2011) revealed that the open-habitat Fiscal Shrike (*Lanius collaris*) comprised phylogeographically discrete populations within its eastern African range, whereas the southern African populations exhibited lower level of geographical structuring. This study seeks to investigate phylogeographic structuring in two additional open-habitat woodland-associated bird species, the Southern Puffback (*Dryoscopus cubla* Latham 1801, Figs. 1.8 & 5.1) with one subspecies *Dryoscopus cubla hamatus* (Hartlaub 1863) and the White-browed Robin-chat *Cossypha heuglini heuglini* (Hartlaub 1866, Figs. 1.7 & 5.2) also with one subspecies distributed across the Malawi Rift.

For both *Dryoscopus cubla* and *Cossypha heuglini*, I obtained DNA sequence data from a mitochondrial (mtDNA) marker (NADH dehydrogenase subunit 2 [ND2]) in order to examine the genetic relationships and population structure in these species. Considering the temporal changes to the environment and extent of phylogeographic structure that has been uncovered in some avian lineages distributed between eastern and southern Africa (Bowie et al. 2004; 2005), I hypothesise that: phylogeographically there is reduced geographic structuring among members of *Dryoscopus cubla* and *Cossypha*

heuglini within the Malawi Rift relative to forest-associated species. To test this hypothesis, I sought to answer the following questions: (i) Does genetic turnover between east and southern African lineages occur among populations of open-habitat and specifically woodland-associated birds distributed across the Malawi Rift? (ii) Are detected geographical breaks congruent with those in forest-associated species? (iii) Where are the common phylogeographic breaks in Malawi and how do they relate to the current placement of national parks?

5.3 MATERIALS AND METHODS

5.3.1 Population Sampling

Tissue samples were obtained from 111 *Dryoscopus cubla* and 53 *Cossypha heuglini* individuals collected during research expeditions conducted in Malawi from 2001 to 2009 (see appendix J & K). Sampling was done from highlands (Misuku Hills, Nyika Plateau, Mount Ntchisi, Mount Dedza, Mount Zomba and Mount Mulanje) and lowlands (Nkhotakota, Mangochi, Mwanza, Chitipa, Vwaza and Lilongwe). In order to understand whether the faunal turnover of lineages occurred in the Malawi Rift, populations of *Dryoscopus cubla* from Mozambique and South Africa as well as *Cossypha heuglini* from South Africa were also sampled. For the sites I could not visit, I obtained preserved tissue from the Museum of Vertebrate Zoology, The Field Museum of Natural History, and the National Museum of Natural History of Denmark. The congener *Dryoscopus gambensis* was used as an outgroup for *Dryoscopus cubla*, and *Cossypha semirufa* was used as an outgroup for *Cossypha heuglini* (Voelker & Bowie unpub. data).

5.3.2 Laboratory procedures

Total genomic DNA was extracted from 0.25 g or less of muscle tissue or blood using a DNeasy Kit (QIAGEN, Germantown, MD, USA) following the manufacturer's animal tissue protocol with an overnight proteinase K digestion at 55°C. The entire 1041 base pair (bp) ND2 gene was amplified by polymerase chain reaction (PCR) with primers L5204 (5'-GCTAACAAAGCTATCGGGCCCAT-3') and H6312 (5'-CTTATTTAAGGCTTTGAAGGCC-3') (Cicero & Johnson 2001). Double-stranded PCR-amplifications were carried out in 10 µl reaction volumes containing: 2.5 µl 10 x buffer, 0.5 µl of 10 mM dNTPs, 0.5 µl of 10 mg/ml of bovine serum albumin, 0.75 µl of 50 mM $MgCl_2$, 1.25 µl of 10 µM of the forward and reverse primer in the presence of 0.25 µl of Taq polymerase (Perkin-Elmer) and genomic DNA.

ND2 was PCR-amplified with an initial denaturation step at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 7 minutes. Standard precautionary measures included negative controls (template or DNA free PCR reactions) that were used to test for the presence of any contamination.

I electrophoresed 5 µl of the PCR product on a 1% agarose gel mixed with ethidium bromide and observed it under ultraviolet light to check for the correct fragment size and to control for specificity of the PCR-amplification. The amplified DNA was purified by using either ExoSAP-IT (USB; Cleveland, OH, USA) or with GELase (Epicentre; Madison, WI), and cycle-sequenced using the ABI PRISM BigDye Terminator kit v.3.1 (Perkin-Elmer). Sephadex spin columns were utilised to clean-up the cycle-sequencing reactions (CureHunter, Oregon, USA). For each locus both the reverse

and forward directions were sequenced, using the same primers as used in the PCR-amplification, on an ABI 3730 automated DNA Analyser (Applied Biosystems).

5.3.3 Data analysis

Sequence alignment

Sequences were obtained from both strands of DNA for each individual and some individuals were sequenced several times in the event of any base ambiguity encountered. Sequence alignment was performed by computation using MAFFT (Katoh et al. 2009) and checked by eye. For ND2, sequences were checked for insertions or deletions, as well as to ensure that stop-codons were not present.

Phylogenetic analyses

Parsimony and Maximum Likelihood analyses were performed on the mitochondrial DNA (ND2) and were conducted in PAUP*10b (Swofford 2002) using a heuristic search with TBR branch-swapping and 1000 random addition replicates. Parsimony bootstrap values were obtained from 1000 pseudoreplicates, with five random addition replicates being performed for each bootstrap pseudoreplicate.

Haplotype network construction

Due to the problems that arise in the construction of intraspecific phylogenies (e.g. Posada & Crandall 2001) TCS v.1.01 was used to construct a statistical parsimony network of haplotypes (Clement et al. 2000) for each locus. The connection limit was set at 95%. The network analysis included only the ingroup samples.

Analysis of molecular variance (*AMOVA*)

In order to determine molecular variation within and among populations as well as among larger geographical regions, several hierarchical analyses of molecular variance (AMOVA) were conducted. This enabled the determination of how genetic variability was partitioned within and among major lineages by using Φ_{ST} (Excoffier et al. 1992). The levels of significance for AMOVA were obtained by using a non-parametric permutation with 10000 iterations, and was carried out using ARLEQUIN v.3.0 (Excoffier et al. 2005).

Mismatch distributions and tests of selective neutrality

Pairwise mismatch distributions and two tests of selective neutrality, Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997) were calculated to determine if populations were in mutation drift equilibrium under an infinite sites model. These indices were used as indicators of recent demographic change that could result from population expansion or contraction (Fu 1997; Rodgers & Harpending 1992). Both tests were conducted using ARLEQUIN v.3.0 (Excoffier et al. 2005).

5.4 RESULTS

5.4.1 Sequence variation

A final alignment of the entire 1041 bp of the ND2 gene was obtained from all 111 individuals of *Dryscopus cubla* included in this study. Removal of identical sequences recovered 56 unique haplotypes (Table 5.1a). Of the 1041 characters, 929 (89.24%) were constant, 22 (2.11%) were variable but parsimony uninformative, and 90 (8.65%) were parsimony informative.

A final alignment of the entire 1041 bp of the ND2 gene was obtained from all 53 individuals of *Cossypha heuglini* included in the study. Removal of identical sequences recovered 25 unique haplotypes (Table 5.1b). Of the 1041 characters, 1006 (96.64%) were constant, 12 (1.15%) were variable but parsimony uninformative, and 23 (2.21%) were parsimony informative.

5.4.2 Phylogenetic analysis

The Maximum Parsimony analyses of *D. cubla* recovered a largely unresolved polytomy of haplotypes from populations distributed across the Malawi Rift. The only exceptions were the three sampled individuals from South Africa and one individual from Ntchisi in central Malawi that formed a separate clade (tree not shown, see network below). Similarly the Maximum Parsimony analyses for *C. heuglini* also recovered a large polytomy of haplotypes from populations distributed across the Malawi Rift (tree not shown, see network below).

5.4.3 Haplotype network construction

The TCS analysis of ND2 for *Dryoscopus cubla* recovered two subnetworks (Fig. 5.4) with (1) Misuku, Nyika, Nkhotakota, Ntchisi, Lilongwe, Dedza, Mangochi, Mwanza, Zomba and Mulanje grouping together, and (2) the samples from South Africa and one individuals from Ntchisi grouping together (Figs. 5.4, A & B). There was no clear indication of substructure present across populations sampled from the Malawi Rift. On average there was considerably haplotype diversity within sampled populations of *Dryoscopus cubla*: Misuku (= 10), Nyika Plateau (= 3), Nkhotakota (= 14), Ntchisi (=

11), Dedza (= 5), Mangochi (= 2), Mwanza (= 5), and Mulanje (= 17); both individuals from Lilongwe shared the same haplotype (Table 5.1a).

The TCS analysis of ND2 for *Cossypha heuglini* recovered one subnetwork (Fig. 5.3) with Misuku, Chitipa, Nyika, Vwaza, Nkhotakota, Ntchisi, Lilongwe, Dedza, Mulanje and South Africa grouped together. Individuals from all the populations shared haplotypes with no clear indication of substructure. There was generally high haplotype diversity within populations of *Cossypha heuglini*: Misuku (= 3), Chitipa (= 2), Nyika (= 5), Vwaza (= 3) Nkhotakota (= 4), Ntchisi (= 6), Lilongwe (= 4), Dedza (= 4) and Mulanje (= 3) (Table 5.1b).

5.4.4 Analysis of molecular variance

An AMOVA for *Dryoscopus cubla* was conducted among the ten sampled populations (Table 5.1a). The genetic variation within groups was 0% (-13.33%), variation within populations was 93.37%, and variation among groups was 19.95%. Population substructure was limited as indicated by the low Φ_{ST}-value (0.066, P = 0.037). For an AMOVA conducted with the six populations with larger sample sizes (Nyika, Lilongwe, Mangochi and Zomba were removed, Table 5.1a), genetic variation within groups was 15.19%, variation within populations was 92.05%, and variation among groups was 0% (-7.24%). The Φ_{ST}-value (0.080, P < 0.001) remained low and this is supported by the pairwise Φ_{ST}-values, which are mostly significant (Table 5.2) indicating a shift in the distribution of allele frequencies between populations from northern and central Malawi relative to those among sampled populations in southern Malawi.

An AMOVA for *Cossypha heuglini* was conducted among the nine sampled populations (Table 5.1b). The genetic variation within groups was 0% (-9.55%), variation within populations was 76.84%, and variation among groups was 32.71%. Some population structure is present (Φ_{ST} = 0.232, P < 0.0001). For an AMOVA conducted on the four populations with larger sample sizes (Misuku, Chitipa, Vwaza, Nkhotakota and Lilongwe were removed, Table 5.1b) the genetic variation among groups was 29.57%, variation within groups was 0% (-3.23%), and variation within populations was 73.66%. The Φ_{ST}-value (0.263, P < 0.001) remained significant. Pairwise comparisons (Table 5.3) suggest that the southern population on Mount Mulanje retains distinct alleles relative to the central and northern populations of *C. heuglini* distributed across the Malawi Rift.

The AMOVA conducted for highland populations (Misuku, Nyika, Ntchisi, Dedza and Mulanje) in *C. heuglini*; the genetic variation within groups was 2.48%, variation within populations was 68.90%, and variation among groups was 28.62% with Φ_{ST} = 0.311, P < 0.0001. The AMOVA for the lowland populations (Chitipa, Lilongwe, Vwaza and Nkhotakota), the genetic variation within groups was 24.87%, variation within populations was 77.30%, and variation among groups was 0% (-2.17%) with Φ_{ST} = 0.227, P < 0.0001.

5.4.5 Mismatch distributions and tests of selective neutrality

Mismatch profiles which follow a modified Poisson distribution (a unimodal bell shaped curve when population expansion is recent), are thought to be associated with past events of population growth, for instance range expansion (Rogers & Harpending 1992; Harpending et al. 1993). In the six *Dryoscopus cubla* mismatch distribution profiles (Fig.

5.5) conducted for populations with large sample sizes (Misuku, Nkhotakota, Ntchisi, Dedza, Mwanza and Mulanje) and sufficient variation (Nyika and Mangochi had three individuals, Lilongwe had one haplotype and Zomba had one individual), all followed a Poisson distribution, with the possible exception of Mount Mulanje. Tajima's D and Fu's Fs statistics for Misuku, Ntchisi, Dedza and Mwanza were not significant indicating that if these populations have undergone a range expansion, it occurred a long time ago. The populations of Nkhotakota and Mulanje have Fu's Fs values that were negative and significant (Table 5.4), consistent with a more recent change in population size, but it is possible this may be a consequence of population decline rather than expansion.

In the four *Cossypha heuglini* mismatch distribution profiles (Fig. 5.6) conducted for populations with larger sample sizes (Nyika Plateau, Mount Ntchisi, Mount Dedza and Mount Mulanje) and sufficient variation (Misuku, Nkhotakota, Vwaza and Lilongwe had four individuals, and Chitipa had two individuals) a Poisson distribution could not be rejected although some of the mismatch plots appear bimodal, a result indicative of complex population dynamics.

5.5 DISCUSSION

Dryoscopus cubla

The mtDNA phylogenetic analyses recovered a large polytomy with the exception of three samples from South Africa and one sample from Ntchisi. This suggests that although isolation-by-distance may be present, southern and central Africa populations likely continue to exchange genes. Greater sampling from intervening populations in Zimbabwe and southern Mozambique is required to further test this hypothesis.

The mtDNA ND2 network analyses of the *D. cubla* populations resulted into two subnetworks that comprised Misuku-Nyika-Nkhotakota-Ntchisi-Lilongwe-Dedza-Mangochi-Mwanza-Zomba and Mulanje, respectively once more, South Africa and one Ntchisi haplotype grouped together in a distinct subnetwork (Figs. 5.4, A & B), a result consistent with the phylogeny reconstruction. The sharing of haplotypes among the sampled populations of Misuku, Nyika, Nkhotakota, Ntchisi, Lilongwe, Dedza, Mangochi, Mwanza, Zomba and Mulanje may be due to gene flow as a result of population connectivity via the more broadly distributed woodland habitats distributed across the Malawi Rift. Among the highland populations sampled, there is geographical structuring that suggests reduced gene flow. This could be due to recent fragmentation of a once more continuous range, possibly in response to anthropogenetic activities. The phylogeographic structuring observed among the highland populations as revealed by the pairwise Φ_{ST}-values (Table 5.2), is in agreement with the montane 'sky islands' across the Malawi Rift acting as important centres of faunal diversification (Carleton & Stanley 2012; Lawson 2013; Bryja et al. 2014).

Both the mismatch distribution for the *D. cubla* populations (Fig. 5.5) of Nkhotakota and Mount Mulanje, and their Fu's Fs tests were significant (Table 5.4), suggesting that the populations were either expanding, or some other complex population dynamic is in operation. Other highlands sampled (Table 5.4) revealed that the Tajima's D and Fu's Fs test were not significant suggesting that these populations are likely demographically stable.

Cossypha heuglini

The mtDNA phylogenetic analyses of *C. heuglini* haplotypes recovered a large polytomy, a result indicative of recurrent gene flow throughout the range of the species across the Malawi Rift. The mtDNA network analyses of *C. heuglini* individuals recovered one subnetwork that comprised Misuku-Chitipa-Nyika-Vwaza-Nkhotakota-Ntchisi-Lilongwe-Dedza-Mulanje and South Africa. It is interesting to note that individuals from Mount Mulanje do not share haplotypes with other populations, re-enforcing the view that the southern highlands exhibit fine scale phylogeographic structure. The mismatch distribution for *C. heuglini* populations of Nkhotakota and Mount Mulanje and their associated Fu's Fs tests were significant, suggesting that the populations are likely to be presently expanding.

Faunal turnover and comparative phylogeography in D. cubla and C. heuglini across the Malawi Rift.

The sampled populations of *D. cubla* and *C. heuglini* distributed across the Malawi Rift are not reciprocally monophyletic. This is an indication of the expected more continuous distribution of woodland-associated species across the Malawi Rift. However, there is some phylogeographic structuring in both species among the highland populations. For instance, the Mount Mulanje population of *C. heuglini* does not share haplotypes with any other population, indicating that the two woodland-associated species studied may be subject to different historical processes, although this requires further investigation, especially with respect to the use of nuclear DNA markers.

Both *D. cubla* and *C. heuglini* exhibit reduced phylogeographic structure compared to forest-associated species (chapter 2-4 of this thesis). This is an indication that studies of faunal turnover within the Malawi Rift are more directly reflected in forest species, and that the fragmentary "sky island" nature of the montane biome across the Malawi Rift may lead to higher species diversification in allopatry as compared to within bird species more continuously distributed across woodland biomes.

In Malawi there are four national parks Lengwe and Liwonde in the south, Kasungu in central Malawi and Nyika in northern Malawi. Most of these national parks are located in the lowlands which are characterised by *Brachystegia* woodland. They are generally dry and do not encompass other important biomes such as the montane forests that are known to promote the accumulation of recently diverged species (Roy 1997; Fjeldså et al. 2012; this study). However, only Nyika National Park, which is the largest park, is strategically located to encompass important evolutionary processes north of the Malawi Rift. This park comprises varied habitats that encompass both montane as well as woodland biomes (Cater et al. 1993).

The proclamation of most national parks in Malawi has largely been the consequence of an ad hoc approach that has not typically followed modern conservation planning strategies (Margules & Pressey 2000). The existing protected areas fail to include all species or evolutionary lineages which effective conservation planning seeks to achieve (Pressey 1994). Most of the places where the phylogeographic breaks occur are forest reserves that are not adequately protected with the exception of Nyika National Park where both *D. cubla* and *C. heuglini* occur. Deforestation and uncontrolled fires are threatening faunal populations, for both birds and small mammals (Mzumara et al. 2012;

Bryja et al. 2014) within the rift, thereby compromising the conservation of fauna and important evolutionary units in general.

The results of this study suggest that molecular DNA data has an important role to play in helping identify phylogeographic breaks within *D. cubla and C. heuglini*. Looking at the distribution of taxa within the Malawi Rift, important habitats for the conservation of evolutionary units are identified, which will contribute to modern conservation planning in Malawi.

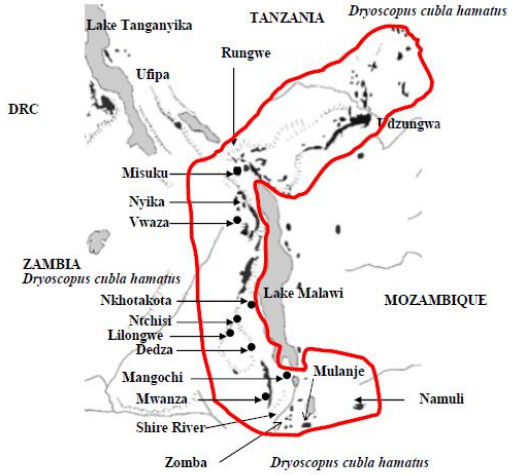


Figure 5.1 The Malawi Rift depicting the distribution of *Dryoscopus cubla* and sampling sites.

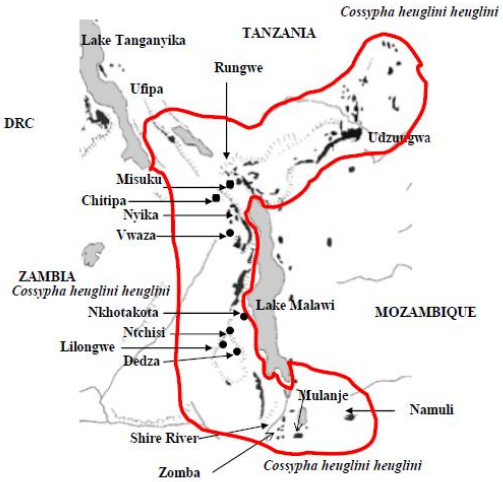


Figure 5.2 The Malawi Rift depicting the distribution of *Cossypha heuglini* and sampling sites.

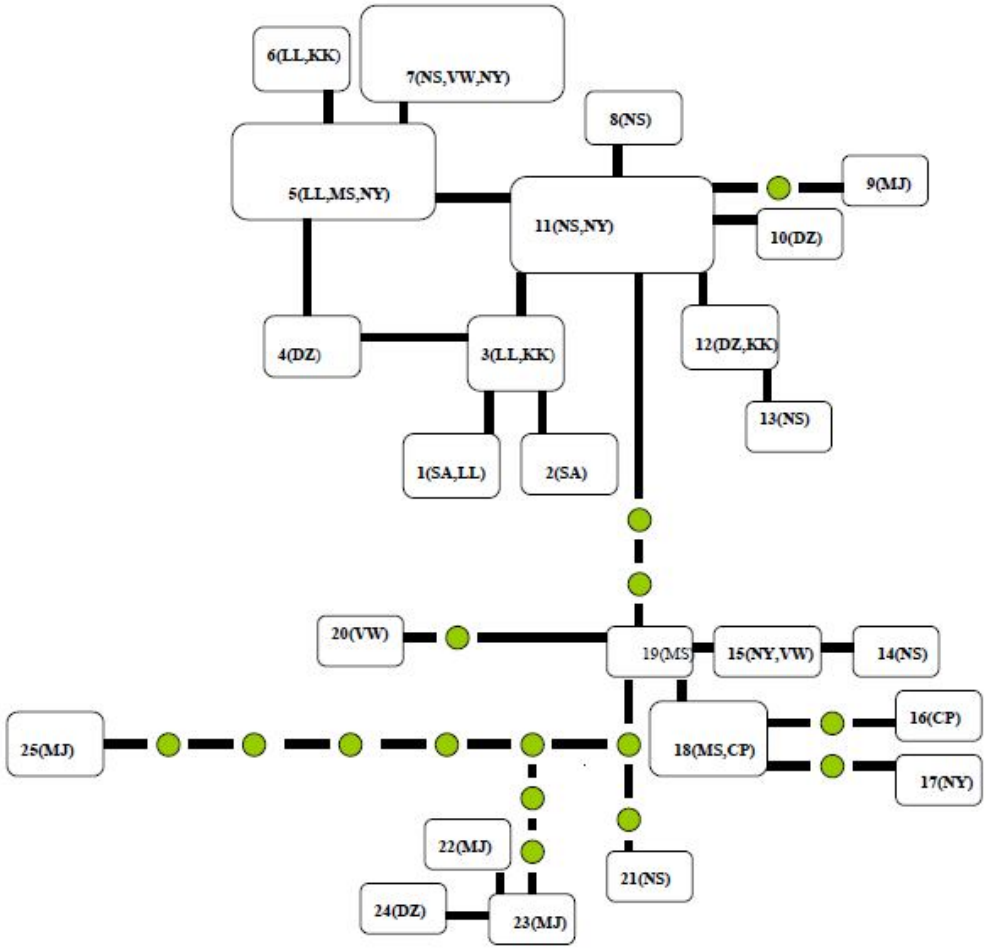


Figure 5.3 Statistical parsimony network obtained with ND2 for the 25 haplotypes of *Cossypha heuglini* (Table 5.1b). The subnetworks are connected to each other having not exceeded the 95% confidence limit of 14 steps. Dots indicate unsampled or extinct haplotypes. The size of each box is proportional to the frequency of the haplotype. Haplotype codes correspond to the population of origin: MS = Misuku, CP = Chitipa, NY = Nyika, VW = Vwaza, KK = Nkhotakota, NS = Ntchisi, LL = Lilongwe, DZ = Dedza, MJ = Mulanje, SA = South Africa.

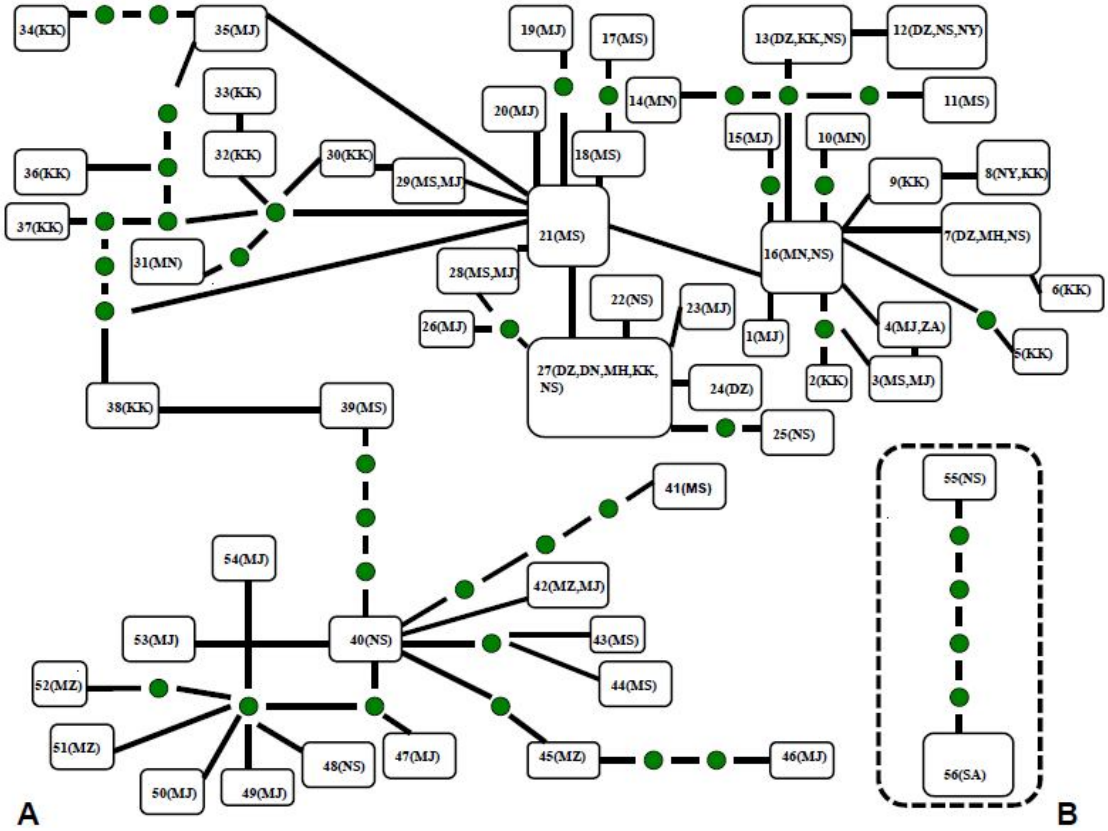
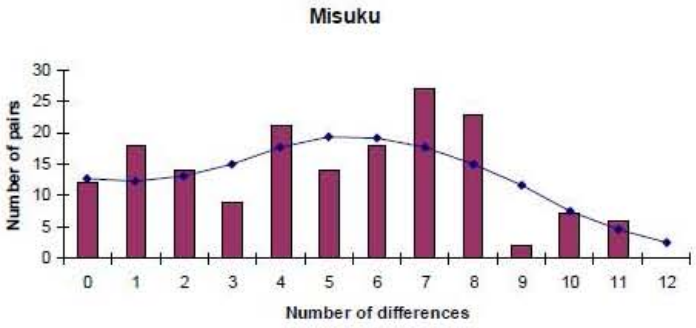
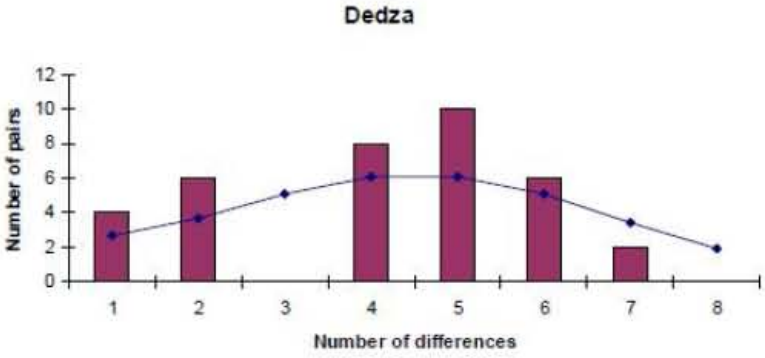
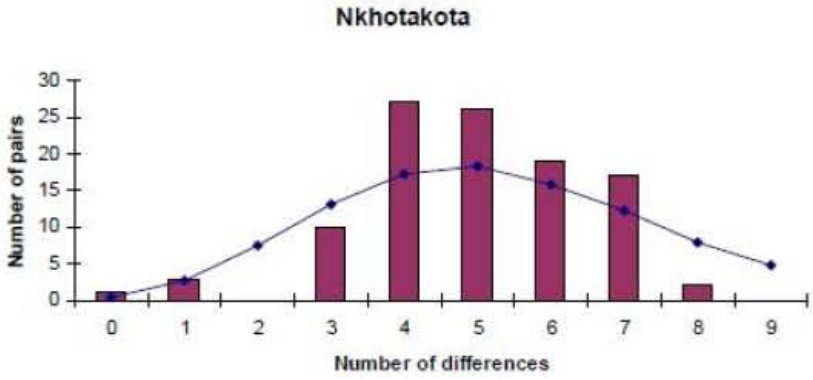


Figure 5.4 Statistical parsimony network obtained with ND2 for the 25 haplotypes of *Dryoscopus cubla* (Table 5.1a). The subnetworks are not connected to each other having exceeded the 95% confidence limit of 14 steps. Dots indicate unsampled or extinct haplotypes. The size of each box is proportional to the frequency of the haplotype. Haplotype codes correspond to the population of origin: MS = Misuku, NY = Nyika, KK = Nkhotakota, NS = Ntchisi, LL = Lilongwe, DZ = Dedza, MH = Mangochi, MN = Mwanza, ZA = Zomba, MJ = Mulanje, MZ = Mozambique, SA = South Africa.


Misuku
Number of pairs
30
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Number of differences


Dedza
Number of pairs
12
10
8
6
4
2
0
1 2 3 4 5 6 7 8
Number of differences


Nkhotakota
Number of pairs
30
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9
Number of differences

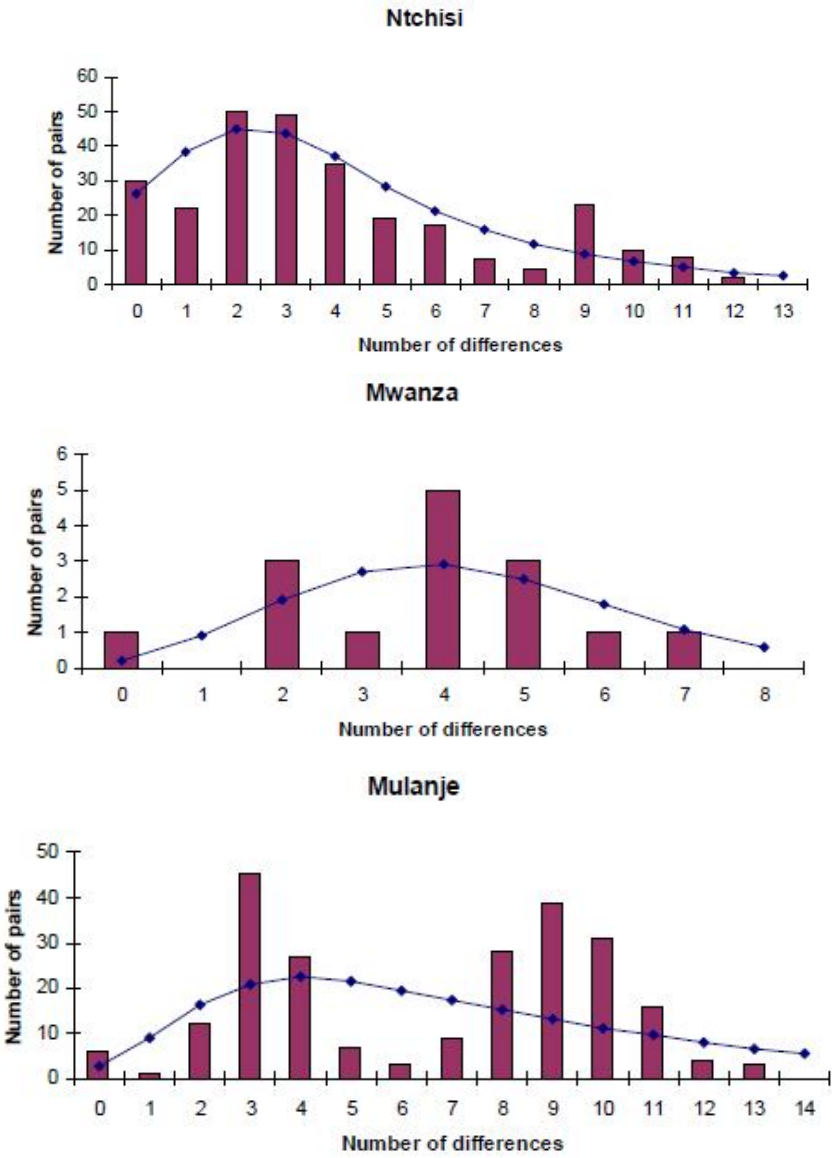


Figure 5.5 Mismatch distributions for selected *Dryoscopus cubla* populations. Histograms represent the observed distributions and the line the expected distribution for a growing population under the same mean.

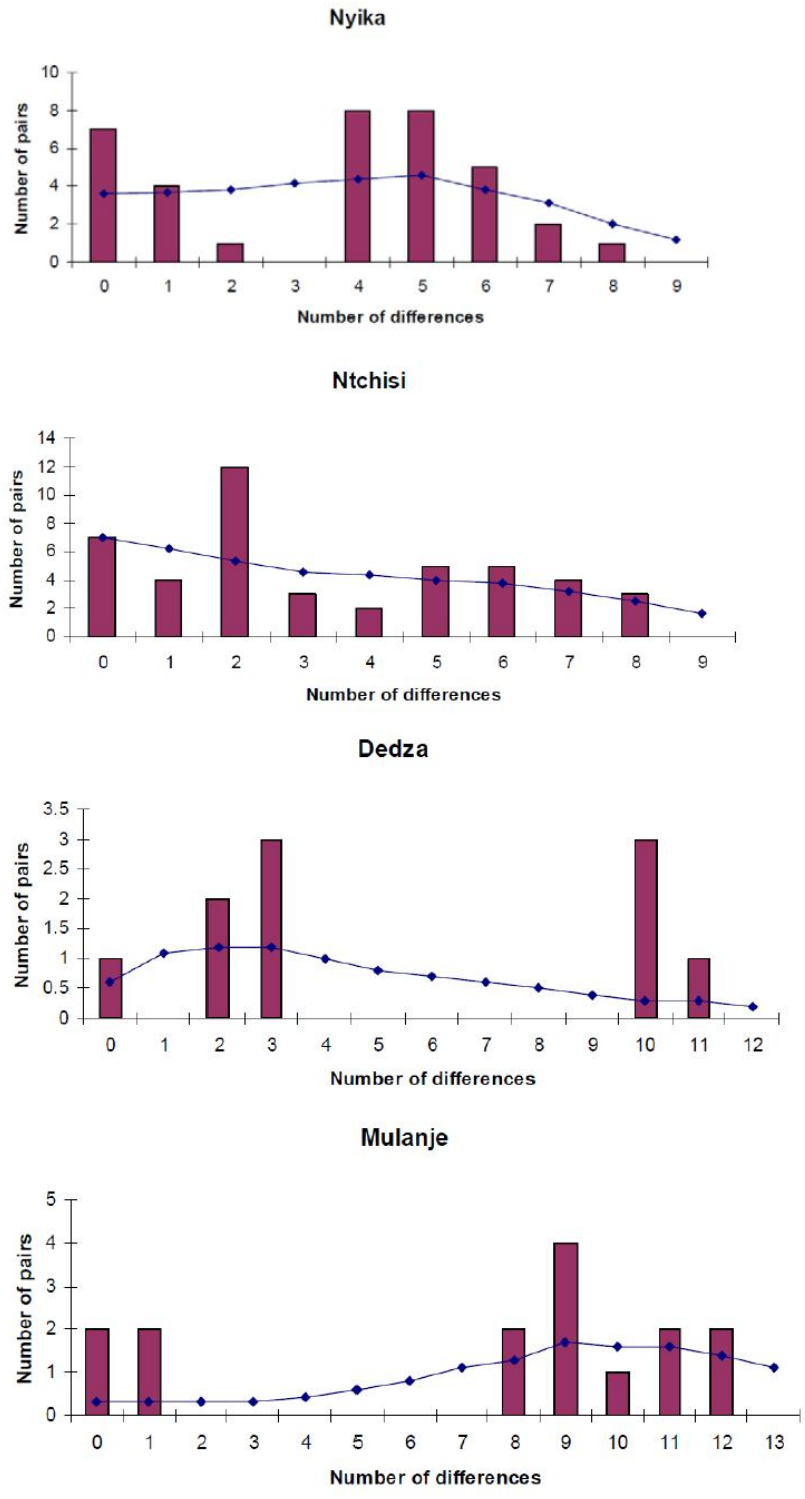


Figure 5.6 Mismatch distributions for selected *Cossypha heuglini* populations. Histograms represent the observed distributions and the line the expected distribution for a growing population under the same mean.

Table 5.1a Taxa analysed in this study for ND2, sampling localities, geographical coordinates and frequencies of haplotypes. [ ] = haplotypes restricted to a specific population, ( ) = occurrence in more than one population.

Species	Locality	Country	Sample size	Latitude	Longitude	Haplotypes mtDNA (ND2)
Dryoscopus cubla	Misuku	Malawi	19	09.68S	33.50E	3(1) 11[2] 17[1] 18[3] 21[3] 28(3) 39[1] 41[2] 43[1] 44[2]
	Nyika	Malawi	3	10.57S	33.70E	8(1) 12(1) 29(1)
	Nkhotakota	Malawi	15	12.50S	34.02E	2[1] 5[1] 6[1] 8(1) 9[1] 13(1) 27(1) 30[1] 32[2] 33[1] 34[1] 36[1] 37[1] 38[1]
	Ntchisi	Malawi	25	13.38S	34.00E	7(3) 12(1) 13(2) 16(2) 22[2] 25[2] 27(7) 29(2) 40[1] 48[2] 55[1]
	Lilongwe	Malawi	2	14.18S	33.33E	27(2)
	Dedza	Malawi	9	11.26S	33.55E	7(2) 12(1) 13(2) 24[2] 27(2)
	Mangochi	Malawi	3			7(1) 27(2)
	Mwanza	Malawi	6			10[1] 14[1] 16(1) 29(2) 31[1]
	Zomba	Malawi	1	15.40S	35.30E	4(1)
	Mulanje	Malawi	22	15.55S	35.38E	1[1] 3(1) 4(1) 15[1] 19[1] 20[1] 23[1] 26[1] 28(1) 35[2] 42(1) 46[1] 47[2] 49[1] 50[3] 53[2] 54[1]

Table 5.1b Taxa analysed in this study for ND2, sampling localities, geographical coordinates and frequencies of haplotypes. [ ] = haplotypes restricted to a specific population, ( ) = occurrence in more than one population.

Species	**Locality**	**Country**	**Sample size**	**Latitude**	**Longitude**	**Haplotypes mtDNA (ND2)**
Cossypha heuglini	Misuku	Malawi	4	09.68S	33.50E	5(1) 18(2) 19[1]
	Chitipa	Malawi	2	9.58S	33.20E	16[1] 18(1)
	Nyika	Malawi	9	10.57S	33.70E	5(2) 7(1) 11(1) 15(4) 17[1]
	Vwaza	Malawi	4	11.08S	33.39E	7(1) 15(1) 20[2]
	Nkhotakota	Malawi	4	12.50S	34.02E	3(1) 6(1) 7(1) 12(1)
	Ntchisi	Malawi	10	13.38S	34.00E	7(2) 8[1] 11(4) 13[1] 14[1] 21[1]
	Lilongwe	Malawi	4	14.18S	33.33E	1(1) 3(1) 5(1) 6(1)
	Dedza	Malawi	5	11.26S	33.55E	4[1] 10[1] 12(2) 24[1]
	Mulanje	Malawi	6	15.55S	35.38E	9[1] 22[2] 23[1] 25[2]

Table 5.2 Pairwise Φ_{ST}-values of six sampled populations of *Dryoscopus cubla* mtDNA (ND2).

	Misuku	Nkhotakota	Ntchisi	Dedza	Mulanje	Mwanza
Misuku	-					
Nkhotakota	0.08**	-				
Ntchisi	0.04*	0.04*	-			
Dedza	0.16**	0.10*	-0.01	-		
Mulanje	0.06*	0.16**	0.09**	0.23**	-	
Mwanza	0.05	0.01	-0.02	0.08	0.14*	-

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**P < 0.001
*P < 0.01

Table 5.3 Pairwise Φ_{ST}-values of four sampled populations of *Cossypha heuglini* mtDNA (ND2).
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	Nyika	Ntchisi	Dedza	Mulanje
Nyika	-			
Ntchisi	0.07	-		
Dedza	0.10	-0.03	-	
Mulanje	0.35**	0.40**	0.25*	-

**P < 0.001
*P < 0.01

Table 5.4 Measures of haplotype diversity, nucleotide diversity and results for tests of selective neutrality (Tajima's D and Fu's Fs) based on ND2 for *Dryoscopus cubla*.

	Number of individuals	**Haplotype diversity**	**Nucleotide diversity**	**Tajima D**		**Fu's Fs**	
				D	P	Fs	P
Dryoscopus cubla							
Misuku	19	0.9298 ± 0.0309	0.004842 ± 0.002749	-0.458	0.360	-1.031	0.313
Nyika	3	1.0000± 0.2722	0.0043830 ± 0.003746	000	0.756	0.308	0.381
Nkhotakota	15	0.9905 ± 0.0281	0.004757 ± 0.002750	-1.366	0.076	-8.993	0.001**
Ntchisi	25	0.9000 ± 0.0411	0.007925 ± 0.004235	-2.111	0.005	0.977	0.689
Lilongwe	2	0	0	0	1.000	-	-
Dedza	9	0.8889 ± 0.0713	0.002989 ± 0.001939	0.928	0.836	0.127	0.506
Mangochi	3	0.6667 ± 0.3143	0.001921 ± 0.001811	0	0.878	1.609	0.693
Mwanza	6	0.9333 ± 0.1217	0.003650 ± 0.002465	-0.795	0.271	-0.893	0.212
Zomba	1	1.0000	0	0	1.000	-	-
Mulanje	22	0.9740 ± 0.0217	0.006371 ± 0.003489	0.943	0.177	-6.647	0.007**

**Significant values based on 10000 permutations indicate that the population is out of equilibrium, suggestive of recent demographic change.

Table 5.5 Measures of haplotype diversity, nucleotide diversity and results for tests of selective neutrality (Tajima's D and Fu's Fs) based on ND2 for *Cossypha heuglini*.

	Number of individuals	**Haplotype diversity**	**Nucleotide diversity**	**Tajima D**		**Fu's Fs**	
				D	P	Fs	P
Cossypha heuglini							
Misuku	4	0.8333 ± 0.2224	0.002562 ± 0.002040	-0.212	0.560	0.556	0.548
Chitipa	2	1.0000± 0.5000	0.001921 ± 0.002353	0	1.000	0.693	0.370
Nyika	9	0.8056 ± 0.1196	0.003469 ± 0.002201	0.417	0.689	0.458	0.578
Vwaza	4	0.8333 ± 0.2224	0.004163 ± 0.003099	-0.069	0.604	1.285	0.677
Nkhotakota	4	1.0000 ± 0.1768	0.002562 ± 0.002040	-0.212	0.571	-1.414	0.061
Ntchisi	10	0.8444 ± 0.1029	0.003245 ± 0.002051	-1.206	0.126	-0.533	0.342
Lilongwe	4	1.0000 ± 0.1768	0.002241 ± 0.001826	0.650	0.804	-1.622	0.045
Dedza	5	0.9000 ± 0.1610	0.005187 ± 0.003515	-0.978	0.183	0.612	0.530
Mulanje	6	0.8667 ± 0.1291	0.007045 ± 0.004438	0.288	0.616	1.931	0.805

**Significant values based on 10000 permutations indicate that the population is out of equilibrium, suggestive of recent demographic change.

Appendix J: *Cossypha heuglini* specimens examined in this study

Genus/Species	Locality	Coordinates	Museum number
Cossypha heuglini	Dedza, Malawi	11.26S 33.55E	FMNH444276
Cossypha heuglini	Lilongwe, Malawi	14.18S 33.33E	FMNH444273
Cossypha heuglini	Lilongwe, Malawi	14.18S 33.33E	FMNH444280
Cossypha heuglini	Ntchisi, Malawi	13.38S 34.00E	FMNH444272
Cossypha heuglini	Dedza, Malawi	11.26S 33.55E	FMNH444268
Cossypha heuglini	Ntchisi, Malawi	13.38S 34.00E	FMNH444278
Cossypha heuglini	Ntchisi, Malawi	13.38S 34.00E	FMNH444269
Cossypha heuglini	Ntchisi, Malawi	13.38S 34.00E	FMNH444279
Cossypha heuglini	Dedza, Malawi	11.26S 33.55E	FMNH444267
Cossypha heuglini	Lilongwe, Malawi	14.18S 33.33E	FMNH444274
Cossypha heuglini	Ntchisi, Malawi	13.38S 34.00E	FMNH444277
Cossypha heuglini	Ntchisi, Malawi	13.38S 34.00E	FMNH444270
Cossypha heuglini	Dedza, Malawi	11.26S 33.55E	FMNH444265
Cossypha heuglini	Ntchisi, Malawi	13.38S 34.00E	FMNH444271
Cossypha heuglini	Lilongwe, Malawi	14.18S 33.33E	FMNH444275
Cossypha heuglini	Dedza, Malawi	11.26S 33.55E	FMNH444266
Cossypha heuglini	Mulanje, Malawi	15.55S 35.38E	FMNH447528
Cossypha heuglini	Mulanje, Malawi	15.55S 35.38E	FMNH447529
Cossypha heuglini	Chitipa, Malawi	09.58S 33.20E	FMNH452723
Cossypha heuglini	Chitipa, Malawi	09.58S 33.20E	FMNH452722
Cossypha heuglini	Nkhotakota, Malawi	12.50S 34.02E	MLW009NK
Cossypha heuglini	Nkhotakota, Malawi	12.50S 34.02E	MLW010NK
Cossypha heuglini	Nkhotakota, Malawi	12.50S 34.02E	MoM2007.2.121
Cossypha heuglini	Nkhotakota, Malawi	12.50S 34.02E	GAV3110
Cossypha heuglini	Mulanje, Malawi	15.55S 35.38E	DHB4329
Cossypha heuglini	Mulanje, Malawi	15.55S 35.38E	JK02-353
Cossypha heuglini	Mulanje, Malawi	15.55S 35.38E	GMS265
Cossypha heuglini	Mulanje, Malawi	15.55S 35.38E	JMD557
Cossypha heuglini	Ntchisi, Malawi	13.38S 34.00E	RB1089
Cossypha heuglini	Ntchisi, Malawi	13.38S 34.00E	MLW-B16
Cossypha heuglini	Ntchisi, Malawi	13.38S 34.00E	MoM2007.2.186
Cossypha heuglini	Ntchisi, Malawi	13.38S 34.00E	MoM2007.2.209
Cossypha heuglini	South Africa		GO228
Cossypha heuglini	South Africa		AR36
Cossypha heuglini	South Africa		AR35
Cossypha heuglini	South Africa		JF2610
Cossypha heuglini	South Africa		HS258
Cossypha heuglini	Misuku Hills, Malawi	09.68S 33.50E	FMNH439314
Cossypha heuglini	Misuku Hills, Malawi	09.68S 33.50E	FMNH439313
Cossypha heuglini	Misuku Hills, Malawi	09.68S 33.50E	FMNH439311
Cossypha heuglini	Misuku Hills, Malawi	09.68S 33.50E	FMNH439312
Cossypha heuglini	Nyika, Malawi	10.57S 33.70E	FMNH440622
Cossypha heuglini	Nyika, Malawi	10.57S 33.70E	FMNH440623
Cossypha heuglini	Nyika, Malawi	10.57S 33.70E	FMNH440624
Cossypha heuglini	Nyika, Malawi	10.57S 33.70E	FMNH440621
Cossypha heuglini	Nyika, Malawi	10.57S 33.70E	FMNH440625

Appendix K: *Dryoscopus cubla* specimens examined in this study

Genus/Species	Locality	Coordinates	Museum number
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	FMNH444477
Dryoscopus cubla	Dedza, Malawi	11.26S 33.55E	FMNH444474
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	FMNH444482
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	FMNH444476
Dryoscopus cubla	Dedza, Malawi	11.26S 33.55E	FMNH444479
Dryoscopus cubla	Lilongwe, Malawi	14.18S 33.33E	FMNH444478
Dryoscopus cubla	Dedza, Malawi	11.26S 33.55E	FMNH444480
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	FMNH444485
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	FMNH444484
Dryoscopus cubla	Dedza, Malawi	11.26S 33.55E	FMNH444481
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	FMNH444483
Dryoscopus cubla	Dedza, Malawi	11.26S 33.55E	FMNH444475
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	FMNH447878
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	FMNH447879
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	FMNH447880
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	FMNH447881
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	FMNH447882
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	FMNH447883
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	FMNH447884
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	FMNH447885
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	FMNH447886
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	FMNH447887
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	FMNH447888
Dryoscopus cubla	Misuku, Malawi	09.68S 33.50E	FMNH452945
Dryoscopus cubla	Misuku, Malawi	09.68S 33.50E	FMNH452937
Dryoscopus cubla	Misuku, Malawi	09.68S 33.50E	FMNH452947
Dryoscopus cubla	Misuku, Malawi	09.68S 33.50E	FMNH452948
Dryoscopus cubla	Misuku, Malawi	09.68S 33.50E	FMNH452940
Dryoscopus cubla	Misuku, Malawi	09.68S 33.50E	FMNH452939
Dryoscopus cubla	Misuku, Malawi	09.68S 33.50E	FMNH452942
Dryoscopus cubla	Misuku, Malawi	09.68S 33.50E	FMNH452941
Dryoscopus cubla	Misuku, Malawi	09.68S 33.50E	FMNH452943
Dryoscopus cubla	Misuku, Malawi	09.68S 33.50E	FMNH452944
Dryoscopus cubla	Misuku, Malawi	09.68S 33.50E	FMNH452946
Dryoscopus cubla	Misuku, Malawi	09.68S 33.50E	FMNH452938
Dryoscopus cubla	Nkhotakota,Malawi	12.50S 34.02E	MLW008NK
Dryoscopus cubla	Nkhotakota,Malawi	12.50S 34.02E	MLW030NK
Dryoscopus cubla	Nkhotakota,Malawi	12.50S 34.02E	MLW052NK
Dryoscopus cubla	Nkhotakota,Malawi	12.50S 34.02E	MLW171NK
Dryoscopus cubla	Nkhotakota,Malawi	12.50S 34.02E	MLW106NK
Dryoscopus cubla	Nkhotakota,Malawi	12.50S 34.02E	MLW119NK
Dryoscopus cubla	Nkhotakota,Malawi	12.50S 34.02E	MLW122NK
Dryoscopus cubla	Nkhotakota,Malawi	12.50S 34.02E	MLW132NK
Dryoscopus cubla	Nkhotakota,Malawi	12.50S 34.02E	MoM2007.2.18
Dryoscopus cubla	Nkhotakota,Malawi	12.50S 34.02E	MoM2007.2.62

Dryoscopus cubla	Nkhotakota,Malawi	12.50S 34.02E	MoM2007.2.71
Dryoscopus cubla	Nkhotakota,Malawi	12.50S 34.02E	MoM2007.2.87
Dryoscopus cubla	Nkhotakota,Malawi	12.50S 34.02E	MoM2007.2.97
Dryoscopus cubla	Nkhotakota,Malawi	12.50S 34.02E	MoM2007.2.119
Dryoscopus cubla	Nkhotakota,Malawi	12.50S 34.02E	MoM2007.2.129
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	GAV3170
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	GAV3174
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	FMNH390099
Dryoscopus cubla	Nyika, Malawi	10.57S 33.70E	FMNH441071
Dryoscopus cubla	Nyika, Malawi	10.57S 33.70E	FMNH441070
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	MLW984
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	MLW1042
Dryoscopus cubla	Dedza, Malawi	11.26S 33.55E	MLW834
Dryoscopus cubla	Dedza, Malawi	11.26S 33.55E	MoM2005.1.7
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	MoM2005.1.180
Dryoscopus cubla	Misuku, Malawi	09.68S 33.50E	FMNH439240
Dryoscopus cubla	Mozambique, Zambezia		FMNH438578
Dryoscopus cubla	Misuku	09.68S 33.50E	FMNH439234
Dryoscopus cubla	Mozambique, Zambezia		FMNH438575
Dryoscopus cubla	South Africa, Kwazulu Natal		FMNH390100
Dryoscopus cubla	Dedza, Malawi	11.26S 33.55E	MLW767
Dryoscopus cubla	Misuku, Malawi	09.68S 33.50E	MoM2003.2.91
Dryoscopus cubla	Misuku, Malawi	09.68S 33.50E	FMNH439241
Dryoscopus cubla	South Africa, Kwazulu Natal		FMNH390101
Dryoscopus cubla	Mozambique, Zambezia		FMNH438576
Dryoscopus cubla	Lilongwe, Malawi	14.18S 33.33E	MoM2005.1.201
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	MLW1137
Dryoscopus cubla	Nyika, Malawi	10.57S 33.70E	MLW447
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	MoM2005.1.150
Dryoscopus cubla	Misuku, Malawi	09.68S 33.50E	FMNH439239
Dryoscopus cubla	Misuku, Malawi	09.68S 33.50E	FMNH439235
Dryoscopus cubla	Misuku, Malawi	09.68S 33.50E	FMNH439238
Dryoscopus cubla	Mozambique, Zambezia		FMNH438577
Dryoscopus cubla	Dedza, Malawi	11.26S 33.55E	MoM2005.1.41
Dryoscopus cubla	Misuku, Malawi	09.68S 33.50E	FMNH439236
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	MLW1132
Dryoscopus cubla	Nyika, Malawi	10.57S 33.70E	FMNH441072
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	GAV2575
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	DHB4838
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	GAV2554
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	GMS314
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	JSB059
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	DHB4858
Dryoscopus cubla	Mangochi, Malawi	14.50S 35.25E	DHB4944
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	CAH073
Dryoscopus cubla	Mwanza, Malawi	15.36S 34.31E	GAV2587
Dryoscopus cubla	Mangochi, Malawi	14.50S 35.25E	JSB060
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	JSB052

Dryoscopus cubla	Mwanza, Malawi	15.36S 34.31E	JSB006
Dryoscopus cubla	Mwanza, Malawi	15.36S 34.31E	DHB4322
Dryoscopus cubla	Mwanza, Malawi	15.36S 34.31E	DHB4317
Dryoscopus cubla	Mangochi, Malawi	14.50S 35.25E	GAV2583
Dryoscopus cubla	Mangochi, Malawi	14.50S 35.25E	JSB063
Dryoscopus cubla	Mwanza, Malawi	15.36S 34.31E	JK02-310
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	JSB058
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	GAV2601
Dryoscopus cubla	Mwanza, Malawi	15.36S 34.31E	JSB034
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	MoM2007.2.241
Dryoscopus cubla	Misuku, Malawi	09.68S 33.50E	MLW059
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	MLW B9
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	MLW B32
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	MLW B13
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	RB1129
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	RB1347
Dryoscopus cubla	Zomba, Malawi	15.40S 3530E	RCKB1471
Dryoscopus cubla	Mulanje, Malawi	15.55S 35.38E	RCKB1415
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	MoM2007.2.214
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	MoM2007.2.218
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	MoM2007.2.225
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	MoM2007.2.243
Dryoscopus cubla	Ntchisi, Malawi	13.38S 34.00E	MoM2007.2.201

Chapter 6

Conclusions and future prospects

The Malawi Rift is important as it acts as the crossroads between eastern and southern African evolutionary lineages. Many eastern African species reach their southern limits in northern Malawi and many species typical of southern Africa reach their northern limit in the southern half of the Malawi Rift. This prospect for lineage turnover makes the Malawi Rift important to sample for biogeographic studies, especially if the processes underlying phylogeographic patterns operating at large spatial scales are to be inferred across the African continent.

The rift depression is occupied by Lake Malawi forming a physical barrier to the east, and to the west highlands rise to almost 3000 m a.s.l. (Clarke 1983; Thompson et al. 2011). The rift falls within the Zambezian ecozone vegetation community (White 1983) that is dominated by broadleaved-woodland. The climate of the Malawi Rift is tropical with maritime influences from the Mozambique Channel. It is also influenced by Lake Malawi. The rift experiences three main seasons: the cool and dry period from May to August, the warm and dry period from September to December, and the warm and wet period from December to April. Annual rainfall ranges from about 600 to 3000 mm (Clarke 1983).

The variable climatic conditions and complex topography create a biogeographic barrier between the faunas of eastern and southern Africa (Bromage et al. 1995a). During periods of aridity the Malawi Rift appears to have harboured several important montane refugia, with these habitats partly buffered by orographic rainfall from local cloud formation off Lake Malawi. The presence of these refugia is reflected by the high

endemic species diversity (Danley et al. 2000; Dudley 2005), as well as phylogeographic structure and high genetic diversity that several montane populations harbour (Chapters 2-4, Lawson 2013, Bryja et al. 2014).

This study sought to detect, and if possible unravel, the processes that have contributed to the formation of genetic structure and turnover in Malawian birds and small mammals. Using morphological data from the literature, this study also re-evaluated the species status of putative endemic taxa distributed within the Malawi Rift.

Given the rapid changes in the African environment in response to high-latitude glaciation, several authors have detected turnover among linages within several bird and mammal species/species-complexes distributed between eastern and southern Africa (Vrba 1985; Partridge et al. 1995; Bowie et al. 2005; Table 1.1). Therefore, I hypothesised that: phylogeographically there are genetic differences among some (if not many) forest-associated species in Malawi, with potential breaks occurring: 1) on either side of the Shire River in southern Malawi, 2) in the central highlands, splitting Malawi into two halves, and 3) across the lowland gap that separates Nyika from the Misuku Hills in northern Malawi (Fig. 1.1). I expected the forest-associated species to show greater phylogeographic structure than the woodland-associated species, due to the disjunct distribution of montane highlands across the Malawi Rift.

To test these hypotheses, I sought to address the following questions.

4. Can genetic turnover in animal species distributed between east and southern Africa be detected and does it occur across the Malawi Rift?
5. Are geographically restricted taxa endemic to Malawi, and what are their distributions in Malawi?

6. Where are the common phylogeographic breaks in Malawi and how do they relate to the current placement of national parks?

6.1 Can genetic turnover in animal species distributed between east and southern Africa be detected and does it occur across the Malawi Rift?

In *Praomys delectorum* the results demonstrate geographical structuring among all the sampled populations. There was no sharing of haplotypes between the northern and southern populations of the Malawi Rift, indicating that these populations are reciprocally monophyletic. The populations on Rungwe, Misuku and Nyika are distinct from the southern populations of Mulanje and Namuli in agreement with the findings by Carleton and Stanley (2012) and Bryja et al. (2014).

In *Lophuromys aquilus* there is strong geographical structuring among all the sampled populations and the mismatch distributions suggest that sampled populations are stable. Therefore the sharing of haplotypes between the northern populations (Mt. Rungwe, Misuku Hills and Nyika Plateau) and southern populations (Mt. Zomba, Mt. Mulanje and Mt. Namuli) is likely indicative of ancestral polymorphism. This suggests that the northern populations and southern populations of *L. aquilus* may not as yet comprise distinct species.

The *Andropadus milanjensis* results revealed strong geographical structuring among all the sampled populations and that they are demographically stable. The Udzungwa Mountains correspond to *A. milanjensis striifacies*; the Misuku Hills, Nyika Plateau, Viphya Plateau, Mount Ntchisi and Mount Zomba correspond to *A. milanjensis olivaceiceps*, and Mount Mulanje and Mount Namuli correspond to *A. milanjensis*

milanjensis, of which each taxon should be accorded species status as suggested in recent field guides (e.g. Sinclair & Ryan 2003). The sharing of haplotypes between Nyika and Viphya Plateaus, as well as Mount Mulanje and Mount Namuli may be due to incomplete lineage sorting.

In *Batis dimorpha* two well-supported clades, one comprising the Eastern Arc distributed *B. crypta* and the other members of the South African species *B. capensis*, that together with the Malawi Rift populations (*B. dimorpha*) forms a species complex. The Malawi Rift populations comprising Nyika Plateau and Mount Ntchisi correspond to *B. dimorpha sola*, whereas those populations on Mount Zomba, Mount Mulanje and Mount Namuli correspond to *B. dimorpha dimorpha*. Therefore, the central highlands of Malawi are inhabited by *B. d. sola* and form its southern most extent. Mount Zomba is closer to Mount Mulanje and Mount Namuli suggesting that Mount Zomba is the most northern extent of *B. dimorpha dimorpha*. This result is contrary to earlier survey results that suggest this taxon extends onto the central highlands of Malawi (Newman et al. 1992; Erard & Fry 1997). These taxa may warrant species status.

The *Apalis thoracica* complex results revealed that *A. thoracica murina* distributed through much of the Eastern Arc, extending to the Misuku Hills, is distinct. The taxon *A. thoracica youngi* that occurs on Nyika Plateau is distinct, as is *A. thoracica flavigularis* from Mount Zomba and Mount Mulanje, and *A. thoracica lynesi* from Mount Namuli.

The two woodland-associated species *Dryoscopus cubla* and *Cossypha heuglini* exhibit limited phylogeographic structure across the Malawi Rift.

6.2 Are geographically restricted taxa endemic to Malawi, and what are their distributions in Malawi?

Of the taxa I studied across the Malawi Rift, *A. thoracica flavigularis* has been observed to have a distribution range that is restricted to Malawi, inhabiting only Mounts Malosa, Zomba and Mulanje (Mzumara et al. 2012; this study). The distribution range of *B. dimorpha sola* also falls entirely within Malawi, as it is restricted to Nyika Plateau in the north and Mount Ntchisi in the central highlands. Additional research is required to determine if this taxon should be accorded species status.

6.3 Where are the common phylogeographic breaks in Malawi and how do they relate to the current placement of national parks?

From the results of the fauna studied in the Malawi Rift the common phylogeographic breaks have been identified to occur along the lowland gap between the Udzungwa Mountains and Misuku Hills; between the Misuku Hills and Nyika Plateau; between Nyika and Mount Ntchisi within the central highlands of Malawi; central highlands of Malawi and Mount Zomba, and between Mount Zomba and Mount Mulanje, and between Mount Mulanje and Mount Namuli (Fig. 6.1). This is in accordance with the expectation of Malawi being a transition zone (faunal turnover) between eastern and southern African taxa.

The results also demonstrate that most national parks in Malawi are situated on each side of the identified phylogeographic breaks, with the northern Malawi Rift having Nyika National Park north of the central break. This park comprises varied habitats that encompass both montane as well as woodland flora (Cater et al. 1993). Kasungu National Park is located within the lowlands of central Malawi Rift and is generally dry with

Brachystegia woodland, and does not encompass important ecosystems such as the montane forests that are known to promote the accumulation of recently diverged species (Roy 1997; Fjeldså et al. 2012; this study). Lengwe National Park occurs in the southern Malawi Rift, and is also generally dry with *Brachystegia* and Accacia woodlands.

These national parks were mostly proclaimed without considering modern conservation planning approaches (Margules & Pressey 2000). Therefore, the existing protected areas do not include all species that effective conservation planning seeks to achieve (Pressey 1994). Most of the places where the phylogeographic breaks occur are outside of national parks and fall within the jurisdiction of forest reserves, and as such they are not adequately protected. Uncontrolled fires and illegal logging are threatening these habitats thus compromising the conservation of the fauna and important evolutionary units.

Comparative phylogeography of birds and small mammals

The taxa studied comprised forest-associated bird and small mammal species (*Andropadus milanjensis*, *Batis dimorpha*, *Apalis thoracica*, *Praomys delectorum* and *Lophuromys aquilus*) and two woodland-associated species (*Dryoscopus cubla* and *Cossypha heuglini*). All the forest species revealed phylogeographic breaks within the Malawi Rift, mainly in the central highlands of Malawi, and exhibited strong geographical structuring and no evidence of recurrent gene flow between northern and southern highlands. Thus, the results strongly suggest the presence of a common major break dividing Malawi into at least two halves in accordance with the expectations of Malawi being a transition zone (faunal turnover) between eastern and southern African taxa. The small mammals showed stronger geographical structure compared to the forest

bird species due to the fact that animals with small dispersal distances are more likely to show phylogeographic breaks than animals with larger dispersal distances (Avise 1994). In the woodland species there is less geographical structuring indicating that these birds have high dispersal distances and that these habitats show greater connectivity across the Malawi Rift.

The results of this study suggest that molecular DNA data has an important role to play in helping identify the phylogeographic breaks within the fauna distributed in the Malawi Rift. Important habitats for the conservation of evolutionary units have also been identified which would contribute to modern conservation planning (Margules & Pressey 2000) and management of birds and small mammals, and more broadly the fauna of Malawi

Future prospects

It appears that Mount Ntchisi harbours a population of *B. dimorpha sola*. However, this distribution range needs further investigation as this subspecies was not previously thought to occur on Ntchisi, but rather to be restricted to 'northern' Malawi (Newman et al. 1992; Erard & Fry 1997).

Given that the Mount Dedza population has individuals that are both genetically close to those from Nyika in the northern highlands and others closer to Zomba in the southern highlands, it is important to have greater sample sizes from Mount Dedza in order to fully understand the dynamics of observed patterns of structure in *A. thoracica whitei* in the Malawi Rift.

The *A. thoracica flavigularis* populations of Mount Mulanje and Mount Zomba are distinct. Therefore Mount Malosa, which is also inhabited by *A. t. flavigularis*

requires sampling in order to fully understand the underlying phylogeographic pattern if this newly recognized Malawian endemic taxon is to be effectively managed and conserved.

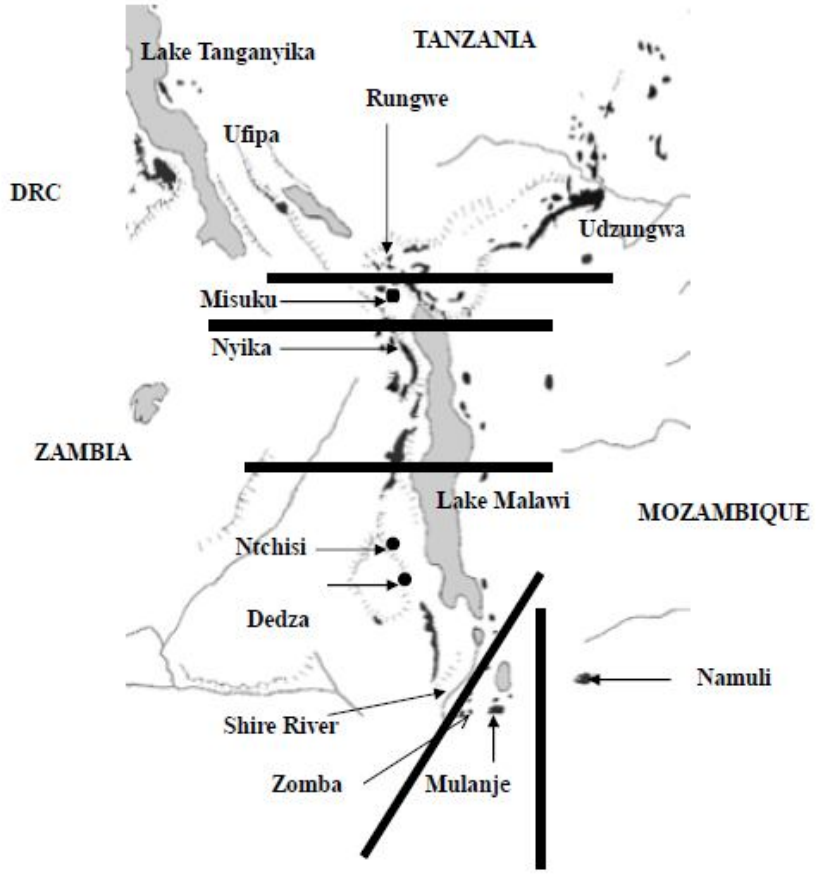

Figure 6.1 Summary of major phylogeographic breaks identified across taxa studied in the Malawi Rift.

Chapter 7

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