Deciphering the taxonomic status of parasitic sucking lice occurring on the *Aethomys* and *Micaelamys* rodent species complex: a comparative phylogenetic and phylogeographic approach.

by

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Declaration

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Abstract

The present study investigated the phylogenetic and phylogeographic structures of parasites and their hosts in an attempt to better understand the mechanisms involved in parasite evolution. Phylogenetic or phylogeographic co-divergence between a parasite and its host would support a hypothesis that the evolution of parasites are closely linked to the evolution of host species. The lack of co-divergences would support the prediction that parasite evolution is the result of, amongst others, a complex interaction between host life history, parasite life history and biogeography. To provide more clarity on the factors influencing parasite evolution, the present study used mitochondrial - and nuclear DNA sequence data to investigate genetic co-divergences between obligate permanent lice species occurring on four rodent taxa associated with the *Aethomys*/*Micaelamys* species complex. Recent genetic investigations provided new taxonomic insights into the phylogeny of the host species and supported that within South Africa the subgenera *Micaelamys* and *Aethomys* should be recognized as distinct genera. It also provided evidence that the cryptic *A. chrysophilus* and *A. ineptus* should be recognized as two different species. The taxonomic descriptions of the lice that are associated with these rodents did not take into account the recent vacillations in host taxonomy and parasite species descriptions were exclusively based on morphology. It can thus be proposed that the parasite-host species lists are outdated and that a taxonomic revision for parasites occurring on these rodents are needed. Nonetheless, it has been reported that *M. namaquensis* and *A. chrysophilus* are both parasitized by the same species of lice, namely *Hoplopleura patersoni* and *Polyplax praomydis.* For *M. granti* and the newly erected species, *A. ineptus*, there are no data on the lice species that are associated with them. The aims of this project were to i) identify the sucking lice associated with *Aethomys* and *Micaelamys* species occurring in South Africa, ii) investigate phylogenetic co-diverge between parasites and hosts for all the lice species sampled on the four host lineages, iii) conduct a fine scale co-divergence analyses by testing for phylogeographic congruence between one widely distributed host species, *M. namaquensis*, and its associated lice species.

COI mitochondrial DNA haplotype networks and Bayesian and Maximum likelihood phylogenetic analyses drawn from 24 host and 74 louse specimens supported four genetically distinct *Hoplopleura* taxa each associated with a different rodent species. Two genetically distinct *Polyplax* taxa were also detected on the two *Micaelamys* species. No *Polyplax* individuals were sampled from the *Aethomys* individuals included herein. Within the widely distributed *M. namaquensis*, there was also indications that *Hoplopleura* - and *Polyplax* lineages trapped in the north east and south west of South Africa are significantly differentiated from each other. In total, this study identified eight genetically distinct louse lineages associated with the *Aethomys/Micaelamys* rodent complex in South Africa. Superficial morphological investigations on these eight lineages revealed at least two morphologically distinct *Hoplopleura* - and two morphologically distinct *Polyplax* taxa occurring on the two *Micaelamys* species respectively. Based on morphological differences, some nuclear DNA differentiation, and more than

20% mitochondrial DNA sequence distances between these lineages, a strong argument can be made that these four lineages represent at least four distinct parasite species, two of them new to science. The phylogeny of the lice species showed marked congruences with the phylogeny of the rodent hosts and divergence dating also showed a fair amount of overlap in the timing of the divergences between the host lineages and those of the parasites. Topology based reconciliation analyses in Jane significantly supported the notion of co-divergence between parasite and host lineages as the most parsimonious solution. In this instance the latter provides support for the hypothesis that the evolution of permanent host specific parasites are closely linked to the evolution of their host species.

The influence of host evolution on parasite evolution is also partly reflected in the finer scale phylogeographic analyses of the two species of lice occurring on *M. namaquensis.* The COI mitochondrial DNA haplotype networks along with Bayesian and Maximum likelihood phylogenetic analyses supported cryptic diversity within *P. praomydis* and *H. patersoni* collected from *M. namaquensis* individuals throughout South Africa. Both the host and the parasites show significant differentiation between lineages in the north-eastern and south-western parts of South Africa. Analyses of molecular variance supported this differentiation and also suggested low levels of gene flow among most sampling localities. Significant population differentiation was present for both *M. namaquensis* and the two lice species occurring permanently on the host. At the phylogeographic level, however, co-divergence analyses indicated limited phylogeographic congruence between *M. namaquensis* and *H. patersoni* throughout the sampled range. Incongruences were mainly confined to the lineages occurring in the north-eastern regions of South Africa. Phylogenetic reconciliation indicated that this incongruence is most likely as a result of a host switch. This partial congruence suggests that alternative factors such as host life history also play a role in the dispersal and subsequent evolution of parasites. In this specific instance it was argued that male bias dispersal over shorter distances in the host can cause incongruent patterns by allowing more opportunities for host switching.

Opsomming

Die huidige studie ondersoek die filogenetiese en filogeografiese strukture van parasiete en hul gashere in ‘n poging om die meganismes betrokke by parasietevolusie beter te verstaan. Filogenetiese of filogeografiese ooreenkomste tussen 'n parasiet en sy gasheer sal die hipotese ondersteun dat die evolusie van die parasiete nou verband hou met die evolusie van die gasheerspesies. Verskille in die evolusionêre patrone van die parasiete en die gasheer, sal op sy beurt die voorspelling ondersteun dat parasiet evolusie eerder die resultaat is van onder andere 'n komplekse interaksie tussen die lewensgeskiedenis van gashere, die lewensgeskiedenis van parasiete, asook biogeografie. Om meer duidelikheid te verkry oor die faktore wat parasietevolusie beïnvloed, gebruik die huidige studie mitokondriale - en kern DNA data om genetiese ko-evolusie te ondersoek tussen permanente luis taksa wat voorkom op vier knaagdier spesies wat geassosieer word met die *Aethomys*/*Micaelamys* spesie kompleks in Suid Afrika. Onlangs gepubliseerde genetiese studies het veranderinge in die taksonomie van die groep voorgestel en gewys dat die subgenera *Micaelamys* en *Aethomys* binne Suid-Afrika as afsonderlike genera erken moet word. Die studies het ook bewys dat die kriptiese *A. chrysophilus* en *A. ineptus* as twee afsonderlike spesies erken moet word. Die taksonomiese beskrywings van die luise wat met hierdie knaagdiere geassosieer word het nie hierdie veranderinge in gasheer-taksonomie in ag geneem nie, en die parasiet taksonomie was uitsluitlik gebaseer op morfologie. As gevolg hiervan is dit waarskynlik dat die parasiet-gasheer beskrywings verouderd is. Nietemin is daar gerapporteer dat *M. namaquensis* en *A. chrysophilus* albei deur dieselfde spesies luise, naamlik *Hoplopleura patersoni* en *Polyplax praomydis* geparasiteer word. Vir *M. granti* en *A. ineptus* is daar geen inligting oor die luise wat met hulle geassosieer word nie. Die doelwitte van hierdie projek was om i) die luise wat op die *Aethomys* en *Micaelamys* species in Suid-Afrika voorkom te identifiseer; ii) filogenetiese ko-divergensie tussen parasiete en gashere te ondersoek vir al die luise wat op die vier gasheer spesies gevind word; iii) om ‘n fynskaalse analise te onderneem om te toets vir filogeografiese kongruensie tussen een wydverspreide gasheerspesie, *M. namaquensis*, en sy verwante luise.

COI mitokondriale DNA haplotipe netwerke en Bayesiaanse en Maksimum waarskynlikheid fylogenetiese ontledings op 24 gasheer- en 74 luismonsters ondersteun vier geneties verskillende *Hoplopleura* taksa wat elk met een van die knaagdierspesies geassosieer word. Twee geneties verskillende *Polyplax* taksa is ook op die twee *Micaelamys* spesies aangetref. Geen *Polyplax* individue is op enige van die *Aethomys* spesies gekry nie. Daar was ook aanduidings dat *Hoplopleura* en *Polyplax* individue wat verwyder is van *M. namaquensis* vanaf die noordooste en suidweste van Suid-Afrika beduidend gedifferensieer is. In totaal het hierdie studie agt geneties gedifferensieerde stamme geïdentifiseer wat met die *Aethomys*/*Micaelamys* knaagdier kompleks in Suid-Afrika geassosieer word. Voorlopige morfologiese ondersoeke van hierdie agt stamme het ten minste twee morfologies verskillende *Hoplopleura* taksa en twee morphologies verskillende *Polyplax* taksa geopenbaar wat op die twee *Micaelamys* spesies onderskeidelik voorkom. Op grond van morfologiese verskille, 'n mate van

kern DNA differensiasie, en meer as 20% mitokondriale DNA-volgorde afstande tussen hierdie stamme kan 'n sterk argument aangevoer word dat hierdie vier stamme ten minste vier verskillende parasiet spesies verteenwoordig, waarvan twee onbeskryf is. Die filogenie van die luis spesies toon sterk ooreenkomste met die filogenie van die gashere en die tydsberekening van skeidings tussen die gasheerstamme het ook beduidende oorvleueling getoon met die van die parasiete. Topologie gebaseerde versoenings ontledings in Jane ondersteun die idee van ko-divergensie tussen die luise en die gashere as die mees parsimoniese oplossing. In hierdie geval ondersteun die studie die hipotese dat die evolusie van permanente gasheer spesifieke parasiete nou verband hou met die evolusie van hul gasheerspesies.

Die invloed van gasheer evolusie op parasiet evolusie is ook deels weerspieël in die filogeografiese ontledings van die twee luise wat op *M. namaquensis* voorkom. Die COI mitokondriale DNA haplotipe netwerke saam met Bayesiaanse en Maksimum waarskynlikheid filogenetiese ontledings ondersteun kriptiese diversiteit binne *P. praomydis* en *H. patersoni* wat van *M. namaquensis* individue regoor Suid-Afrika versamel is. Beide die gasheer en die parasiete toon 'n beduidende differensiasie tussen stamme in die noordoostelike en suidwestelike dele van Suid-Afrika. Analise van molekulêre variansie ondersteun hierdie differensiasie en stel ook lae vlakke van geenvloei tussen die meeste lokaliteite voor. Beduidende bevolkingsdifferensiasie was teenwoordig vir beide *M. namaquensis* en die twee luis spesies wat permanent op die gasheer voorkom. Op die filogeografiese vlak het ko-divergensie analises egter 'n onvolledige filogeografiese ooreenkoms tussen *M. namaquensis* en *H. patersoni* aangedui. Filogeografiese verskille tussen gasheer en parasiet was hoofsaaklik beperk tot die stamme wat in die noordoostelike streke van Suid-Afrika voorkom. Filogenetiese versoening het aangedui dat hierdie verskille waarskynlik die gevolg is van 'n gasheerruiling. Hierdie onvolledige ooreenkomste dui daarop dat alternatiewe faktore soos die lewensgeskiedenis van die gasheer ook 'n rol speel in die verspreiding en daaropvolgende evolusie van parasiete. In hierdie spesifieke geval is geargumenteer dat manlik bevoordeelde beweging oor korter afstande in die gasheer onvolledige filogeografiese ooreenkoms patrone kan veroorsaak deur meer geleenthede vir gasheerruiling toe te laat.

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Chapter 1: General Introduction

1.1 The relationship between Anopluran lice and their Murid hosts

Lice of the suborder Anoplura, also known as sucking lice, are blood-feeding ectoparasites of almost all major groups of eutherian mammals (Kim & Ludwig, 1978). They are obligate parasites, and all life stages are permanently associated with the bodies of their hosts. As such, they complete their entire life cycle on the host and are thus dependent on their hosts for survival, reproduction and dispersal (Marshall 1981; Durden & Musser 1994). The transmission of lice between hosts (intra- or interspecifically) occur through direct bodily contact (e.g. grooming, suckling and fighting) and it is reasonable to predict that most bodily contact among host individuals will be between conspecific individuals and also between individuals in the same geographic space.

The close association between lice and their hosts have led to the prediction that lice will show strong co-evolutionary patterns with their hosts (Page & Hafner 1996; Nieberding & Morand 2006). Light & Hafner (2008) reported significant co-phylogenetic relationships between heteromyid rodents and their sucking lice of the genus *Fahrenholzia.* The study also revealed that the timing of divergence events in the hosts and parasites corresponds to one another, thus further supporting co-divergence between these rodents and their sucking lice (Light & Hafner 2008). The authors ascribed the congruent pattern to the low vagility of the lice along with the asocial behaviour of the hosts (Light & Hafner 2008). A further study, which analysed the genealogy, population structure and population dynamics of the sucking louse *Polyplax serrata* across four host species in the genus *Apodemus*, revealed a clear structure with three distinct louse clades displaying different host specificities (Stefka & Hypsa 2008). Two of these clades share the same host and live in sympatry, which means that the genetic barrier between these clades is not as a result of present day geographical isolation (Stefka & Hypsa 2008). Based on this the authors concluded that these lice clades must represent cryptic species (Stefka & Hypsa 2008). What is interesting to note is that one of these louse lineages parasitize two different host taxa (*A. sylvaticus* and *A. flavicollis*), while the other louse lineage was strictly specific to one host taxon (*A. flavicollis*) (Stefka & Hypsa 2008). A divergence time estimate between these two lineages showed that they may have arisen as a result of parasite duplication on *A. flavicollis* (Stefka & Hypsa 2008). In a third study the sucking louse *Polyplax arvicanthis*, occurring on the rodent genus *Rhabdomys* (du Toit et al. 2013a) was studied. In this system, the host *Rhabdomys* can be divided into four geographically separate species (du Toit et al. 2012) and a prediction was made that each of the four species will harbour their own unique parasite lineage. Surprisingly in this study the authors found two cryptic species on the same host (du Toit et al. 2013 a) and both these species show only partial phylogeographic congruence between the parasite lineages and the host lineages (du Toit et al. 2013 b). As a result, the evolutionary relationship between the parasite and the host were ascribed to several duplication,

sorting and host switching events (du Toit et al. 2013 b). The authors concluded that the larger effective population sizes of the lice lineages, the vagility and social behaviour of the host species, and the lack of host specificity by the lice in areas of host sympatry is the reason for the incongruence (du Toit et al. 2013 b).

From the above it is clear that host speciation can exert strong evolutionary forces on permanent obligatory parasites such as lice. It is, however, also evident that evolutionary co-divergences between parasites and hosts are complex and cannot simply be ascribed to one factor only (Baker 1990; du Toit et al. 2013 b). By also comparing studies using parasites who are less dependent on their hosts for the completion of their life cycle, it is evident that more specialist parasites (parasites with a narrow host range) will generally show greater phylogeographic congruence with their hosts as supposed to generalist parasites (Page & Hafner 1996; Gomez-Diaz et al. 2007; van der Mescht et al. 2015). If generalist parasites are exposed to host vicariance events, then abiotic factors will also affect their evolutionary patterns (van der Mescht et al. 2015; Sands et al. 2017).

1.2 Lice taxonomy and host associations

Historically much of the taxonomic descriptions of ectoparasites have been based on morphology only, and extrapolations on host associations of parasites are based on outdated species-host and host-species lists (e.g. Zumpt 1961, Ledger 1980, Segerman 1995). A case in point relates to a study that investigated the diversity, co-phylogenetic relationships and biogeography of hoplopleurid sucking lice and their rodent hosts in the Manu National Park and Biosphere Reserve in Peru. In this study it was revealed that 15 distinct louse species parasitize 19 different rodent host species (Smith et al. 2008). Of these 15 louse species, three were new to science, and 13 louse/host associations were previously unknown (Smith et al. 2008). What is interesting to note about this study is the fact that none of the lice species examined appeared to be host-specific as they were recorded on multiple host genera (Smith et al. 2008). The authors ascribed this lack of specificity to be as a result of cryptic parasite species or the occurrence of host switching without speciation (Smith et al. 2008). Poorly recognized ectoparasite diversity is also highlighted by recent studies conducted on the rodent genus *Rhabdomys* and their ectoparasitic lice (du Toit et al. 2013 a) and mites (Engelbrecht et al. 2014). du Toit et al. (2013 a) found two cryptic species of the sucking louse, *Polyplax arvicanthis*, which occur on *Rhabdomys* individuals in southern Africa. What is also striking about the result is the fact that the two lice species have a sympatric distribution and also occasionally occur sympatrically on the same host individual (du Toit et al. 2013 a). With regard to mites, Engelbrecht et al.

(2014) found two cryptic lineages of the mite *Laelaps giganteus*, of which one lineage was confined to *Rhabdomys dilectus* and the other to its close relative *Lemniscomys rosalia*.

Based on the above, it is reasonable to suggest that the Anoplurid biodiversity on small mammals are underestimated (also see Kim 2006). From a medical and veterinary perspective, it is important to confirm the taxonomy and phylogenetic relationship of sucking lice as some species are known to be able to acts as vectors that can transmit louse-borne pathogens to their hosts (Reeves et al. 2006). In turn, rodents have also been found to act as reservoirs of pathogens (e.g. bacteria and protozoa) that can be transmitted by lice (Reeves et al. 2006). It is however also important to note that although certain parasite taxa are associated with disease and mortality, this is not always the case.

1.3 The study region and species investigated

1.3.1 Murid rodent species complexes of southern Africa

The southern African region is characterized by a rich faunal composition that includes amongst others several recognized endemic vertebrate species which belong to various species complexes. Traditional species complexes were often unresolved from an evolutionary perspective since their phylogenetic relationships were mostly inferred from morphological data alone (Dippenaar & Rautenbach 1986; Ellison et al. 1993). In the past 20 to 30 years molecular data revolutionized the field of systematics and resulted in the revision of several taxonomic hypotheses (Maree 2002; Maputla 2007; Mouline et al. 2008). One group that is particularly problematic from a morphological perspective is the Rodentia (rodents) and more pertinent to this study, also the rodents confined to the Muridae family. For example, some Murid species have been shown to be morphologically similar whilst being genetically distinct (Gordon & Watson, 1986) and recent molecular studies have also facilitated the clarification of the species relationships within several Murid species complexes (Veyrunes et al. 2004; Bastos et al. 2011). The latter led to the identification of several cryptic species within genera such as *Mastomys* (Lecompte et al. 2005), *Otomys* (Engelbrecht et al. 2011), *Mus* (Lamb et al. 2014), *Rhabdomys* (Rambau et al. 2003; du Toit et al. 2012) and pertinent to this study *Aethomys* (Russo et al. 2006; Phukuntsi et al. 2016) and *Micaelamys* (Russo et al. 2006; Phukuntsi et al. 2016).

1.3.2 The *Aethomys*/*Micaelamys* rodent species complex.

1.3.2.1 *Aethomys*

Aethomys are referred to as veld rats and members of this genus are widely distributed throughout east, central, and southern Africa with some marginal distribution in West Africa (Monadjem et al. 2015). These murid rodents predominantly occur in Grassland vegetation with some shrub cover but they have also been documented in Savanna woodlands. *Aethomys* species tend to excavate burrows under the cover of shrubs or places with greater cover such as rocky crevices, piles of boulders or debris, fallen trees, thick shrub or clumps of grass (Skinner & Chimimba 2005). Species within the genus are nocturnal, and although limited information is available, it appears that they are generally solitary in nature but can form small family groups (Skinner & Chimimba 2005).

Currently there are nine species recognized in this genus which include *A. chrysophilus, A. ineptus, A. bocagei, A. hindei, A. kaiseri, A. nyikae, A. silindensis, A. stannarius* and *A. thomasi* (Monadjem et al. 2015). Only two of these are endemic to the southern African subregion: the red veld rat, *A. chrysophilus*, and the tete veld rat, *A. ineptus*. These two species are indistinguishable when using existing taxonomic identification keys but they differ karyologically (Gordon & Rautenbach 1980). The upper parts of both these species range from brown to dark brown and are sprinkled with very dark brown or black hairs (Skinner & Chimimba 2005). *Aethomys chrysophilus* and *A. ineptus* can be distinguished from similar species such as *Micaelamys* (see below), by being more heavily built and having shorter tail lengths relative to their body length. Their tails are also thicker and more heavily scaled (Skinner & Chimimba 2005). Within South Africa, *A. chrysophilus* has a small range in the furthest northern part of the country and they may overlap with the larger range of *A. ineptus*. (Fig 1.1). The latter species has a larger range that span from central South Africa toward the east coast boundary and northwards almost reaching the northern boundary. The range of *A. chrysophilus* falls within the Savanna biome and the range of *A. ineptus* spans across the Savanna biome as well as the Grassland biome.

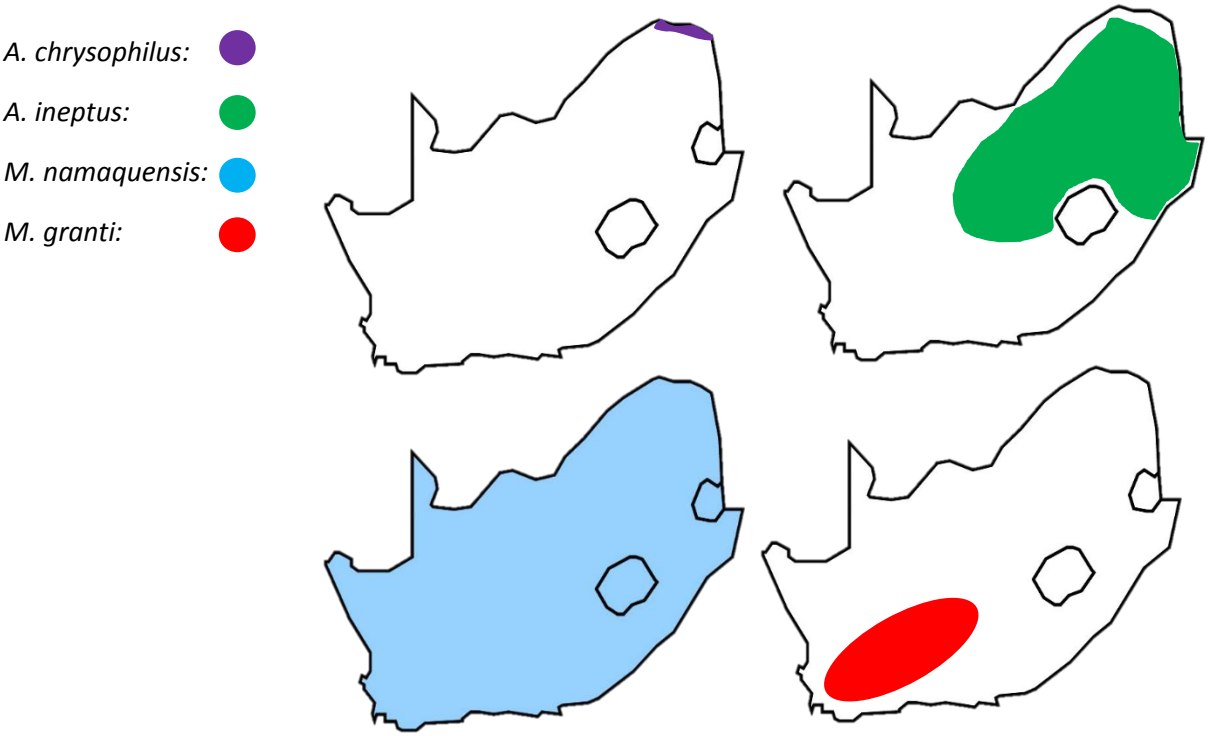

Figure 1.1-The respective ranges of *A. chrysophilus* (purple), *A. ineptus* (green), *M. namaquensis* (Blue) and *M. granti* (red) in South Africa. Approximate species distributions derived from Monadjem et al. 2015.

1.3.2.2 *Micaelamys*

Micaelamys, previously regarded as part of the *Aethomys* genus (Davis 1975; Musser & Carleton 1993), is a group of rodents also referred to as lesser veld rats. The genus has a large geographic range that spans almost the whole of the southern African subregion, extending north into Angola, Zambia, Malawi and Mozambique (Skinner & Chimimba 2005). Although *Micaelamys* also occur over a large variety of habitats (woodland, grassland, on the fringe of pans and in open scrub) they prefer rocky outcrops or boulder-strewn hillsides, where they live in rock crevices, holes in trees, under fallen logs or in piles of debris (Skinner & Chimimba 2005). These rodents are also nocturnal but seem to be more group-living and usually live in small colonies (Skinner & Chimimba 2005). Currently there are two annotated species that belong to this genus: Grant's rock mouse, *M. granti*, and the Namaqua rock mouse, *M. namaquensis* (Monadjem et al. 2015). The two species differ morphologically and in the number of chromosomes (Visser & Robinson 1986; Chimimba et al. 1999). *Micaelamys namaquensis* individuals have a longer tail relative to their body length (with the tail length being approximately one and a half times the body length) and a pure white

ventral pelage. They also have three pairs of nipples and a chromosome number of 2n=24. *Micaelamys granti* individuals have a shorter tail relative to their body length (with the tail being approximately as long as the body length) and a more greyish ventral pelage and five pairs of nipples. This species has a chromosome number of 2n=32 (Visser & Robinson 1986; Chimimba et al. 1999). Within South Africa, the range of *M. namaquensis* spans almost the whole of the country while the range of *M. granti* is restricted to the Karoo in south-central South Africa (Monadjem et al. 2015) (Fig 1.1). *Micaelamys namaquensis* occurs in all of the biomes in South Africa (Forest, Fynbos, Grassland, Nama Karoo, Savanna, Succulent Karoo and Thicket), while the range of *M. granti* spans across the Nama Karoo, Fynbos, Succulent Karoo and Grassland biomes.

1.3.3 Systematics of the *Aethomys/Micaelamys* rodent species complex

The four species mentioned above are characterized by vacillations in their taxonomy. As mentioned above, *M. granti* and *M. namaquensis* were previously classified to be part of the *Aethomys* genus (Davis 1975; Musser & Carleton 1993) and *A. chrysophilus* and *A. ineptus* were previously classified as conspecific (Gordon & Rautenbach 1980). Based on mtDNA (mitochondrial DNA) cytochrome b gene analyses, *A. namaquensis* was placed distantly related to *A. chrysophilus* and paraphyletic from other *Aethomys* species (Ducroz et al. 2001). In a subsequent study, the uniqueness of these lineages were supported by a sequence divergence value of 24.5% between the genera, justifying their separate generic status (Chimimba 2005). These results saw that the subgenera *Micaelamys* and *Aethomys* were elevated to full generic rank. The previously conspecific cryptic *A. chrysophilus* and *A. ineptus* have been recognized as two different species as a result of morphometric analysis as well as DNA sequence data (Chimimba et al. 1999; Russo et al. 2006). The latter two species are however closely related from a genetic perspective.

1.3.4 Speciation history of *Aethomys* and *Micaelamys*

Fossil evidence suggests that members of the *Aethomys* and *Micaelamys* genera were present in southern Africa since the late Miocene (Lecompte et al. 2008). The exact mechanisms at play that gave rise to the establishment of the members of the *Aethomys/Micaelamys* rodent species complex is not well researched. A recent study, however, linked three major periods of diversification within *M. namaquensis* to periods of aridification and the expansion of savanna habitats in southern Africa (Russo et al. 2010). The authors concluded that the intra-lineage phylogeographic patterns suggests differences in adaptation and responses to Plio-Pleistocene climatic and vegetation changes (Russo et al. 2010). At the generic level, it is noteworthy that the late Miocene (6.7-6.5 Ma) was associated with the onset of xeric conditions in southern

Africa that was brought about by the increased upwelling of cold water by the Benguela current, following a glaciation event of Antarctica which lead to the rapid cooling of ocean temperatures (Marlow et al. 2000; Tyson & Partridge 2000). This restricted the amount of evaporation for onshore precipitation, leading to the aridification of southern Africa (Marlow et al. 2000). In addition there were tectonic uplift events (5 Ma) along the margins of the Great Escarpment that lead to the sloping topology of southern Africa from east to west, which resulted in the rain shadow effect across this region (Patridge 1997; du toit et al. 2012). These two events were responsible for significant vegetation changes and the resulting establishment of the modern biomes in southern Africa (Coetzee 1978; Scott et al. 1997; du Toit et al. 2012). These vegetation changes led to the diversification of several small mammals in this period (Taylor et al. 2009; Willows-Munro et al. 2011; du Toit et al. 2012) and most likely also contributed to the diversification of the *Aethomys/Micaelamys* species complex.

1.4 Ectoparasitic lice occurring on rodents belonging to the *Aethomys/Micaelamys* rodent species complex

From a taxonomic perspective, it can be argued that the South African *Aethomys/Micaelamys* rodent species complex has now been resolved and is stable. However, the taxonomy of the lice that are found on these rodents is peculiar. Previous investigations based on morphological characteristics have showed that *M. namaquensis* is parasitized by three lice species which include, *Hoplopleura patersoni, Hoplopleura aethomydis* and *Polyplax praomydis.* (Durden & Musser 1994; Fagir et al. 2014). For *M. granti* there is currently no data on the lice species that are associated with it. The distantly related *A. chrysophilus* is parasitized by two species of lice also occurring on *M. namaquensis*: *Polyplax praomydis* and *Hoplopleura patersoni* (Durden & Musser 1994). As a result of the recent revision of the *Aethomys* genus (Chimimba et al. 1999; Ducroz et al. 2001; Chimimba 2005; Russo et al. 2006), and given the close evolutionary association among *A. ineptus* and *A. chrysophilus*, it is predicted that these two rodent species share the same lice species but there is no current data to confirm this prediction.

Hoplopleura patersoni can be distinguished morphologically by apical setae on its paratergites 4-6 and these are minute. The 7th paratergite has a ventral lobe that is acute rather than truncate (Johnson 1960). Other morphological features which can be used to identify this species include features of the head and sternal plate (Johnson 1960). The head of this species is acutely rounded before the antennae and the head is longer than broad (Johnson 1960). The sternal plate of this species is narrowed, apically rounded and has a posterior prolongation, with the rest of the plate being oval in shape (Johnson 1960).

Hoplopleura aethomydis can be distinguished from other species by the features of its head, sternal plate and paratergal plates (Kleynhans 1969). The head of this species is truncate in front and longer than it is wide, with preantennal margins that are straight and converging while postantennal margins are smoothly rounded (Kleynhans 1969). The sternal plate of this species is about one and a half times longer than wide (Kleynhans 1969). The first sternal plate of segment 3 has two enlarged setae on either side (Kleynhans 1969). The paratergal plates are scaly with the apical lobes slender in both plates 2 and 3, both lobes broad and slightly biblobate in plates 4 and 5, both lobes slender in plate 6, and neither of the apical lobes developed in plate 8 (Kleynhans 1969). *Hoplopleura aethomydis* shows the most similarity in terms of morphology to *H. patersoni* (Kleynhans 1969), these two species do however differ in that the two paramedian setae on the first sternal plate of the third abdominal segment are very small *in H. aethomydis* (Kleynhans 1969). *Hoplopleura aethomydis* also lacks a distinct constriction anteriorly on paratergal plates 3-7 (Kleynhans 1969). A further difference between these two species is the fact that in *H. aethomydis* both of the apical lobes of paratergal plate 6 are slender (Kleynhans 1969).

Polyplax praomydis show some morphological overlap with *P. spinulosa* (Johnson 1960). These species are however readily separable as the paratergal plates 3-6 in *P. praomydis* have neither of the posterior angles prolonged (Johnson 1960). The two apical setae on each of these plates are set very near the posterior apical angles in *P. praomydis* (Johnson 1960). In *P. praomydis* the head is also much longer compared to the width when compared to *P. spinulosa*. The head of these species are also rounded in front of the antennae (Paterson & Thompson 1953).

1.5 Aims, Objectives and Hypotheses

The present study focussed on providing additional data to decipher the mechanisms involved in louse speciation, and also to provide more insights into the taxonomy of lice occurring on the *Aethomys*/*Micaelamys* rodent species complex.

The aims of the present study were:

1) to document the species diversity of lice occurring on the South African *Aethomys/Micaelamys* rodent species.
2) to test the validity of the single species descriptions for *H. patersoni* and *P. praomydis* who occur on members of the *Aethomys/Micaelamys* rodent species complex.

3) to test whether there is any co-divergence between permanent ectoparasitic lice and their rodent hosts.
4) to test for cryptic geographic diversity in *H. aethomydis*, *H. patersoni*, and *P. praomydis* that are all known to occur on the geographically widespread *M. namaquensis.*
5) to investigate the mechanisms affecting parasitic lice evolution.

The objectives of the present study were:

1) to sample, identify and sequence sucking lice obtained from the four rodent species belonging to the South African *Aethomys/Micaelamys* species complex.
2) to use phylogenetic-, co-divergence and dating analyses of DNA sequence data to infer potential co-divergence patterns between parasitic lice and rodent host lineages.
3) to use broad scale geographic sampling, sequence data derived from mtDNA and nuDNA genes, and comparative phylogeographic techniques, to test for geographic cryptic speciation and evolutionary co-divergence in lice occurring on the widely distributed *M. namaquensis.*
4) to compare the outcomes of the present study to recently published works in the same geographic area in an attempt to advance our knowledge on the factors responsible for parasite dispersal and evolution.

It was hypothesised that:

1) the species diversity of the lice found on South African *Aethomys* and *Micaelamys* species is underestimated.
2) the species-host lists for the lice found on South African *Aethomys* and *Micaelamys* is outdated.
3) at least three rodent host species studied herein will harbour genetically distinct lice species.
4) the phylogeny of the lice collected from the different hosts will show congruence to the phylogeny of the hosts.
5) the lice collected from *M. namaquensis* across South Africa will show significant phylogeographic congruence with their host.

1.6 Predictions

It is anticipated that this study will provide new insights into the taxonomy of lice on the *Aethomys*/*Micaelamys* rodent species complex and will offer new insights into the mechanisms involved in ectoparasite speciation. For example, the outcome of the present study can provide more clarity on the effect of host dispersal on parasite gene flow by comparing the data to the recently published work on *Rhabdomys* (du Toit et al. 2013 b). Compared to *Rhabdomys*, which is a generalist occupying mostly plains regions, *M. namaquensis* is mainly confined to rocky outcrops, and is thus more restricted in their dispersal (Russo et al. 2010). Since a higher level of host dispersal and host contact was put forward as the reason why there were only partial phylogeographic congruence between *P. arvicanthus* species and their hosts *Rhabdomys* (du Toit et al. 2013 b) the outcome of the current study can be used to test whether restrictions in host movement can facilitate better congruence between parasite and host structures (Matthee et al. 2018).

Chapter 2: Deciphering the evolutionary history of parasitic sucking lice and their *Aethomys* and *Micaelamys* rodent hosts: a case for evolutionary co-divergence*

* The format of this chapter is governed by the intention to submit it for publication in the peer review literature. It should be regarded as a publication unit and for this reason some repetition with text presented in Chapter 1 may occur.

2.1 Introduction

Close associations between parasites and their hosts have led to the widely held believe that parasites should show some level of phylogenetic congruence with their host (Morand & Krasnov 2010). More in depth studies, however, have shown that a number of factors can influence the level of evolutionary congruence between parasites and their hosts (Nieberding et al. 2008; Althoff et al. 2014; Engelbrecht et al. 2016). These factors typically include an interaction between parasite - and host life history characteristics and biogeography (du Toit et al. 2013 b). Predicting phylogenetic congruence between parasites and their hosts is thus complex, although some generalizations are evident. For example, host-specific permanent parasites are more likely to have evolutionary co-divergence with their host as they are more dependent on their specific host for food and dispersal when compared to generalist parasites (Patton 1984). Indeed, it has been shown that permanent parasites that spend their entire lifecycle on their host often show full or partial evolutionary co-divergence with their host (Hafner & Page 1995; Page & Hafner 1996; Gomez-Diaz et al. 2007). Host life history characteristics, such as social behaviour and dispersal ability, also influence parasite-host co-divergence. Asocial behaviour of a host is generally associated with a greater dispersal ability and more contact among different individuals over larger geographic scales (Matthee et al. 2018) and this would result in a higher probability for host switching. The latter will result in the lack of evolutionary congruence between parasites and their hosts (Martinu et al. 2015). Despite the strong influence of parasite and host life history characteristics on co-divergences, biogeography (and particularly host vicariance) can play and equally powerful role in shaping the evolution of hosts and their parasites, even in generalist species (Sands et al. 2017).

The complexity of parasite-host co-divergence was recently highlighted in the sucking louse *Polyplax arvicanthis* that parasitize the rodent genus *Rhabdomys* (du Toit et al. 2013 b). *Polyplax arvicanthis* individuals are specific to their host genus (Ledger 1980) and permanently associated with the body of their host, i.e. they complete their entire lifecycle on their host (Durden & Musser 1994). Based on this, and the above, it was predicted that evolutionary congruence would be recorded between *P. arvicanthis* and its host. The authors however, found cryptic diversity on the host (du Toit et al. 2013 a) and partial evolutionary co-divergence (du Toit et al. 2013 b). They ascribed the lack of complete congruence to the larger effective population size of the parasite lineages, the vagility and social behaviour of *Rhabdomys* and the lack of host-specificity in areas where the congeneric hosts co-occur (du Toit et al. 2013 b). From this it is reasonable to propose that evolutionary co-divergence between parasites and their hosts cannot be assumed even in documented cases where there is a strong association. Rather, evolutionary co-divergence is a result of the complex relationship between biogeographic-, host- and parasite related factors (Page 2003; Clayton et al. 2003; du Toit et al. 2013 b).

The *Aethomys*/*Micaelamys* rodent species complex in South Africa provides an ideal model to further unravel co-divergences between hosts and their parasites. Within the genus *Aethomys,* there are two species that are endemic to the southern African subregion: the Red veld rat, *A. chrysophilus*, and the Tete veld rat, *A. ineptus* (Monadjem et al. 2015). *Aethomys chrysophilus* is restricted to a small range on the northern boundary of South Africa, whilst *A. ineptus* has a larger range that span from central South Africa towards the east coast and northwards almost reaching the northern boundary of the country (Monadjem et al. 2015) (Fig 2.1). These two species are indistinguishable when using existing taxonomic identification keys but they differ karyologically (Gordon & Rautenbach 1980). They are genetically closely related with an mtDNA (mitochondrial DNA) cytochrome b sequence divergence of 1.9% separating the two monophyletic taxa (Russo et al. 2006). The genus *Micaelamys* consist of two species which are both present in South Africa: Grant's rock mouse, *M. granti*, and the Namaqua rock mouse, *M. namaquensis* (Monadjem et al. 2015). The range of *M. namaquensis* spans almost the whole of the country whilst the range of *M. granti* is restricted to the Karoo in south-central South Africa (Monadjem et al. 2015) (Fig 2.1). These two species differ morphologically and in chromosome number (Visser & Robinson 1986; Chimimba et al. 1999), however, no molecular sequence divergence data are currently available. Interestingly, based on morphological analyses, *M. granti* and *M. namaquensis* were previously classified to be part of the *Aethomys* genus (Davis 1975; Musser & Carleton 1993) and *A. chrysophilus* and *A. ineptus* were previously classified as conspecific (Gordon & Rautenbach 1980). Molecular sequence data however recommended that the subgenera *Micaelamys* and *Aethomys* be elevated to full generic rank (Ducroz et al. 2001; Chimimba 2005), and that the previously conspecific cryptic *A. chrysophilus* and *A. ineptus* be recognized as two different species (Chimimba et al. 1999; Russo et al. 2006). *Micaelamys* and *Aethomys* are separated by an mtDNA cytochrome b sequence divergence of 24.5 % (Chimimba 2005). What makes the *Aethomys/Micaelamys* rodent species complex an ideal model to test for co-divergences between parasites and hosts is the fact that i) the host phylogeny and taxonomy is resolved, ii) the species differ in their genetic relationships to one another (timing of divergences differ markedly among taxa), iii) some host species overlap partially in distribution, while others are allopatric, making host switching possible or less likely.

Previous investigations focussing on the parasites occurring on *Aethomys*/*Micaelamys* rodents did not take into account the vacillations in host taxonomy and were exclusively based on external morphology (Durden & Musser 1994). In this context it also important to realize that the four rodent species included in this study are difficult to distinguish morphologically and the original parasite-host species lists may thus be incorrect also (e.g. Zumpt 1961, Ledger 1980; Segerman 1995). Nonetheless, from the parasite taxonomic literature it can be deduced that three sucking lice species, *Hoplopleura patersoni, Hoplopleura aethomydis* and *Polyplax praomydis* occur on *M. namaquensis* (Durden & Musser 1994; Fagir et al. 2014). Two of these species, *P. praomydis* and *H. patersoni*, in addition to a third species, *Polyplax solivaga* have been

recorded on *A. chrysophilus* (Durden & Musser 1994). With regard to the later species (*P. solivaga*), only one male individual was found on *A. chrysophilus* (Kleynhans 1969). Given that the two rodent genera are reported to share conspecific lice, based on morphology, and taking into consideration the recent revision of the *Aethomys* genus (Chimimba et al. 1999; Ducroz et al. 2001; Chimimba 2005; Russo et al. 2006), it is predicted that the closely related *A. ineptus* and *A. chrysophilus* will either share conspecific or closely related lice taxa. For *M. granti*, however, there is no lice species data, but given the divergence between the two *Micaelamys* species, it is hypothesise that they will harbour genetically distinct, and possibly cryptic *P. praomydis* and *H. patersoni* lineages. A thorough investigation of the parasites occurring on the *Aethomys* - and *Micaelamys* species in South Africa is however critically needed. Specifically a molecular approach is needed to test the validity of the single species hypotheses for *P. praomydis* and *H. patersoni* reported to occur on *M. namaquensis* and *A. chrysophilus*. Since these two lice species complete their entire lifecycle on their hosts they are likely to show evolutionary congruence with their hosts (Hafner & Page 1995; Page & Hafner 1996; Gomez-Diaz et al. 2007). If this prediction holds several new lice species/taxa exist on these rodents and given that they are reportedly morphologically similar, these lineages should at least then be cryptic. Cryptic diversity is rife in parasites as their reduced bodily features, small size and morphological stasis often limits finding variable morphological characters (Perkins et al. 2011). This is especially true for closely related species (Nadler & de Leon 2011). The present study will facilitate a better understanding of the extent of cryptic diversity in parasite fauna (de Leon & Nadler 2010; Nadler & de Leon 2011; Perkins et al. 2011; du Toit et al. 2013 a; Engelbreght et al. 2014).

By sampling all the lice occurring on all four *Aethomys/Micaelamys* species occurring in South Africa, and by making use of mitochondrial - and nuclear DNA sequence data, the aims of the study were to: 1) test the validity of the single species descriptions for *H. patersoni* and *P. praomydis*; 2) test whether there is any co-divergence between permanent ectoparasitic lice and their rodent hosts; and 3) investigate the mechanisms affecting lice evolution. The hypotheses were that: 1) the four rodent species would harbour genetically distinct lice taxa; and 2) the phylogeny of the lice collected from the different hosts would show significant congruence to the phylogeny of the hosts.

2.2 Materials and Methods

2.2.1 Host sampling

Representatives of all four host species were collected within their respective ranges (Fig 2.1) and where possible identified based on morphological grounds. The widely distributed *M. namaquensis* were trapped at three localities, Loeriesfontein (LF) Elandskuil (EK) and Mogalakwena (MO). *Micaelamys granti* individuals were trapped at Eselfontein (EF), while *A. chrysophilus* samples originate from Mogalakwena (MO) and *A. ineptus* from Groot Marico (GM) (Fig 2.1). All trapping and animal handling was conducted after obtaining the necessary permits from local authorities (Permit numbers: Limpopo, ZA/LP/90994; North West, NW 7705; Eastern Cape, CRO 150/17CR and CRO 11/17CR; Northern Cape, FAUNA 0942/2017 and FAUNA 0949/2017) and also ethical clearance from Stellenbosch University (SU-ACUD16-00190). Sherman-type live traps, baited with a mixture of peanut butter and oats, were used. Between 70 and 300 traps were used per locality and up to 100 traps were set in each of the replicated trap lines. Within the trap lines, traps were spaced approximately 10m apart. Sampling was conducted in habitats that looked similar to the described preferred habitats of the individual rodent species (Skinner and Chimimba 2005). As the rodents in this study are all nocturnal, the traps were set out late afternoon and then checked and closed again early in the morning. Trapped hosts of the targeted species were placed in individual plastic bags and euthanized using an intraperitoneal injection of sodium pentobarbitone (200mg/kg). Each bag was tagged with a unique animal reference sampling code. The carcasses were frozen in the field at -20^{o}C. Host tissue (muscle/tongue) was collected once back in the laboratory and this was placed in 100% ethanol to confirm species authenticity based on molecular sequences. All other non-targeted rodent species were identified, recorded and released.

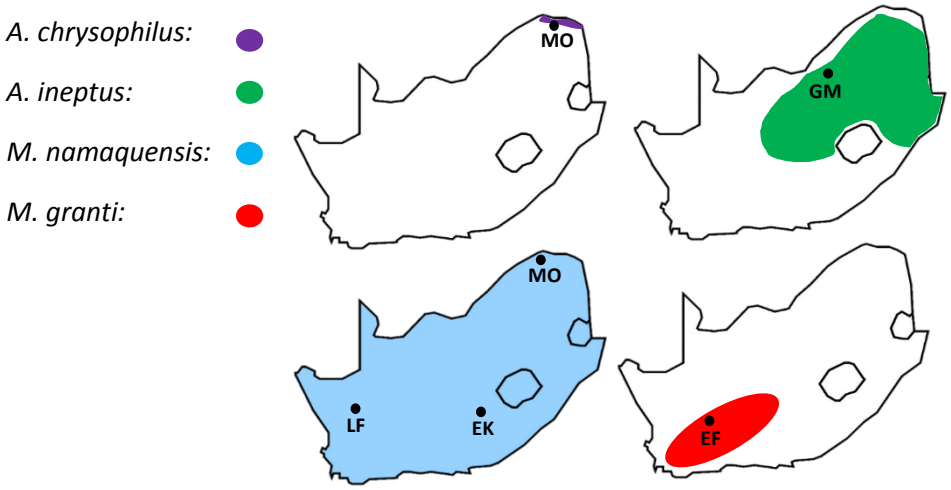

Figure 2.1-Trapping localities within the respective ranges of *A. chrysophilus* (purple), *A. ineptus* (green), *M. namaquensis* (blue) and *M. granti* (red) in South Africa. Localities include: Mogalakwena (MO), Elandskuil (EK), Loeriesfontein (LF), Eselfontein (EF) and Groot Marico (GM). Distribution ranges derived from Monadjem et al. 2015.

2.2.2 Parasite removal and identification

In the laboratory, host individuals were thawed and all lice were removed using a stereoscopic microscope (Leica Microsystems, Wetzlar, Germany) and fine point forceps, after which lice were placed in 100% ethanol. After lice DNA was extracted the exoskeleton of each louse was recovered for morphological identification using published species descriptions (Johnson 1960; Kleynhans 1969) as well as taxonomic reference keys (Paterson & Thompson 1953; Johnson 1960; Kleynhans 1969). Lice species identification were also confirmed by an expert taxonomist (Prof L.A. Durden, Department of Biology, Georgia Southern University, USA).

2.2.3 Sampling, DNA extraction and sequencing

The aim for this study was to include at least five individual sequences per host species per locality, and 10 individual sequences per lice species, per locality. This was not possible in all circumstances and in some instances smaller sample sizes were included as a result of: i) unsuccessful trapping of hosts at various localities; ii) the low prevalence or absence of lice on the hosts; and iii) the unsuccessful sequencing of some lice individuals (See Table 2.1). In the absence of replicate geographic sampling for all species and to obtain some insights into intraspecific diversity, the widely distributed *M. namaquensis* was sampled at three localities. Total genomic DNA was extracted from host and louse individuals using the Nucleospin

Tissue kit according to the protocol set out by the manufacturer (Macherey-Nagel, Duren, Germany). The mitochondrial Cytochrome Oxidase subunit I (COI) gene was amplified for all hosts and lice using published and newly designed primers (Table 2.2). Nuclear DNA data were also generated for the carbamoyl-phosphate synthetase 2, aspartate transcarbamylase and dihydroorotase (CAD), nuclear elongation factor 1 alpha (EF-1α) and interphotoreceptor retinoid binding protein (IRBP) for *Polyplax* spp., *Hoplopleura* spp. and all hosts respectively using primers outlined in (Table 2.2). Despite several attempts, the same nuclear gene fragments could not be used across all taxa due to failed attempts to amplify. The PCR protocols for the amplification of the COI gene fragment for all of the lice species included five minutes of initial denaturation at 95°C, followed by 10 cycles of denaturation for 30 seconds at 95°C, one minute of annealing at 48°C and two minutes of extension at 72°C this was then followed by 30 cycles of denaturation for 30 seconds at 93°C, one minute of annealing at 48°C and two minutes of extension at 72°C, this was then followed by a final extension period for 10 minutes at 72°C. The PCR protocols for the amplification of the COI fragment for host individuals included five minutes of initial denaturation at 94°C followed by 35 cycles of denaturation for one minute at 94°C, one minute of annealing at 48°C and 1minute of extension at 72°C, this was followed by a final extension of 10 minutes at 72°C. The PCR amplification of the nuclear CAD and Ef-1α genes included three minutes of initial denaturation at 94°C followed by 35 cycles of denaturation for 30 seconds at 94°C, 45 seconds of annealing at 55°C and 45 seconds of extension at 72°C, this was followed by a final extension of five minutes at 72°C. For the amplification of the nuclear IRBP gene the PCR protocols included five minutes of initial denaturation at 94°C followed by 35 cycles of denaturation for one minute at 94°C, one minute of annealing at 65°C and one minute of extension at 72°C, this was followed by a final extension of 10 minutes at 72°C. Sequencing of the various gene fragments were performed following standard BigDye chemistry and analyses were performed on an automated sequencer (ABI 3730 XL DNAAnalyzer, Applied Biosystems). Sequences were visualized in Geneious v. 9.1 (https://www.geneious.com) and aligned with Clustal W (Thompson et al. 1994) implemented in the software. To confirm taxonomic identity of the hosts, their sequences were blasted against Genbank using the nucleotide BLAST (BLASTN) function. In the case of *A. chrysophilus*, *A. ineptus* and *M. namaquensis*, however, only cytochrome b sequences were available on Genbank (Mazoch et al. 2017; Russo et al. 2006; Russo et al. 2010). To confirm species authenticity, the cytochrome b gene regions was also amplified and sequenced for subsamples of the hosts using the published primer pair L14724 and H15915 (Pääbo et al. 1988; Irwin et al. 1991). The same protocol as outlined for the COI region was used.

Table 2.1-The number of host trapped, the number of host screened, the lice that were associated with these hosts and the number of these hosts and lice used for molecular analyses for each locality (locality abbreviations correspond to those given in Fig 2.1).

Locality/host	Number of hosts caught	Number of hosts screened	Number of hosts with lice	Number of lice (*Hoplopleura*/*Polyplax*)	Number of host used in analyses	Number of lice used in analyses (*Hoplopleura*/*Polyplax*)
MO/*M. namaquensis*	15	15	11	93/19	5	10/10
LF/*M. namaquensis*	8	8	2	0/15	2	0/8
EK/*M. namaquensis*	35	35	31	69/362	5	10/10
	21	21	11	10/70	5	4/10
	2	2	1	2/0	2	2/0
	11	11	3	23/0	5	10/0

Table 2.2-Primers and annealing temperatures used for the PCR amplification of the various gene fragments for the different taxonomic groups. The taxa used, gene fragments sequenced, primer abbreviations and source of the primers are also indicated

Taxon names	Genes sequenced	Primer abbreviations	F/R	Primer sequences (5'-3')	PCR annealing temperatures (°C)	Source of the primers
Polyplax spp.	COI	C1-J-1718	F	GGAGGATTTGGAAATTGATTAGTTCC	48°C	Simon et al. 1994
		HCO2198	R	TAAACTTCAGGGTGACCAAAAAATCA		Folmer et al. 1994
	CAD	CADPfor	F	ACGACAACTGCATTACCGTTTGCA	55°C	Du Toit et al. 2013 b
		CADPrev	R	CCACCGGGGAATTTTGACAAC		Du Toit et al. 2013 b
Hoplopleura spp.	COI	COIHoplopleura	F	GAACCGGATGAACTGTGTACC	48°C	This study
		COIFHoploNN	F	GGTCAACAAATCATAAAGATATT		This study, adapted from LCO 1490, Folmer et al. 1994
		HCO2198	R	TAAACTTCAGGGTGACCAAAAAATCA		Folmer et al. 1994
	EF-1α	EF-1αFor3	F	GGGGACAAYGTTGGTTTCAACG	55°C	Danforth & Ji 1998
		Cho10	R	ACGGCVACKGTYTGHCKCATGTC		Danforth & Ji 1998
Rodents	COI	LCO1490	F	GGTCAACAAATCATAAAGATATTGG	48°C	Folmer et al. 1994
		HCO2198	R	TAAACTTCAGGGTGACCAAAAAATCA		Folmer et al. 1994
	IRBP	IRBP-F1	F	GAATGACCCACGTCTCTTCATCT	65°C	Phukuntsi et al. 2016
		IRBP-R1	R	AGGTCCTCTTCAGAGACCA		Phukuntsi et al. 2016

2.2.4 Data analysis

2.2.4.1 Phylogenetic relationships

To investigate the intraspecific relationship among the mitochondrial and nuclear haplotypes of *Polyplax* and *Hoplopleura* species, and to confirm the genetic distinctiveness among the four rodent host species, statistical parsimony haplotype networks were constructed in Popart 1.7 (Clement et al. 2002). To test statistical significance of the connections among distantly related haplo-groups, statistical parsimony networks were also drawn in TCS 1.21 (Templeton et al. 1992; Clement et al. 2000). Standard molecular diversity measures were calculated in DNAsp 6.12 (Rozas et al. 2017). Since clades forming part of the haplotype networks for *Polyplax* - and *Hoplopleura* species could not be connect with statistical certainty, the deeper evolutionary relationships among clades were examined by constructing Bayesian phylogenetic trees in MrBayes 3.2.6 (Ronquist et al. 2012) and Maximum likelihood trees in RaxMl 1.5 (Stamatakis

2006). Based on the outcome of the network analyses, mitochondrial and nuclear DNA sequence data were combined and analysed together in a partitioned fashion to determine the phylogenetic associations between the *Polyplax* spp., *Hoplopleura* spp. and the four rodent species respectively. *Hoplopleura biseriata* found on G*erbilliscus leucogaster* and *Hoplopleura capensis* found on *Desmodillus auricularis* were used as distantly related outgroups to root the *Hoplopleura* phylogenies (Durden & Musser 1994; Ducroz et al. 2001; Chevret & Dobigny 2005). For the *Polyplax* phylogeny, *Polyplax praomydis* occurring on *Rhabdomys pumilio* was used outgroup (Durden & Musser 1994; Ducroz et al. 2001; du Toit et al. 2013 b). For the host phylogenies *Gerbillus nanus* and *Rhabdomys pumilio* were included as outgroups, where *R. pumilio* has a closer relationship with *Aethomys* and *Micaelamys* than the more distantly related *G. nanus* (Ducroz et al. 2001; Chevret & Dobigny 2005). *Rhabdomys pumilio* was included as it is the host of the outgroup species included in the *Polyplax* phylogeny. The hosts of outgroup taxa in the *Hoplopleura* phylogeny (*G. leucogaster* and *D. auricularis*) are represented by *G. nanus* in the host phylogeny as this species is closely related to both these hosts (Ducroz et al. 2001; Chevret & Dobigny 2005) and the relevant sequences of the original host were not available on Genbank.

Best-fit models of sequence evolution was calculated using jModelTest 3.7 (Guidon & Gascuel 2003; Darriba et al. 2012) in PAUP 4 (Swafford 2002) and by applying the AIC criterion (Akaike 1973; Burnham & Anderson 2004). The Bayesian analyses were all based on data that were partitioned by codon and the parameters were unlinked across partitions. Each analysis included two parallel Markov Chain Monte Carlo (MCMC) simulations that ran for 10 million generations where 25% of the total number of generations were discarded as burn-in. The remaining trees were visualized in Figtree 1.4.3 (Rambaut 2015) to obtain posterior probabilities for nodes. The same partitions were employed for the Maximum Likelihood analyses but in this instance 1000 bootstrap repetitions were performed to obtained confidence in the nodes. The best-fit models of evolution were again specified for different partitions and trees were also visualized in Figtree 1.4.3 (Rambaut 2015).

2.2.4.2 Co-phylogeny

Co-phylogeny between the hosts and both parasite taxa was investigated by topology-based reconciliation in Jane v.4 (Conow et al. 2010). Tree topologies based on the Bayesian and Maximum likelihood topologies were constructed for the host as well as both louse taxa using tree editor imbedded in Jane v.4 (Conow et al. 2010). In these co-phylogeny analyses a cost is assigned to different evolutionary events whilst the analyses attempt to find the most parsimonious (lowest cost) solution to map the parasite phylogeny onto that of the host (Conow et al. 2010). The Vertex cost model with the cost scheme: failure to diverge = 1,

loss = 1, duplication followed by host-switch = 2, duplication = 1, co-divergence = 0, was implemented (Conow et al. 2010). The Genetic algorithm was set to 1000 generations and a population size of 300. Statistical significance of the solutions was evaluated by random tip mapping and the randomization of the parasite topology, where the statistical algorithm was again set to 1000 generations, a population size of 300 and including a sample size of 1000 (as in Engelbrecht et al. 2016).

2.2.4.3 Divergence dating

In order to investigate whether the evolutionary timing of divergence events between the parasite lineages corresponded to that of the hosts, the COI datasets were used to estimate divergence dates in BEAST 2.5.1 (Bouckaert et al. 2014). In the absence of fossil dates for the species under consideration, fossil dating from other Murid taxa were used in the analyses. For the rodent host analysis the split between *Mus* and *Rattus* approximately 11-12.3 Ma (Benton & Donoghue 2007; du Toit et al. 2013 b) was included as a minimum prior. Similarly, the split between human and chimpanzee *Pediculus* lice, approximately 5-7 Ma (Stauffer et al. 2001; Light et al. 2010), was included as a prior for both lice. For all of the divergence dating analyses the optimal model as determined by jModelTest 3.7 (Guidon & Gascuel 2003; Darriba et al. 2012) was specified, with the calibrated Yule speciation process as tree prior. The MCMC simulation ran for 50 million generations whilst sampling every 5,000 generations. To ensure that all effective sample size (ESS) values were more than 200, convergence and mixing were analysed in Tracer 1.7.1 (Rambaut et al. 2018). This was followed by the estimation of the maximum clade credibility (MCC) tree in TreeAnnotator 2.5.1 (Bouckaert et al. 2014), where the first 1,000 tree samples were discarded as burn-in. The MCC trees were then then visualised and edited in Figtree 1.4.3 (Rambaut 2015).

2.3 Results

2.3.1 Identification of sucking lice

A total of 162 *Hoplopleura* individuals were collected from 58 *M. namaquensis* hosts whilst 10 *Hoplopleura* individuals were collected from 21 *M. granti* hosts. A total of 23 *Hoplopleura* individuals were also collected from 11 *A. ineptus* hosts whilst two *Hoplopleura* individuals were collected from two *A. chrysophilus* hosts. For *Polyplax* a total of 396 individuals were removed from 58 *M. namaquensis* hosts, whilst 70 *Polyplax* individuals were removed from 21 *M. granti* hosts. For *Hoplopleura*, all specimens collected from *M. namaquensis* and the two *Aethomys* species matched the description of *H. patersoni* (Johnson 1960) while those collected from *M. granti* did not match any currently described *Hoplopleura* species. The latter undescribed species differed morphologically from *H. patersoni* in that the last paratergal plate was more elongate and pointy, there were no dorsal marginal head setae, the setae on the paratergal plates were different lengths and in the male genitalia there was an extra scleratized area basal to the parameres. Within *Polyplax*, two morphologically distinct species were collected from the two *Micaelamys* species. *Polyplax* species were also completely absent from the *Aethomys* hosts collected herein. The *Polyplax* species found on *M. namaquensis* matched the description of *P. praomydis* (Johnson 1960), but the lice on *M. granti* did not match any description currently available for *Polyplax* species. The latter species differed from *P. praomydis* in that the principle head setae was elongated and the shape of the sternal plate was also noticeably different. The morphological uniqueness of the two undescribed lice taxa was confirmed by L.A. Durden (Department of Biology, Georgia Southern University, USA).

2.3.2 Molecular analyses

2.3.2.1 Genetic relationships.

2.3.2.1.1 Rodent host associations

MtDNA and nuDNA sequence data were analysed for 24 host and 74 louse specimens (Table 2.1). The results obtained in this study confirm the genetic differentiation between the two rodent genera (*Aethomys* and *Micaelamys*) with 14.1% (±3.5%) mtDNA and 1.3% (±0.4%) nuDNA sequence divergence. Within genera the close association between the two *Aethomys* species was confirmed, as sequence divergences of 1.4% (±0.1%) and 0.3% (±0.2%) for mtDNA and nuDNA, respectively, were recorded. The *Micaelamys* species were more distantly related with an mtDNA COI sequence divergence of 11.3% (±2.7%) and 0.5% (±0.2%) nuDNA sequence divergence. What is noteworthy however is that sequence diversity values (although based on very limited sample sizes and in most instances sampled at single localities) was very low at mtDNA and nuclear DNA level (Table 2.3) but within *M. namaquensis* there was a 3.7% (±1.1%)

Table 2.3-The number of mtDNA and nuDNA base pairs sequenced, the nucleotide diversity and haplotype diversity for the different haplo-groups that could not be connected with 95% confidence in the mtDNA TCS haplotype network. Species names in brackets refer to the host the different lice haplo-groups were collected from.

	Base pairs sequenced	Nucleotide diversity	Haplotype diversity
Haplo-groups	**mtDNA/nDNA**	**mtDNA/nDNA**	**mtDNA/nDNA**
***M. namaquensis* (N)**	465/640	0.003±0.004/0.002±0.002	0.7±0.2/0.6±0.2
***M. namaquensis* (S)**	465/640	0.005±0.005/0.002±0.002	0.9±0.1/0.5±0.2
M. granti	465/640	0.003±0.003/0	0.7±0.2/0
A. ineptus	465/640	0/0	0/0
A. chrysophilus	465/640	0.002±0.002/0	1±0.5/0
***Hoplopleura* (*M. namaquensis* (N))**	222/307	0.02±0.01/0	0.6±0.1/0
***Hoplopleura* (*M. namaquensis* (S))**	222/307	0/0	0/0
***Hoplopleura* (*M. granti*)**	222/307	0/0	0/0
***Hoplopleura* (*A. chrysophilus*)**	222/307	0/0	0/0
***Hoplopleura* (*A. ineptus*)**	222/307	0/0	0/0
***Polyplax* (*M. namaquensis* (N))**	318/295	0.006±0.001/0	0.2±0.2/0
***Polyplax* (*M. namaquensis* (S))**	318/295	0.004±0.006/0.0004±0.0009	0.4±0.1/0.1±0.09
***Polyplax* (*M. granti*)**	318/295	0.009±0.009/0	0.5±0.2/0

mtDNA and 0.3% (±0.1%) nuDNA sequence divergence between populations sampled in the northern regions of South Africa (Fig 2.1; Mogalakwena (MO)) versus in the southern parts of South Africa (Fig 2.1; Elandskuil (EK) and Loeriesfontein (LF)).

The mtDNA TCS haplotype networks confirmed the differentiations among the four rodent species. Haplo-groups representing the two genera differed by at least 53 mutational steps and could not be connected within the 95% confidence interval. Only the two *Aethomys* species could be connected within a 95%

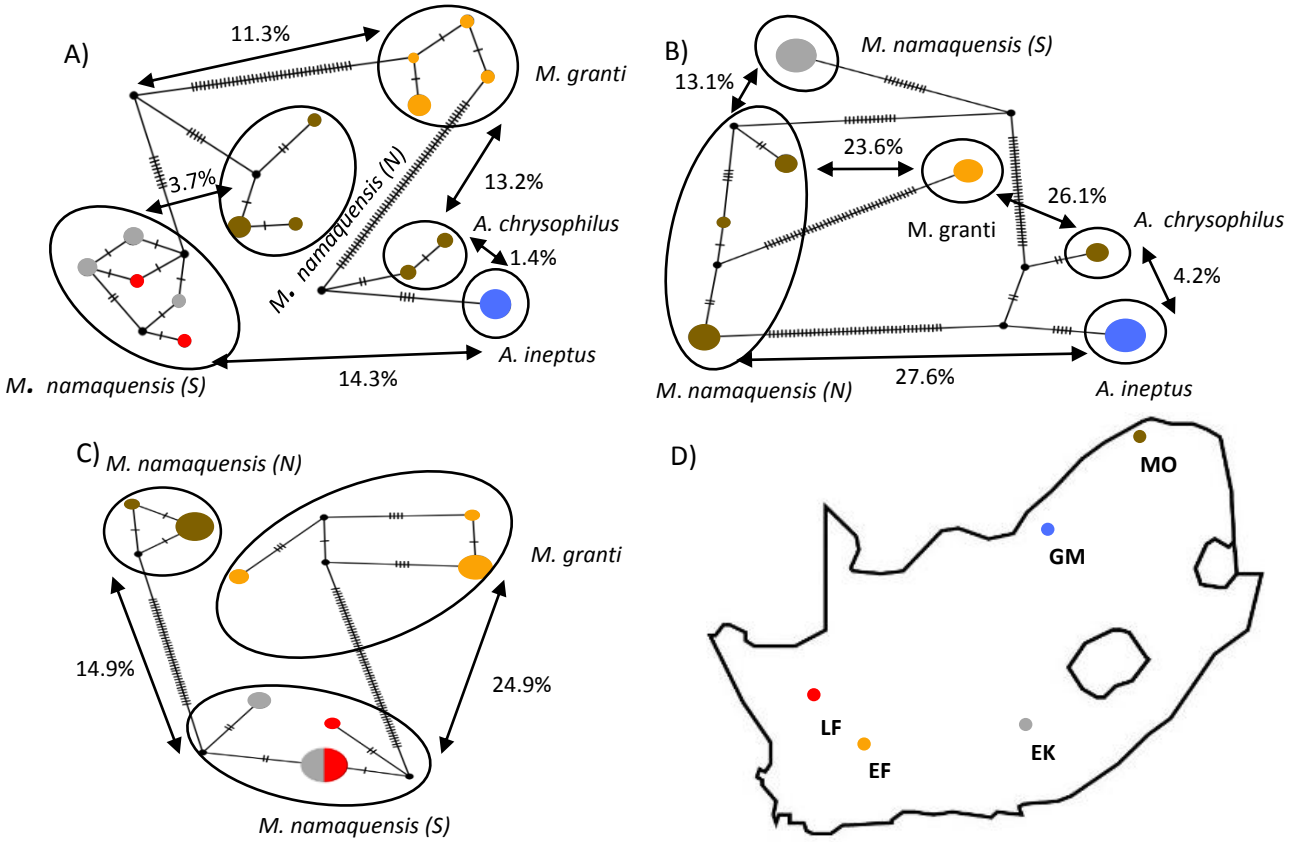

Figure 2.2-MtDNA haplotype networks for A) all hosts; B) *Hoplopleura* spp. and C) *Polyplax* spp. Species names in the parasite networks (B & C) refer to the host species from which the lice were collected. Percentages represent the average percentage mtDNA COI sequence divergence between the different haplotypes. D) Represents the sampling map where samples were obtained from and the haplotype colours in A-C correspond to the colours of the different localities on the map.

confidence interval (Fig 2.2; A). The two *Micaelamys* species differed by 46 mutational steps (Fig 2.2; A) and within *M. namaquensis*, the northern (*M. namaquensis* (N)) and the southern (*M. namaquensis* (S)) haplo-groups differed by 14 mutational steps (Fig 2.2; A). For the nuDNA TCS haplotype networks, all hosts could be connected with 95% confidence (Fig 2.3; A). In congruence with the pattern obtained with the mtDNA, the two different genera were separated by six mutational steps (Fig 2.3; A), whilst within species both the two *Micaelamys* species as well as the two *Aethomys* species were separated by two mutational steps each (Fig 2.3; A). Although the nuclear DNA results did not support a clear geographic

differentiation between north and south for *M. namaquensis* each locality was characterised by a distinct haplotype that differed as much from each other than the two recognized *Aethomys* species (Fig 2.3; A).

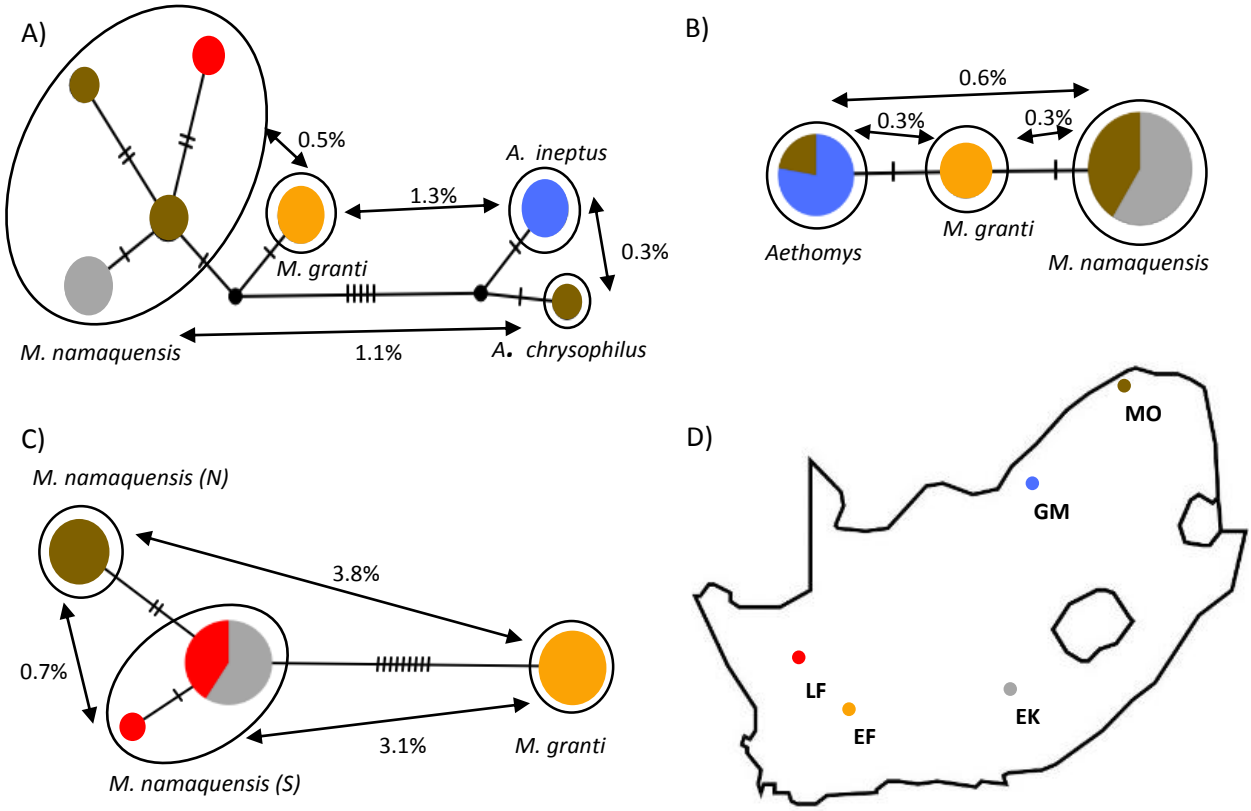

Figure 2.3-NuDNA haplotype networks for A) all hosts; B) *Hoplopleura* spp. and C) *Polyplax* spp. Species names in the networks refer to the host species from which the lice were collected. Percentages represent the average percentage nuDNA sequence divergence between the different haplotypes. D) Represents the sampling map where samples were obtained from and the haplotype colours in A-C correspond to the colours of the different localities on the map.

The evolutionary differentiation between these haplo-groups were supported by the Bayesian and Maximum likelihood analyses. The TrNef+G model (nst = 6, rates = gamma) was assigned to the first and third codon, whilst the TIMef model (nst = 6, rates = equal) was assigned to the second codon. Since there were no obvious conflict between the mtDNA and nuclear DNA haplotype networks the data were combined for the phylogenetic analyses. Combined data derived from the mtDNA datasets in combination with the nuDNA datasets revealed significant posterior probability and high bootstrap support for the monophyly of the four host species and the two distinct genera (Fig 2.4). The intraspecific divergence of the *M. namaquensis* (N) and *M. namaquensis* (S) lineages was also significantly supported in the ML analyses but not supported at the 0.05 level in the Bayesian analyses (Fig 2.4).

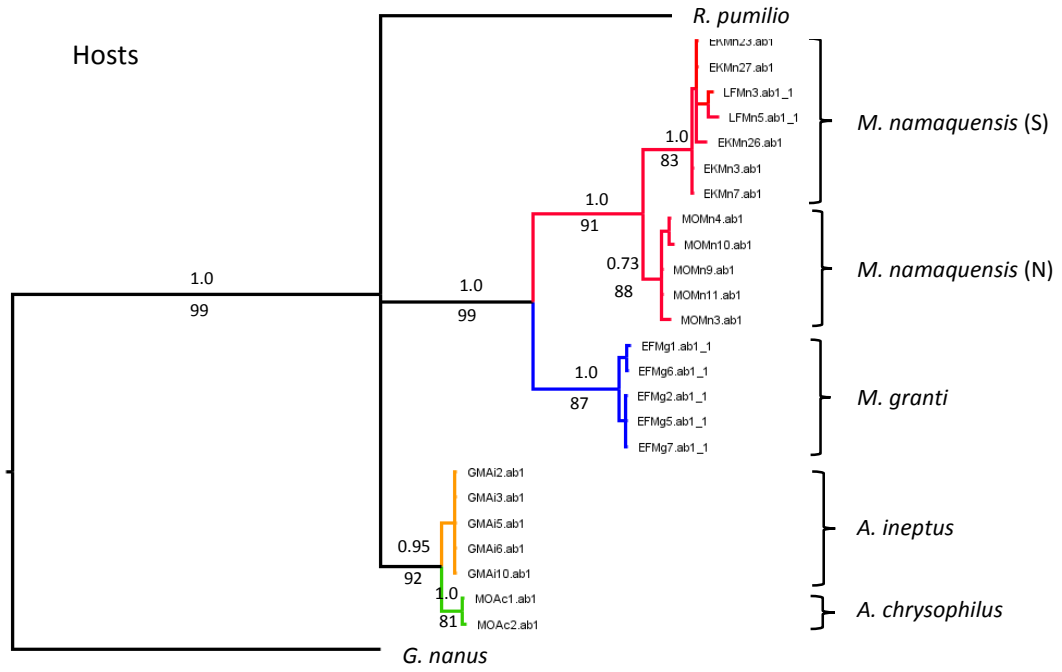

Figure 2.4-The combined Bayesian and maximum likelihood topology of the hosts, with nodal support indicated by posterior probabilities above and bootstrap values below branches. Colours indicated the different hosts included with: *M. namaquensis* in red, *M. granti* in blue, *A. ineptus* in orange and *A. chrysophilus* in green. Names on the right indicated the lineages separated by high nodal support in at least one of the analyses. *Gerbillus nanus* was used as outgroup

2.3.2.1.1 *Hoplopleura* evolutionary associations

The mtDNA sequence divergence between the *Hoplopleura* individuals collected from the four different rodent species were much higher than that seen between the rodents themselves. *Hoplopleura* individuals collected from all of the *Micaelamys* hosts differed by 27.3% (±6.2%) mtDNA and 0.6% (±0.2) nuDNA sequence divergence from *Hoplopleura* individuals collected from the two *Aethomys* hosts. *Hoplopleura* individuals collected from *M. namaquensis* and *M. granti* had a 23.6% (±8.5%) mtDNA and 0.3% (±0.2%) nuDNA sequence divergence, whilst *Hoplopleura* individuals collected from *A. chrysophilus* and *A. ineptus* had a mtDNA sequence divergence of 4.2% (±2.9%), whilst they could not be differentiated based on nuDNA sequences (Fig 2.2; B & Fig 2.3; B). Interestingly, there was again also a 13.1% (±4.8%) mtDNA sequence divergence between the *H. patersoni* individuals collected from *M. namaquensis* (N) and *M. namaquensis* (S) (Fig 2.2; B & Fig 2.3; B), whilst they could not be differentiated by nuDNA.

The *Hoplopleura* spp. mtDNA TCS haplotype network confirmed the differentiation between the lice sampled on the different rodent genera (Fig2.2; B). Five distinct haplo-groups were obtained in what has traditionally been described as a single morpho-species, *H. patersoni*. The evolutionary distances among these groups were so large that none of the haplo-groups could be connected with a 95% confidence interval. The *Hoplopleura* individuals occurring on the two distinct host genera differed by at least 47 mutational steps (Fig 2.2; B). Within the genus, the *Hoplopleura* individuals collected from the two *Micaelamys* species differed by at least 42 mutational steps (Fig 2.2; B) while the *Hoplopleura* individuals occurring on the two *Aethomys* species differed by nine mutational steps (Fig 2.2; B). The haplo-groups of the *H. patersoni* individuals collected from *M. namaquensis* individuals trapped in the north (*M. namaquensis* (N)) and individuals trapped in the south (*M. namaquensis* (S)) differed by 26 mutational steps (Fig 2.2; B). The nuDNA haplotype network of the *Hoplopleura* individuals collected from all hosts showed less variation and could be connected within the 95% confidence interval. The *Hoplopleura* individuals occurring on both *Aethomys* species shared a single haplotype that differed by one mutational step from the *Hoplopleura* individuals occurring on *M. granti* and two mutational steps from *Hoplopleura* individuals collected from *M. namaquensis* (Fig 2.3: B). The *Hoplopleura* individuals sampled from the two *Micaelamys* species differed by one mutational step (Fig 2.3; B).

The Bayesian and Maximum likelihood analyses supported the five distinct haplo-groups found for *Hoplopleura* individuals collected from the rodent hosts (Fig 2.5). The TrNef+G model (nst = 6, rates = gamma) was assigned to the first and third codon, whilst the K81 model (nst = 6, rates = equal) was assigned to the second codon. *Hoplopleura* individuals found on the two *Aethomys* species are strongly supported as a monophyletic entity (BI = 0.99; BS = 99; Fig 2.5) and the same holds true for the *Hoplopleura* individuals sampled on *M. namaquensis* (BI = 1.0; BS = 91; Fig 2.5). The clustering of the two *Hoplopleura* haplo-groups occurring on the two distantly related *Micaelamys* species, however, did not receive strong nodal support (BI = 0.70; BS = 51; Fig 2.5).

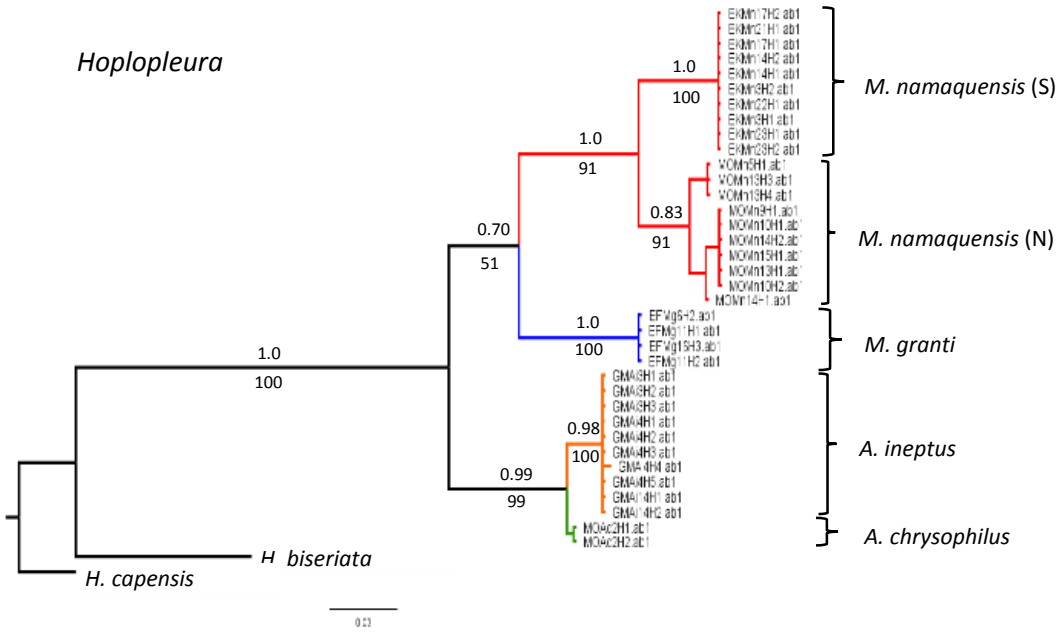

Figure 2.5-The mtDNA and nuDNA Bayesian and maximum likelihood topology for *Hoplopleura* spp. with nodal support indicated by posterior probabilities above and bootstrap values below nodes. Colours indicate lice collected from the different hosts with: *M. namaquensis* in red, *M. granti* in blue, *A. ineptus* in orange and *A. chrysophilus* in green. Names on the right indicated the host lineages separated by high nodal support in at least one of the analyses. *Hoplopleura capensis* and *H. biseriata* were used as outgroups.

2.3.2.1.1 *Polyplax* evolutionary associations

The *Polyplax* individuals collected from the two *Micaelamys* species had a 24.9% (±5.7%) mtDNA and 3.3% (±1.0%) nuDNA sequence divergence (Fig 2.2; C). There was also a 14.9% (±4.7%) mtDNA and 0.7% (±0.3%) nuDNA sequence divergence between *P. praomydis* individuals collected from *M. namaquensis* (N) and *M. namaquensis* (S) (Fig 2.2; C).

The *Polyplax* spp. mtDNA TCS haplotype network confirmed the presence of at least three distinct haplo-groups that could not be connected within the 95% confidence interval (Fig 2.2; C). The morphologically different *Polyplax* individuals collected from the two *Micaelamys* species differed by 60 mutational steps (Fig 2.2; C) while the haplo-groups of the *P. praomydis* individuals collected from *M. namaquensis* individuals trapped in the north (*M. namaquensis* (N)) and individuals trapped in the south (*M. namaquensis* (S)) differed by 42 mutational steps (Fig 2.2; C). In the nuDNA haplotype network *Polyplax* individuals collected from the two *Micaelamys* species could also not be connected with a 95% confidence interval as they differed by nine mutational steps (Fig 2.3; C). The haplo-groups of the *P. praomydis* individuals

collected from *M. namaquensis* individuals trapped in the north (*M. namaquensis* (N)) and individuals trapped in the south (*M. namaquensis* (S)) could be connected with 95% confidence interval and they differ by two mutational steps (Fig 2.3; C).

Bayesian and Maximum likelihood phylogenetic analyses strongly support the three monophyletic haplo-groups previously obtained (Fig 2.6). The TrNef+G model (nst = 6, rates = gamma) was assigned to the first and third codon, whilst the TIMef model (nst = 6, rates = equal) was assigned to the second codon. Each *Micaelamys* lineage harbours a unique parasite assemblage and there is also significant support for the two haplo-groups found on *M. namaquensis* to form a monophyletic entity (BI = 1.0; BS = 96; Fig 2.6) to the exclusion of the lice found on *M. granti*.

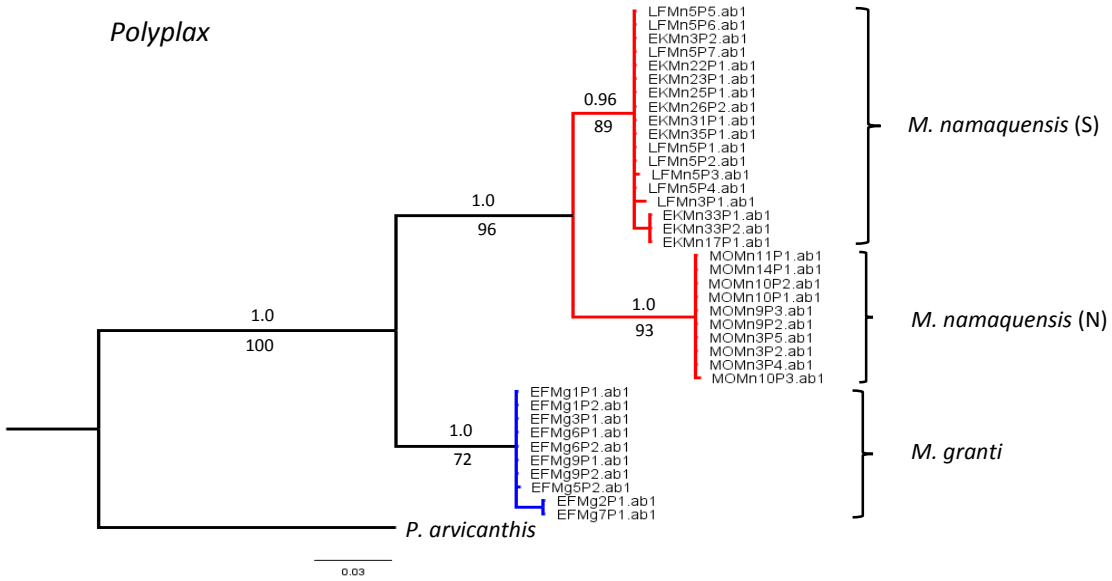

Figure 2.6-The mtDNA and nuDNA Bayesian and maximum likelihood topology for *Polyplax* spp. with nodal support indicated by posterior probabilities above and bootstrap values below nodes. Colours indicate lice collected from the different hosts with: *M. namaquensis* in red and *M. granti* in blue. Names on the right indicated the host lineages separated by high nodal support in at least one of the analyses. *Polyplax arvicanthis* was used as outgroup.

2.3.2.3 Co-phylogeny.

When comparing the phylogeny of the host species to that of the *Hoplopleura* - and *Polyplax* lineages, congruent phylogenetic patterns were clearly visible. This is highlighted by the clustering analyses and sequence divergence values among the various taxa. This congruency is evident for both *Hoplopleura* (Fig 2.7) and *Polyplax* individuals (Fig 2.8). Significant posterior probabilities were obtained for nearly all the associations (Fig 2.7; Fig 2.8). The Jane co-phylogenetic reconstruction between all of the rodent hosts and the *Hoplopleura* lineages revealed that the most parsimonious solution with zero cost includes four co-divergences (Fig 2.9, A). Both of the statistical analyses indicated significant co-phylogeny between the host lineages and the *Hoplopleura* lineages, with $p = 0.04$ and $p = 0.02$ for random tip mapping and random parasite tree respectively. The Jane co-phylogenetic reconstruction between the host lineages and *Polyplax* lineages revealed that the most parsimonious solution with zero cost includes two co-divergences (Fig 2.9, B). Both of the statistical analyses however indicated non-significant co-phylogeny between the hosts lineages and the *Polyplax* lineages, with $p = 0.31$ and $p = 0.35$ for random tip mapping and random parasite tree respectively. These non- significant statistical values are due to the low number of tree tips as a result of the fact that no *Polyplax* spp. were collected on either of the *Aethomys* species and as a result those braches and tree tips could not be included.

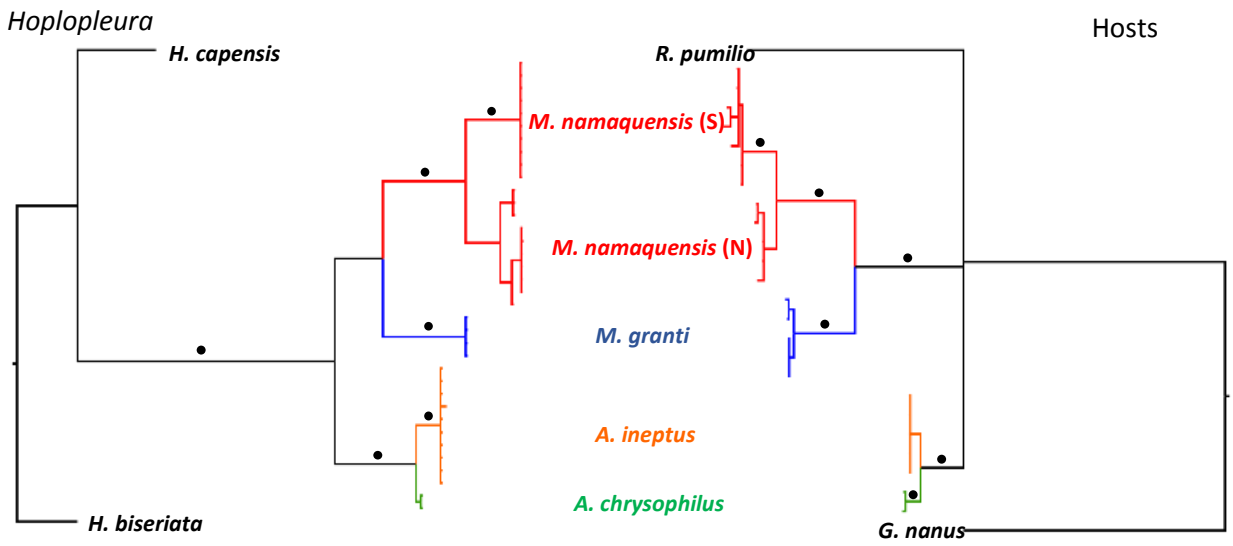

Figure 2.7-Comparative phylogenies of *Hoplopleura* spp. and the hosts they were collected from. Matching colours indicate *Hoplopleura* individuals collected from the corresponding host with: *M. namaquensis* in red, *M. granti* in blue, *A. ineptus* in orange and *A. chrysophilus* in green. Dots indicate significant posterior probabilities for nodes. Outgroup species names are coloured black in both trees.

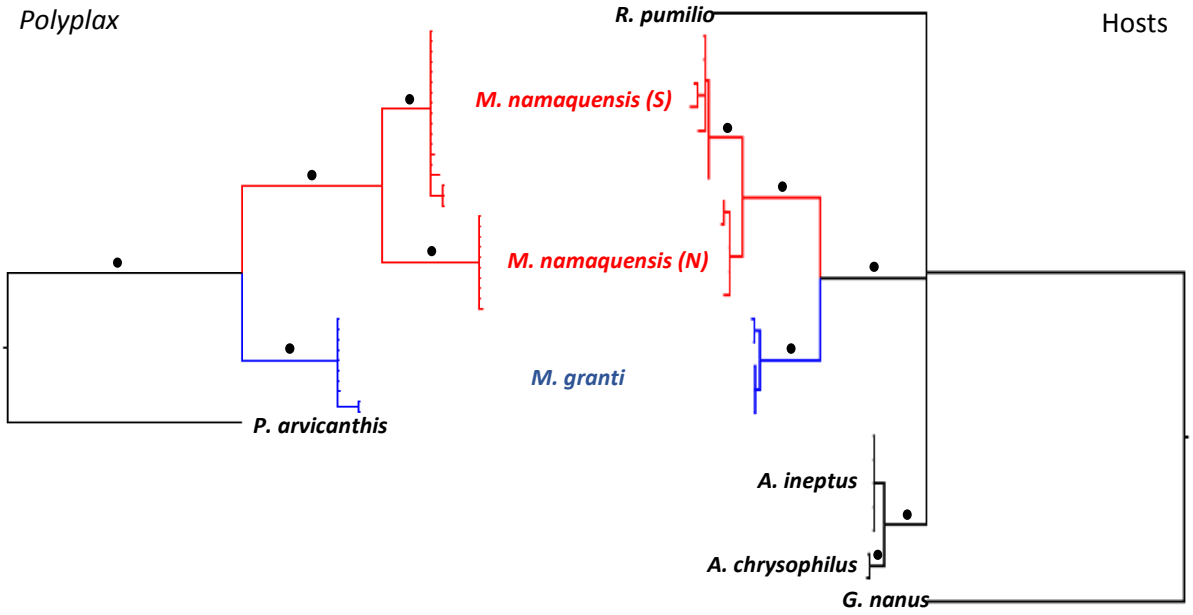

Figure 2.8-Comparative phylogenies of *Polyplax* spp. and the hosts they were collected from. Matching colours indicate *Polyplax* individuals collected from the corresponding host with: *M. namaquensis* in red and *M. granti* in blue. Dots indicate significant posterior probabilities for nodes. Outgroup species names are coloured black in both trees.

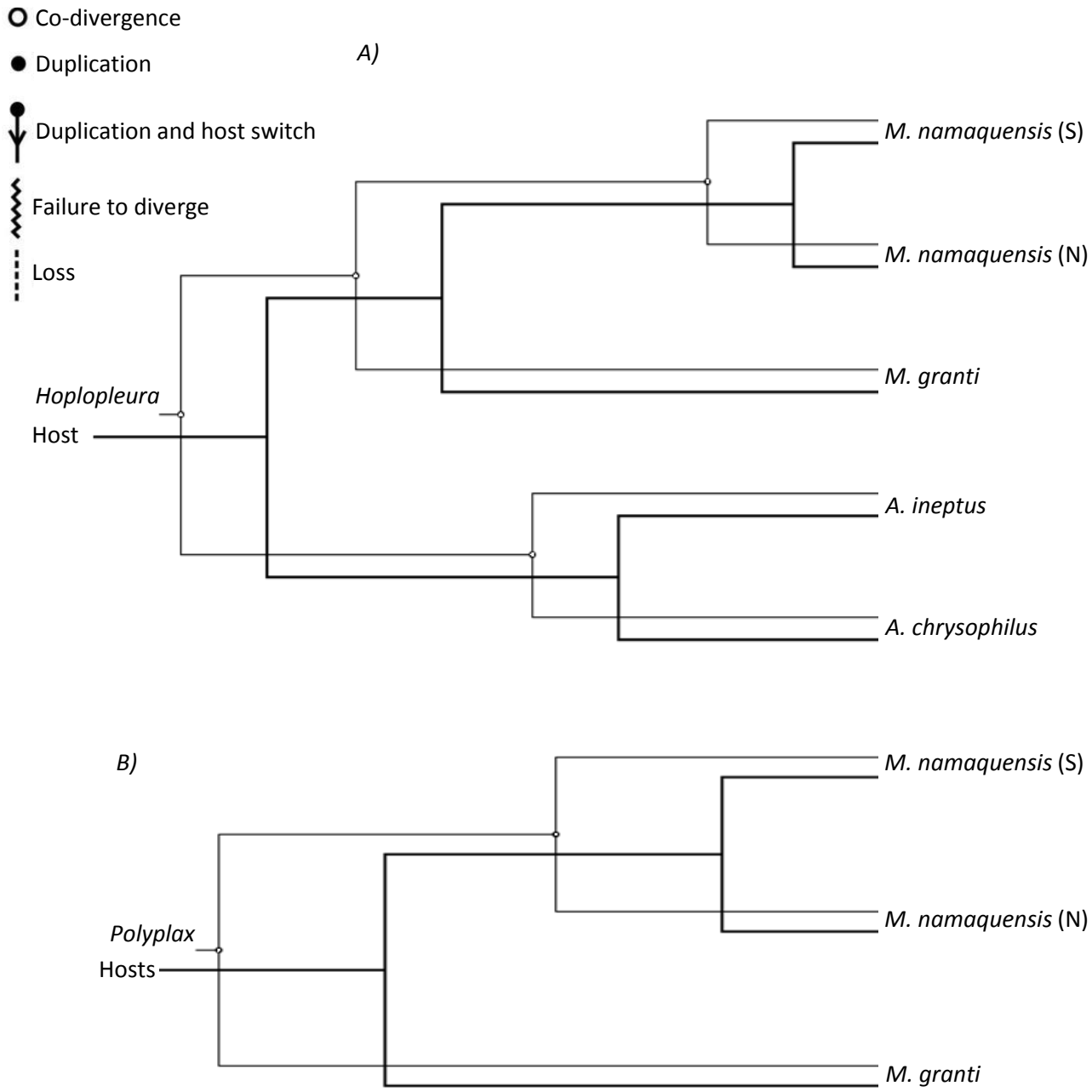

Figure 2.9-Phylogenetic reconciliation of the hosts with *Hoplopleura* spp. (A) and *Polyplax* spp. (B) retrieved from Jane after the five types of evolutionary events (legend) were tested for. Tip labels refer to the hosts from which the lice were collected from.

2.3.2.4 Divergence dates

There is overall congruence between the 95% confidence interval divergence dating between the parasite and the host lineages. In the host phylogeny the split between the two rodent genera, *Micaelamys* and *Aethomys*, occurred roughly 10 Ma (Fig 2.10, A; Table 2.4). Since these two genera are not monophyletic (Ducroz et al. 2001) the present analyses could also not provide 95% confidence divergence values for this monophyletic clustering of *Micaelamys* and *Aethomys*, (Fig 2.10, A; Table 2.4). The 95% credibility intervals for the corresponding split in the *Hoplopleura* spp. phylogeny, although very broad, overlapped with the split between the *Aethomys* - and *Micaelamys* species and was roughly estimated to have occurred 7.7 Ma (Fig 2.10, A; Table 2.4). The split between *M. namaquensis* and *M. granti* was estimated to have occurred 5.8 Ma (Fig 2.10, B; Table 2.4). The 95% credibility intervals for these two hosts overlapped substantially with the corresponding split in the *Hoplopleura* lineages that was roughly estimated to have occurred 6.4 Ma (Fig 2.10, B; Table 2.4). As a result of the monophyly between these two lineages not being supported the analyses could not provide 95% confidence divergence values for this split in the *Hoplopleura* lineages (Fig 2.10, B; Table 2.4). The lineage split for *M. namaquensis* (S) and *M. namaquensis* (N) occurred roughly 2.3 Ma (Fig 2.10, C; Table 2.4), in *H. patersoni* the 95% credibility interval overlapped substantially with this lineage split in the host and was estimated to have occurred roughly 3.3 Ma (Fig 2.10, C; Table 2.4). The divergence date for the split between *A. ineptus* and *A. chrysophilus* was fairly recent and was estimated to be roughly 1.2 Ma in the hosts (Fig 2.10, D; Table 2.4). Similarly, the corresponding split between the *Hoplopleura* lineages occurred roughly 1.2 Ma (Fig 2.19, D; Table 2.4). The lineage split between the *Polyplax* individuals collected from *M. namaquensis* and *M. granti* was estimated to have occurred roughly 5.4 Ma (Fig 2.10, B; Table 2.4), the 95% credibility interval overlapped substantially with the corresponding split in the host phylogeny that was estimated to have occurred roughly 5.8 Ma (Fig 2.10, B; Table 2.4). In the host phylogeny the split between *M. namaquensis* (S) and *M. namaquensis* (N) was estimated to have occurred roughly 2.3 Ma (Fig 2.10, C; Table 2.4), for

P. praomydis the 95% credibility intervals for the corresponding split overlapped substantially to that seen in the host and was estimated to have occurred roughly 2.8 Ma (Fig 2.10, C; Table 2.4).

Table 2.4-95% confidence and mean lineage divergence dates for all rodent genera and lice taxa.

Taxa	divergence between lineages (Ma)							
	Aethomys* - *Micaelamys		***M. granti* – *M. namaquensis***		***M. namaquensis* (N) - *M. namaquensis* (S)**		***A. chrysophilus* – *A. ineptus***	
	95% confidence interval	**Mean**	**95% confidence interval**	**Mean**	**95% confidence interval**	**Mean**	**95% confidence interval**	**Mean**
Rodents	Not supported	10.3	3.9 – 7.8	5.8	1.4 – 3.4	2.3	0.5 – 2.1	1.2
Hoplopleura spp.	4.7 – 11.2	7.7	Not supported	6.4	1.8 – 5.0	3.3	0.5 – 2.0	1.2
Polyplax spp.	Not present on *Aethomys*	Not present on *Aethomys*	3.5 – 7.5	5.4	1.7 – 4.0	2.8	Not present on *Aethomys*	Not present on *Aethomys*

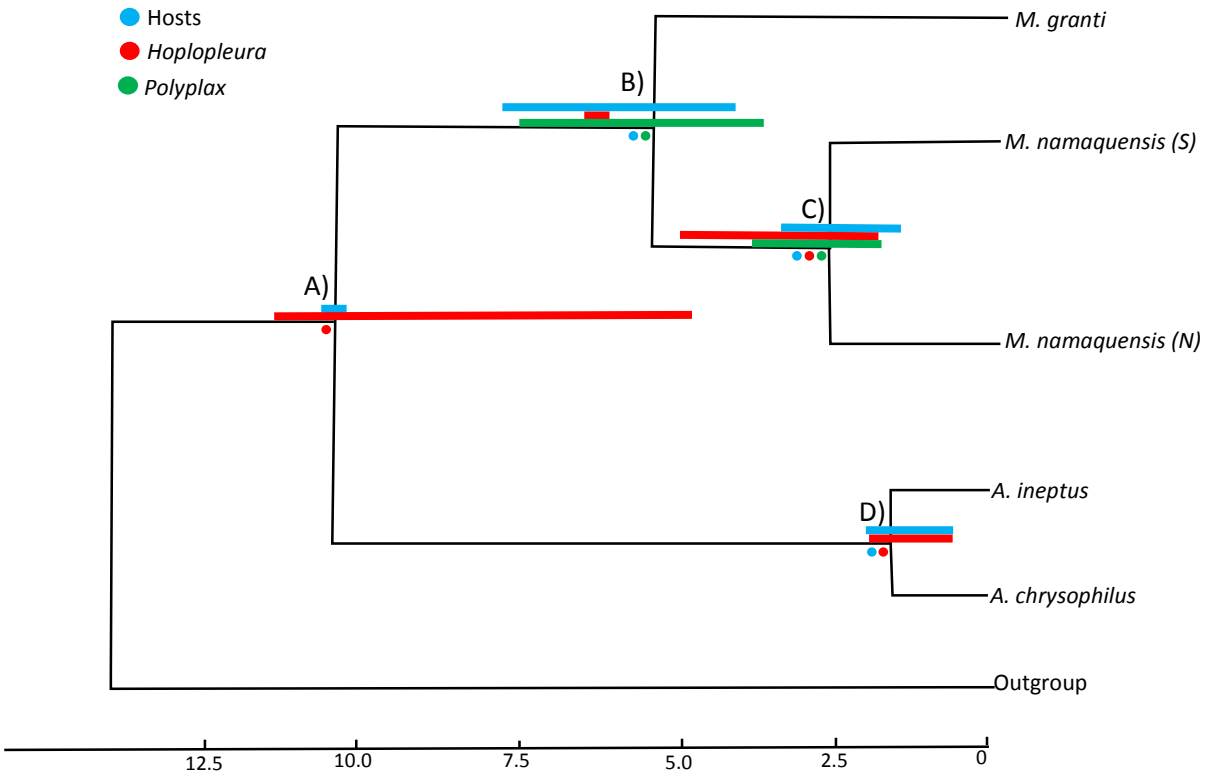

Figure 2.10-Representative tree showing the 95% credibility divergence dates where significant posterior probabilities were obtained and mean divergence dates where non-significant posterior probability values were obtained for the rodents (blue), *Hoplopleura* spp. (red) and *Polyplax* spp. (green) individuals included in this study. Dots under the nodes indicate significant posterior probabilities for the different genera. A-D refer to lineage splits: *Aethomys*/*Micaelamys*, *M. granti*/*M. namaquensis*, *M. namaquensis* (S)/*M. namaquensis* (N) and *A. ineptus*/*A. chrysophilus* separately.

2.4 Discussion

The present study highlights the notion that the current ectoparasites species descriptions on rodents is in need of revision. The failure of the present study to detect any *H. aethomydis* on the rodents sampled (Durden & Musser 1994; Fagir et al. 2014) or any *Polyplax* lice on members of the *Aethomys* genus (Durden & Musser 1994), may indicate that the species lists are outdated. However, this could of course also simply be attributed to sampling bias (the prevalence of *H. aethomydis* is known to be low (Fagir et al. 2014) and only two *Aethomys* genus-specific sampling localities were included herein). What is more important, however, is that only three species of ectoparasitic lice were previously recognized to occur on members of the *Aethomys/Micaelamys* species complex (Durden & Musser 1994; but also see Kleynhans et al. 1969) and that the present study identified at least two additional undescribed species based on crude morphological comparisons. In addition, eight distinct genetic lineages were identified of which six seems cryptic based on crude morphological comparisons. This finding contributes further to emphasize the high level of undetected ectoparasites diversity (du Toit et al. 2013 b; Engelbrecht et al. 2014) and also support the hypothesis that each genetically distinct rodent lineage is characterised by its own lice taxon.

The exact taxonomic designation for each of the new cryptic parasite lineages is not yet certain and in need of further investigation. However, given the obvious morphological differences between the *Hoplopleura* - and *Polyplax* individuals sampled on the evolutionary divergent *M. namaquensis* and *M. granti,* coupled to the large mtDNA sequence divergences between the parasite taxa, 23.6% (±8.5%) and 24.9% (±5.7%) respectively, it seems reasonable to argue that there are at least one undescribed *Hoplopleura* and one undescribed *Polyplax* species on *M. granti.* These two putative species are in need of morphological description. Should this hold, then it would imply that the morphologically similar *H. patersoni* occurring on *M. namaquensis* is paraphyletic with respect to those occurring on the two species of *Aethomys,* and a third cryptic taxon is thus also in need of a new species description. In addition, the COI sequence divergence of 27.3% (±6.2%) between the *Hoplopleura* haplo-groups occurring on *Aethomys* and *Micaelamys* respectively and the 24.9% (±5.7%) mtDNA divergence between the *Polyplax* lice collected from the two *Micaelamys* species is comparable to the 24% COI sequence divergence between other rodent associated *Hoplopleura* species, such as *H. onychomydis* and *H. emphereia,* that parasitize *Onychomys* and *Peromyscus* hosts separately (Sanchez-Montes et al. 2016). Likewise, within *M. namaquensis* the northern *H. patersoni* haplo-group is separated from the southern *M. namaquensis* haplo-group by 13.1% (±4.8%) COI sequence divergence and this is also comparable to the 15% interspecific divergences seen between *H. reithro* and *H. emphereia*, that parasitize *Reithrodontomys* and *Peromyscus* hosts separately (Sanchez-

Montes et al. 2016). The nuDNA haplotype network showed less resolution but did differentiate between *Hoplopleura* individuals collected from *Aethomys, M. granti* and *M. namaquensis,* giving some additional support for the recognition of these taxa as distinct species.

Apart from emphasizing the need to revise the taxonomy on the parasitic lice occurring on *Aethomys/Micaelamys,* the present study also provide additional insights into the evolution of ectoparasites in general. First, the genetic analyses based on mtDNA and nuDNA supported the systematics and accepted taxonomy for the four rodent host lineages studied herein (Visser & Robinson 1986; Chimimba et al. 1999; Ducroz et al. 2001; Chimimba 2005; Russo et al. 2006). The timing of divergences suggest a radiation within *Micaelamys* roughly 5.8 Ma (Fig 2.8) in the late Miocene, which corresponds to the timing of speciation in other southern African mammals (de Menocal 2004; Lecompte et al. 2008; du Toit et al. 2012). This period was characterised by a change from sub-tropical vegetation which dominated the Miocene to the establishment of the modern biomes in South Africa (Scott et al. 1997). It is reasonable to conclude that the establishment of biomes in South Africa was most likely influential in driving the divergence in the *Micaelamys* lineages. Within *M. namaquensis* the timing of divergence suggests a radiation roughly 2.3 Ma, whilst the timing of divergence within *Aethomys* suggests a radiation 1.2 Ma. Both of these divergence dates also fall within periods of aridification which could have led to the divergence between rodent lineages as a result of isolation of suitable habitats (de Menocal 2004; Russo et al. 2010).

The genetic analyses for the lice species associated with these host showed marked congruences with the host phylogeny of the rodents. These findings are derived from analysing both nuclear and mtDNA markers and although the nuclear DNA provide less resolution, general congruence was obtained. Our results also supported statistically significant co-phylogeny between the host - and *Hoplopleura species*. Additionally, there is also a fair amount of overlap in the timing of the divergences between the host lineages and those of the parasites (Fig 2.10). The phylogenetic congruences between parasite and host topologies, coupled to a large overlap in host and parasite divergence times, tend to give strong support for the second hypothesis tested in this chapter in that the phylogeny of the lice collected from the different hosts will show significant congruence to the phylogeny of the hosts. Although adaptive divergence is not indicated in the present study, the most probable explanation for the co-divergence pattern obtained herein would also suggest co-evolution between the parasites studied herein and their hosts (Page 2003). In this respect it can be argued that the close association of the permanent parasite facilitated a pattern of co-divergence between the hosts and their parasites (Hafner et al. 1994; Light & Hafner 2007).

Recent studies on parasite co-divergences have however shown that despite a very close association between parasites and their host, congruence in patterns are often the result of more than one factor (Paterson et al. 2000; Mizukoshi et al. 2012; du Toit et al.2013 b; Engelbrecht et al. 2016). Although host

association certainly played a big role in the co-phylogenies that were obtained between *Aethomys* and *Micaelamys* and their parasites, it is also possible that the co-divergence patterns could have been partly re-enforced by host distribution. In a previous study, *Polyplax* lice that differed genetically by more than seven mutational steps and that occurred on different host species showed several instances of host switching among the different host species (du Toit et al. 2013 b). This was particularly prevalent in the zones of contact between the hosts (du Toit et al. 2013 b). In the present study, three of the four species have an allopatric distribution (Monadjem et al. 2015; Fig 2.1). In addition, more extensive sampling throughout the range (see chapter 3) revealed that at the ecological scale, different species do not really share similar micro-habitat (but see Linzey et al. 2003). A total of 13 sampling sites were included where overlap in distribution should occur and of these, only at a single locality (Mogalakwena (MO); Fig 2.1), there was overlap in distribution between two host species included herein. It is thus proposed that at the micro-ecological scale the species do not overlap since no single trap line recovered more than one species at any sampling site. The lack of extensive overlap in distribution and thus contact between species, will also facilitate co-divergence between the parasites and their *Aethomys* and *Micaelamys* hosts. A second factor that is host related that may contribute to the parasite host co-divergence pattern obtained herein is confined in host dispersal ability. *Aethomys* - and *Micaelamys* individuals are mostly confined to rocky terrain and this will cause a high level of host population differentiation. Indeed, for *M. namaquensis*, significant host population structure has been observed (Russo et al. 2010) and it is thus likely that the restriction in host movement may also contribute to stronger signals of co-divergence (Matthee et al. 2018).

In conclusion this study provide strong evidence that at least two additional *Hoplopleura* and one additional *Polyplax* species are in need of formal species descriptions. Additional sampling from more localities, and a thorough morphological investigation will be required to determine the robustness of the patterns obtained for the ectoparasites found on the two *Aethomys spp.* and also on *M. granti*. The striking genetic difference between *M. namaquensis* (north) and *M. namaquensis* (south) is indicative of large intraspecific variation within a widespread rodent species and necessitate a more thorough geographic sampling approach, something that is addressed in chapter 3 of this thesis. At first glance it may also be interpreted that host specificity (parasite life history of permanent parasites) is the reason for the co-divergences observed but with the data at hand, additional factors such as host distribution and host dispersal may have also contributed to the patterns obtained.

Chapter 3: Comparative phylogeography between parasitic sucking lice and their host, the Namaqua rock mouse, *Micaelamys namaquensis.*

3.1 Introduction

The genetic differentiation between populations of the same species across its geographic range is known as phylogeography. The extent of phylogeographic differentiation can be affected by a number of factors that among others include, the dispersal ability of the specific organism, the habitat requirements of an organisms and or historical biogeographic influences (Avise et al. 1987; Zamudio et al. 2016; Kumar & Kumar 2018). Of these, the dispersal ability of a species is probably most important since taxa with a good dispersal ability are able to disperse over a large part of their geographic range, usually resulting in high levels of geneflow and thus also comparatively low levels of phylogeographic structure (Avise et al. 1987; Arbogast & Kenagy 2001; Dawson et al. 2002; Zamudio et al. 2016). The opposite is then that a species with a poor dispersal ability should have low geneflow and high genetic structure between populations (Avise et al. 1987; Arbogast & Kenagy 2001; Dawson et al. 2002; Zamudio et al. 2016). The habitat preference of a species can also influence the amount of genetic structure between populations (Dawson et al. 2002; Zamudio et al. 2016). For instance, a habitat specialist species often occurs in fragmented patches and will thus show more genetic structure than a habitat generalist that has less impediments to geneflow (Dawson et al. 2002). The influence of historic biogeographical events such as vicariance can also play a major role in the extant phylogeography of a species (Wooding & Ward 1997; Arbogast 1999; Arbogast & Kenagy 2001; Lorenzen et al. 2012; Zamudio et al. 2016; Kumar & Kumar 2018). Vicariance events are associated with similar phylogeographic breaks between different populations as a result of historical fragmentation and can for example be linked to the contraction and expansion events of suitable habitat accompanying glacial and interglacial periods (Rogers et al. 1991; Arbogast 1999; Arbogast & Kenagy 2001; Kumar & Kumar 2018).

Much of the theory behind phylogeographic structure outlined above is based on case studies derived from free-living organisms. In the case of parasites, some additional factors related to parasite life history can also play a role in phylogeographic structures. For example, generalist parasites who are only dependent on their host for a short period of time (for example some ticks, mites and fleas) will often show weak phylogeographic congruence with their hosts (Cangi et al. 2013; van der Mescht et al. 2015). In contrast, permanent, obligate species-specialist parasites who depend on their host for food, reproduction, and dispersal often show stronger congruent patterns with their host species (Price 1980; Hafner et al. 1994; Nieberding et al. 2004; Light & Hafner 2007; du Toit et al. 2013 b). For example, a species-specific endoparasitic nematode *Heligmosomoides polygyrus* shows phylogeographic congruence with its rodent host *Apodemus sylvaticus* even though the life history of the nematode includes a free-living larval stage (Nieberding et al. 2004). The effect of parasite life history on the phylogeography of parasites is also further refined by van der Mescht et al. (2015) who indicated that generalist fur fleas (*Listropsylla agrippinae*)

who spend slightly more time on these rodent hosts, showed greater phylogeographic congruence with its hosts compared to the nest flea (*Chiastopsylla rossi*) who spend more time off its hosts. From these studies and others, it is evident that the phylogenetic and phylogeographic congruences between parasites and their hosts can often be ascribed to the life history of the parasite and in particular also the dispersal potential of the hosts.

The complexity of matching phylogeographic structures between parasites and their hosts are probably best exemplified by a recent study investigating the phylogeographic congruence of a permanent obligate *Polyplax* lice associated with the rodent genus *Rhabdomys.* Despite the hypothesis that the louse and the host should show similar phylogeographic structures, only limited phylogeographic congruence were observed between the parasite and the host (du Toit et al. 2013 b). The authors ascribed this lack of congruence to the larger effective population sizes of the parasite lineages, the vagility and social behaviour of the hosts and the lack of host-specificity in areas of host sympatry (du Toit et al. 2013 b). More research are thus needed to better understand the mechanisms involved in parasite phylogeography.

Micaelamys namaquensis, the Namaqua rock mouse, occur across multiple biomes and diverse habitats (woodland, grassland, on the fringe of pans and in open scrub) in southern Africa but they prefer rocky outcrops or boulder-strewn hillsides (Skinner & Chimimba 2005). At the micro-evolutionary scale, their habitat is thus patchy. These rodents are nocturnal, and they possibly occur in small colonies (Skinner & Chimimba 2005). The phylogeographic structure in the host is reasonably well studied. Historically it was believed that *M. namaquensis* consisted of 16 subspecies (Roberts 1951; Meester et al. 1964). This research was based on morphological and/or morphometric data only. However, a more recent study analysing morphometric and morphological patterns of intraspecific variation in *M. namaquensis* proposed that the number of subspecies be decreased to only four (Chimimba 2001). In this study they found that the ranges of these four subspecies coincided with the phytogeographical zones within southern Africa: one restricted to the Succulent Karoo, Fynbos and the southern coastal Savanna /Grassland region of the Eastern Cape, Kwazulu-Natal and eastern Mpumalanga Provinces of South Africa; the second to the Nama-Karoo biome; the third to the Savanna biome of southern Africa excluding the southern Savanna/Grassland region of the Eastern Cape, Kwazulu-Natal and eastern Mpumalanga; the fourth subspecies was restricted to the grassland biome within southern Africa (Chimimba 2001). Recent molecular DNA studies have however revealed a complex and pronounced phylogeographic structure present in *M. namaquensis* (Russo et al. 2010). At least eight mtDNA Cytochrome b lineages have been identified (Russo et al. 2010) and these were similarly associated with different vegetation types (Russo et al. 2010).

Previous investigations based on morphological characteristics have showed that *M. namaquensis* is parasitized by three sucking lice species which include, *Hoplopleura patersoni*, *Hoplopleura aethomydis*

and *Polyplax praomydis* (Durden & Musser 1994; Fagir et al. 2014). In the present study however no *H. aethomydis* individuals were found to be present on *M. namaquensis* (see chapter 2). Some preliminary evidence presented in chapter 2 suggest that *H. patersoni* and *P. praomydis* show significant phylogeographic structure throughout their range with one lineage of each parasite species confined to the northern part of South Africa country and another lineage of each parasite species to the southern part, (see chapter 2 Fig 2.5 & 2.6). There was phylogeographic congruence between the host and parasite lineages. The two host lineages *M. namaquensis* (N) and *M. namaquensis* (S) differed by a mtDNA COI sequence divergence of 3.7 % (±1.1%) while the each of the two parasite lineages (*H. patersoni* and *P. praomydis*) have a mtDNA COI sequence divergence of 13.1 % (±4.8%) and 14.9 % (±4.7%) respectively (see chapter 2).

Given the preponderance of cryptic ectoparasite diversity recently discovered on southern African rodents (du Toit et al. 2013 a, b; Engelbrecht et al. 2014) and the finding of four genetic assemblages for lice occurring on *M. namaquensis* (chapter 2), a more thorough molecular approach is clearly needed. Specifically, a study needs to test the validity of the morphological hypotheses that *M. namaquensis* is parasitized by monophyletic *H. patersoni* and *P. praomydis* throughout their range (Durden & Musser 1994). Based on the outcome of chapter 2, it is predicted that multiple cryptic lineages will be present within both *H. patersoni* and *P. praomydis* and if so, this finding will lend further credence that lice diversity of rodents in Southern Africa is grossly underestimated (also see du Toit et al. 2013 a).

The aim of the study was to sample, identify and sequence sucking lice from *M. namaquensis* from multiple localities and biomes in South Africa. This study will provide insights into the mechanisms that are responsible for parasite speciation and from these data it will be possible to document the true geographic taxonomic diversity for *H. patersoni* and *P. praomydis* occurring on *M. namaquensis*. Specifically, the outcome of the present study may provide more clarity on the effect of host dispersal on parasite gene flow by also comparing the data to the recently published work on *Rhabdomys* (du Toit et al. 2013 b). Compared to *Rhabdomys*, which is a more generalist rodent occupying mostly plains regions, *M. namaquensis* is mostly confined to rocky outcrops, and is thus more restricted in local dispersal (Russo et al. 2010). Importantly however, *Rhabdomys* species show a high level of interspecific geographic differentiation and some taxa are more social and restricted in their movement (*R. pumilio*) than others (*R. dilectus*; du Toit et al. 2012 and references therein). Since higher levels of host movement can facilitate parasite gene dispersal (Matthee et al. 2018) the outcome of the current study can be used to test whether restrictions in host movement (due to the host's restriction to rocky outcrops) can facilitate stronger congruence between parasite and host structures. The *Rhabdomys* species that most closely resembles the life history of *M.*

namaquensis is most likely *R. pumilio* (showing social flexibility but are predominantly group living and occur in fragmented habitat patches; Schradin et al. 2012).

By using mtDNA and nuclear DNA markers the aims of this study were to: 1) test for cryptic diversity in both lice species that occur on *M. namaquensis*; 2) assess the phylogenetic and phylogeographic congruence between *M. namaquensis* and both lice species associated with it and 3) provide more insights into the mechanisms affecting parasitic lice evolution on rodents. The hypotheses were that: 1) the taxonomy of the two lice species (*P. praomydis* and *H. patersoni*) found on *M. namaquensis* is in need of revision and 2) both lice species collected from *M. namaquensis* across South Africa will show significant phylogeographic congruence with their host.

3.2 Materials and Methods

3.2.1 Host sampling

Micaelamys namaquensis individuals were successfully sampled at 13 different localities across its geographic range in South Africa (Fig 3.1), after obtaining the needed permission from local authorities (Permit numbers: Limpopo, ZA/LP/90994; North West, NW 7705; Eastern Cape, CRO150/17CR and CRO 11/17CR; Northern Cape, FAUNA 0942/2017 and FAUNA 0949/2017; Gauteng, CPF6-0194; Free state, NC. 672/2017; Western Cape, 0056-AAA007-00140) and also ethical clearance from Stellenbosch University (ethical clearance reference number SU-ACUD16-00190). The localities represented the following biomes: Fynbos, Succulent Karoo, Nama Karoo, Savanna and Grassland. Trapping was conducted following the methods set out in chapter 2.

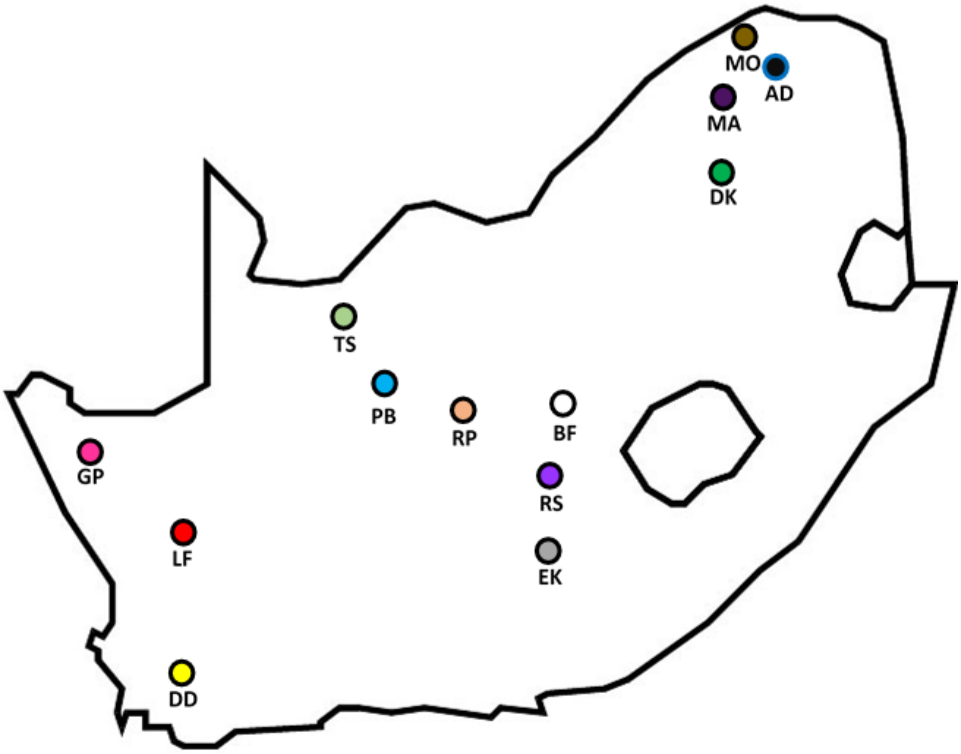

Figure 3.1-Localities where *M. namaquensis* individuals were trapped: De doorns (DD), Loeriesfontein (LF), Goegap (GP), Elandskuil (EK), Rusplaas (RS), Bloemfontein (BF), Rooipoort (RP), Postmasburg (PB), Tswalu (TS), Dinokeng (DK), Marken (MA), Alldays (AD) and Mogalakwena (MO).

3.2.2 Parasite removal and identification

Parasite removal and identification was done as described in chapter 2 by using published species descriptions and taxonomic reference keys (Paterson & Thompson 1953; Johnson 1960; Kleynhans 1969). Species identification was confirmed by an expert taxonomist (Prof L.A. Durden, Department of Biology, Georgia Southern University, USA).

3.2.3 DNA extraction and sequencing

Total genomic DNA was extracted from 98 *M. namaquensis* individuals and 218 louse individuals using the Nucleospin Tissue kit according to the protocol set out by the manufacturer (Macherey-Nagel, Duren, Germany). The mitochondrial Cytochrome Oxidase subunit I (COI) gene was amplified for *M. namaquensis*, *H. patersoni* and *P. praomydis* using published and newly designed primers (Table 2.2, chapter 2). Nuclear DNA data were generated for the carbamoyl-phosphate synthetase 2, aspartate transcarbamylase and dihydroorotase (CAD), nuclear elongation factor 1 alpha (EF-1α) and interphotoreceptor retinoid binding protein (IRBP) markers for *P. praomydis*, *H. patersoni* and *M. namaquensis* respectively using primers outlined in chapter 2, Table 2.2. The PCR protocols set out in chapter 2 was also followed for the amplification of these gene fragments (see Table 2.2, chapter 2). Sequences were visualized in Geneious v. 9.1 (https://www.geneious.com) and aligned with Clustal W (Thompson et al. 1994). All the *M. namaquensis* sequences were blasted against Genbank using the nucleotide BLAST (BLASTN) function to confirm the field identification. For *M. namaquensis*, however, only cytochrome b sequences were available on Genbank (Russo et al. 2010). To confirm species authenticity, the cytochrome b gene region was also amplified and sequenced using the published primer pair L14724 and H15915 (Pääbo et al. 1988; Irwin et al. 1991). The same protocol as outlined for the COI region was used as in chapter 2. All the COI sequences generated were also translated to proteins to confirm the absence of stop codons.

3.2.4 Data analysis

3.2.4.1 Phylogenetic relationships

To investigate the intraspecific relationship among the mitochondrial and nuclear haplotypes of *P. praomydis*, *H. patersoni* and to confirm the genetic differentiation within *M. namaquensis*, statistical parsimony haplotype networks were constructed in Popart 1.7 (Clement et al. 2002). To test the statistical significance of the connections among distantly related haplo-groups, statistical parsimony networks were also drawn in TCS 1.21 (Templeton et al. 1992; Clement et al. 2000). Due to the absence of variation in the nuclear DNA data the alleles were not subjected to allelic phasing. Standard molecular diversity measures were calculated in DNAsp (Rozas et al. 2017). Since clades forming part of the haplotype networks for *M. namaquensis*, *P. praomydis* and *H. patersoni* could not be connect with statistical certainty, the deeper evolutionary relationships among clades were examined by constructing Bayesian phylogenetic trees in MrBayes 3.2.6 (Ronquist et al. 2012) and Maximum likelihood trees in RaxMl 1.5 (Stamatakis 2006).

Mitochondrial and nuclear DNA sequence data were combined and analysed to determine the phylogenetic and phylogeographic associations between *P. praomydis*, *H. patersoni* and *M. namaquensis* respectively. *Micaelamys granti* and its associated *Hoplopleura* - and *Polyplax* lice were used as closely related outgroups in the analyses (derived from chapter 2).

Best-fit models of sequence evolution was calculated using jModelTest 3.7 (Guidon & Gascuel 2003; Darriba et al. 2012) in PAUP 4 (Swafford 2002) and by applying the AIC criterion (Akaike 1973; Burnham & Anderson 2004). The Bayesian analyses were all partitioned by codon and parameters were unlinked across partitions. Each analysis included two parallel Markov Chain Monte Carlo (MCMC) simulations that ran for 10 million generations where 25% of the total number of generations were discarded as burn-in. The remaining trees were visualized in Figtree 1.4.3 (Rambaut 2015) to obtain posterior probabilities for nodes. The same partitions were employed for the Maximum Likelihood analyses but in this instance 1000 bootstrap repetitions were performed to obtained confidence in the nodes. The best-fit models of evolution were again specified for different partitions. Trees were again visualized in Figtree 1.4.3 (Rambaut 2015).

For the mtDNA data of *M. namaquensis*, *P. praomydis* and *H. patersoni*, a three-level hierarchical analyses of molecular variance (AMOVA; Excoffier et al. 1992) were conducted in Arlequin v 3.5.2.2 (Excoffier & Lischer 2010). This analyses tests for significant differentiation in Φst values among groups indentified by the network analyses, among sampled localities within these groups and within sampling localities.

3.2.4.2 Co-phylogeny

Co-phylogeny between *M. namaquensis* and *H. patersoni* was investigated by topology-based reconciliation in Jane v.4 (Conow et al. 2010). Tree topologies based on the mtDNA haplo-groups were constructed for the host as well as *H. patersoni* using tree editor imbedded in Jane v.4 (Conow et al. 2010). In these co-phylogeny analyses a cost is assigned to different evolutionary events whilst the analyses attempt to find the most parsimonious (lowest cost) solution to map the parasite phylogeny onto that of the host (Conow et al. 2010). The Vertex cost model with the cost scheme: failure to diverge = 1, loss = 1, duplication followed by host-switch = 2, duplication = 1, co-divergence = 0, was implemented (Conow et al. 2010). The Genetic algorithm was set to 1000 generations and a population size of 300. Statistical significance of the solutions was evaluated by random tip mapping and the randomization of the parasite topology, where the statistical algorithm was again set to 1000 generations, a population size of 300 and including a sample size of 1000 (as in Engelbrecht et al. 2016).

3.3 Results

3.3.1 Identification of sucking lice associated with *M. namaquensis*

Two lice species were recorded on *M. namaquensis*: *Hoplopleura patersoni* and *Polyplax praomydis* (Johnson 1960). *Hoplopleura patersoni* was distributed across most of the country, but was absent from some of the south-western and especially the extreme south-western localities in South Africa (Table 3.1; Fig 3.2). *Polyplax praomydis* was also widely distributed but absent at some of the north-eastern localities (Table 3.1; Fig 3.2). This is interesting as *P. praomydis* is also more prevalent on *M. namaquensis* in the south-western parts of South Africa, whilst *H. patersoni* is more prevalent in the north-eastern parts (Table 3.1). Further, all localities south-west of Bloemfontein (BF) has a greater prevalence of *P. praomydis* than

Table 3.1-The number of hosts trapped and screened, the total abundance and prevalence of lice per locality and the number of hosts and lice used for molecular analyses for each locality. The locality abbreviations refer to the localities in Fig 3.1.

Locality	Number of *M. namaquensis* caught and screened	Number of hosts with lice	Number of lice (*H. patersoni/P. praomydis*	Lice prevalence (*H. patersoni/P. praomydis*) %	Number of *M. namaquensis* used in analyses	Number of lice used in analyses(*H. patersoni/P. praomydis*)
DD	2	2	0/6	0 /100	2	0/5
LF	8	2	0/15	0 /25	2	0/8
GP	6	6	0/6	0/100	1	0/6
EK	35	31	69/362	46 /83	10	15/19
RS	36	31	82/436	44 /69	16	14/18
RP	23	13	0/34	0 /57	13	0/16
PB	29	23	69/157	41 /62	15	9/19
TS	9	4	0/8	0 /44	4	0/8
BF	18	14	83/6	72 /6	14	22/6
DK	25	9	49/0	32 /0	4	7/0
MA	20	7	61/0	35 /0	4	13/0
AD	7	3	10/0	43 /0	3	7/0
MO	15	11	93/19	73 /40	10	10/12

H. patersoni and all the other localities in the north-west, including BF, has a greater prevalence of *H. patersoni* than *P. praomydis* (Table 3.1; Fig 3.2).

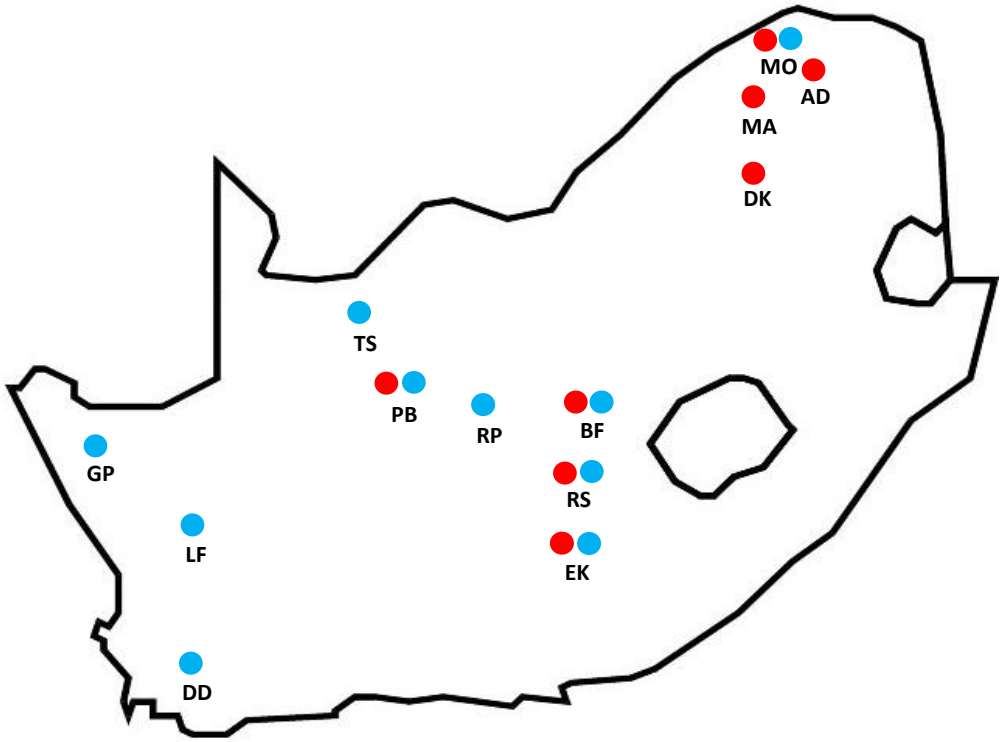

Figure 3.2-*M. namaquensis* trapping localities where *H. patersoni* (red) and/or *P. praomydis* (blue) was present. Abbreviations correspond to locality names in Fig 3.1.

3.3.2 Molecular analyses

MtDNA and nuDNA sequence data were generated for 98 *M. namaquensis*, 121 *P. praomydis* and 97 *H. patersoni* individuals across the range of *M. namaquensis* (Table 3.2). Stop codons were absent from all the generated COI sequences.

Table 3.2-Sequence data generated for *M. namaquensis*, *H. patersoni* and *P. praomydis*.

	mtDNA	number of individuals sequenced	Base pairs sequenced	nuDNA	Number of individuals sequenced	Base pairs
M. namaquensis	COI	98	465	IRBP	74	640
H. patersoni	COI	96	222	EF-1α	80	307
P. praomydis	COI	121	318	CAD	87	295

3.3.2.1 Phylogenetic and phylogeographic relationships

Our results confirmed the findings of chapter 2 indicating significant intraspecific differentiation within *M. namaquensis*. The mtDNA haplotype network showed that there are three haplo-groups within *M. namaquensis* that could not be connected with 95% confidence (Fig 3.3; A & D). The first haplo-group (Central/SW) comprised of all the *M. namaquensis* individuals trapped at localities in central and south-western localities in South Africa: De doorns (DD), Loeriesfontein (LF), Goegap (GP), Elandskuil (EK), Rusplaas (RS), Bloemfontein (BF), Rooipoort (RP), Postmasburg (PB) and Tswalu (TS) (Fig 3.3; A & D). The second haplo-group (NE 1) comprised of *M. namaquensis* individuals trapped in the most north-eastern locality (Mogalakwena (MO)) (Fig 3.3; A & D). The third haplo-group (NE 2) comprised of *M. namaquensis* individuals also trapped in the north-eastern localities at Dinokeng (DK), Marken (MA) and Alldays (AD) (Fig 3.3; A & D). The central/SW haplo-group differed by an mtDNA sequence divergence of 3.7 % (±0.5; 11 mutational steps) from the NE 1 haplo-group and 3.6 % (±0.4; 13 mutational steps) from the NE 2 haplo-group (Fig 3.3; A). Haplo-groups NE 1 and NE 2 had a mtDNA sequence divergence of 4.0% (±1.1) and haplotypes belonging to these groups differed by at least 18 mutational steps. The nuDNA TCS network for *M. namaquensis* shows a large amount of haplotype sharing among sampling sites and all haplotypes could be connected with 95% confidence (Fig 3.4; A).

The mtDNA TCS network for *H. patersoni* also showed three haplo-groups that could not be connected with 95% confidence (Fig 3.3; B & D). Similar to *M. namaquensis*, the first haplo-group (central/SW) comprised of the *H. patersoni* individuals collected from the *M. namaquensis* individuals trapped at localities in central and southwest South Africa these localities included: Bloemfontein (BF), Rusplaas (RS), Elandskuil (EK) and Postmasburg (PB) (Fig 3.3; B & D). As in the host there were also two separate haplo-groups in the north-eastern part of South Africa, but the geographic positioning of these clades differed from *M. namaquensis* (Fig 3.3; B & D). Haplo-group NE 1 comprised of *H. patersoni* individuals from Mogalakwena (MO), Marken (MA) and Alldays (AD), whilst haplo-group NE 2 comprised of *H. patersoni* individuals from Dinokeng (DK) (Fig 3.3; B & D). The differentiation between the *H. patersoni* haplo-groups were however much higher than that seen between the *M. namaquensis* haplo-groups. The central/SW haplo-group had a mtDNA sequence divergence of 13.0 % (±1.4; 35 mutational steps) from the NE 1 haplo-group and 11.0 % (±2.0; 18 mutational steps) from the NE 2 haplo-group (Fig 3.3; A). Haplo-groups NE 1 and NE 2 had an mtDNA sequence divergence of 9.5 % (±2.3) and differed by 17 mutational steps. The nuDNA TCS network were unable to provide any insights as all *H. patersoni* individuals were identical for the EF-1α gene (Fig 3.4; B).

The mtDNA TCS network for *P. praomydis* revealed two haplo-groups that could not be connected with 95% confidence (Fig 3.3; C). Similar to *M. namaquensis*, the first haplo-group (central/SW) comprised of *P. praomydis* individuals from central and south-western localities (De doorns (DD), Elandskuil (EK), Bloemfontein (BF), Goegap (GP), Rusplaas (RS), Rooipoort (RP), Loeriesfontein (LF), Postmasburg (PB) and Tswalu (TS)) (Fig 3.3; C & D). The second haplo-group (NE 1) comprised of *P. praomydis* individuals from Mogalakwena (MO) (Fig 3.3; C & D). These two haplo-groups were separated by an mtDNA sequence divergence of 14.6 % (±2.3; 40 mutational steps). Although all *P. praomydis* individuals could be connected with 95% confidence in the nuDNA TCS network, the two haplo-groups (retrieved in the mtDNA data) differed by two mutational steps (Fig 3.4; C).

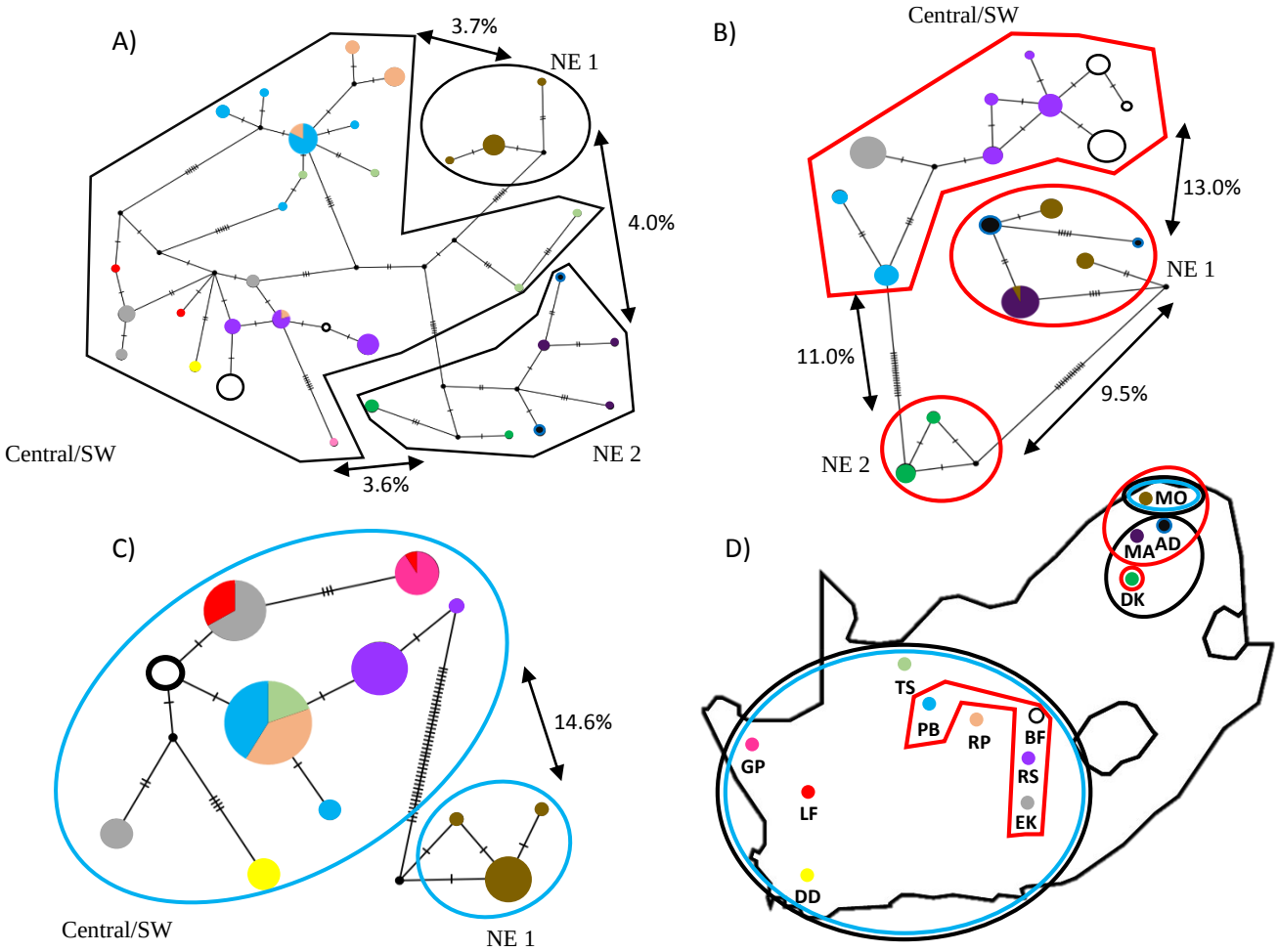

Figure 3.3-MtDNA haplotype networks for A) *M. namaquensis*; B) *H. patersoni* and C) *P. praomydis*. Shapes and corresponding labels separate the haplo-groups that could not be connected with 95% confidence. Percentages represent the average percentage COI sequence divergence between the different haplotypes. Haplotype colours correspond to the colours of the different localities from where the samples were collected in D. The geography of haplo-groups are indicated in D, where black, red and blue circles indicate the haplo-groups of *M. namaquensis*, *H. patersoni* and *P. praomydis* separately.

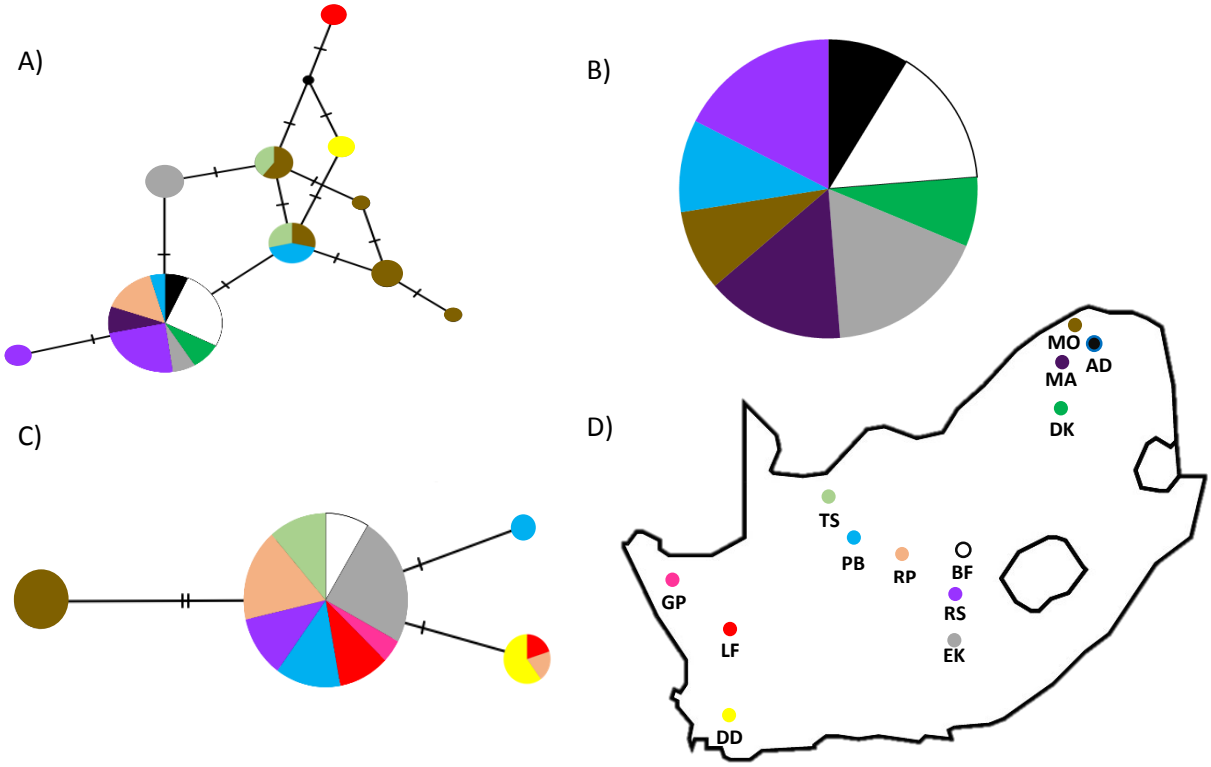

Figure 3.4-NuDNA haplotype networks for A) *M. namaquensis*; B) *H. patersoni* and C) *P. praomydis*. Haplotype colours correspond to the colours of the different localities from where the samples were collected in D.

The nucleotide diversity within the *M. namaquensis* haplo-groups were low with values of 0.02 (±0.004), 0.002 (±0.002) and 0.01 (±0.005) for the central/SW, NE 1 and the NE 2 haplo-groups separately (Table 3.3). *Hoplopleura patersoni* had similar nucleotide diversity values of 0.01 (±0.004), 0.01 (±0.006) and 0.002 (±0.002) for the three separate haplo-groups (Table 3.3). *Polyplax praomydis* had lower within haplo-group nucleotide diversity than *M. namaquensis* and *H. patersoni* (Table 3.3). The central/SW haplo-group had a nucleotide diversity of 0.008 (±0.003) whilst the NE 1 haplotype had a nucleotide diversity of 0.001 (±0.002).

For *M. namaquensis* the haplotype diversity within the Central/SW and NE 2 haplo-groups were high with values of 0.93 (±0.01) and 0.91(± 0.07), respectively, however the haplotype diversity within NE 1 haplo-group was lower (Table 3.3). The latter is most likely because the haplo-group only consists of one locality. For *H. patersoni*, as was the case for the host, the central/SW haplo-groups recorded the highest haplotype diversity (0.85 ±0.02), however, NE 1 recorded the second highest diversity followed by NE 2 (Table 3.3). The haplotype diversity within *P. praomydis* again reported the highest haplotype diversity for the central/SW haplo-group (0.79 ±0.03) with a lower diversity recorded for NE 1 (Table 3.3).

Table 3.3-The nucleotide diversity and haplotype diversity for the different haplo-groups that could not be connected with 95% confidence in the mtDNA TCS haplotype networks for *M. namaquensis*, *H. patersoni* and *P. praomydis*.

Haplo-groups	Nucleotide diversity (mtDNA)	Haplotype diversity (mtDNA)
M. namaquensis		
Central/SW	0.02 ±0.004	0.93 ±0.01
NE 1	0.002 ±0.002	0.38 ±0.18
NE2	0.01 ±0.005	0.91 ±0.07
H. patersoni		
Central/SW	0.01 ±0.004	0.85 ±0.02
NE 1	0.01 ±0.006	0.71 ±0.06
NE 2	0.002 ±0.002	0.48 ±0.17
P. praomydis		
Central/SW	0.008 ±0.003	0.79 ±0.03
NE 1	0.001 ±0.002	0.32 ±0.16

For the *M. namaquensis* phylogeny the Timef model (nst = 6, rates = equal) of sequence evolution was assigned to each partition. Data derived from the mtDNA and nuDNA datasets revealed significant posterior probability and high bootstrap support for the monophyly of the three *M. namaquensis* clades (Fig 3.5). There was also significant support for the NE 1 and NE 2 haplo-group lineages (Fig 3.5). The Central/SW lineage was also significantly supported in the Bayesian analyses but not supported by the ML analyses. The monophyly between the NE 2 and Central/SW haplo-groups were not significantly supported. For the *H. patersoni* phylogeny the TrNef+G model (nst = 6, rates = gamma) of sequence evolution was assigned for the first and third codons, whilst the K81 model (nst = 6, rates = equal) of sequence evolution was assigned to the second codon. The phylogenetic analyses revealed significant nodal support for the monophyly of all the *H. patersoni* individuals collected from *M. namaquensis* (Fig 3.6). There was also significant nodal support for the clustering of Central/SW, NE 1 and NE 2 lineages (Fig 3.6). The monophyly of the NE 2 and Central/SW lineages was also significantly supported (Fig 3.6). For the *P. praomydis* phylogeny the TrNef+G model (nst = 6, rates = gamma) of sequence evolution was assigned for the first and third codons, whilst the K81 model (nst = 6, rates = equal) of sequence evolution was assigned to the second codon. Data derived from the mtDNA and nuDNA datasets revealed significant posterior

probability and high bootstrap support for both the NE 1 lineage and the central/SW lineage (Fig3.7). The monophyly of these two lineages was also significantly supported (Fig 3.7).

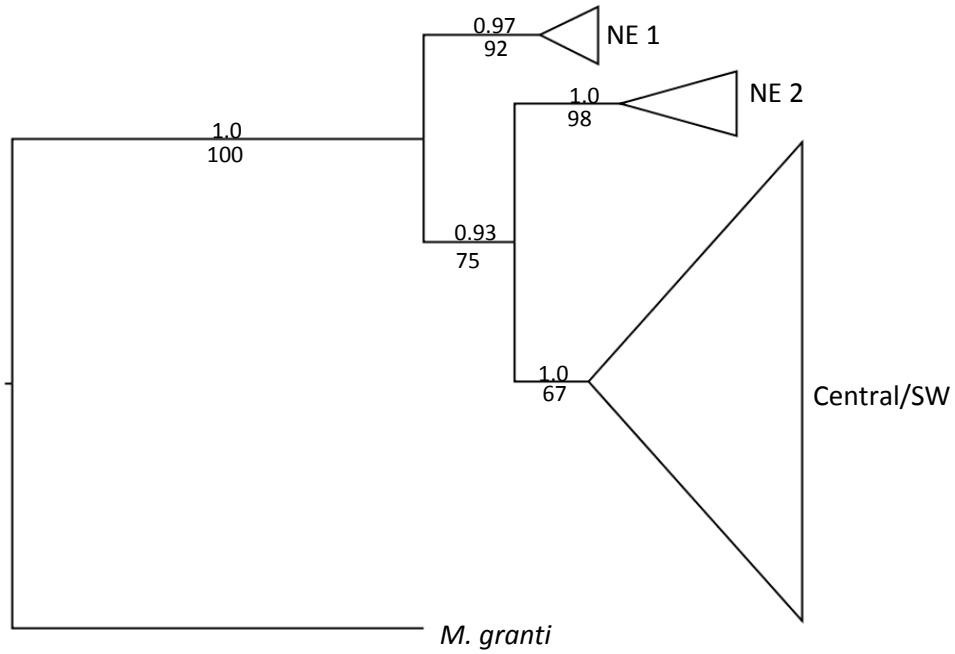

Figure 3.5-Bayesian and maximum likelihood topology for *M. namaquensis*. Nodal support indicated by posterior probabilities above and bootstrap values below nodes. Names on the right indicate haplo-groups that could not be connected with 95% confidence in the TCS network. With *M. granti* as outgroup.

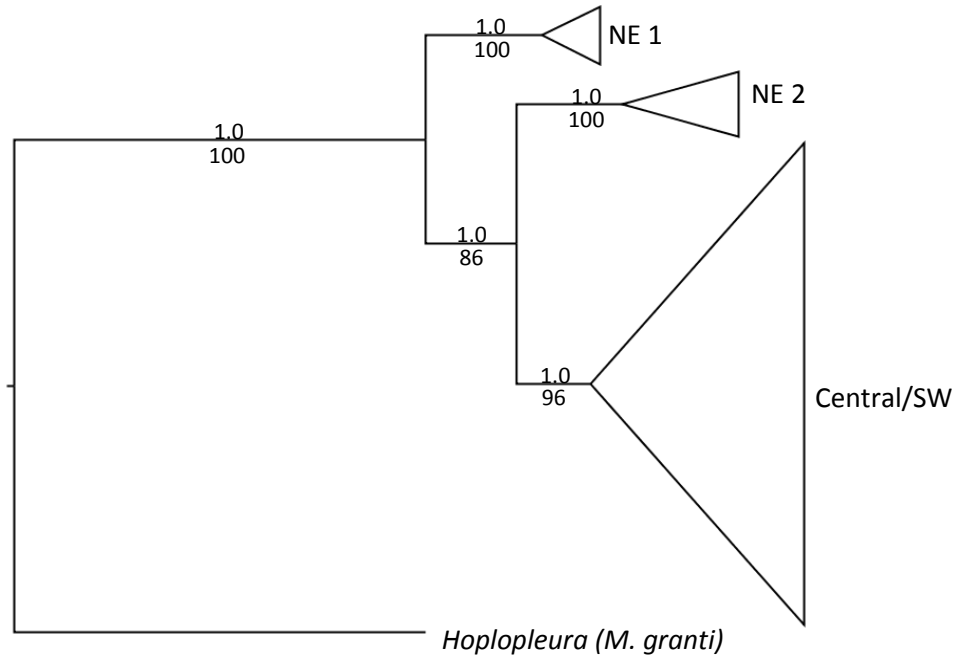

Figure 3.6-Bayesian and maximum likelihood topology for *H. patersoni*. Nodal support indicated by posterior probabilities above and bootstrap values below nodes. Names on the right indicate haplo-groups that could not be connected with 95% confidence in the TCS network. With the *Hoplopleura* species collected from *M. granti* as outgroup.

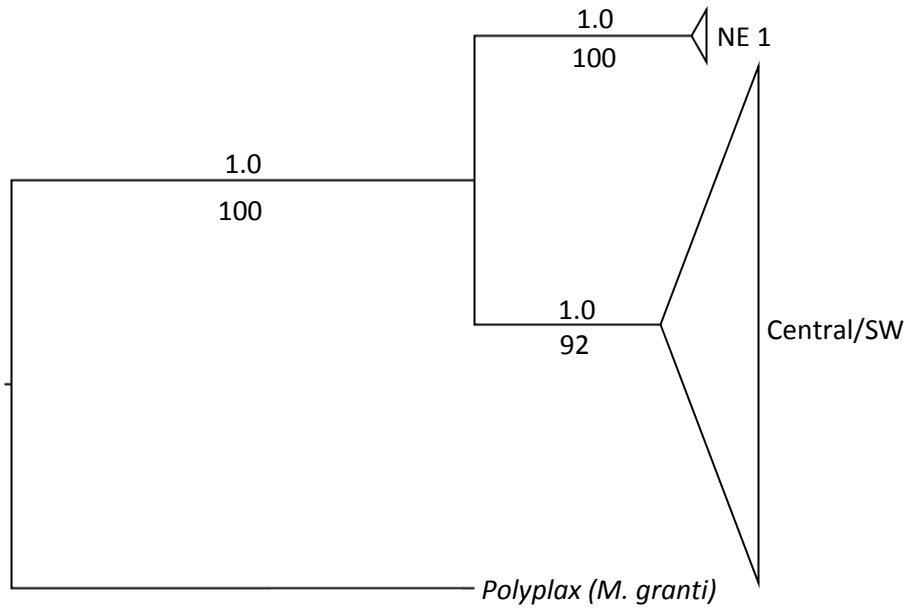

Figure 3.7-Bayesian and maximum likelihood topology for *P. praomydis*. Nodal support indicated by posterior probabilities above and bootstrap values below nodes. Names on the right indicate haplo-groups that could not be connected with 95% confidence in the TCS network. With the *Polyplax* species collected from *M. granti* as outgroup.

The three-level hierarchical analyses of molecular variance indicated that the haplo-groups within *M. namaquensis*, *H. patersoni* and *P. praomydis* are significantly differentiated at the mtDNA level (Table 3.4). The variation among the *M. namaquensis* haplo-groups was 54.1 %, whereas the variation amongst the haplo-groups for *H. patersoni* was higher at 85.7 %, and even higher in *P. praomydis* at 93.6 % (Table 3.4). The variation of 35.0 % among localities within these haplo-groups was the highest in *M. namaquensis*, compared to the 10.2 % and 5.5 % variation seen in *H. patersoni* and *P. praomydis*, respectively (Table 3.4). The variation of 10.9% within these sampling localities for *M. namaquensis* was again the highest in *M. namaquensis*, compared to the 4.1 % and 0.9 % variation seen in *H. patersoni* and

Table 3.4-Results from 3 level hierarchical analyses of molecular variance for the mtDNA datasets of *M. namaquensis*, *H. patersoni* and *P. praomydis*. Statistically significant values (P<0.05) are indicated with *.

Species	Fixation index			Variation (%)		
	ΦST	ΦSC	ΦCT	Among haplo-groups	Among localities within haplo-groups	Within localities
M. namaquensis	0.89*	0.76*	0.54*	54.1	35.0	10.9
H. patersoni	0.96*	0.72*	0.86*	85.7	10.2	4.1
P. praomydis	0.99*	0.85*	0.94	93.6	5.5	0.9

P. praomydis separately (Table 3.4). For *M. namaquensis* significant pairwise Φst values supported the differentiation among almost all sampling localities (Table 3.5). Non-significant pairwise Φst values were mostly retrieved between localities that had low sampling sizes (DD, LF, GP, TS, AD, MA, and DK) for *M. namaquensis* (Table 3.5). For *H. patersoni* significant pairwise Φst values supported the differentiation among all sampling localities (Table 3.6) and for *P. praomydis* significant pairwise Φst values supported the differentiation among almost all sampling localities (Table 3.7). Non-significant pairwise Φst values were only retrieved between Tswalu (TS) and Postmasburg (PB), Tswalu (TS) and Rooipoort (RP), Postmasburg (PB) and Rooipoort (RP) and Loeriesfontein (LF) and Elandskuil (EK) (Table 3.7).

Table 3.5-MtDNA Φst values among *M. namaquensis* sampled localities. Statistically significant values (P<0.05) are bold and indicated with *.

	MO	AD	MA	DK	EK	BF	RS	RP	DD	LF	GP	PB	TS
MO	0.00												
AD	**0.93***	0.00											
MA	**0.93***	0.10	0.00										
DK	**0.94***	**0.68***	**0.66***	0.00									
EK	**0.92***	**0.87***	**0.86***	**0.88***	0.00								
BF	**0.97***	**0.96***	**0.95***	**0.96***	**0.75***	0.00							
RS	**0.93***	**0.89***	**0.89***	**0.91***	**0.62***	**0.63***	0.00						
RP	**0.89***	**0.83***	**0.82***	**0.85***	**0.77***	**0.87***	**0.81***	0.00					
DD	**0.96***	0.90	0.87	0.92	**0.63***	**0.90***	**0.73***	**0.80***	0.00				
LF	**0.93***	0.80	0.79	0.84	0.10	**0.80***	**0.62***	**0.72***	0.50	0.00			
GP	0.94	0.87	0.85	0.89	0.80	0.95	0.82	0.77	1.00	0.60	0.00		
PB	**0.95***	**0.90***	**0.90***	**0.90***	**0.84***	**0.92***	**0.87***	**0.33***	**0.90***	**0.83***	0.90	0.00	
TS	**0.74***	**0.54***	**0.58***	**0.60***	**0.61***	**0.79***	**0.71***	**0.42***	0.42	0.26	0.29	**0.49***	0.00

Table 3.6-MtDNA Φst values among *H. patersoni* sampled localities. Statistically significant values (P<0.05) are bold and indicated with *.

	DK	MA	MO	AD	PB	EK	RS	BF
DK	0.00							
MA	**0.99***	0.00						
MO	**0.86***	**0.44***	0.00					
AD	**0.95***	**0.81***	**0.21***	0.00				
PB	**0.96***	**0.98***	**0.88***	**0.95***	0.00			
EK	**0.99***	**1.00***	**0.93***	**0.98***	**0.92***	0.00		
RS	**0.97***	**0.98***	**0.91***	**0.96***	**0.83***	**0.86***	0.00	
BF	**0.96***	**0.98***	**0.92***	**0.96***	**0.83***	**0.85***	**0.44***	0.00

Table 3.7-MtDNA Φst values among *P. praomydis* sampled localities. Statistically significant values (P<0.05) are bold and indicated with *.

	MO	DD	BF	EK	LF	GP	TS	PB	RP	RS
MO	0.00									
DD	**0.99***	0.00								
BF	**0.99***	**1.00***	0.00							
EK	**0.97***	**0.79***	**0.32***	0.00						
LF	**0.99***	**0.93***	**0.69***	0.13	0.00					
GP	**1.00***	**1.00***	**1.00***	**0.75***	**0.87***	0.00				
TS	**0.99***	**1.00***	**1.00***	**0.58***	**0.84***	**1.00***	0.00			
PB	**0.99***	**0.97***	**0.86***	**0.65***	**0.85***	**0.97***	0.01	0.00		
RP	**1.00***	**1.00***	**1.00***	**0.66***	**0.89***	**1.00***	0.00	0.04	0.00	
RS	**1.00***	**0.96***	**0.96***	**0.75***	**0.90***	**0.98***	**0.93***	**0.87***	**0.94***	0.00

3.3.2.2 Co-phylogeny

The Jane co-phylogenetic reconstruction between *M. namaquensis* and *H. patersoni* revealed that the most parsimonious solution with a total cost of three, includes three co-divergences, one host switch and one loss (Fig 3.8). Both of the statistical analyses indicated non-significant co-phylogeny between the *M. namaquensis* and *H. patersoni*, with p = 0.39 and p = 0.27 for random tip mapping and random parasite tree respectively. This analyses was not conducted to test for co-phylogeny between *M. namaquensis* and *P. praomydis* as these two taxa showed perfect congruence with a split between the north-eastern lineage and the south-western lineage.

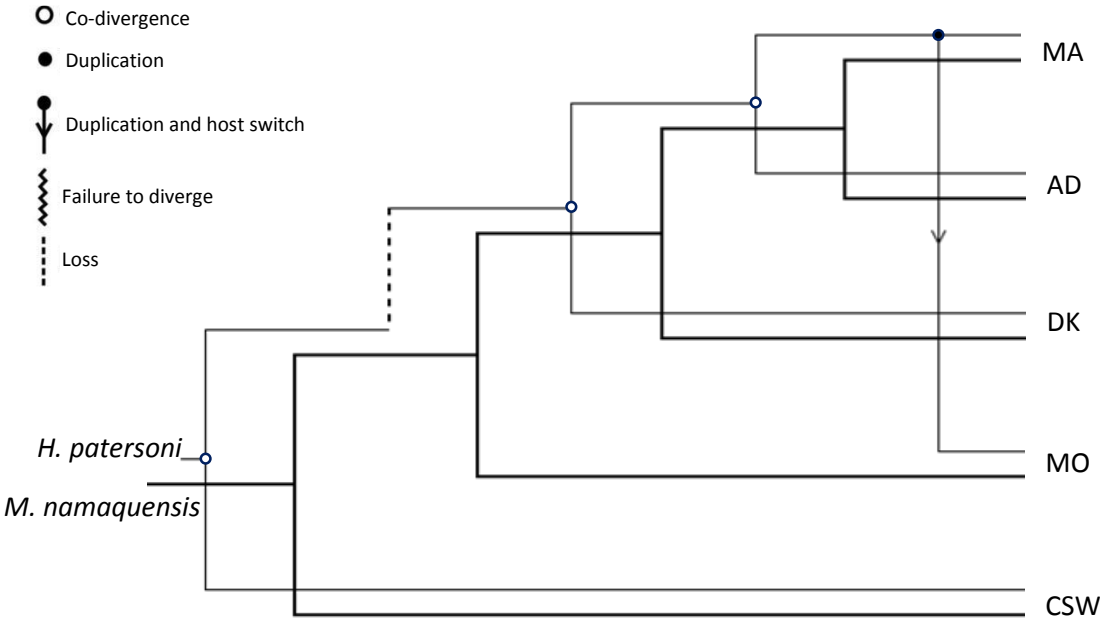

Figure 3.8-Phylogenetic reconciliation of *H. patersoni* and *M. namaquensis* retrieved from Jane after the five types of evolutionary events (legend) was tested for. The locality abbreviations refer to the localities in Fig 3.1. And CSW refers to the Central/southwest haplo-group.

3.4 Discussion

In the present study, cryptic taxonomic diversity is confirmed in both *H. patersoni* and *P. praomydis* collected from *M. namaquensis* (See chapter 2) and further evidence is provided for a cryptic *H. patersoni* lineage occurring on *M. namaquensis* individuals collected at Dinokeng (DK) (Fig 3.3, B & D). This lineage had mtDNA sequence divergences of 11.0 % and 9.5 % from the other two *H. patersoni* lineages (Fig 3.3, B & D). Although these high mtDNA sequence distances approach for example the 15 % interspecific divergences seen between *H. reithro* and *H. emphereia* that parasitize the *Reithrodontomys* and *Peromyscus* genera, respectively (Sanchez-Montes et al. 2016), the exact taxonomic designation of the cryptic *H. patersoni* lineages cannot be established at this time. In the present study, cryptic diversity is well supported by the statistical TCS analyses as well as generally high bootstrap and posterior probability values for both lice species. The mtDNA cryptic diversity is however only supported by nuclear DNA data in the case of *P. praomydis* (unfortunately the EF-1α fail to differentiate the three *H. patersoni* lineages). Additional data from faster evolving nuclear markers, coupled to a thorough morphological investigation would be required to fully resolve the taxonomy of *H. patersoni* and *P. praomydis* collected from *M. namaquensis.*

In this study the mtDNA genetic patterns obtained for the lice lineages occurring on *M. namaquensis* show some congruence to that of the host as there is strong differentiation between individuals from the south-west and individuals from the north-east in all taxa (both parasites and their host show the same pattern).

Although this differentiation is not consistently supported at the nuclear DNA level for all clades, the mtDNA sequences among the genetic assemblages are high. The northern and southern clades are separated by at least 3.7 % in the host and 11.0 % and 14.6 % within *H. patersoni* and *P. praomydis*, respectively (Fig 3.3). The vicariance pattern found separating the northern clades from the southern clades, can most likely be attributed to host distribution and host dispersal patterns (also see Russo et al. 2010) where three higher level *M. namaquensis* clades were found that could not be connected with statistical support. In the present study, the mean estimated timing of the divergence for host and parasites range between 2.3 Ma to 3.3 Ma (chapter 2, Table 2.4; Figure 2.10) and these dates coincide with the range of 2.03 - 4.06 Ma reported for the divergences among host lineages in Russo et al. (2010). Host divergence was most likely driven by climatic and vegetation changes during this period (de Menocal 2004; Russo et al. 2010). Since geneflow was restricted in the host over large geographic distances, the movement of the permanent *H. patersoni* and *P. praomydis* was most likely also restricted and hence distinct maternal regionally confined genetic clades evolved for both parasites.

The situation is however more complex, since within the northern region there is no clear geographic congruence between host differentiation and *H. patersoni* differentiation. In the host, one haplo-group consists of individuals from Mogalakwena (MO) whilst the other north-eastern haplo-group consists of individuals from Marken (MA), Alldays (AD) and Dinokeng (DK) (Fig 3.3; A & D; broadly similar to what has been detected in Russo et al. 2010 and described therein as lineage A2 and lineage H). In *H. patersoni* however, Dinokeng (DK) forms a separate lineage whilst Mogalakwena (MO), Alldays (AD) and Marken (MA) form a second distinct north-eastern lineage (Fig 3.3; B & D). The present analyses shows that this mtDNA incongruence is due to a duplication and hosts switching event in the *H. patersoni* phylogeny (Fig 3.8, A). Unfortunately, the nuclear DNA did not support the mtDNA pattern and rather show shared haplotypes between the north and south and also among mtDNA host haplo-groups. Although this can be ascribed to incomplete lineage sorting that is known to predominate phylogenetic divergences with shallow time depths (Maddison & Knowles 2006), the potential for strong male biased dispersal that is present in *M. namaquensis* cannot be excluded (Fleming & Nicolson 2004). It has been recorded that *M. namaquensis* males disperse across a wide area during the breeding season whilst females remain in their relatively small, discrete, contiguous areas (Fleming & Nicolson 2004). As a result, it is possible that mtDNA may not reveal the true gene flow particularly over shorter geographic distances and in the absence of geographic barriers. The resulting conflict in structure between the nuclear and the mtDNA in *M. namaquensis* can thus also be attributed to male biased dispersal. The conflict in co-divergence between *M. namaquensis* and *H. patersoni* over short geographic distances can thus possibly be attributed to host switching facilitated by dispersing males during the breeding season. Supplementary sampling and the addition of faster evolving nuclear markers are clearly needed to resolve this pattern fully. Although the two haplo-groups in *P. praomydis*

(Central/SW and NE 1) corresponded perfectly to that of *M. namaquensis* (Fig 3.3) the failure to collect *P. praomydis* individuals from both *M. namaquensis* haplo-groups in the north-east impacted the study's ability to fully compare the parasite and host phylogenies in this area. This is problematic as the north-eastern parts of South Africa is where incongruences were found in the *H. patersoni* and *M. namaquensis* phylogenies. As a result, further sampling would have to be undertaken in the north-eastern parts of South Africa to truly test for co-phylogeny between *P. praomydis* and *M. namaquensis*.

The present study partly confirms the notion that sucking lice who are obligate parasites of their host, are also dependent on their host for dispersal, and they thus will show evolutionary co-divergence with their host (Hafner & Page 1995; Page & Hafner 1996; Gomez-Diaz et al. 2007). It, however, also highlights that the situation at the phylogeographic level is complex and the mechanisms responsible for co-divergence or the lack thereof is not easy to decipher (du Toit et al. 2013a). By using a comparative approach between the present study and what has been reported in the literature, more insights may be gained. It was predicted that because *M. namaquensis* is mostly confined to rocky outcrops, and should thus be more restricted in their dispersal when compared to for example *Rhabdomys* species (that is a generalist occupying mostly plains regions; Skinner & Chimimba 2005), greater genetic diversity and structure should be seen over the geographical range for the sucking lice associated with *M. namaquensis* than that reported for *Polyplax* that occurs on *Rhabdomys* (du Toit et al. 2013 b). The host, *M. namaquensis* had comparable mtDNA genetic structure throughout its range (Φst = 0.89) with *R. bechuanae* (Φst = 0.79; Matthee et al. 2018). The latter *Rhabdomys* species is known to be restricted in their dispersal since they are confined for more fragmented habitat patches when compared to the highly mobile solitary *R. dilectus* (Φst = 0.18; Matthee et al. 2018). Irrespective the restrictions to host dispersal in the present study, it is noteworthy that the genetic structure for both lice species associated with *M. namaquensis* was also marked (most localities are characterized by a set of closely related mtDNA haplotypes and differed significantly in the Φst pairwise comparisons). In fact similar global Φst values of 0.96, 0.99 were found for *H. patersoni* and *P. praomydis* and these values correlate very well with what has been found for lice on *Rhabdomys* (*P. arvicanthis* 1 and *P. arvicanthis* 2 each had Φst values of 0.96 and 0.90 respectively) (du Toit et al. 2013 b). In this study the correlation between host population divergence and parasite divergence among sites (for both parasite species) coupled to the broad phylogeographic congruence found between parasite and host suggest that the permanency of the parasite on the hosts is the main contributing factor for the co-divergence. On the other hand, host switching events are also evident in the present data set and this can best be explained by lack of adaptation. Previous studies have shown that certain morphological characters of lice evolve to adapt to changes in the host. One such study showed that the body size of the louse correlates positively with the body size its host (Morand et al. 2000). Similarly, another study concluded that the diameter of the rostral groove, which lice use to grasp the hairs of their host, significantly correlates to the hair diameter of their host (Reed at al.

2000). Such adaptations could result in lice being unable to switch hosts as the adaptive features would be detrimental to their survival on other hosts. However, in this study the close intraspecific evolutionary history between the host lineages would not have resulted in such adaptational differences in the lice, making host switching and survival possible.

In conclusion this study highlights the striking intraspecific genetic difference in *M. namaquensis* (north-east and south-west) and also in its associated lice species, as identified in chapter two of this thesis. This study further highlights intraspecific variation in the north-eastern parts of South Africa in both *M. namaquensis* and *H. patersoni*. As a result of this cryptic variation in both *H. patersoni* and *P. praomydis* this study also provides support for the growing body of literature that show that cryptic diversity is rife in parasites (de Leon & Nadler 2010; Nadler & de Leon 2011; Perkins et al. 2011; du Toit et al. 2013 a; Engelbreght et al. 2014). Furthermore, this study provided evidence that co-divergence between a parasitic sucking louse and its host cannot be assumed, and that evolutionary events such as host switching can influence the extent of co-divergence.

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