Phylogeography and co-evolution of ectoparasitic mites that live on the rodent host species *Lemniscomys rosalia* (Rodentia: Muridea)

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Abstract

Lemniscomys rosalia is a widespread, possibly cryptic species of murid grass mouse. Cytogenetic evidence points towards cryptic speciation in *L. rosalia* as the eastern African karyotype (2N = 54; FNA = 62) differs from the southern African karyotype (2N = 48; FNA = 62). Further resolution through phylogeographic analysis is necessary to corroborate this hypothesis; however, there is little to no molecular data on *L. rosalia*. The present study sought to determine the phylogeographic patterns found in southern African populations of *L. rosalia* using mitochondrial and nuclear markers. Parasites are commonly used to complement the genealogy of their host species since both species may undergo co-phylogeny. *Laelaps giganteus* is an ectoparasite found on *L. rosalia* and it may be a good candidate to test co-phylogeny, since it is thought to be a specialist mite. DNA extracted from museum specimens and a few supplementary field samples were sequenced for cytochrome oxidase 1 (COI) and internal transcribed spacer 1 (ITS1). The sequences were analysed using Bayesian Inference and Maximum Parsimony to represent the phylogenies of *L. rosalia* and *L. giganteus*, respectively.

Haplotype networks were constructed and Analyses of Molecular Variance was run for both host and parasite. The haplotype network and Bayesian Inference phylogram for COI separate southern African populations of *L. rosalia* from Tanzania. A co-phylogeny was run using CO1 and all 85 solutions had a low cost of 15, suggesting strong congruence.

There are many examples of widespread mammal species with a phylogeographic split between eastern and southern Africa. Differences in congruence between these hosts of *L. giganteus* is likely attributable to life history traits. It is plausible limited dispersal in *L. giganteus* may have caused the low number of host switching events during host distribution changes. This study provides additional evidence for cryptic speciation within *L. rosalia*, but wider sampling is

necessary to make more robust conclusions. It would be useful to construct a phylogeny of *L. giganteus* as a whole to determine if there is a co-phylogeny in eastern African populations.

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Chapter 1: Introduction

1.1 Phylogeography

Phylogeography is the analysis of the phylogeny of an organism in the context of its geographical distribution (Avise 2000; Hickerson *et al.* 2010; Avise *et al.* 2016). Essentially, phylogeography makes use of molecular markers to explain biogeographic patterns. The field is relatively new as it developed in the late 20th century (Avise 2009). The advent of polymerase chain reactions and the accessibility of mitochondrial DNA lead to an increase in the use of phylogeography as many modern phylogeographic studies rely on DNA analyses (Avise 2000; Emerson & Hewitt 2010). Evolutionary significant units are used in conservation and are defined by phylogeographic analyses (Moritz 1994). Phylogeography can be used to define the species of widely spread terrestrial small mammalian species, murid rodents

There are several examples of widespread murid species that have been shown to be composed of multiple cryptic species. Recent phylogeographic studies of widely distributed species, such as the vlei rat *Otomys irroratus* (Engelbrecht *et al.* 2011), Namaqua rock mouse *Micaelamys namaquensis* (Russo *et al*. 2010), bush karoo rat *Myotomys unisulcatus* (Edwards *et al.* 2011), and four striped mouse *Rhabdomys pumilio* (Rambau *et al.* 2003; Du Toit *et al.* 2012) have depicted cryptic speciation.

In contrast, phylogeographic studies can also show that species boundaries are maintained in widespread species. There is a high degree of haplotype sharing amongst both *Mastomys coucha* and *Mastomys natalensis* females within the southern African range (Sands *et al.* 2015). Rodent diversification is influenced not only by phylogeographic barriers but also by paleoclimatic factors and life history traits (Sands *et al.* 2015). Molecular data has shown

evidence of mammal species cycling between isolation (within refugia) during unfavourable conditions and population expansion once conditions became favourable (Nicolas *et al.* 2008; Sands *et al.* 2015). Many of these refugia for these mammal species are found in the eastern regions of Africa (Nicolas *et al.* 2008). *Lemniscomys rosalia* is a widely distributed, murid rodent in the eastern region of sub-Saharan Africa (Happold 2013). Very little is known about the factors possibly influencing speciation in *L. rosalia* (Castiglia *et al.* 2002; Monadjem *et al.* 2016).

1.2 Single striped mouse taxonomy

The species of the single striped grass mouse *Lemniscomys rosalia* (Thomas 1904) is classified to the family Muridae in the order Rodentia and belongs to a monophyletic genus of Arvicanthine murids. Many members of the Muridae family are considered pest species, as they damage crops, destroy man-made infrastructure, and are known to transmit disease (Begon *et al.* 1999; Bastos *et al.* 2005; Nicholas *et al.* 2012). The genus *Lemniscomys* (Trouessart 1881) contains either 11 (Happold 2013) or 9 (Monadjem *et al.* 2015) recognized species of small to medium sized grass mice. Pelage pattern and colouration separates *Lemniscomys* into three groups (Happold 2013). The *griselda* species group with absent or faint lateral stripes, the *barbarus* species group with unbroken lateral stripes, and lastly the *striatus* species group with continuous lateral lines to the mid-dorsal line but laterally broken into spots (Happold 2013). *Lemniscomys rosalia* is the most widely distributed species of the *griselda* species group (Happold 2013).

Further resolution of *L. rosalia* as a single species is necessary as cytogenetic analysis of *L. rosalia* shows two distinct karyotypes within the species (Castiglia *et al.* 2002). The karyotype of the Tanzanian *L. rosalia* (2N = 54; FNA = 62) differed considerably from the South African karyotype (2N = 48; FNA = 62) (Ducroz *et al.* 1999; Castiglia *et al.* 2002). Contemporarily, no molecular based studies have been done for this small mammal species despite the fact that the sister species of *L. rosalia*, *R. pumilio* is very well studied (Rambau *et al.* 2003; Castiglia *et al.* 2011; Du Toit *et al.* 2012; Ganem *et al.* 2012; Le Grange *et al.* 2015). Co-phylogeny studies involving small mammals have been done on fossorial mammals and their parasites (Hafner *et al.* 2003), the field mouse *Apodemus sylvaticus* and the endoparasitic intestinal roundworm *Heligmosomoides polygyrus* (Nieberding *et al.* 2004), and the four striped mouse *Rhabdomys* and its ectoparasitic louse *Polyplax arvicanthis* (Du Toit *et al.* 2013), to name a few. The Ixodid tick *Rhipicephalus* and two species of the ectoparasitic flea genus *Crocidura* have been reported on *L. rosalia* (Horak *et al.* 2005; Shihepo *et al.* 2008).

1.3 Parasites and co-phylogeny

Several host-parasite interaction studies have been published due to the potential impact of parasites on the health of wildlife, domesticated animals, and humans (Hafner *et al.* 2003; Nieberding & Olivieri 2006; Lareschi *et al.* 2019). In conjunction, parasites are commonly used to complement the genealogy of their host species since both species may undergo co-phylogeny (Hafner *et al.* 2003; Nieberding *et al.* 2004; Nieberding & Olivieri 2006; Du Toit *et al.* 2013; Engelbrecht *et al.* 2016). Parasites have also been known to drive ecology in host species (Matthee *et al.* 2007). Parasites control natural population fluctuations in the host species (Matthee *et al.* 2007). In the European Green Crab (*Carcinus maenas*), native

population growth was kept under control by parasites while introduced populations suffered less from parasitism (both endoparasitic and ectoparasitic) as well as predation (Torchin *et al.* 2001).

In most host-parasite relationships the parasite takes energy from the host while the host tries to prevent this energy loss through an evolutionary 'arms race' (Hafner *et al.* 2003). Parasites have a shorter generation time and, under certain circumstances, a smaller effective population size compared to that of their host species thus acting as a magnifying glass of the host evolutionary trajectory through time (Nieberding & Olivieri 2006). Host-parasite co-evolution is dependent on the strength of the relationship between host and parasite (Hafner *et al.* 2003; Nieberding & Olivieri 2006). The host-parasite relationship is likely congruent, provided the parasite is specific and obligate (Hafner *et al.* 2003). Host specificity is one of the main influences on the congruence of host-parasite genealogies since the life histories of both species are linked (Clayton & Johnson 2003; Nieberding & Olivieri 2006). The presence or absence of a free-living stage and an intermediate host also influences congruence (Clayton & Johnson 2003; Nieberding & Olivieri 2006).

1.4 *Laelaps* life history

Laelapine mites (Order: Mesostigmata) complete the majority of their life cycle in the nest of the mammalian/vertebrate host (Radovsky 1994). Host specificity in Laelapine mites can be conflicting (Martins-Hatano *et al.* 2002). Laelapine mites are mostly monoxenous (parasite development is restricted to a single host species), and each mite species has a separate host species (Martins-Hatano *et al.* 2002). However, there are reported cases of polyxenous laelapine species (Engelbrecht *et al.* 2014). Based on the mitochondrial Cytochrome Oxidase

I (COI) and nuclear Internal Transcribed Spacer (ITS) genes, *Laelaps giganteus* has shown cryptic speciation (Engelbrecht *et al.* 2016). Since *L. giganteus* has shown host specificity within *Rhabdomys* spp., it may be a good candidate to test host-parasite co-phylogeny (Engelbrecht *et al.* 2016).

Laelaps giganteus is possibly a specific parasite of *L. rosalia* while it is not an obligate parasite. These mites have a nidicolous lifestyle as the offspring stay within the nest of host for a long time, much like most Laelapine mites (Martins-Hatano *et al.* 2002). Males and the immature mites are often restricted to the nest of the host in nidicolous Laelapine mites (Radovsky 1985; 1994). In nidicolous mites, dispersal is primarily vertical and mite populations tend to be homogenous and specific (Martins-Hatano *et al.* 2002).

The poultry mite genus *Dermanysus,* is closely related to free-living Laelapine mites (Roy & Chauve 2007). Female *Dermanysus* adults, deutonymphs and protonymphs have thin and elongated chelicerae adapted to haematophagy (Roy & Chauve 2007). The chelicerae of Laelapine mites are also somewhat adapted to haematophagy, yet they are mostly adapted for grasping and tearing cuticles (Martins-Hatano *et al.* 2002). *Laelaps giganteus* has similar life history traits to *Dermanysus,* with females primarily adapted for haematophagy (Martins-Hatano *et al.* 2002). For these reasons, the ratio of *L. giganteus* found on rodents is heavily sex-biased towards female mites (Matthee *et al.* 2007).

1.5 Museum material and field sampling

There are certain advantages and disadvantages to using museum material in phylogeographic studies. Museum material preserved in 100% ethanol can yield the same quantities of total genomic DNA in comparison to fresh specimens (Dillon *et al.* 1996; Jaksch *et al.* 2016; Schäffer *et al.* 2017). Using museum samples benefits live specimens, especially in vulnerable taxa (Barbanera *et al*. 2016). However, older museum specimens may have been misidentified, leading to confounding results (Phukuntsi *et al.* 2016). It can often be very difficult to find good quality bio-banked tissue samples when using museum material (Haring *et al.* 2012). In some cases, with degraded historical museum material, long sequences of DNA cannot be amplified (Haring *et al.* 2012; Jaksch *et al.* 2016; Lalonde & Marcus 2020).

1.6 Genetic markers

The ITS nuclear gene marker has often been used in genus and species delimitation in fungi (Glass & Donaldson 1995; Homan *et al.* 1997; Vu *et al.* 2019). Roy *et al.* (2009) used ITS1 and CO1 as part of a total evidence approach with the mite *Dermanyssus*. However, many identical ITS1 populations were separated by using the mitochondrial DNA markers CO1 and 16S (Roy *et al.* 2009). Roy *et al*. (2010) used another nuclear marker Tropomyosin (TropoM) to test reproductive isolation in *Dermanyssus.*

Mitochondrial DNA is the genetic marker of choice in phylogeographic studies of Baker *et al.* (1993) and Ball *et al.* (1988). Mitochondrial DNA replication is asynchronous with cell division and occurs more frequently than nuclear DNA replication, making mtDNA more suitable for detecting intraspecific variation (Avise 2009). Mitochondrial DNA lacks

recombination as it is inherited maternally (Avise 2009). Cytochrome oxidase subunit I (CO1) barcoding is generally used to separate species of the same genus (Hebert *et al.* 2003; Dawnay *et al.* 2007).

DNA barcoding uses a short gene from the CO1 locus of the mitochondrial genome to identify species (Nicolas *et al.* 2012). While CO1 is the barcoding gene, cytochrome *b* is effective at separating species and has been widely used for mammalian DNA analysis (Baker & Bradley 2006; Nicolas *et al.* 2012). Nicolas *et al.* (2012) compared the effectiveness of CO1 and cyt *b* at separating genera of the Murinae subfamily. These included seven of the eight genera in the Praomyini tribe (*Colomys*, *Zelotomys*, *Heimyscus*, *Hylomyscus*, *Mastomys*, *Myomyscus*, *Praomys*) (Nicolas *et al.* 2012). Both these markers were found to differentiate between Praomyini species better when compared to 16s. Congeneric species exhibit substantial divergences in the sequence of the COI gene (Hebert *et al.* 2003). Both CO1 and cyt *b* are considered for DNA barcoding purposes.

1.7 Aims and objectives

The current study aims to:

- Elucidate the phylogeographic patterns of *L. rosalia* based on COI marker and using Maximum Parsimony and Bayesian Inference analyses
- Describe the co-phylogeography of *L. rosalia* and *L. giganteus* based on COI and ITS1 markers and using the co-phylogeny software Jane.

The mitochondrial DNA marker COI will be compared to the nuclear DNA markers ITS1 and TropoM (Engelbrecht *et al.* 2014). We do not expect a difference in the patterns between the markers due to the dispersal of male and female parasites i.e., female parasite bias. The COI marker will be used in the current study to compare host and parasite sequences in the co-phylogeny. Engelbrecht *et al.* (2014) reported the occurrence of *L. giganteus* on *L. rosalia*. This study showed evidence of cryptic diversity among a lineage of *L. giganteus* associated with *Rhabdomys* species. There is a possibility that a similar pattern can be seen in the lineage found on *L. rosalia* (Engelbrecht *et al*. 2014); however, limited sampling prevented conclusions that are more robust.

Chapter 2: Phylogeography of *Lemniscomys rosalia*

The single striped mouse, *Lemniscomys rosalia*, is a widely distributed herbivorous murid species, which occurs throughout much of sub-Saharan Africa (Castiglia *et al.* 2002; Monadjem *et al.* 2016). *Lemniscomys rosalia* is a seasonal breeder; it is reproductively active during the summer rain season and uses tall grass for cover and grass nests to safely bear its young (Monadjem & Perrin 1997; Hagenah *et al.* 2009). The removal of tall grass cover by fires leads to the evacuation of *L. rosalia* from an area (Monadjem & Perrin 1997). However, a study done by Fitzherbert *et al.* (2007) showed no association between *L. rosalia* and the tall grass habitat but rather showed an association with a variety of grass habitats. *Lemniscomys rosalia* is crepuscular with individuals most active during twilight (Perrin 1981). The distribution of *L. rosalia* is mostly restricted to the eastern regions of southern Africa (Fig. 2.1).

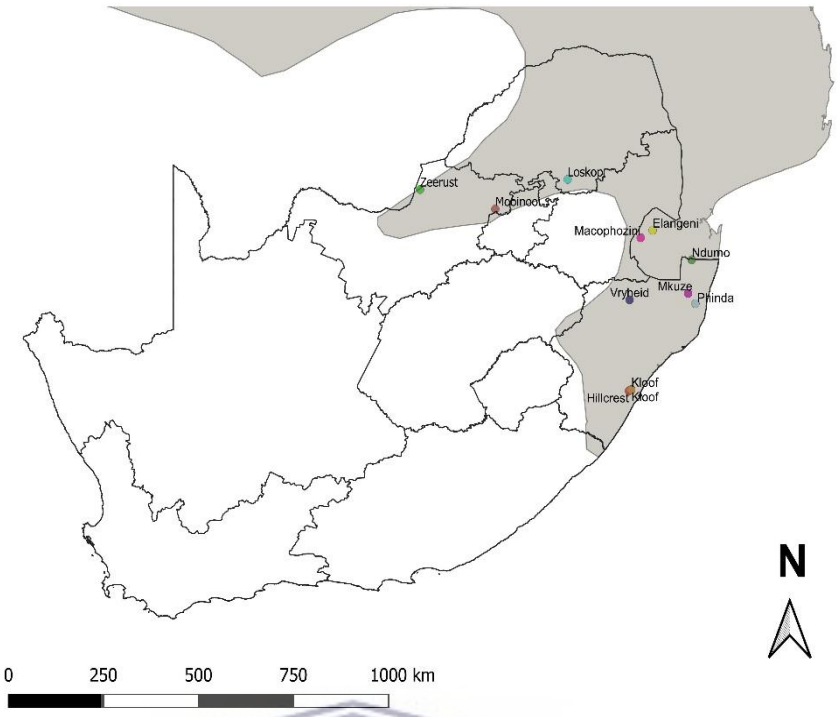

Figure 2.1: Map of the distribution of *Lemniscomys rosalia* within southern Africa (depicted by the grey area). Each colour dot on the map represents a different locality used in this study, as follows. Mooinooi = Orange; Zeerust = Dark Green; Loskop = Light Blue; Hillcrest = Red; Phinda = Blue; Ndumo = Light Green; Vryheid = Dark Blue; Kloof = Brown; Elangeni = Yellow; Macophozini = Purple; Mkuze = Violet.

Recent phylogeographic studies have been done on other rodent species complexes within the same distribution as *L. rosalia*. Two similarly widespread species of the genus *Mastomys* have a high haplotype sharing across their distribution (Sands *et al.* 2015). *Mastomys coucha* and *M. natalensis* were found to survive unfavourable conditions in refugia and extend their distributions during times of grassland expansions (Sands *et al.* 2015). Multiple lineages are found within *Rhabdomys dilectus* as it has different karyotypes within the species (Castiglia *et al.* 2011). *Rhabdomys dilectus*, as well as multiple other mammal species, have a

phylogeographic split between eastern and southern Africa (Castiglia *et al.* 2011). The presence of refugia in these widespread species during grassland expansion and shrinking events could affect *L. rosalia* as well (Russo *et al.* 2006; Castiglia *et al.* 2011; Sands *et al.* 2015).

Lemniscomys rosalia may represent a species complex as it has two different karyotypes. Usually, a species will have a single karyotype or chromosome number and when there is a marked difference, they might be different species (Castiglia *et al.* 2011). The Tanzanian karyotype (2N = 54; FNA = 62) differs from the southern African karyotype (2N = 48; FNA = 62). Castiglia *et al.* (2002) suggested the name *L. rosalia* remains with Tanzanian species while the southern African species should be changed to *L. calidior* Thomas & Wroughton, 1908. The Zambezi River is a known barrier of gene flow between these karyotypes (Hánová *et al.* 2020). Hánová *et al.* (2020) has suggested that the two populations are distinct parapatric subspecies.

2.1 Study site and species sampling

Samples of *L. rosalia* and *Laelaps giganteus* were obtained from Durban Natural Science Museum collected throughout the distribution of the species (Fig. 2.1). *Lemniscomys rosalia* were caught across the remaining distribution in South Africa, according to Monadjem *et al.* (2016), with Sherman live traps (Fig. 2.1). Forty traps per site were set in trap quadrats, 5 m apart, with a peanut butter and oats mixture as bait. Traps were checked twice a day, baited, and set at night. Sampling took place over the span of a week at Loskop, Limpopo during 2019. The hosts had fur clipped in order to prevent recapture. Host DNA samples were taken by scraping the inside of the mouth to collect buccal epithelial cells (Ethical clearance number 15/2/20) (Meldgaard *et al.* 2004). The host DNA were preserved and extracted in a similar

method to Meldgaard *et al.* (2004). Field samples were taken from the Loskop, Limpopo locality while the rest of the samples were museum specimens.

2.2 Localities

Localities within South Africa included Mooinooi and Zeerust in the North West Province, Loskop in Limpopo, and Hillcrest, Phinda, Ndumo, Vryheid, Kloof and Mkuze in KwaZulu-Natal. Locations outside of South Africa include Mcaphozini and Elangeni (eSwatini) and a single locality within Tanzania (Fig. 2.1). Mostly female mites were used in the study due to the sex ratio being female biased (Engelbrecht 2016).

2.3 DNA extraction, PCR and genetic sequencing

Total genomic DNA were extracted from the body of the mite and the tissue of the host using a commercial DNA extraction kit (Macherey-Nagel DNA kit) following the protocol of the manufacturer. Tissue samples were digested at 56 ˚C overnight. Extracted DNA was stored at -20 °C. The mitochondrial COI gene was amplified using the universal primers LCO1490 and HCO2198 to amplify 708base pairs of the COI gene (Folmer *et al.* 1994). PCR reactions were carried out using 25 µl reaction volumes in a BioRad® T100 thermal cycler. COI regions were amplified via a 'cold start' reaction consisting of a denaturation cycle of 1 min at 94 °C followed by a 10-cycle loop of 1 min at 95 °C, 45 °C and 72 °C, respectively. A 35-cycle loop was then followed using the exact same conditions apart from increasing the annealing temperature to 50 °C. The ITS1 nuclear gene was amplified with a 'hot start' of 95 °C for 1 min followed by a 10-cycle loop of 1 min at 95 °C, 45 °C and 72 °C, respectively. A 35-cycle

loop was then followed using the exact same conditions apart from increasing the annealing temperature to 59 °C. Amplification of TropoM was attempted with the same conditions as ITS1. All reactions ended with a final 5 min extension period at 72 °C. Five microlitres of the PCR products were visualized on a 1% agarose gel. The remaining 20 µl of the PCR product was cleaned and cycle sequenced through BigDye Chemistry and analysed at Stellenbosch University Central Analytics Facilities with an ABI 3730 XL DNA Analyzer (Applied Biosystems, Inc.).

2.4 Statistical analysis for phylogenetics

The best-fit model was chosen using the program MODELTEST ver. 3.06 (Posada and Crandall 1998). The sequences were aligned through the multiple sequence alignment software MAFFT (Katoh *et al.* 2019). Phylogenetic relationships within the hosts and parasites were inferred using Maximum Likelihood (ML) and Maximum Parsimony (MP) as implemented in PAUP*4 ver. beta 10 (Swofford 2002). Confidence in nodes were determined with the aid of bootstrapping. One thousand bootstrap replications were performed for MP. Bootstrap values above 75% are considered well supported, while bootstrap values below 75% are considered poorly supported (Felsenstein 1985). Further Bayesian analysis was done using Cipres (http://www.phylo.org/) with the MrBayes v3.2.6 extension (Ronquist & Huelsenbeck 2003). A total of 10 million generations were used while four chains were sampled every hundred generations. The parameter values were summarized with sump and trees were summarized with sumt to visualise the statistical support of nodes, both according to the burn-ins of the mcmc command. The trees were viewed in FigTree version 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

2.5 Population genetics analysis

Haplotype networks were constructed using the method of Templeton *et al.* (1992), using TCS ver. 1.21. Haplotype and nucleotide diversity along with neutrality tests and mismatch distribution were calculated by using DnaSP ver. 6.12 (Rozas *et al.* 2017). The neutrality tests are there to determine whether a population fits the neutral model (Fu & Li 1993), while mismatch distribution tests whether the population is expanding or contracting. Tajima's D compares the average pairwise differences with the number of segregated sites (Tajima 1989). The population genetic structure was analyzed using an Analysis of Molecular Variance (AMOVA) with one thousand permutations in Arlequin v3.5 (Excoffier & Lischer 2010).

2.6 General gene sequencing

DNA was extracted from 28 samples of *L. rosalia* and 40 samples of *L. giganteus*. *Lemniscomys rosalia* had 25 successful COI sequences of 652 base pairs while multiple attempts were made to sequence ITS1 and TropoM, without success. *Laelaps giganteus* had a total of 59 COI sequences of 576 base pairs. The ITS1 gene for *L. giganteus* had 27 sequences of 459 base pairs. Ramp-up PCRs were attempted for ITS1 with degraded samples.

Table 2.1: Locality data and number of samples for cytochrome oxidase 1 (COI) per locality for *Lemniscomys rosalia*.

Locality	Location	Coordinates S (°)	Coordinates E (°)	No. of samples
Elangeni	eSwatini, Elangeni.	-26.424986	31.210921	3
Hillcrest	RSA, KwaZulu-Natal Province: Hillcrest, Springside Nature Reserve.	-29.782296	30.776242	5
Kloof	RSA, KwaZulu-Natal Province: Kloof, Krantzkloof Nature Reserve.	-29.772489	30.830554	2
Loskop	RSA, Limpopo Province.	-25.313645	29.324668	2
Macophozini	eSwatini, Mcaphozini.	-26.418100	31.191400	1
Mkuze	RSA, KwaZulu-Natal Province: Mkuze, uMkuze Game Reserve.	-27.644785	32.151463	2
Ndumo	RSA, KwaZulu-Natal Province: Ndumo, Ndumo Game Reserve.	-26.911196	32.264083	3
Phinda	RSA, KwaZulu-Natal Province: Phinda, Phinda Private Game Reserve.	-27890257	32.209745	5
Tanzania	Tanzania, BEA CMR grid.			1
Vryheid	RSA, KwaZulu-Natal Province: Vryheid; Vryheid Hill Nature Reserve.	-27.750835	30.795585	1

Table 2.2: Locality data and number of samples for internal transcribed spacer (ITS1) and cytochrome oxidase 1 (COI) per locality for *Laelaps giganteus*.

Locality	Location	Coordinates S (°)	Coordinates E (°)	No. of samples COI	No. of samples ITS1
Hillcrest	RSA, KwaZulu-Natal Province: Hillcrest, Springside Nature Reserve.	-29.782296	30.776242	9	26
Loskop	RSA Limpopo Province.	-25.313645	29.324668	6	6
Mooinooi	RSA, North West Province.	-25.753620	27.557250	23	0
Ndumo	RSA, KwaZulu-Natal Province: Ndumo, Ndumo Game Reserve.	-26.911196	32.264083	0	1
Zeerust	RSA, North West Province.	-25.544580	26.101110	21	0

2.7 Haplotype network for *Lemniscomys rosalia*

Only one grouping shares a haplotype over multiple regions (Phinda, Elangeni and Vryheid in KwaZulu-Natal) (Fig. 2.2). There were two branches separated from the main multi-region haplotype. Firstly, two Phinda sequences and secondly Hillcrest and Elangeni formed separate branches. There were three more separate groupings of haplotypes. Namely, an Elangeni (eSwatini) and Phinda (RSA: KwaZulu-Natal) haplotype group, two Loskop sequences and lastly, two Hillcrest sequences with a single Ndumo sequence. The remaining sequences showed no haplotype sharing.

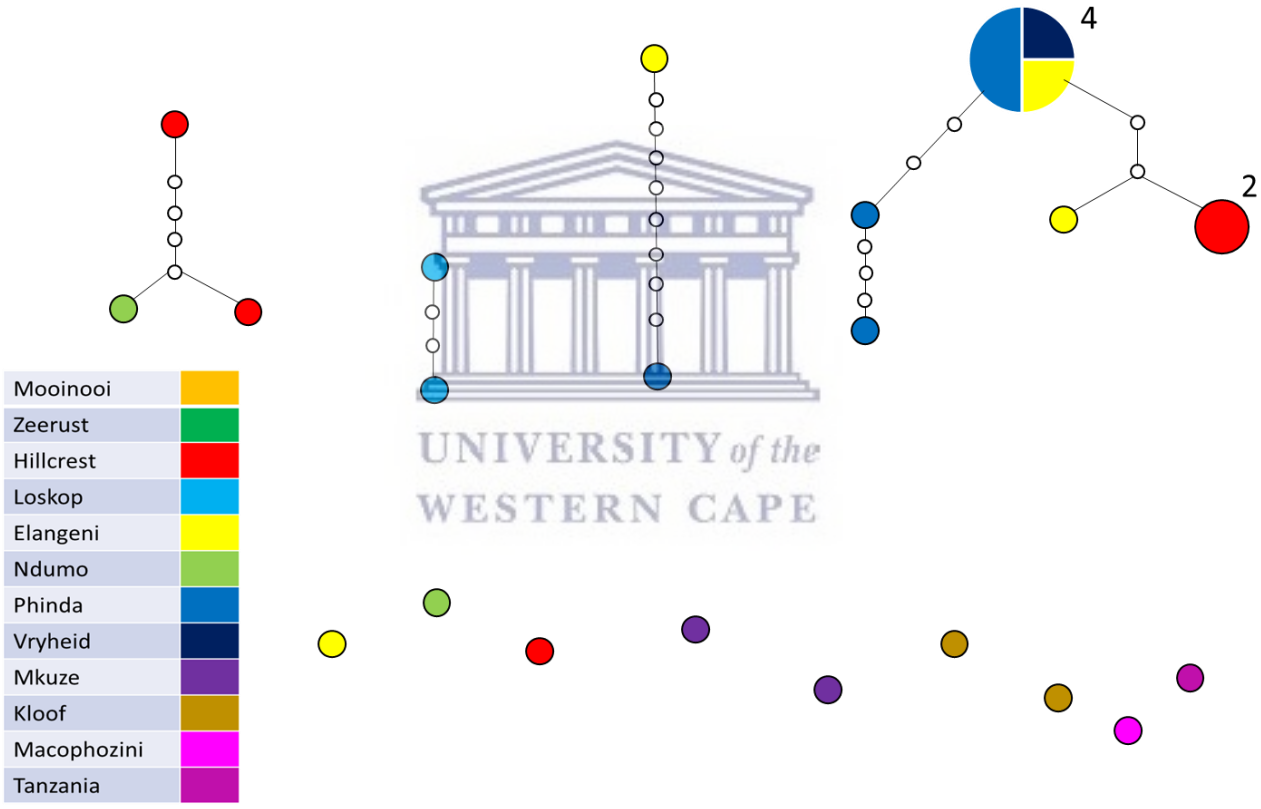

Figure 2.2: Redrawn minimum spanning haplotype network of *Lemniscomys rosalia* for the mtDNA cytochrome oxidase 1 (CO1) gene marker at 95% confidence levels. Each colour indicates a separate locality while blank circles represent mutational steps between observed haplotypes. The size of the circles corresponds with the number of sequence repetitions per haplotype.

2.8 Phylograms for *Lemniscomys rosalia*

Both the Maximum Parsimony (MP) (Fig. 2.3) and Bayesian Inference (BI) (Fig. 2.4) phylograms were used since they each exhibited a unique structure. Specifically, the large amount of polytomy in the MP derived phylogram and the lack of separation of the main grouping in the BI derived phylogram that differs from MP. Both phylograms had Kloof placed loosely together with Mcaphozini (eSwatini) and Tanzania.

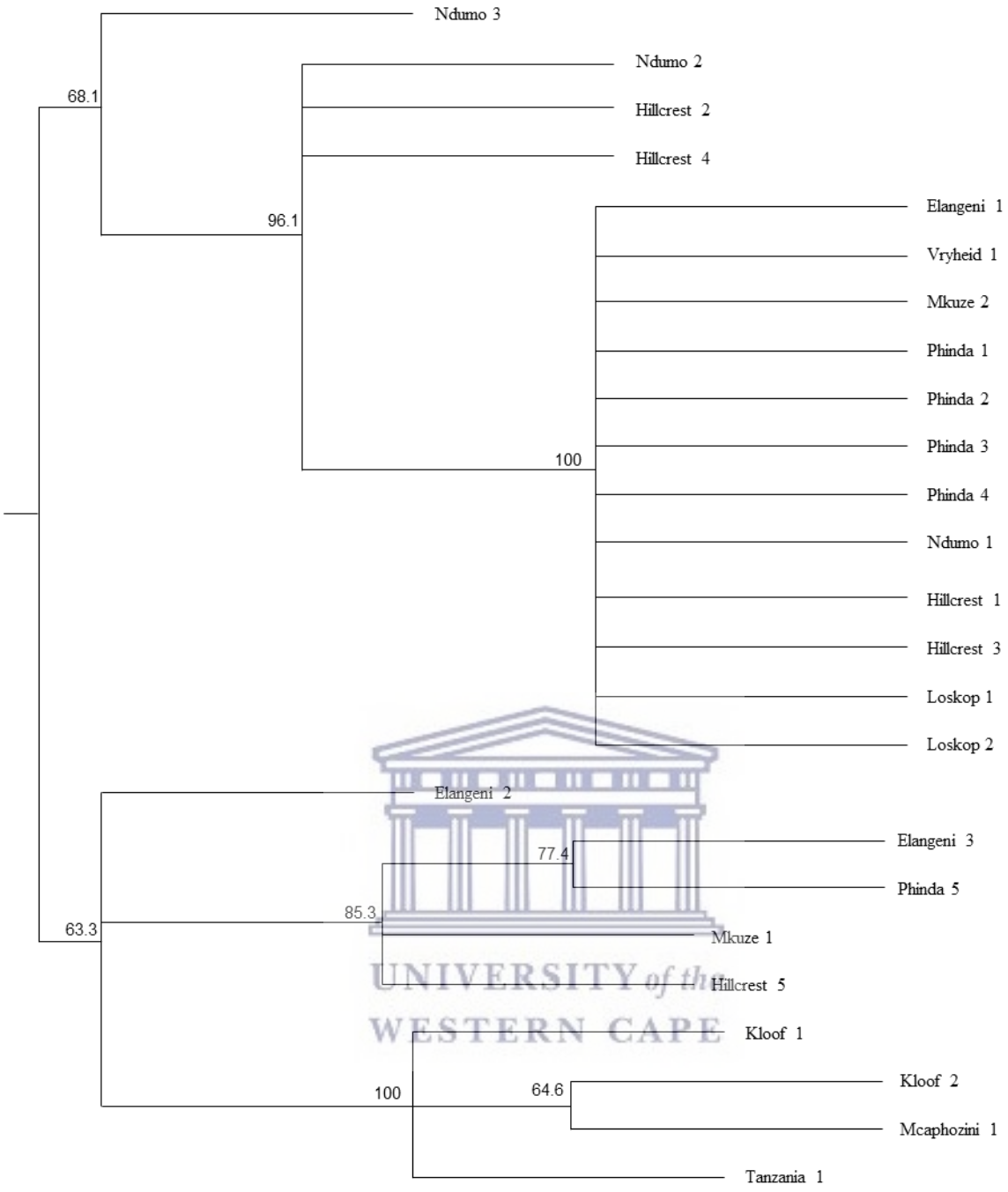

Figure 2.3: The Maximum Parsimony phylogram for *Lemniscomys rosalia* using the cytochrome oxidase 1 (CO1) gene (652 base pairs). The percentages represent support values for nodes.

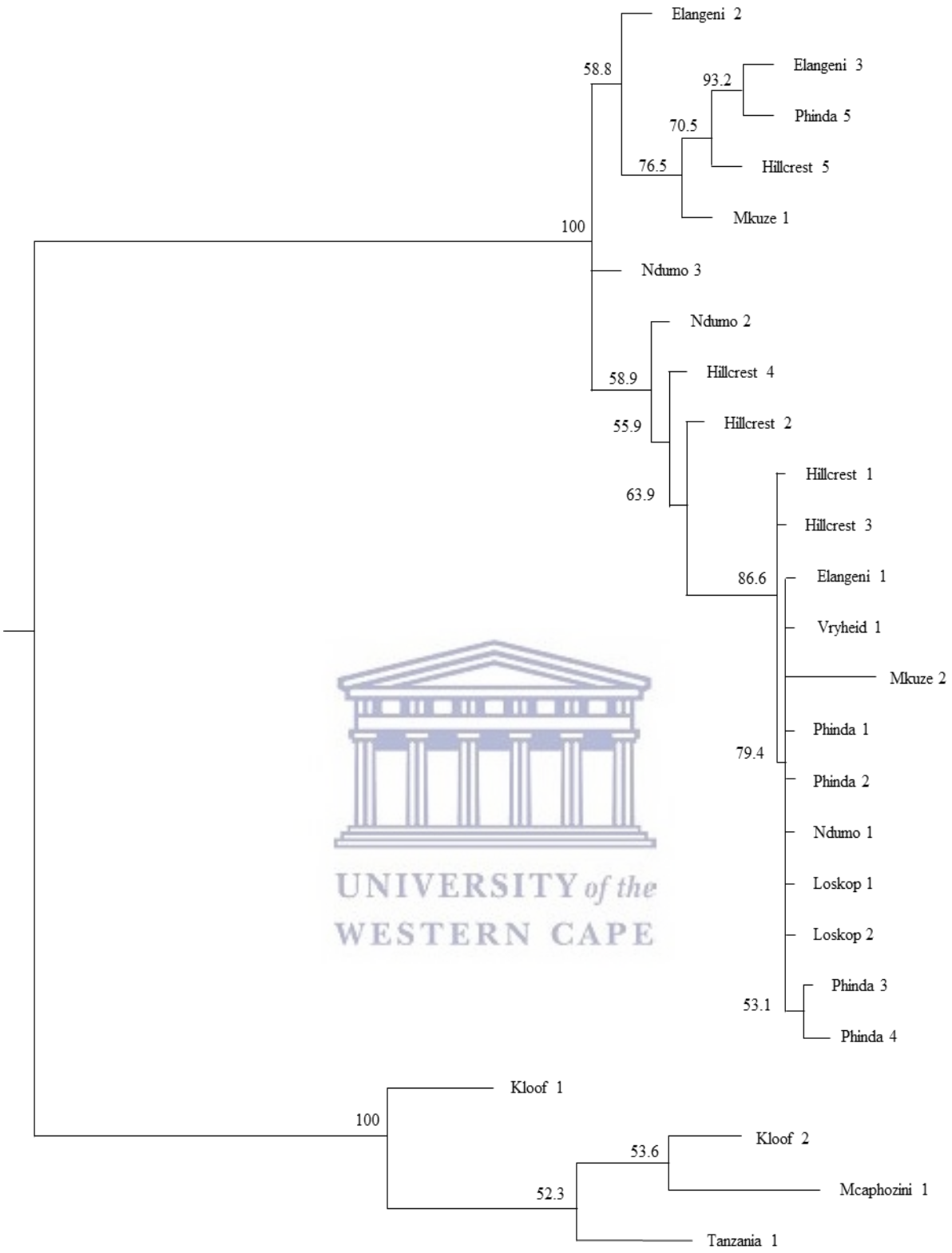

Figure 2.4: The Bayesian Inference phylogram for *Lemniscomys rosalia* using the cytochrome oxidase 1 (CO1) gene (652 base pairs). The percentages represent support values for nodes.

2.9 Population genetics of *Lemniscomys rosalia*

The positive Tajima's D value of 1.37827 shows a population in stasis as the pairwise differences are more than the number of segregating sites (Table 2.3). The AMOVA revealed a Φst of 76.55% (Va = 64.44073). The mismatch distribution had several peaks of varying sizes (Fig. 2.5).

Table 2.3: Results of the neutrality tests.

	Laelaps giganteus		*Lemniscomys rosalia*	
	COI	ITS1	COI	ITS1
Haplotype diversity	0.939	0.919	0.943	
Variance of haplotype diversity	0.00036	0.00097	0.00099	
Nucleotide diversity	0.083	0.034	0.185	
Fu's F	-14.042	-3.86	8.018	
Fu and Li's D	-1.522	-0.657	-0.577	
Fu and Li's F	-1.896	-1.467	-0.984	
Tajima's D	-1.699	-2.138	1.378	

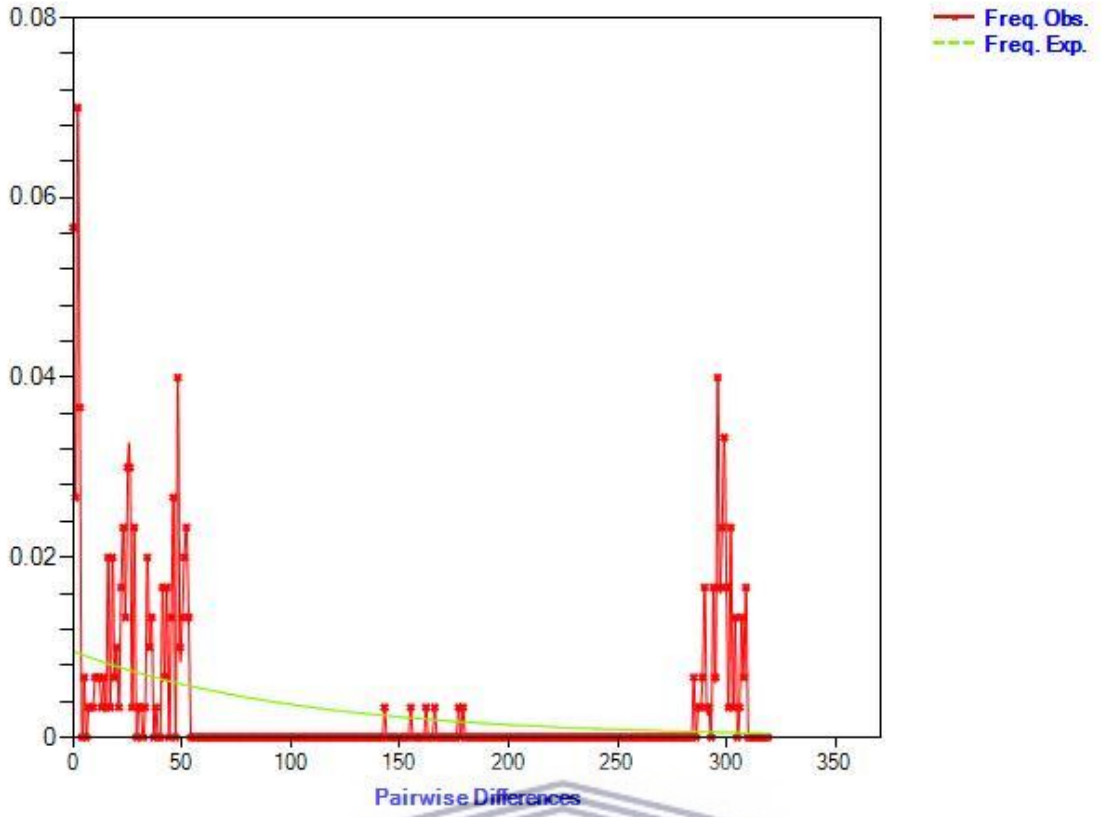

Figure 2.5: Mismatch distribution of *Lemniscomys rosalia* for cytochrome oxidase 1 (CO1).

Table 2.4: Analysis of molecular variance of *Lemniscomys rosalia* based on cytochrome oxidase 1 (CO1) gene. F_{SC} indicates genetic variation among populations within groups, F_{ST} overall genetic variation among populations; F_{CT} genetic differentiation between groups.

Source of Variation	d.f	Sum of squares	Variance components	Percentage of variance
Among groups	6	1164.936	65.681 Va	77.86
Among populations within groups	3	40.529	-2.308 Vb	-2.74
Total	24	1520.2	84.355	
Fixation indices				
F_{SC}:	-0.124			
F_{ST}:	0.751			
F_{CT}:	0.779			

2.10 Discussion for *Lemniscomys rosalia*

The genetic structure of *L. rosalia* is very likely shaped by life history traits. Tall dense grass is essential for *L. rosalia* and it is not found in montane forests or grasslands (Monadjem 1999). It is commonly found in agricultural areas provided there is enough tall grass cover (Monadjem 1999). The distribution of *L. rosalia* is affected by disturbance of grasslands, mostly by fire but also by agricultural activities, since individuals avoid areas without ample grass cover (Monadjem & Perrin 1997). *Lemniscomys striatus*, a closely related species to *L. rosalia*, has been shown to survive in refugia during unfavourable forest expansions and spread during grassland expansions during the late Pleistocene (Nicolas *et al.* 2008). Geographic barriers in the form of the Volta and Niger rivers may have limited gene flow in *L. striatus* (Nicolas *et al.* 2008). Therefore, rivers could be barriers of gene flow for *L. rosalia*.

A lack of tall grasslands due to aridification prevents movement of *L. rosalia* to the more arid Western regions of South Africa, with the exception of the North West Province (Bryja *et al.* 2010). However, there are no major rivers limiting gene flow within South Africa since the Orange River, much like its wide western African counterparts the Niger, Volta and Senegal rivers, would only effect western distributions (Bryja *et al.* 2010). *Lemniscomys rosalia* is not distributed in the western region of southern Africa (Fig. 2.1). It is more likely that the life history traits of *L. rosalia* are the cause of gaps in haplotype sharing (Sands *et al.* 2015). *Lemniscomys rosalia* individuals are solitary in nature and live in singular grass nests which could explain the lack of haplotype sharing across long ranges (Monadjem & Perrin 1997). This coupled with relatively small, not overlapping, home ranges of 15 m^2 and possible territorial behaviour towards other *L. rosalia* individuals could explain limited haplotype sharing (Monadjem 1999).

There are studies that have provided evidence for separating *L. rosalia* from Tanzania with populations from South Africa. Cytogenetic analysis of South African *L. rosalia* showed a marked difference in the karyotype compared to the Tanzanian type specimen (Castiglia *et al.* 2002). The difference in the karyotype was enough for Castiglia *et al.* (2002) to claim that *L. rosalia* from the two regions are separate species which supports the findings of the current study. However, more sampling is required.

Chapter 3: *Laelaps* and *Lemniscomys* co-phylogeography

The cosmopolitan family Laelapidae (Berlese 1892) is commonly found on Rodentia species, especially in the Neotropics (Fonseca 1958; Martins-Hatano *et al.* 2002). The family is well studied worldwide and new species have been described recently. In Iran, two new species of *Gaeolaelaps* were described by Nemati & Mohseni (2013) and Joharchi & Halliday (2011) described new species of Laelapidae found on Coleoptera. New species of the honeybee parasite *Tropilaelaps* on Asian *Apis* spp. have been described or redefined (Anderson & Morgan 2007). Comprehensive studies have been done on Neotropical Laelapid mites (Martins-Hatano *et al.* 2002). There is still confusion in whether species of Laelapidae are monoxenous (specific to a single host) or polyxenous (multiple host species) (Gettinger 1992; Martins-Hatano *et al.* 2002). Host specificity experiments were done by Esbérard *et al.* (2005) on ectoparasites and *Laelaps* spp. preferred their specific host species over a novel host. *Laelaps giganteus* has a large variety of widespread, small mammal host species (Engelbrecht *et al.* 2014). Lineages of *L. giganteus* were found on southern African *Lemniscomys rosalia* and *Rhabdomys dilectus* (Engelbrecht *et al.* 2014; 2016).

The general life cycle of Mesostigmatid mites includes egg, larva, protonymph, deutonymph followed by either a male or female adult (Martins-Hatano *et al.* 2002). In Laelapine mites the egg stage or possibly the larva could be suppressed and the female gives birth to either larvae or an octopod protonymph (Martins-Hatano *et al.* 2002). Many Laelapine mites have a nidicolous lifestyle where most of the life cycle takes place inside the nests or burrows of the host species (Martins-Hatano *et al.* 2002). Only the inseminated female Laelapine mites are found on hosts, most likely to disperse to the nest of another host (Martins-Hatano *et al.* 2002).

Sex-biased infestation is a common occurrence in parasites, especially in sexually dimorphic species (Matthee *et al.* 2009).

3.1 Sampling and DNA Extraction

Live hosts were trapped in the same manner as in chapter 2.1. Hosts were placed in a plastic bag to be screened for parasites before being brushed. Samples of the ectoparasites were collected from the field by gently brushing the mites off the mice (Ignoffo 1958; Burger *et al.* 2012). The brushes were cleaned with 100% ethanol between uses to prevent cross contamination. Mites were preserved in 100% ethanol.

DNA was extracted from the mites in the same way as in Chapter 2.3. The body of the mite does not digest during extraction and was stored in ethanol to be identified based on the morphological characters. Similar PCR protocols were used to extract ITS1 and COI as in Chapter 2.3. *Laelaps giganteus* had a total of 59 COI sequences of 576 base pairs, as in Chapter 2.6. Internal transcribed spacer 1 for *L. giganteus* had 27 sequences of 459 base pairs (Chapter 2.6).

3.2 Population genetics of *Laelaps giganteus* and Co-phylogeny of *Lemniscomys rosalia* and *Laelaps giganteus*

Haplotype networks and tests of population genetic structure were run as in Chapter 2.5. The COI and ITS1 phylograms were run as in Chapter 2.4.

A co-phylogeny was run through Jane v4 (Conow *et al.* 2010). A host tree and parasite tree were both mapped using the COI phylograms. The phylograms were redrawn and compressed in the Jane v4 tree editor for the program into a tree file. Interactions between the host tree and parasite tree were added and *L. giganteus* samples without a host were removed. The default cost scheme was used with the following costs: cospeciation = 0, duplication = 1, duplication & host switch = 2, loss = 1 and failure to diverge = 1. The program ran for 1000 generations with a population size of 300.

3.3 Haplotype network

The COI region haplotype network for *L. giganteus* (Fig. 3.1) consists of sequences from Mooinooi, Zeerust, Hillcrest and Loskop only (see Fig. 2.1 & Table 2.2 for locality data). There is a large central grouping of Zeerust and Mooinooi with a few more branches of Zeerust and Mooinooi from the central group. Loskop sequences branch at two points – one with a Mooinooi sequence and one with a double branching segment. The second branch is grouped with Hillcrest sequences as well as a Mooinooi sequence. One Mooinooi sequence and a Hillcrest grouping is separate from the rest of the network.

The network for ITS1 (Fig. 3.2) is made up mostly by Hillcrest with a few Ndumo sequences. The Ndumo sequences form a large central haplotype with many Hillcrest sequences. Three groups of two Hillcrest sequences are separated from the network.

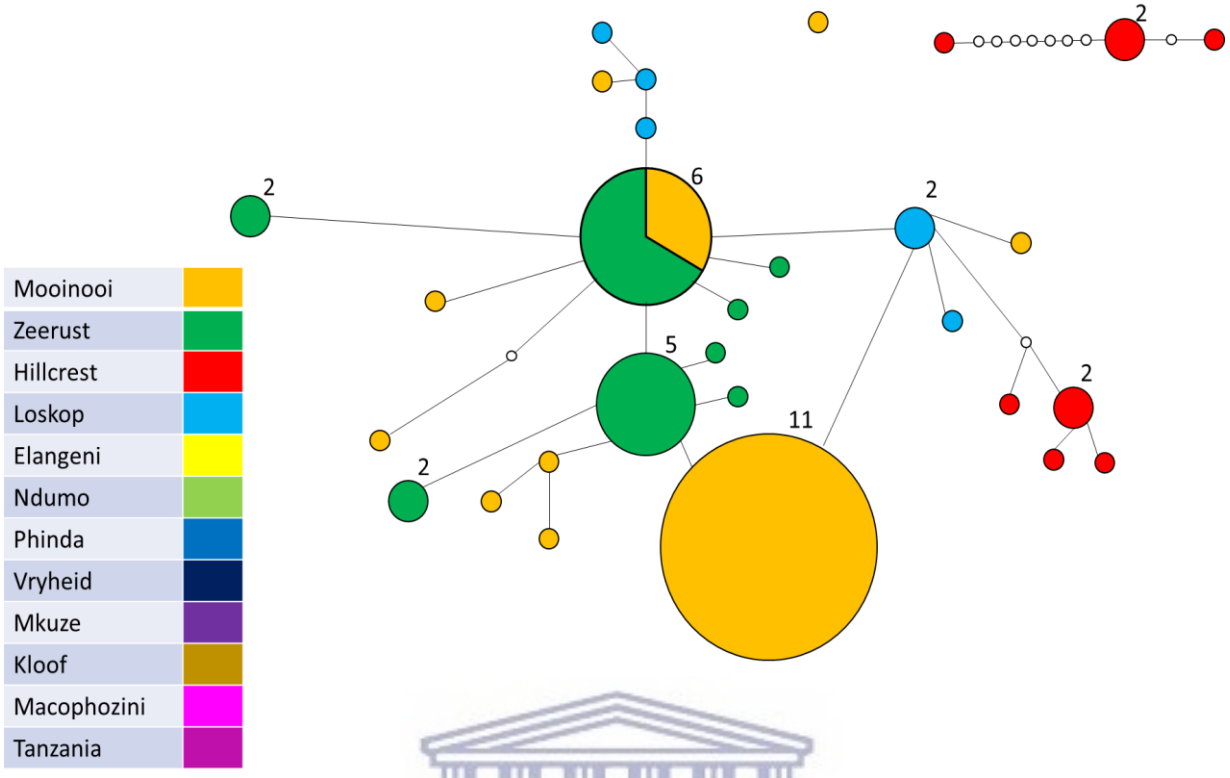

Figure 3.1: Redrawn minimum spanning haplotype network for *Laelaps giganteus* for the cytochrome oxidase 1 (COI) gene marker at 95% confidence levels. Each colour indicates a separate locality while blank circles represent mutational steps between observed haplotypes. The size of the circles corresponds with the number of sequence repetitions per haplotype.

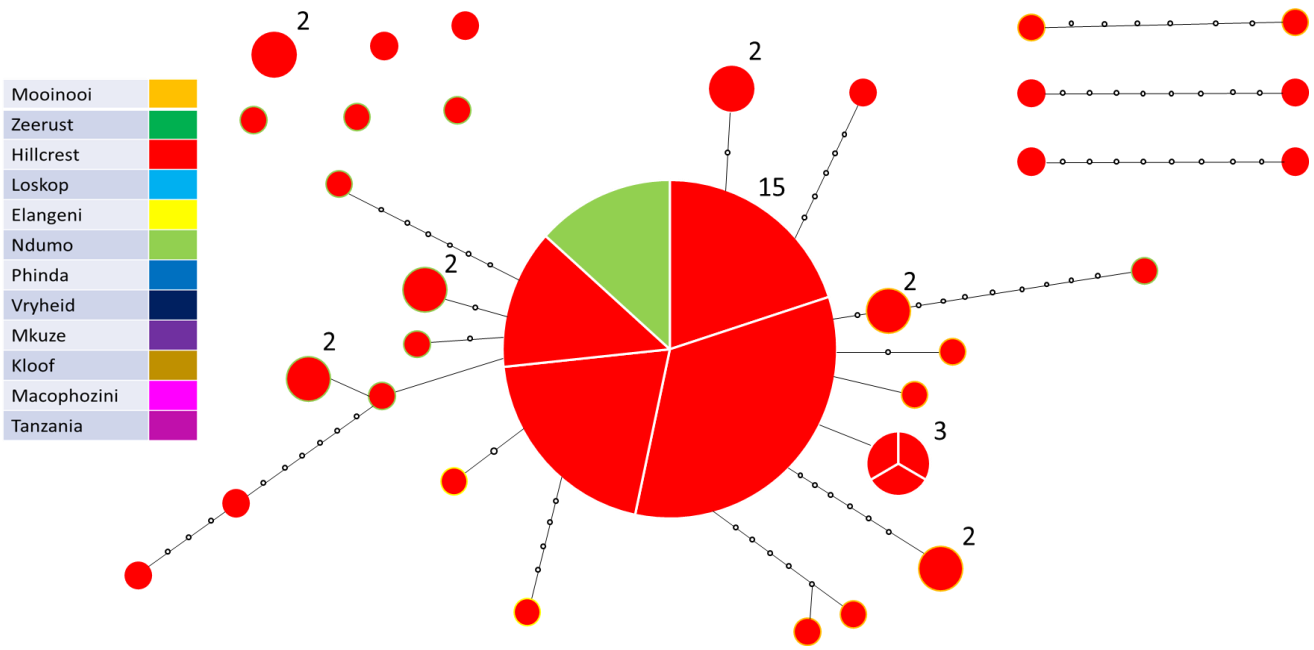

Figure 3.2: Redrawn minimum spanning haplotype network for *Laelaps giganteus* for the internal transcribed spacer 1 (ITS1) gene marker at 95% confidence levels. Each colour indicates a separate locality while blank circles represent mutational steps between observed haplotypes. The size of the circles corresponds with the number of sequence repetitions per haplotype.

3.4 Phylograms for *Laelaps giganteus*

There was a large close group of Mooinooi, Loskop, and Zeerust with a polytomy found in the MP phylogram (Fig. 3.3). Hillcrest sequences were also grouped together with each other but not as close. Similar groupings were present in the BI phylogram but with more separation and more defined groupings (Fig. 3.4). The sequence of a *L. giganteus* sampled from *R. pumilio* from Kaalplaas was used as an outgroup for the COI phylogram.

Only the BI phylogram was used for the ITS1 region of *L. giganteus* (Fig. 3.5). There were multiple sequences for the same mites as phase was used in DNASP since ITS1 is a nuclear DNA marker. There were also large polytomous groupings, similar to those found in the COI region.

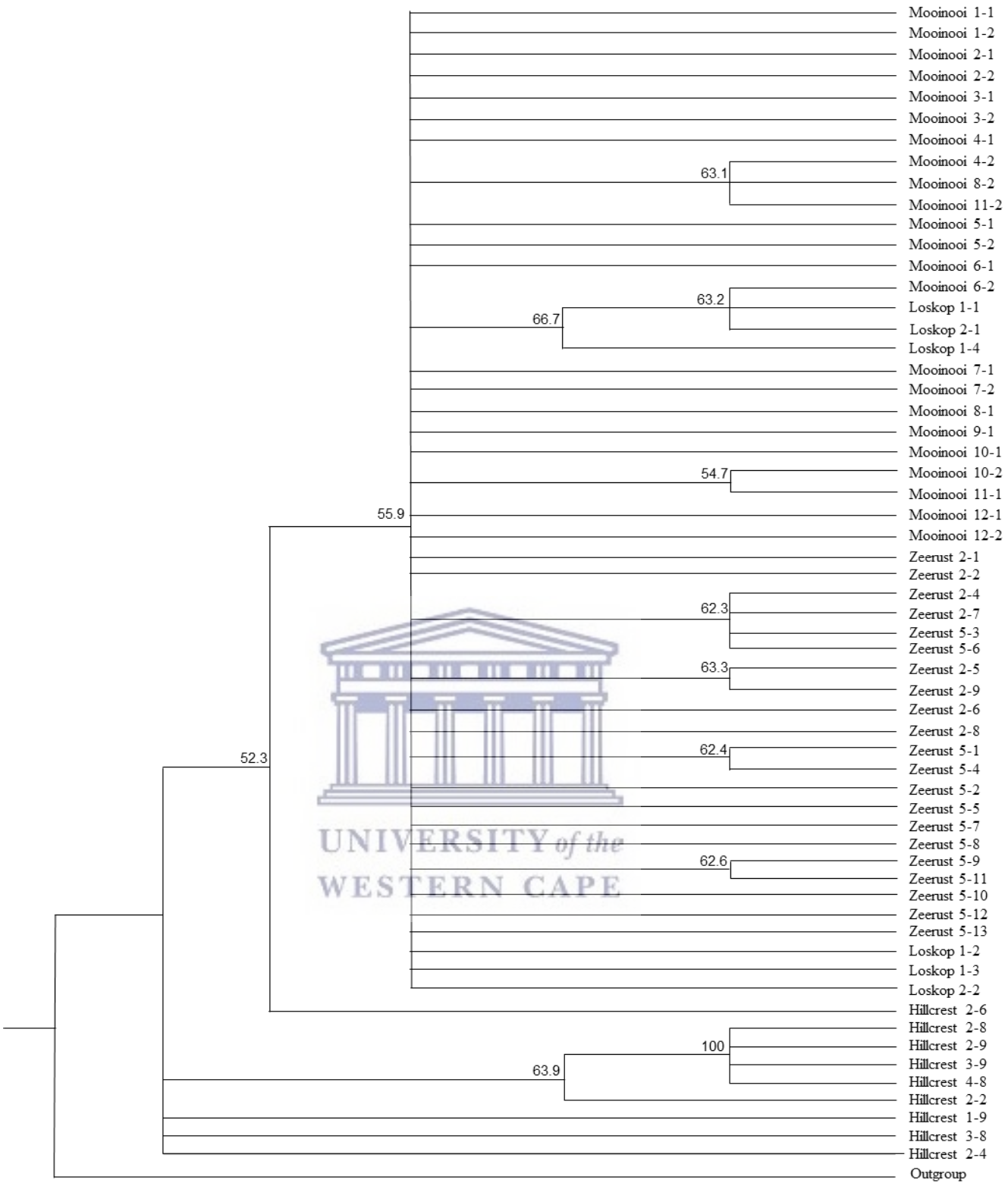

Figure 3.3: The Maximum Parsimony phylogram for *Laelaps giganteus* using the mtDNA cytochrome oxidase 1 (CO1) gene marker (576 base pairs). The percentages represent support values for nodes.

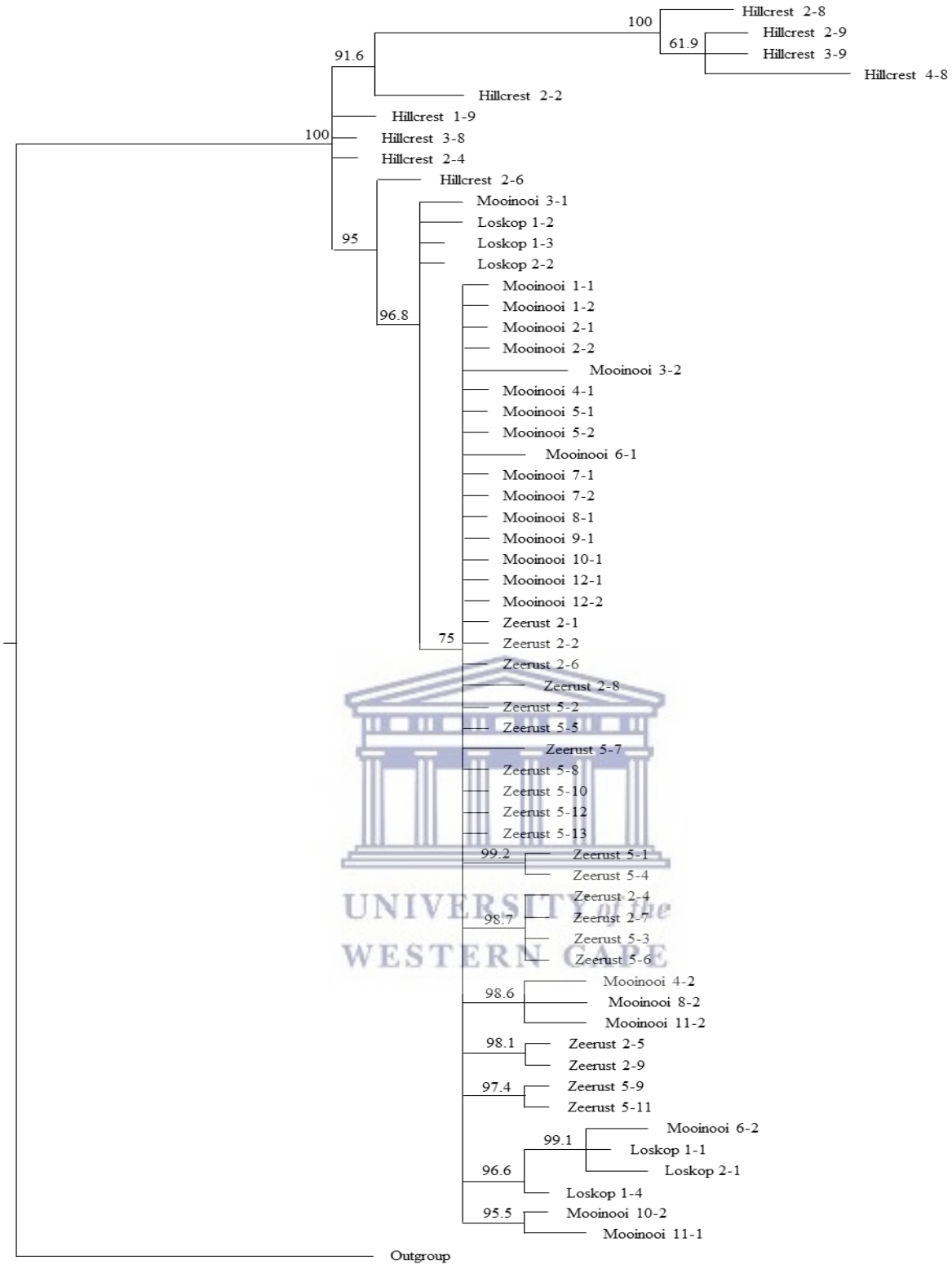

Figure 3.4: The Bayesian phylogram for *Laelaps giganteus* using the mtDNA cytochrome oxidase 1 (CO1) gene marker (576 base pairs). The percentages represent support values for nodes.

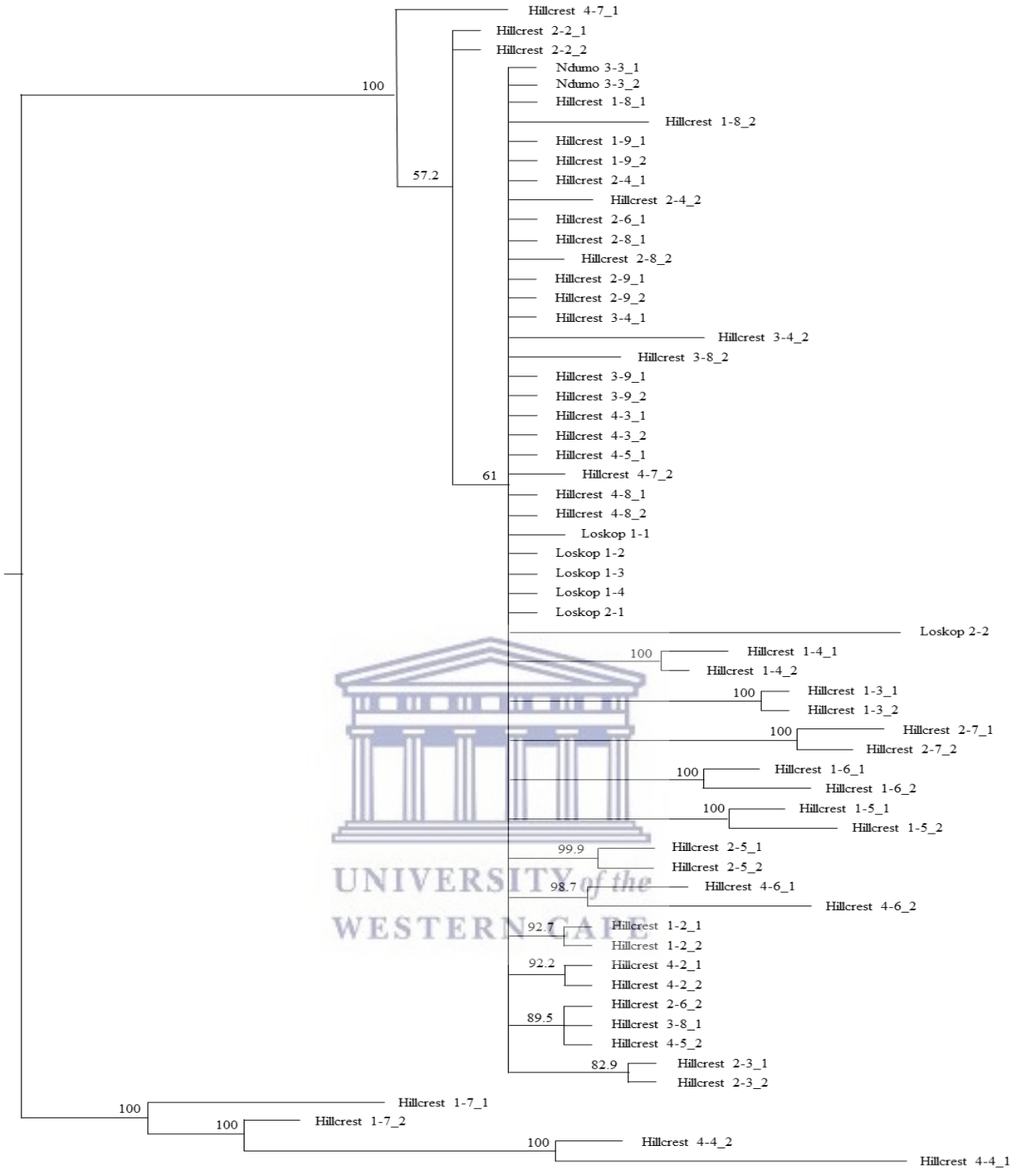

Figure 3.5: The Bayesian phylogram for *Laelaps giganteus* using the internal transcribed spacer 1 (ITS1) gene (459 base pairs). The percentages represent support values for nodes.

3.5 Population genetics of *Laelaps giganteus*

Both COI and ITS1 had negative Tajima's D values of -1.69917 and -2.13778, respectively, which indicates that the gene markers are under selection (Table 2.3). The AMOVA for COI revealed a Φst of 31.76% (Va = 1.97513) (Table 3.1) and a Φst of -27.44% (Va = -1.82175) for ITS1 (Table 3.2). The mismatch distribution for COI had two peaks (Fig. 3.6) while ITS1 had several peaks that follow the expected curve (Fig. 3.7).

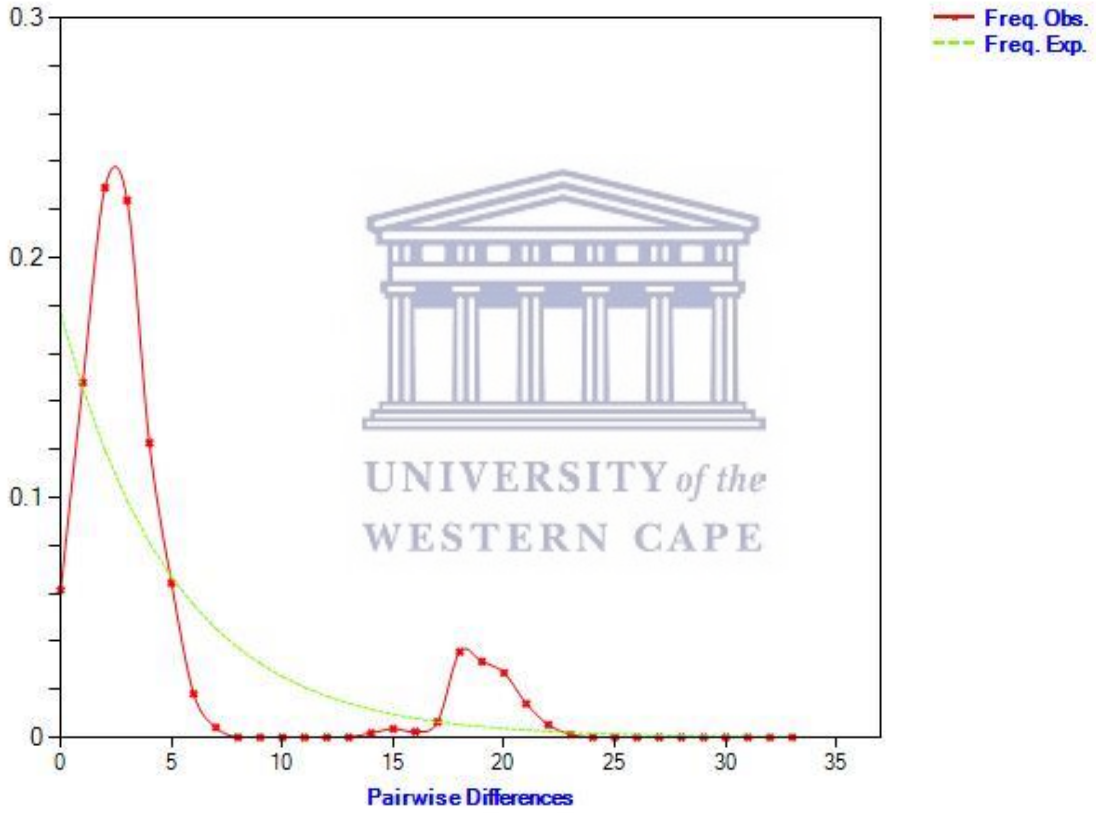

Figure 3.6: Mismatch distribution for cytochrome oxidase 1 (CO1) for *Laelaps giganteus*.

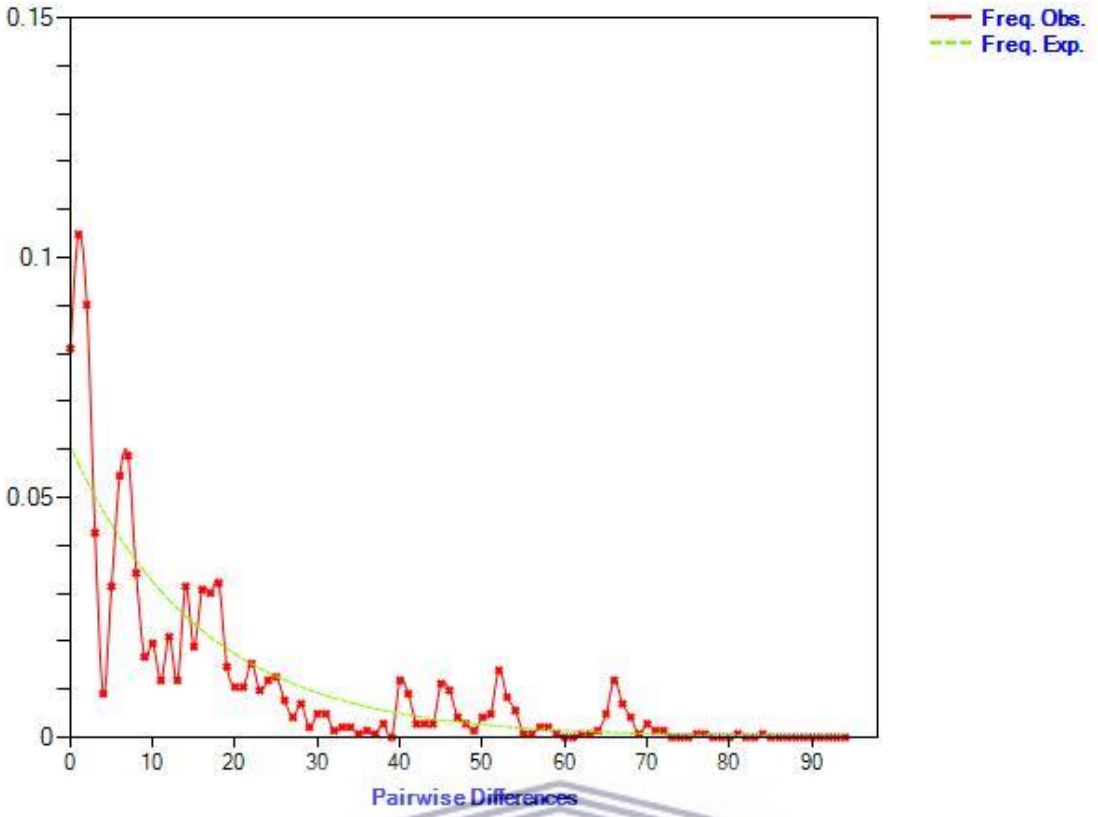

Figure 3.7: Mismatch distribution for internal transcribed spacer 1 (ITS1) for *Laelaps giganteus*.

Table 3.1: Analysis of molecular variance of *Laelaps giganteus* for the cytochrome oxidase 1 (CO1) gene. F_{SC} - genetic variation among populations within groups, F_{ST} - overall genetic variation among populations; F_{CT} - genetic differentiation between groups.

Source of Variation	d.f	Sum of squares	Variance components	Percentage of variance
Among groups	2	56.293	0.454 Va	7.63
Among populations within groups	1	48.744	2.064 Vb	34.67
Total	58	293.949	5.953	
Fixation indices				
F_{SC}:	0.375			
F_{ST}:	0.423			
F_{CT}:	0.076			

Table 3.2: Analysis of molecular variance of *Laelaps giganteus* for the internal transcribed spacer 1 (ITS1) gene. F_{SC} - genetic variation among populations within groups, F_{ST} - overall genetic variation among populations; F_{CT} - genetic differentiation between groups.

Source of Variation	d.f	Sum of squares	Variance components	Percentage of variance
Among groups	1	1.444	-1.907 Va	-28.86
Among populations within groups	3	32.119	0.19 Vb	2.87
Total	53	441.444	6.607	
Fixation indices				
F_{SC}:	0.022			
F_{ST}:	-0.26			
F_{CT}:	-0.289			

3.6 Co-phylogeny results

The Jane output, run for 1000 generations with a population size of 300, included 85 solutions from the default cost scheme (cospeciation = 0, duplication = 1, duplication & host switch = 2, loss = 1 and failure to diverge = 1). All solutions had a low cost of 15. Out of the 85 solutions, 73 had four cospeciations, six duplications, four duplication and host switches, and a single loss event. The 12 remaining solutions had five cospeciations, five duplications, four duplication and host switches, and two loss events. None of the 85 solutions had any failures to diverge.

3.7 Tests of neutrality

Tests of neutrality find out whether polymorphism in a population can be explained by the Wright-Fisher neutral model (Fu 1997). The mismatch distribution for *L. giganteus* COI (Fig. 3.6) shows two distinct peaks on graph which is evidence of a non-expanding population (Rogers & Harpending 1992).

3.8 Discussion for *Laelaps giganteus*

The co-phylogeny results are similar to *L. giganteus* linages found on *Rhabdomys* (Engelbrecht *et al*. 2016), as expected. The costs of the co-phylogeny run in Jane were low in both cases. Engelbrecht *et al.* (2016) used Jane, AXPARAFIT (Legendre *et al*. 2002), and CORE-PA v0.3a (Merkle *et al.* 2010) during co-phylogeny testing. In this instance, Jane was sufficient to test co-phylogeny between *L. rosalia* and *L. giganteus*. The limited dispersal of *L. giganteus* has led to strong congruence with *L. rosalia*. This is further evidence for specialization of *L. giganteus* lineages on small mammal species. The patterns found in the COI haplotype networks also seem to match the co-phylogeny (Fig. 3.1). Hillcrest samples, which make up a large portion of *L. giganteus* samples, form part of two separate clusters in both the *L. rosalia* and *L. giganteus* COI haplotype networks (See Fig. 2.2 & Fig. 3.1). This provides evidence for high specificity in the *L. giganteus* lineage found on *L. rosalia*.

Lemniscomys rosalia shares a distribution with *Rhabdomys.* except in the western winter rainfall region of southern Africa (see Fig. 2.1). Therefore, any differences in congruence between these hosts of *L. giganteus* is likely down to life history traits. *Lemniscomys rosalia* lives in above ground grass nests compared to the underground burrows in *Rhabdomys*

(Engelbrecht *et al.* 2016). *Rhabdomys* live in more solitary nests within the shared distribution, similar to *L. rosalia*. The possible territorial nature of *L. rosalia* could be another limiting factor of dispersal of *L. giganteus*. Even with these differences in life history, the co-phylogenies are similar. Congruence is likely influenced by these factors coupled with *L. giganteus* life history traits. *Laelaps giganteus* is mostly restricted to the nests of the hosts (Radovsky 1985; 1994; Martins-Hatano *et al.* 2002). Limited dispersal in *L. giganteus* likely caused the low number of host switching events during host distribution changes (Engelbrecht *et al.* 2016).

Chapter 4: Conclusion

The current study is the first in-depth look at the phylogeographic structure of *Lemniscomys rosalia* within South Africa and Swaziland. Cytogenetic analysis of *L. rosalia* shows two distinct karyotypes which is abnormal for a single species (Castiglia *et al.* 2002). The study aimed to provide phylogeographic evidence for species delimitation within southern African *L. rosalia*. The ectoparasite *Laelaps giganteus* was also used to complement the phylogeography of its host. This study aimed to describe the co-phylogeography of *L. rosalia* and a possible lineage of *L. giganteus* found on *L. rosalia*.

Similar phylogeographic studies have been done in southern Africa on the murid species including *Rhabdomys pumilio* (Rambau *et al.* 2003; Du Toit *et al.* 2012), *Otomys irroratus* (Engelbrecht *et al.* 2011), *Micaelamys namaquensis* (Russo *et al*. 2010), and *Myotomys unisulcatus* (Edwards *et al.* 2011). *Laelaps giganteus* also has lineages found on *R. dilectus* (Engelbrecht *et al.* 2016).

Museum specimens of both *L. rosalia* and *L. giganteus* and a few supplementary field samples were extracted and subsequently sequenced for cytochrome oxidase 1 (COI) and internal transcribed spacer 1 (ITS1). The sequences were analysed through Bayesian Inference and Maximum Parsimony to represent the phylogenies of *L. rosalia* and *L. giganteus*, respectively.

4.1 Key findings

The phylogeographic structure of *Lemniscomys rosalia* complements the cytogenetic evidence provided by Castiglia *et al.* (2002). This evidence along with future studies of Tanzanian *L. rosalia* can provide insight into the species boundaries. *Laelaps giganteus* found on southern African *L. rosalia* shows congruence with its host, most likely due to limited dispersal of *L. giganteus*. Jane was sufficient to test co-phylogeny between *L. rosalia* and *L. giganteus* as the costs of the co-phylogeny were low. The patterns found in the COI haplotype networks also seem to match the co-phylogeny. This is further evidence for specialization of *L. giganteus* lineages on small mammal species.

4.2 DNA markers

A phylogeographic study on congeneric species *Lemniscomys striatus* used cytochrome *b* as the mtDNA marker of choice (Nicolas *et al.* 2012). However, COI and ITS1 were used in the study to compare *L. rosalia* and *L. giganteus* with the same markers. Tropomyosin marker was used with little to no success in this study. This has been documented in previous studies, see Engelbrecht *et al.* (2014). Cytochrome oxidase 1 provided more insight into evolutionary history of both taxa when compared to ITS1. Since museum materials were used, degradation could be an issue. To resolve this problem internal primers and nested PCR methods could have yielded better results. Additional markers could have been used to resolve polytomies and markers that can be used on both taxa should be explored in future research.

4.3 Limitations and future research

This study mainly used samples from South Africa and Swaziland with a single sample from Tanzania. In a future study extensive sampling should be done outside of southern Africa particularly populations within the pockets of the distribution of *L. rosalia*. Low trapping rates, limited sampling of the host species, and in turn the sampling the parasite. Several different DNA markers should be used to test compatibility with the host and parasite species for co-phylogenies. Distribution models could have been run to find possible range shifts in *L. rosalia*. A full phylogeny of *L. giganteus* found on *L. rosalia* could provide evidence of co-phylogeny in eastern African populations.

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