Evaluating the effects of biogeography and fragmentation on the taxonomic, functional, and genetic diversity of forest-utilising bats in a South African biodiversity hotspot

by

Monika Ilka Moir

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Supervisors:

Dr. Ramugondo V. Rambau

Prof. Michael I. Cherry

Dr. Leigh R. Richards

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Declaration

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This dissertation includes one original paper published in a peer-reviewed journal with me as lead author, and three articles submitted and under peer-review. The development and writing of the papers (published and unpublished) were the principal responsibility of myself.

Monika Ilka Moir
August 2020

Abstract

Bats are a highly diverse mammalian order and are some of the most economically important non-domesticated vertebrates, providing many ecosystem services that contribute to the global economy. Yet, they remain a largely understudied taxon, particularly in the Eastern Cape province of South Africa, in which basic surveys of bat assemblages utilising indigenous forests are lacking. Indigenous forests constitute South Africa's smallest and most fragmented biome yet support disproportionally high biodiversity. They have been fragmented throughout most of their evolutionary history due to global palaeoclimatic shifts; the responses of bats to forest fragmentation and historical climatic shifts in this habitat have been poorly studied.

This study addresses these gaps with the broad aims of compiling a species inventory from 17 forests across the Eastern Cape and KwaZulu-Natal provinces; assessing the effects of fragmentation and biogeography on taxonomic and functional diversity of bat assemblages; and determining how genetic diversity and population genetic structure are informed by forest habitat associations and fragmentation. A multi-faceted approach of sampling methods, including capture and acoustic recording, and species identification techniques (morphology, acoustics, and DNA barcoding) were used to assemble an inventory of 25 species, with range extensions noted for six species. The first reference call library of hand released bats for forests in this region is presented, which may be used for species identification in further acoustic surveys. A minimum acoustic monitoring period of 6 to 7 nights per forest is recommended for future surveys.

Forest biogeography was an important determinant of the functional diversity of insectivorous bat assemblages. Forest edge effects were found to demonstrate a positive relationship with functional evenness, thus motivating for maintenance and conservation of forest edges, particularly in temperate regions. Larger forearm length and low wing loading were identified as morphological traits exhibiting greater sensitivity to fragmentation, flagging species exhibiting these traits as potentially vulnerable to habitat fragmentation.

The effect of historical climate-induced fluctuations of forest extent on population genetic structuring and demographic histories for six species was investigated using two mitochondrial markers, cytochrome *b* and D-loop. Population genetic trends were not informed by forest habitat associations, but rather by species-specific traits of dispersal ability, philopatry, and roost utilisation. Low genetic diversity and high population structure identify two species, *Rhinolophus swinnyi* and *Laephotis botswanae*, for conservation priority. Demographic

responses to the Last Glacial Maximum (LGM) were not detected, with all six species displaying population expansions over this time. It appears that volant insectivores in eastern South Africa were less affected by the harsh conditions of the LGM than elsewhere.

The dusky pipistrelle (*Pipistrellus hesperidus*) was used as a model organism to investigate the gene flow, genetic diversity, and migration of a forest-utilising species across the region with the use of eight microsatellite markers. The effects of urbanisation and agricultural development on gene flow were also examined. Findings of low population structure, low migration rates, and two genetic discontinuities were presented. This species does not depict dependence on forested habitats to maintain genetic connectivity on the landscape. The data also suggest that agricultural development and urbanisation have not yet had an impact on gene flow, thus providing a baseline with which to monitor the effects of future anthropic development on this species.

Overall, this study has provided novel insights into the taxonomic, functional, and genetic diversity of forest-utilising bats in relation to biogeographical history and fragmentation within eastern South Africa.

Opsomming

Vlermuise is 'n baie diverse soogdierorde en is van die mees ekonomies belangrike nie-domestiese gewerwelde diere, wat baie ekosisteemdienste lewer en sodoende bydra tot die wêreldekonomie. Tog bly hulle 'n grootliks onderbestudeerde takson, veral in die Oos-Kaap en KwaZulu-Natal provinsies van Suid-Afrika, waar basiese opnames oor vlermuis samestellings ontbreek. Inheemse woude vorm die kleinste en mees gefragmenteerde bioom van Suid-Afrika, maar nogtans ondersteun dit ‘n buitensporige hoë biodiversiteit. Hulle is deur die grootste deel van hul evolusionêre geskiedenis gefragmenteer as gevolg van wêreldwye paleoklimatiese verskuiwings; die reaksie van vlermuise op woudfragmentering en historiese klimaatverskuiwings in hierdie habitat is swak bestudeer.

Hierdie studie spreek hierdie leemtes aan met die breë doelstellings om 'n spesies-inventaris vanaf 17 woude regoor die Oos-Kaap en KwaZulu-Natal provinsies saam te stel; die gevolge van fragmentasie en biogeografie op taksonomiese en funksionele diversiteit van vlermuis samestellings te evalueer; en vas te stel hoe genetiese diversiteit en genetiese struktuur van die populasie bepaal kan word deur woud habitat assosiasies en fragmentasie. 'n Veelsydige benadering van monsternemingsmetodes, insluitend vang- en akoestiese opname, asook spesie-identifikasietegnieke (morfologie, akoestiek en DNS-strepieskodering) is gebruik om 'n inventaris van 25 spesies saam te stel met uitbreidings in geografiese omvang aangeteken vir ses spesies. Die eerste biblioteek met roep verwysings vir hand vrygestelde vlermuise van woude in hierdie streek word aangebied wat in verdere akoestiese opnames gebruik kan word vir die identifisering van spesies. 'n Minimum akoestiese moniteringstydperk van 6 tot 7 nagte per woud word aanbeveel vir toekomstige opnames.

Die biogeografie van ‘n woud was 'n belangrike bepalende faktor van die funksionele diversiteit van insekvretende vlermuise. Daar is gevind dat woud rand-effekte 'n positiewe verwantskap met funksionele egaligheid toon en bied dus motivering vir die instandhouding en bewaring van woud rande veral in gematigde streke. Groter voorarmlengte en lae vlerklading is geïdentifiseer as morfologiese eienskappe wat meer sensitiwiteit getoon het vir fragmentasie wat sodoende spesies wat hierdie eienskappe toon as potensieel kwesbaar vir fragmentasie van die habitat uitlig.

Met behulp van twee mitokondriale merkers, sitokroom b en D-lus, is die effek van historiese klimaat-geïnduseerde wissellinge van die omvang van die woud op die genetiese strukturering en demografiese geskiedenis van die populasie vir ses spesies ondersoek. Populasie genetiese

neigings is nie deur woud habitat assosiasies gevorm nie, maar eerder deur spesiespesifieke eienskappe van verspreidingsvermoë, filopatrie en nes gebruik. Lae genetiese diversiteit en hoë populasie struktuur identifiseer twee spesies, *Rhinolophus swinnyi* en *Laephotis botswanae*, vir prioriteitsbewaring. Demografiese reaksies op die Laaste Glasiale Maximum (LGM) is nie opgespoor nie met al ses spesies wat gedurende hierdie tyd uitbreiding in populasie getoon het. Dit lyk asof vlieënde insekvreters in oos Suid-Afrika minder geraak is deur die haglike toestande van die LGM as elders.

Die Kuhl-vlermuis (*Pipistrellus hesperidus*) is as 'n modelorganisme gebruik om die geenvloei, genetiese diversiteit en migrasie van 'n woud-bruikende spesie oor die hele streek te ondersoek met behulp van agt mikrosatellietmerkers. Die gevolge van verstedeliking en landbou ontwikkeling op geenvloei is ook ondersoek. Bevindinge van lae populasie struktuur, lae migrasietempo, en twee genetiese diskontinuïteite word aangebied. Hierdie spesie toon nie ‘n afhanklikheid van bewoude habitatte om genetiese konnektiwiteit in die landskap te handhaaf nie. Die data dui ook daarop dat landbou ontwikkeling en verstedeliking nog nie 'n invloed op geenvloei gehad het nie wat dus 'n basis bied om die gevolge van toekomstige antropiese ontwikkeling te montior op hierdie spesie.

Hierdie studie lewer as geheel nuwe insigte tot die taksonomiese, funksionele en genetiese diversiteit van woud-bruikende vlermuise in verband met biogeografiese geskiedenis en fragmentasie in oos Suid-Afrika.

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List of published or submitted articles

Chapter 2

Moir M, Rambau RV, Cherry MI, Richards LR. (**in press**). Bats of Eastern Cape and southern KwaZulu-Natal forests, South Africa: diversity, call library and range extensions. *Acta Chiropterologica*.

Chapter 3

Moir M, Richards LR, Rambau RV, Cherry MI. (**in review**). Functional diversity and trait filtering of insectivorous bats in response to forest biogeography and fragmentation in South Africa. *Journal of Biogeography*.

Chapter 4

Moir M, Richards LR, Cherry MI, Rambau RV. (2020). Demographic responses of forest-utilising bats to past climate change in South Africa. *Biological Journal of the Linnean Society* 130(4): 850-868. DOI: 10.1093/biolinnean/blaa048

Chapter 5

Moir M, Richards LR, Rambau RV, Wannenburgh A, Cherry MI. (**in press**). Fragmentation does not affect gene flow in forest populations of the dusky pipistrelle bat on the eastern seaboard of South Africa. *Journal of Mammalogy*.

Conference presentations of the PhD work

Moir M, Richards LR, Cherry MI, Rambau RV. (2019). The effects of dispersal ability on genetic diversity and population structure of six forest associated bats in the eastern Cape, South Africa. Oral presentation at 18th *International Bat Research Conference*, Phuket, Thailand.

Moir M, Rambau RV, Cherry MI, Richards LR. (2019). Who's out there? Bat diversity of forests in the Eastern Cape and southern KwaZulu-Natal. Oral presentation at 39th *Zoological Society of southern Africa National Congress*, Skukuza, South Africa.

Moir M, Richards LR, Cherry MI, Rambau RV. (2018). Comparison of genetic structure of two forest bats across the Eastern Cape province. Oral presentation at 4th Biodiversity Information Management Forum (*BIMF)–Foundational Biodiversity Information Programme (FBIP) Forum*, Cape St Francis, South Africa.

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Chapter 1. General Introduction

1.1 The economic and ecological value of bats

Bats form the highly successful mammalian order Chiroptera consisting of over 1,400 species across the globe (Mammal Diversity Database, 2020). They are the most widely distributed terrestrial mammals on earth, comprising one fifth of all mammalian diversity (Burgin *et al.*, 2018). The African Chiroptera Report (2019) currently recognises 14 extant families of 331 species across Africa, with 69 species occurring in South Africa. With this high diversity, comes many different foraging strategies allowing bats to perform several ecosystem services, contributing not only to ecosystem function but also substantially to the global economy (Kunz *et al.*, 2011). Bats are considered one of the most economically important non-domesticated mammals in the world (Boyles *et al.*, 2013). For example, fruit bats are essential seed dispersers for widespread and endemic plants in Afrotropical forests, allowing for forest regeneration (Seltzer, Ndangalasi and Cordeiro, 2013). They serve as pollinators of approximately 528 plant species worldwide, of which many are agriculturally important crop plants (Kunz *et al.*, 2011). A global review of vertebrate pollination systems found bat-pollinated plants exhibit higher dependence on their pollinators than bird-pollinated plants, with an 83% reduction in fruit and seed production when bats are excluded from the pollination system (Ratto *et al.*, 2018). Lastly, insectivores provide what is considered the most economically valuable ecosystem service of bats by suppressing insect crop pest populations (Boyles *et al.*, 2013). The insect biocontrol service of bats was estimated at $22.9 billion per year for the agricultural industry in USA (Boyles *et al.*, 2011). Locally, the annual insect pest biocontrol services of bats and nocturnal birds in macadamia orchards was valued at approximately US $2,500 per hectare (Linden *et al.*, 2019).

Bats may also be used as key biological indicators of habitat quality as they are sensitive to anthropogenic disturbances to ecosystems (Kalcounis-Rueppell *et al.*, 2007; Jones *et al.*, 2009; Park, 2015). They can be used successfully as bio-indicators due to their widespread distribution, their taxonomy is mostly stable, they occupy higher trophic levels, and their population trends are easy to monitor (Jones *et al.*, 2009). Considering the economic and ecological contributions of bats, and their use as bio-indicators, many authors argue for their inclusion in conservation and management plans across the world (Lacki, Hayes and Kurta, 2007; Weller, 2008; Wordley *et al.*, 2017; Farneda *et al.*, 2018). However, a major limiting factor for management planning and conservation action is the shortfall of knowledge of the

status of bat populations. For example, globally 18% of species assessed by the International Union for Conservation of Nature (IUCN) are data deficient and 57% of species have unknown population trends (Frick, Kingston and Flanders, 2019). They remain as a largely understudied vertebrate taxon (Lindecke *et al.*, 2019), with many unknown aspects of their ecology, behaviour, and population trends.

1.2 Bats in forests

Bats exhibit flight morphology and echolocation systems adapted to the habitats they utilise (Norberg and Rayner, 1987). Clutter, defined as the number of obstacles a bat has to detect and avoid, is an important environmental constraint on flight and echolocation (Fenton, 1990; Monadjem, Taylor, *et al.*, 2010). The relative clutter of a habitat is mostly determined by its horizontal and vertical vegetation structure, in this context, forests are highly cluttered habitats. Wing morphology allowing for adaptation to the environment is predominantly informed by measures of wing loading and aspect ratio. Wing loading is a measure of body mass relative to wing area; high wing loading allows for fast flight speeds while manoeuvrability is associated with low wing loading (Norberg and Rayner, 1987). Aspect ratio is a measure of the size and shape of wings, where a higher aspect ratio correlates to greater flight efficiency with lower energy losses (Norberg and Rayner, 1987). African bats are grouped into four functional or foraging guilds that is dependent on their wing morphology and echolocation adaptations to foraging and navigating within different habitats. These guilds include frugivores, open-air insectivores, clutter-edge insectivores, and clutter insectivores (Monadjem, Taylor, *et al.*, 2010; Webala *et al.*, 2019).

Frugivores are larger species that rely on sight and smell for navigation and foraging, and exhibit broad wings with high wing loading and low aspect ratio (Monadjem, Taylor, *et al.*, 2010). The open-air functional guild consists of fast flying species, such as those belonging to the Molossidae and Emballonuridae families, capable of foraging high above the vegetation canopy and adjacent open areas. Open-air foragers typically have long, narrow wings with high wing loading allowing for speed and agility (Monadjem, Taylor, *et al.*, 2010). They also use narrowband, low frequency and long duration echolocation calls optimised for long distance detection of prey items in open space (Norberg and Rayner, 1987; Schnitzler and Kalko, 2001). The clutter-edge functional group (e.g. Vespertilionidae and Miniopteridae families) forage near and along vegetation edges (Monadjem, Taylor, *et al.*, 2010), such as along forest edges and gaps in the forest canopy. This guild exhibits variable wing morphology with mostly

intermediate sized wings, wing spans and wing loading. Clutter-edge bats utilise a mix of echolocation signal types, typically low duty-cycle shallow frequency-modulated (QCF) search phase calls alternated with frequency modulated (FM) calls (Norberg and Rayner, 1987; Schnitzler and Kalko, 2001; Monadjem, Taylor, *et al.*, 2010). The clutter foraging group consists of highly manoeuvrable bats that can navigate and hunt within dense vegetation and near to the ground. Clutter foragers display short, broad wings with low wing loading for slow manoeuvring within dense vegetation (Norberg and Rayner, 1987). This guild consists of two subgroups based on echolocation systems. Hipposideridae, Rhinolophidae and Rhinonycteridae families utilise constant frequency high duty-cycle (HD-CF) calls of long duration with medium to high peak frequency. While the Nycteridae family utilises broadband, low duty-cycle, frequency-modulated calls (LD-FM), with shorter duration and lower intensities (Schnitzler and Kalko, 2001; Monadjem, Taylor, *et al.*, 2010). Clutter bats dominate the species assemblage in forest interiors (Senawi and Kingston, 2019).

Across Africa, forested regions host high bat activity, species richness and diversity (Duncan and Chapman, 1999; Linden *et al.*, 2014; Brinkley, 2018; Webala *et al.*, 2019). Forests provide a myriad of roosting places for foliage, crevice and hollow-roosting species (Monadjem, Taylor, *et al.*, 2010), reliable food sources for fruit bats (Richter and Cumming, 2008), and high foraging potential for insectivores (Nurul-Ain, Rosli and Kingston, 2017). Some forested areas have even been recognised as of 'exceptional importance for bat diversity' such as Mount Nimba in West Africa (Monadjem, Richards and Denys, 2016). In southern Africa, bat species richness is highest in the eastern parts of the subregion (Schoeman *et al.*, 2013; Cooper-Bohannon *et al.*, 2016), with the Afromontane and coastal forest mosaic identified as high species richness zones (Cooper-Bohannon *et al.*, 2016). These findings suggest forested areas, particularly the Afrotemperate and coastal forests in the south-east region of South Africa, are important bat biodiversity areas.

Forests in the south-east of South Africa do not host any strictly forest-dependent bat species (Monadjem, Taylor, *et al.*, 2010), apart from *Rhinolophus swinnyi s.s.* (Taylor *et al*., 2018). Several species make effective use of forest habitats in the form of foraging within the forest interior or along its edges, drinking and foraging along streams within the forests, roosting in trees and foliage, or commuting through pathways within the forest. In this study, these species are considered as forest-utilising as they actively occur within and on the edges of forests but are not exclusively dependent on forest habitats. Forest-utilising species may then be further described as forest associated or habitat generalists. Forest associated species, such as the dusky

pipistrelle (*Pipistrellus hesperidus*) and the banana bat (*Neoromicia nana*), occur in well-wooded habitats being forest, riparian vegetation, and savanna woodland (Monadjem, Taylor, *et al.*, 2010). Habitat generalists occur in forests but also inhabit open habitat biomes such as savanna and grasslands, for example the lesser long-fingered bat (*Miniopterus fraterculus*) and Botswana long-eared bat (*Laephotis botswanae*) (Monadjem, Taylor, *et al.*, 2010).

1.3 Forests in South Africa

Indigenous forests in South Africa are highly fragmented occurring as small, disjoint patches embedded within large, open biomes of savannah, grassland, fynbos and Albany thicket (Low and Rebelo, 1996). Forests form the smallest biome in the country (Mucina and Geldenhuys, 2006) constituting only 7,177 km^2 of land surface (Low and Rebelo, 1996). Although they are small and fragmented, forests support immense biodiversity and have high conservation value. They exhibit high botanical diversity (Berliner, 2009), and support a high proportion of the region's faunal biodiversity (Geldenhuys and Macdevette, 1989). For example, forests host the highest number of endemic vertebrate species of all biomes in the country (Castley, 1997), with 13% of IUCN red list vertebrates dependent on forests (Endangered Wildlife Trust, 2002).

They form a discontinuous belt along the eastern and southern escarpment ranges and coastal lowlands (Mucina and Geldenhuys, 2006), and are distributed across a range of climatic, altitudinal and topographical gradients. South African forests are classed into inland Afrotemperate forests and Indian Ocean Coastal Belt forests (von Maltitz *et al.*, 2003; Mucina and Geldenhuys, 2006). Afrotemperate forests occur inland along the main escarpment and are part of the Afromontane Region which occurs further north in Zimbabwe, Malawi, the East Africa mountain arch and Ethiopia (Mucina and Geldenhuys, 2006). Mistbelt forests were sampled in this study, they are a type of Afrotemperate forest. The coastal forests form part of the Tongaland-Pondoland Regional Mosaic and share some subtropical elements with the Zanzibar-Inhambane Regional Mosaic (White, 1983). A narrow band of Scarp forests is located between these two forest types, demonstrating overlapping floral and faunal community composition with both Afrotemperate and coastal forests, as well as paleoendemic and relict tropical elements (Macdevette *et al.*, 1989; Lawes, 1990; Lawes, Mealin and Piper, 2000).

1.4 Forest biogeographical history

Southern Africa has experienced numerous climatic oscillations in response to global paleoclimatic shifts (Tyson, 1986), with forests as one of the most affected biomes. Most

forests have been fragmented throughout their evolutionary history due to the climatic history of South Africa (Eeley, Lawes and Piper, 1999). Repeated oscillations between glacial and interglacial periods have taken place over the Quaternary (Tyson, 1986). Glacial periods were characterised by cool, xeric conditions from glacier advances with a resultant contraction of the forest biome to limited areas of high precipitation. Whereas, glacial retreat during interglacial periods brought about higher temperatures and increased rainfall resulting in forest expansion (Tyson, 1986; Dupont, Caley and Castañeda, 2019). The most recent interglacial period spanned from 130,000 to 40,000 years before present, subsequently receding into cold and dry conditions towards the Last Glacial Maximum (LGM) ~21,000 – 18,000 years before present (BP) (Deacon, 1983; Tyson, 1986). Afrotemperate forests are biogeographically ancient, some having persisted since the Miocene (Eeley, Lawes and Piper, 1999). With temperatures at their lowest limits in 125,000 years during the LGM, and a significantly drier climate than at present, Afrotemperate forests underwent severe contraction and fragmentation (Deacon, 1983; Tyson, 1986). Precipitation levels increased during the Holocene altitherm, from 17,000 – 15,000 years BP, with warmer conditions re-established by 8,000 years ago. These conditions were once again conducive to forest growth and expansion (Tyson, 1986) resulting in the Indian Ocean Coastal belt forests extending south along the eastern coastline from Mozambique (Lawes, 1990; Eeley, Lawes and Piper, 1999). Forest expansion during the Holocene altitherm created the potential for mixing of Afrotemperate and Indian Ocean Coastal belt forests along the current day Scarp forest belt (Eeley, Lawes and Piper, 1999).

The biotic communities of Afrotemperate forests have undergone more than one historical climate change cycle with severe fragmentation effects causing climatic extinction filtering. Contemporary Afrotemperate faunas are relatively species-poor, unsaturated, and dominated by generalist species, while the Indian Ocean Coastal belt forests have not endured such an event due to their more recent evolutionary history (Lawes, 1990; Lawes *et al.*, 2007). Scarp forests are of Afrotemperate origin, but located on coastal gorges, scarps and krantzes nearer to the Indian Ocean than Afrotemperate forests. Their proximity to the warm ocean acted as a buffer to the extreme climate of the LGM, allowing patches to survive as important forest remnants (Eeley, Lawes and Piper, 1999). These remnant patches served as refugia for Afrotemperate fauna and after the LGM, Afrotemperate communities were recolonised from Scarp refugia (Lawes *et al.*, 2007). The largest area of contiguous forest in Africa is located in tropical Central Africa with three main forest blocks: Upper Guinea, Lower Guinea, and the Congolian block (Kenfack *et al*., 2007). Studies have shown the possible linkage of forests in

southern Africa to this main equatorial block was maintained over biogeographical history via eastern African coastal forests for forest mammals (Rodgers *et al.*, 1982) and hominins (Joordens *et al.*, 2019). It seems coastal forests in southern Africa were colonised from refugia in tropical East Africa along the eastern coastline (Lawes *et al.*, 2007). As a result, Scarp forest faunas exhibit high diversity, many forest-dependent species, and demonstrate strong affinities with Afrotemperate assemblages and an element of coastal forest communities (Lawes, 1990; Lawes *et al.*, 2007). Thus, the large-scale climatic processes informing the biogeographical history and structure of forests have largely shaped the composition of faunal communities inhabiting them (Lawes, 1990; Lawes, Mealin and Piper, 2000; Lawes *et al.*, 2007). The cyclic changes in habitat extent have created complex patterns of isolation and interconnectivity among populations, which has substantially influenced the distributions and diversity patterns of the extant faunal communities (Lawes, 1990; Lawes *et al.*, 2007).

1.5 Anthropogenic pressures on forests and bat populations

More recently, forests were subjected to anthropogenic fragmentation upon the arrival of Dutch settlers in the Cape in 1652. Mostly unregulated logging continued through the colonial era (King, 1938) until it was terminated at the start of the Second World War. Large areas of indigenous forest were lost to this logging and to clearing for sugar plantations in KwaZulu-Natal following colonisation in 1840 (du Bois, 2016). The total extent of forest lost across the country is unknown, but Olivier, Van Aarde and Lombard (2013) estimate a clearance of 82% of indigenous forest in KwaZulu-Natal, and Lawes, Macfarlane and Eeley (2004) estimated 5.7% of forest was cleared from the Karkloof-Balgowan area in KwaZulu-Natal. Currently, all South African forests are protected under the National Forests Act (NFA) No. 84 of 1995. Current anthropogenic impacts mostly consist of small-scale harvesting of forest products by local communities causing habitat degradation (Leaver and Cherry, 2020) rather than deforestation.

The Eastern Cape and KwaZulu-Natal provinces, located in the south-east of South Africa, have the highest proportions of national forest in the country, 46% and 29%, respectively (Berliner, 2009). However, the Eastern Cape has the lowest proportion of forest (4.75%) under strict Type 1 protection in the country (Berliner, 2009) [areas declared as protected under the National Environmental Management Protected Areas Act (South African National Biodiversity Institute, 2018) and are effectively managed as protected areas]. These forests are located within the Maputaland-Pondoland-Albany global biodiversity hotspot, but also within

the most impoverished and rural regions of the country (Berliner, 2009). The Eastern Cape Provincial Government has published a development plan to work towards alleviating poverty and empowering local communities (Eastern Cape Planning Commission, 2014). The development plan proposes to expand the economic potential of under developed areas by growing small towns into industrial hubs, increasing agricultural production, and improving built infrastructure (Eastern Cape Planning Commission, 2014). Therefore future land use change, in the form of urbanisation and agricultural development, is a possible threat to South African biodiversity through habitat destruction and degradation (Bomhard *et al.*, 2005; O'Connor and Kuyler, 2009), with the Eastern Cape development plan possibly encroaching on forested areas of the region and the biotic communities that inhabit them.

Bat populations across the world appear to be decreasing in response to human induced changes to their environment (Voigt and Kingston, 2016). Rodhouse *et al.* (2019) and Ingersoll, Sewall and Amelon (2013), documented long-term population declines of several species in the Pacific North-western and eastern United States, respectively. Similar studies have not been done in Africa where population trends are not known but assumed to be similar to those in the United States of America. Bats exhibit exceptionally long lifespans for their body size and low reproductive rates (Munshi-South and Wilkinson, 2010), meaning their populations are susceptible to crashes from disturbances and are slow to recover from such losses. Reviews of the global conservation status of bats have identified a number of common anthropogenic burdens on biodiversity as threats relevant to bat conservation, such as pressure on roosting and foraging resources from expanding human populations, roost disturbances, persecution due to negative public image, and overexploitation for food (Mickleburgh, Hutson and Racey, 2002; Mickleburgh, Waylen and Racey, 2009; Frick, Kingston and Flanders, 2019).

Adding to these pressures, new threats have subsequently emerged such as temperature extremes causing large die-offs of fruit bats in Australia (Welbergen *et al.*, 2008), the infectious fungal disease (white-nose syndrome) killing cave bats in North America (O'Shea *et al.*, 2016), and high mortality rates emanating from wind energy facilities being developed worldwide (Thaxter *et al.*, 2017). More recently, the current global pandemic of the disease COVID-19 from the SARS-CoV-2 virus, is another looming threat to bat conservation (Fenton *et al.*, 2020). The discovery that SARS-CoV-2 is most closely related to bat coronaviruses (Zhou *et al.*, 2020) suggests the virus causing COVID-19 originated in bats (Andersen *et al.*, 2020). The virus is wreaking havoc on economies, and people's health and livelihoods. Global media is reporting bats as the source of the spill over, which has and continues to cause increased

negative stigmas associated with them, leading to greater public persecution and extermination (Fenton *et al.*, 2020). In April 2020, the IUCN Species Survival Commission Bat Specialist Group recommended that all field work involving contact with bats be suspended while the risk of human to bat transmission of SARS-CoV-2 is assessed (Nuñez *et al.*, 2020). With the suspension of further field-based research and the risk of greater public persecution, it has become increasingly important to mobilise available data informing bat species distributions, diversity assessments, population trends, and responses to environmental variables such as habitat fragmentation and urbanisation. This knowledge will aid in conservation status assessments of species and to gauge vulnerability of their populations to the disease and further anthropogenic disturbances.

In the South African context, wind farm related mortality of insectivorous and fruit bats is a cause for concern (Doty and Martin, 2013; MacEwan, 2016). Currently 22 wind farms, with a total of 1061 wind turbine generators, are fully operational across the country with a further 11 facilities under construction (https://sawea.org.za/stats-and-facts-sawea/). The Eastern Cape province is a high wind resource area in the country, with more than half of the currently operational wind farms scattered across the province. Furthermore, several operational wind farms and proposed renewable energy developments are within the greater surrounds of forests. The first known incident of mortality of fruit bats from wind farms in the world was recorded in the Eastern Cape (MacEwan, 2016). Considering the importance of fruit bats for forest regeneration ecosystem services in Africa (Seltzer, Ndangalasi and Cordeiro, 2013), wind farm development may not only be a risk to stability of bat populations but also to forest conservation.

1.6 Knowledge gaps and thesis aims

In terms of terrestrial ecological surveys, Africa is poorly surveyed for its size (Martin, Blossey and Ellis, 2012). This is true in the case of South African forests, particularly within the Eastern Cape province, where many forest fauna have not previously been surveyed. Small mammalian fauna of this province have been overlooked in past field-based inventories (De Graaff and Nel, 1970; Klein, 1976; Hayward *et al.*, 2005). This is further true for a small, flying, nocturnal taxon that proves difficult to study, such as bats, where many species are known to allude traditional capture methods (Berry *et al.*, 2004). For many southern African bat species in particular, basic information such as the full extent of their distribution ranges is often lacking (Monadjem, Taylor, *et al.*, 2010; Happold and Happold, 2013). It is only in recent years that

an increasing number of ecological studies of bat communities have been carried out in both natural and disturbed ecosystems in South Africa. However, basic species inventories and diversity assessments of bat assemblages within forests of the Eastern Cape and KwaZulu-Natal are limited or have not yet been recorded.

Bats are traditionally surveyed by active capture methods which entail the use of mist nets and harp traps (Francis, 1989); and more recently, by passively recording their echolocation calls with acoustic recorders or bat detectors (O'Farrell and Gannon, 1999). Active capture of bats allows for the collection of detailed morphological data and genetic samples, and is important for the survey of non-echolocating fruit bats (Collins, 2016). Species identification of captured bats may be performed using external morphological traits, however this may prove difficult for cryptic sympatric species (Stoffberg, Jacobs and Miller-Butterworth, 2004). Field identification may then be validated via the molecular method of DNA barcoding (Clare *et al.*, 2007), which is the sequencing of a short, standardised DNA segment (COI marker). The DNA barcode sequence may be referenced against taxonomically verified specimens in the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert, 2007) and GenBank (Benson *et al.*, 2013). Barcoding has proven very useful for species identification of highly diverse bat assemblages (Clare *et al.*, 2007).

The passive recording of echolocation calls with bat detectors is widely used by researchers to detect habitat use by bats, for comparison of relative activity levels between sites, and to estimate population trends (Russo and Voigt, 2016). Verified reference call libraries are required for accurate species identification of acoustic recordings (Monadjem *et al.*, 2017). Reference calls are collected from captured individuals of which the species identification has been validated. Several call libraries have been developed for southern African species (Monadjem, Taylor, *et al.*, 2010; Taylor *et al.*, 2013; Monadjem *et al.*, 2017; Brinkley, 2018; Parker and Bernard, 2019). However, bats exhibit intraspecific call variation in response to habitat structure and between different ecoregions (Aspetsberger, Brandsen and Jacobs, 2003; Mutumi, Jacobs and Henning, 2016). Creating accurate and reliable call libraries for different ecoregions across the country is a necessary foundation for standardising acoustic analysis of South African bat calls. An echolocation call library has not yet been compiled for forested habitats and for the south-eastern region of the country. This study addresses the lack of baseline biological and biogeographical data of bats inhabiting forests in southern KwaZulu-Natal and Eastern Cape provinces of South Africa. A combination of all available sampling methods (capture with mist nets and harp traps, and passive acoustic recording), as well as

species identification techniques (morphology, DNA barcoding, release echolocation calls), are employed to sample these communities and compile a reference call library.

Forests in South Africa present an interesting case of fragmentation due to their historical climate induced fragmentation and the more recent anthropogenic pressures of fragmentation and degradation. Habitat fragmentation is well known to reduce the diversity of vital ecological components which may disrupt ecosystem processes and function (Bregman, Sekercioglu and Tobias, 2014). Bats are essential components of ecosystems (Kunz *et al.*, 2011) and considering their ecological and economic importance, the response and vulnerability of bats to fragmentation has been poorly studied, particularly in Africa. Traditionally, taxonomic diversity and species richness were the measures used to investigate the effects of fragmentation on species assemblages (Bernard and Fenton, 2007; Heer *et al.*, 2015; Muylaert, Stevens and Ribeiro, 2016). However, functional diversity has been shown to be a better estimate of biodiversity as it links the mechanistic effect of species functional traits with ecosystem processes (Hooper *et al.*, 2006). Functional diversity is an important driver of ecological processes that shape the functionality, resistance and resilience of ecosystems (Villéger, Mason and Mouillot, 2008), and is defined as the range, abundance, and distribution of functional traits within a community (Tilman, 2001). As community assembly processes operate on ecological traits, functional diversity is a more suitable indicator of changes to community structure in response to habitat disturbances than taxonomic diversity (Mouillot *et al.*, 2013; Cisneros, Fagan and Willig, 2015). In this study, I examine the interaction of forest biogeographical structure and fragmentation with bat functional diversity and functional traits. This allows us to understand the effects of fragmentation on bat communities and to identify species traits that may be sensitive to fragmentation in a South African context.

Historical global climatic shifts have had important consequences for extant forest composition and configuration in South Africa, as repeated habitat contraction and expansion cycling promote diversification and extinction events of biota (Hewitt, 2000, 2004). Fluctuations of forest habitats within South Africa have caused range changes or spatial discontinuity of forest fauna with subsequent oscillations in their population sizes and genetic diversification. Work has been done on forest biogeography and associated faunal phylogeography for invertebrates, birds, non-volant mammals, frogs, and reptiles (Lawes, 1990; Lawes, Mealin and Piper, 2000; Lawes *et al.*, 2007; Mcdonald and Daniels, 2012; Busschau *et al.*, 2017; Busschau, Conradie and Daniels, 2019; Coetzer *et al.*, 2019; Kushata *et al.*, 2020). However, to date, very few studies have focussed on forest utilising bats in South Africa, with none investigating how their

habitat associations may inform population genetic structuring and fluctuations in their historical population demographics. With the use of molecular data, such as mitochondrial DNA sequences, I investigate the biogeographic history of six forest-utilising bat species by comparing the relative timing of changes to their effective population sizes with the timing of large-scale historical fluctuations of forest extent. Forest reductions causing habitat fragmentation may result in isolation of populations and/or decreases in effective population size, while forest expansion would have caused a similar population expansion effect. Simultaneously, evidence of past habitat shifts may be depicted by trends of genetic diversity and population structure demonstrated by the study species. Understanding of population genetic trends, and their link to habitat association, is facilitated by the comparison of three forest associated species with three habitat generalist species.

Furthermore, the urban and agricultural development planned for the Eastern Cape presents a potential threat to the forested region and its biodiversity. Anthropogenic development alters the composition and configuration of a landscape creating a mosaic of suitable and unsuitable habitats for fauna to inhabit and traverse. Anthropogenic changes to the landscape subsequently affects ease of movement, dispersal routes for gene flow, and the resultant population structuring of native fauna (Manel *et al.*, 2003). As forests are highly fragmented in South Africa (von Maltitz *et al.*, 2003), it is likely that there is naturally limited habitat connectivity which may impede gene flow of forest-utilising species across the region. Currently, little is known of the abundance and current population trends of several forest-utilising bats in South Africa. Therefore, it is imperative to assess the possible impacts anthropic development may have on gene flow and population genetic structure of these species. Due to its dependence on wooded habitats, clutter-edge foraging strategy and lower dispersal ability (inferred from wing morphology), the dusky pipistrelle (*Pipistrellus hesperidus*) provides a good model system to investigate the potential impacts urbanisation and agricultural intensification may have on forest-utilising bats in the region. Microsatellites are molecular markers extensively used to estimate genetic diversity within populations and for differentiation amongst populations (Abdurakhmonov, 2016). They exhibit high mutation rates, obey Mendelian inheritance, have high polymorphism rates, and occur in high abundance (Morgante, Hanafey and Powell, 2002). They have been broadly applied in conservation genetics, landscape genetics, and studies of genetic population structure (Funk *et al.*, 2012). Therefore, I employed microsatellites in the current study to investigate genetic diversity, population structuring and permeability of contemporary land cover types to gene flow for *P. hesperidus*.

The main objectives and hypotheses of this study are:

i. To compile the first species inventory and call library for bats in forests across southern KwaZulu-Natal and Eastern Cape provinces of South Africa. More specifically, to compare the species richness, taxonomic diversity, and similarity of bat communities between sampled forests, and forest types to investigate the distribution of taxonomic diversity. In line with Lawes *et al.* (2007), I hypothesize that bat species richness and diversity would be highest in the Scarp forest group. From the historical dispersal routes of fauna from Scarp to Mistbelt and Coastal forest groups, I expect the similarity between Scarp assemblages and both Mistbelt and Coastal forest assemblages to be higher than the similarity between Mistbelt and Coastal forest assemblages (Chapter 2).

ii. To quantify the functional diversity of insectivorous bats in the study forests and to assess the effect of forest fragmentation and biogeographical structure on bat functional diversity. Furthermore, to investigate the interaction of bat functional traits with forest type and fragmentation metrics to determine which traits contribute most to species fragmentation sensitivity. I hypothesize that forest type will be a predominant predictor of functional diversity as was found for forest birds in the Eastern Cape province (Leaver *et al.*, 2019). Also, I hypothesize forest cover (measured by patch size) to positively affect functional richness (García-Morales *et al.*, 2016). Lastly, species with a reduced dispersal capacity, inferred by wing morphology of low wing loading, are predicted to be more vulnerable to reduced forest cover and increased fragmentation as they are less able to commute large distances between forests (Chapter 3).

iii. To test if habitat associations played an important role in determining the genetic diversity and population genetic structure of three forest associated and three habitat generalist bat species. Furthermore, to test whether their habitat associations informed past demographic histories in response to changes in forest extent that occurred during the Last Glacial Maximum (LGM). Species with similar habitat associations are expected to depict similar demographic histories due to shared biogeographic histories (Chen *et al.*, 2010). I hypothesize concordance in population genetic trends for species utilising similar habitats (Campbell *et al.*, 2006): forest associated species should show high levels of population structure as their distribution is dependent on fragmented forests, while habitat generalists should demonstrate low genetic structuring as suitable habitats are

more widely available. Additionally, forest associated species are expected to display demographic histories characteristic of past population contractions in response to forest contraction at the time of the LGM. Habitat generalists are expected to show stable genetic histories with overall population expansion (Chapter 4).

iv. To study the genetic diversity and population genetic structuring of a forest-utilising bat, the dusky pipistrelle (*Pipistrellus hesperidus*) across the region. The distribution of genetic diversity and structure across the region is investigated, to identify and conserve areas hosting high diversity and facilitating gene flow. Also, to examine the permeability of contemporary land cover types to gene flow for *P. hesperidus*; and assess whether fragmentation and urbanisation affect population structuring (Chapter 5).

These primary aims are proposed with the goal of generating baseline species inventories, a call library, measures of diversity and activity levels of the forest bat communities. Furthermore, I will generate novel insights of the response of functional diversity, genetic diversity, and population genetic structure of forest-utilising bats to the effects of forest fragmentation, biogeographical history, and associated structure. Findings of this study will contribute to and motivate for bat conservation efforts in forests of south-east South Africa to mitigate potential ecological and economic costs associated with the loss of bat diversity from the study region.

Chapter 2. Bats of Eastern Cape and southern KwaZulu-Natal forests, South Africa: diversity, call library and range extensions

This chapter is in press with Acta Chiropterologica. Chapters have been formatted in accordance with thesis layout.

Moir M, Rambau RV, Cherry MI, Richards LR. (in press). Bats of Eastern Cape and southern KwaZulu-Natal forests, South Africa: diversity, call library and range extensions. *Acta Chiropterologica*.

2.1 Abstract

Bats are a highly diverse order with substantial economic and ecological value. Similarly, forests in South Africa form a valuable biome supporting unique biotic diversity, yet forest bat communities have not previously been surveyed. We sampled 17 forests, of seven forest types and three forest groups, in the Eastern Cape and southern KwaZulu-Natal provinces, located within the Maputaland-Pondoland-Albany global biodiversity hotspot. We utilised capture and acoustic survey methods to compile the first bat species inventory for these forests. Species identification was performed with a combined approach of morphology, echolocation, and DNA barcodes. With this we contributed novel DNA barcodes to the Barcode of Life Data System. A total of 25 species was recorded, with range extensions southward into the Eastern Cape for six species, indicating the region to be more diverse than previously thought. Updated modelled distribution maps for these species are presented. We compiled the first reference call library of hand released bats for South African forest habitats, and the southeast region of the country. We compared species richness, diversity, and dissimilarity of the forest types and found that Scarp forests host the highest species richness and diversity. Patterns of species assemblage similarities between forest groups are attributed to forest biogeographical history and historical dispersal routes of forest fauna. A comprehensive survey, such as this, may assist in the compilation and implementation of forest conservation management plans and future monitoring programs.

2.2 Introduction

Chiroptera is a successful mammalian order comprising over 1,400 species across the globe (Mammal Diversity Database, 2020). Along with this high diversity, bats provide important ecosystem services that amount to substantial ecological and economic value (Kunz *et al.*, 2011). Insect crop pest suppression is considered the most valuable ecosystem service they

perform (Boyles *et al.*, 2013). The annual insect pest biocontrol services of bats and nocturnal birds in South African macadamia orchards was valued at just under USD 2,500 per hectare (Linden *et al.*, 2019). They also serve as key plant pollinators and seed dispersers vital for forest regeneration (Hodgkison *et al.*, 2003). African fruit bats exclusively disperse seeds of approximately 20% of submontane tree flora in the Afrotropical forests of Tanzania (Seltzer, Ndangalasi and Cordeiro, 2013). Bats are also recognised as bioindicators useful for monitoring climate change and habitat deterioration (Jones *et al.*, 2009). Despite their high diversity and high ecological and economic value, foundational knowledge of bat ecology, such as the full extent of species distributions, is often lacking, particularly for African species (Monadjem, Taylor, *et al.*, 2010; Happold and Happold, 2013). This is the case for bat communities of forests in South Africa. Bat surveys have been conducted in riverine forests and woodlands of the Kruger National Park (Rautenbach, Whiting and Fenton, 1996; Brinkley, 2018) and Mapungubwe National Park (Parker and Bernard, 2019) however, comprehensive studies of true forested habitat are limited to the Soutpansberg mountain range of northern South Africa (Taylor *et al.*, 2013; Linden *et al.*, 2014).

Forests in South Africa are biogeographically ancient (White, 1981) and have undergone historical reductions, fragmentations and expansions (Eeley, Lawes and Piper, 1999), resulting in unique evolutionary histories and compositions of their biotic communities (Lawes *et al.*, 2007). South African forests have high botanical diversity (Berliner, 2009), and the highest diversity of threatened vertebrate species of all biomes in the country (Castley, 1997). The Eastern Cape and KwaZulu-Natal provinces, located in the south-east of the country, hold the highest proportions of national forest in South Africa (Berliner, 2009). This region is known for its high biodiversity as it is situated within the Maputaland-Pondoland-Albany global biodiversity hotspot (Bertzky *et al.*, 2013). Furthermore, forests of this south-east region are likely important for bat conservation as they host a relatively high species richness and a large proportion of narrow niche species (Cooper-Bohannon *et al.*, 2016). Despite the significant ecological value of the forest habitats and potentially high bat diversity, basic bat surveys are not known to have been performed here.

South African forests have been broadly classified into three main groups: inland Afrotemperate (also known as Mistbelt), subtropical Indian Ocean Coastal, and Scarp forests which are geographically positioned between, and consist of floral and faunal elements of both Mistbelt and Coastal forests (Macdevette *et al.*, 1989; von Maltitz *et al.*, 2003). The contemporary faunal assemblages of these forests have been shaped in part by palaeoclimatic

events. Mistbelt forests are more ancient and were most affected by climatic extinction filtering during the last glacial maximum (LGM), which occurred approximately 18,000 years ago (Eeley, Lawes and Piper, 1999; Lawes *et al.*, 2007). Forest fauna inhabiting Scarp forests were less affected by the cooling and drying of the LGM (Lawes *et al.*, 2007), as these forests were buffered by their proximity to the warm Indian Ocean (Eeley, Lawes and Piper, 1999). Three faunal groups (birds, non-volant mammals and frogs) survived the LGM in Scarp forest refugia with subsequent re-dispersal back to Mistbelt forests (Lawes *et al.*, 2007). Coastal forests developed approximately 8,000 years ago (Macdevette *et al.*, 1989) and faunal colonisation occurred from Scarp forests (Lawes *et al.*, 2007). The three faunal groups showed highest species richness in Scarp forests; and faunal assemblages of Scarp forests overlapped with both Coastal and Mistbelt communities as a result of the historical dispersal emanating from Scarp forests (Lawes *et al.*, 2007).

Bats are typically surveyed by active capture methods involving mist nets and harp traps (Francis, 1989); or by passively recording their echolocation calls with acoustic recorders (O'Farrell and Gannon, 1999). Species identification of captured bats may be performed with external morphological characters, but this may be difficult for morphologically similar sympatric species (Stoffberg, Jacobs and Miller-Butterworth, 2004). Molecular techniques, such as DNA barcoding, are useful in validating or correcting field identification (Clare *et al.*, 2007). DNA barcoding entails the sequencing of a short, standardised DNA segment that may be referenced against taxonomically validated specimens in the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert, 2007) and GenBank (Benson *et al.*, 2013). Similarly, accurate species identification of recordings from acoustic studies is dependent on verified reference call libraries. Several studies have developed call libraries for southern African species (Monadjem, Taylor, *et al.*, 2010; Taylor *et al.*, 2013; Monadjem *et al.*, 2017; Brinkley, 2018; Parker and Bernard, 2019). However, bats are known to exhibit intraspecific call variation in different regions and habitats (Aspetsberger, Brandsen and Jacobs, 2003; Mutumi, Jacobs and Henning, 2016), such that reference calls may be less accurate when applied to different areas, and a call library has not yet been developed for bats in South African forests.

In this study we aimed to address this knowledge gap by surveying bat communities, to compile the first inventory and call library for forests in the Eastern Cape and southern KwaZulu-Natal provinces of South Africa. Species that were captured have been barcoded and sequenced to contribute to the BOLD and GenBank databases. Additionally, we sought to update modelled distribution maps for six species with new occurrence records emanating from this study.

Lastly, we compared species richness, diversity, and dissimilarity of bat communities between seven forest types and three forest groups: Southern Mistbelt, Scarp and Southern Coastal (after von Maltitz *et al.*, 2003). In line with the findings of Lawes *et al.* (2007), we hypothesized bat species richness and diversity would be highest in the Scarp forest group. From the historical dispersal routes of fauna from Scarp to Mistbelt and Coastal forest groups, we further hypothesized the similarity between Scarp assemblages and both Mistbelt and Coastal forest assemblages to be higher than the similarity between Mistbelt and Coastal forest assemblages.

2.3 Materials and Methods

2.3.1 Study area

We surveyed bats from 17 forests across the southern KwaZulu-Natal and Eastern Cape regions of South Africa (Figure 2.1, Appendix S2.1). Xumeni State Forest (29.926 °S, 29.882 °E) was the northernmost forest we visited, with The Island Nature Reserve (33.989 °S, 25.366 °E) as the southernmost. The forest classification scheme of von Maltitz *et al*. (2003) clusters the 17 forests into three broad forest groups and further into seven forest types. From these we visited six forests of the Eastern, Transkei and Amatole Mistbelt forest types from the Southern Mistbelt Group. These forests are located inland on the main escarpment on south and south-east facing slopes at altitudes of 850 – 1600 m (von Maltitz *et al.*, 2003). Southern Mistbelt forests stand tall (15 – 20 m) on deep, nutrient rich soils and are characterised by heavy summer mists (von Maltitz *et al.*, 2003). We sampled seven forests of the Pondoland and Transkei Coastal Scarp forest types that are classed as the Scarp Group. Forests of this group are tall (15 – 25 m), botanically speciose, structurally diverse and typically associated with coastal gorges, platforms and scarps (von Maltitz *et al.*, 2003). We also surveyed four forests of the Eastern Cape Dune and Albany Coastal forest types that are of the Southern Coastal Group. These coastal forests are low to middle-grown and found on stabilised coastal dunes or undulating coastal plains; with a dominating subtropical floral element (von Maltitz *et al.*, 2003). Sampling was conducted from late August 2017 to December 2018. Each forest was surveyed for 6 - 7 nights, following Law *et al.* (2015) and Skalak *et al.* (2012). We limited field work as much as possible to the warm, wet season and periods of low lunar illumination. Since some insectivorous species are sensitive to small variations in rainfall (Appel *et al.*, 2019), we limited sampling to nights of less than 3 mm precipitation (Fischer *et al.*, 2009). Data were collected for five nights from Umtamvuna and Xumeni forests due to high rainfall. We actively caught bats and passively recorded their echolocation calls over the same nights.

2.3.2 Active capture

The study was approved by the Stellenbosch University Animal Use and Care Research Ethics Committee (protocol #0409) and licensed by Eastern Cape Parks and Tourism Agency (RA0237, CRO 59/17CR, CRO 60/17CR), Department of Agriculture, Forestry and Fisheries (WIFM 04-2016, WIFM 09-2017, WIFM 06-2018), Ezemvelo KZN Wildlife (OP 143/2018, OP 3847/2018) and South African National Parks (CHER-MI/2018-004). Private landowner permission was acquired where necessary. We caught bats using four ground-level mist nets (Ecotone, Sopot, Poland) of 3 - 12 m lengths for approximately 4 hours after sunset. We also deployed three-bank harp traps (Faunatech, Victoria, Australia) where possible. We sampled across roads, pathways, and water courses within the forests as they were likely to be commonly used flight paths. We alternated trapping location every night and maintained a minimum distance of 50 m from nearby acoustic monitoring locations. The capture and handling of bats was done in accordance with Sikes and the Animal Care and Use Committee of the American Society of Mammalogists (2016). Upon capture, morphological data for species identification, sex, reproductive condition, and approximate life stage (subadult/adult) were collected. Sub-adults were detected by the incomplete ossification of epiphyseal joints. A 2 - 3 mm wing membrane biopsy was taken from each individual and stored in 96% ethanol. Bats were released at the site of capture after processing. Upon release, echolocation calls were recorded with an EchoMeter 3 (EM3) detector (Wildlife Acoustics, Maynard, USA) for compilation of the call library. Initial species identification was performed with external morphology following Monadjem, Taylor, *et al.* (2010), and later finalised with release calls and DNA barcodes.

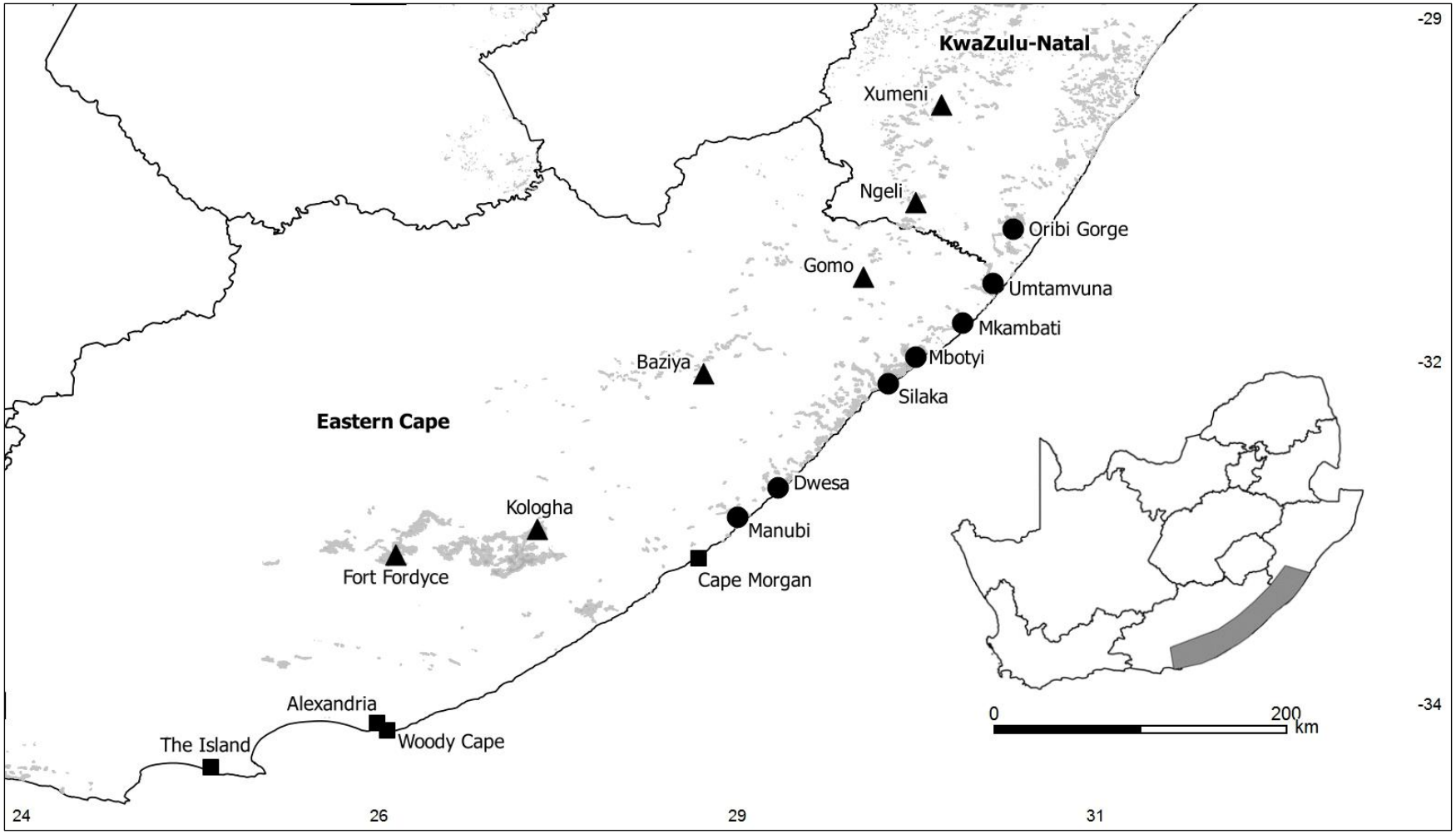


Figure 2.1 Map of seventeen forest sampling sites; locality shapes correspond with forest groups (Southern Mistbelt group – triangles, Scarp group – circles, Southern Coastal – squares). The inset of South Africa shows the study area extent as a grey filled polygon. Shading displays the extent of indigenous forest cover (Thompson, 2019).

2.3.3 Passive acoustic monitoring and sound analysis

Five Wildlife Acoustics Song Meter SM4BAT acoustic recorders and one Wildlife Acoustics SM2+BAT recorder were deployed at each forest to record echolocation calls. The recorders were positioned along pathways, roads, or watercourses within the forests. Each recorder was set to trigger at 16 kHz (lower trigger settings caused too many false triggers from insects resulting in issues of data storage), with 384 kHz sample rate and 12 dB gain in full spectrum. Recording was performed throughout the night, starting 30 minutes before sunset until one hour after sunrise. Recorders were placed a minimum distance of 250 - 500 m apart to reduce pseudo-replication (Hurlbert, 1984; Kingston, 2016; Colegrave and Ruxton, 2018). Microphones were placed at a height of 2 – 3 m above the ground and pointed in the direction of fewest trees as recommended by Weller and Zabel (2002).

Echolocation calls were visualised as spectrograms with Wildlife Acoustics Kaleidoscope Pro software. We examined recordings of hand released bats for search-phase calls showing the full bandwidth, nearer to the end of the recording, to utilise calls that were the best approximation of natural calls (Kearney *et al.*, 2019). Approximately 10 consecutive calls were used to measure parameters for compilation of the call library (Monadjem *et al.*, 2017). Four parameters are useful in identifying southern African bat species: duration of call (Dur), minimum frequency (Fmin), frequency of flattest section of call (Fc), and knee frequency (Fk) (Taylor *et al.*, 2013). We measured and reported these parameters as well as maximum frequency (Fmax) for the call library. The passive acoustic recordings were run through Wildlife Acoustics Kaleidoscope Pro to scrub noise files and apply the automated species identification filter, Bats of South Africa 5.1.0, on the -1 more sensitive setting. All files were then manually verified for species identification using the call library we developed for the region, and other published works for South Africa and Swaziland (Monadjem, Taylor, *et al.*, 2010; Taylor *et al.*, 2013; Monadjem *et al.*, 2017). The species identification had an average of 68% accuracy as the filter did not encompass ten species recorded here. Files with less than two calls and calls that could not be confidently identified to species level (N = 2,066) were discarded from further analyses.

2.3.4 Barcoding

The cytochrome c oxidase subunit 1 mitochondrial gene (COI) is used for barcoding purposes, but cytochrome *b* is also reliable for species identification (Branicki, Kupiec and Pawlowski, 2003). We employed a combination of both markers to verify species identification as barcodes

were not available for all species. DNA extraction from wing punches and cytochrome *b* amplification procedures are described in Moir *et al.* (2020). The same PCR reactions and conditions were employed for amplification of COI, with the exception of an annealing temperature of 42.5°C. The universal primers LCO1490 and HCO2198 (Folmer *et al.*, 1994) were utilised. PCR products were separated in 1% agarose gels and gene fragments were purified with a BioSpin Gel Extraction Kit (Bioer Technology, Hangzhou, China). The products were sequenced at the Core Sequencing Facility, Stellenbosch University. Sequences were cross-referenced on https://blast.ncbi.nlm.nih.gov/Blast.cgi to ascertain species identification. Barcodes were deposited with Barcode of Life Data Systems under the Foundational Biodiversity Information Programme (FBIP) in the Foundational Biodiversity Eastern Cape Forest project. Cytochrome *b* sequences were deposited with GenBank (accession numbers MN790784 – 790977).

2.3.5 Activity, richness, diversity, and dissimilarity

Capture rates were standardised between forest types by summing the number of hours nets were open per meter length for mist netting; and by the sum of hours the harp traps were deployed with the square meter size of the trap (Kingston, 2016). Two measures of bat activity were calculated. Firstly, the mean number of passes (of all species) per recording hour, where a pass was defined as one or more echolocation pulses with <1 s between sequential pulses (Fenton, 1970). This measure of bat activity was utilised for comparison of results with that of Sowler *et al.* (2020). Secondly, the acoustic activity index (AI) of Miller (2001) was calculated whereby the presence of bat activity (for all species) is recorded per one-minute interval. This method is less biased by multiple recordings of the same individual. Both measures of activity were standardised by total recording time. Incidence data from active capture, passive acoustic recording, and a combined dataset were pooled per forest type. EstimateS (Colwell, 2013) was used to calculate the Chao (1987) species richness estimates and Shannon-Weiner diversity index (*H'*) (Magurran, 2004). The acoustic survey design and effort were standardised for each forest site, while active capture was not, as such only data from acoustic surveys were used to generate pairwise Jaccard dissimilarity matrices. We employed incidence data from 30 acoustic nights per forest as this was the minimum sampling effort per site. We then grouped the data for forest sites into the seven forest types using a total of 60 acoustic nights, and further into the three forest groups using 120 acoustic nights per group. We used the 'betapart' package (Baselga and Orme, 2012) in R (R Core Team, 2019) to calculate total dissimilarity with Jaccard indices (a monotonic transformation of beta diversity) with the respective turnover and

nestedness components (Baselga and Orme, 2012).

2.3.6 Species distribution models

We updated the potential distribution maps of six species (*Epomophorus crypturus*, *Hypsugo anchietae* (*Neoromicia anchietae* as per Monadjem *et al.*, 2020), *Kerivoula argentata*, *Laephotis botswanae*, *Myotis bocagii* and *Otomops martiensseni*), with the addition of new locality records emanating from the captures and acoustic monitoring of this study. Georeferenced presence data was gathered from the Global Biodiversity Information Facility (www.gbif.org), the African Chiroptera Report (2019), and South African natural history museums namely, Amatole, Ditsong, Iziko, McGregor and Durban Natural Science museums. The R package 'spThin' (Aiello-Lammens *et al.*, 2015) was used to reduce presence data to one point per kilometre. Potential distributions were modelled using 19 bioclimatic variables (www.worldclim.org) that are derivations of monthly temperature and precipitation data. Thirty arc second (~ 1 km) resolution data were clipped to the coordinates -4.0°, 9.0° and -35.5°, 42.0° such that the distribution models cover southern Africa. The point sampling tool was used in QGIS (QGIS Development Team, 2014) to extract bioclimatic variables at the occurrence points for each species. These data frames were then tested for collinearity between the bioclimatic variables with the 'cor' function in R Studio, using $r < 0.7$ threshold (Dormann *et al.*, 2013). One of a pair of highly correlated variables was excluded from the trial model runs. Variables that reduced model performance, or contributed less to it, were removed. Final variables used are listed in Appendix S2.5.

The potential distributions were created with maximum entropy species distribution modelling software - MaxEnt (Phillips, Dudik and Schapire, 2006). We used occurrence data with the presence-only model as few locality records are mostly available for these species and absence information is unavailable. We set the software to use a 75 to 25% split of the data for training and testing, with a regularisation multiplier of 1. We used 10 replicates for the Jackknife validation function to assess the contributions of bioclimatic variables for each species during trial runs. Variables that reduced model performance, or contributed minimally to it, were removed. Model performance was assessed with AUC values (area under the curve), highly predictive models showed values closer to 1.0 and models with lower predictive performance showed values below 0.7 (Metz, 1978).

2.4 Results

2.4.1 Species inventory

Twenty-five species were detected, with both capture and acoustic methods, from the 17 forests (Table 2.1). A total of 519 individuals belonging to 19 species were caught with 2,138 m^2 harp trap hours and 35,096 mist net meter hours. A total of 116,818 bat passes of 21 species were recorded over 6,220 recording hours (Table 2.1 and 2.3). Two fruit bat species, *Epomophorus crypturus* and *E. wahlbergi*, were sampled exclusively by means of mist netting as they do not echolocate. Male fruit bat mate attraction calls were heard in Baziya and Manubi forests. The audible calls of these two species are similar and could not be reliably discerned, so we assumed them to have been individuals of *E. wahlbergi* as this species was caught in these forests/forest types whereas *E. crypturus* was not. Both *Epomophorus* species were captured from the same sampling point or within very similar localities within Oribi Gorge and Mbotyi forests (Table 2.1), indicating these species may be syntopic. Two insectivorous species, *Hipposideros caffer* and *Hypsugo anchietae* (*Neoromicia anchietae* as per Monadjem *et al*., 2020), were also sampled only via active capture.

Field identification of captured bats, based on morphology and release call characteristics, was relatively accurate when referenced against DNA barcodes, as most morphologically similar species had easily differentiated echolocation calls. The cytochrome *b* sequences allowed us to differentiate *Hypsugo achietae* (*Neoromicia anchietae* as per Monadjem *et al*., 2020) from *Pipistrellus hesperidus*, and *Epomophorus crypturus* from *E. wahlbergi* as these species display overlapping morphology and/or call parameters. *Hypsugo anchietae* was mistakenly identified as *P. hesperidus* in the field. These two species depict largely overlapping call parameters and the previously furthest south locality of *H. anchietae* (*Neoromicia anchietae* as per Monadjem *et al*., 2020) was Port Edward (Durban Natural Science Museum no. 13403) (ACR, 2019). Cytochrome *b* sequences of *H. anchietae* recovered 99% BLAST identity match. *Epomophorus crypturus* can be easily distinguished from *E. wahlbergi* by the presence of post-dental palatal ridges (Taylor and Monadjem, 2008). This character was not noted during sampling as distribution maps did not indicate *E. crypturus* to occur that far south (Monadjem, Taylor, *et al.*, 2010). *Epomophorus* species sequences retrieved 99% match, with approximately 5% differentiation between them. The decision to identify *E. crypturus* from *E. wahlbergi* in more southern forests, such as The Island and Alexandria, with cytochrome *b* sequences was facilitated by a Yale Peabody Museum voucher (MAM 007552) locality from

Cypris, Port Elizabeth (33° 58'S, 25° 36'E) (ACR, 2019). Our study benefitted from DNA barcoding as we confirmed the range extension alluded to in the African Chiroptera Report (ACR) by this museum voucher.

2.4.2 Reproductive condition

Reproductively active (pregnant, lactating, or post-lactating) females of all species were captured from the onset of the spring season throughout most of the summer months, which coincides with the wet season (August – February; Appendix S2.4). The fruit bats, *E. crypturus* and *E. wahlbergi*, are known to birth pups throughout the year, with peaks in the wet summer months (Monadjem, Taylor, *et al.*, 2010). We did not detect reproductively active females during the dry season as we limited the sampling to the wet season. Sub-adults of the two fruit bat species and *Scotophilus dinganii* were also captured in the summer months (Appendix S2.4).

Table 2.1 Twenty-five species of eight families sampled in forests of the Eastern Cape and KwaZulu-Natal provinces, South Africa. Forests are grouped in line with von Maltitz *et al*. (2003). The number of individuals captured with mist nets and harp traps are indicated, * depicts detection with acoustic detectors, bold indicates new records extending known species ranges, and # indicates audible male mate attraction calls that were noted.

	Southern Mistbelt Group						Scarp Group							Southern Coastal Group			
	Eastern Mistbelt		Transkei Mistbelt		Amatole Mistbelt		Pondoland Scarp				Transkei Coastal Scarp			Eastern Cape Dune		Albany Coastal	
	Xumeni	Ngeli	Gomo	Baziya	Kologha	Fort Fordyce	Oribi Gorge	Umtamvuna	Mkambati	Mbotyi	Silaka	Dwesa	Manubi	Cape Morgan	Woody Cape	The Island	Alexandria
Pteropodidae																	
Epomophorus																	
crypturus							**2**	**4**		**1**						**3**	**1**
E. wahlbergi		1	1	#			1			4		1	1 #	1			
Hipposideridae																	
Hipposideros																	
caffer							2										
Rhinolophidae																	
Rhinolophus																	
capensis					*	1*									*	*	*
R. clivosus	*	*	1*	*	*	4*	9*	*	1*	*	*		*	*	*	*	3*
R. simulator	1*	*	*	*			*	*	*	*							
R. swinnyi	*	4*	1*	13*	2*	6*		*		2*	*	1*					
Emballonuridae																	
Taphozous																	
mauritianus													*				
Nycteridae																	
Nycteris																	
thebaica									*								
Molossidae																	
Chaerephon																	
pumilus							*	*		*		*					

	Southern Mistbelt Group						Scarp Group							Southern Coastal Group			
	Eastern Mistbelt		Transkei Mistbelt		Amatole Mistbelt		Pondoland Scarp				Transkei Coastal Scarp			Eastern Cape Dune		Albany Coastal	
	Xumeni	Ngeli	Gomo	Baziya	Kologha	Fort Fordyce	Oribi Gorge	Umtamvuna	Mkambati	Mbotyi	Silaka	Dwesa	Manubi	Cape Morgan	Woody Cape	The Island	Alexandria
Otomops martiensseni							*****			*****			*****				
Tadarida aegyptiaca	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Miniopteridae																	
Miniopterus fraterculus	5*	*	7*	2*	10*	17*	1*			*	*	*	*			13*	1*
M. natalensis	*	*	13*	*	*	1*	*	*	*	*	*	2*	*	*	*	3*	1*
Vespertilionidae																	
Eptesicus hottentotus	*	*	*	*	*	*	*	*	*			*	*	*	*	*	*
Hypsugo anchietae (*Neoromicia anchietae* as per Monadjem *et al*., 2020)													**1**		**6**		**2**
Kerivoula argentata	*****								**1***	*****							
K. lanosa		*	*	*	1*	*											
Laephotis botswanae	2*	**1***	**3***	**2***	*****	**2***	*****					*****	**3***				
Myotis bocagii							**1***	**2***		**1**							
M. tricolor	*	7*	*	3*	*	4*	6*	*		2*	5*	*	3*			*	*
Neoromicia capensis (*Laephotis capensis* as per	4*	2*	*	5*	2*	8*	*	12*	*	*	*	*	1*	9*	24*	2*	9*

	Southern Mistbelt Group						Scarp Group							Southern Coastal Group			
	Eastern Mistbelt		Transkei Mistbelt		Amatole Mistbelt		Pondoland Scarp				Transkei Coastal Scarp			Eastern Cape Dune		Albany Coastal	
	Xumeni	Ngeli	Gomo	Baziya	Kologha	Fort Fordyce	Oribi Gorge	Umtamvuna	Mkambati	Mbotyi	Silaka	Dwesa	Manubi	Cape Morgan	Woody Cape	The Island	Alexandria
Monadjem *et al*., 2020) *N. nana* (*Afronycteris nana* as per Monadjem *et al*., 2020)							3*	*	*	1*	16*	2*	13*	13*			
Pipistrellus hesperidus	10*	10*	32*	10*	1*	26*	3*	2*	3*	11*	4*	12*	45*	8*	*	*	6*
Scotophilus dinganii							1*	4*	*	*	1*	*	*	*	*		*

2.4.3 Call library

We measured release call parameters from 449 of the 519 captured individuals from 17 species to compile the reference call library (Table 2.2). A typical call of each species is shown on spectrograms in Appendix S2.2 and 2.3. Calls of the four families can be discerned by the parameters presented in Table 2.2, but there is considerable overlap between a few confamilial species. *Rhinolophus capensis* and *R. simulator* display largely overlapping parameters (Table 2.2). These species have overlapping predicted distributions at the edges of their ranges (Monadjem, Taylor, *et al.*, 2010), and accurate identification of acoustic recordings from the overlapping area is complicated by the geographic variation of *R. capensis* calls across its west to east range (Odendaal, Jacobs and Bishop, 2014). The acoustic recordings of approximately 80 kHz from Amatole Mistbelt forests were identified as *R. capensis*, as this species was caught in this forest type and *R. simulator* has not been previously recorded that far south (Monadjem, Taylor, *et al.*, 2010; ACR, 2019). The Vespertilionidae species, *Pipistrellus hesperidus* and *Hypsugo anchietae*, are acoustically indistinguishable based on the release call parameters (Table 2.2); the captured *H. anchietae* individuals were positively identified by cytochrome *b* sequences. All passive acoustic recordings with these species' parameters were identified as *P. hesperidus* as it was the most abundant species across all forest types (Table 2.1). Thus, *H. anchietae* may be considerably under-represented in this study. Calls of *Myotis* species are easily distinguished from other species by their vertical shape (Appendix S2.3). However, the differentiation of *Myotis bocagii* and *M. tricolor* may be unreliable due to the considerable variation in their Fc and Fk measurements. *Myotis* calls from passive acoustic recordings, with Fc and Fk values within the ranges listed in Table 2.2, were identified as *M. bocagii* only at localities in which it was captured. This species may thus also be under-represented here.

Table 2.2 Release call parameters (mean ± SD, with range in italics below) of 17 bat species recorded from forests of the Eastern Cape and southern KwaZulu-Natal. N_1 – number of individual bats, N_2 – total number of calls, Dur – duration, Fmax – maximum frequency, Fmin – minimum frequency, Fc – frequency of flattest section of call, Fk – knee frequency.

Family and species	N_1 (N_2)	Dur (ms)	Fmax (kHz)	Fmin (kHz)	Fc (kHz)	Fk (kHz)
Hipposideridae						
Hipposideros caffer	2 (49)	5.06 ± 1.29	148.64 ± 0.03	136.46 ± 3.04	145.93 ± 0.54	148.1 ± 0.08
		4.15 - 5.97	*148.62 - 148.67*	*134.31 - 138.61*	*145.55 - 146.31*	*148.04 - 148.16*
Rhinolophidae						
Rhinolophus capensis	1 (19)	8.49 ± 1.41	81.72 ± 1.09	78.87 ± 0.96	79.83 ± 0.98	80.41 ± 1.01
		7.09 - 9.90	*80.63 - 82.81*	*77.91 - 79.83*	*78.85 – 80.84*	*79.39 – 81.43*
R. clivosus	15 (335)	16.66 ± 4.09	94.39 ± 0.74	84.99 ± 3.54	92.76 ± 0.8	92.61 ± 0.7
		10.42 - 25.11	*93.34 - 96*	*76.30 - 88.77*	*91.73 - 94.66*	*91.27 - 93.86*
R. simulator	1 (28)	11.20 ± 0.87	82.31 ± 0.10	77.24 ± 0.08	81.06 ± 0.08	81.12 ± 0.09
		9.47 – 11.20	*82.11 – 82.31*	*77.24 – 77.39*	*80.90 – 81.06*	*80.93 – 81.12*
R. swinnyi	29 (811)	13.11 ± 5.44	107.29 ± 1.11	95.93 ± 3.98	105.4 ± 0.91	105.79 ± 1.18
		5.78 - 30.09	*105.29 - 109.61*	*88.12 - 104.39*	*103.56 - 107.61*	*103.02 - 108.41*
Miniopteridae						
Miniopterus fraterculus	52 (970)	2.07 ± 0.51	83.21 ± 8.26	58.59 ± 1.65	58.59 ± 1.65	59.35 ± 1.43
		1.33 - 3.78	*68.35 - 101.11*	*53.43 - 61.19*	*56.43 - 61.19*	*57.80 - 61.46*
M. natalensis	20 (373)	2.19 ± 0.49	71.72 ± 5.90	48.28 ± 1.65	50.37 ± 2.14	52.79 ± 2.71
		1.46 - 3.48	*62.55 - 85.55*	*45.99 - 53.83*	*47.26 - 53.25*	*49.07 - 61.60*
Vespertilionidae						
Hypsugo anchietae	8 (125)	2.73 ± 0.32	87.09 ± 8.74	46.06 ± 1.26	46.63 ± 1.29	50.5 ± 2.08

(*Neoromicia anchietae* as per Monadjem *et al*., 2020)		*2.16 - 3.16*	*74.02 – 92.60*	*44.46 - 47.95*	*44.83 - 48.38*	*46.88 - 52.85*
Kerivoula argentata	1 (6)	1.55 ± 0.70	151.48 ± 6.60	84.06 ± 4.29	108.02 ± 12.76	104.50 ± 3.69
		0.70 - 2.54	*141.22 - 157.871*	*77.67 - 90.28*	*93.81 - 122.56*	*100.37 - 110.46*
K. lanosa	1 (10)	1.22 ± 0.2	147.28 ± 5.53	70.28 ± 2.95	95.41 ± 3.31	95.58 ± 5.68
		0.66 - 1.60	*137.19 - 152.32*	*67.59 - 74.65*	*89.28 - 98.86*	*90.79 - 107.94*
Laephotis botswanae	10 (269)	1.54 ± 0.21	59.63 ± 3.62	33.25 ± 0.66	33.73 ± 0.75	38.85 ± 2.43
		1.07 - 1.72	*53.43 - 62.9*	*32.02 – 34.00*	*32.88 - 35.38*	*35.54 - 44.38*
Myotis bocagii	2 (69)	2.27 ± 0.13	64.98 ± 2.71	35.69 ± 0.16	39.68 ± 1.08	59.01 ± 6.41
		2.18 - 2.36	*58.57 – 69.35*	*35.31 – 35.69*	*38.31 – 46.99*	*50.35 - 65.79*
M. tricolor	28 (510)	2.50 ± 0.47	77.20 ± 5.14	35.59 ± 3.51	56.38 ± 6.64	75.09 ± 5.76
		2.08 – 3.45	*70.53 – 86.49*	*27.35 – 38.95*	*48.32 – 69.75*	*68.16 – 85.59*
Neoromicia capensis (*Laephotis capensis* as per Monadjem *et al*., 2020)	78 (2384)	3.09 ± 0.65	68.15 ± 9.29	39.2 ± 1.36	39.63 ± 1.35	42.5 ± 2.20
		1.93 - 4.87	*46.23 - 82.68*	*35.64 - 42.26*	*35.85 - 43.09*	*36.79 - 48.17*
N. nana (*Afronycteris nana* as per Monadjem *et al*., 2020)	47 (1097)	2.09 ± 0.55	91.8 ± 11.23	66.97 ± 2.26	67.28 ± 2.27	71.17 ± 2.89
		1.21 - 3.63	*71.92 - 118.39*	*60.34 - 71.49*	*61.02 - 71.68*	*65.27 - 77.96*
Pipistrellus hesperidus	129 (3871)	2.59 ± 0.66	73.11 ± 7.87	45.63 ± 1.44	46.04 ± 1.42	49.65 ± 2.27
		1.43 - 4.31	*50.96 - 91.36*	*42.60 - 49.81*	*42.79 - 49.92*	*44.11 - 54.55*
Scotophilus dingani	5 (132)	2.65 ± 0.52	53.63 ± 8.39	34.68 ± 1.58	34.68 ± 1.58	35.33 ± 1.72
		2.30 - 3.55	*51.57 - 74.49*	*33.15 - 36.94*	*33.15 - 36.94*	*33.57 - 37.95*

Table 2.3 Standardised number of captures from active sampling and mean activity levels from acoustic monitoring of bats in forests on the eastern seaboard of South Africa. Mist netting, harp trapping and acoustic recording sampling effort is detailed with the total number of captures and passes per method. Total taxonomic species richness and Shannon-Weiner diversity from the combined capture and acoustic datasets are also indicated.

Forest type	Total mist net meter hours (Total captures)	Captures/ mist net meter hour	Total m^2 harp trap hour (Total captures)	Captures/m^2 harp trap hour	Total acoustic nights (Total passes)	Mean passes/ recording hour	Mean activity index (AI)	Species richness	Shannon diversity (*H'*)
Eastern Mistbelt	6,714 (42)	0.006	588 (5)	0.009	66 (9,996)	15.15	0.13	14	2.50
Transkei Mistbelt	6,180.75 (93)	0.015	0	-	80 (12,976)	16.22	0.15	13	2.38
Amatole Mistbelt	1,832.25 (50)	0.027	252 (37)	0.147	80 (15,784)	19.73	0.15	12	2.46
Pondoland Scarp	7,528.5 (83)	0.011	210 (2)	0.010	132 (23,589)	17.87	0.16	21	2.82
Transkei Coastal Scarp	6,471.75 (44)	0.007	710 (77)	0.108	118 (19,530)	16.55	0.14	17	2.54
Eastern Cape Dune	2,964 (65)	0.022	126 (6)	0.048	76 (27,182)	35.77	0.22	11	2.17
Albany Coastal	3,405 (46)	0.014	252 (4)	0.016	70 (7,761)	11.09	0.11	12	2.34

2.4.4 Activity, richness, diversity, and dissimilarity

The Amatole Mistbelt forests had the highest mist net (0.027 bats/mhr) and harp trap (0.147 bats/m^2hr) capture rates, while the Eastern Cape Dune forests displayed the highest activity levels from acoustic recorders (35.77 passes/hour, AI = 0.22) (Table 2.3). The Eastern Mistbelt and Albany Coastal forest types had the lowest capture rate and acoustic activity levels. Species richness and diversity were highest for the Pondoland Scarp forests (21 species, *H'* = 2.82) followed by Transkei Coastal Scarp (17 species, *H'* = 2.54) (Table 2.3). Species richness estimates were also highest for both Scarp types for the acoustic and combined datasets (Figure 2.2). The Eastern Cape Dune and Albany Coastal forests consistently had the lowest species richness, diversity (Table 2.3) and richness estimates (Figure 2.2). The active capture datasets began to plateau after 10 nights of sampling for all forest types, except Amatole Mistbelt and Transkei Coastal Scarp (Figure 2.2). We detected 12 species using both monitoring methods for the Amatole Mistbelt forest type (Table 2.3), although the Chao richness estimator from capture data predicted a total of 17 species may occur in this forest type (Figure 2.2). This may be due to high capture rates from Fort Fordyce Nature Reserve. The richness estimators of the acoustic datasets mostly plateaued after 6 - 7 nights of monitoring. The combined datasets showed 10 nights of sampling presented a 90 – 100% complete survey of all forest types, except for Transkei Coastal Scarp which was 70% complete. The Transkei Coastal Scarp combined dataset estimator did not plateau even after 22 nights (Figure 2.2).

With the Jaccard dissimilarity index, we found the bat communities of the three forest groups to be relatively comparable (Table 2.4). The greatest dissimilarity was between the Scarp and Southern Coastal groups (0.47), with a high nestedness (0.31). Looking at the forest type level results between these two groups, the Eastern Cape Dune forest type had the greatest dissimilarity index with Pondoland Scarp (0.56), with nestedness values exceeding those of turnover between the four Coastal and Scarp forest types (Table 2.5). The analysis on the forest level depicts Mbotyi and Woody Cape forests to be most dissimilar (0.63, Appendix 2.6). The Scarp and Southern Mistbelt groups were the most similar (0.40) (Table 2.4), however the overall highest dissimilarity values between individual forests was of Mkambati (Scarp) with Kologha and Fort Fordyce (Southern Mistbelt) (0.65), largely due to high turnover (Appendix S2.7). This points to the importance of quantifying beta diversity at multiple spatial scales of sampling to identify site specific trends as well as the scales at which diversity responds to environmental gradients (Veech *et al.*, 2010). The dissimilarity indices between forest types of the same forest group were consistently lower than between forest types of different groups

(Table 2.4), with the lowest dissimilarity between Eastern and Transkei Mistbelt types (0.08) resulting solely from nestedness (Table 2.5). Eastern Cape Dune forest depicted the highest dissimilarity values with Eastern and Transkei Mistbelt types (0.62 and 0.60, respectively) (Table 2.5), with this trend evident at the individual forests level too (Appendix S2.6). It is interesting to note that the Amatole Mistbelt and Albany Coastal types depict the lowest value of dissimilarity between forest types of different groups (0.31), with similarly low values between individual forests of these types (Appendix S2.6).

Table 2.4 Jaccard dissimilarity matrix of bat communities sampled from three forest groups in the Eastern Cape and KwaZulu-Natal provinces. Turnover and nestedness values are presented in parentheses.

Forest groups	Jaccard dissimilarity (turnover/nestedness)
Scarp – Southern Mistbelt	0.40 (0.25/0.15)
Scarp – Southern Coastal	0.47 (0.16/0.31)
Southern Mistbelt – Southern Coastal	0.44 (0.31/0.13)

Table 2.5 Jaccard dissimilarity matrix of bat communities sampled from seven forest types in the Eastern Cape and KwaZulu-Natal provinces with turnover and nestedness values in parentheses (turnover/nestedness).

	Eastern Mistbelt	Transkei Mistbelt	Amatole Mistbelt	Pondoland Scarp	Transkei Coastal Scarp	Eastern Cape Dune
Transkei Mistbelt	0.08 (0.00/0.08)					
Amatole Mistbelt	0.21 (0.15/0.06)	0.154 (0.15/0.00)				
Pondoland Scarp	0.33 (0.14/0.19)	0.38 (0.15/0.23)	0.47 (0.29/0.18)			
Transkei Coastal Scarp	0.44 (0.37/0.07)	0.41 (0.29/0.12)	0.41 (0.29/0.12)	0.22 (0.13/0.09)		
Eastern Cape Dune	0.62 (0.50/0.12)	0.60 (0.50/0.10)	0.50 (0.36/0.14)	0.56 (0.20/0.36)	0.50 (0.20/0.30)	
Albany Coastal	0.46 (0.33/0.13)	0.43 (0.33/0.10)	0.31 (0.18/0.13)	0.50 (0.18/0.32)	0.44 (0.18/0.26)	0.27 (0.20/0.07)

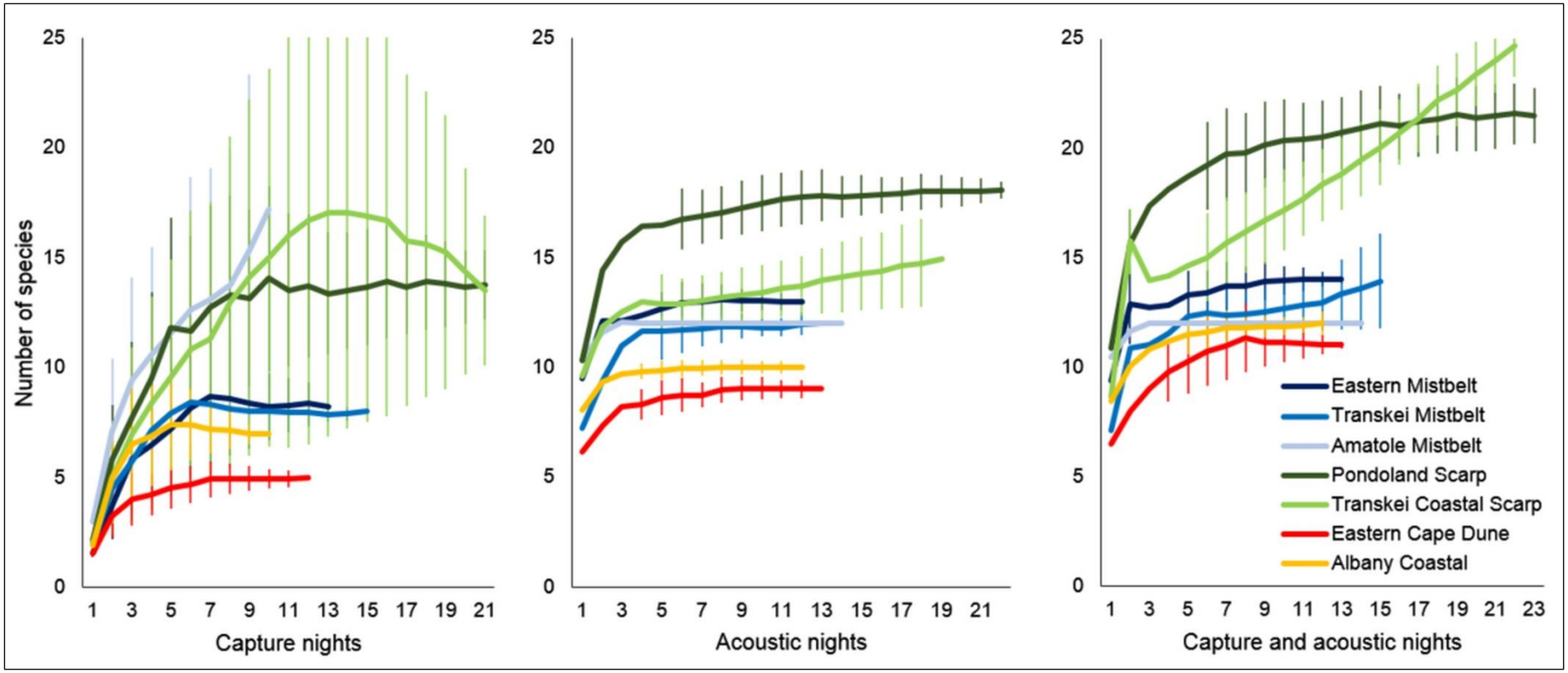


Figure 2.2 Species richness estimates (Chao, 1987) per forest type per monitoring method (capture, acoustic and combined) calculated with EstimateS (Colwell, 2013). Standard deviations shown by error bars.

2.4.5 Species distribution models

The updated modelled distribution maps for six species are presented in Figure 2.3. Five to 10 bioclimatic variables were used in each model and AUC test values ranged between 0.822 and 0.971 (Appendix S2.5). When compared to the map of Monadjem, Taylor, *et al.* (2010), the potential distribution area for *Epomophorus crypturus* was extensively increased along the south east coastline due to our new distribution record from The Island Nature Reserve in Port Elizabeth (Figure 2.3A). The model predicts *E. crypturus* to occur as far south as the Southern Afrotemperate forests along the south east coast (Figure 2.3A). Our captures of *Hypsugo anchietae* (*Neoromicia anchietae* as per Monadjem *et al*., 2020) from Alexandria and Woody Cape Nature Reserves are currently the furthest south records for this species (Monadjem, Taylor, *et al.*, 2010; ACR, 2019). The addition of these localities did not extend the modelled range when compared with the map of (Monadjem, Taylor, *et al.*, 2010) (Figure 2.3B). We collected an acoustic recording of *Kerivoula argentata* from Xumeni forest (Table 2.1): it has not been previously recorded inland from the South African coastline (Monadjem, Taylor, *et al.*, 2010; ACR, 2019). Also, this species has not been previously recorded from localities as far south as Mkambati and Mbotyi forests (Table 2.1). The potential distribution modelled here shows a reduced range along the south east coast compared to that of Monadjem, Taylor, *et al.* (2010) (Figure 2.3C), this is likely due to the use of different bioclimatic variables and model fit. We provide eight new records for *Laephotis botswanae*, substantially increasing its predicted extent into the Eastern Cape and along the south east coast (Figure 2.3D). The distribution of *Myotis bocagii* was not previously modelled in Monadjem, Taylor, *et al.* (2010), as there were too few records available at the time of publication. We present three new locality records: Oribi Gorge, Umtamvuna and Mbotyi forests (Table 2.1). The potential range of this species extends along the coastline and into the interior of the KwaZulu-Natal and Eastern Cape provinces (Figure 2.3E). The distribution map of *Otomops martiensseni* within southern Africa, was not modelled in Monadjem, Taylor, *et al.* (2010) as too few localities were available at that time. We recorded *O. martiensseni* calls from Manubi forest, currently the furthest south record for this species. This species is modelled to have a very localised distribution along the coastline of KwaZulu-Natal and partly into the Eastern Cape province (Figure 2.3F).

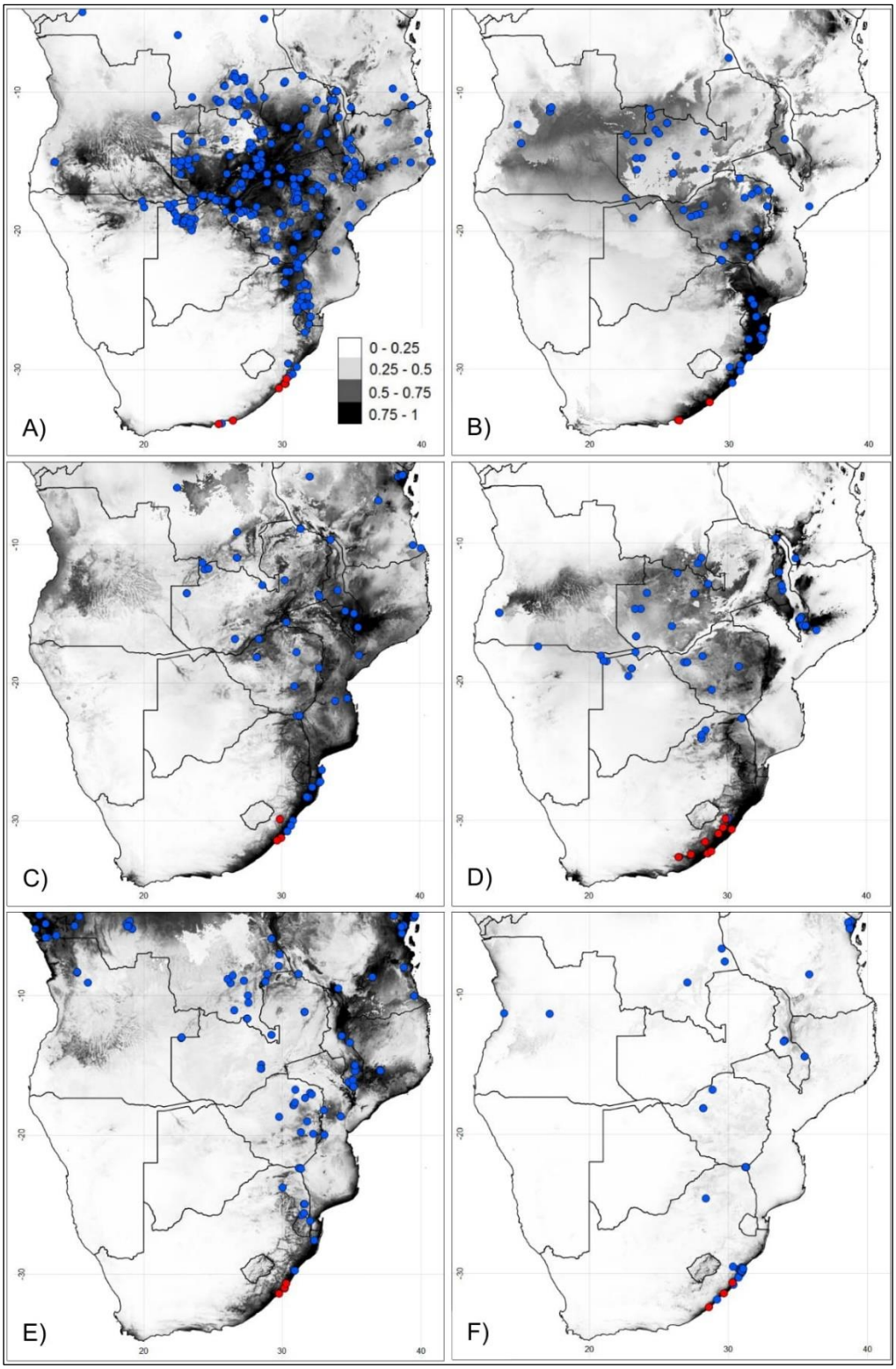


Figure 2.3 Species distribution maps updated with new occurrence records for A) *Epomophorus crypturus* B) *Hypsugo anchietae* (*Neoromicia anchietae* as per Monadjem *et al*., 2020) C) *Kerivoula argentata* D) *Laephotis botswanae* E) *Myotis bocagii* and F) *Otomops martiensseni*. Distribution maps were generated with presence data in MaxEnt (Phillips *et al*., 2006). Red dots indicate new records from this study; blue dots are from other sources. Key indicates probability of occurrence.

2.5 Discussion

This study presents the first bat species inventory for forests in the Eastern Cape and southern KwaZulu-Natal provinces of South Africa, based on a comprehensive survey of 17 forests. Utilising capture and acoustic methods, we sampled a total of 25 species (Table 2.1). Bearing in mind the sampling methods and effort varied between surveys, our study yielded a similar richness to those of Shapiro and Monadjem (2016) (26 species) and Taylor *et al.* (2013) (24 species). We have identified forest habitats hosting high bat species richness and diversity, with range extensions for six species indicating the region to be more diverse than previously thought. Two of the range extended species, *K. argentata* and *O. martiensseni*, have Near Threatened regional red list status (Monadjem, Cohen, *et al.*, 2016; Richards *et al.*, 2016). These two species are rare and difficult to sample, and much is unknown of their population trends and distributions (Monadjem, Cohen, *et al.*, 2016; Richards *et al.*, 2016). The new occurrence records and updated distribution maps may facilitate future conservation assessments and action plans for these species. A comprehensive survey, such as this, may also assist in the compilation and implementation of forest conservation management plans and monitoring programs.

2.5.1 Call library for forests in the south east of South Africa

We present the first reference call library for forested habitats in the KwaZulu-Natal and Eastern Cape provinces, as recorded by a Wildlife Acoustics EM3 detector (Table 2.2). It is relatively complete for four families, but reference calls for high flying species of the region are still needed. Call parameters for rare species based on few individuals, such as *K. argentata*, *K. lanosa* and *M. bocagii*, should be used with caution. The library is useful for easy identification of families and functional groups, but we could not distinguish between three species pairs based on their call parameters. This problem is often encountered for sympatric species that occupy similar foraging niches (Russo and Jones, 2002), and has been found in other acoustic studies for South Africa (Taylor *et al.*, 2013) and Eswatini (Monadjem *et al.*, 2017). The overlap in parameters complicates surveys based solely on acoustic methods and may substantially increase species identification error, rendering reference call libraries essential for accurate identification. The library we have developed may be applied to future acoustic surveys within the south east region of South Africa and forested habitats across the country.

2.5.2 The utility of DNA barcoding

Cryptic diversity is frequently found in bats, as many species display highly similar morphological feature, hampering field identification (Clare *et al.*, 2007). DNA barcoding has performed well even for highly diverse bat assemblages, and was successfully used to differentiate 165 species from Southeast Asia (Francis *et al.*, 2010). In this study, we utilised barcoding and cytochrome *b* sequencing to confirm and correct field identifications between four species, two of which (*Epomophorus crypturus* and *Hypsugo anchietae* - *Neoromicia anchietae* as per Monadjem *et al*., 2020) may have been overlooked if we had not employed these molecular methods. This study further demonstrates the utility of barcoding for ecological surveys, particularly when vouchers cannot be taken. However, barcoding should be employed with care, as COI sequences did not reliably differentiate two morphologically distinct African fruit bat species (Nesi *et al.*, 2011). We found more accurate identity matching, and in some cases, more reference sequences available for cytochrome *b* than COI. For more accurate species identification, we recommend barcoding be used in conjunction with sequencing of other markers, morphology, or acoustic identification.

Most taxa demonstrate one dominant barcode sequence with a few rare variants (Hebert *et al.*, 2004; Ward *et al.*, 2005; Hajibabaei *et al.*, 2006), but Guyanese bats showed substantial levels of variation wherein each individual had a different sequence (Clare *et al.*, 2007). This highlights the importance of compiling regional barcode libraries, not only for reliable identification, but also for detection of cryptic diversity across species ranges (Clare *et al.*, 2007). We have contributed novel COI sequences for four species: *Rhinolophus swinnyi*, *Kerivoula lanosa*, *Miniopterus fraterculus* and *Myotis tricolor*. We also contributed COI sequences for *Rhinolophus clivosus* and *Laephotis botswanae*, for which barcodes from South Africa were not previously available. The assembly of local barcode libraries is crucial for further taxonomic and phylogenetic work.

2.5.3 Relative activity levels

Overall activity levels are comparable across the forest types (11.09 – 19.73 passes/hour, AI = 0.11 – 0.16), apart from the Eastern Cape Dune forests, in which a much higher activity level was found (35.77 passes/hour, AI = 0.22) (Table 2.3). There is, however, a substantial disparity between the relative activity levels of each forest of the Eastern Cape Dune type. We recorded the highest number of passes of all forests in Cape Morgan Nature Reserve with mean activity at 62.48 passes/hour and AI of 0.36, while we recorded only 6.08 passes/hour (AI = 0.07) in

Woody Cape Nature Reserve. This difference is likely due to site-specific habitat variability, for example acoustic recorders were stationed near two large water sources in Cape Morgan while there were no fresh water sources in Woody Cape at the time of survey. Sowler *et al.* (2020) present acoustic activity levels for South African ecoregions from environmental impact assessment surveys for wind farms (ecoregions according to Dinerstein *et al.*, 2017). The Scarp and Coastal forests fall within the KwaZulu-Cape coastal forest mosaic ecoregion. Sowler *et al.* (2020) report relative activity, at ground level recording height, between 8.31 and 40.39 passes/hour for this ecoregion. The activity levels reported here fall mostly within the lower half of this activity range. Species richness decreases in a north to south direction within this ecoregion (Cooper-Bohannon *et al.*, 2016). Our survey sites are situated within the southern half of the ecoregion; perhaps bat activity follows a similar trend to richness and decreases in the ecoregions' southern extent. The Mistbelt forests we surveyed are located within the Drakensberg montane grasslands, woodlands, and forest ecoregion. Relative activity levels for this ecoregion are lacking in Sowler *et al.* (2020): this study addresses that gap.

2.5.4 Similarity of species assemblages

As expected, bat species richness and diversity were highest in Scarp forests (Table 2.3). Lawes *et al.* (2007) showed Scarp forests served as palaeorefugia to faunal extinctions during the LGM from trends of high species richness of forest birds, non-volant mammals and frogs. Furthermore, Lawes *et al.* (2007) found post-LGM dispersal of forest fauna in the direction from Scarp to both Coastal and Mistbelt forests, which led us to hypothesize that Scarp assemblages would demonstrate high similarity with both Mistbelt and Coastal forest assemblages while Mistbelt and Coastal forest assemblages should be the most dissimilar. Although the species assemblages of the three forest groups depicted relatively comparable dissimilarity values (Table 2.4), aligning with our hypothesis, we found the Scarp and Southern Mistbelt forest groups to be most similar. Mistbelt and Scarp assemblages likely show greater similarity as both of these forest groups are of Afrotemperate forest origin, with Scarp forest more recently evolved than Mistbelt (Eeley, Lawes and Piper, 1999), and thus derive from broadly similar faunal assemblages. Their assemblage similarity was then further developed with Mistbelt fauna finding refuge in Scarp forests during the LGM, with recolonisation of Mistbelt forests from Scarp refugia after the LGM (Lawes *et al.*, 2007).

However, contrary to our hypothesis, Southern Mistbelt and Southern Coastal assemblages were more similar than Scarp and Southern Coastal groups. Amatole Mistbelt and Albany

Coastal forest types depicted the greatest similarity amongst forest types (Table 2.5), and is likely driving the similarity between Mistbelt and Coastal assemblages. Lawes *et al.* (2007) found a similar trend wherein the Amatole community of non-volant mammals better aligned with forests in the Southern Cape than with Scarp forests. Since the Amatole Mistbelts are the closest geographically to Albany Coastal forests, it may infer exchange is occurring between these forest types. Also, the Albany Coastal assemblage showed relative similarity with all other forest types despite a well-known faunal biogeographic barrier, the Bedford Gap (Lawes, 1990; Willows-Munro and Matthee, 2011; du Toit *et al.*, 2012; Taylor *et al.*, 2019), separating the Albany Coastal sites from other forest types. The forest-dependent bird community of Alexandria also depicted similarity with Eastern Cape coastal forests (von Maltitz *et al.*, 2003). This suggests volant animal communities, such as bats and birds, are better able to bridge the gap.

Lastly, the dissimilarity values between Scarp and Coastal groups, as well as the relevant forest types was due to nestedness. As such the species-poor Coastal forest assemblages form subsets of the species in the species-rich Scarp forests, which indicates coupled gradients of species traits and site environmental characteristics (Ulrich, Almeida-Neto and Gotelli, 2009). Darlington (1957) proposed the nested pattern of species on islands is due to the 'immigrant pattern' whereby better dispersers colonise the most islands and the furthest islands, while poor dispersers are restricted to less isolated islands. Coastal faunas derive from colonisation along the eastern seaboard from Scarp refugia (Lawes *et al.*, 2007). If forests are considered as an archipelago of terrestrial islands surrounded by open biomes (Low and Rebelo, 1996), the Coastal assemblages depict the 'immigrant pattern' of nestedness of better dispersing species from Scarp assemblages.

2.5.5 Complementarity of capture and identification methods

Intraspecific variability and interspecific overlap of call parameters and external morphology is a common problem with bats that complicates species identification. This study shows the utility of a combined identification approach utilising morphology, echolocation calls and DNA barcoding. Our results also demonstrate the value of employing both capture and acoustic survey methods, as we captured four species that we did not, or could not in the case of the two *Epomophorus* species, acoustically detect and recorded six species we did not capture (Table 2.1). Overall, the acoustic survey detected greater activity and richness than capture methods (Table 2.3) however, both methods have their limitations. For example, we captured

Hipposideros caffer but did not record it acoustically. This is likely due to its low acoustic detection probability as it has a medium intensity call with a maximum call detection distance of 0.3 m (Monadjem *et al.*, 2017). Conversely, only the acoustic survey detected *Tadarida aegyptiaca*, as it is a high-flying species which makes it difficult to capture. We advise using both methods in conjunction for forest surveys to complement the biases of each. Additionally, we recommend a minimum acoustic survey period of 6 to 7 nights to sample bat species assemblages of forest habitats in South Africa (Figure 2.2).

2.6 Study limitations and recommendations for future research

Although we acoustically detected *Otomops martiensseni* in three new localities, these recordings were incidental as the recorders were set to trigger at 16 kHz and this species peak frequency range is 11.5 – 12.1 kHz (Schoeman and Jacobs, 2008). Thus, it is likely that it occurs more widely across the region. Acoustic studies designed to specifically record *O. martiensseni* would be useful to elucidate the full extent of its range and frequency of occurrence. Furthermore, five species (*Eidolon helvum*, *Rousettus aegyptiacus*, *Rhinolophus blasii*, *Glauconycteris variegata* and *Chaerephon pumilus*) occur in the region (Monadjem, Taylor, *et al.*, 2010), but were not recorded here. *Eidolon helvum* is a seasonal, yet infrequent migrant to South Africa (Monadjem, Taylor, *et al.*, 2010); perhaps our sampling did not coincide with its occurrence in or near our sampling localities and as such we did not sample it. The modelled distribution map presented for the species depicts it to be more associated with savanna and grassland biomes than forests in South Africa (Monadjem, Taylor, et al. 2010. *Rousettus aegyptiacus* feeds on fig trees in the canopy of forests (Jacobsen, Viljoen and Ferguson, 1986), so it is likely we did not capture this species because we did not make use of canopy nets. *Rhinolophus blasii* has been found just north of Xumeni with a modelled distribution through our study area - we may have not sampled this species as it is more associated with savanna woodland habitat (Monadjem, Taylor, *et al.*, 2010). *Glauconycteris variegata* was also recorded north of our study area and has a modelled distribution along the coastline into the Eastern Cape (Monadjem, Taylor, *et al.*, 2010). It is unclear why we did not detect this species as it is associated with coastal and riparian forest.

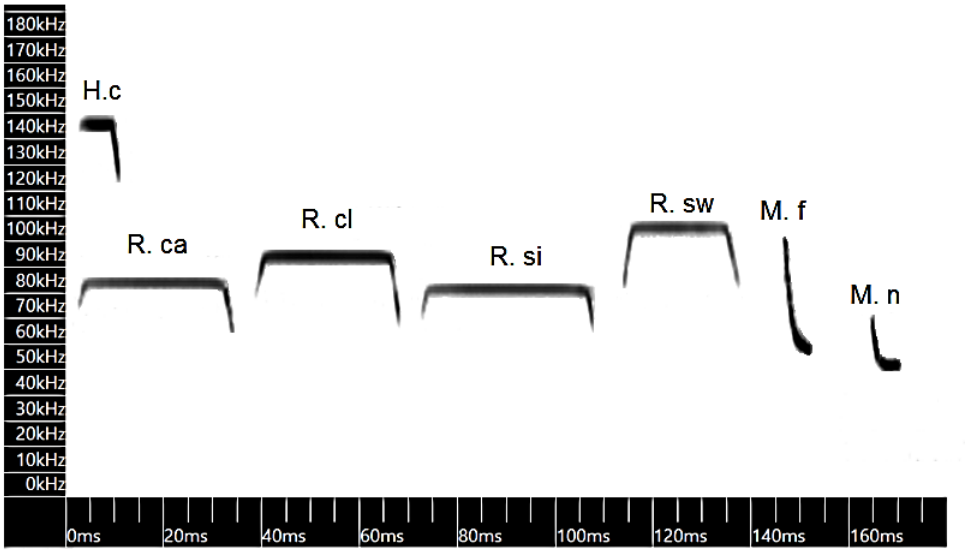
A limitation of this study is that we did not employ canopy nets and canopy acoustic recorders. We recommend they be used for future forest surveys as this should increase the number of species detected. Additionally, repeat surveys of an area typically record additional species (Brinkley, 2018) such that further studies of the 17 forests are recommended to increase the

total species count; particularly for the Transkei Coastal Scarp forest type as the species richness is predicted to be substantially higher than recorded here.

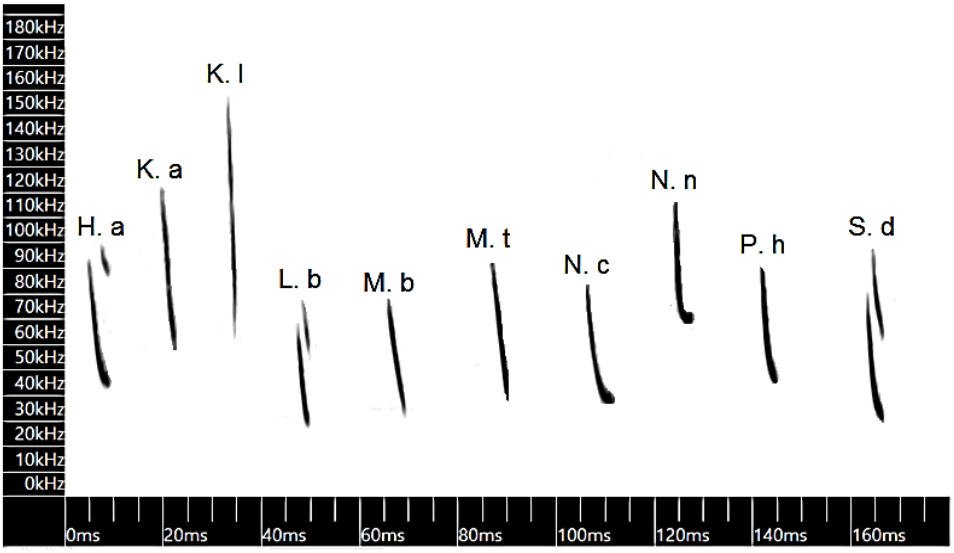
The species-area relationship (Arrhenius, 1921) informs the relationship of species richness based on habitat area, that larger areas contain greater species richness. As such, larger forest patches may be expected to host higher bat species richness. This relationship was not addressed here but is investigated in relation to bat functional diversity in Chapter 3. Lastly, different species demonstrate differences in detectability by acoustic recorders which limits the ability to compare relative activity levels between species, families, or functional groups (Adams *et al.*, 2012). Detection distances, and the relevant correction factors, have been developed for bats in savanna habitats in Eswatini (Monadjem *et al.*, 2017) but not yet for forested habitats in South Africa or for the acoustic recorders and associated omni-directional microphones utilised in this study. Therefore, we recommend future work be done to address this knowledge gap.

Appendix S2.1 The forests sampled classified per group and type according to von Maltitz *et al*. (2003), with their respective IUCN categories (Berliner, 2009) and co-ordinates.

Forest group	Forest type	IUCN category	Forest	Latitude (°S)	Longitude (°E)
Southern Mistbelt	Eastern Mistbelt	Endangered	Xumeni	29.926	29.882
			Ngeli	30.542	26.681
	Transkei Mistbelt	Vulnerable	Gomo	31.011	29.349
			Baziya	31.575	28.407
	Amatole Mistbelt	Near Threatened	Kologha	32.537	27.364
			Fort Fordyce	32.684	26.490
Scarp	Pondoland Scarp	Critically Endangered	Oribi Gorge	30.706	30.269
			Umtamvuna	31.065	30.177
			Mkambati	31.304	29.964
			Mbotyi	31.446	29.737
	Transkei Coastal Scarp	Critically Endangered	Silaka	31.651	29.505
			Dwesa	32.264	28.858
			Manubi	32.445	28.607
Southern Coastal	Eastern Cape Dune	Near Threatened	Cape Morgan	32.705	25.355
			Woody Cape	33.745	26.326
	Albany Coastal	Vulnerable	The Island	33.989	25.366
			Alexandria	33.717	26.383



Appendix S2.2 Release echolocation calls from species of the Hipposideridae, Rhinolophidae and Miniopteridae families (H. c – *Hipposideros caffer*; R. ca – *Rhinolophus capensis*; R. cl – *R. clivosus*; R. si – *R. simulator*; R. sw – *R. swinnyi* s.l; M. f – *Miniopterus fraterculus*; M. n – *M. natalensis*).



Appendix S2.3 Release echolocation calls from species of the Vespertilionidae family (H. a – *Hypsugo anchietae* (*Neoromicia anchietae* as per Monadjem *et al*., 2020); K. a – *Kerivoula argentata*; K. l – *K. lanosa*; L. b – *Laephotis botswanae*; M. b – *Myotis bocagii*; M. t – *M. tricolor*; N. c – *Neoromicia capensis* (*Laephotis capensis* as per Monadjem *et al*., 2020); N. n – *N. nana* (*Afronycteris nana* as per Monadjem *et al*., 2020); P. h – *Pipstrellus hesperidus*; S.d – *Scotophilus dinganii*).

Appendix S2.4 Time periods of reproductive condition of bats captured from forests of the Eastern Cape and KwaZulu-Natal provinces. Males were assessed as abdominal or scrotal, and females as pregnant, lactating, or post-lactating. Sub-adults were detected by the incomplete ossification of epiphyseal joints.

	Sub-adult	Abdominal	Scrotal	Pregnant	Lactating	Post-lactating
Epomophorus crypturus	Feb 18		Feb 18	Dec 18	Dec 18	Nov 18
E. wahlbergi	Oct 18			Nov, Dec 18	Aug 18	Aug 17
Hipposideros caffer		Dec 18		Oct 17		
Rhinolophus clivosus			Dec 18	Dec 18	Dec 18	Dec 18
R. swinnyi		Feb, Oct-Nov 18	Apr 18	Feb 18	Feb 18	
Miniopterus fraterculus		Feb-Apr, Aug 18	Feb 18	Dec 18		
M. natalensis		Apr, Aug, Oct 18				
Hypsugo anchietae (*Neoromicia anchietae* as per Monadjem *et al*., 2020)		Oct 18		Oct 18		
Laephotis botswanae		Oct 17 and 18, Feb 18	Apr 18		Dec 18	
Myotis bocagii			Feb, Nov, Dec 18			
M. tricolor		Feb, Oct, Nov 18	Mar, Apr 18	Dec 18	Dec 18	Dec 18
Neoromicia capensis (*Laephotis capensis* as per Monadjem *et al*., 2020)		Feb, Apr, Oct 18	Feb, Mar 18	Aug, Oct 18	Oct 18	
N. nana (*Afronycteris nana* as per Monadjem *et al*., 2020)		Oct 17; Aug, Dec 18	May, Aug 18	Sep, Oct 17; Dec 18	Nov, Dec 18	
Pipistrellus hesperidus		Oct-Dec 18	Sep-Oct 17; Feb-May, Aug-Dec 18	Oct-Dec 18	Nov, Dec 18	
Scotophilus dinganii	Dec 18		Dec 18			Dec 18

Appendix S2.5 The number of records, bioclimatic variables and AUC (area under curve) values of the species distribution models created with MaxEnt software (Phillips, Dudik and Schapire, 2006).

Species	Number of records	Number of bioclim variables used in models (in order of their contribution)	AUC training; test values
Epomophorus crypturus	356	9 (BIO16, 3, 6, 15, 2, 17, 12, 11, 19)	0.850; 0.870
Otomops martiensseni	53	10 (BIO17, 2, 3, 18, 19, 14, 15, 16, 12, 13)	0.943; 0.971
Hypsugo anchietae (*Neoromicia anchietae* as per Monadjem *et al*., 2020)	69	5 (BIO9, 18, 17, 7, 13)	0.864; 0.856
Kerivoula argentata	50	10 (BIO17, 4, 2, 19, 18, 9, 6, 15, 11 ,5)	0.875; 0.936
Laephotis botswanae	56	10 (BIO4, 16, 19, 6, 9, 2, 8, 18, 3, 1)	0.908; 0.890
Myotis bocagii	104	10 (BIO2, 19, 16, 7, 8, 4, 5, 15, 18, 13)	0.859; 0.822

The environmental variables are coded as follows: BIO1 = Annual Mean Temperature; BIO2 = Mean Diurnal Range; BIO3 = Isothermality (BIO2/BIO7) (×100); BIO4 = Temperature Seasonality (standard deviation ×100); BIO5 = Max Temperature of Warmest Month; BIO6 = Min Temperature of Coldest Month; BIO7 = Temperature Annual Range (BIO5-BIO6); BIO8 = Mean Temperature of Wettest Quarter; BIO9 = Mean Temperature of Driest Quarter; BIO10 = Mean Temperature of Warmest Quarter; BIO11 = Mean Temperature of Coldest Quarter; BIO12 = Annual Precipitation; BIO13 = Precipitation of Wettest Month; BIO14 = Precipitation of Driest Month; BIO15 = Precipitation Seasonality (Coefficient of Variation); BIO16 = Precipitation of Wettest Quarter; BIO17 = Precipitation of Driest Quarter; BIO18 = Precipitation of Warmest Quarter; BIO19 = Precipitation of Coldest Quarter (https://worldclim.org/data/bioclim.html).

Appendix S2.6 Pairwise Jaccard dissimilarity matrix of fourteen surveyed forests in the Eastern Cape and southern KwaZulu-Natal, based on acoustic data.

	Xumeni	Ngeli	Gomo	Baziya	Kologha	Fort Fordyce	Oribi Gorge	Umtamvuna	Mkambati	Mbotyi	Silaka	Dwesa	Manubi	Cape Morgan	Woody Cape	The Island
Xumeni																
Ngeli	0.08															
Gomo	0.15	0.08														
Baziya	0.08	0.00	0.08													
Kologha	0.21	0.15	0.23	0.15												
Fort Fordyce	0.21	0.15	0.23	0.15	0.00											
Oribi Gorge	0.41	0.38	0.33	0.38	0.47	0.47										
Umtamvuna	0.44	0.40	0.36	0.40	0.50	0.50	0.27									
Mkambati	0.50	0.56	0.53	0.56	0.65	0.65	0.44	0.36								
Mbotyi	0.41	0.47	0.44	0.47	0.56	0.56	0.25	0.27	0.44							
Silaka	0.47	0.43	0.38	0.43	0.43	0.43	0.40	0.31	0.50	0.29						
Dwesa	0.44	0.40	0.36	0.40	0.40	0.40	0.27	0.29	0.56	0.38	0.31					
Manubi	0.47	0.44	0.40	0.44	0.44	0.44	0.20	0.44	0.50	0.41	0.36	0.33				
Cape Morgan	0.60	0.57	0.54	0.57	0.57	0.57	0.43	0.33	0.27	0.53	0.36	0.46	0.38			
Woody Cape	0.60	0.57	0.54	0.57	0.46	0.46	0.53	0.46	0.42	0.63	0.50	0.57	0.50	0.22		
The Island	0.43	0.38	0.33	0.38	0.25	0.25	0.47	0.50	0.57	0.56	0.42	0.50	0.43	0.45	0.30	
Alexandria	0.47	0.43	0.38	0.43	0.31	0.31	0.40	0.43	0.50	0.50	0.33	0.43	0.36	0.36	0.20	0.10

Appendix S2.7 Pairwise values of turnover (below diagonal) and nestedness (above diagonal) fourteen surveyed forests in the Eastern Cape and southern KwaZulu-Natal.

	Xumeni	Ngeli	Gomo	Baziya	Kologha	Fort Fordyce	Oribi Gorge	Umtam-vuna	Mkambati	Mbotyi	Silaka	Dwesa	Manubi	Cape Morgan	Woody Cape	The Island	Alexandria
Xumeni		0.08	0.15	0.08	0.06	0.06	0.04	0.04	0.07	0.04	0.13	0.04	0.00	0.20	0.20	0.23	0.13
Ngeli	0.00		0.08	0.00	0.00	0.00	0.09	0.00	0.03	0.07	0.10	0.00	0.04	0.17	0.17	0.18	0.10
Gomo	0.00	0.00		0.08	0.06	0.06	0.17	0.05	0.00	0.13	0.05	0.05	0.09	0.14	0.14	0.13	0.05
Baziya	0.00	0.00	0.00		0.00	0.00	0.09	0.00	0.03	0.07	0.10	0.00	0.04	0.17	0.17	0.18	0.10
Kologha	0.15	0.15	0.17	0.15		0.00	0.07	0.00	0.02	0.06	0.10	0.00	0.04	0.17	0.24	0.25	0.13
Fort Fordyce	0.15	0.15	0.17	0.15	0.00		0.07	0.00	0.02	0.06	0.10	0.00	0.04	0.17	0.24	0.25	0.13
Oribi Gorge	0.38	0.29	0.17	0.29	0.40	0.40		0.11	0.13	0.00	0.22	0.11	0.06	0.43	0.31	0.27	0.22
Umtamvuna	0.40	0.40	0.31	0.40	0.50	0.50	0.15		0.05	0.11	0.13	0.00	0.04	0.33	0.24	0.14	0.10
Mkambati	0.43	0.53	0.53	0.53	0.63	0.63	0.31	0.31		0.13	0.04	0.03	0.07	0.27	0.19	0.07	0.04
Mbotyi	0.38	0.40	0.31	0.40	0.50	0.50	0.25	0.15	0.31		0.29	0.09	0.04	0.31	0.23	0.20	0.17
Silaka	0.33	0.33	0.33	0.33	0.33	0.33	0.18	0.18	0.46	0.00		0.13	0.18	0.14	0.10	0.05	0.00
Dwesa	0.40	0.40	0.31	0.40	0.40	0.40	0.15	0.29	0.53	0.29	0.18		0.05	0.24	0.17	0.14	0.10
Manubi	0.47	0.40	0.31	0.40	0.40	0.40	0.14	0.40	0.43	0.38	0.18	0.29		0.38	0.28	0.23	0.18
Cape Morgan	0.40	0.40	0.40	0.40	0.40	0.40	0.00	0.00	0.00	0.22	0.22	0.22	0.00		0.00	0.05	0.14
Woody Cape	0.40	0.40	0.40	0.40	0.22	0.22	0.22	0.22	0.22	0.40	0.40	0.40	0.22	0.22		0.08	0.20
The Island	0.20	0.20	0.20	0.20	0.00	0.00	0.20	0.36	0.50	0.36	0.36	0.36	0.20	0.40	0.22		0.10
Alexandria	0.33	0.33	0.33	0.33	0.18	0.18	0.18	0.33	0.46	0.33	0.33	0.33	0.18	0.22	0.00	0.00	

Chapter 3. Functional diversity and trait filtering of insectivorous bats in response to forest biogeography and fragmentation in South Africa

This chapter is in review with Journal of Biogeography.

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3.1 Abstract

Forest fragmentation is considered one of the major drivers of biodiversity loss, with important implications for ecosystem functioning. Forests in South Africa present an interesting case of fragmentation, in that they occur as naturally fragmented patches due to historical climatic fluctuations but have also undergone more recent anthropogenic fragmentation. Bats are essential components of ecosystems and provide a myriad of ecosystem services. Considering their ecological and economic importance, the response and vulnerability of bats to fragmentation has been poorly studied, particularly in Africa. To this end, we aim to assess the effects of forest biogeographical history and fragmentation on functional diversity of bats and their functional traits. We utilised insectivorous bat communities from seventeen forests in Eastern Cape and southern KwaZulu-Natal provinces, of South Africa. We derived four functional diversity indices and used generalised linear models to assess the response of diversity indices to biogeographical history and fragmentation, represented by forest type and five landscape fragmentation metrics. RLQ and fourth-corner analysis were used to investigate the interaction of traits with fragmentation metrics and forest type. Pondoland Scarp forests displayed high functional richness, whilst Eastern Cape Dune forests exhibited high functional divergence but low functional richness and dispersion. Two fragmentation metrics affected functional diversity dynamically: edge density had a positive effect on functional evenness; and dispersion was negatively affected by river length through forests. Results showed stronger interactions of functional traits with forest type than fragmentation metrics, with greater filtering effects on traits of body size and wing morphology than echolocation characteristics. The large-scale historical processes of forest biogeography and associated structure are important determinants of functional richness, divergence, and dispersion of insectivorous bat communities. Scarp forests showed the highest species and functional richness as they experienced less extreme climatic extinction filtering than Mistbelt forests during the Last

Glacial Maximum, whereas the low diversity of Eastern Cape Dune forests results from their younger evolutionary history and homogenous vegetation structure. Little is known of the sensitivity of bats to habitat fragmentation in Africa: here we show larger-sized species and species exhibiting low wing loading may be more vulnerable to fragmentation.

3.2 Introduction

Forest fragmentation is considered one of the major drivers of biodiversity loss, with important implications for ecosystem functioning in forested regions (Fahrig, 2003). Forests in South Africa present an interesting case of fragmentation, in that they occur as naturally small, disjoint patches due to past climatic fluctuations (Eeley, Lawes and Piper, 1999). They constitute the smallest biome in the country, covering only 7,177 km^2 of land surface (Low and Rebelo, 1996), and are often described as an archipelago of forest islands surrounded by open habitat biomes (Mucina and Geldenhuys, 2006). There are two main types of forest: Afrotemperate, of which the Mistbelt forests are (von Maltitz *et al.*, 2003) an ancient forest type that has persisted since the Miocene (White, 1981); and the Indian Ocean Coastal belt forests which arose approximately 8,000 years ago (Macdevette *et al.*, 1989). Located between these two types is a narrow band of Scarp forests, also of Afromontane origin but younger than mistbelt forests (Eeley, Lawes and Piper, 1999) and comprising both Mistbelt and coastal flora and fauna (Macdevette *et al.*, 1989; Lawes, 1990). Historically, Mistbelt forests occurred as extensive tracts across the mid-altitude region above the eastern seaboard of South Africa, but underwent substantial contractions and fragmentation during cold and dry conditions of glacial periods, with the Last Glacial Maximum (LGM) (~21,000 - 18,000 years before present) being the most recent (Eeley, Lawes and Piper, 1999). These climate-induced fragmentation effects caused climatic extinction filtering of forest biota (Eeley, Lawes and Piper, 1999; Lawes *et al.*, 2007). Soon after the southward expansion of the younger Indian Ocean Coastal belt forests, the warm and wet conditions of the Holocene altitherm (~7,000 years ago) caused forest expansion with the resultant mixing of Mistbelt and Indian Ocean Coastal belt forests along the current-day scarp belt (Eeley, Lawes and Piper, 1999). These forests thus demonstrate a complex biogeographical history arising from climate-induced expansions and contractions which have substantially influenced the distributions and diversity patterns of the extant biotic communities that inhabit them (Lawes, 1990; Lawes *et al.*, 2007). Subsequently, forests have been subjected to anthropogenic fragmentation with mostly unregulated logging continuing from 1652 through the colonial era (King, 1938). Large areas of indigenous forest were lost, both to this logging and to clearing for sugar plantations in KwaZulu-Natal (du Bois, 2016).

Post-1994 anthropogenic disturbances are chiefly limited to small-scale harvesting of forest products by local communities that cause habitat degradation (Leaver and Cherry, 2020), rather than deforestation.

Habitat fragmentation reduces the diversity of vital ecological components which may disrupt ecosystem processes and function (Bregman, Sekercioglu and Tobias, 2014). Bats are essential components of ecosystems as they exhibit high diversity, fill multiple niches and provide a myriad of ecosystem services (Kunz *et al.*, 2011). Crop pest suppression by insectivores is considered the most valuable ecosystem service bats provide (Boyles *et al.*, 2013), for example, insectivorous bats prevented the loss of 9-23% of the annual damage caused by stinkbugs to South African macadamia orchards (Taylor *et al.*, 2018). Bats have also been recognised as useful bio-indicators to assess the response of biodiversity to habitat fragmentation as they occur widely around the world and trends in their populations can be monitored (Jones *et al.*, 2009). Considering their ecological and economic importance, the response and vulnerability of bats to fragmentation has been poorly studied, particularly in Africa.

Functional diversity has been shown to be a useful estimate of biodiversity as it links the mechanistic effect of species functional traits with ecosystem processes (Hooper *et al.*, 2006). Functional diversity is an important driver of ecological processes that shape the functionality, resistance and resilience of ecosystems (Villéger, Mason and Mouillot, 2008). As community assembly processes operate on ecological traits, functional diversity is a suitable indicator of changes to community structure in response to habitat disturbances (Mouillot *et al.*, 2013). Environmental filters select for specific functional traits that determine species persistence within the habitat (Luck *et al.*, 2012). Understanding which traits are selected for by environmental filters may inform how changes to the landscape impact diversity and community assembly processes (Mayfield *et al.*, 2010). Forest fragmentation has been found to cause changes to bat species communities (García-Morales *et al.*, 2016; Farneda *et al.*, 2018), with the configuration of the landscape, such as forest cover, edge ratio and fragment connectivity, acting as environmental filters on functional traits (Farneda *et al.*, 2015).

Here we present the first investigation of functional diversity and functional trait filtering of insectivorous bats in African forests. We quantify the functional diversity of bats in forests across the southern reaches of KwaZulu-Natal and the Eastern Cape provinces of South Africa and assess the effects of fragmentation using five landscape fragmentation metrics. We also consider the effects of historical fragmentation and biogeographical structure of forests,

represented by forest type, on functional diversity. Lastly, we investigate the interaction of functional traits with forest type and fragmentation metrics to determine which traits contribute most to species' fragmentation sensitivity. We anticipated forest type to be a predominant predictor of functional diversity, as was found for forest birds in the Eastern Cape (Leaver *et al.*, 2019). Also, we hypothesized forest cover (measured by patch size) to positively affect functional richness (García-Morales *et al.*, 2016). Lastly, we predicted species with a reduced dispersal capacity, inferred by wing morphology of low wing loading, to be more vulnerable to reduced forest cover and increased fragmentation as they are less equipped to commute large distances between forests.

3.3 Materials and Methods

3.3.1 Study area and acoustic surveys

We conducted the study in the Eastern Cape and KwaZulu-Natal provinces of South Africa as they hold the highest proportions of indigenous forest in the country: 46% and 29%, respectively (Berliner, 2009), and are located within the Maputaland-Pondoland-Albany global biodiversity hotspot. Acoustic surveys were performed at 17 forests spanning from the northern most site, Xumeni State Forest (29.926 °S, 29.882 °E), in southern KwaZulu-Natal, south-westwards through the Eastern Cape to The Island Nature Reserve (33.989 °S, 25.366 °E) (Figure 3.1, Table 3.1). Von Maltitz *et al.* (2003) classifies the 17 forests into seven forest types within three groupings. We sampled in six Mistbelt forests; seven Scarp forests and four Coastal forests (Table 3.1). Bat communities of each forest were surveyed, using active and passive sampling methods, as described in Chapter 2. Passive acoustic datasets were used to calculate the functional diversity indices.

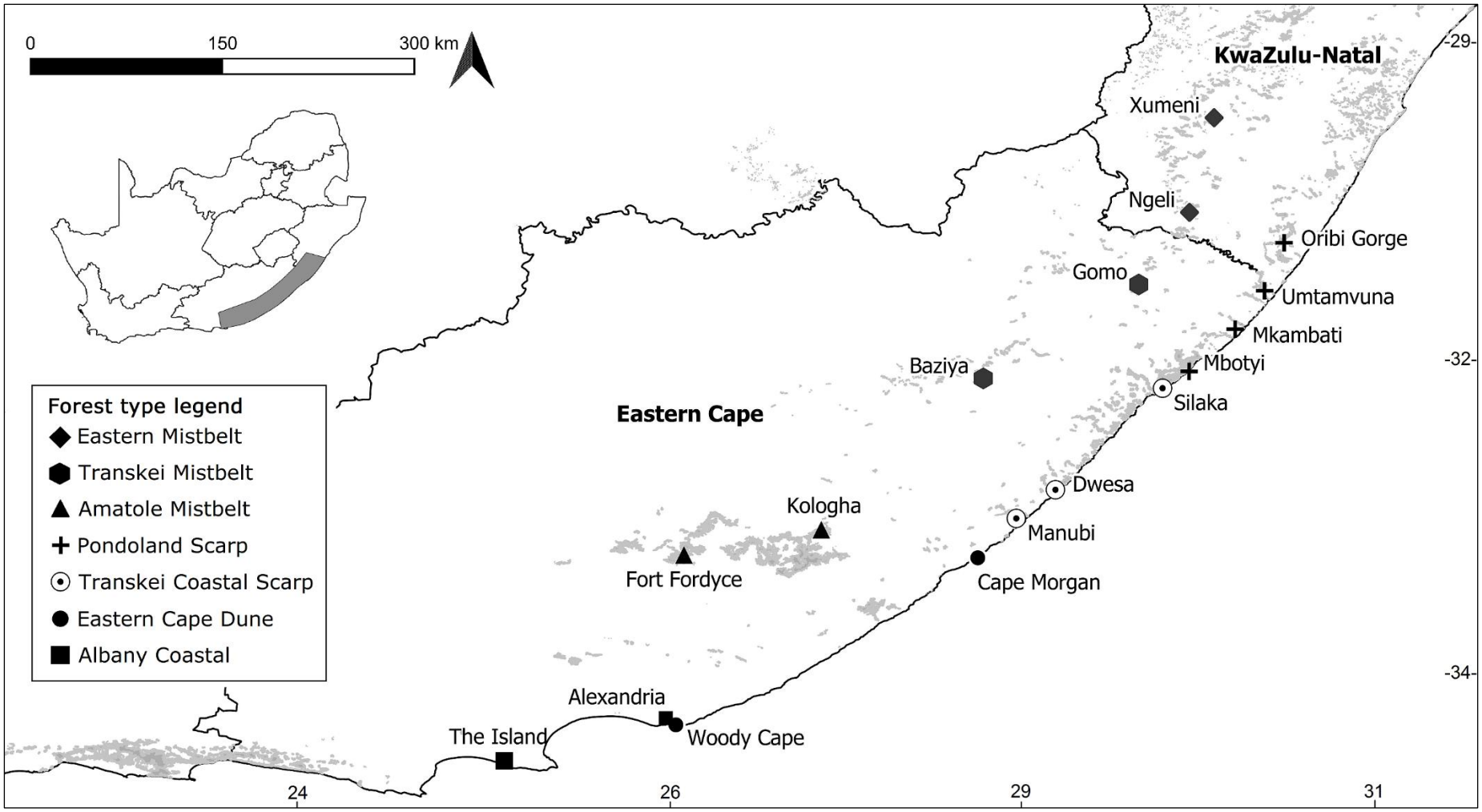

Figure 3.1 Map of indigenous forests surveyed in southern KwaZulu-Natal and Eastern Cape provinces of South Africa. Forest types (as per von Maltitz *et al*., 2003) are depicted with different shapes as listed in the legend. Grey shading displays the fragmented nature of forest cover across the country. Extent of study region is depicted with the grey polygon on the inset map of South Africa.

Table 3.1 List of surveyed forests, of seven forest types (as per von Maltitz *et al.*, 2003), across southern KwaZulu-Natal and Eastern Cape provinces of South Africa with corresponding Internation Union of Conservation of Nature (IUCN) conservation category (Berliner, 2009). Species richness and mean values of functional diversity indices of bat communities are listed per forest (FRic – functional richness; FEve – functional evenness; FDiv – functional divergence; FDis – functional dispersion; SF – state forest; NR – nature reserve).

Forest type	IUCN category	Study forest	Co-ordinates	Species richness	FRic	FEve	FDiv	FDis
Eastern Mistbelt	Endangered	Xumeni SF	29.926°S 29.882°E	12	0.477	0.575	0.711	0.784
		Ngeli SF	30.542°S 26.681°E	12	0.334	0.640	0.850	0.977
Transkei Mistbelt	Vulnerable	Gomo SF	31.011°S 29.349°E	12	0.722	0.301	0.574	0.805
		Baziya SF	31.575°S 28.407°E	12	0.471	0.272	0.711	0.652
Amatole Mistbelt	Near Threatened	Kologha SF	32.537°S 27.364°E	12	0.482	0.351	0.676	0.684
		Fort Fordyce NR	32.684°S 26.490°E	12	0.482	0.597	0.673	1.061
Pondoland Scarp	Critically Endangered	Oribi Gorge NR	30.706°S 30.269°E	15	1.307	0.531	0.779	0.899
		Umtamvuna NR	31.065°S 30.177°E	13	0.639	0.507	0.804	0.806
		Mbotyi SF	31.446°S 29.737°E	14	1.229	0.369	0.800	0.861
		Mkambati NR	31.304°S 29.964°E	11	0.329	0.288	0.758	1.439

Transkei Coastal Scarp	Critically Endangered	Dwesa NR	32.264°S 28.858°E	12	0.565	0.479	0.759	0.729
		Manubi SF	32.445°S 28.607°E	13	1.208	0.253	0.852	0.569
		Silaka NR	31.651°S 29.505°E	10	0.237	0.261	0.883	0.763
Eastern Cape Dune	Near Threatened	Cape Morgan NR	32.705°S 25.355°E	8	0.101	0.391	0.933	0.523
		Woody Cape NR	33.745°S 26.326°E	8	0.099	0.281	0.948	0.793
Albany Coastal	Vulnerable	The Island NR	33.989°S 25.366°E	9	0.113	0.535	0.713	1.048
		Alexandria NR	33.717°S 26.383°E	10	0.197	0.244	0.749	1.053

3.3.2 Functional traits and diversity indices

Diversity was quantified by species richness and four functional diversity indices: functional richness (FRic), functional evenness (FEve), functional divergence (FDiv) and functional dispersion (FDis). The functional diversity indices are derived from quantitative values for traits, with species distributed in a multidimensional functional trait space (Villéger, Mason and Mouillot, 2008). FRic is the volume of functional space occupied by the community; and is calculated with the convex hull volume index (Villéger, Mason and Mouillot, 2008). FEve quantifies the regularity with which species abundances are distributed in a functional trait space (Mason *et al.*, 2005). FDiv quantifies the divergence of species in their distances (weighted by abundance) along the range of the functional trait space (Villéger, Mason and Mouillot, 2008), whereas FDis measures the mean distance of individual species to the centroid of all species in the trait space where the centroid is weighted towards the most abundant species (Laliberte and Legendre, 2010).

Species were plotted in a functional space using the following traits (after Wordley *et al.*, 2017): echolocation call type, characteristic frequency of call, forearm length, aspect ratio and wing loading (see Appendix S3.2). These traits were employed as they inform the ecological niches filled by species; and as such are linked to the ecosystem services they provide (Luck *et al.*, 2012). The structure and frequency of echolocation calls are adaptations to preferred habitat type and foraging mode (Siemers and Schnitzler, 2004). Forearm length was used as an indicator of body size, which is an important consideration as it has been linked to forest fragmentation in the Amazon (Farneda *et al.*, 2015). Wing morphology is a predictor of foraging habitat and strategy, dispersal ability and size of home range (Norberg and Rayner, 1987). Bats with long, narrow wings are fast, high-flying species that demonstrate high wing loading and high aspect ratio values. Species with short, broad wings (low wing loading and low aspect ratio) demonstrate greater manoeuvrability for flight within cluttered/dense vegetation habitats and typically have lower dispersal abilities (Norberg and Rayner, 1987). We included wing morphology as it infers dispersal ability within and between forests and may thus inform sensitivity of species to fragmentation.

We captured bats in the forests with mist nets (Ecotone, Poland) and three-bank harp traps (Faunatech Austbat, Australia) (described in Chapter 2), to acquire morphological measurements and release call parameters for the sampled species. The capture and handling of bats was done in accordance with Sikes and the Animal Care and Use Committee of the

American Society of Mammalogists (2016). Body mass, forearm length and adult status were measured and recorded from captured individuals (Appendix S3.1). Sub-adults were detected by the incomplete ossification of epiphyseal joints. Measurements of sub-adults were not included in analyses. Bats were placed with their ventral side down on laminated graph paper, with the right wing and tail membrane extended. Photographs were taken at a 90° angle to the bat with a 16-megapixel Canon SX600 HS camera. Wing photographs were calibrated and measured with ImageJ2 (Rueden *et al.*, 2017). Wingspan (B), handwing (l_{hw}) and armwing length (l_{aw}), wing area (S), handwing (S_{hw}) and armwing area (S_{aw}) were measured as described in Norberg and Rayner (1987). Aspect ratio was calculated by $A = B^2/S$, and wing loading by WL = mg/S (Norberg and Rayner, 1987). Characteristic frequency values of echolocation calls were taken from the call library in Chapter 2. For species that we recorded acoustically but did not capture, values of wing loading, aspect ratio and characteristic frequency were taken from Norberg and Rayner (1987); Schoeman and Jacobs (2003, 2008); and Monadjem, Taylor, *et al.* (2010). Trait values are listed in Appendix S3.2.

Continuous traits were standardised to a mean of zero and standard deviation of one for comparison of their relative effects on the same scale (Farneda *et al.*, 2015). Relative abundances of mean passes per recording hour were used to account for variation in sampling effort across forests. Trait values were used to generate species-species Gower dissimilarity matrices for each forest (Gower, 1971). Per site singletons were excluded from the analysis, as very rare species are unlikely to be pertinent to ecosystem functionality (McConkey and O'Farrill, 2015). Gower matrices were reduced with principal coordinate analysis to develop the multidimensional trait space. Functional diversity indices were calculated with the 'FD' package (Laliberte, Legendre and Shipley, 2014) in R (R Core Team, 2019).

3.3.3 Fragmentation metrics

We used five class level landscape metrics to calculate the extent of forest fragmentation and habitat connectivity (Schumaker, 1996). We employed two area-edge metrics: patch size and edge density. Patch size is the extent of forest cover of the larger, connected patch within which we sampled delineated by abutting open habitats. Edge density is a measure of total forest edge length divided by total forest area: it facilitates the comparison of the edge effect among forests of varying sizes (McGarigal and Marks, 1995). We also used two core area metrics: patch cohesion index (PCI) and effective mesh size (EMS). Patch cohesion is a measure of the physical connectedness of forest patches as would be perceived by organisms dispersing within

a binary landscape (Schumaker, 1996), and thus increases as patches become more aggregated. EMS characterises anthropogenic penetration of a landscape using a geometric approach (Jaeger, 2000). It is based on the ability of two animals, placed in different areas of a region, to find one another within the landscape. EMS takes into account the patch size relative to the total landscape area and is useful for comparison of forests differing in size (Jaeger, 2000). The size and connectivity of waterways are well-known to affect the distribution and movement of bats (Campbell *et al.*, 2009; Lookingbill *et al.*, 2010), so we included the total river length (km) running through the forests as a measure of habitat connectivity (Berliner, 2009) for bats as they are likely to commute along these water ways.

Fragmentation metrics (Appendix S3.3) were calculated using the South African National Land Cover 2018 dataset (Thompson, 2019), with the LecoS spatial analysis tool (Jung, 2016) in Quantum GIS (QGIS Development Team, 2014). We employed classes of indigenous forest, woodland, and planted forest from the Land Cover dataset. Total river length per forest was extracted from the 2018 National Biodiversity Assessment spatial rivers dataset (Van Deventer *et al.*, 2019). As bats are volant and have a higher dispersal ability relative to non-volant small mammals, we calculated patch cohesion index, effective mesh size and total river length within a 5 km wide buffer around the forests. The buffer size was selected to encompass the home ranges of different-sized African insectivorous bat species (Fenton and Rautenbach, 1986; Noer *et al.*, 2012).

3.3.4 Statistical analyses

We assessed the response of the diversity indices to forest type and fragmentation metrics using generalised linear models (GLMs). We tested for spatial autocorrelation of species richness and functional diversity values between forests using Moran's I test with the 'ape' package (Paradis and Schliep, 2018) in R. Moran's I test revealed the four functional diversity metrics were independent of forest location but that species richness displayed significant autocorrelation (Appendix S3.4), so it was removed from further analyses. Fragmentation metrics and diversity indices were visualised and tested for normality with the 'shapiro.test' function in R. Diversity and fragmentation metrics were transformed where necessary. We tested for correlations between fragmentation metrics with the 'cor' function in R. No significant correlations were found indicating independence of predictor variables for use in the GLMs. The functional diversity indices were modelled separately, with the 'glm' function in R, employing additive and interactive models of forest type and fragmentation metrics as

predictor variables. Forest type was a categorical variable, with Albany Coastal as the reference site based on alphabetical order. Optimal models were chosen by backward selection of Akaike Information criterion (AIC) values, using the 'stepAIC' function in the 'MASS' package (Venables and Ripley, 2002). A Gaussian distribution was employed for all models. Collinearity between predictor variables was tested for with variance inflation factors (VIFs) in the 'car' package (Fox, 2002) of R. Patch size and PCI independently had VIF values greater than five and were removed from the models (Zuur, Ieno and Elphick, 2010). We then ran univariate models separately for these two metrics. The coefficient of determination (R^2) was used to assess the goodness-of-fit for each model with the 'rsq' package (Zhang, 2018).

We analysed the interaction of functional traits with forest type and fragmentation metrics using RLQ analysis and the fourth-corner approach (Dolédec *et al.*, 1996; Dray *et al.*, 2014). RLQ utilises ordination to assign scores to species abundances, functional traits, and environmental variables. A graphical summary is generated to display the joint structure of these inputs (Dray *et al.*, 2014). The fourth-corner approach measures and tests the associations between each trait and environmental variable; the output is a coefficient matrix indicating how variation in environmental responses was mediated by species functional traits (Dray *et al.*, 2014). A combination of these two methods is recommended for analysis of trait – environment associations (Kleyer *et al.*, 2012). Three datasets were used as inputs for the RLQ analysis: species traits table (Q), forest type and fragmentation metrics (R), and species abundances (L). Separate ordinations were performed on each dataset: Hill-Smith PCA was applied to Q and R as they contain qualitative and quantitative variables (Hill and Smith, 1976), and correspondence analysis was applied to L. We weighted the ordinations of the R and Q matrices with scores from the correspondence analysis. We used a combination of model 2 (tests that distributions of species with site-independent traits are not influenced by environmental conditions) and model 4 (tests that species composition of sites with fixed environmental conditions are not influenced by species traits) (Dray *et al.*, 2014). Model significance was assessed with 50,000 permutations. RLQ analysis was performed with 'ade4' package (Dray and Dufour, 2007) in R. We performed the fourth-corner analysis with the 'traitglm' function in the 'mvabund' R package (Wang *et al.*, 2018). A GLM was constructed for species abundance (L) with additive terms of R and Q, quadratic terms for continuous variables, and interaction terms between traits and environmental variables. We used a LASSO (least absolute shrinkage and selection operator) penalty which automatically performs model selection, with interaction coefficients that do not reduce the Bayesian information criterion (BIC) set to zero

(David, 2015). Phylogenetic correction was not incorporated as per de Bello *et al.* (2015).

3.4 Results

3.4.1 Effect of forest type and fragmentation on functional diversity

A total of 116,818 passes of 21 species was recorded over 6,220 recording hours. Oribi Gorge of Pondoland Scarp forest type depicted the highest species richness (15), while both sites of the Eastern Cape Dune forest type had the lowest species richness (8). A total of 12 species was detected for each of the six Mistbelt forests. The survey completeness was assessed with Chao (1987) species richness estimator per forest type as presented in Chapter 2: richness estimates generally plateaued after 6 – 7 nights per forest type. Oribi Gorge and Mbotyi, of the Pondoland Scarp type, displayed the highest FRic (1.307 and 1.229, respectively). FEve was greatest for Ngeli forest (0.640) and lowest for Alexandria (0.244). The Eastern Cape Dune forests exhibited the highest values of FDiv (0.933 – 0.948) and the lowest FDis (0.523), while Mkambati of the Pondoland Scarp demonstrated the highest FDis (1.439) (Table 3.1). There is noteworthy variability of the functional diversity indices between forests of the same forest type, pointing to possible local scale effects on community assembly processes.

Backward AIC selection of the GLMs found forest type to be the best predictor of FRic with no fragmentation metrics retained in the top model (Table 3.2). GLM estimates showed FRic of the Transkei Mistbelt ($\beta = 0.59 \pm 0.23$, $p = 0.02$), Pondoland Scarp ($\beta = 0.71 \pm 0.20$, $p = 0.006$), and Transkei Coastal Scarp forest type ($\beta = 0.56 \pm 0.21$, $p = 0.02$) differed from Albany Coastal as the reference type (Table 3.2). FRic per forest type is displayed in Figure 3.2a with Pondoland Scarp demonstrating a notably higher richness while Transkei Coastal Scarp shows a wide interquartile range. Forest type was retained in the top GLM model of FEve, but without any significant differences from the reference forest type (Table 3.2). Two fragmentation metrics, edge density and river length, were also retained in the model. The effect of river length on FEve was small and non-significant ($\beta = 0.04 \pm 0.03$, $p = 0.18$) while edge density depicted a significant positive effect ($\beta = 28.15 \pm 10.57$, $p = 0.02$) (Table 3.2). The relationship of edge density with FEve is displayed in Figure 3.3a, wherein points cluster around the trendline at lower values of evenness with slightly higher spread as values increase.

Table 3.2 Response of bat functional diversity indices to forest type and fragmentation from generalised linear models. AIC and R^2 values are shown for best fit models. Significant *p* values are bolded.

Functional diversity	Predictor	Estimate	Standard error	T	*p*
Functional richness	Intercept	-0.83	0.17	-5.00	**<0.01**
AIC = 5.79	Eastern Mistbelt	0.43	0.23	1.83	0.09
R^2 = 0.74	Transkei Mistbelt	0.59	0.23	2.53	**0.02**
	Amatole Mistbelt	0.51	0.23	2.18	**0.05**
	Pondoland Scarp	0.71	0.20	3.50	**<0.01**
	Transkei Coastal Scarp	0.56	0.21	2.64	**0.02**
	Eastern Cape Dune	-0.17	0.23	-0.75	0.47
Functional evenness	Intercept	-0.98	0.26	-3.789	**<0.01**
AIC = -18.85	Eastern Mistbelt	0.24	0.11	2.10	0.07
R^2 = 0.71	Transkei Mistbelt	-0.31	0.16	-1.93	0.09
	Amatole Mistbelt	-0.35	0.25	-1.40	0.20
	Pondoland Scarp	-0.14	0.14	-0.99	0.35
	Transkei Coastal Scarp	-0.28	0.15	-1.80	0.11
	Eastern Cape Dune	-0.07	0.12	-0.62	0.55
	Edge density	28.15	10.57	2.66	**0.02**
	River length	0.04	0.03	1.46	0.18
Functional divergence	Intercept	0.58	0.08	6.73	**<0.01**
AIC = -46.42	Eastern Mistbelt	0.07	0.05	1.35	0.21
R^2 = 0.87	Transkei Mistbelt	-0.09	0.06	-1.45	0.18
	Amatole Mistbelt	-0.09	0.08	-1.18	0.27
	Pondoland Scarp	0.07	0.06	1.18	0.27
	Transkei Coastal Scarp	0.09	0.06	1.57	0.15
	Eastern Cape Dune	0.25	0.05	4.62	**<0.01**
	River length	0.01	0.01	1.09	0.31
	Effective mesh size	0.04	0.02	1.57	0.16
Functional dispersion	Intercept	0.40	0.18	2.17	0.06
AIC = -30.55	Eastern Mistbelt	-0.09	0.08	-1.15	0.28
R^2 = 0.73	Transkei Mistbelt	0.03	0.11	0.31	0.77
	Amatole Mistbelt	0.26	0.18	1.51	0.17
	Pondoland Scarp	0.13	0.10	1.32	0.22
	Transkei Coastal Scarp	0.006	0.11	0.05	0.96
	Eastern Cape Dune	-0.25	0.08	-3.00	**0.02**
	Edge density	-9.60	7.49	-1.28	0.24
	River length	-0.05	0.02	-2.51	**0.03**

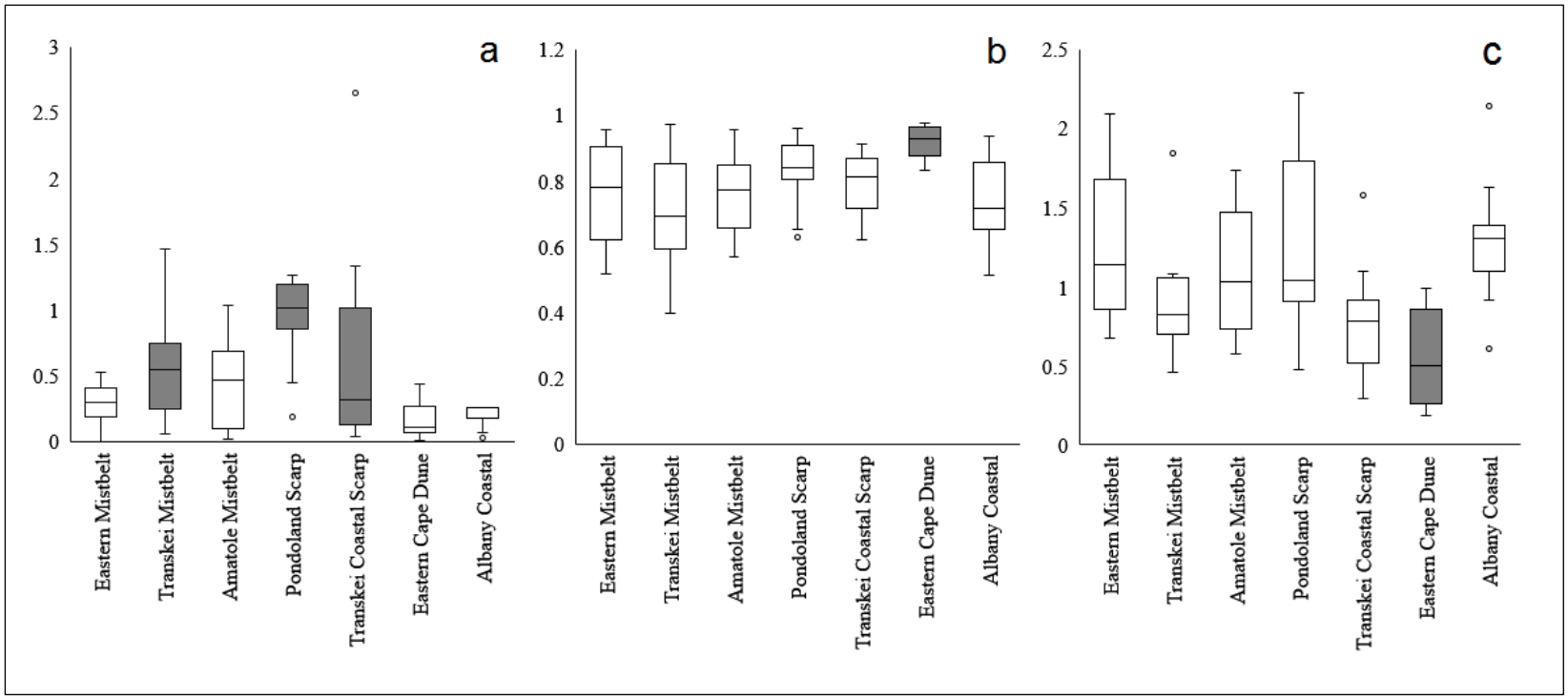

Figure 3.2 Boxplots displaying a) functional richness b) functional divergence c) functional dispersion of bat communities per forest type. Grey shaded plots indicate forest types depicting significant differences from reference forest type as determined with generalised linear models. Small circles depict outliers. Significant variation of functional evenness amongst forest types was not found.

Forest type, river length and effective mesh size were retained in the best model of FDiv however, both river length (β = 0.01 ± 0.01) and EMS (β = 0.04 ± 0.02) showed small, non-significant effects (Table 3.2). The Eastern Cape Dune forests depicted higher FDiv (β = 0.25 ± 0.05, p = 0.002) than other forest types (Table 3.2; Figure 3.2b). Forest type, edge density and river length were the best predictors of FDis (Table 3.2), with the Eastern Cape Dune forest type having lower FDis than other forest types (β = -0.25 ± 0.08, p = 0.02; Figure 3.2b). Edge density exhibited a large negative but non-significant effect (β = -9.60 ± 7.49), while river length demonstrated a significantly negative relationship (β = -0.05 ± 0.02, p = 0.03) with FDis. The weak negative relationship of river length with FDis is displayed in Figure 3.3b, with a relatively wide dispersion of points around the trend-line. Lastly, the univariate models of forest patch size and PCI with each of the four functional diversity metrics did not return any significant relationships.

3.4.2 Trait – environment associations

The first axis of the RLQ accounted for 66.5% of total co-inertia between traits (Q) and environmental variables (R), with the cumulative projected inertia of the first three axes at 99.66% (Table 3.3). The inertia and co-inertia ratios were high for both R (82% and 75%) and Q (81% and 82%) matrices, indicating the variances were well preserved. From the first axis, the correlation between Q and R was 15%, near the 34% of the maximum possible correlation from the correspondence analysis of L (Table 3.3). The permutation test found the inertia was not significant for model 2 (observation = 0.21, standard observation = 0.97, p = 0.16) showing species distributions were not influenced by the considered environmental conditions; as well as model 4 (observation = 0.21, standard observation = -0.37, p = 0.59) portraying species composition across sites was not influenced by species traits. Despite the models not recovering statistical significance, several notable trends were revealed by the RLQ. The three main forest types Coastal (Albany Coastal and Eastern Cape Dune), Mistbelt and Scarp forests clustered in separate quadrants of ordination space (Figure 3.4a). All species of the *Rhinolophus* genus were associated with Mistbelt forests (Figure 3.4a and c). Species with a high wing loading and low-duty cycle call types (*Scotophilus dinganii*, *Tadarida aegyptiaca*, *Chaerephon pumilus* and *Otomops martiensseni*) clustered on the lower left quadrant, aligning with Coastal and Scarp forest types (Figure 3.4a and c).

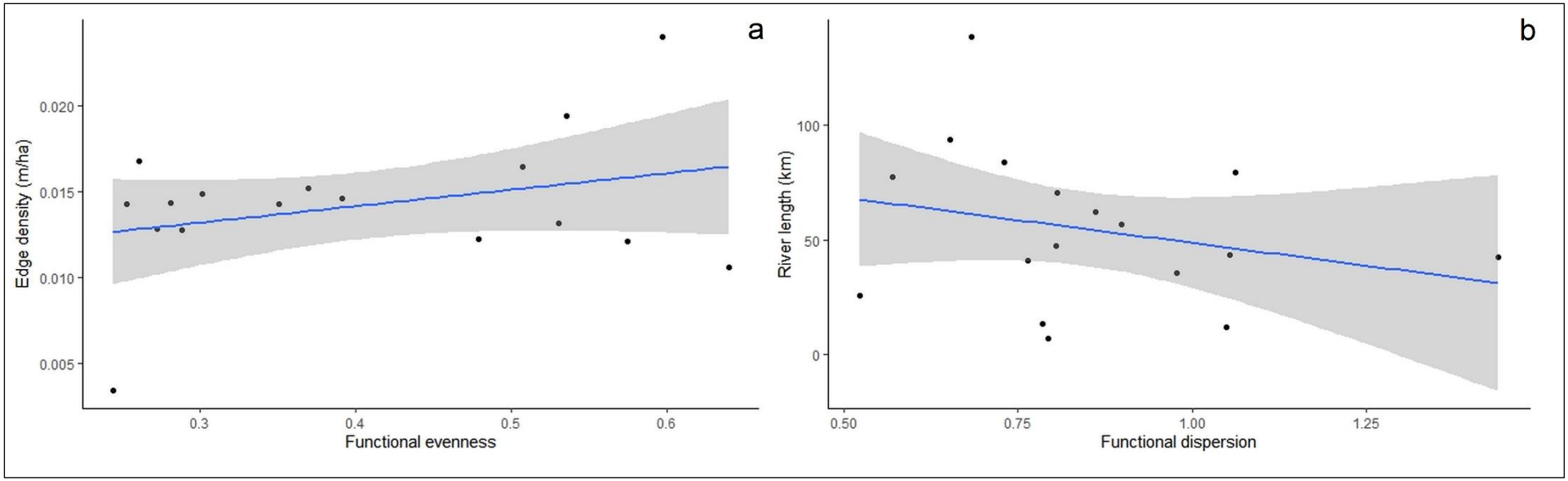

Figure 3.3 Graphical display of generalised linear model effects of a) edge density on functional evenness; and b) river length on functional dispersion (with 95% confidence intervals) for forests across southern KwaZulu-Natal and Eastern Cape, South Africa.

Table 3.3 Summary results of the RLQ analysis of bat communities with forest type and fragmentation metrics. Decomposition of the total inertia by the first three axes are shown under the eigenvalues. The inertia, maximum inertia and their ratio is shown for each axis. The correlation L shows the comparison between the trait-based species scores and environmental scores.

Eigenvalues	Axis 1	Axis 2	Axis 3
Inertia	0.14	0.05	0.01
Cumulative projected inertia (%)	66.50	90.29	96.66
Inertia and co-inertia	Inertia	Max	Ratio
R: Axis 1	2.32	2.82	0.82
Axis 2	3.74	4.97	0.75
Q: Axis 1	2.71	3.33	0.81
Axis 2	4.13	5.03	0.82
Correlation L: Axis 1	0.15	0.43	0.34
Axis 2	0.16	0.35	0.44

The fourth-corner approach complements the RLQ by assessing the strength of observed trait - environment associations using a regression-based method. Results showed stronger interactions of traits with forest type than fragmentation metrics (Figure 3.5). The fourth-corner further supported the association of high wing loading with Coastal and Scarp forests seen from the RLQ, by depicting a strong positive effect between wing loading and the Eastern Cape Dune and Pondoland Scarp forest types (Figure 3.5). Mistbelt forest types showed a moderate to strong negative association with wing loading. Of the fragmentation metrics, patch size displayed the strongest associations with species traits: positively interacting with aspect ratio and forearm length; and negatively with wing loading and characteristic frequency (Figure 3.5).

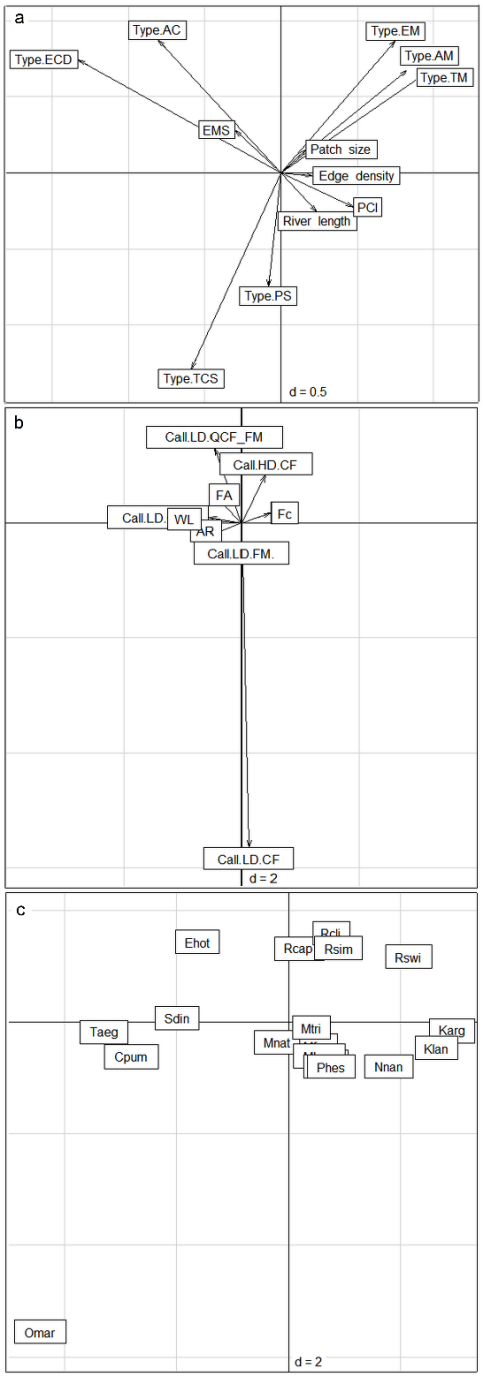

Figure 3.4 RLQ ordination of a) forest type and fragmentation metrics; b) bat functional traits; and c) bat species of forests in southern KwaZulu-Natal and Eastern Cape, South Africa (AC - Albany Coastal; AM - Amatole Mistbelt; ECD - Eastern Cape Dune; EM - Eastern Mistbelt; PS - Pondoland Scarp; TCS - Transkei Coastal Scarp; TM - Transkei Mistbelt; EMS - Effective mesh size; PCI - Patch Cohesion Index; AR - aspect ratio; FA - forearm length; Fc - characteristic frequency; WL - wing loading; HD - high duty-cycle; LD - low duty-cycle; CF - constant frequency; QCF - quasi-constant frequency; FM - frequency modulated; species names abbreviations in Appendix S3.2).

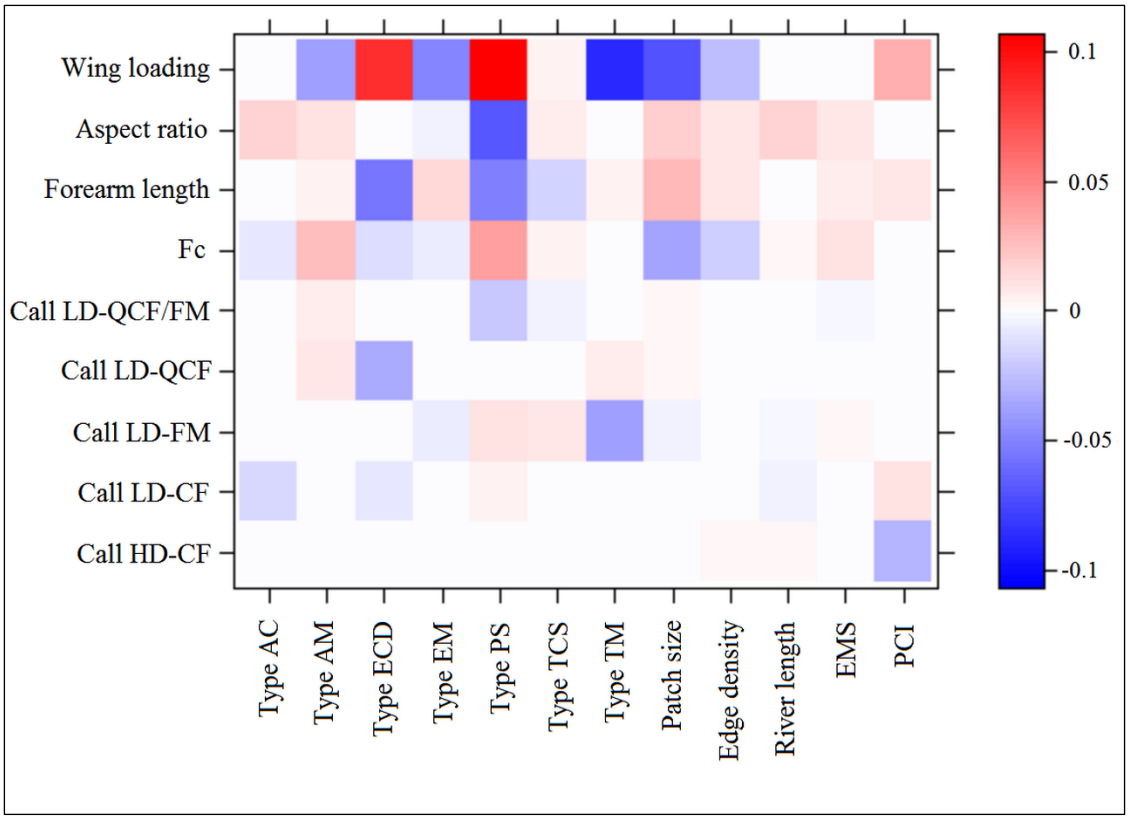

Figure 3.5 Fourth-corner analysis coefficients of interactions of forest type and fragmentation variables with bat functional traits. Blue shades depict a negative interaction between variable and trait; red indicates a positive effect. Shade of colour displays strength of interaction (see Figure 3.4 legend for abbreviations).

3.5 Discussion

We show that the large-scale historical processes of forest biogeography and structure are important determinants of the contemporary insectivorous bat communities by the effects on functional richness (FRic), functional divergence (FDiv) and functional dispersion (FDis). Although we did not find evidence for the effects of fragmentation on functional richness, two metrics affected bat functional diversity dynamically: edge density demonstrated a positive relationship with functional evenness (FEve) and FDis was negatively affected by river length. We also found forest type to demonstrate stronger filtering of functional traits than fragmentation metrics. Specifically, Eastern Cape Dune and Pondoland Scarp forest types were linked to faster flying species with high wing loading, and Mistbelt forest types were associated with slower flying species better able to manoeuvre within forest vegetation. Of the fragmentation metrics, patch size (measure of forest cover) displayed the greatest interaction effect with functional traits. As we had anticipated, increased forest cover filtered for species

with slow manoeuvrable flight with a reduced dispersal capacity, indicated by the negative interaction with wing loading. Furthermore, both forest type and fragmentation exhibited greater filtering effects on traits of body size (forearm length) and wing morphology (wing loading and aspect ratio) than echolocation type and frequency (Figure 3.5).

3.5.1 Historical climate-induced patterns of functional richness

As hypothesized, forest type was the predominant predictor of FRic. Our results align with those found for Eastern Cape forest birds by Leaver *et al.* (2019). Pondoland scarp forests demonstrated the highest species richness and FRic, while forests of the Transkei Coastal Scarp showed high FRic but with large variability between forests. Scarp forests are of Afrotemperate origin (Lawes, 1990) but occur geographically closer to the Indian Ocean than Mistbelt forests. The closer proximity of Scarp forests to the warm ocean allowed for them to be buffered from extreme palaeoclimatic events, such as the LGM, that resulted in extinction filtering of Mistbelt biota. These extinction events likely removed ecologically specialised and sensitive species from Mistbelt communities, while Scarp forests served as refugia for fauna during these cold and dry periods (Lawes *et al.*, 2007). With the onset of the Holocene altitherm post LGM, Scarp fauna re-colonised Mistbelt forests, as well as colonised the younger Indian Ocean Coastal forests (Eeley, Lawes and Piper, 1999; Lawes *et al.*, 2007). Thus, Scarp forests demonstrate higher species richness and more ecologically specialised species as they experienced less extreme climatic filtering than Mistbelt forests. This finding is supported by the high genetic diversity of bats from Scarp forests in the study region (Moir *et al.*, 2020). Scarp forests are also known to exhibit high floral endemicity and are considered to have the most significant biodiversity and biogeographical conservation value of all forest types in South Africa (von Maltitz *et al.*, 2003).

The Transkei Mistbelt type also demonstrated high FRic. Transkei Mistbelt forests are geographically closer to both Scarp forest types than Eastern and Amatole Mistbelt forests, so post-LGM faunal recolonisation of Transkei Mistbelts from Scarp forests by ecologically specialised species was most likely facilitated by their closer proximity (Figure 3.1), as shown by the close association of the relevant mammal communities in Lawes *et al.* (2007).

3.5.2 Eastern Cape Dune forests

The Eastern Cape Dune forests presents a unique case in that they exhibit the lowest species richness, FRic and FDis, with the highest functional divergence (FDiv), of all forest types. This

indicates a low effective number of functionally distinct and specialised species in the bat assemblage, while the most abundant species exhibit traits located at the extremities of the trait range. The RLQ indicates the assemblage to be dominated by common habitat generalist species such as the Egyptian free-tail bat (*Tadarida aegyptiaca*) and long-tailed serotine (*Eptesicus hottentotus*), with a strong selection for faster flying species demonstrating higher wing loading (Figure 3.5). The low species and functional diversity of Eastern Cape Dune forests is likely a result of their younger evolutionary history and homogenous vegetation structure, since they have not had as much evolutionary time to develop as many ecological niches for specialised species to fill. Also, Eastern Cape Dune forests have short, dense canopies with low plant diversity (von Maltitz *et al.*, 2003); and unlike Mistbelt forests that occur as more extensive bands or belts, these dune forests are limited to small pockets on a narrow cordon of coastal dunes (von Maltitz *et al.*, 2003). The small patch size and low vegetation diversity presents limited roosting potential for foliage or tree roosting bats, while the low and dense canopy limits flight, and therefore foraging opportunities, below or within the canopy for several species. Foraging space is mostly restricted to over the canopy by the open-air foraging guild (bats that forage high above the ground away from vegetation) and along forest edges by clutter-edge foragers (bats that forage on the edges of dense vegetation). This is evidenced by dominance of species with high wing loading, inferring faster flight, typical of the open-air foraging guild (Monadjem, Taylor, *et al.*, 2010).

3.5.3 Edge effects

Contrary to expectations, forest cover (measured by patch size) did not exhibit a significant effect on FRic. The lack of significant effects of fragmentation metrics on FRic indicates bat communities maintain functional diversity across forests with varying degrees of fragmentation, probably because the bat community has been subjected to fragmented conditions over the evolutionary history of these forests (Kotze and Lawes, 2007). However, we found a positive relationship of edge density with FEve. High FEve is indicative of a robust community with greater regularity of trait abundance distribution in functional space (Mason *et al.*, 2005). Petchey and Gaston (2006) predict ecosystem processes are largely influenced by FEve, independently of FRic. The positive relationship of FEve with edge density shown here may indicate efficient resource use of forest edges by insectivorous bats. In contrast, Meyer *et al.* (2008) found edge-sensitivity as the key trait of vulnerability to forest fragmentation for Neotropical bats, with gleaning species which prey on vertebrate animals showing particular sensitivity to edge effects and fragmentation (Meyer *et al.*, 2008; Farneda *et al.*, 2018). Forest

fragmentation, through the effects of patch size and edge effect, positively affected avian insectivory and bird FEve in France and New Zealand, as foliage-gleaning avian insectivores forage disproportionately more along edges than in forest interiors (Barbaro *et al.*, 2014). Our finding of increased functional diversity with edge density aligns with other studies of temperate forest insectivores as the assemblage consists of hawking and gleaning insectivores.

Forest edges surrounded by open habitats, as is the case for South African forests, demonstrate high structural heterogeneity and habitat diversity with resources to support different functional groups (Barbaro *et al.*, 2014). Forest edges, and roads that cut through forests, are thus valuable habitats, often demonstrating high bat species richness and activity (Jantzen and Fenton, 2013; Caldwell, Carter and Doll, 2019). High bat activity is found along forest edges as insect abundance is typically supported by edge habitats (Heim *et al.*, 2018). Bats preferentially travel along treelines which serve as commuting corridors (Kalcounis-Rueppell *et al.*, 2013), providing protection from high wind conditions (Verboom and Spoelstra, 1999). Sensitivity of bats to edge effects varies with the degree of contrast between forest fragments and the adjoining matrix, with greater negative edge effects in high-contrast landscapes (Estrada and Coates-Estrada, 2002; Bernard and Fenton, 2003).

3.5.4 River length and clutter-edge guild

The area and connectedness of waterways are important predictors of activity (Campbell *et al.*, 2009; Lookingbill *et al.*, 2010) as bats utilise them for drinking purposes; for their high foraging potential (Monadjem & Reside, 2008); and as navigational aids (Cortes and Gillam, 2020). Despite their importance, no studies have assessed how river length within a habitat type affects functional diversity of bat communities. We found the dispersion of species in functional trait space (FDis) decreased with an increase in river length within forests. This implies that the effective number of functionally distinct species decreased as river length increased, with a reduction in niche space with dominance of the assemblage by species of a particular niche. Lloyd, Law and Goldingay (2006) similarly found species-specific utilisation of riparian zones in Australian timber landscapes. This study demonstrated the association of miniopterid and vespertilionid bats with river length, these species utilise LD-FM echolocation calls, and are relatively small in size with intermediate to low aspect ratio and wing loading values. They fall within the clutter-edge foraging guild (Monadjem, Taylor, *et al.*, 2010) that hunt for insect prey along the edges of riparian vegetation. Forest interior habitat would typically be dominated by the clutter foraging guild (species that forage within highly cluttered vegetation), with open-air

foragers dominating the assemblage above the forest canopy. Thus, increased river length allows for penetration into the forest interior and dominance of the bat community by the clutter-edge functional guild, that may otherwise be occupied by clutter foraging species.

3.5.5 Trait selection

Both the RLQ and fourth-corner analyses revealed selection for fast flying species, with high wing loading, in Eastern Cape Dune and Pondoland Scarp forest types. The opposite was found for the Mistbelt forest type with selection for species with low wing loading. This infers species with slower flight speeds and higher manoeuvrability, useful for navigating through spatially complex environments such as forest interiors, are more prevalent in Mistbelt forests, while open-air species are more dominant in Coastal and Scarp forests. Mistbelt forests are characterised by extensive tracts of forest along the Main Escarpment with tall canopies and well-developed tree layers (von Maltitz *et al.*, 2003). Scarp forests also have high canopies but occur as smaller scattered patches in gorges, scarps, and krantzes; whereas Eastern Cape Dune forests are low-stature, dense-canopied forests occurring in small pockets (von Maltitz *et al.*, 2003). The high canopies and larger tracts of Mistbelt forests may be better able to support slow-hawking or gleaning bats of the clutter and clutter-edge foraging guilds that typically demonstrate low wing loading, as these species are adapted for flight within and around dense vegetation. While fast-hawking species of the open-air guild may be more predominant in the smaller sized patches of Scarp and Eastern Cape Dune forests as they can more easily navigate between forest patches while utilising neighbouring open biomes.

Furthermore, the four representatives of the *Rhinolophus* genus, that are of the clutter foraging guild, overlapped in ordination space with the three mistbelt forest types (Figure 3.4). This association is likely also due to an interplay of the selection of suitable caves by these species for roosting and breeding sites (Monadjem, Taylor, *et al.*, 2010), which are more abundant in mountainous regions. The Eastern and Transkei Mistbelt forests are situated within the greater Drakensberg Mountains region and the Amatole Mistbelt forests are located within the Amatole Mountain range. The close association of the *Rhinolophus* species with mistbelt forests is likely a result of their close association with mountainous terrain due to their cave roosting requirements.

Of the fragmentation metrics, patch size exhibited the strongest filtering effect on the functional traits of wing morphology and forearm length. Edge density displayed the same interactions with these traits, but the unimodal association of edge density and patch size makes inferences

of their relative effects difficult to extricate (Fletcher *et al.*, 2007). As anticipated, increased forest cover selected for slow-flying, manoeuvrable species demonstrating low wing loading. Patch size also filtered for larger species, with its positive interaction on forearm length (Figure 3.5). Meyer *et al.* (2008) and Farneda *et al.* (2015) similarly found forest fragmentation to filter traits of body size and wing morphology. Species of larger body size are typically at higher trophic levels and tend toward higher vulnerability in response to fragmentation (Henle *et al.*, 2004). Several studies have demonstrated bats with shorter and broader wings to be more vulnerable both to forest fragmentation and to urbanisation (Threlfall *et al.*, 2011; Hanspach *et al.*, 2012; Farneda *et al.*, 2015), as well as habitat modification from agricultural intensification (Wordley *et al.*, 2017). Similarly, wing morphology was a good predictor of extinction risk, both for temperate insectivorous bats (Safi and Kerth, 2004) and on a global scale, based on IUCN threat criteria (Jones, Purvis, & Gittleman, 2003). Little is known of the sensitivity of bats to habitat fragmentation in Africa: here we show larger insectivorous species and species exhibiting low wing loading may be more vulnerable to forest fragmentation.

Appendix S3.1 Morphological measurements (mean ± SD) of insectivorous bat species sampled from forests in southern KwaZulu-Natal and Eastern Cape provinces, South Africa. *Eptesicus hottentotus*, *Chaerephon pumilus*, *Otomops martiensseni* and *Tadarida aegyptiaca* are excluded from this table as they were not captured during this study.

Family and species	N	Forearm length (mm)	Mass (g)	Wingspan (cm)	Wing area (cm^2)	Aspect ratio	Wing loading (N/m^2)
Rhinolophidae							
Rhinolophus capensis	1	53.00	16.00	33.97	170.04	6.79	9.23
R. clivosus	16	54.43 ± 1.56	19.32 ± 2.15	34.83 ± 1.36	204.70 ± 17.06	5.95 ± 0.31	9.27 ± 0.81
R. simulator	1	50.49	16.00	33.84	189.79	6.03	8.27
R. swinnyi	39	43.42 ± 0.22	8.31 ± 0.21	28.12 ± 0.2	130.97 ± 1.51	6.02 ± 0.51	6.31 ± 0.17
Miniopteridae							
Miniopterus fraterculus	55	43.75 ± 0.09	8.85 ± 0.13	30.12 ± 0.18	137.09 ± 1.27	6.64 ± 0.06	6.35 ± 0.08
M. natalensis	25	46.12 ± 1.04	12.49 ± 2.13	32.14 ± 1.63	153.81 ± 16.45	6.74 ± 0.37	7.90 ± 1.34
Vespertilionidae							
Kerivoula argentata	1	37	6	27.15	140.92	5.23	4.18
K. lanosa	1	32.79	4.5	24.06	103.86	5.57	4.25
Laephotis botswanae	11	37.02 ± 0.37	7.64 ± 0.29	26.60 ± 0.32	121.32 ± 3.44	5.8 ± 0.10	6.04 ± 0.09
Myotis bocagii	4	38.49 ± 1.14	8.80 ± 0.31	27.31 ± 0.83	127.67 ± 5.29	5.84 ± 0.12	6.76 ± 0.12
M. tricolor	37	49.25 ± 0.22	13.09 ± 0.51	33.17 ± 0.25	186.14 ± 2.58	5.96 ± 0.10	6.93 ± 0.17
Neoromicia capensis (*Laephotis capensis* as per Monadjem *et al*., 2020)	43	33.23 ± 1.41	6.54 ± 1.17	23.16 ± 1.14	97.67 ± 7.73	5.80 ± 0.31	6.92 ± 1.03
N. nana (*Afronycteris nana* as per Monadjem *et al*., 2020)	47	30.27 ± 0.12	3.88 ± 0.09	20.95 ± 0.16	74.33 ± 1.23	5.94 ± 0.05	5.07 ± 0.13
Pipistrellus hesperidus	135	32.89 ± 0.10	6.19 ± 0.12	23.18 ± 0.13	89.55 ± 0.87	6.01 ± 0.03	6.90 ± 0.10
Scotophilus dinganii	7	54.10 ± 1.81	29.36 ± 2.97	36.74 ± 1.63	21.70 ± 20.28	6.25 ± 0.20	13.29 ± 0.63

Appendix S3.2 Bat species, name abbreviations and functional traits used to calculate functional diversity indices (HD - high duty-cycle; LD - low duty-cycle; CF - constant frequency; QCF- quasi-constant frequency; FM - frequency modulated; Fc - characteristic frequency). Superscript numbers correspond with references used for measurements (listed below table). *Measurements of *Otomops martiensseni* may correspond to *O. harrisoni* that has subsequently been described, but these measurements are the only currently available for *O. martiensseni* and *O. harrisoni* may be considered a proxy for the species.

Species	Species name abbreviations	Call type	Fc (kHz)	Forearm length (mm)	Aspect ratio	Wing loading (N/m^2)
Rhinolophus capensis	Rcap	HD-CF	79.83	53.00	6.79	9.23
R. clivosus	Rcli	HD-CF	92.76	54.43	5.95	9.27
R. simulator	Rsim	HD-CF	81.06	50.49	6.03	8.27
R. swinnyi	Rswi	HD-CF	105.4	43.42	6.02	6.31
Chaerephon pumilus	Cpum	LD-QCF	25.6 [1]	37.60 [2]	8.60 [3]	11.80 [3]
Otomops martiensseni	Omar	LD-CF	11 [1]	63.00 [2]	*9.30 [3]	*14.90 [3]
Tadarida aegyptiaca	Taeg	LD-QCF	22.7 [1,4]	46.50 [2]	8.10 [1,4]	13.10 [1,4]
Miniopterus fraterculus	Mfra	LD-FM	58.59	43.75	6.64	6.35
M. natalensis	Mnat	LD-FM	50.37	46.12	6.74	7.90
Eptesicus hottentotus	Ehot	LD-QCF and LD-FM	30.6 [1,4]	50.00 [2]	6.30 [1,4]	10.30 [1,4]
Kerivoula argentata	Karg	LD-FM	108.02	37.00	5.23	4.18
K. lanosa	Klan	LD-FM	95.41	32.79	5.57	4.25
Laephotis botswanae	Lbot	LD-FM	33.73	37.02	5.80	6.04
Myotis bocagii	Mboc	LD-FM	39.68	38.49	5.84	6.76
M. tricolor	Mtri	LD-FM	56.38	49.25	5.96	6.93

Neoromicia capensis (*Laephotis capensis* as per Monadjem *et al*., 2020)	Ncap	LD-FM	39.63	33.23	5.80	6.92
N. nana (*Afronycteris nana* as per Monadjem *et al*., 2020)	Nnan	LD-FM	67.28	30.27	5.94	5.07
Pipistrellus hesperidus	Phes	LD-FM	46.04	32.89	6.01	6.90
Scotophilus dinganii	Sdin	LD-FM	34.68	54.10	6.25	13.29

[1] Schoeman, M. C., & Jacobs, D. S. (2008). The relative influence of competition and prey defenses on the phenotypic structure of insectivorous bat ensembles in southern Africa. *PloS One*, *3*(11), e3715–e3715. https://doi.org/10.1371/journal.pone.0003715

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Appendix S3.3 Landscape fragmentation variables as measures of forest connectivity and fragmentation used in generalised linear models.

Study forest	Patch size (ha)	Edge density (m/ha)	Patch cohesion index	Effective mesh size (ha)	River length (km)
Xumeni	1164.20	0.0121	49.98	53.50	13.36
Ngeli	1607.20	0.0106	48.31	324.55	35.49
Gomo	1980.84	0.0148	53.80	22.66	47.59
Baziya	6017.12	0.0128	52.07	156.37	93.62
Kologha	8906.44	0.0142	52.22	38.95	138.08
Fort Fordyce	4581.76	0.0239	45.27	164.28	79.42
Oribi Gorge	2723.16	0.0131	53.74	33.06	56.71
Umtamvuna	1075.24	0.0164	53.45	10.56	70.24
Mbotyi	2232.60	0.0152	53.15	281.58	62.21
Mkambati	1205.84	0.0128	54.02	7.97	42.27
Dwesa	4383.40	0.0122	45.05	78.12	83.87
Manubi	2194.12	0.0142	46.22	71.75	77.13
Silaka	718.44	0.0168	46.47	111.80	41.15
Cape Morgan	333.88	0.0146	34.52	10.24	25.58
Woody Cape	954.12	0.0143	36.31	630.49	6.88
The Island	1437.68	0.0194	38.91	148.14	12.10
Alexandria	7779.92	0.0034	45.10	944.62	43.27

Appendix S3.4 Results from Global Moran's I test for bat species richness and functional diversity indices (significant *p* value in bold depicts spatial autocorrelation between forests).

Moran's I	Observed	Expected	Z	*p*
Species richness	0.246	-0.063	0.099	**0.002**
FRic	0.104	-0.063	0.098	0.089
FEve	0.026	-0.063	0.102	0.386
FDiv	-0.128	-0.063	0.098	0.505
FDis	-0.073	-0.063	0.092	0.905

Chapter 4. Demographic responses of forest-utilising bats to past climate change in South Africa

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4.1 Abstract

Historical forest contractions may have restricted the distributions of forest-utilising fauna while providing opportunities for range expansions for open-habitat species. We aimed to test if habitat associations have played an important role in determining population genetic structure and demographic responses of six bats to oscillations in forest extent since the Last Glacial Maximum (LGM). We hypothesized that forest associated species would display high levels of population structure and past population contractions as their distribution is dependent on fragmented forests. By contrast, habitat generalists would demonstrate low geographical structuring and historical population stability as suitable habitats are widely available. We used mitochondrial DNA to generate genetic diversity and population structure metrics of three forest associated species and three habitat generalists in South Africa. Neutrality tests and Bayesian skyline plots were used to investigate demographic histories. A forest habitat association did not inform the population genetics of the study species. Rather, species-specific traits of roosting requirements, philopatry to the natal range and dispersal ability informed the observed structure. All species demonstrated population expansions during the Pleistocene, with no apparent decline during the LGM. It appears that the lower climate change footprint and refuge-status of eastern South Africa prevented population declines of insectivorous bats during the LGM.

4.2 Introduction

South African forests occur today as isolated fragments (Low and Rebelo, 1996), forming the smallest biome in the country (Rutherford and Westfall, 1994), yet they support a high proportion of the country's faunal biodiversity (Geldenhuys and Macdevette, 1989). Forests are distributed from the southern seaboard of the Cape Peninsula, north along the Eastern Cape and KwaZulu-Natal Provinces to the borders of Mozambique and Zimbabwe (Midgley *et al.*, 1997). They are broadly categorised as inland Afrotemperate Mistbelt, subtropical Indian

Ocean Coastal Belt, and Scarp forests (von Maltitz *et al.*, 2003). The global climatic fluctuations of the Quaternary caused historical habitat changes to forests (Partridge, 1997): cooler drier conditions during the Last Glacial Maximum (LGM) ~21 – 18 kya caused forest contraction (Tyson, 1986), which was followed by forest expansion at the onset of warmer and wetter conditions during the Holocene Climatic Optimum from 17 – 15 to ~7 kya (Partridge *et al.*, 1990).

Historical forest contractions may have restricted the distributions of forest-dependent fauna while providing opportunities for range expansion in open habitat species (Campbell *et al.*, 2006). Similarly, forest expansion during glacial retreat would have allowed for radiation of forest fauna (Agarwal and Karanth, 2015). Fauna that undergo range expansions may display genetic diversity characteristic of founder effects (Hewitt, 2004), while species that experience habitat fragmentation or range restriction typically show population bottlenecks (Knowles, Carstens and Keat, 2007). Fluctuations of forest coverage within South Africa have caused range changes or spatial discontinuity of forest fauna with subsequent oscillations in their population sizes and genetic diversification. For example, historical climate change contributed to the diversification of the Cape velvet worm (*Peripatopsis capensis*) in the Western Cape (Mcdonald and Daniels, 2012), and to historical population declines of the Cape parrot (*Poicephalus robustus*) (Coetzer *et al.*, 2019).

Climate-induced oscillations of forest coverage have contributed to phylogeographical structuring of non-volant small mammals in central and West Africa (Huhndorf, Kerbis Peterhans and Loew, 2007; Nicolas *et al.*, 2008). Forest biogeography and associated small mammal phylogeography have not been well studied for southern Africa, and to date, very few studies have focused on forest-utilising bats in South Africa. Thus, we compared the genetic diversity, population genetic structure and demographic history of six forest-utilising insectivorous bat species across South Africa. The species utilise forest habitats to varying degrees and were grouped as either forest associated or habitat generalist. *Rhinolophus swinnyi s.l.* (suborder Pteropodiformes, family Rhinolophidae) occurs in savanna woodland in the north of its range (Skinner and Chimimba, 2005) and Afrotemperate forest in the south of its range (Bronner, 1990), and was classed as forest associated. *Miniopterus fraterculus* (suborder Vespertilioniformes, family Miniopteridae) is a temperate habitat generalist species associated with montane grassland (Monadjem, Taylor, *et al.*, 2010). *Laephotis botswanae* (family Vespertilionidae) is a habitat generalist as it has been recorded from open woodland and savanna habitats (Monadjem, Taylor, *et al.*, 2010). *Myotis tricolor* (Vespertilionidae) has a

wide distribution that is restricted to mountainous areas due to its dependency on caves for roosting (Monadjem, Taylor, *et al.*, 2010) and was grouped as a habitat generalist. *Neoromicia nana* (*Afronycteris nana* as per Monadjem *et al*., 2020) and *Pipistrellus hesperidus* (Vespertilionidae) are both considered forest associated species, but not forest-dependent, as they utilise well-wooded habitats such as forest and riparian vegetation (Happold, Happold and Hill, 1987; Monadjem and Reside, 2008). The six species were selected as they have similar distributions in the eastern part of South Africa and have similarly low predicted dispersal abilities (Monadjem, Taylor, *et al.*, 2010). Dispersal ability in bats is often inferred from wing shape and size. The study species have short, broad wings with low wing loading and low aspect ratios, traits typical of species foraging within and around vegetation that require high manoeuvrability and lower flight speeds. Species with low wing loading and aspect ratios are generally regarded to have a lower dispersal ability relative to other bat species (Norberg and Rayner, 1987).

We aimed to test if habitat associations have played an important role in determining bat species' responses to changes in forest that occurred during the LGM. Species with similar habitat associations are expected to depict similar demographic histories due to shared biogeographical histories (Chen *et al.*, 2010). We hypothesized concordance in population genetic trends for species utilising similar habitats (Campbell *et al.*, 2006): forest associated species should show high levels of population structure as their distribution is dependent on fragmented forests, while habitat generalists should demonstrate low geographical structuring as suitable habitats are more widely available. Additionally, we hypothesized that forest associated species would display demographic histories characteristic of past population contractions in response to forest contraction at the time of the LGM. Habitat generalists were expected to show stable genetic histories with overall population expansion.

4.3 Materials and Methods

4.3.1 Sample collection

Bats were sampled from 20 forests across the Eastern Cape and KwaZulu-Natal Provinces from August 2017 to December 2018 (Figure 4.1; Table 4.1). The study was approved by the Stellenbosch University Animal Use and Care Research Ethics Committee (protocol #0409),

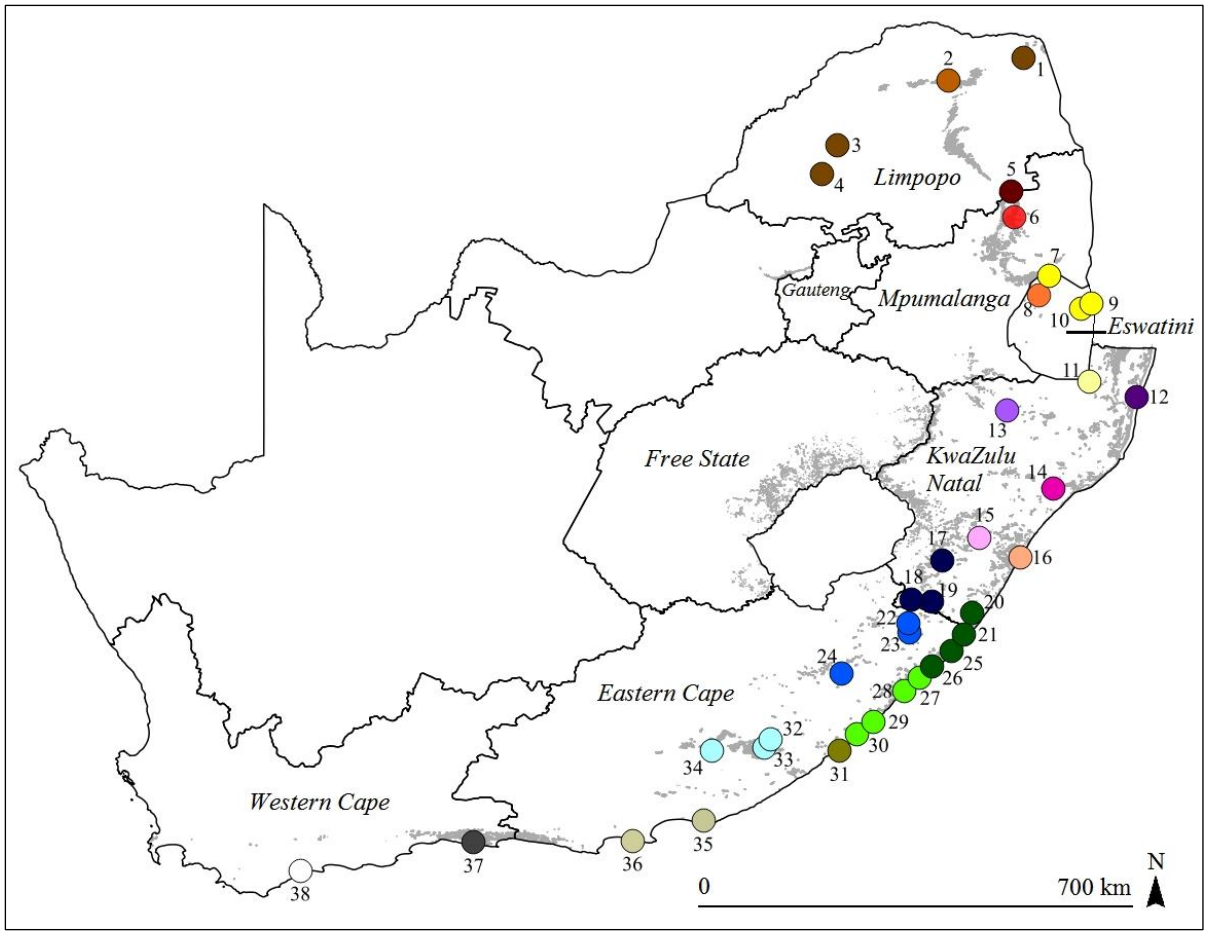


Figure 4.1. Sample localities (listed in Table 4.1) with province names in italics. Grey shading displays the extent of indigenous forest cover across South Africa. Locality colours correspond to haplotype networks in Figure 4.2.

licensed by the Eastern Cape Parks and Tourism Agency (RA0237, CRO 59/17CR, CRO 60/17CR), Department of Agriculture, Forestry and Fisheries (WIFM 04-2016, WIFM 09-2017, WIFM 06-2018), Ezemvelo KZN Wildlife (OP 143/2018, OP 3847/2018) and South African National Parks (CHER-MI/2018-004). The capture and handling of bats was performed in accordance with the 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education (Sikes and the Animal Use and Care Committee of American Society of Mammalogists, 2016). A 2 – 3 mm wing membrane biopsy was taken and stored in 96% ethanol. Supplementary tissue samples were sourced from museum collections to represent as much of the species distributions within South Africa as possible. However, only few museum specimens proved to be available from which DNA could be extracted and amplified. Consequently, sampling is biased towards forest habitats, with fewer samples from other habitat types. Samples were acquired from the following South African mammal collections: Durban Natural Science Museum, Bloemfontein National

Museum and McGregor Museum (Appendix S4.1).

4.3.2 Laboratory procedure

Total genomic DNA was extracted from wing biopsies and tissue samples using a Nucleospin Tissue kit (Macherey-Nagel, Germany). Cytochrome *b* (1140 bp) was amplified using tRNA-GluA (5'-TGACTTGAARAACCAYCGTTG-3') and H15915R (5'-TTCATTACTGGTTTACAAGAC-3') (Irwin, Kocher and Wilson, 1991). The polymerase chain reaction (PCR) consisted of an initial 5 min denaturation at 95 °C, 35 cycles of 30 s at 94 °C, 30 s annealing at 43.5 °C, 30 s at 72 °C, and a final extension at 72 °C for 10 min. The D-loop of the mitochondrial control region was amplified with primers C (5'-TGAATTGGAGGACAACCAGT-3') and E (5'-CCTGAAGTAGGAACCAGATG-3') (Wilkinson and Chapman, 1991) for *Miniopterus fraterculus.* PCR conditions for these primers required 40 cycles of 95 °C for 1 min, 55 °C for 1.5 min and 72 °C for 2 min, followed by 7 min at 72 °C. Forward primers RF (5'-TTCCACCATCAGCACCCAAAGC-3') and VF (5'-CCCCACCATCAACACCCAAAGC-3') were used for *R. swinnyi* s.l. and the Vespertilionidae species, respectively, with the reverse primer 5'-GTTGCTGGTTTCACGGAGGTAG-3' (Wilkinson and Chapman, 1991). The PCR protocol followed initial denaturation of 94 °C for 5 min, 30 cycles of 30 s at 94 °C, 30 s annealing at 50 °C for RF and 60 °C for VF, 30 s at 72 °C, and a final extension cycle at 72 °C for 10 min. PCRs contained 14.9 µL of millipore water, 2.5 µL of 25 mM $MgCl^2$, 2.5 µL of 10×Mg^{2+}- free buffer, 0.5 µL of a 10 mM dNTP solution and 0.5 µL (10 mM) primer solution, 0.1 µL of Supertherm *Taq* polymerase and template DNA. All reactions included a negative, without template DNA, to control for contamination. Products were separated in 1% agarose gels and excised with a sterile surgical blade. A BioSpin Gel Extraction Kit (Bioer Technology, China) was used to purify gene fragments from the agarose gel. The products were sequenced at the Core Sequencing Facility, Stellenbosch University, on an ABI 3100 device (Applied Biosystems, Perkin Elmer, USA).

4.3.3 Population genetics analyses

Additional sequences were downloaded from GenBank to increase sample sizes and localities encompassed in this study (Appendix S4.2). Mitochondrial cytochrome *b* and D-loop markers were concatenated as longer sequences to provide higher resolution (Jacobsen, Friedmann and Omland, 2010). Intraspecific haplotype diversity and nucleotide diversity (Nei, 1987) were calculated with DnaSP v.6 (Rozas *et al.*, 2017). Global population structure was estimated by F_{ST} and Φ_{ST} and population pairwise structure Φ_{ST} in Arlequin (Excoffier and Lischer, 2010).

Table 4.1 Sample localities and forest or vegetation type per South African province and Eswatini (Figure 4.1 – colours correspond with map). Species collected from each locality are listed with respective sample sizes. The occurrence of *Laephotis botswanae* and *Myotis tricolor* in various habitats is evident in this table by their collection in different vegetation types. *Pipistrellus hesperidus* and *Neoromicia nana* (*Afronycteris nana* as per Monadjem *et al*., 2020) are grouped as forest associated as they occur in well wooded areas of other vegetation types (Happold *et al*., 1987; Monadjem and Reside, 2008). *Rhinolophus swinnyi* was collected only from forest habitats.

Number on Figure 4.1	Province	Locality	Latitude (° S)	Longitude (° E)	Forest/vegetation type (von Maltitz *et al*., 2003; Mucina and Rutherford, 2011)	Species (sample size)
1	Limpopo	Witsand Dam	22.683	31.033	Bushveld	*Laephotis botswanae* (3)
2		Soutpansberg	22.973	29.933	Northern Afrotemperate forest	*Pipistrellus hesperidus* (2)
3		Lapalala Wilderness Area	23.947	28.440	Bushveld	*Laephotis botswanae* (1)
4		Vaalwater	24.312	28.121	Bushveld	*Laephotis botswanae* (2)
5	Mpumalanga	Blyderivierspoot Nature Reserve	24.854	30.850	Grassland	*Myotis tricolor* (2)
6		Feather River	24.955	30.891	Bushveld	*Pipistrellus hesperidus* (1)
7	Eswatini	Ngonini Estates	25.801	31.405	Lowveld	*Neoromicia nana* (1)
8		Maguga	26.088	31.250	Bushveld	*Neoromicia nana* (3)
9		Mlawula Nature Reserve	26.200	32.004	Lowveld	*Pipistrellus hesperidus* (1)
10		Hlane Nature Reserve	26.260	31.785	Lowveld	*Neoromicia nana* (1)

11	KwaZulu-Natal	Hlatikulu Forest Reserve	27.324	31.989	Eastern Scarp forest	*Myotis tricolor* (1), *Pipistrellus hesperidus* (1)
12		Sodwana Bay National Park	27.636	32.582	KwaZulu-Natal Coastal forest	*Neoromicia nana* (1)
13		Vryheid Hill Nature Reserve	27.747	30.789	Northern KwaZulu-Natal Mistbelt forest	*Neoromicia nana* (1)
14		Eshowe	28.894	31.462	Eastern Scarp forest	*Rhinolophus swinnyi* (1), *Myotis tricolor* (1), *Neoromicia nana* (1), *Pipistrellus hesperidus* (1)
15		Ferncliff Nature Reserve	29.564	30.348	Northern KwaZulu-Natal Mistbelt forest	*Rhinolophus swinnyi* (1), *Miniopterus fraterculus* (1)
16		Durban	29.873	30.971	Grassland	*Neoromicia nana* (1), *Pipistrellus hesperidus* (1)
17		Xumeni State Forest	29.929	29.849	Eastern Mistbelt forest	*Miniopterus fraterculus* (5), *Laephotis botswanae* (2), *Pipistrellus hesperidus* (4)
18		Kokstad	30.509	29.406	Eastern Mistbelt forest	*Rhinolophus swinnyi* (4)
19		Ngeli State Forest	30.526	29.694	Eastern Mistbelt forest	*Rhinolophus swinnyi* (4), *Laephotis botswanae* (1), *Myotis tricolor* (7), *Pipistrellus hesperidus* (4)
20		Oribi Gorge Nature Reserve	30.691	30.292	Pondoland Scarp forest	*Miniopterus fraterculus* (1), *Myotis tricolor* (3), *Neoromicia nana* (3), *Pipistrellus hesperidus* (4)
21		Umtamvuna Nature Reserve	31.006	30.153	Pondoland Scarp forest	*Pipistrellus hesperidus* (2)
22	Eastern Cape	Ntsizwe Mine	30.805	29.280	Transkei Mistbelt forest	*Miniopterus fraterculus* (1)
23		Gomo State Forest	30.981	29.373	Transkei Mistbelt forest	*Rhinolophus swinnyi* (1), *Miniopterus fraterculus* (4), *Laephotis botswanae* (3), *Pipistrellus hesperidus* (4)
24		Baziya State Forest	31.574	28.390	Transkei Mistbelt forest	*Rhinolophus swinnyi* (5), *Miniopterus fraterculus* (2), *Laephotis botswanae* (2), *Myotis tricolor* (3), *Pipistrellus hesperidus* (4)
25		Mkambathi Nature Reserve	31.264	29.986	Pondoland Scarp forest	*Pipistrellus hesperidus* (3)

26		Mbotyi State Forest	31.475	29.692	Pondoland Scarp forest	*Rhinolophus swinnyi* (2), *Myotis tricolor* (2), *Neoromicia nana* (1), *Pipistrellus hesperidus* (4)
27		Silaka Nature Reserve	31.636	29.523	Transkei Coastal Scarp forest	*Myotis tricolor* (5), *Neoromicia nana* (5), *Pipistrellus hesperidus* (4)
28		Hluleka Nature Reserve	31.824	29.298	Transkei Coastal Scarp forest	*Myotis tricolor* (1), *Neoromicia nana* (5), *Pipistrellus hesperidus* (1)
29		Dwesa Nature Reserve	32.276	28.846	Transkei Coastal Scarp forest	*Rhinolophus swinnyi* (1), *Neoromicia nana* (2), *Pipistrellus hesperidus* (4)
30		Manubi State Forest	32.457	28.599	Transkei Coastal Scarp forest	*Laephotis botswanae* (2), *Myotis tricolor* (3), *Neoromicia nana* (3), *Pipistrellus hesperidus* (5)
31		Cape Morgan Nature Reserve	32.705	28.355	Eastern Cape Dune forest	*Neoromicia nana* (4), *Pipistrellus hesperidus* (5)
32		Kologha State Forest	32.535	27.351	Amatole Mistbelt forest	*Rhinolophus swinnyi* (2), *Miniopterus fraterculus* (5), *Pipistrellus hesperidus* (1)
33		Sandile's Cave	32.660	27.271	Amatole Mistbelt forest	*Rhinolophus swinnyi* (5), *Myotis tricolor* (4)
34		Fort Fordyce Nature Reserve	32.688	26.498	Amatole Mistbelt forest	*Rhinolophus swinnyi* (6), *Miniopterus fraterculus* (6), *Laephotis botswanae* (3), *Myotis tricolor* (4), *Pipistrellus hesperidus* (8)
35		Alexandria Forest of Addo National Park	33.717	26.383	Albany Coastal forest	*Miniopterus fraterculus* (1), *Pipistrellus hesperidus* (6)
36		The Island Nature Reserve	33.987	25.341	Albany Coastal forest	*Miniopterus fraterculus* (5)
37	Western Cape	Knysna	34.035	23.046	Southern Cape Afrotemperate forest	*Pipistrellus hesperidus* (1)
38		De Hoop Guano Cave	34.430	20.532	Western Cape Milkwood forest	*Myotis tricolor* (1)

jModeltest v.2.1.10 (Posada, 2008) was used to identify the best-fitting substitution models for each species under the corrected Akaike information criterion. Pairwise Φ_{ST} values were estimated under the Tamura and Nei model (Tamura and Nei, 1993) with alpha gamma shape value of 0.05 for all species (except *Miniopterus fraterculus* - the Tamura model was used). Localities with one sequence were removed from the alignment before computing population pairwise Φ_{ST}. Haplotype networks were created using DnaSP v.6 and POPART (Leigh and Bryant, 2015).

4.3.4 Demographic history

Fu's F_S (Fu, 1997) and the R_2 statistic (Ramos-Onsins and Rozas, 2002) were used to test for historical population expansion. An excess of single nucleotide polymorphisms relative to expectation under the standard neutral model indicates recent population expansion, which is demonstrated by significantly negative Fu's F_S values and significantly positive R_2 values. Fu's F_S is particularly useful for large sample sizes (~50) and R_2 for small sample sizes (~10). Fu's F_S was generated with Arlequin with 1000 simulated samples, and the R_2 statistic was generated in DnaSP. Fu and Li's D^* was used to test for historical population contractions as it detects an excess of old mutations (Fu and Li, 1993), and was generated in DnaSP.

Mismatch distributions were generated in Arlequin utilising cytochrome *b* alignments as it has a known mutation rate (Nabholz, Gle and Galtier, 2007), whereas the mutation rate for the concatenated mitochondrial DNA (mtDNA) dataset was unknown (Lin *et al.*, 2013). Populations experiencing long-term demographic stability under the stochastic influences of genetic drift, or that are declining slowly, display a ragged multimodal distribution, and recently expanded populations produce a smooth unimodal distribution (Rogers and Harpending, 1992). The Raggedness Index (H_r) and sum of squared deviations (SSD) were computed to test for significant deviations from a sudden expansion model in Arlequin with 5000 replicates. The time of expansion (t) was estimated from the mismatch distribution with $t = \tau/2u$, where τ is the date of population growth measured in units of mutational time and u is mutation rate per generation for the entire sequence (Rogers and Harpending, 1992). The mutation rate per generation was computed by $u = \mu gk$, where μ is the mutation rate per nucleotide, k is sequence length and g is generation time (2 years; Xu *et al.*, 2010). A mutation rate of 1.30×10^{-8} substitutions/site/year was used (Thong *et al.*, 2012).

Bayesian skyline plots (BSPs) reconstructed changes in effective population size through time and were generated with Beast v.2.6.1 (Bouckaert *et al.*, 2019). Significant population structure

has a confounding effect on BSP inferences (Heller, Chikhi and Siegismund, 2013). Population structure identified from the pairwise population Φ_{ST} and haplotype networks was typically generated by a few sequences from more distant localities from the main sampling area. These sequences were removed from alignments for the BSP analysis. The analysis was performed with the concatenated alignments using the model results provided by jModeltest. A strict molecular clock was used with a mutation rate of 1.30×10^{-8} substitutions/site/year for cytochrome *b* (Thong *et al.*, 2012) and 1.73×10^{-7} substitutions/site/year for the D-loop (Ratrimomanarivo *et al.*, 2009). The Markov chain Monte Carlo search was run for 40 million generations, sampling every 1,000 chains, with 10% discarded as burn-in. The results were summarized using Tracer v.1.7.1 (Rambaut *et al.*, 2018); all effective sample size values exceeded 700 for all parameters.

4.4 Results

A total of 222 sequences (ranging from 19 for *L. botswanae* to 71 for *P. hesperidus*) from 38 localities (ranging from nine for *L. botswanae* to 23 for *P. hesperidus*) were employed in the analyses. Cytochrome *b* sequence length was between 1,122 and 1,140 nucleotides, D-loop sequence length was between 246 and 537 nucleotides, and concatenated length ranged over 1,368 – 1,660 nucleotides. The number of polymorphic sites varied between 26 and 76 with 10 – 34 haplotypes. The sequences generated in this study were accessioned on GenBank (accession numbers MN790784 – 791086).

4.4.1 Population genetics of forest associated species

Rhinolophus swinnyi had a comparatively high haplotype diversity (0.96 ± 0.02) but the lowest nucleotide diversity (0.00403 ± 0.00067) (Table 4.2). Both F_{ST} and Φ_{ST} were comparatively low, suggesting limited population structure across the sample localities. Pairwise population analysis found seven of 28 (25%) significant Φ_{ST} values ranging from 0.23 to 0.89. Six significant values indicate genetic differentiation of Sandile's Cave and Fort Fordyce from other localities (Appendix S4.2). Sandile's Cave, Fort Fordyce and Kologha are located within the Amatole Mistbelt complex and contained central haplotypes within the network (Figure 4.2A). Despite low F_{ST} and Φ_{ST} values, geographical groupings were apparent in the network. Haplotypes from KwaZulu-Natal (Eshowe to Kokstad) were private and clustered separately from central haplotypes by several mutations. Haplotypes from the two coastal localities (Dwesa and Mbotyi) were divergent with numerous mutations and showed high pairwise population Φ_{ST} values from the other localities (0.68 – 0.89) indicating reduced gene flow

between these areas.

Neoromicia nana (*Afronycteris nana* as per Monadjem *et al*., 2020) displayed high haplotype diversity (0.96 ± 0.02) and the highest nucleotide diversity (0.00761 ± 0.0008) (Table 4.2), indicating a large stable population. It demonstrated the highest global Φ_{ST} (0.574) (Table 4.2), with seven of 21 (33%) significant pairwise Φ_{ST} estimates ranging from 0.34 to 0.76 (Appendix S4.2). Five of the seven significant pairwise Φ_{ST} values displayed differentiation of Silaka from most localities. There was considerable structuring within the haplotype network, particularly between haplotypes from Eswatini and other South African localities (Figure 4.2A). The Ngonini haplotype showed several mutations from the neighbouring haplotypes of Magagu, despite there being only ~35 km straight line distance between the localities. Silaka haplotypes clustered with Hluleka, which is situated ~27 km south-east along the Cape coastline from Silaka. However, there were no shared haplotypes between Silaka and Mbotyi, which is equidistant north-east along the coastline.

The diversity indices of *P*. *hesperidus* revealed relatively high haplotype diversity (0.91 ± 0.03) with comparatively low nucleotide diversity (0.00491 ± 0.00043) (Table 4.2). *Pipistrellus hesperidus* had high population structure estimates of F_{ST} (0.324) and Φ_{ST} (0.525) (Table 4.2), with the highest proportion of significant pairwise Φ_{ST} values (39/91, 42%) of the study species (Appendix S4.2). The northernmost localities (Limpopo, Mpumalanga and Eswatini) were divergent from other localities based on virtual haplotypes and several mutations in the network (Figure 4.2A). There was low-level structuring between localities within the Eastern Cape, evident from the clustering of haplotypes from Gomo, Baziya, Manubi, Fort Fordyce and Alexandria.

4.4.2 Population genetics of habitat generalists

Miniopterus fraterculus contained the highest haplotype diversity (0.99 ± 0.01) with a comparatively high nucleotide diversity (0.00703 ± 0.00061) (Table 4.2), representative of a large panmictic population. Global population structure F_{ST} (0.029 - non-significant) and Φ_{ST} (0.247) were lowest of all species (Table 4.2), with 7/21 (33%) significant pairwise Φ_{ST} values ranging from 0.16 to 0.42 (Appendix S4.2). Four of the seven significant pairwise Φ_{ST} were due to geographical differentiation of Gomo from most other localities. High haplotype diversity was evident in the haplotype network due to a high proportion of unique haplotypes with no geographical structure between localities, depicting the habitat generalist nature of this species (Figure 4.2B).

Table 4.2 Details of concatenated cytochrome *b* and D-loop sequences, genetic diversity indices with standard deviation and measures of population structure. Statistical significance denoted as ** $p < 0.001$, * $p < 0.05$.

	Forest associated			Habitat generalists		
	R. swinnyi	*N. nana* (*Afronycteris nana* as per Monadjem *et al*., 2020)	*P. hesperidus*	*M. fraterculus*	*L. botswanae*	*M. tricolor*
Sample size	32	32	71	31	19	37
Number of localities	11	14	23	10	9	13
Haplotypes (h)	19	21	34	26	10	21
Haplotype diversity (Hd)	0.96 ± 0.02	0.96 ± 0.02	0.91 ± 0.03	0.99 ± 0.01	0.89 ± 0.05	0.94 ± 0.03
Nucleotide diversity (π)	0.00403 ± 0.00067	0.00761 ± 0.0008	0.00491 ± 0.00043	0.00703 ± 0.00061	0.00599 ± 0.00123	0.00562 ± 0.00097
F_{ST}	0.049*	0.149*	0.324**	0.029	0.428**	0.218**
Φ_{ST}	0.180	0.574**	0.525**	0.247**	0.559*	0.519**

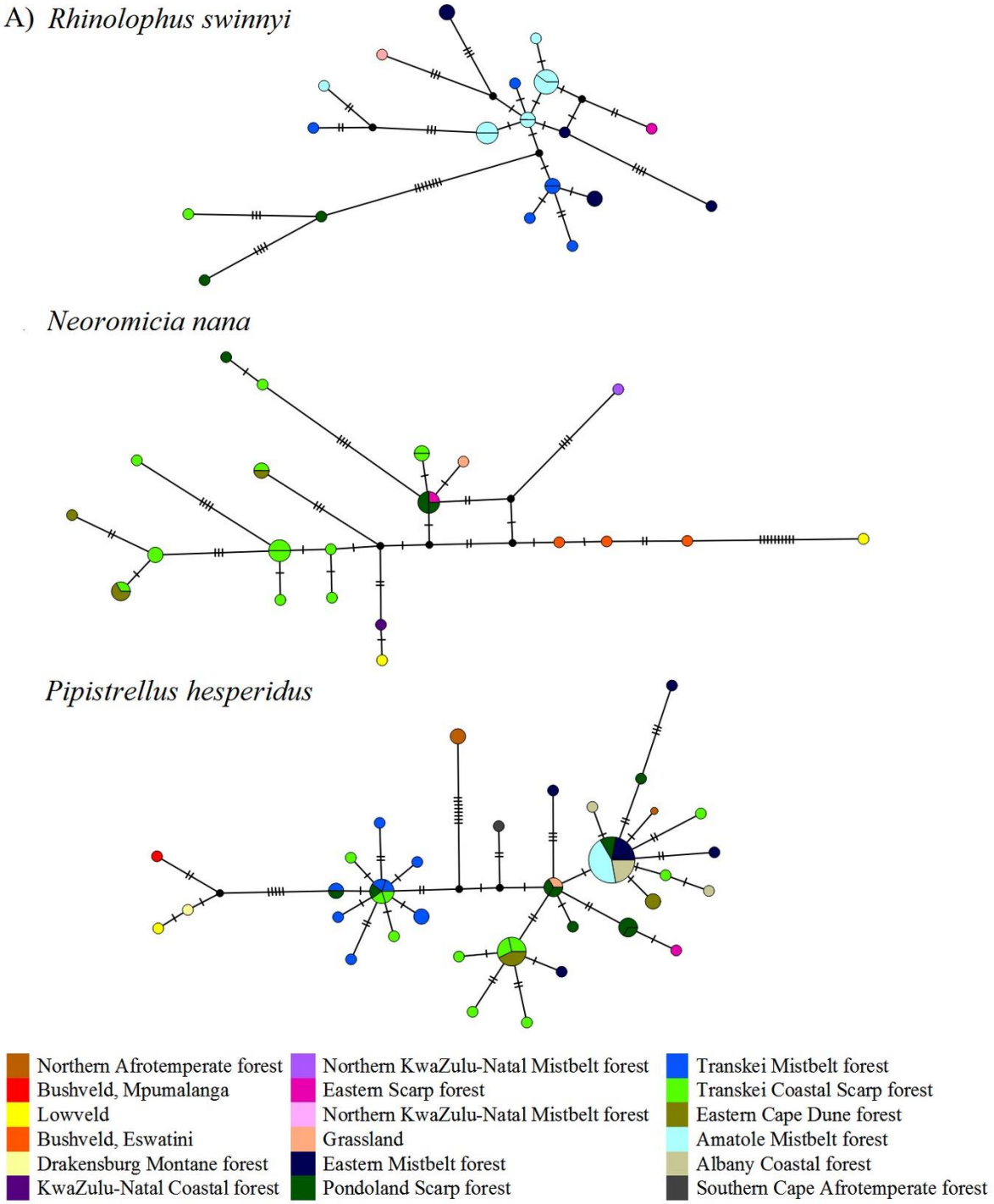


Figure 4.2 Haplotype networks of (A) forest associated species and (B) habitat generalist species, with corresponding colour keys. Haplotype sizes represent relative frequency, hatch marks represent mutations and black haplotypes are virtual haplotypes. The number of sequences is represented by haplotype proportions.

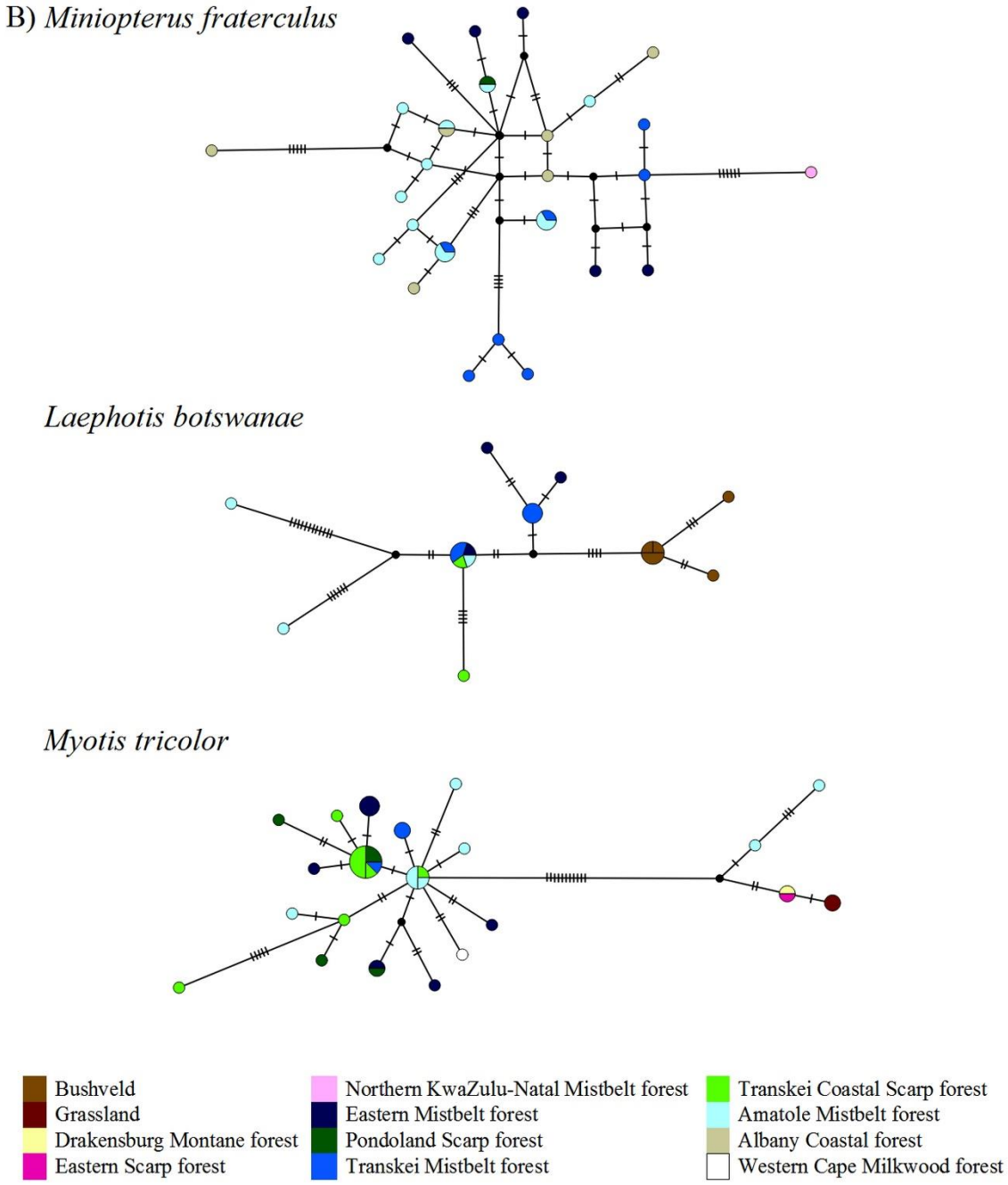


Figure 4.2 Continued.

Laephotis botswanae had the lowest haplotype diversity (0.89 ± 0.05) with a relatively low nucleotide diversity (0.00599 ± 0.00123) (Table 4.2). This species had the highest global F_{ST} value (0.428) with a comparatively high Φ_{ST} (0.559) (Table 4.2), but no significant pairwise Φ_{ST} values (Appendix S4.2). This is probably due to the low sample size. Witsand and Vaalwater localities (Limpopo) demonstrated the highest pairwise Φ_{ST} with localities of the Eastern Cape and KwaZulu-Natal provinces. This divergence was evident in the haplotype network by separation with a virtual haplotype and four mutations (Figure 4.2B). Haplotypes

of Gomo and Ngeli (straight line distance of ~50 km between localities) were divergent from other Eastern Cape localities that are ~100 km away. Private haplotypes from Fort Fordyce and Manubi were differentiated substantially from the shared central haplotype.

Myotis tricolor demonstrated high haplotype diversity (0.94 ± 0.03) and relatively low nucleotide diversity (0.00562 ± 0.00097). Relatively high global population structure was evident with F_{ST} (0.218) and Φ_{ST} (0.519) (Table 4.2), but there were only 7/36 significant (19%) pairwise Φ_{ST} values (range 0.12 – 0.85) (Appendix S4.2). Spatial genetic divergence was evident in the haplotype network; the northernmost localities (Blyderivierspoot, Hlatikulu and Eshowe) were one virtual haplotype and several mutational steps divergent from shared haplotypes (Figure 4.2B). Structuring between haplotypes of KwaZulu-Natal and Eastern Cape provinces was not evident, with the haplotype from De Hoop Guano Cave in the Western Cape (~600 km from the nearest sampling locality) having only two mutations difference from a central haplotype. However, as seen for *L. botswanae*, haplotypes from Manubi and Fort Fordyce were substantially divergent and accounted for four of the seven significant pairwise Φ_{ST} values.

4.4.3 Demographic history

Neutrality tests indicated *R. swinnyi* has undergone historical expansion with significant Fu's F_S (−17.000) and R_2 values (0.125) (Table 4.3). However, the mismatch distribution analysis uncovered a multimodal distribution (Appendix S4.3) indicative of stable population dynamics. The significant SSD value (0.02) suggests this species underwent a sudden expansion, and thus expansion time could not be calculated (Table 4.3). The BSP indicates a marked increase in population size occurring from ~30 kya and entering a plateau from ~5 kya (Figure 4.3A).

Neoromicia nana (*Afronycteris nana* as per Monadjem *et al*., 2020) depicted past population expansion (Fu's F_S −19.566) (Table 4.3). The mismatch distribution was multimodal, typical of populations experiencing demographic stability (Appendix S4.3). However, multimodal distributions may also result from populations that have undergone geographical structuring or have secondary contact (Liebers, Helbig and De Knijff, 2001). Accumulation of low-frequency mutations in the mismatch distribution is characteristic of non-equilibrium population dynamics. The sudden expansion model was not rejected (H_r 0.005; SSD 0.005), and population expansion was dated to 191 kya [95% confidence interval (CI): 98 – 270 kya]. The BSP shows a gradual increase over the approximate period of 150 – 5 kya, followed by a slight decline from 5 kya to the present (Figure 4.3A).

Table 4.3 Results of neutrality tests, mismatch distribution analysis and estimated time of population expansion. Statistical significance denoted as ** $p < 0.001$, * $p < 0.05$.

	Forest associated			Habitat generalists		
	R. swinnyi	*N. nana* (*Afronycteris nana* as per Monadjem *et al.*, 2020)	*P. hesperidus*	*M. fraterculus*	*L. botswanae*	*M. tricolor*
Fu and Li's *D**	-2.477	-1.338	-4.775*	-0.602	-2.319	-3.191*
Fu's F_s	-17.000**	-19.566**	-25.441**	-15.142**	-0.862	-9.645**
R_2	0.125**	0.081	0.035**	0.089	0.087*	0.050**
Raggedness index (H_r)	0.072	0.005	0.011	0.006	0.028	0.014
Sum of squared deviations (SSD)	0.020*	0.005	0.005	0.002	0.010	0.005
Mismatch distribution	Multimodal	Multimodal	Unimodal	Multimodal	Multimodal	Bimodal
τ (95% CI)	-	11.305 (5.803-16.027)	6.404 (2.873-9.797)	3.219 (1.969-14.688)	6.518 (2.654-10.725)	2.285 (1.639-11.219)
Expansion time (95% CI) (years)	-	~191,000 (97,977-270,598)	~109,000 (49,023-167,172)	~55,000 (33,7180-251,524)	~109,000 (44,536-179,973)	~39,000 (27,917-191,098)

Significant values of Fu and Li's D^* (−4.775), Fu's F_S (−25.441) and R_2 (0.035) indicated traces of both historical contraction and expansion for *P. hesperidus* (Table 4.3). The sudden expansion model was not rejected by the mismatch distribution analysis (H_r 0.011; SSD 0.005), which was unimodal (Appendix S4.3). The population expansion was estimated to have occurred around 109 kya (95% CI: 49 – 167 kya) (Table 4.3). A relatively constant population size from ~125 to 25 kya is evident from the BSP, followed by a substantial increase until ~5 kya. Population size then plateaus until the present (Figure 4.3A). Population contraction is supported by the low nucleotide diversity found for this species, although timing of contraction is unknown as it is not evident in the BSP.

Miniopterus fraterculus demonstrated demographic expansion by a significant negative Fu's F_S (−15.142) (Table 4.3), although the multimodal mismatch distribution inferred stable demographic dynamics (Appendix S4.3). The sudden expansion model was not rejected (H_r 0.0006; SSD 0.002), and the expansion was dated to ~55 kya (95% CI: 45 – 180 kya) (Table 4.3). A gradual population increase is shown on the BSP from 100 kya to ~5 – 10 kya, with a greater incline to the present (Figure 4.3B).

Historical population expansion was indicated for *L. botswanae* by a significant R_2 statistic (0.087) (Table 4.3). The mismatch distribution was multimodal, but the accumulation of low-frequency mutations was typical of a population in non-equilibrium dynamics (Appendix S4.3). Sudden population expansion could not be rejected as the raggedness index (0.028) and SSD (0.010) were not significant. Population expansion was estimated to have occurred ~109 kya (95% CI: 45 – 180 kya) (Table 4.3) with the BSP indicating a slow but steady increase in population size over the last 80 kya (Figure 4.3B).

All three selective neutrality test values were significant for *Myotis tricolor*, indicating both historical population contraction and expansion (Table 4.3). The bimodal mismatch distribution suggested expansions at different times (Appendix S4.3). An expansion was estimated to have occurred ~39 kya (95% CI: 28 – 191 kya) (Table 4.3). A gradual population increase, occurring from ~80 to 20 kya, is evident in the BSP, followed by a more constant population size to the present (Figure 4.3B). Population contraction found with the neutrality tests was supported by the low nucleotide diversity of this species but was not evident in the BSPs, and thus the time of contraction is unknown.

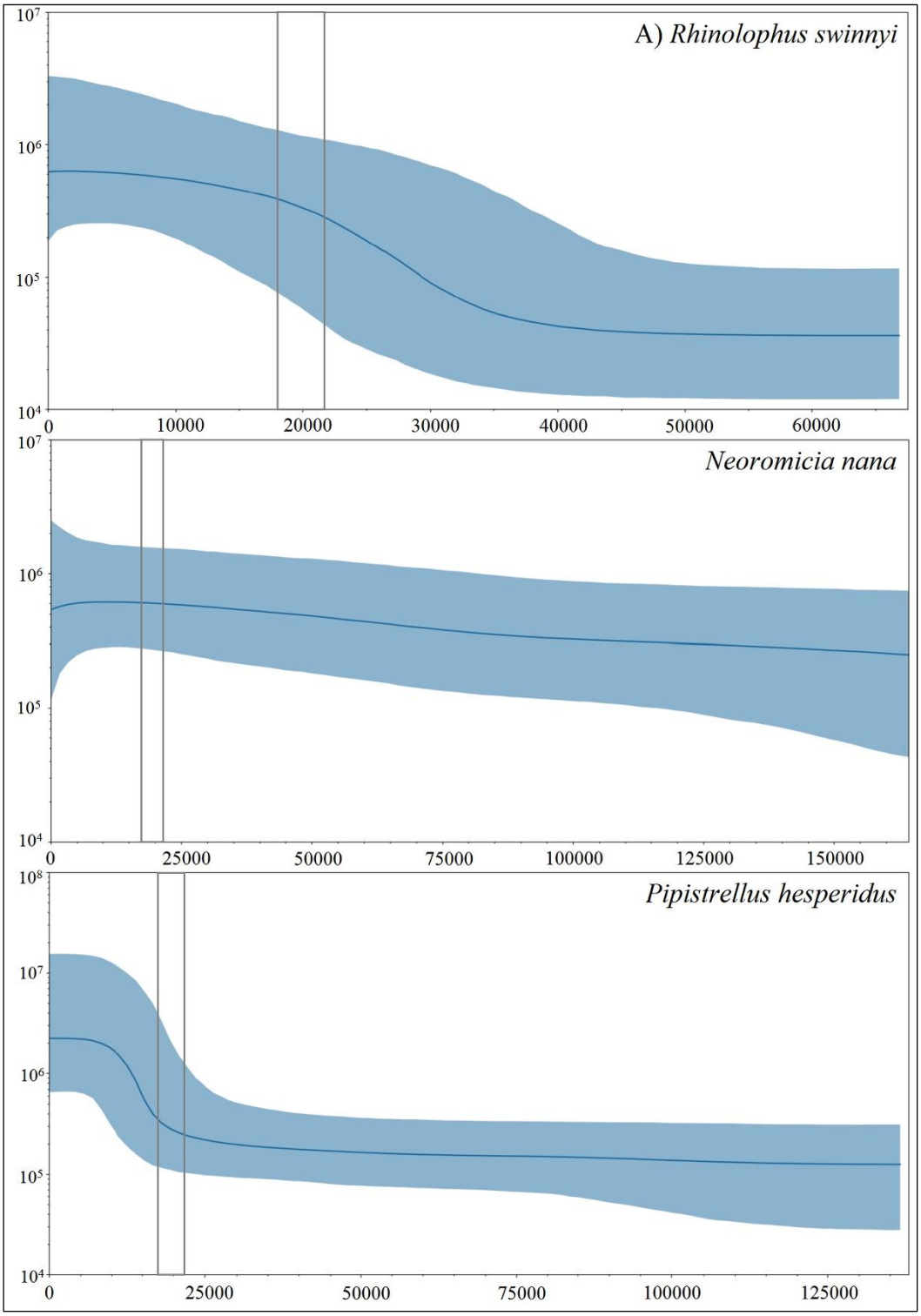


Figure 4.3 Bayesian skyline plots of (A) forest associated species and (B) habitat generalist species. The estimated effective population size (Nef) is displayed on the *y*-axis and time before present in years on the *x*-axis. Time scale on the *x*-axis varies between species as it represents coalescence time from the most recent common ancestor. The median estimate is shown (solid line) with 95% posterior density intervals (shaded area). The grey box depicts the approximate time of the LGM.

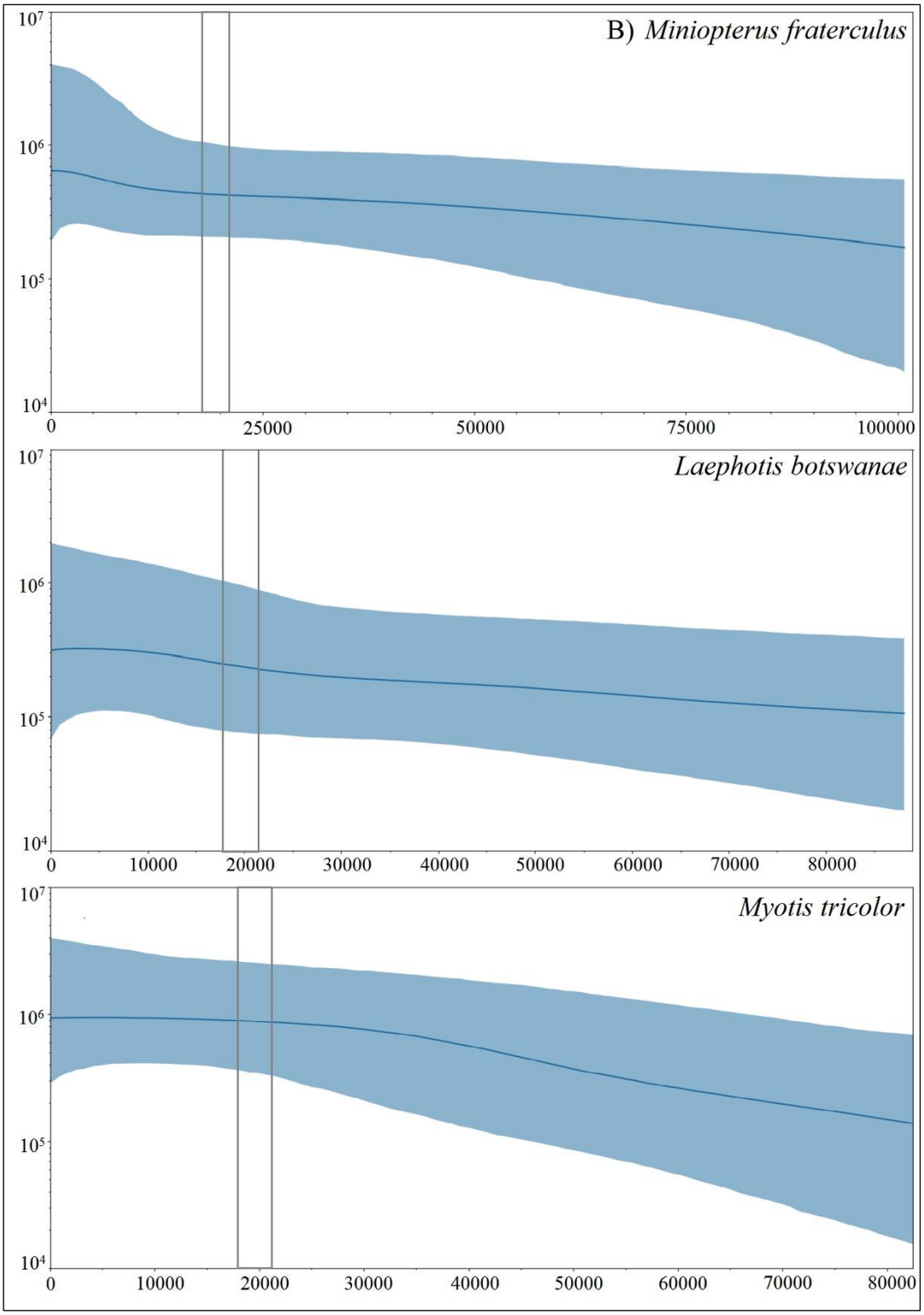


Figure 4.3 Continued.

4.5 Discussion

4.5.1 Gene flow independent of forest utilization

Contrary to our expectations based on forest habitat associations, the genetic diversity and scale of population structure were not concordant across ecologically similar species. Instead, all species, except *Miniopterus fraterculus*, displayed notable genetic structuring along eastern South Africa. Rather than habitat utilisation, genetic structure appears to relate to species-specific behaviours, and to ecological traits such as roosting requirements, philopatry to the natal range and dispersal ability. This highlights the need to consider life history characteristics when comparing genetic diversity and structure across taxa, and the utility of comparative studies to elucidate species-specific population trends.

Rhinolophus swinnyi s.s. has a narrow distribution limited to the Eastern Cape and KwaZulu-Natal provinces of South Africa (Taylor *et al.*, 2018). Despite the species' limited distribution, population structuring was evident within its range. Genetic structuring across the range is common in the genus *Rhinolophus* (Chen *et al.*, 2006; Chakravarty *et al.*, 2018; Budinski *et al.*, 2019). *Rhinolophus clivosus* showed genetic differentiation corresponding to South Africa's ecoregions (Stoffberg, Schoeman and Matthee, 2012). The population differentiation of *R. swinnyi* may result from its dependence on two naturally disjunct resources: forests for foraging (Bronner, 1990) and suitable caves for roosting (Cotterill, 1996). Its wing morphology suggests a comparatively low dispersal ability that may be limiting movement between suitable sites geographically separated by large distances.

Both *N. nana* and *P. hesperidus* displayed genetic structuring across the study region, and between proximal neighbouring localities (Figure 4.2A). *Neoromicia nana* roost in furled leaves of *Strelitzia* sp. and banana plants in KwaZulu-Natal and Eastern Cape provinces. The roosting resource is ubiquitous but ephemeral, requiring bats to locate new suitable leaves frequently. Although this species regularly moves between suitable plants, males show fidelity to a clump of plants within a small area (Happold and Happold, 1996): it is a sedentary species that persists within a small home range of ~300 m (Laval and Laval, 1977). The maintenance of a small home range and resulting philopatry to the natal area may result in the population structuring evident for *N. nana*. The mating system of *P. hesperidus* is unknown (Monadjem, Taylor, *et al.*, 2010), but other *Pipistrellus* species demonstrate a mating system based on female resource polygyny (Gerell and Lundberg, 1985). Males establish courting territories along the females' flight routes (Barak and Yom-Tov, 1991), suggesting females remain in

natal areas and that gene dispersal is male-biased, which may inform the population structuring observed using maternally inherited mitochondrial markers.

Myotis tricolor showed population structuring with strong haplotypic segregation between Mpumalanga and northern KwaZulu-Natal, and between southern KwaZulu-Natal and Eastern Cape (Figure 4.2B). This species undertakes seasonal migrations between winter hibernacula and summer maternity caves (McDonald, Rautenbach and Nel, 1990), but the population structuring observed infers females may be philopatric to natal caves demonstrating limited gene flow during migratory events. Additionally, a phylogenetic study of Afrotropical *Myotis* species partitioned South African *Myotis tricolor* mtDNA sequences into two clades (Patterson *et al.*, 2019). This subdivision of the species within the country may relate to the population structuring reported here. Strong population structuring of northern localities from southern localities was also evident for *L. botswanae*. As this species is difficult to capture or may be naturally rare (Taylor *et al.*, 2016), and is poorly represented in museums (Monadjem, Taylor, *et al.*, 2010), the results presented here are based on a low sample size (N = 19) and should be interpreted with caution. *Laephotis botswanae* is a slow-flying species that typically prefers vegetation clutter for cover during flight: a light-tagged individual did not fly above trees or over open water (Kearney, 2013). As the interior regions of Gauteng and northern Free State comprise mostly grassland-dominated vegetation types (Mucina and Geldenhuys, 2006), the predominantly open vegetation may pose a barrier to a species with low dispersal capabilities.

However, the roosting and mating behaviour of *L. botswanae* is currently poorly documented (Monadjem, Taylor, *et al.*, 2010; Kearney, 2013) but could better inform the observed population differentiation. The population structure of the vespertilionid species (*N. nana*, *P. hesperidus* and *Myotis tricolor*) has been mostly attributed to philopatry, which is a common strategy in this family (Burland and Wilmer, 2001; Rivers, Butlin and Altringham, 2005; Santos *et al.*, 2016). Additionally, the vespertilionid species showed equally high Φ_{ST} values (Table 4.2), whereas values for the Rhinolophidae and Miniopteridae representatives were relatively lower. The similarities in population structuring for the vespertilionid species may have been shaped by their shared evolutionary history.

Miniopterus fraterculus displayed high genetic diversity and high gene flow between localities, as was anticipated for this generalist species, and indicates a stable, continuous population. However, the congeneric *Miniopterus natalensis*, despite being migratory, exhibited strong population substructure within South Africa, which was attributed to philopatry in both sexes.

Furthermore, the subpopulations correlated with the major biomes of the country (Miller-Butterworth, Jacobs and Harley, 2003). Perhaps population structuring was not evident for *Miniopterus fraterculus* as only a portion of its range was represented here, which corresponds to the range of only one subpopulation (and only one biome) that Miller-Butterworth, Jacobs and Harley (2003) identified for *Miniopterus natalensis*. Inclusion of samples across its complete range could reveal a different trend.

4.5.2 South-east region of South Africa as a refuge during the LGM

The mismatch distributions (Table 4.3) and BSPs (Figure 4.3) revealed population expansions at varying times of the Tarantian stage of the Pleistocene (0.0117 – 0.1260 Mya) and Holocene (0 – 0.0017 Mya) (Gibbard *et al.*, 2010), with no apparent contraction at the time of the LGM. The dated expansions mostly correspond with the latter periods of interglacial cycles (Bintanja, Wal and Oerlemans, 2005) which consisted of warmer and wetter climates (Castañeda *et al.*, 2016). Conditions of moderately warm waters in the Indian Ocean (Simon *et al.*, 2015) and high humidity along the KwaZulu-Natal coast (Dupont *et al.*, 2011) would have facilitated the prevalence of forest along the east coast of South Africa. Pollen assemblages have shown that evergreen and deciduous forests were the predominant vegetation type in the region during interglacial periods (Dupont *et al.*, 2011). The interglacial climate and subsequent forest growth may have then facilitated population expansions of the study species.

Again, contrary to expectations, neither forest associated, nor generalist species appeared to experience population declines associated with the LGM (Figure 4.3). South African forests underwent oscillations in coverage extent due to climate change (Eeley, Lawes and Piper, 1999), but the African continent experienced less dramatic climate change than the rest of the world during the Late Quaternary, with fewer extinctions of small mammal species (Nogués-Bravo *et al.*, 2010). Vegetation shifts during glacial-interglacial periods were less pronounced in subtropical south-east Africa than further north at Lake Malawi, which indicated a more stable climate in southern Africa than in tropical Africa (Castañeda *et al.*, 2016). Models of forest coverage indicate three African refugia that persisted during the LGM: the Cameroon Highlands, Congo Basin, and south-east South Africa. Furthermore, high mammal and bird species richness was maintained in the south-east part of South Africa during the LGM (Levinsky *et al.*, 2013). It appears the lower climate footprint of Africa and climate stability, particularly in southern Africa, maintained forest coverage, and the refugia of eastern South Africa prevented large-scale population declines of the study species during the LGM.

Three of the study species even depicted population expansions during the LGM. A global study of bat responses to past climate change found larger nectivores and frugivores were more likely to have experienced bottlenecks during the LGM, and that some temperate insectivores underwent expansions during this time (Carstens *et al.*, 2018). They attributed these patterns to differences in dietary niche (Carstens *et al.*, 2018). Evidence suggests African fruit bats were impacted largely by climate-induced oscillations of forest coverage: Myonycterini bats diversified during periods of decreased tree cover (Nesi *et al.*, 2013); speciation of Scotonycterini bats was caused by isolation in forest refugia in western and central Africa (Hassanin *et al.*, 2015); and *Rousettus* bats showed population expansions in response to forest expansions (Stribna *et al.*, 2019). Insectivorous species may have been at an advantage during climatic fluctuations given that the three study species, as well as the little free-tailed bat (*Chaerephon pumilus*), underwent expansions in South Africa coincident with the LGM (Taylor *et al.*, 2009). Interestingly, two insectivorous Eastern Cape forest birds, Cape batis (*Batis capensis*) and yellow-throated woodland-warbler (*Phylloscopus ruficapilla*), also experienced population growth over the LGM (J. Mulvaney, unpublished data). It appears volant insectivores may have experienced advantageous conditions during the LGM, at least in eastern South Africa.

Mistbelt forests are biogeographically ancient and were substantially reduced during the LGM (Eeley, Lawes and Piper, 1999). Climatic extinction filtering was more severe for mistbelt forests; forest-specialist fauna persisted in refugia in the scarp belt during the LGM as the scarp forests were buffered from climate extremes by their proximity to the coast (Lawes *et al.*, 2007). Mistbelt and younger coastal forests (established following the LGM at 8 kya) were subsequently re/colonised from scarp refugia (Lawes *et al.*, 2007). The haplotype networks of *P. hesperidus*, *L. botswanae* and *Myotis tricolor* (Figure 4.2) depict a historical persistence in mistbelt and scarp forests as their haplotypes occupy shared and central positions in the networks, depicting them as the most ancestral of the sampled haplotypes. Haplotypes of coastal forests depict a more recent ancestry given their external positions in the networks of *N. nana* and *P. hesperidus*. The study species show traces of historical of ancient mistbelt and scarp forests with more recent occurrence in coastal forests. This supports Lawes *et al.*'s (2007) hypothesis that scarp forests served as refugia during the LGM with subsequent colonisation of younger coastal forests.

4.5.3 Egossa Interval of the Pondoland Centre

The Pondoland Centre is an epicentre of botanical diversity associated with the Msikaba Formation, a narrow wedge of sandstone extending from the Umzimkulu River in southern KwaZulu-Natal to the Egossa Fault (just north of Mbotyi State Forest) in the Eastern Cape (Van Wyk, 1990). The Pondoland Centre is unique in that the sandstone deposits host botanical elements of the present-day Cape Floral Kingdom (Van Wyk, 1990). Smaller outcrops of sandstone occur at Port St Johns and Uvongo. A 30 km wide interval of mainly Karoo sediments occurs between the Egossa fault and Port St Johns, referred to as the Egossa interval (Van Wyk, 1990). The haplotype network of *N. nana* (*Afronycteris nana* as per Monadjem *et al*., 2020) revealed a central haplogroup from Mbotyi, Oribi Gorge and Eshowe, and a separate haplogroup from Silaka and Hluleka (Figure 4.2A). The sequence from Mbotyi clustered more closely with samples from localities situated at least 100 km north than with samples collected from Silaka, ~20 km south; the Egossa Interval occupies the gap between Mbotyi and Silaka. This gap has been found to cause genetic subdivision for a variety of other taxa with widely varying dispersal abilities: the Pondoland cannibal snail (*Natalina beyrichi*) (Moussalli, Herbert and Stuart-Fox, 2009), Pondo flat-gecko (*Afroedura pondolia*) (Busschau, Conradie and Daniels, 2019) and the bush squeaker frog (*Arthroleptis wahlbergii*) (Kushata, 2018).

4.6 Conclusion

Trends relating to both gene flow and demographic history of six bat species did not reflect forest habitat utilization. Five of the six species showed population structuring across the region. Interspecific differences in population structuring may be interpreted in the context of species-specific traits of roosting requirements, philopatry to the natal range and dispersal ability. All study species revealed population expansions during the Tarantian stage of the Pleistocene and the Holocene, without contraction at the time of the LGM. It is likely that the lower climate footprint of Africa, combined with relative climate stability in southern Africa and the forest refugia of eastern South Africa in particular, prevented large-scale population declines during the LGM. It appears volant insectivores may have experienced advantageous conditions during the LGM, at least in this region.

Appendix S4.1 Museum samples and GenBank sequence numbers employed (DM – Durban Natural Science Museum, NMB – National Museum of Bloemfontein).

Species	Locality	Museum number	GenBank number
Laephotis botswanae	Lapalala Wilderness Area		EU797431.1
	Vaalwater		EU797432.1 - 797433.1
	Witsand Dam		EU797430.1, 797444.1, 797445.1
Miniopterus fraterculus	Pietermaritzburg	DM14046	
Myotis tricolor	Blyderivierspoot Nature Reserve		AJ504409.1, AJ841952.1
	De Hoop Guano Cave		AJ841953.1
	Eshowe	DM14295	
	Hlatikulu Forest Reserve	DM14329	
Neoromicia nana	Durban	DM14964	
	Eshowe	DM14300	
	Hlane Nature Reserve	DM8425	
	Maguga	NMB11560 - 11562	
	Mlawula Nature Reserve	DM8427	
	Ngonini Estates	DM13465	
	Sodwana Bay National Park	DM14564	
	Vryheid Hill Nature Reserve	DM13013	
Pipistrellus hesperidus	Durban	DM14243	
	Eshowe	DM14301	
	Feather River		KM886097.1
	Hlatikulu Forest Reserve	DM14330	
	Knysna		AJ841968.1
	Soutpansberg	DM13539, DM13582	
Rhinolophus swinnyi	Eshowe	DM14292	
	Kokstad		KU531370.1, KU531391
	Pietermaritzburg	DM14036	

Appendix S4.2 Pairwise population Φ_{ST} for each species. Significant values are highlighted in bold (p <0.05).

Rhinolophus swinnyi

	Mbotyi	Kokstad	Ngeli	Baziya	Gomo	Sandile's Cave	Fort Fordyce
Kokstad	0.99529						
Ngeli	0.72805	0.47979					
Baziya	**0.67923**	0.98896	0.10244				
Gomo	0.70847	0.66724	-0.36188	-0.93807			
Sandile's Cave	**0.7923**	0.49403	**0.29854**	0.14658	0.20829		
Fort Fordyce	**0.88932**	**0.80027**	**0.38378**	**0.23401**	0.60743	-0.04449	
Kologha	0.8018	0.64773	0.14257	-0.00632	0.34682	-0.06708	-0.04804

Neoromicia nana (*Afronycteris nana* as per Monadjem *et al*., 2020)

	Cape Morgan	Maguga	Dwesa	Hluleka	Manubi	Oribi Gorge
Maguga	0.5942					
Dwesa	0.25506	0.5082				
Hluleka	0.22053	**0.437**	-0.03448			
Manubi	-0.12821	0.86667	0.69585	**0.41418**		
Oribi Gorge	0.49013	0.61905	0.06557	0.08245	0.77778	
Silaka	**0.3434**	**0.75806**	**0.47842**	0.04639	**0.72477**	**0.62793**

Pipistrellus hesperidus

	Alexandria	Baziya	Cape Morgan	Dwesa	Fort Fordyce	Gomo	Manubi	Mbotyi	Mkambati	Ngeli	Oribi Gorge	Silaka	Umtamvuna
Baziya	**0.82472**												
Cape Morgan	**0.38546**	**0.72652**											
Dwesa	**0.486**	0.28622	0.18295										
Fort Fordyce	0.04858	**0.92106**	**0.54502**	**0.62048**									
Gomo	**0.80639**	0.08599	**0.70752**	0.24972	**0.90455**								
Manubi	**0.68797**	**0.79232**	0.16912	**0.28699**	**0.81336**	**0.77601**							
Mbotyi	**0.487**	0.25087	**0.35221**	-0.10251	**0.63575**	0.17818	**0.51931**						
Mkambati	0.08293	**0.73848**	0.1944	0.25483	0.34247	**0.71033**	**0.54903**	0.24785					
Ngeli	-0.02176	**0.7391**	0.2295	**0.3159**	0.18644	**0.71589**	**0.56366**	**0.30529**	-0.08421				
Oribi Gorge	**0.35451**	**0.69371**	0.2663	0.2736	**0.53703**	**0.67139**	**0.51263**	0.25564	-0.18759	0.15604			
Silaka	0.25598	0.24027	0.25855	-0.04787	0.39753	0.2068	**0.46699**	-0.13531	0.06459	0.12998	0.18061		
Umtamvuna	0.35718	0.2629	0.25652	-0.20501	**0.61814**	0.20467	0.509	-0.30832	0.03681	0.11775	0.14553	-0.41784	
Xumeni	0.17148	**0.61317**	0.0526	0.17024	**0.29329**	**0.59409**	**0.31787**	**0.21444**	-0.0439	-0.01949	0.03948	0.12704	0.01976

Miniopterus fraterculus

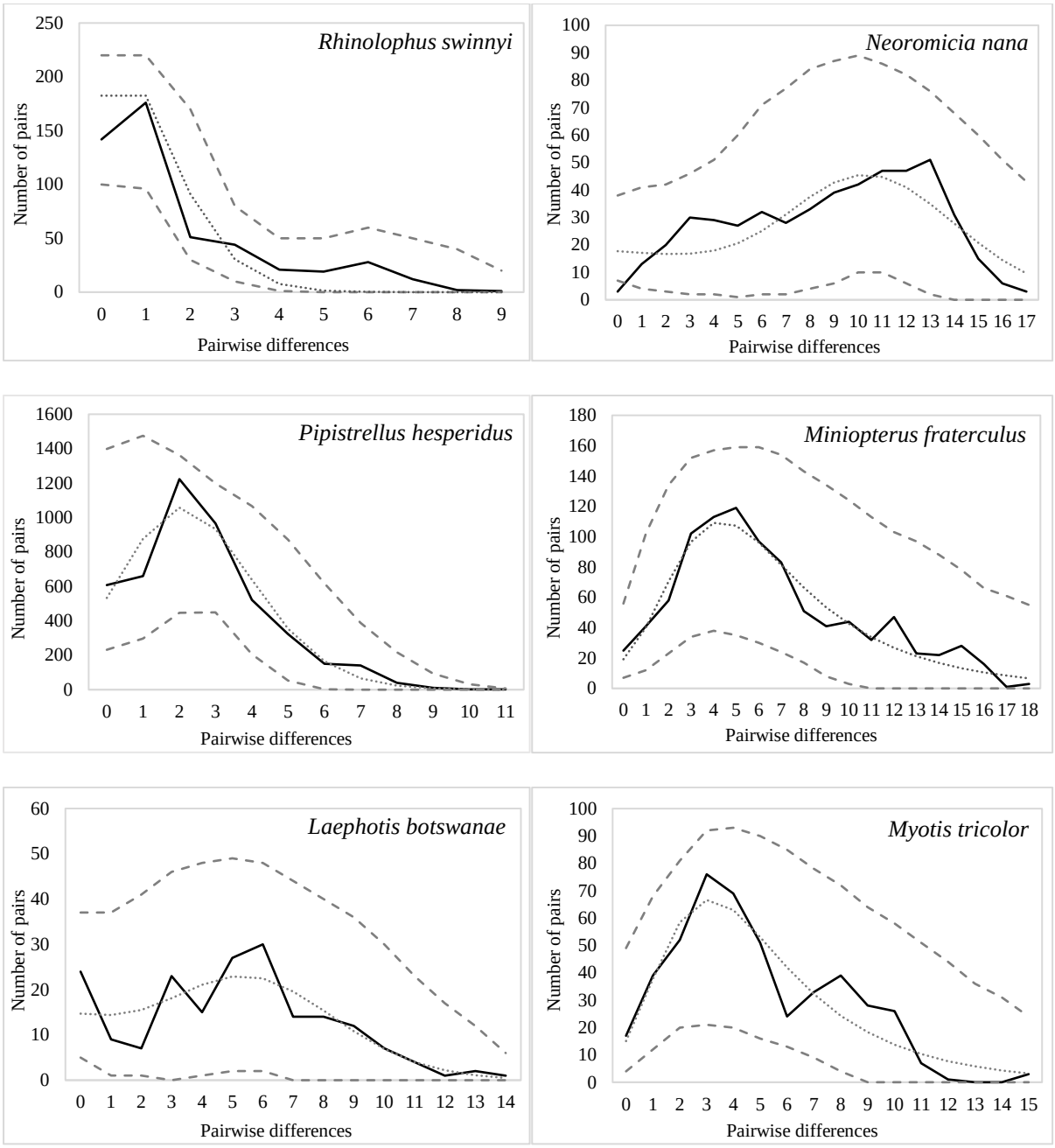
	Gomo	The Island	Oribi Gorge	Xumeni	Kologha	Fort Fordyce
The Island	**0.3468**					
Oribi Gorge	0.18676	-0.01852				
Xumeni	**0.39408**	**0.16068**	-0.19677			
Kologha	**0.31988**	0.03281	0.07001	**0.25026**		
Fort Fordyce	**0.42323**	0.11365	0.1697	**0.30149**	-0.01495	
Baziya	0.219	0.11433	-0.12743	0.23343	-0.18279	-0.15817

Laephotis botswanae

	Baziya	Fort Fordyce	Gomo	Manubi	Xumeni	Witsand Dam
Fort Fordyce	-0.10482					
Gomo	1	0.37032				
Manubi	0	-0.00567	0.72906			
Xumeni	0	0.01516	0.23111	0.02828		
Witsand Dam	0.72831	0.49394	0.757	0.65867	0.5403	
Vaalwater	1	0.39811	1	0.76287	0.62211	-0.21856

Myotis tricolor

	Blyderivier-spoot	Baziya	Fort Fordyce	Sandile's Cave	Manubi	Mbotyi	Ngeli	Oribi Gorge
Baziya	0.95488							
Fort Fordyce	0.27365	0.21324						
Sandile's Cave	0.94121	0.19379	0.24145					
Manubi	0.8514	0.26889	0.18001	0.25575				
Mbotyi	1	0.36842	**0.12006**	0.4129	0.29131			
Ngeli	**0.85935**	0.0527	0.36481	0.10531	**0.28993**	-0.16513		
Oribi Gorge	0.82241	-0.00304	**0.16263**	0.03222	-0.03745	-0.10806	0.00762	
Silaka	0.98404	0.51555	0.39511	**0.55205**	**0.54538**	-0.29385	0.08358	**0.2453**



Appendix S4.3 Mismatch distributions for each species, with observed values (solid line), modelled values (dotted line), and 95% confidence intervals (dashed lines).

Chapter 5. Fragmentation does not affect gene flow in forest populations of the dusky pipistrelle bat on the eastern seaboard of South Africa

This chapter is in review with Journal of Mammalogy. Feedback from two rounds of journal review has been implemented and the manuscript is awaiting final decision from the Editor-in-Chief.

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5.1 Abstract

The Eastern Cape province harbors almost half of the indigenous forest in South Africa, but these forests are threatened by large-scale agricultural and urban development planned for the coming decade. Additional anthropic development is likely to cause further fragmentation and degradation of forests inhabited by the dusky pipistrelle bat (*Pipistrellus hesperidus*). We utilised eight microsatellite markers to study the genetic diversity, population structure and migration of *P. hesperidus* (N = 120) across 14 sites in the Eastern Cape and KwaZulu-Natal provinces of South Africa. We examined the effect of contemporary land cover types on genetic differentiation to assess whether current levels of urbanisation and agricultural development affect gene flow. High gene flow and low population structure were evident across sampled sites, apart from genetic discontinuities at the northern (Oribi Gorge Nature Reserve) and southern (Alexandria Forest) ends of the seaboard. Genetic discontinuity at Oribi Gorge may relate to anthropogenic modification of two rivers surrounding the forest, while the Alexandria-linked barrier is a climatic break known as the Bedford gap. Migration rates were generally low between sites except for one scarp forest, Manubi State Forest, from which individuals dispersed to other sites. The Amatole Mistbelt forests supported high genetic diversity, and likely served as a refugium for *P. hesperidus* during the Last Glacial Maximum. The composition of land cover classes between sites was a poor predictor of genetic differentiation, although it seems likely that *P. hesperidus* uses riparian habitats and wetlands for dispersal. Lastly, urban and agricultural development did not have a significant effect on genetic differentiation, which may reflect the wide niche breadth and intermediate distribution range of the species. This study provides the first insights into genetic diversity and gene flow of *P. hesperidus* across the study region prior to agricultural intensification and large-scale urbanisation.

5.2 Introduction

South Africa's indigenous forests form the smallest biome in the country, covering less than one percent of the land surface (Mucina and Geldenhuys, 2006), but supporting the highest diversity (~14%) of threatened terrestrial vertebrates of all biomes (Castley, 1997). Most forests are smaller than 100 ha in size and often occur as small fragmented patches (<10 ha) (Mucina and Geldenhuys, 2006). They are distributed along the eastern and southern seaboard and on south facing slopes of the Great Escarpment (von Maltitz *et al.*, 2003; Berliner, 2009), with the Eastern Cape and KwaZulu-Natal provinces holding the largest proportions of indigenous forest (46% and 29% respectively) in the country. Of all the provinces, the Eastern Cape has the lowest proportion of forest (4.75%) under strict Type 1 protection (Berliner, 2009), as determined by the National Environmental Management Protected Areas Act (NEMPAA) (SANBI, 2018). These forests are located within the Maputaland-Pondoland-Albany global biodiversity hotspot but also within the poorest rural areas of the country (Berliner, 2009). In order to alleviate poverty and empower local communities, the Eastern Cape Provincial Government has compiled a development plan for completion by 2030 (Eastern Cape Planning Commission, 2014). The development plan seeks to grow the economic potential of rural areas by transforming small towns into industrial hubs, expanding and intensifying agricultural production, and increasing built infrastructure (Eastern Cape Planning Commission, 2014). Future land use change, in the form of urbanisation and agricultural intensification, albeit necessary for economic development, poses a threat to South African biodiversity through habitat destruction and degradation (Bomhard *et al.*, 2005; O'Connor and Kuyler, 2009).

Bats are important faunal components of ecosystems as they constitute the second largest order of mammalian species and comprise many functional guilds (Wilson and Reeder 2005). Bats provide several vital ecological services: frugivores maintain and regenerate forest by dispersing seeds over large distances, nectivorous bats perform plant pollination services, and insectivores are crucial for the suppression of insect pest populations (Kunz *et al.*, 2011). Additionally, bats are useful bioindicators that show sensitivity to habitat loss, fragmentation, and agricultural intensification (Jones *et al.*, 2009). The highest bat species richness in southern Africa extends along the eastern seaboard of south-east South Africa, encompassing the Afromontane and coastal forest mosaic, northwards along the coast of Mozambique (Cooper-Bohannon *et al.*, 2016). Considering both the prospective development for the Eastern Cape and the high bat diversity associated within its forested region, it is important to assess the

possible impacts anthropic development may have on bats in this area.

Understory insectivorous and carnivorous bats, with a conservation status of Least Concern, are sensitive to human induced disturbances in Neotropic forests (García-Morales, Badano and Moreno, 2013). Smith *et al.* (2016) found clutter and clutter-edge functional groups (species that fly within and on the edge of dense/cluttered vegetation), were most impacted by anthropogenic development. Additionally, tree-roosting insectivores are more susceptible to genetic erosion from forest fragmentation than cave-roosting species (Rossiter *et al.*, 2012; Meyer, Struebig and Willig, 2016). *Pipistrellus hesperidus* (Chiroptera: Vespertilionidae), known as the dusky or African pipistrelle (Monadjem, Schoeman, *et al.*, 2010; Monadjem, Jacobs, *et al.*, 2016), was selected for this study as it is a clutter-edge insectivore, occurring in the understory of forests in the eastern region of southern Africa (Monadjem, Taylor, *et al.*, 2010). It is listed as Least Concern conservation status at the regional and global scale (Monadjem, Jacobs, *et al.*, 2016; Piraccini, 2016). Although the roosting habits of *P. hesperidus* are not well known, it has been found roosting beneath loose bark of dead trees (Smithers, 1971). Thus, it provides a good model system to investigate the impact of future land use in the study region.

Habitat composition of a landscape affects dispersal and resultant population structuring of a species. Gene flow through a landscape, and spatial population differentiation, may be affected by anthropogenic changes to a region and by the configuration of suitable and unsuitable habitats (Manel *et al.*, 2003). The relationship between landscape variables and genetic differentiation can be used to infer which landscape components enable or inhibit gene flow (Storfer *et al.*, 2010). *Pipistrellus hesperidus* is restricted to habitats with woody vegetation (Monadjem, Taylor, *et al.*, 2010), utilising savanna woodlands, and Afromontane and coastal forests (Cooper-Bohannon *et al.*, 2016). As forests are highly fragmented in South Africa (von Maltitz *et al.*, 2003), it is likely that there is limited habitat connectivity which may impede gene flow of *P. hesperidus* across the region. Also, agricultural intensification is known to alter bat communities (Cleary, Waits and Finegan, 2016) and is thought to have been the cause of severe population declines for many bat species in Europe (Wickramasinghe *et al.*, 2003). Although urbanisation is generally viewed as the most ecologically damaging change to land use (Jung and Threlfall, 2016), species of the Vespertilionidae family are generally considered to be urban adapters (Jung and Kalko, 2011), with some pipistrelles being highly successful in urban areas (Gehrt and Chelsvig, 2004; Ancillotto, Tomassini and Russo, 2016; Schoeman, 2016).

Pipistrellus hesperidus is a small bat of approximately 6 g with short, broad wings and low wing loading (Monadjem, Taylor, *et al.*, 2010). Short and broad wings allow for maneuvrability within dense vegetation habitats but are typically not well adapted for long distance flights (Norberg and Rayner, 1987). Bats with this wing morphology and associated flight capabilities are considered to have a reduced dispersal potential, typically resulting in lower genetic diversity and greater genetic structuring across their ranges (Burns and Broders, 2014). Bat species exhibiting this wing morphology and high population structuring are more vulnerable to habitat fragmentation and localised extinction (Safi and Kerth, 2004; Burns and Broders, 2014). Little is known of the abundance and current population trends of *P. hesperidus* (Monadjem, Jacobs, *et al.*, 2016; Piraccini, 2016). We aimed to study the genetic diversity and population structuring of *P. hesperidus* across the Eastern Cape and KwaZulu-Natal provinces of South Africa, as it is characterised by an inferred reduced dispersal ability, is dependent on fragmented wooded habitats, and is facing a changing landscape. We investigated the presence of possible genetic barriers and whether contemporary migration is occurring across the region to identify and conserve areas hosting high diversity and facilitating gene flow. Lastly, we examined which contemporary land cover types are permeable to gene flow to evaluate the functional connectivity of the landscape for *P. hesperidus* and assessed whether current levels of urbanisation and agricultural development impact gene flow.

5.3 Materials and Methods

5.3.1 Study area

We utilised samples from 14 sites spanning across the KwaZulu-Natal and Eastern Cape provinces of South Africa (Figure 5.1, Table 5.1). The forests are classified into eight types belonging to three groups (von Maltitz *et al.*, 2003). Samples were used from six mistbelt forests (Table 5.1) of the Southern Mistbelt group. We surveyed six forests of the Scarp group, classed as Pondoland Scarp and Transkei Coastal Scarp types, and two forests of the Southern Coastal group. Sample sizes from individual sites were low. We pooled samples for analyses based on forest type as they are broadly similar in geographic location and habitat structure.

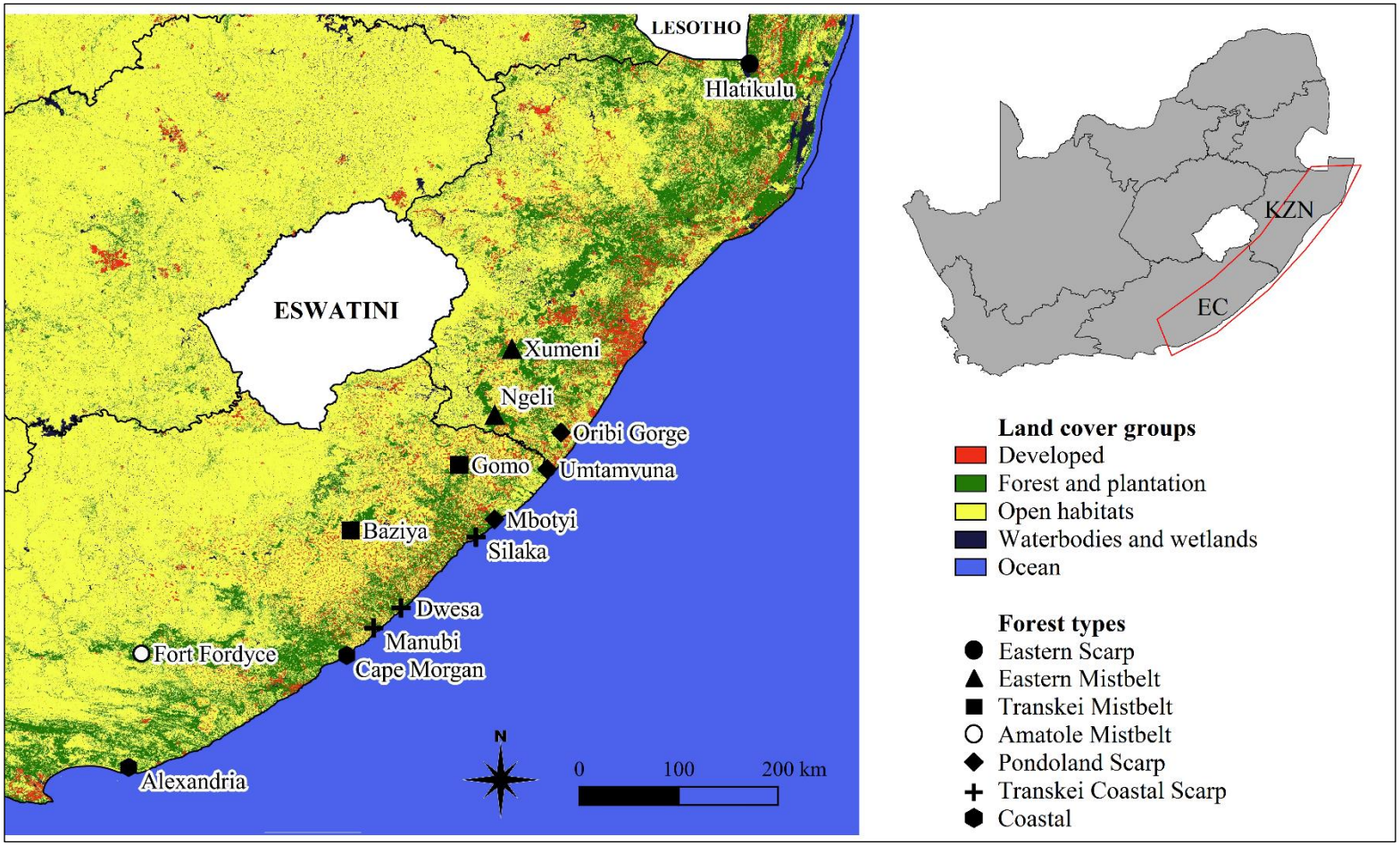


Figure 5.1 Sampling sites of *Pipistrellus hesperidus* (study region depicted in red on insert map of South Africa) in KwaZulu-Natal (KZN) and Eastern Cape provinces (EC) of South Africa. The colour key indicates groupings of land cover types used in analyses. The key of symbols displays classifications of forest type as per von Maltitz *et al*. (2003). The white areas within the map represent the countries of Eswatini and Lesotho (for which land cover data were not used). Only a small portion of land cover at the northernmost edge of the study area was excluded as a result.

Table 5.1 List of study sites, grouped by forest type according to the classification of von Maltitz *et al*. (2003), with the sample sizes, and genetic diversity summary statistics of *Pipistrellus hesperidus*. NR denotes localities that are protected Nature Reserves and SF for unfenced State Forests.

Forest type	Study forest	Co-ordinates	Sample size	Observed heterozygosity (H_O)	Unbiased expected heterozygosity (uH_E)	Allelic richness (AR)	Private alleles (P_A)
Eastern Scarp	Hlatikulu NR	27.324°S 31.989°E	4	0.562	0.652	2.596	1
Eastern Mistbelt	Xumeni SF	29.926°S 29.882°E	10	0.65	0.669	2.668	1
	Ngeli SF	30.542°S 26.681°E	9	0.597	0.677	2.671	1
Transkei Mistbelt	Gomo SF	31.011°S 29.349°E	13	0.599	0.713	2.815	2
	Baziya SF	31.575°S 28.407°E	10	0.638	0.715	2.808	1
Amatole Mistbelt	Fort Fordyce NR	32.684°S 26.490°E	11	0.727	0.712	2.833	3
Pondoland Scarp	Oribi Gorge NR	30.706°S 30.269°E	4	0.594	0.612	2.527	0
	Umtamvuna NR	31.065°S 30.177°E	5	0.625	0.708	2.804	0
	Mbotyi SF	31.446°S 29.737°E	10	0.64	0.713	2.828	2
Transkei Coastal	Dwesa NR	32.264°S 28.858°E	4	0.562	0.679	2.855	0
Scarp	Manubi SF	32.445°S 28.607°E	12	0.599	0.701	2.779	1
	Silaka NR	31.651°S 29.505°E	15	0.583	0.671	2.671	3
Coastal	Cape Morgan NR	32.705°S 25.355°E	8	0.623	0.673	2.709	0
Coastal	Alexandria NR	33.717°S 26.383°E	5	0.600	0.645	2.555	0
Mean/Total			120	0.614	0.678	2.728	15

5.3.2 Sample collection

Sampling was conducted from August 2017 to December 2018. Mist nets (14-mm mesh size; Ecotone, Sopot, Poland) and three-bank harp traps (Faunatech, Victoria, Australia) were used to sample 116 *P. hesperidus* individuals in study forests (Figure 5.1, Table 5.1). A 2-mm wing punch was collected from each individual and stored in 96% ethanol, after which bats were released at site of capture. The capture and handling of bats was done in accordance with the 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education (Sikes and The Animal Care and Use Committee of the American Society of Mammalogists 2016). The study was approved by the Stellenbosch University Animal Use and Care Research Ethics Committee (protocol #0409) and licensed by Eastern Cape Parks and Tourism Agency (RA0237, CRO 59/17CR, CRO 60/17CR), the Department of Agriculture, Forestry and Fisheries (WIFM 04-2016, WIFM 09-2017, WIFM 06-2018), Ezemvelo KZN Wildlife (OP 143/2018, OP 3847/2018) and South African National Parks (CHER-MI/2018-004). Supplementary tissue samples were sourced from the Durban Natural Science Museum.

5.3.3 DNA extraction, PCR, and genotyping

Total genomic DNA was extracted from wing punches and museum tissues using a Nucleospin® Tissue kit (Macherey-Nagel, Germany). Microsatellite loci are versatile among bat species (Burland *et al.*, 1999) and cross-species amplification from libraries developed for Vespertilionidae has been successful for other *Pipistrellus* species (Kaňuch *et al.*, 2007). We tested the amplification of 22 microsatellite loci developed for six species of the Vespertilionidae family, of which 11 successfully amplified. Primers were 5'-end labelled with a florescent dye for amplification between three multiplexes. Details of primers and labels used are provided in Appendix S5.1. Polymerase chain reactions (PCRs) were carried out with 5 µL KAPA2G Fast Multiplex Mix (Kapa Biosystems, United States), 1 – 2 µL template DNA (5 ng/µL), varying volumes of forward and reverse primers (Appendix S5.1) and distilled water to a total reaction volume of 10 µL. The PCR protocol consisted of an initial 5 min denaturation at 94°C, followed by 30 cycles of denaturation at 94°C for 30 s, annealing for 30 s at 51°C for multiplex 1, 57.5°C for multiplex 2 and 60°C for multiplex 3, elongation at 72°C for 30 s, with a final extension at 72°C for 10 min. PCR products were quantified on an ABI3730xl Genetic Analyser (Applied Biosystems, California) at the Central Analytical Facility of Stellenbosch University. Microsatellite fragments were visualised and scored using Geneious 9.0

(https://www.geneious.com). Approximately 20% of all samples were re-amplified and re-scored for error checking.

5.3.4 Data quality and genetic diversity

Micro-checker 2.2.3 was used to detect data input errors, allelic dropout, null alleles and stuttering (Van Oosterhout *et al.*, 2004). Tests for deviations from Hardy-Weinberg equilibrium and linkage disequilibrium were performed in Genepop 4.2 (Raymond and Rousset, 1995). We corrected *p*-values for multiple hypothesis testing using sequential Bonferroni correction with a threshold α of 0.05. Observed (H_O) and unbiased expected heterozygosity (uH_E), and allelic richness (*AR*) were calculated with the 'diveRsity' package (Keenan *et al.*, 2013) in the R statistical environment (R Core Team 2019). The number of private alleles (P_A) per site was calculated in GenAlEx v6.5 (Peakall and Smouse, 2012).

5.3.5 Genetic clustering

Two Bayesian clustering analyses were used to ascertain the spatial structure of the genetic data: STRUCTURE (Pritchard, Stephens and Donnelly, 2000) and TESS v2.3 (Chen *et al.*, 2007). Both programs utilise Markov chain Monte Carlo (MCMC) simulations with hierarchical Bayesian modelling to assign individuals to clusters by minimizing linkage disequilibrium and maximizing Hardy-Weinberg equilibrium (Francois and Durand, 2010). We utilised both programs as STRUCTURE relies only on individual genotypes without use of a spatial prior distribution, whereas TESS utilises a spatially explicit model with geographic coordinates to estimate ancestry coefficients (Francois and Durand, 2010). It is often useful to employ different genetic clustering approaches to detect underlying structure. Closely related individuals within populations have been reported to bias Bayesian clustering methods (Rodriguez-Ramilo and Wang, 2012). The degree of relatedness between pairs of individuals was assessed using TrioML (Wang, 2007) in Coancestry 1.0.1.9 (Wang, 2011). Relatedness densities were plotted to determine the exclusion threshold as described by Halczok *et al.* (2017). We manually selected the threshold of 0.32 from the plotted distribution, as it best separated closely related individuals from unrelated individuals. One individual of a pair, sampled from the same site, exceeding the relatedness threshold was removed from the dataset for both Bayesian clustering methods.

We ran STRUCTURE assuming an admixture model with correlated allele frequencies (Hubisz *et al.*, 2009). Twenty independent runs were performed with K = 1 to 7 (number of pooled

sample sites + 1). The most likely number of clusters was determined using the Evanno Δ*K* statistic, which is the second-order rate of change of log probability between successive values of *K* (Evanno, Regnaut and Goudet, 2005). All runs used 10 million iterations after a burn-in of 100,000. Results were plotted with Pophelper (Francis, 2016). We utilised the conditional autocorrelation (CAR) admixture model in TESS (Chen *et al.*, 2007), with the default spatial interaction parameter (ψ) of 0.6. TESS requires a range of *Kmax* values which are the assumed maximum number of clusters (Safner *et al.*, 2011), hence clusters $K = 2 – 7$ were tested. We employed 200 runs per *K*, a burn-in of 50,000 sweeps and a total run time of 100,000 sweeps. The Deviance Information Criterion (DIC) scores were used to determine the most likely number of clusters. Twenty percent of the lowest DIC scores were retained to identify *K* with the highest likelihood and lowest DIC. The DIC was also averaged over all runs as the most appropriate *K* may coincide with the plateau of the DIC curve.

Furthermore, we employed a nonparametric clustering method, AWCLUST (Gao and Starmer, 2008), to complement the two parametric methods described above as it is robust to small samples (<10) and violations of assumptions of the Hardy-Weinberg and linkage equilibrium (Deejai *et al.*, 2010). We generated pairwise allele sharing distance matrices and multidimensional scaling plots with AWCLUST. The plots allowed for visualisation of putative clusters and identification of outliers. We calculated gap statistics for each value of *K* with 100 null simulations. We tested $K = 1 – 14$ (total number of sites) as this method is not sensitive to sample sizes. Individuals were assigned to clusters based on hierarchical clustering plots.

5.3.6 Population structure

Pairwise population structure was investigated with D_{est} (the harmonic mean across loci for each pairwise population; Jost (2008)) and G''_{ST} (expected heterozygosity and corrected for sampling a small number of populations; Meirmans and Hedrick (2011)) calculated in GenAlEx v6.5 (Peakall and Smouse, 2012). Sites with $N < 5$ samples were excluded from the pairwise D_{est} and G''_{ST} analyses. Significance levels for pairwise tests were adjusted using Bonferroni correction.

5.3.7 Barriers and migration rates

Barrier v2.2 (Manni, Guérard and Heyer, 2004) was used to detect possible genetic barriers across the landscape. Barrier utilises a computational geometry approach with Monmonier's

maximum difference algorithm on a Delaunay triangulation to detect an abrupt rate of change in genetic differentiation (Manni, Guérard and Heyer, 2004). The R package 'diveRsity' (Keenan *et al.*, 2013) was used to bootstrap 1,000 pairwise D_{est} distance matrices for input into Barrier. Bayesian inference of recent migration rates, within the last two generations, between the sites was performed with BayesAss v3.04 (Wilson and Rannala, 2003). We ran BayesAss for 50 million generations with a burn-in of 10 million and sampled every 1,000 generations. The step sizes of migration rates (*m*) were adjusted to 0.6, allele frequencies (*a*) to 1.0 and inbreeding coefficients (*f*) to 0.3 in order to correct acceptance rates such that they remained between 20% and 60% throughout the run (Rannala, 2015). As this method is sensitive to low and uneven sample sizes, we pooled samples into three forest groups (Mistbelt, Scarp and Coastal) and re-ran the analysis. Tracer v1.7.1 (Rambaut *et al.*, 2018) was used to visually evaluate adequate mixing and whether convergence had been reached prior to the end of the burn-in.

5.3.8 Effect of distance and land cover on gene flow

We tested for the effects of land cover type and Euclidean distances between sites on gene flow. G''_{ST} was used as a measure of genetic differentiation and was calculated with GenAlEx v6.5 (Peakall and Smouse, 2012). Straight line Euclidian distances were measured between sampling locations. The home range size or distance *P. hesperidus* travels to move between roosts is currently unknown. Feyerabend and Simon (2000) found *P. pipistrellus* to have travelled a maximum distance of 15 km between roosts in Hessen, Germany. *Pipistrellus hesperidus* is similar in size to *P. pipistrellus* such that the two species may possibly have similar dispersal capabilities; thus, a buffer radius of 15 km was employed. The South African National Land Cover dataset (Thompson, 2019) was used to characterise land cover types within the buffers extending between forest localities. The proportion of land cover types was calculated in ArcGIS Pro v2.4 (ESRI Inc., 2019). Land cover types were grouped into four classes that are expected to affect the movement of *P. hesperidus*: developed (all types of built up areas, mines and quarries), forest (indigenous and planted forest), open habitats (shrubland, grassland, barren and cultivated land), waterbodies and wetlands (natural and artificial waterbodies, and herbaceous and woody wetlands). Wetlands were grouped with waterbodies, and not in the open habitat category, as bat activity and species richness is markedly higher in wetlands than surrounding open habitats in South Africa (Sirami, Jacobs and Cumming, 2013). We thought it best to group wetlands with watercourses that are also known to support high bat activity (Zeale, Davidson-Watts and Jones, 2012). Agricultural areas were combined with

natural open areas, as we considered the dispersal resistance of these open habitats to be comparable for the study species. Ocean and beach areas were excluded from the analysis.

To compare the effects of land cover and Euclidean distance on genetic distance we utilised multiple regression on distance matrices (MRM function) with the 'ecodist' package (Goslee and Urban, 2019) in R. Collinearity between predictor variables was tested for with variance inflation factors (VIFs) using the 'car' package (Fox, 2002). Land cover variables showed collinearity which may hinder the interpretation of multivariate regressions, thus only univariate models were used. The univariate models included additive and interaction terms of land cover variables with Euclidean distance. Landscape genetics analyses typically utilise a resistance matrix approach to evaluate how land use affects gene flow wherein each land cover type is assigned a resistance value based on specialist opinion or ecological field data (Hall and Beissinger, 2014). Keeley *et al.* (2017) reported animals to be more sensitive to habitat suitability in the home range than during dispersal and mating movements. We opted to utilise proportions of the land cover classes (developed, forest, open, and waterbodies and wetlands) in large linear buffers over the resistance matrix approach, as basic information on roost utilization and movement ecology of *P. hesperidus* is not available and the assignment of resistance values is likely to be error prone. Talbot *et al.* (2017) favour this approach, for volant animals as opposed to those with lower dispersal capabilities, as they may easily traverse small high resistance areas and respond to land cover on a broader landscape scale.

5.4 Results

A total of 120 individuals from 14 sample sites were genotyped for 11 microsatellite loci, with no individuals unscored at more than two loci. Difficulties in amplification and/or scoring of historic museum material were encountered, so only four museum samples, from Hlatikulu Forest Reserve (Figure 5.1), were included in the analyses (museum accession numbers DM 14331 – 14334, collected in 2014). For error checking, all samples re-amplified successfully and 97% scored the same. Locus D15 was found to be monomorphic at 91 nucleotides and was discarded from all analyses. Evidence of null alleles and significant departures from HWE were detected for loci Ppip02 and P13; accordingly, both loci were excluded from all analyses. We found no evidence of linkage disequilibrium. Thus, we utilised a total of eight loci across all analyses. The number of alleles ranged from 4 (locus F19) to 24 (locus Ppip03) (Appendix S5.1). Observed heterozygosity ranged from 0.562 (Hlatikulu and Silaka) to 0.650 (Xumeni) across all loci, and unbiased expected heterozygosity ranged from 0.612 (Oribi Gorge) to 0.715

(Baziya) (Table 5.1). Average allelic richness was 2.728, ranging from 2.527 (Oribi Gorge) to 2.855 (Silaka) (Table 5.1). A total of 15 private alleles were found, with nine of the 14 sites possessing at least one private allele. Manubi and Fort Fordyce were the sites with the highest number of private alleles (Table 5.1).

5.4.1 Genetic clustering

Six pairs of individuals were found to exceed the relatedness threshold; one individual of each pair was removed from the dataset. All genetic clustering analyses were run with eight loci for 114 individuals. The optimal values for K were not consistent across the three clustering methods. The DIC plot for K_{MAX} from TESS did not plateau, and K = 4 was selected with the lowest DIC value (6489.59). The proportions of all individuals assigned to each genetic cluster were mostly symmetrical in the resulting plot from TESS, with the exception of the Amatole Mistbelt forest type (Figure 5.2A). This illustrates a lack of population structuring between all forest types with a slight differentiation of Fort Fordyce in the Amatole Mistbelt. Two genetic clusters were selected based on the mean LnP(K) = -3948.12 and ΔK from the results of STRUCTURE (Appendix S5.2). There was no clear assignment of any individuals to either of these genetic clusters, thus indicating a lack of population structure (Figure 5.2B). Gap statistics of AWCLUST depicted the appropriate number of clusters was K = 3 (Appendix S5.3). The results of AWCLUST also portray a lack of genetic clustering, indicating general dispersion of all individuals within the multidimensional scaling plot (Figure 5.2C).

5.4.2 Population structure

Pairwise D_{est} and G''_{ST} values were generally low between sites (Appendix S5.4). Pairwise D_{est} ranged from -0.045 to 0.114 with 4 of the 35 values (11.4%) significant after Bonferroni correction. Pairwise G''_{ST} ranged from -0.064 to 0.161, also with 4 of 35 values significant. The highest significant D_{est} and G''_{ST} values were between Alexandria and Ngeli (0.161 and 0.114 respectively), with Alexandria demonstrating all significant pairwise values (Appendix S5.4).

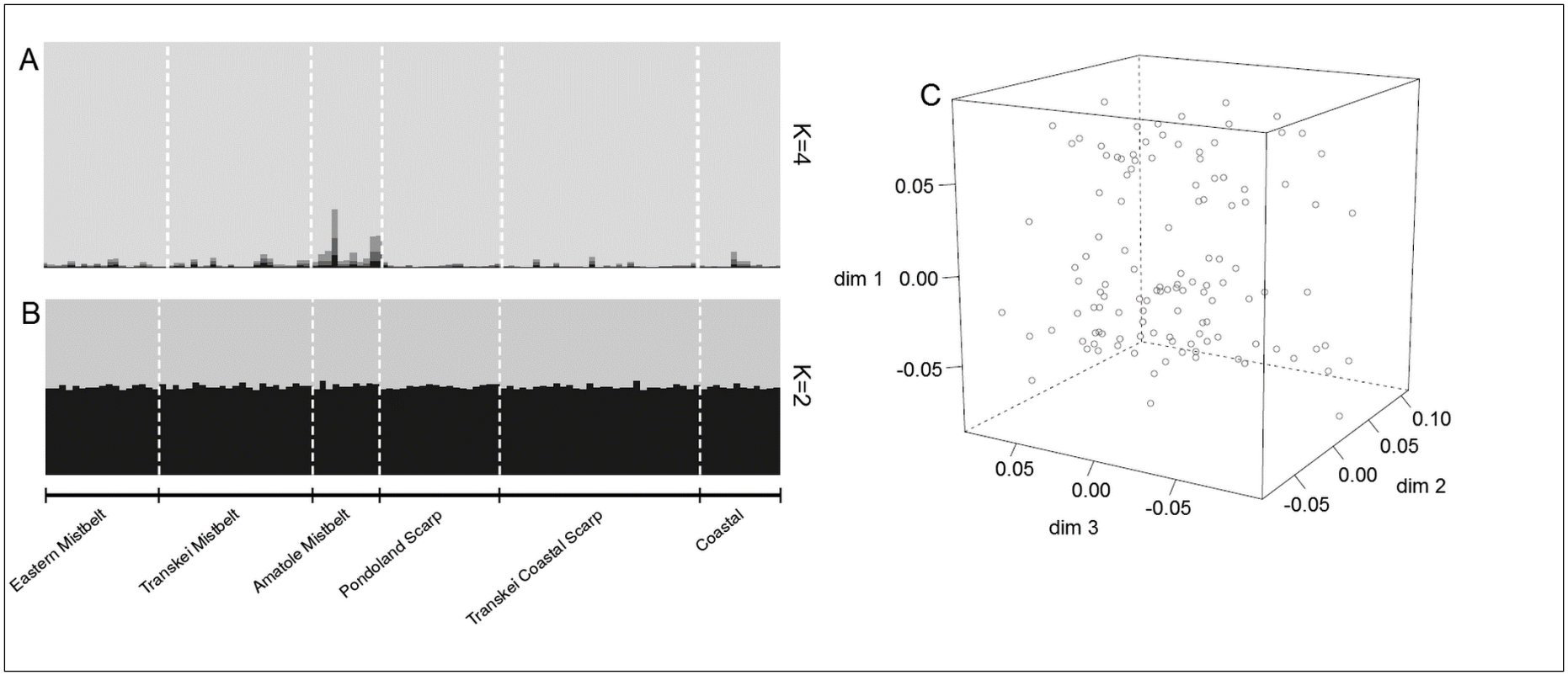


Figure 5.2 Inferred population structure of *Pipistrellus hesperidus* from the Eastern Cape and KwaZulu-Natal provinces of South Africa generated using eight polymorphic microsatellite loci. A) Population structure inferred with the use of TESS (Chen *et al.*, 2007) where $K = 4$, and B) Results from STRUCTURE (Pritchard, Stephens and Donnelly, 2000) where $K = 2$. The y-axis depicts the ancestry coefficient (Q) and grey shades represent the proportion of assignment of each individual to the genetic clusters (K). Samples were pooled per forest type for figure components A and B. C) Multidimensional scaling plot from AWCLUST (Gao and Starmer, 2008) with circles displaying the distribution of individuals along three principal coordinate axes.

5.4.3 Barriers and migration rates

Two main barriers were identified: the barrier of highest bootstrap support depicts genetic discontinuity of Oribi Gorge from surrounding sites, with a further barrier differentiating Alexandria (Appendix S5.5). The remaining barriers were not reported, as they had lower bootstrap support values and were not supported by other analyses. The BayesAss analysis displayed limited contemporary migration between sites over the past two generations (Appendix S5.6). The proportion of migrant individuals range from 0.0117 to 0.0195 between all sites, except Manubi. Albeit low, the migration rate of individuals emanating from Manubi is between 7 - 14 times greater than all other sites with the rate between 0.090 (migration from Manubi to Umtamvuna) and 0.171 (migration from Manubi to Gomo). The analysis with pooled samples illustrated considerably higher migration rates originating from scarp forest towards the other forest groups (0.317 to Mistbelt, 0.288 to Coastal), while migration between the other forest groups was low (0.006 – 0.023) (Figure 5.3).

5.4.4 Effect of distance and land cover on gene flow

The test for isolation by distance (IBD) using the straight-line distance between sites was not significant (R^2 = 0.0357 p = 0.343, Table 5.2). The IBD plot displays a slight positive trend of increased genetic differentiation with Euclidean distance, but with high scatter around the line (Appendix S5.7). The proportions of developed, forest, and open habitat land cover classes had non-significant, negative relationships with G''_{ST}. The proportion of waterbodies and wetlands between sites was the only land cover class found to be related to genetic differentiation (p = 0.024, Table 5.2). However, Figure 5.4 shows variability in the data with high scatter of points around the regression line. The low R^2 (0.0161) value indicates the proportion of wetlands and waterbodies between sites explains only 1.61% of the variation of G''_{ST}. The additive and interaction models, encompassing combinations of predictor land cover variables with Euclidean distance, only returned significant results for models including the waterbodies and wetlands land cover class. All combinations of additive and interaction terms of the waterbodies and wetlands class with distance showed significant relationships. However, these models returned lower coefficient of determination (R^2) values than the univariate model using only the waterbodies and wetlands land cover class. Thus, the significant results were driven by the effects of the land cover class.

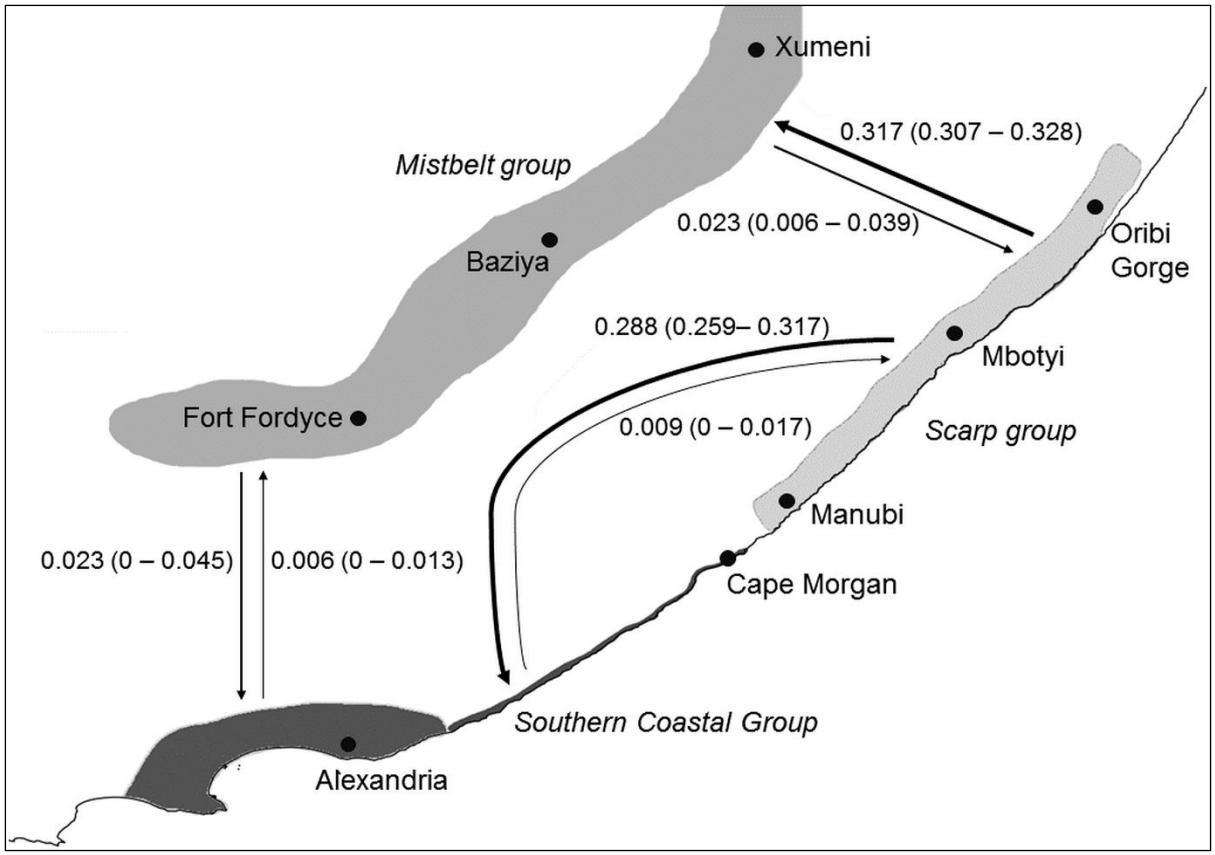


Figure 5.3 Schematic representation of the migration rates (and 95% credible intervals) of *Pipistrellus hesperidus* between three forest groups (Southern Coastal, Mistbelt and Scarp, forest classification after von Maltitz *et al*. 2003) along the eastern seaboard of South Africa estimated with BayesAss. The size and direction of arrows represent the magnitude and direction of migration rates, with higher rates portrayed by thicker lines. The size, shape and position of forest types are schematic and not accurate representations of their actual size and distribution. A few select sites were presented to orientate the reader to schematic location.

Table 5.2 Results from multiple regression on distance matrices of genetic distance of *Pipistrellus hesperidus* with Euclidean distance and the proportion of four land cover types between sites in south-eastern South Africa. Significant *p* value in bold.

	Slope	R^2	F test	*p*
Euclidean distance (km)	6.648×10^{-5}	0.0357	3.3000	0.343
Developed	-0.0009	0.0019	7.9835	0.314
Forest	-0.0001	0.0003	1.3570	0.671
Open habitats	-0.0002	0.0006	2.4224	0.559
Waterbodies and wetlands	-0.0140	0.0161	67.058	**0.024**

5.5 Discussion

5.5.1 Main findings

Despite *P. hesperidus* bearing a wing morphology indicative of low dispersal potential and a dependence on fragmented wooded habitats as a clutter-edge forager, we found a lack of population structuring and high gene flow across Eastern Cape and KwaZulu-Natal provinces of South Africa (Figure 5.2). This general finding was with the exception of genetic discontinuities for Oribi Gorge and Alexandria from other sampled forests (Appendix S5.5). The diversity metrics do not depict a loss of heterozygosity among sites (Table 5.1); nearly all pairwise D_{est} and G''_{ST} values were not significant (Appendix S5.4) and there was no isolation-by-distance effect (Appendix S5.7). Our results show that *P. hesperidus* is thus likely not dependent on forested habitats to maintain genetic connectivity on the landscape. We found contemporary migration to be originating from Manubi, with few migrants emanating from other forests (Appendix S5.6). The generally low population structure between sites indicates that migrants from Manubi may be the largest contributors to gene flow across the region. Additionally, Fort Fordyce was identified as one of the localities with the highest expected heterozygosity, allelic richness and number of private alleles (Table 5.1), indicating significant genetic diversity is held within the Amatole mistbelt forests, which may have served as a historical refugium for *P. hesperidus*.

Furthermore, we found land cover composition between sites to be poor predictors of genetic differentiation for this species. The proportions of forest, open habitats (encompassing agricultural areas), and anthropogenically developed land had no effect on gene flow. Therefore, we found no measurable effect of the current extent of anthropic development (urban and agricultural) on genetic differentiation of *P. hesperidus*. The proportion of waterbodies and wetlands was the only land cover class to demonstrate a significant relationship, indicating it to be permeable to gene flow (Table 5.2, Figure 5.4). Albeit a weak association, riparian habitats and wetlands are known for their importance to bats for drinking, foraging and commuting (Lookingbill *et al.*, 2010; Sirami, Jacobs and Cumming, 2013). Additionally, *P. hesperidus* shows a strong preference for utilisation of riparian forest over open savanna habitat in Eswatini (Monadjem and Reside, 2008). Thus, it seems likely this species utilises riparian habitats and wetlands for dispersal to maintain the genetic connectivity across the landscape.

5.5.2 Infrequent gene flow and female philopatry

Not much is known of the movement and dispersal of *P. hesperidus* within South Africa. The low population structure observed indicates high gene flow. However, the migration rate between sites over the past two generations, as inferred from the BayesAss analysis, suggests gene flow events may not be occurring frequently (Appendix S5.6). *Pipistrellus pipistrellus* can live for approximately 16 years in the wild (Gaisler *et al.*, 2003). If *P. hesperidus* has a similar lifespan, it would have a generation time of multiple years such that gene flow events would not need to occur every year to maintain genetic connectivity and low population structure between sites. Alternately, persistent low-level migration may be sufficient to homogenize population structure. In addition, little is known of this species' mating system. Other *Pipistrellus* species demonstrate female resource polygyny (Gerell and Lundberg, 1985), with males holding courting territories along the females' flight routes (Barak and Yom-Tov, 1991). This suggests females remain in natal areas and dispersal is male biased. Genetic analyses with mitochondrial DNA observed population structuring between these forests (Moir *et al.*, 2020), yet the present study showed low structuring with nuclear markers. This indicates gene dispersal may be male mediated and females are philopatric, a common system in vespertilionid bats (Burland *et al.*, 2001; Santos *et al.*, 2016).

5.5.3 Oribi Gorge and Alexandria

To the best of our knowledge, this study provides the first indication of a genetic discontinuity between Oribi Gorge with Umtamvuna and Ngeli for any vertebrate taxon. It may be a result of anthropogenic modification of the Mzimkhulu and Mzimkhuluwana rivers that surround Oribi Gorge and connect it to Ngeli. Sugarcane farming is extensive across coastal KwaZulu-Natal. Sugarcane farms straddled both rivers (Lewis, 1990), with the establishment of a sugarcane mill on the Mzimkhulu river in the early 1900's (du Bois, 2016). Sugarcane agriculture is reliant on a good water supply and causes deterioration of water courses (Matavire, 2015), which is likely impacting the lower reaches of these rivers. The Weza-Ngeli forest, situated inland along these rivers, has long been exploited for timber with the construction of the Ngeli sawmill in the 1890's (McCracken, 1986). Alien timber trees are notorious for high levels of water abstraction in South Africa (Albaugh, Dye and King, 2013). These plantations, which now comprise *ca.* 145 km^2 of the Weza-Ngeli forest (Grieve and Downs, 2015), negatively affect the upper reaches of the river system. In the 2018 National Biodiversity Assessment both the Mzimkhulu and its tributary, the Mzimkhuluwana, on which

Oribi Gorge is located, were identified as modified rivers with most reaches classed either as Critically Endangered or Endangered (Skowno *et al.*, 2019). The removal of riparian vegetation by traditional sugarcane farming and plantation forestry practices may deter *P. hesperidus* (a clutter-edge specialist) from utilising such watercourses, thereby hindering possible dispersal events. However, the small sample size presented in this study is an important limitation to the inferences that can be made. Preliminary results of a population genetics study of three forest bird species: white-starred robin (*Pogonocichla stellata*), Cape batis (*Batis capensis*) and chorister robin-chat (*Cossypha dichroa*) show similar genetic structure between Oribi Gorge and Ngeli (J. Mulvaney, unpublished data). This study identifies the Oribi Gorge area and its surrounds as worthy of further investigation.

The barrier of Alexandria is a well-known climatic break called the Bedford gap (Lawes, 1990) and is of biogeographical importance in southern Africa (Willows-Munro and Matthee, 2011). The Bedford gap is a semi-arid area with xeric vegetation that separates the forests of the eastern and south-eastern Cape. The climate and vegetation of the Bedford gap are a result of the intruding ingression of the Karoo sub-desert biome (Lawes, 1990). The gap has been identified as a barrier to gene flow for the Cape mole-rat (*Georychus capensis*) (Visser, Bennett and Vuuren, 2018), limiting the distribution of the samango monkey (*Cercopithecus albogularis*) (Lawes, 1990) and dark-footed forest shrew (*Myosorex cafer*) (Willows-Munro, 2008), and speciation of the common slug eating snake (*Duberria lutrix lutrix*) (Kulenkampff *et al.*, 2019). This is the first record of the suggested effect of the Bedford gap on a volant taxon.

5.5.4 Migrants from Manubi

Mistbelt forests are biogeographically ancient and were most severely affected by climatic extinction filtering during the last glacial maximum (LGM) (Eeley, Lawes and Piper, 1999). Scarp forests were mostly unaffected by the cooling and drying effect of the LGM as they were buffered by their proximity to the warm Indian Ocean. Scarp forests served as refugia to forest fauna with subsequent recolonisation from scarp refugia to mistbelt forests. Coastal forests have a more recent origin and were also colonised by fauna from scarp forests (Lawes *et al.*, 2007). The sampled sites were pooled to visualise migration rates between forest groups (Figure 5.3). This study may represent the first genetic work in support of Lawes *et al*.'s (2007) findings, as migration of *P. hesperidus* emanates primarily from scarp forests towards the mistbelt and coastal forests.

When the BayesAss analysis was run with individual sites, we found the scarp forest migration originated solely from Manubi. It is unclear as to why migrants disperse from Manubi to other sites, but do not disperse from other sites towards Manubi using the same routes. We recommend this as the basis for further work on *P. hesperidus* in the region. The migration estimates may be biased by small and uneven sample sizes with Manubi, as the better sampled site, being identified as the source of migrants. Nonetheless, this forest also appears to be a source of genetic diversity for forest-utilising bats and birds. The Temminck's myotis (*Myotis tricolor*) and Botswana long-eared bat (*Laephotis botswanae*) had unique, highly differentiated mtDNA haplotypes from this forest (Moir *et al.*, 2020). The yellow-throated woodland warbler (*Phylloscopus ruficapilla*) demonstrated high allelic richness, while the chorister robin-chat displayed the highest genetic diversity from Manubi than other forests in the Eastern Cape (J. Mulvaney, unpublished data). Manubi also holds high bird species and functional richness relative to other Eastern Cape forests (Leaver *et al.*, 2019).

Manubi has been identified as a highly productive forest (Ham, 2000; Tshaduli, Geldenhuys and Chirwa, 2018), and was recognized as "of much higher quality than other in the Transkeian coastal belt" by (King, 1940) due to its rich doleritic soil, mild winters and evenly distributed annual rainfall. The ideal weather conditions and high productivity of the forest may inform the observed trends of high species and genetic diversity. Although under protection from the Department of Environment, Forestry and Fisheries, Manubi's tree stock is illegally harvested (Leaver and Cherry, 2020). However, this harvesting is on a scale resulting in habitat degradation rather than deforestation. To the authors' knowledge, the effect of this harvesting on bats has not yet been studied, but it has been found to negatively impact the functional diversity of forest birds (Leaver *et al.*, 2019). It is evident that Manubi is an important forest for genetic diversity and species richness across different taxa thus, sustainable management of harvesting practices is needed to conserve this important source of biodiversity.

5.5.5 Amatole mistbelt as a refugium

The high genetic diversity of *P. hesperidus* found at Fort Fordyce, in the Amatole mistbelt forest complex, indicates it has been able to support sizeable populations such that it was not likely to have been recently founded from a scarp refuge. The diversity metrics (Table 5.1) suggest *P. hesperidus* endured past climate change in small sheltered refugia within the mistbelt forest (Lawes, 1990), which has been reported for the mesophytic, flowering herb

genus *Streptocarpus* within the same forest complex (Hughes *et al.*, 2005). The Amatole mistbelt forests are relatively extensive, covering an area of approximately 35,000 ha (von Maltitz *et al.*, 2003). The large size of the complex may have contributed to its resilience during the LGM and facilitated the survival of fauna within smaller refugia. The Fort Fordyce population also demonstrated a greater observed than expected heterozygosity (Table 5.1), indicative of the isolate-breaking effect which is the mixing of two previously isolated populations. This may suggest the subsequent mixing of two populations that were separated within small refugia of the forest complex. This finding of *P. hesperidus* taking refuge within the complex is supported by mtDNA analyses in which Fort Fordyce haplotypes showed a more ancient status (Moir *et al.*, 2020).

5.5.6 Lack of effect of agricultural development and urbanisation on gene flow

Generally, a higher degree of urbanisation and agricultural development decreases habitat use by bats (Jung and Threlfall, 2016), but bats have shown mixed responses to anthropic changes to the environment. The effects are typically highly species specific with sympatric species demonstrating different responses to urbanisation and habitat fragmentation (Duchamp, Sparks and Whitaker, 2004; Klingbeil and Willig, 2009). Sensitivity of a species to fragmentation and urbanisation is often dependent on traits informing mobility; niche breadth (Safi and Kerth, 2004); ability to exploit matrix habitats (Farneda *et al.*, 2015); and flexibility in roosting and foraging behavior (Jung and Threlfall, 2016). Agricultural and urban areas did not have a measurable effect on gene flow of *P. hesperidus* (Table 5.2), possibly due to the species having a relatively wide niche breadth and intermediate range (Cooper-Bohannon *et al.*, 2016). Not much is known of the roosting ecology and preferences for dispersal habitat of the study species. Our results may suggest a greater flexibility in roost selection; a lower dependency on well-wooded habitats than recorded to date; or the ability to disperse through matrix habitats (or a combination of these traits).

It is important to note that the observed lack of effects of agricultural development and urbanisation may be due to the geographic scale at which we performed the analysis (Klingbeil and Willig, 2009). Analysis on a finer scale, with smaller buffer zones, may detect a significant relationship of land cover with genetic differentiation. We recommend future studies to utilise a range of ecologically relevant buffer scales. A further contributing factor to the lack of observed effects may be that the Eastern Cape is considerably less developed than other regions of South Africa, such that anthropogenic disturbances have not yet reached a threshold beyond

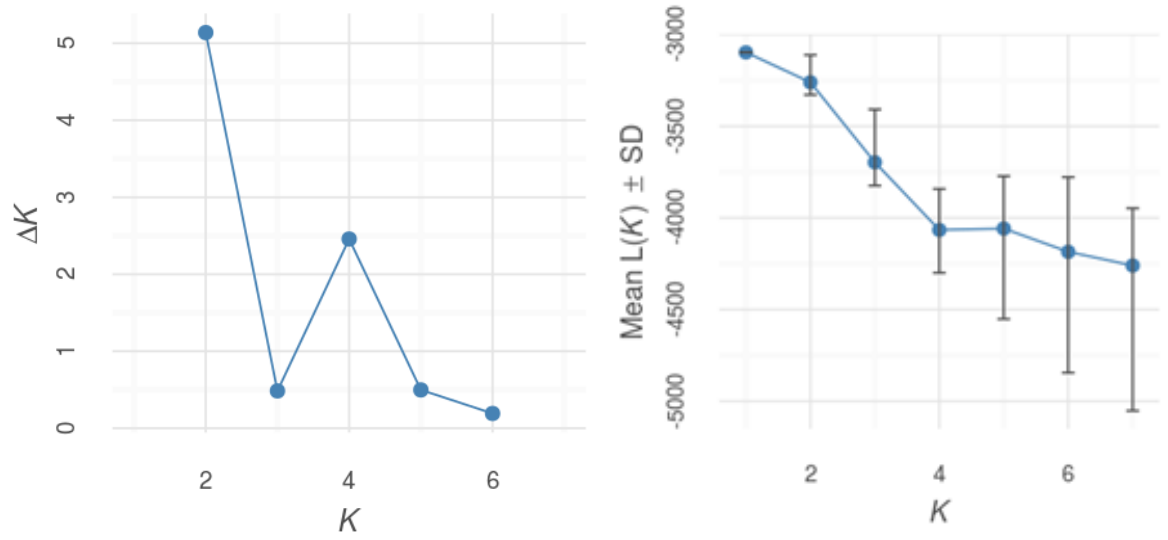
which an effect is detectable. Species exposed to recent habitat transformations provide the opportunity to study the mechanisms of contemporary gene flow and adaptation to habitat changes (Stockwell, Hendry and Kinnison, 2003). This study provides the first insights into genetic diversity and gene flow of *P. hesperidus* across the Eastern Cape and KwaZulu-Natal provinces prior to agricultural intensification and large-scale urbanisation of the region. This study may be used as a baseline with which to measure the impacts of future development on this species.

Appendix S5.1 PCR conditions of cross-species amplification of vespertilionid microsatellite loci in *Pipistrellus hesperidus*.

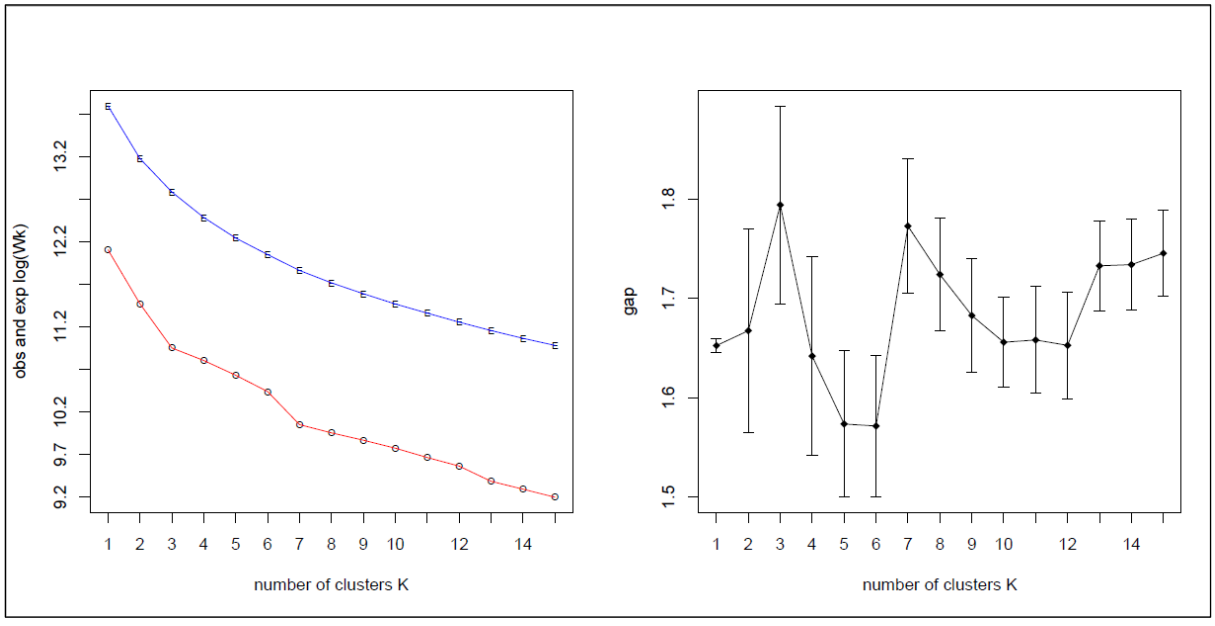
Locus	Reference	Size range (bp)	Multiplex; Label	Primer concentration (µM)	Number of alleles
EF4	(Vonhof *et al.*, 2002)	210 - 238	2; FAM	0.1	12
D15	(Castella and Ruedi, 2000)	91	3; VIC	0.1	1
F19	(Castella and Ruedi, 2000)	177 - 197	2; PET	0.1	4
NN8	(Petri *et al.*, 1997)	160 - 188	1; FAM	0.15	11
P13	(Mayer, Schlotterer and Tautz, 2000)	129 - 157	1; VIC	0.25	12
P20	(Mayer, Schlotterer and Tautz, 2000)	92 - 106	1; VIC	0.07	6
Ppip01	(Racey *et al.*, 2007)	142 - 166	2; FAM	0.6	11
Ppip02	(Racey *et al.*, 2007)	106 - 128	1; NED	0.05	9
Ppip03	(Racey *et al.*, 2007)	188 - 242	3; PET	0.1	24
Ppip05	(Racey *et al.*, 2007)	139 - 175	3; NED	0.1	17
Ppip06	(Racey *et al.*, 2007)	101 - 181	3; PET	0.7	14

Primer concentration – final concentration of primers in multiplex PCRs

Primers that did not amplify with several rounds of PCR troubleshooting: b22 (Kerth, Safi and König, 2002); EF1 and EF15 (Vonhof *et al.*, 2002); NN18 (Petri *et al.*, 1997); P217 (Mayer, Schlotterer and Tautz, 2000); Paur02 and Paur05 (Burland, Barratt and Racey, 1998); Ppip04 (Racey *et al.*, 2007). Primers that did amplify but were thought to be monomorphic after analysis of the LabChip PerkenElmer 3K assay (Central analytical Facility, Stellenbosch University): EF6 (Vonhof *et al.*, 2002); H29 (Castella and Ruedi, 2000); P219 (Mayer, Schlotterer and Tautz, 2000).



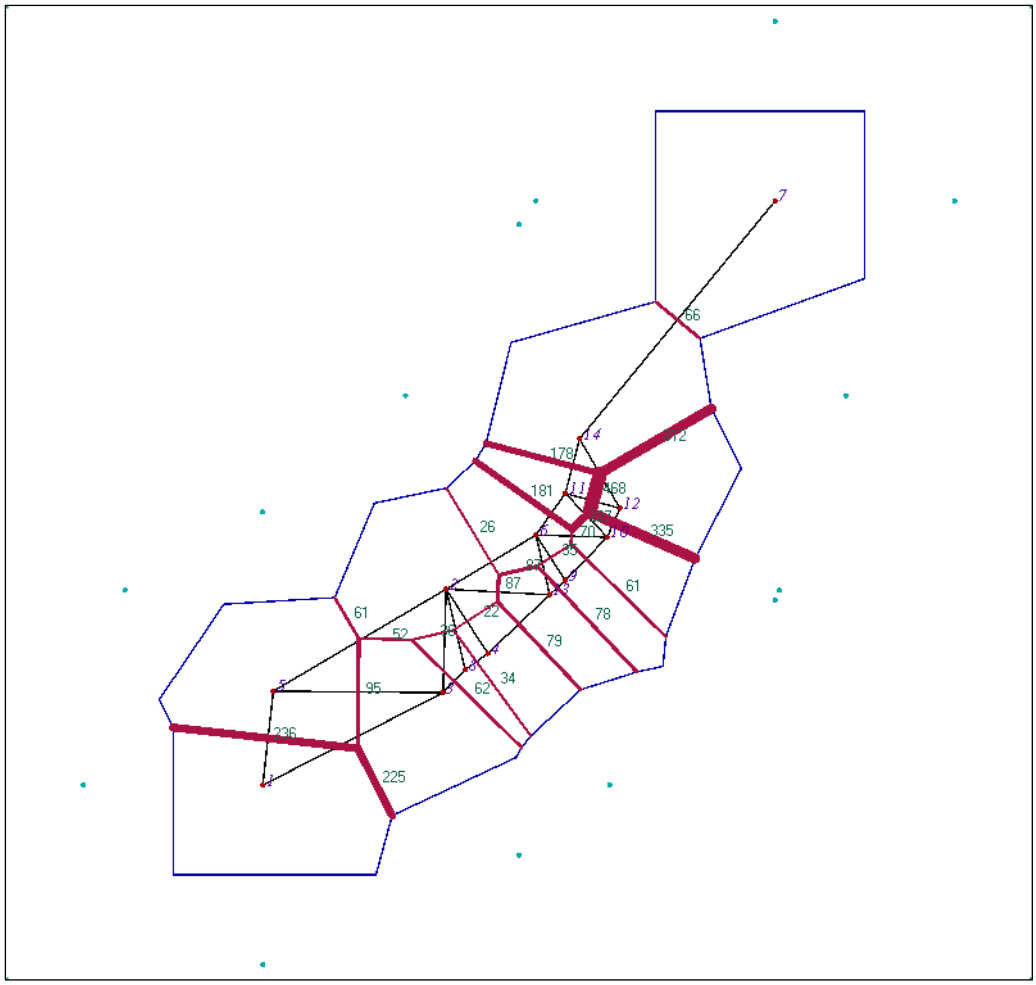
Appendix S5.2 Determination of the most likely number of genetic clusters (*K*) with the method of Evanno *et al*. (2005), with Δ*K* depicting *K* = 2 (Δ*K* = 5.104) as the most likely number of clusters.



Appendix S5.3 Gap statistics calculated with AWCLUST, with 100 null simulations, for selection of *K*.

Appendix S5.4 Population pairwise D_{est} (above the diagonal) and G''_{ST} (below the diagonal) values for *Pipistrellus hesperidus* across the Eastern Cape and KwaZulu Natal provinces of South Africa. Significant values indicated in bold following Bonferroni correction with a threshold α of 0.05.

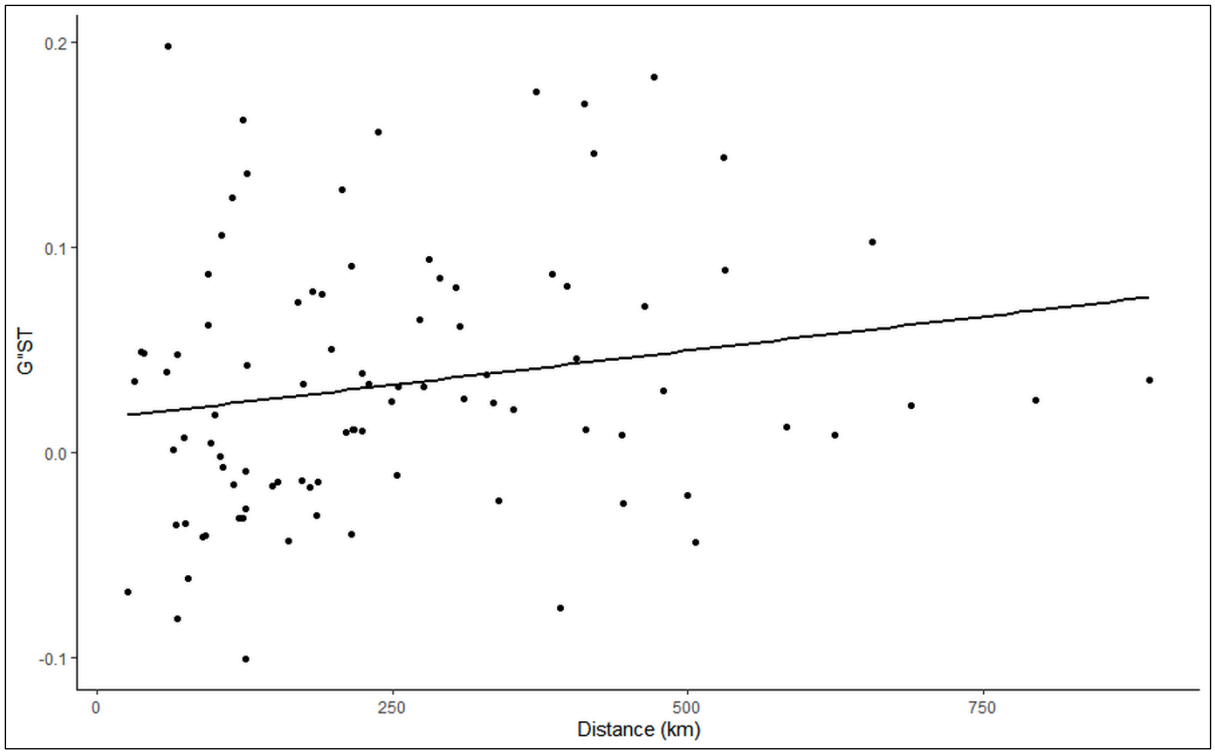
	Xumeni	Ngeli	Gomo	Baziya	Fort Fordyce	Mbotyi	Dwesa	Manubi	Cape Morgan	Alexandria
Xumeni		0.024	-0.016	0.015	0.006	-0.017	0.013	0.035	-0.022	**0.092**
Ngeli	0.035		0.012	0.038	0.056	0.062	-0.009	0.099	0.032	**0.114**
Gomo	-0.024	0.016		-0.028	0.020	-0.014	-0.026	0.044	-0.042	**0.104**
Baziya	0.021	0.053	-0.039		0.002	-0.037	-0.045	0.001	-0.032	0.040
Fort Fordyce	0.009	0.079	0.028	0.003		0.013	0.001	0.031	0.020	**0.084**
Mbotyi	-0.024	0.087	0.061	-0.052	0.018		-0.037	-0.023	-0.022	0.041
Dwesa	0.019	-0.013	-0.037	-0.064	0.001	-0.053		0.012	-0.038	0.048
Manubi	0.051	0.140	0.061	0.001	0.044	-0.033	0.017		0.023	0.002
Cape Morgan	-0.032	0.046	-0.062	-0.047	0.029	-0.032	-0.056	0.033		0.049
Alexandria	**0.133**	**0.161**	**0.145**	0.057	**0.119**	0.059	0.069	0.002	0.072	



Appendix S5.5 Genetic barriers, detected by Barrier (Manni *et al*., 2004), depicted with red lines and numbers showing bootstrap support for barriers. Voronoï tessellation of population localities in blue and Delaunay triangulation in black.

Appendix S5.6 Posterior mean migration rates of *Pipistrellus hesperidus* between forest localities in South Africa as generated with BayesAss. Standard deviation shown in parentheses. The migration rates display the fraction of individuals in the population (row values) that are migrants (per generation) derived from the forest location (column values).

	Alexandria	Baziya	Cape Morgan	Dwesa	Fort Fordyce	Gomo	Hlatikulu	Manubi	Mbotyi	Ngeli	Oribi Gorge	Silaka	Umtamvuna	Xumeni
Alexandria	0.6841 (0.0166)	0.0177 (0.0169)	0.0176 (0.0165)	0.0175 (0.0165)	0.0178 (0.0169)	0.0176 (0.0167)	0.0177 (0.0168)	0.1046 (0.0350)	0.0174 (0.0165)	0.0178 (0.0168)	0.0174 (0.0166)	0.0175 (0.0166)	0.0176 (0.0168)	0.0177 (0.0167)
Baziya	0.0140 (0.0134)	0.6804 (0.0131)	0.0139 (0.0134)	0.0138 (0.0132)	0.0141 (0.0136)	0.0139 (0.0133)	0.0140 (0.0134)	0.1524 (0.0333)	0.0139 (0.0134)	0.0140 (0.0135)	0.0140 (0.0134)	0.0140 (0.0133)	0.0138 (0.0132)	0.0138 (0.0133)
Cape Morgan	0.0153 (0.0145)	0.0151 (0.0144)	0.6819 (0.0146)	0.0152 (0.0144)	0.0151 (0.0144)	0.0152 (0.0146)	0.0152 (0.0146)	0.1362 (0.0343)	0.0151 (0.0146)	0.0150 (0.0144)	0.0151 (0.0145)	0.0152 (0.0145)	0.0151 (0.0144)	0.0152 (0.0145)
Dwesa	0.0128 (0.0123)	0.0127 (0.0122)	0.0127 (0.0121)	0.6793 (0.0122)	0.0130 (0.0125)	0.0128 (0.0123)	0.0127 (0.0122)	0.1676 (0.0318)	0.0126 (0.0122)	0.0127 (0.0122)	0.0128 (0.0124)	0.0128 (0.0124)	0.0127 (0.0121)	0.0128 (0.0123)
Fort Fordyce	0.0136 (0.0131)	0.0134 (0.0130)	0.0136 (0.0130)	0.0137 (0.0130)	0.6806 (0.0135)	0.0136 (0.0131)	0.0136 (0.0130)	0.1564 (0.0329)	0.0138 (0.0132)	0.0137 (0.0131)	0.0136 (0.0131)	0.0135 (0.0130)	0.0136 (0.0130)	0.0134 (0.0129)
Gomo	0.0124 (0.0119)	0.0124 (0.0120)	0.0125 (0.0120)	0.0124 (0.0121)	0.0131 (0.0126)	0.6792 (0.0120)	0.0125 (0.0121)	0.1707 (0.0319)	0.0126 (0.0120)	0.0125 (0.0120)	0.0124 (0.0120)	0.0125 (0.0119)	0.0123 (0.0119)	0.0125 (0.0120)
Hlatikulu	0.0185 (0.0175)	0.0187 (0.0177)	0.0186 (0.0176)	0.0186 (0.0177)	0.0187 (0.0178)	0.0186 (0.0175)	0.6852 (0.0175)	0.0919 (0.0342)	0.0184 (0.0175)	0.0186 (0.0176)	0.0185 (0.0174)	0.0187 (0.0176)	0.0187 (0.0178)	0.0184 (0.0175)
Manubi	0.0117 (0.0113)	0.0119 (0.0114)	0.0120 (0.0115)	0.0120 (0.0117)	0.0151 (0.0143)	0.0123 (0.0117)	0.0123 (0.0116)	0.8406 (0.0319)	0.0120 (0.0117)	0.0119 (0.0115)	0.0121 (0.0117)	0.0120 (0.0117)	0.0122 (0.0117)	0.0120 (0.0115)
Mbotyi	0.0138 (0.0133)	0.0139 (0.0132)	0.0138 (0.0131)	0.0139 (0.0132)	0.0141 (0.0134)	0.0138 (0.0132)	0.0138 (0.0132)	0.1530 (0.0333)	0.6806 (0.0135)	0.0138 (0.0132)	0.0139 (0.0133)	0.0138 (0.0132)	0.0139 (0.0134)	0.0139 (0.0132)
Ngeli	0.0175 (0.0167)	0.0176 (0.0167)	0.0175 (0.0166)	0.0174 (0.0164)	0.0177 (0.0168)	0.0175 (0.0166)	0.0176 (0.0166)	0.1052 (0.0347)	0.0176 (0.0168)	0.6842 (0.0164)	0.0175 (0.0166)	0.0175 (0.0166)	0.0175 (0.0166)	0.0177 (0.0167)
Oribi Gorge	0.0144 (0.0139)	0.0146 (0.0139)	0.0145 (0.0138)	0.0145 (0.0140)	0.0148 (0.0141)	0.0145 (0.0138)	0.0144 (0.0138)	0.1446 (0.0333)	0.0144 (0.0138)	0.0145 (0.0140)	0.6812 (0.0139)	0.0146 (0.0139)	0.0146 (0.0139)	0.0145 (0.0138)
Silaka	0.0187 (0.0176)	0.0186 (0.0174)	0.0185 (0.0175)	0.0187 (0.0177)	0.0186 (0.0176)	0.0186 (0.0175)	0.0185 (0.0175)	0.0917 (0.0346)	0.0185 (0.0174)	0.0186 (0.0175)	0.0186 (0.0176)	0.6854 (0.0179)	0.0186 (0.0176)	0.0185 (0.0175)
Umtamvuna	0.0187 (0.0177)	0.0188 (0.0177)	0.0187 (0.0177)	0.0188 (0.0177)	0.0195 (0.0184)	0.0189 (0.0179)	0.0188 (0.0178)	0.0896 (0.0346)	0.0186 (0.0176)	0.0186 (0.0175)	0.0187 (0.0176)	0.0185 (0.0175)	0.6852 (0.0175)	0.0187 (0.0177)
Xumeni	0.0139 (0.0133)	0.0140 (0.0133)	0.0139 (0.0133)	0.0139 (0.0133)	0.0140 (0.0134)	0.0140 (0.0135)	0.0138 (0.0133)	0.1524 (0.0335)	0.0138 (0.0133)	0.0139 (0.0134)	0.0139 (0.0134)	0.0139 (0.0133)	0.0140 (0.0134)	0.6805 (0.0134)



Appendix S5.7 Isolation by distance plot with G''_{ST} values.

Chapter 6: General discussion and conclusion

This thesis aimed to describe and investigate taxonomic, functional, and genetic diversity of bat communities in forests in south-east South Africa, and their interactions with forest biogeographical history and fragmentation. The key objectives and hypotheses were:

i. To compile a species list and call library to describe and assess the species richness and taxonomic diversity across study forests and forest types. Species richness and diversity were hypothesized to be highest in forests of the Scarp group, and that Scarp assemblages would show greater similarity to both Mistbelt and Coastal forest assemblages than between Mistbelt and Coastal forest assemblages.

ii. To quantify the functional diversity of insectivorous bats in study forests, and to evaluate the effects of forest fragmentation and biogeography on functional diversity and functional traits. It was hypothesized that forest type would be a predominant predictor of functional diversity and that forest cover would demonstrate a positive relationship with functional richness. Species with a reduced dispersal capacity were expected to be more vulnerable to fragmentation.

iii. To test if genetic diversity, population genetic structure and demographic histories of six bat species are informed by their forest habitat associations. The hypotheses were that forest associated species should depict high population genetic structure due to dependence on fragmented habitats, while habitat generalists would show low structuring in response to their widely available habitat use. Additionally, I hypothesized that forest associated species would demonstrate population contractions coinciding with forest contractions during the LGM.

iv. To investigate how the genetic diversity and population structuring of the dusky pipistrelle (*Pipistrellus hesperidus*) is distributed across the region and whether forest fragmentation and urbanisation have affected genetic differentiation.

6.1 Study approach and key findings

In Chapter 2, active capture and passive acoustic monitoring methods were used to survey bat assemblages from 17 forests, of seven forest types and three forest groups, in the Eastern Cape and southern KwaZulu-Natal provinces. Bat community surveys were not known to have been conducted in forested habitats of this region or were poorly recorded. This was the first

knowledge gap filled by this study. A species inventory is presented, consisting of a total of 25 species from eight families. New distribution records were found, extending known distribution ranges further south into the Eastern Cape, for six species. These novel occurrence records and the updated species distribution maps presented here are useful in the compilation of conservation assessments and management action plans for the relevant species. The range extensions further validate the high bat species richness of forested habitats in eastern South Africa as posed by Schoeman *et al.* (2013) and Cooper-Bohannon *et al.* (2016). It also highlighted the conservation value of South African forests, as they host high botanical and faunal biodiversity (Geldenhuys and Macdevette, 1989). This basic inventory may be used towards biodiversity assessments of each surveyed site by forest and nature reserve managers and the compilation of conservation management plans with the knowledge of which bat species occur in the area and their preferred roost and habitat use in mind.

In line with the hypothesis, bat species richness and diversity was highest in Scarp forests which agrees with Lawes *et al.* (2007) that showed Scarp forests served as palaeorefugia to faunal extinctions during the Last Glacial Maximum (LGM). With regards to similarity of species assemblages of the three forest groups, and aligning with the hypothesis, Scarp and Southern Mistbelt assemblages were most similar. This is likely due to their shared evolutionary history being of Afrotemperate origin (Eeley, Lawes and Piper, 1999), as well as post-LGM recolonisation of fauna from Scarp refugia to Mistbelt forests (Lawes *et al.*, 2007). The Albany Coastal assemblage depicted similarity with other sites despite the presence of a biogeographic barrier, the Bedford Gap (Lawes, 1990; Willows-Munro and Matthee, 2011; du Toit *et al.*, 2012; Taylor *et al.*, 2019), isolating this forest type from others. This finding suggests the powered flight of bats allows them to cross this gap more easily than non-volant small mammals.

The first reference call library for forested habitats in the south-eastern region of South Africa is presented. Reference calls are essential for accurate species identification, the library forms the foundations to facilitate future acoustic surveys of forested habitats in South Africa and is relevant for surveys in the south-east of the country. Species identification was performed with a combined approach of morphology, echolocation, and DNA barcodes. The utility of DNA barcoding, using the COI marker for identification, is presented here with emphasis for its use for cryptic species and in ecological surveys where voucher specimens are not taken. Novel barcodes were contributed to the Barcode of Life Data system for several species and the study region. These sequences are valuable for future molecular taxonomic and phylogenetic work

pertaining to the relevant species. This study demonstrates the importance of using a combined approach for both identification and sampling techniques. I recommend employing all available tools, such as morphology, echolocation calls and barcoding, for accurate species identification; as well as active capture and passive acoustic monitoring methods to ensure survey completeness. Lastly, I recommend a minimum acoustic monitoring period of 6 to 7 nights per forest for bat surveys within forested habitats.

In Chapter 3, acoustic surveys and morphological traits were used to quantify four functional diversity indices of the insectivorous bat assemblage from each forest and across forest types. The effects of forest biogeography and fragmentation metrics on functional diversity and species functional traits were assessed. Pondoland Scarp forests were found to host high functional richness, whilst Eastern Cape Dune forests exhibited high functional divergence but low functional richness and dispersion. One of the main findings of this investigation, in line with the hypothesis, was that forest biogeography and the associated vegetation structure is an important determining factor of functional diversity of contemporary insectivorous bat assemblages. Forest biogeography also demonstrated stronger filtering of traits than fragmentation metrics: slow flying and manoeuvrable species were associated with more extensive Mistbelt forest tracts, while fast flying species were selected for by Scarp and Coastal forest types characterised by smaller, scattered patches. This emphasises the large-scale effects of historical processes on shaping contemporary faunal assemblages.

Contrary to the predictions, forest cover did not positively affect functional richness. Rather, a positive relationship of forest edge effects with functional evenness was demonstrated. This finding is likely relevant to temperate forest bat communities as it contrasts with studies conducted in the Neotropics (Meyer *et al.*, 2008; Farneda *et al.*, 2018). Sensitivity of bats to edge effects varies with the degree of contrast between forest and the adjoining matrix, with negative edge effects in high-contrast landscapes (Estrada and Coates-Estrada, 2002; Bernard and Fenton, 2003). The results presented here could inform forest management plan development to benefit bat conservation, through the practical application of maintaining the integrity, structural heterogeneity, and low contrast of forest edges.

Of the fragmentation metrics, forest cover displayed the greatest interaction effect with functional traits. As predicted, increased forest cover selected for species with a lower wing loading, as well as selection for bigger species with a greater forearm length. Our results align with a number of studies demonstrating the importance of forest cover for its selection on bat

functional traits, particularly for larger species and species with a reduced dispersal capacity (Farneda *et al.*, 2015; García-Morales *et al.*, 2016; Wordley *et al.*, 2017). These findings may be used to flag species with these morphological traits as potentially vulnerable to habitat fragmentation. This should be considered when assessing species conservation status and when conducting environmental impact assessments.

In Chapter 4, genetic diversity and population genetic structuring of six species were investigated in light of their forest habitat associations. The comparison was made between three forest associated species and three habitat generalists. Two mitochondrial markers, cytochrome *b* and D-loop, were sequenced from a total of 38 localities across South Africa to generate genetic diversity and population structure metrics, and to investigate demographic histories. Contrary to the hypotheses, common trends of population structure were not evident between species with similar habitat preferences, nor did demographic histories link to historical changes in forest extent. Rather than habitat utilisation, genetic structure was informed by species-specific behaviours and ecological traits. These results emphasise the need to consider life history characteristics when comparing genetic diversity and population structure across taxa. Our study also demonstrates the utility of comparative studies to illuminate species-specific traits that inform population genetics trends. Recently, there have been several studies seeking to clarify the systematics of some of the study species (Taylor *et al.*, 2018; Demos *et al.*, 2019; Hutterer *et al.*, 2019; Monadjem *et al.*, 2020). Understanding the genetic variation and population demographics of these species is a further step towards resolving their taxonomy.

Genetic structuring was evident for *Rhinolophus swinnyi* s.s. despite its limited distribution range in the Eastern Cape and KwaZulu-Natal (Taylor *et al.*, 2018; however see Demos *et al.*, 2019 for argument against the elevation of *R. swinnyi rhodesia*). The low genetic diversity and low gene flow is of concern as *R. swinnyi s.s* is generally considered rare across its range (Monadjem, Taylor, *et al.*, 2010). This species demonstrates wing morphology inferring a comparatively low dispersal ability that may be limiting movement between suitable sites, which suggests limited rescue effects between geographically separate populations. This species is suspected to be in decline, was classed as Vulnerable on the regional red list in 2016 and Endangered on the 2004 national red list (Jacobs *et al.*, 2016). The results presented here flags this species for conservation priority and recommend further molecular work be carried out with microsatellite or single nucleotide polymorphism markers to further assess the state of genetic diversity and gene flow in this species.

Laephotis botswanae presented low genetic diversity and strong population structuring of localities in northern South Africa from southern localities, indicating a lack of genetic exchange across the country as it also demonstrates a low dispersal ability (arising from wing morphology). Addressing the problem of low diversity and restricted gene flow for this species is hampered by the lack of information pertaining to its behaviour and ecology. Roosting behaviour of *L. botswanae* is poorly understood; no information on reproductive behaviour is available (Monadjem, Taylor, *et al.*, 2010); and the abundance of this species is unknown (Taylor *et al.*, 2016). Furthermore, its taxonomy remains unresolved due to the phylogenetic position of *Laephotis* within the *Neoromicia* genus (Goodman *et al.*, 2017; Hutterer *et al.*, 2019). There is an urgent need for further studies to investigate these aspects of this species taxonomy and ecology and to assess its population viability.

Haplotypic segregation was found between Mpumalanga and northern KwaZulu-Natal with southern KwaZulu-Natal and Eastern Cape localities for *Myotis tricolor*. A phylogenetic study partitioned sequences of *M. tricolor* from South Africa into two clades based on mitochondrial DNA (Patterson *et al.*, 2019). There appears to be diversification of this species within South Africa that warrants further study with comprehensive molecular and morphological assessment. Additionally, little is known of the migratory routes and distances of this species. Further study of migratory behaviours may clarify population genetic trends.

Analyses of demographic histories of the six study species provided novel insights of historical growth of effective population sizes for all species. Forest associated species were anticipated to display demographic histories characteristic of past population contractions corresponding to forest contraction during the LGM. However, all species demonstrated population expansions. We provide the dating of these expansions and show that they occurred despite the cold and dry conditions that were prevalent during the LGM, with three species even demonstrating expansions in this glacial period. The maintenance of effective population sizes during the LGM is attributed to the lower climate footprint, maintained forest coverage and refugia of south-east South Africa. I postulate that volant insectivorous bats, at least in eastern South Africa, were less affected by the harsh conditions of the LGM and at an advantage for population growth over phytophagous species. This is an important outcome applicable to the understanding of phylogeographic trends for different taxa across Africa.

In Chapter 5 the genetic diversity, gene flow and migration of *Pipistrellus hesperidus* across 14 forest sites was investigated with the use of eight microsatellite loci. The effect of

contemporary land cover types on genetic differentiation was also examined to assess whether current levels of urbanisation and agricultural development affect gene flow. The data shows gene flow is occurring across sampled sites which infers the novel insight that *P. hesperidus* is not dependent on forested habitats to maintain genetic connectivity on the landscape. Furthermore, not much is known of the gene dispersal and reproductive strategy of *P. hesperidus* within South Africa. Genetic analyses with mitochondrial DNA showed population structuring (Moir *et al.*, 2020), while nuclear markers indicated low structuring. This suggests that gene dispersal is male mediated, and females are philopatric, a critical contribution to the understanding of the ecology and behaviour of this species.

This study provides the first indication of a genetic discontinuity at Oribi Gorge Nature Reserve, possibly resulting from anthropogenic modification of the Mzimkhulu and Mzimkhuluwana rivers that surround this forest. With similar findings for three forest bird species, I motivate for further work to be conducted on these taxa in the Oribi Gorge area and its surrounds. A further genetic break was identified for *P. hesperidus* near Alexandria Forest, coinciding with the biogeographically important break known as the Bedford gap (Willows-Munro and Matthee, 2011). This is the first record of the suggested effect of the Bedford gap on a volant taxon. However, the similarity of Albany Coastal forest assemblages with other forest types (presented in Chapter 2) demonstrates this effect of the Bedford Gap is specific to *P. hesperidus,* and potentially other ecologically similar species. Future research should focus on identifying species and their traits that inform sensitivity of bats to biogeographic breaks.

Migration rates of *P. hesperidus* were generally low between sites except for one scarp forest, Manubi State Forest. I present evidence of the importance of Manubi Forest for the genetic diversity of bats (Moir *et al.*, 2020), in addition to forest bird species and functional richness (Leaver and Cherry, 2020), and motivate for the sustainable management of harvesting practices to conserve this important source of biodiversity. Lastly, agricultural development and urbanisation was found to not yet have a measurable effect on gene flow of *P. hesperidus*. This is likely due to the species having a relatively wide niche breadth and intermediate range (Cooper-Bohannon *et al.*, 2016). Thus, suggesting this species demonstrates a greater flexibility in roost selection, a lower dependency on well-wooded habitats than recorded to date, the ability to disperse through matrix habitats, or a combination of these traits. As not much is known of the roosting ecology and dispersal habitats of this species, I recommend this be investigated further.

Table 6.1 Chapter-wise summary of novel insights and scope of use.

Chapter	Novel insights	Scope
1. Species inventory	List of forest-utilising bats per site; call library for forested habitats; species range extensions; novel DNA barcodes; required survey period	Future bat surveys in forests; species conservation assessments; taxonomic and phylogenetic studies
2. Biogeography and fragmentation effects on functional diversity and functional traits	Biogeography is key determinant of functional diversity and traits; edge effects positively affect functional diversity; large species and/or species with low wing loading are more vulnerable to fragmentation	Bat functional diversity studies; temperate forest management plans; species conservation assessments; environmental impact assessments
3. Population genetic structure and demographic response of six forest-utilising bats	Population genetic trends for each study species; genetic structure informed by species-specific traits; south-east South Africa as a refuge for volant insectivores	Population genetics; species conservation assessments and action plans; phylogeographic trends
4. Gene flow of *Pipistrellus hesperidus*	Population genetics, behavioural, and ecological insights for *P. hesperidus*; locations of genetic breaks	Conservation assessment of *P. hesperidus*; phylogeographic and biogeographic studies

Overall, this thesis used a suite of methods and analytical approaches to understand the distribution of taxonomic, functional, and genetic diversity of bat communities in forests across the Eastern Cape and southern KwaZulu-Natal of South Africa. It has contributed the foundations from which all future bat surveys in forests should work. The study generated novel insights which are transferable to several other contexts (Table 6.1). Findings relevant to bat conservation and forest management have been identified, with an overall significant contribution to existing knowledge gaps in South African bat ecology and population genetics.

6.2 Study limitations and future recommendations

A limitation of this study is that survey methods sampling for species within or above the forest canopies were not used (Chapter 2). As a result, thorough survey of canopy utilising species and the open-air foraging guild is lacking for species richness, taxonomic diversity, and functional diversity estimates. Additionally, reference calls for high flying species are absent from the call library. I recommend canopy nets and canopy acoustic recorders be used for future forest surveys to complete the species inventory and generate reference calls of high-flying species for the region. Furthermore, the surveys consisted of once off visits to study forests while repeat surveys of an area have been shown to record additional species (Brinkley, 2018). Further sampling should be conducted in the 17 forests of this study, as well as further forests not covered in this study, to increase the total species count and thoroughly quantify diversity and activity indices. This study recorded acoustic monitoring data from several point localities within each study forest. The effects of bioclimatic variables on overall and species-specific activity indices was not considered here but can be explored in future given the current data set.

Quantification of functional diversity indices in Chapter 3 was performed with only the results of acoustic monitoring, not considering data generated by the active capture survey methods. As such frugivorous bats, a significant component of forest-utilising bat communities, were excluded. I chose to confine the analysis to the results of the acoustic monitoring program, and hence insectivorous bats, as the sampling design was stringent and the sampling effort standardised, whereas netting and trapping sampling effort was not consistent for all forests. This approach was further justified by the findings of previous studies that phytophagous bats are more tolerant of edge and matrix habitats and therefore fragmentation effects (Klingbeil and Willig, 2009; Farneda *et al.*, 2015). Although fruit bats are not a speciose group in the study region, they occupy a distinct niche and present functional traits largely varying from

those of insectivorous species. This gap should be addressed by future studies whereby fruit bats are used in the assessment of functional diversity in relation to forest fragmentation. This will provide a useful understanding of their sensitivity to fragmentation and determine which functional traits inform this sensitivity. It would be useful to compare those results with studies from the Neotropics and the western Ghats that encompassed frugivores (Meyer *et al.*, 2008; Farneda *et al.*, 2015, 2018; Meyer, Struebig and Willig, 2016; Wordley *et al.*, 2017) to search for meaningful commonalities.

There was notable variability of the functional diversity indices between forests of the same forest type (Chapter 3). The analysis of the effects of fragmentation and biogeography on functional diversity was performed on a landscape scale however, the variability between forests indicates there may be local scale effects on community assembly processes. Different trends or fragmentation effects on functional diversity may become evident if the analyses were performed on a smaller scale, such as per acoustic monitoring location within each forest. Therefore, further work should be carried out to assess the causes of variability of functional diversity at multiple geographic scales, which could be applied to the development of site-specific forest management plans.

Although the molecular data (both mitochondtrial sequence data in Chapter 4 and microsatellites in Chapter 5) were sufficient to detect demographic patterns, population structuring and gene flow for the study species; the low sample sizes for certain species/localities are notable limitations. The low sample sizes limit inferences that can be made from the available data. For example, the overall low sample sizes for *L. botswanae* (*N* = 19) resulted in pairwise population Φ_{ST} values between localities not being statistically significant (Chapter 4). This may have reduced the probability of detecting shared haplotypes and resulted in less robust conclusions regarding gene flow among localities. *Laephotis botswanae* is a somewhat difficult species to capture using conventional mist-netting, is poorly represented in museums (Monadjem, Taylor, *et al.*, 2010), and seems naturally rare (Taylor *et al.*, 2016). Acquiring sufficient samples sizes within a limited time frame is a common challenge for rare and difficult to sample species.

Although, the total sample size of *P. hesperidus* across all sites was fair (*N* = 116), sample sizes were small for a few individual forest sites. This may limit the reliability of population clustering and ordination analyses, and analyses of the migration rate (BayesAss) and genetic barriers (Barrier) for certain localities (Chapter 5). Future studies should ideally accumulate

larger sample sizes and sequence/genotype more loci to build on the results emanating from this study. This will provide further insights to the degree of interconnectivity and possible gene flow among forests for the study species. Additionally, future studies may then be able to identify new localities important for the maintenance of gene flow among fragmented forest habitats, to be included in protected area networks. More sensitive markers such as next generation sequencing and single nucleotide polymorphisms could be employed to complement our understanding of the processes that shape the population genetics of these species.

As mentioned above, the low sample sizes per locality for *Pipistrellus hesperidus* presents a limitation for the interpretations of results, such as the BayesAss analysis of migration identifying Manubi Forest as the primary source of migrants in the study region. Although limited, these results are useful in that they identify Manubi Forest as a site of interest. It is unclear as to why *P. hesperidus* migrants disperse from Manubi to other sites, but do not disperse from other sites towards Manubi using the same routes. This is recommended as the basis for further work on *P. hesperidus* in the region. Future studies should aim to collect more samples for molecular work from sites surrounding Manubi and generally across the study region to increase sample sizes. Additionally, sampling should be conducted further south of Alexandria Forest and north of Oribi Gorge to ascertain if genetic discontinuities and population structuring is evident across these breaks. The inferred population structure of the study species (in both Chapter 4 and 5) are based on the current sampling extent. Although additional museum materials were sourced as widely as possible and all available sequences were retrieved from GenBank, finer scale sampling while simultaneously sampling broadly across the full distribution range of each species will allow for more reliable results and power of analyses. Samples covering a wider geographic range would more accurately describe the total distribution of genetic diversity for all study species, as well as confirm or reject the inferred genetic breaks and migration rates found here for *P. hesperidus*.

6.3 Forest biogeographical history informing contemporary bat communities

A consistently recurring theme in this thesis is the importance of the large-scale historical processes of forest biogeography and associated structure in shaping the contemporary forest-utilising bat communities in South Africa. Lawes *et al.* (2007) found that Scarp forests served as palaeorefugia to faunal extinctions during the LGM from trends in diversities and distributions of forest birds, non-volant mammals and frogs. Additionally, they described post-

LGM dispersal routes of these taxa from Scarp forests to Mistbelt and Coastal forests. Results presented here are the first in support of their work based on the taxonomic, functional, and genetic diversity distribution of bats. Forest biogeography was the primary determinant of functional richness, functional divergence, and functional dispersion of bat assemblages. Bat species richness and taxonomic diversity was highest in Scarp forests, arising from their historical refugia status. Migrants of *P. hesperidus* emanated primarily from a Scarp forest towards Mistbelt and Coastal forests, aligning with the post-LGM dispersal routes posed by Lawes *et al.* (2007). Higher dissimilarity and high nestedness between Coastal and Scarp forest assemblages follow the immigrant pattern (Darlington, 1957) of faunal dispersal from Scarp towards Coastal forests.

This work has identified specific forests important for hosting and maintaining species and genetic diversity for bats. Scarp forests are known for their biodiversity and biogeographical value (von Maltitz *et al.*, 2003). I build on this by providing evidence that they maintain high bat species richness, taxonomic diversity, and functional diversity. The Amatole Mistbelt is recognised as a forest complex supporting unique genetic diversity for several bat species. Fort Fordyce Nature Reserve presented the highest active capture rates, and highly differentiated mitochondrial haplotypes for two bat species, *Laephotis botswanae* and *Myotis tricolor* (Moir *et al.*, 2020), and the dark-footed forest shrew (*Myosorex cafer*) (E. Matamba, unpublished data). The high genetic diversity of *P. hesperidus* found at Fort Fordyce indicates it has been able to support considerable populations of this species and as such was not likely to have been recently founded from a scarp refuge. The microsatellite diversity metrics suggest *P. hesperidus* endured past climate change cycles in small insulated refugia within the Amatole complex (Lawes, 1990). A similar finding was reported for the yellowwood tree (*Streptocarpus*) in this forest complex (Hughes *et al.*, 2005). The Amatole Mistbelt forests form a comparatively large complex (von Maltitz *et al.*, 2003), its wide extent may have facilitated survival of patches during the LGM which enabled the survival of fauna within smaller refugia. The unique genetic diversity and distinct community composition of both volant and non-volant mammals of the Amatole Mistbelt, and specifically Fort Fordyce Nature Reserve, should flag this forest complex as high conservation priority.

While the Eastern Cape Dune forests displayed the highest relative activity levels and activity index (Miller, 2001) as determined from acoustic recorders, they consistently had the lowest species richness, richness estimates, and taxonomic diversity. Eastern Cape Dune forests also exhibited high functional divergence but low functional richness and dispersion. Coastal forests

have a more recent evolutionary history and demonstrate a low, dense, and homogenous canopy distinguishing its vegetation structure from both Mistbelt and Scarp forest types. Although bat species and functional richness was lower than that of other forests, the community composition and function was unique. The unique biogeographic history, vegetation structure, and bat community composition of coastal forests thus deserves conservation priority.

6.4 Use of rivers and waterbodies by the clutter-edge guild

Water sources are well known as hotspots for bats in South Africa, supporting high species richness and high activity levels (Monadjem and Reside, 2008; Sirami, Jacobs and Cumming, 2013; Shapiro *et al.*, 2020; Taylor *et al.*, 2020). However, most work has been conducted in savanna habitats and agricultural landscapes, with few studies investigating the association of bats with rivers and waterbodies in forested habitats (Rautenbach, Whiting and Fenton, 1996). The effect of river length within forest patches on functional diversity of insectivorous bats was investigated here, with a resultant negative relationship of river length with functional dispersion. The association of functional diversity with river length was particularly driven by miniopterid and vespertilionid bats of the clutter-edge foraging guild that hunt for prey along riparian vegetation of watercourses (Monadjem, Taylor, *et al.*, 2010). Similar use of rivers by the clutter-edge guild has been found for savanna habitats (Shapiro *et al.*, 2020; Taylor *et al.*, 2020). However, this study shows how rivers allow for the penetration of clutter-edge species into forest interiors. Furthermore, this is the first study to demonstrate the association of gene flow via water courses for a bat species in South Africa. I show how waterbodies and wetlands, via the significant negative relationship with genetic differentiation of *P. hesperidus*, was the only land cover to be permeable to gene flow on the landscape for this clutter-edge species. Thus, indicating this species likely disperses along riparian habitats and wetlands for genetic connectivity across the landscape. Its utilization of watercourses for gene flow may be representative of the dispersal mechanism of other species of the clutter-edge insectivore guild with similar flight capabilities.

Also, the genetic discontinuity of *P. hesperidus* between Oribi Gorge and neighbouring sites may be attributed to anthropogenic degradation of its surrounding rivers, which may deter *P. hesperidus* from utilising such watercourses, thereby hindering possible dispersal events. Conservation of these habitats is important for maintaining the genetic connectivity of *P. hesperidus* populations, and possibly other species of this guild, as well as habitat use by the clutter-edge guild, and therefore the important ecosystem services they provide. In the USA,

not only is the extent of wetland habitats an important determinant of bat activity, but also the connectivity of the wetland to the watercourse system (Lookingbill *et al.*, 2010). It is thus important to conserve not only the functionality and integrity of these habitats, but also their connectivity to the greater river system. Conservation efforts should be channelled into the maintenance and improvement of wetlands, rivers and associated riparian habitat, and the linkage of these habitats across barren lands, agricultural expanses and developed areas.

6.5 Towards the conservation of Eastern Cape forests

Several large-scale developments are currently under way or planned for the Eastern Cape province. An upgrade to the N2 Wild Coast Toll Highway is planned to construct new sections of road linking East London in the Eastern Cape with Durban in KwaZulu-Natal, with a section of the upgrade transecting through the Pondoland Centre of Endemism (PCE) (Clarke, De Kock and Carter, 2019). A key mitigation measure for the environmental impacts of the proposed N2 upgrade on the PCE is the development a large consolidated conservation area, Pondoland Park, consisting of Mkambati Nature Reserve, the Mkambati Palms National Monument, a small marine reserve, numerous indigenous forest reserves, and large tracts of state land (Clarke, De Kock and Carter, 2019). However, Xolobeni Heavy Minerals Sand Project proposes to mine sand dunes for titanium along a 22 km stretch of the Wild Coast within an area that would constitute the proposed Pondoland Park (Bennie, 2010). If permitted, this mining would result in the destruction of the Near Threatened coastal dune forests. Lastly, two multi-purpose storage dams, Ntabelanga dam and Laleni dam, are proposed for development on the Tsitsa River in the Tsitsa Catchment Area (Van Tol *et al.*, 2018). All of the aforementioned developments occur within and between the surrounds of forested areas in the Eastern Cape, having either direct or possibly indirect negative impacts on forest habitats. Yet, consideration of the impacts on forests are not reflected in the publicly-available environmental impact assessment reports.

This study is one of many of a research group, spearheaded by Prof. Michael Cherry and funded by the Foundational Biodiversity Information Programme (FBIP), that has sought to survey the biodiversity of forests across the Eastern Cape province. Over the last four years, the research group has generated a wealth of information concerning the diversity, systematics, community ecology, phylogeography, phylogenetics, population genetics etc. of a range of floral, invertebrate and vertebrate faunal groups, as well as the anthropogenic impacts of forest product harvesting (Busschau *et al.*, 2017; Cooper, Wannenburgh and Cherry, 2017; Daniels,

2017; Daniels, Dreyer and Sharma, 2017; Opperman, Cherry and Makunga, 2018; Barnes and Daniels, 2019; Busschau, Conradie and Daniels, 2019; Leaver *et al.*, 2019; Moir *et al.*, 2020; Mulvaney and Cherry, 2020; Deng *et al.*, 2020; Kushata *et al.*, 2020; Leaver and Cherry, 2020). It is clear that these forests support extraordinary biodiversity of both floral and faunal taxa, and that the quality, integrity and interconnectivity of forest patches scattered throughout the province are essential for maintaining the connectivity and viability of populations of these taxa. There is a need to now consolidate the pertinent findings of this body of work relevant to assessing and mitigating the environmental impacts of the aforementioned large-scale developments on forests in the province. Application based results of this research need to be distilled as vital data that may contribute to the development of locally-relevant and ecologically-informed management plans for the conservation of indigenous forest in this region.

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