

***Taxonomy and ecology of parasitic chigger mites (Acari:  
Trombiculidae) on small mammals in South Africa***

by

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## **Abstract**

Within South Africa (SA) parasites have received variable attention with limited research conducted on mites within the family Trombiculidae. They are regarded as temporary parasites with only the larval stage or “chigger” being parasitic. The present study investigated the diversity, ecology and distribution of chiggers associated with small mammal hosts (rodents and insectivores) across SA, with a focus on the Cape Floristic Region (CFR). The study supports the existence of seasonal occurrence of chiggers in a temperate region. Chiggers that occurred on a generalist rodent host were most prevalent during the warm dry months of the year as opposed to wet cold months. Total counts conducted on the bodies of several co-occurring rodent species in the CFR recorded a diverse assemblage of chigger species. The findings support previous studies in that chiggers are host generalist, though there does appear to be a preference for the most abundant host species, *Rhabdomys pumilio*, in the biotype. Host species were parasitized by multiple chigger species of which *Leptotrombidium muridium* was the most abundant species. The study recorded and described three new chigger species (*Austracarus* n. sp., *Microtrombicula* n. sp. and *Schöngastiella* n. sp.). Chigger abundances were found to be higher on reproductively active as opposed to non-active hosts. Twelve chigger species were recorded across SA and the individual species showed variation in extent of their geographic range. On-host distribution of chigger species recorded a preference for the tail area of the host, which was shared by the three most abundant chigger species. This pattern may explain the higher co-occurrence of chigger species than expected by chance that was recorded on *R. pumilio*. It is evident that chiggers of small mammals are a diverse group that vary spatially and temporary across the landscape.

## **Opsomming**

Die verskeie parasiet taksa wat in Suid Afrika (SA) voorkom het ongelyke aandag ontvang tydens parasitologie studies tot dusver. Trombiculidae myte is een van die parasiet groepe wat baie min aandag ontvang het. Die groep myte word beskou as tydelike parasiete, weens die feit dat slegs die larf stadium (ook verwys as “chigger”) van die myt parasities is. Die studie het die diversiteit, ekologie en geografiese verspreiding van chiggers bestudeer wat geassosieer word met klein soogdiere binne SA, met ‘n fokus op die Kaapse Floristiese Ryk (KFR). Die studie het bevind dat chiggers ‘n seisoenale voorkoms het, hul was meer volop tydens die warm droë maande in vergelyking met nat en koel winter maande, soortgelyke resultate is aangeteken in ander dele van die wêreld. Die studie het ook gevind dat die myte wat in die KFR nie gasheer spesifiek was nie, maar dat hul wel ‘n voorkeur getoon het vir die gasheer wat die volopste was, *Rhabdomys pumilio*. Die verskeie gasheer spesies wat ondersoek was was deur verskillende chigger spesies geparasiteer waarvan *Leptotrombidium muridium* die mees volopste was. Drie nuwe chigger spesies is beskryf tydens die studie (*Austracarus* n. sp., *Microtrombicula* n. sp. and *Schöngastiella* n. sp.). Die resultate van die studie het ook gedui dat gashere wat reproduktief was hoër getalle myte gehad het wat op hul geparasiteer het in vergelyking met gashere wat nog nie reproduktief aktief was nie. Twaalf chigger spesies was tydens die studie aangeteken, hul het verskil in terme van hul geografiese verspreidings. Die verspreiding van die myte op die gasheer se lyf was ook bestudeer en daar was bevind dat die myte ‘n voorkeur toon vir die stert area van die gasheer. Die verskillende chigger spesies het ook saam voorgekom op ‘n spesifieke aanhegtings plek op die muis se lyf en geen uitsluitings-kompetisie was gevind nie. Die gevolgtrekking van die studie is dat chiggers wat klein soogdiere parasiteer in SA ‘n baie diverse groep is wat verskil ten opsigte van hul geografiese verspreiding asook in terme van in hul seisoenale teenwoordigheid binne die landskap.

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## Chapter 1

### General introduction

#### Taxonomy of Trombiculid mites

One of the earliest mentionings of Trombiculid mites was by the famous Linnaeus in *Systema Naturae* (1735), where he referred to them as *Acarus batatas* (Goff *et al.*, 1982; Shatrov and Kudryashova, 2006). Almost two hundred years later, in 1905, the genus *Trombicula* was established by Berlese with only six species in this group. *Trombicula* was initially included in the family *Trombidiidae* (Oudemans 1912) and detailed examination of the genus led to the establishment of the subfamily, *Trombiculinae* (Ewing 1929). The subfamily *Trombiculinae* was later upgraded to family level by Ewing (1944). *Trombiculidae* consisted of two subfamilies, *Trombiculinae* and *Hemitrombiculinae*, which included 26 species (Shatrov and Kudryashova, 2006; Krantz & Walter, 2009). Another addition was made to the family in the form of the subfamily *Leeuwenhoekiiinae* (Womersley 1945), which was raised to family level in 1945 (Shatrov and Kudryashova, 2006; Krantz & Walter, 2009). Ewing (1949) included four subfamilies within the *Trombiculidae*: *Hemitrombiculinae*, *Walchiinae*, *Leeuwenhoekiiinae* and *Trombiculinae*. Ewing (1949) was the first to use the external morphology of the larval stages of Trombiculid mites to construct a system of classification (Krantz & Walter, 2009). Wharton *et al.* (1951) suggested *Trombiculidae* be divided into four subfamilies; *Leeuwenhoekiiinae*, *Walchiinae*, *Apoloniinae* and *Trombiculinae*. Later the subfamily *Walchiinae* was renamed to *Gahrlepiinae* (Womersley 1952). Taxonomists were divided in their support of the taxonomic classification of trombiculids, hence there are currently three classifications of trombiculids (Shatrov and Kudryashova, 2006; Krantz & Walter, 2009).

Most recent classification according to Krantz & Walter (2009)

- Order Trombidiformes
- ❖ Suborder Prostigmata
  - \*Superfamily Trombiculoidea (Consists out of 6 families)
  - Family Jhonstonianidae

- Jhonstonianinae (Welbourn, 1991)
- Charadacarinae (Welbourn, 1991)
- Family Trombiculidae (Ewing, 1944)
  - Trombiculinae (Ewing, 1929)
  - Gahrlipeiinae (Womersley, 1952)
- Family Leeuwenhoekidae (Womersley, 1945)
  - Leeuwenhoekinae (Womersley, 1944)
  - Apoloniinae (Wharton, 1947)
- Family Neotrombidiidae (Vercammen-Grandjean, 1973)
  - Neotrombidiinae (Vercammen-Grandjean, 1973)
  - Anomalothrombiinae (Vercammen-Grandjean, 1973)
- Family Trombellidae (Leach, 1918)
  - Trombellinae (Thor, 1935)
  - Moyanellinae (Robaux, 1967)
  - Spelaeonthrombiinae
- Family Audyanidae (monobasic family)
  - Audyninae (Womersley, 1954)

Second classification (Shatrov and Kudryashova, 2006; Krantz & Walter, 2009)

- Family Trombiculidae (Ewing, 1944)
  - Subfamily Trombiculinae (Ewing, 1929)
  - Subfamily Gahrlipeiinae (Womersley, 1952)
- Family Leeuwenhoekidae (Womersley, 1945)
  - Subfamily Leeuwenhoekinae (Womersley, 1944)
    - ❖ Tribe Leeuwenhoekini (Vercammen-Grandjean, 1968)
    - ❖ Tribe Whartoniini (Vercammen-Grandjean, 1968)
  - Subfamily Apoloniinae (Wharton, 1947)
    - ❖ Tribe Apoloniini (Vercammen-Grandjean, 1968)
    - ❖ Tribe Sauracarellini (Vercammen-Grandjean, 1968)

Third classification (Shatrov and Kudryashova, 2006; Krantz & Walter, 2009)

- Family Trombiculidae
  - Subfamily Trombiculinae (Ewing, 1929)
    - ❖ Tribe Trombiculini (Vercammen-Grandjean, 1960)
    - ❖ Tribe Schoengastiini (Vercammen-Grandjean, 1960)
    - ❖ Tribe Gahrliapiini (Nadchatram et Dohany, 1974)
  - Subfamily Leeuwenhoekinae (Womersley, 1944)
    - ❖ Tribe Leeuwenhoekinae (Vercammen-Grandjean, 1968)
    - ❖ Tribe Whartoniini (Vercammen-Grandjean, 1968)
  - Subfamily Apoloniinae (Wharton, 1947)
    - ❖ Tribe Apoloniini (Vercammen-Grandjean, 1968)
    - ❖ Tribe Sauracarellini (Vercammen-Grandjean, 1968)

### **Bionomics of trombiculid mites**

Members of the trombiculid family are regarded as temporary parasites, as only the larval stage or “chigger” is parasitic (Mohr, 1947; Daniel, 1961; Traub & Wisseman, 1974; Balashov, 2006; Krantz & Walter, 2009). The life cycle of trombiculid mites consists of seven distinct stages (Figure 1.1). The larva, deutonymph and adult are all active. The calyptostases include the egg, deutovum, protonymph and tritonymph. A brief outline of each stage in the life cycle follows: Adult female mites deposit eggs, singly or in clumps, in the superficial layers of the soil (Traub & Wisseman, 1974; Simonová, 1983; Shatrov, 1996, 2003). About 1-5 eggs are laid per day for up to twelve weeks, thereafter the female rests for a similar period before resuming oviposition (Traub & Wisseman, 1974). Interestingly the time lag between oviposition events is slightly prolonged for species in montane habitats compared to species in tropical and temperate regions (Traub & Wisseman, 1974). A record 40 000 eggs can be produced per female during the reproductive cycle (Traub & Wisseman, 1974). The inactive egg stage lasts on average five to seven days, but it has been recorded that trombiculid species residing in colder regions can over-winter in the egg stage and emerge when temperatures rise (Daniel, 1961). The egg develops into a quiescent prelarva or deutovum, which is still developing (Traub & Wisseman, 1974; Krantz & Walter, 2009).

After five to seven more days the deutovum cracks open and an active larva emerges (Traub & Wisseman, 1974; Krantz & Walter, 2009). These minute parasitic larvae have a wide host range and will attach to any organism that comes into close proximity to their birth place or “mite foci” (Mohr, 1947; Lawrence, 1949; Daniel, 1961; Traub & Wisseman, 1974; Krantz & Walter, 2009). Due to their limited mobility chiggers do not travel more than a few meters from their hatching site and thus infestation will be highly localized (Traub & Wisseman, 1974; Goff, 1979; Krantz & Walter, 2009; Mariana *et al.*, 2011). Host location is primarily achieved through questing behaviour. Chiggers will become aware of a hosts presence in their immediate surroundings by picking up on the vibrations, smells and carbon dioxide of the host (Traub & Wisseman, 1974; Goff, 1979). Members of the genus *Leptotrombidium* (*L. deliense* and *L. flethceri*) have been documented as climbing on to nearby vegetation forming clusters and awaiting a passing host (Traub & Wisseman, 1974). By climbing onto vegetation they increase the height at which they can attach to a host (Traub & Wisseman, 1974). Direct contact between the chigger and host animal needs to be accomplished for the chigger to be able to attach. Upon achieving contact, the larva will attach by inserting their chelicerae into the skin of the host (Figure 1.2) (Lawrence, 1949; Goff *et al.*, 1982; Krantz & Walter, 2009). Chiggers feed by extracting enzymatically liquefied tissue and epithelial cells via a feeding tube or hypostome that is formed by the incited immune reaction of the host to repeated injection of saliva into the wound (Krantz & Walter, 2009). The duration of attachment varies significantly between species ranging from a few days to months (Traun and Wiseman, 1974; Shatrov and Kudryashova, 2006; Dietsch, 2008 Mariana *et al.*, 2011). After engorgement, the larva detaches from the host and enters the soil where it passes through the quiescent protonymph stage (Traub & Wisseman, 1974; Krantz & Walter, 2009). Upon completion of the protonymph stage, an active deutonymph emerges. The octopod nymph is a free-living predator of small arthropods and their eggs within the soil (Lawrence, 1949; Traub & Wisseman, 1974; Goff, 1982; Krantz & Walter, 2009). The deutonymph stage lasts for up to two weeks, followed by the second inactive, tritonymph stage (Goff, 1982). The adult mite that emerges is a free-living, soil-dwelling predator, much like the deutonymph, except that they are much larger in size and sexually mature (Daniel, 1961; Traub & Wisseman, 1974; Goff, 1982; Krantz & Walter, 2009). No copulation occurs between adult male and female mites; instead males deposit stalked spermatophores in the substrate (within the soil) as soon as a day after emergence (Traub & Wisseman, 1974).

Spermatophore production can either take place for a limited time or for the duration of their lifespan (Simonová, 1983). Females collect the spermatophores and store them within genital valves (Traub & Wisseman, 1974). The number of ovi-production cycles ranges from one to three for the duration of the female's life (Shatrov 1996, 2003). Both sperm and egg production can take place before the adults have fed (Traub & Wisseman, 1974). Laboratory experiments and field observations of tropical and sub-tropical species revealed that the entire life cycle can be completed in two to three months; this is especially true for member of the genus *Leptotrombidium* (Daniel, 1961; Traub & Wisseman, 1974). On the other hand montane species and species occurring in cold climatic regions can take as long as eight months to complete their life cycle (Daniel, 1961; Traub & Wisseman, 1974). In the tropics trombiculid reproduction is incessant, resulting in chiggers being prevalent year round (Traub & Wisseman, 1974; Goff, 1982). In temperate regions however, environmental conditions modify the developmental cycle of chiggers and only one or two generations are produced annually (Goff, 1982). Some species will be prevalent during spring and summer while others will be prevalent during winter and autumn depending on the environmental requirements of the specific chigger species (Goff, 1982).

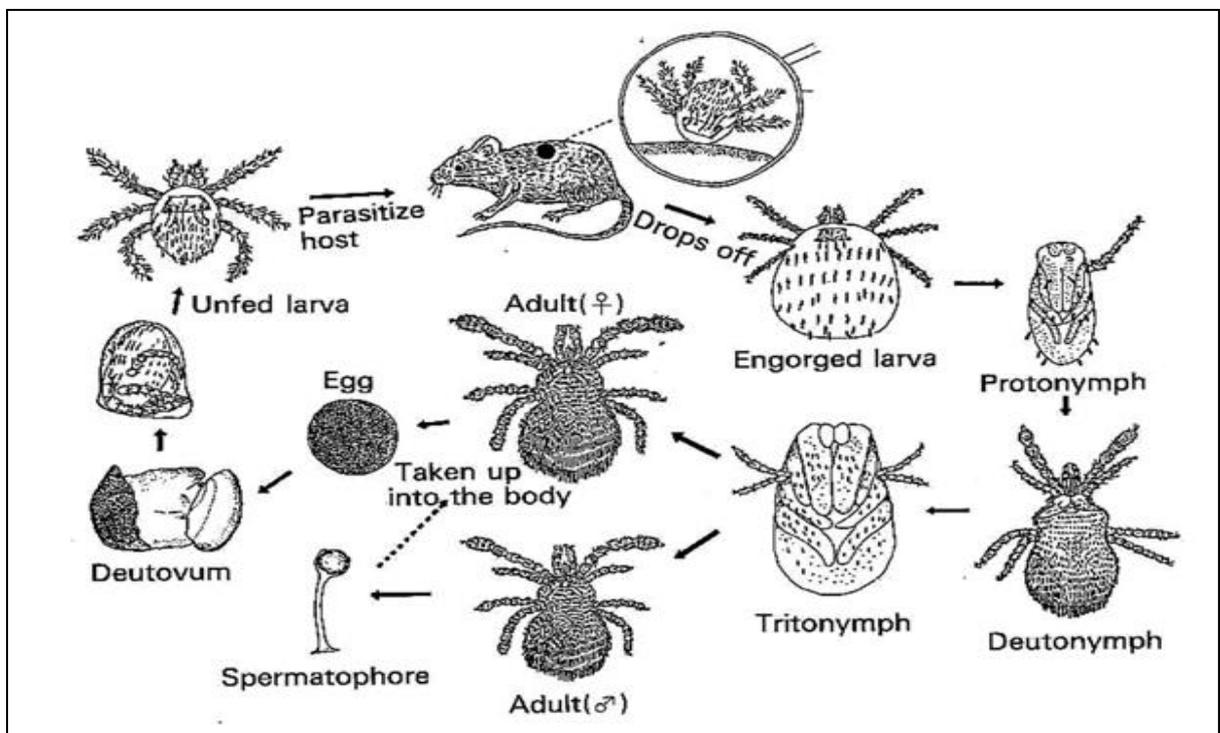


Figure 1.1: Life cycle of Trombiculid mites (taken from Shatrov and Kudryashova, 2006).

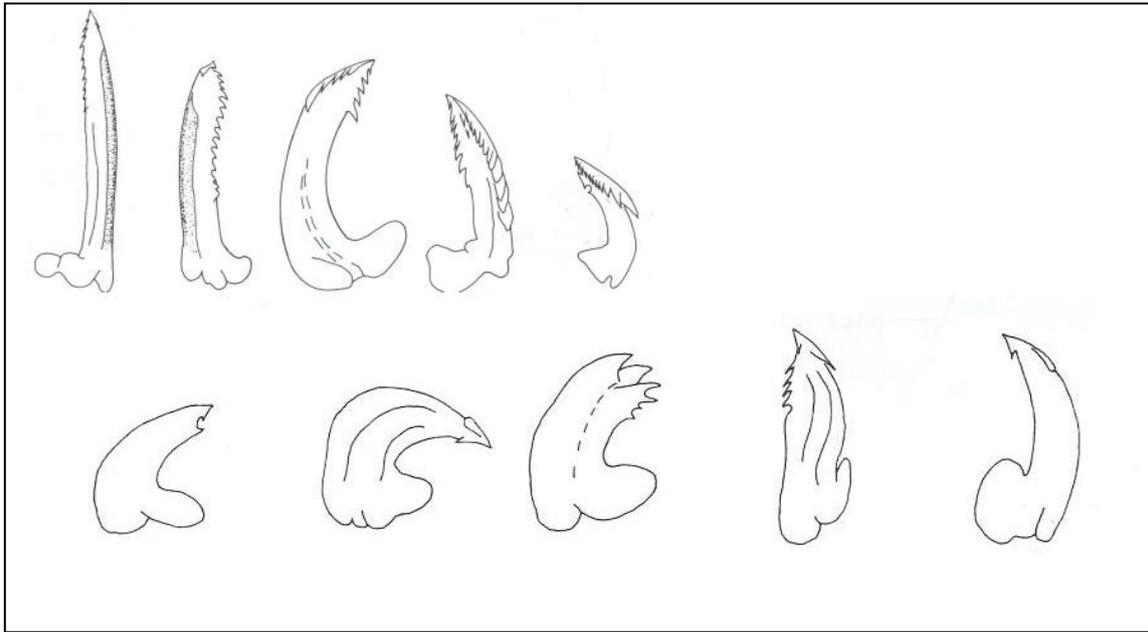


Figure 1.2: Illustration of the different lengths and serratedness of cheliceral blades (taken from Goff *et al.*, 1982).

### Off-host habitat preferences

Trombiculid mites have a widespread geographic distribution and occur on all continents, except Antarctica (Traub & Wisseman, 1974; Clopton & Gold, 1993; Watt & Parola, 2006). However, at a local scale they exhibit a patchy non-uniform distribution within the landscape (Traub & Wisseman, 1974; Walter & Proctor, 2004; Scholer *et al.*, 2006). Chiggers seem to aggregate in patches termed “mite islands”, (Lawrence, 1949; Traub & Wisseman, 1974; Goff *et al.*, 1982) that provide suitable habitat (vegetation structure) and climatic conditions (temperature, humidity and rainfall) for their survival (Traub & Wisseman, 1974; Clopton & Gold, 1993; Scholer *et al.*, 2006; Diaz, 2010). Chiggers are generally considered habitat specialists and host species generalist, as the larval stage can occur on multiple host taxa (Lawrence, 1949; Goff, 1979; Dong *et al.*, 2009; Mariana *et al.*, 2011). Daniel (1961) found a clear trend in chigger species distribution within different biotopes within the landscape; *Trombicula zachvatkini* was dominant in pristine forest patches, while conspecific species *T. talmiensis* and *T. autumnalis* were predominant in disturbed and cultivated biotopes. Chigger species that exhibited general microclimatic requirements had

a wider range of occurrence across different biotopes (Daniel, 1961; Goff, 1979, 1982). From the literature, it seems that chiggers are often associated with transitional zones and disturbed habitats, which may in turn be linked to the presence of reservoir hosts or with high animal movement (Mohr, 1947, 1956; Traub & Wisseman, 1974; Goff, 1979; Clopton & Gold, 1993).

### **Host specificity**

The host spectrum of chiggers is wide-ranging and include mammals, birds, amphibians, reptiles and insects (Mohr, 1957; Traub & Wisseman, 1974; Brennan & Reed, 1975; Goff, 1979, 1982; Arnold, 1986; Krantz & Walter, 2009). Chiggers are highly opportunistic parasites and will parasitize any organism that passes through mite islands within the habitat (Traub & Wisseman, 1974; Kudryashova, 1998; L. Goff, personal communication, 2013). Species exploiting land mammals in Papua New Guinea, occurred on an average of five different host species (Goff, 1979). It is also not uncommon for a single host organism to be infested with multiple chigger species at one time (Mohr, 1956; Traub & Wisseman, 1974; Whitaker & Loomis, 1978; Goff, 1979, 1982; Xing-Yuan *et al.*, 2007; Dong *et al.*, 2008, 2009; Mariana *et al.*, 2011). The maximum number of chigger species recorded from a single host organism during the study by Goff (1979), was 18 species collected from the variable spiny rat (*Rattus ruber*). The study furthermore indicated that hosts were on average exploited by three different chigger species.

Due to the opportunistic nature of chiggers it is not surprising that host selection is not based on the phylogenetic relatedness of the various hosts, but rather on overlap in habitat utilization between the chigger and the host organisms, a phenomenon known as ecological fitting (Daniel, 1961; Sasa, 1961; Kudryashova, 1998; Brooks *et al.*, 2006; Shatrov and Kudryashova, 2006; Agosta & Klemens, 2008; Krantz & Walter, 2009). Eventhough chiggers are regarded as host species generalists it is not unkommon for them to display a preference towards a specific host species within a given biotope (Mohr, 1947, 1956; Daniel, 1961; Traub & Wisseman, 1974; Whitaker & Loomis, 1978, Shatrov and Kudryashova, 2006). Host preference is strongly influenced by host population density, co-habitation of a patch by the chigger and the host species, behaviour of the host (e.g. foraging within the area,

sociality and home-range size) and the ecology of the specific chigger species (Daniel, 1961; Shatrov and Kudryashova, 2006; Dietsch, 2008).

Even though chiggers are highly euryoecious in terms of host preference, evidence suggest that rodents are important hosts for chiggers globally (Mohr, 1947, 1956; Daniel, 1961; Traub & Wisseman, 1974; Brennan & Reed, 1975; Goff, 1982; Shatrov and Kudryashova, 2006; Dong *et al.*, 2008; Mariana *et al.*, 2011). The largest genus within the *Trombiculidae*, *Leptotrombidium*, comprises of 178 species of which more than half are associated with rodents (Shatrov and Kudryashova, 2006). Numerous studies have revealed that a single chigger species is capable of infesting multiple small mammal hosts from different orders (rodentia, chiroptera and insectivora) (Daniel, 1961; Traub & Wisseman, 1974; Goff, 1979, 1982; Dong *et al.*, 2008; Mariana *et al.*, 2011). As an example *L. deliense* was recovered from the red spiny rat (*Maxomys surifer*), whitehead's spiny rat (*Maxomys whiteheadi*), plantain squirrel (*Callosciurus notatu*) and the common tree-shrew (*Tupaia glis*) during a study that investigated the acarine ectoparasite diversity of the Panti Forest Reserve in Johore, Malaysia (Mariana *et al.*, 2011). However, it appears that chigger species infesting bats are more host specific (Shatrov and Kudryashova, 2006; Mariana *et al.*, 2011). Seven genera of chiggers occurring on Papua New Guinea (*Whartonia*, *Bishoplinia*, *Chiroptella*, *Riedlinia*, *Rudnicula*, *Sasatrombicula* and *Trombicula*) were exclusively found on bat hosts (Goff, 1982). *Trombigastia* species occurring in Malaysia were exclusively found on Diadem leaf-nosed bats (*Hipposideros diadema*) (Mariana *et al.*, 2011). However, host switching is a common phenomenon amongst chiggers, which is in part facilitated by their low host specificity. Examples include; members of the genus *Whartonia*, commonly associated with bats, were also recovered from rodents (Shatrov and Kudryashova, 2006). Another host specific chigger *Eutrombicula goeldii* ordinarily associated with frogs has been collected from lizards, small mammals, birds and tapirs (Brennan & Reed, 1975).

### **Chigger diversity within a host population**

There are multiple factors that determine the species diversity and abundance of ectoparasites within a given host population and on a host individual. These factors can be divided into host-, environmental- and parasite-related factors (Morand *et al.*, 2006). From

the literature it seems that the following are important host factors that determine susceptibility to parasites: sex, age, reproductive state, immune system, sociality and behaviour (Hanley *et al.*, 1995; Poulin, 1996, 2007; Zuk, 1996; Zuk & McKean, 1996; Schalk & Forbes, 1997; Krasnov *et al.*, 2010; Matthee *et al.*, 2010; Froeschke *et al.*, 2013; Froeschke & Matthee, 2014). Within a population of hosts, some individuals are more susceptible to infestation by parasites than others (Krasnov *et al.*, 2006; Shatrov and Kudryashova, 2006; Poulin, 2007, 2013). For example, larger hosts are capable of harbouring not only higher parasite loads, but also more diverse assemblages of parasites than smaller hosts (Noble *et al.*, 1963; Dobs & Roberts, 1995; Lo *et al.*, 1998; Poulin, 2007; van der Mescht, 2012). Comparing the body of a host organism to that of an island, larger islands have a wider variety of niches which can accommodate a wider diversity of species. Furthermore, the size of a host can serve as a proxy for its age (Poulin, 2007). Older hosts tend to harbour diverse parasite assemblages and this is due to length of exposure to parasites. Younger hosts have not been exposed for a similar amount of time to parasites within the environment and have thus not had equal time to accumulate different parasites (Lo *et al.*, 1998; Shatrov and Kudryashova, 2006; Poulin, 2007; van der Mescht, 2012). The feeding behaviour of the host also seems to play a role in parasite infestations. A study conducted by Dietsch (2008) revealed that the foraging behaviour of birds can significantly influence the extent of infestation by chiggers. There was a strong correlation between infestation rate and feeding height, with a decrease in chigger abundance with an increase in foraging height off the ground. From this it can be suggested that hosts that are ground dwelling or have nests at ground level have a higher chance of infestation than host species that are arboreal or rock inhabitants. Furthermore the study also found that foraging behaviour is not the only factor that influences infestation by chiggers. Other factors that play an important role in infestation probability include: length of exposure to an environment with active chigger larvae, behaviour of the host (e.g. grooming, activity periods, hibernation) and the ecology and preferred habitat of the chigger species (Dietsch, 2008). Limited information is available on sex-biased parasitism with specific reference to chiggers. Studies that have performed total counts of chiggers on hosts did not find any significant difference in the chigger load of male versus female hosts (Dietsch, 2008; Dong *et al.*, 2008).

When considering the habitat of parasites, it is important not only to take into account the on-host (biotic) environment but also the off-host (abiotic) environment (Krasnov *et al.*, 2004). The composition of parasite communities can be influenced by both environments through host environmental filtering and abiotic environmental filtering (Krasnov *et al.*, 2014). Abiotic environmental filtering suggests that parasites are filtered by temperature, humidity, rainfall and soil composition, whereas host environmental filtering could refer to some hosts being more inhabitable than other or that the presence of a specific host determines whether the host specialist parasite will be able to survive (Traub & Wisseman, 1974; Sutherst, 2001; Krasnov *et al.*, 2002; Krasnov *et al.*, 2004; Shatrov and Kudryashova, 2006; van der Mescht, 2012; Berkhout *et al.*, 2014). The importance of the abiotic environmental filtering is still unknown (Krasnov *et al.*, 2014). When considering the strong association between chiggers and their abiotic environment, it could be suggested that abiotic environmental filtering will play a major role in the species composition of chigger communities.

### **On-host habitat selection**

Hosts can be regarded as habitats for parasites and some areas of the host's body are more inhabitable than others. Various factors can influence the suitability of an attachment site on the host's body. Host-defence mechanisms are grooming and immune responses against specific parasites. Morphological adaptations of chiggers to overcome host defences are cheliceral blade morphology. Competition between con-specific parasites and interspecies competition also plays a role. Host grooming can influence the area where parasites attach on the host's body and species co-occurrence often take place in these sites (Goff, 1979; 1982). From the literature it seems that chiggers prefer certain areas or parasitopes on the host's body. Dong *et al.* (2008) suggests that the attachment site on the host might be correlated to the thickness of the skin in that specific area of the body and that chiggers often occur in areas where the skin is the thinnest. In general chiggers are aggregated in their distribution on the host. On small mammals, specifically rodent and insectivores, chigger clusters have readily been observed in the following parasitopes: ear lobes and fringe, intranasal area, anal area and the scrotum of males (Mohr 1947, 1956; Nadchatram,

1970; Traub & Wisseman, 1974; Goff, 1979; Mariana *et al.*, 2011). *Ascoschoengastia* and *Gahrliopia* species have been recorded in the intranasal parasitope of small mammals in Papua New Guinea (Goff, 1982). It is suggested that some lizard species have evolved specific structures, known as mite pockets where chiggers and other parasitic mites can attach (Arnold, 1986; Klukowski, 2004). These pockets are located in the neck, axilla, groin and postfemoral regions (Klukowski, 2004). These structures may have evolved to limit damage inflicted by ectoparasites (Arnold, 1986), however another possibility is that mites attach in these particular regions because the skin is thinnest in these regions (Klukowski, 2004). Interestingly, chiggers were more frequently recovered from lizards with mite pockets than those without (Arnold, 1986). Chiggers also occur on large mammals and have been recorded on alpacas (*Vicugna pacos*) in Peru (Gomez-Puerta *et al.*, 2012) and Florida black bears (*Ursus americanus floridanus*) in United States of America (Cunningham *et al.*, 2001). Infestations on alpacas were restricted to the head area, whereas chiggers occurred in various parasitopes on black bears, namely the ventral abdomen and thorax, lower regions of the abdomen and on the proximal medial aspect of the extremities. From these studies it does appear that chigger communities are structures in that certain body regions are selected above others. This pattern is not uncommon and has been recorded in other ecto- and endoparasite taxa (Bush and Holmes 1986; Stock & Holmes, 1987; Cohen *et al.*, 1991; Matthee *et al.*, 1997; Behnke *et al.*, 2001; Shatrov and Kudryashova, 2006; Hillegas *et al.*, 2008).

### **Vector competence and disease association**

Certain members of the *Trombiculidae* are known vectors of diseases of medical and veterinary importance (Traub & Wisseman, 1974). Studies investigating the vector competence of chiggers first became of great importance during World War II, when thousands of soldiers became ill with acute fever of unknown origin (Mohr 1947, 1956; Bavaro *et al.*, 2005). The epidemiology of the disease was investigated and it became apparent that chiggers were vectors of the bacteria, *Orientia tsutsugamushi* (Hayashi, 1920; Tamura, *et al.*, 1995) the causative agent of scrub typhus or chigger-borne rickettsiosis (Traub & Wisseman, 1974; Xing-Yuan *et al.*, 2007; Diaz, 2010; Makajan, 2012). Members of

the genus *Leptotrombidium* (*L. scutellare* and *L. deliense*) have been identified as important vectors of *O. tsutsugamushi* (Traub & Wisseman, 1974; Xing-Yuan *et al.*, 2007; Diaz, 2010; Makajan, 2012). Scrub typhus is endemic in Japan, Eastern Russia, Australia, Pakistan and Afghanistan (Watt & Parola, 2006; Kuo *et al.*, 2011). The bacteria is maintained within a trombiculid population through trans-stadial and trans-ovarian transmission (Traub & Wisseman, 1974). As chiggers only feed once, horizontal transmission of the bacteria does not occur (Mohr, 1947; Traub & Wisseman, 1974; Goff, 1982; Bavaro *et al.*, 2005; Dong *et al.*, 2008). Scrub typhus can be successfully treated with broad spectrum antibiotics, such as tetracycline however, complications can occur in individuals with a compromised immune systems (Arlian, 2009; Krantz & Walter, 2009; Diaz, 2010; Kuo *et al.*, 2011).

In addition to the above mentioned disease, certain chigger species are also associated with dermatitis or trombiculosis in humans and animals. The genera *Eutrombicula* and *Schoengastia*, include numerous dermatitis causing chigger species (Goff, 1982). Trombiculosis is characterized by itchy lesions that may become inflamed or infected (Krantz & Walter, 2009). When chigger infestations occur in livestock of economic importance, it can be devastating to the local economy. In 2011 the first infestation of alpacas by chiggers was reported in Peru and resulted in dermatitis, edema, irritation of the infested area and hair loss (Gomez-Puerta *et al.*, 2012). It has been suggested by Nadchatram (1970) that the coloration of the larva's idiosoma could potentially reflect its vector capabilities. Larvae that are orange to red in colour seem to be associated with dermatitis, whilst white to pale yellow larvae may be potential vectors of rickettsiosis (Nadchatram, 1970). As yet, few studies have tested this theory (Goff *et al.*, 1982).

Three case studies have been documented in SA where chigger mites attacked livestock, domestic animals and humans. In the late 1980s chigger infestations were documented in sheep in Bloemfontein, Free State Province. Infected animals displayed orf-like lesions and dermatitis (Heyne *et al.*, 2001). More recently in the same area a localized incidence of dermatitis was reported from a single residence. The household's pet dog and small children were infested with chiggers (Heyne *et al.*, 2001). It is not yet known whether any chigger

species occurring in SA are vectors of rickettsiosis or any other disease. It is postulated that the high diversity of available host species in SA is the main reason for the low incidences of rickettsiosis or scrub-itch in humans (Lawrence, 1949). A study by Palmeirim *et al.* (2014) found that disease virulence drastically increased in areas with diminished biodiversity. Another possibility for the low incidence of scrub typhus in SA could be misdiagnoses due to lack of studies conducted on the medical importance of chiggers in the country (Lawrence, 1949).

### **Studies conducted on Trombiculid mites in South Africa**

South Africa is known for its high level of plant and animal diversity (Cowling *et al.*, 2003; Skinner & Chimimba, 2009). The same pattern is evident in the ectoparasites of vertebrate taxa (Lawrence, 1949; Zumpt, 1965; Ledger, 1980; Segerman, 1995; Matthee *et al.*, 2007, 2010). Within South Africa ectoparasites associated with mammalian hosts have received variable attention with a strong focus on ticks and more recently fleas, lice and mesostigmatoid mites (Lawrence, 1949; Zumpt, 1965; Ledger, 1980; Segerman, 1995; Shatrov and Kudryashova, 2006; Matthee *et al.*, 2007, 2010; van der Mescht, 2012; Archer *et al.*, 2014; Fagir *et al.*, 2014). It is estimated that there are at least 60 chigger species endemic to the country (Lawrence, 1949, 1951; Zumpt, 1965; Goff, 1990). This may be a gross underestimate when compared to the richness recovered from small mammals (rodents and insectivores) alone in other parts of the world (Mohr, 1956; Daniel, 1961; Goff, 1974, 1982; Whitaker & Loomis, 1978; Mariana *et al.*, 2011). For example, 48 species were documented on a single rodent species (*Apodemus chevrieri*) in southwest China (Xing-Yuan *et al.*, 2007) while another study in China identified 109 species from 21 small mammal species (Dong *et al.* 2008). To date, most studies on chiggers in SA focused on reptilian and amphibian hosts and limited attention has been given to small mammals (Lawrence, 1949; Zumpt, 1965). Based on the data available there are eleven chigger species, representing eight genera that have been described from small mammals including bats in SA (Lawrence, 1949; Zumpt, 1961). Given that rodents and insectivores are important hosts to chiggers, it can be predicted that novel species will be recorded in future studies. In addition, most studies have been taxonomic and descriptive with little attention given to ecological aspects

(distribution, host preference, parasite-host-relationship) that can influence chigger populations and communities on small mammals. More recently, mark-recapture studies have reported the occurrence of chiggers on small mammal hosts (Archer *et al.*, 2014; Fagiri *et al.*, 2014). However, the data is qualitative and no species identification was provided.

### **Rodent and insectivore hosts in South Africa**

Within SA, rodents and insectivores differ in terms of geographic range of occurrence, habitat preference, dietary requirements and social systems (Skinner & Chimimba, 2005; Schradin & Pillay, 2005, 2006). Geographic ranges include more localised occurrence to regional distributions (e.g. *Rhabdomys* spp. occur throughout the country, while *Macroscelides proboscideus* is restricted to the western region of sub-Saharan Africa, extending from Namibia to the Western Cape Province in South Cape). However, at a habitat level segregation is noticed in niche utilization between various small mammals for example the Namaqua rock mouse *Micaelamys namaquensis* and sengis (*Macroscelides proboscideus* and *Elephantulus edwardii*) readily occur in rocky areas, whereas the four striped mouse, *Rhabdomys* spp., and vlei rat, *Otomys irroratus*, frequent moist grassy areas (Skinner & Chimimba, 2005). Similarly activity periods vary temporally between the various species: *M. namaquensis* is nocturnal, while *Rhabdomys* spp. and *O. irroratus* are crepuscular and the greater red musk shrew, *Crocidura flavescens*, has alternating periods of rest and activity within a 24-hour cycle (Skinner & Chimimba, 2005). There is also diversity among the dietary requirements between the various small mammals. For example, *Rhabdomys* spp. and *M. namaquensis* are omnivorous, their diet consists out of seeds, plant material and to lesser extent insects, while *C. flavescens* is generally regarded as an insectivore but they do have cannibalistic and predatory tendencies (Skinner & Chimimba, 2005). *Otomys irroratus* is an apt nest builder, but not a burrower and will use burrows made by other rodents e.g. *M. namaquensis*. The nests of *O. irroratus* have distinct runways leading to the above ground saucer shaped nest. Nests and runways made by *O. irroratus* are often used by other small mammals such as *Rhabdomys* spp. and *C. flavescens*. *Rhabdomys* spp. and *C. flavescens* are ecologically tolerant species and thrive in both pristine and disturbed habitats (Skinner & Chimimba, 2005). Several rodent species (such as

*Rhabdomys* spp.) are often associated with agricultural, urban and peri-urban areas and is of economic importance (Skinner & Chimimba, 2005). These various small mammals also exhibit different social structures. The most complex of which is that of *Rhabdomys* spp. This species is socially plastic and adapts its social system in response to food availability and precipitation. *Sengis* and *O. irroratus* are regarded as solitary animals, however it is not uncommon for them to form pairs or family groups, whereas *C. flavescens* are predominantly solitary and only form groups during mating season (Skinner & Chimimba, 2005). Above mentioned species all occur in the Western Cape Province, some more widespread than others.

### **Cape Floristic Region**

The Cape Floristic Region (CFR), in the Western Cape Province of SA, is one of six floristic kingdoms globally and is renowned for its high plant endemism (Cowling *et al.*, 1998; Heelman *et al.*, 2008). The vegetation of the area is known as Fynbos, which comprise a diversity of low scrub-like plant species (Cowling *et al.*, 1998). The area also contains rich assemblages of vertebrate (Skinner & Chimimba, 2005) and invertebrate taxa (Giliomee, 2003). The overarching climate of the region can be characterized as Mediterranean, with hot summers (mean temperature 25 °C) and cold winters (mean temperature 10 °C) (Heelman *et al.*, 2008). The region also receives most of its rain fall during the cold winter months (average annual rainfall 500 - 800 mm) (Cowling *et al.*, 1998; Heelman *et al.*, 2008).

### **The aims of the current study were:**

- 1) To establish temporal variation in occurrence of chiggers associated with a broad niche rodent species within the Cape Floristic Region of South Africa
- 2) Record chigger diversity and abundance on co-occurring rodent and insectivore species at two localities in the Cape Floristic Region
- 3) Provide data on host association and distribution of chigger species associated with rodents and insectivores in South Africa
- 4) Develop taxonomic species descriptions of newly recorded chigger species

- 5) Determine infracommunity dynamics of chigger species on a broad niche rodent species (*Rhabdomys pumilio*).

## Chapter 2

### ***A proposed alternative method for the removal, clearing and mounting of chigger mites (Trombiculidae)***

#### **Introduction**

Trombiculidae have a cosmopolitan distribution, occurring on all continents except for Antarctica (Daniel, 1961; Traub & Wisseman, 1974; Krantz & Walter, 2009). The group is highly specious with more than 3000 described species, with the vast majority known from only the larval stage or “chigger.” The life cycle consists of seven distinct stages: egg, prelarva (deutovum), larva (chigger), protonymph, deutonymph, tritonymph and adult. Of these only the larval stage is parasitic, the deutonymph and adult are active predators of soil-dwelling arthropods and their eggs within the soil, with the remaining four stages being inactive. Chiggers have a wide host range which includes; mammals, lizards, amphibians, birds and insects (Wharton & Fuller, 1952; Daniel, 1961; Traub & Wisseman, 1974; Krantz & Walter, 2009). For a given species of chigger, it is not unusual for the host range to include a number of species and frequently this will cross both family and ordinal lines (Traun & Wisseman, 1974; Goff, 1982; Shatrov and Kudryashova, 2006). Chiggers are also known to exploit multiple attachment sites or parasitopes on the host; however a single parasitope is usually preferred (Goff, 1979, 1982). For a given genus, it is not unusual for species groups to exist, each occupying a specific parasitope. For example in the genera *Ascoschoengastia* and *Gahrliopia* in New Guinea, two species groups have been reported, one from the exposed ear parasitope and the other exclusively in the intranasal parasitope of small mammals (Goff, 1982). In general, chiggers form clusters in specific regions on the host’s body. For example, on lizards, chiggers frequently occupy mite pockets and skin folds (Klukowski, 2004). On small mammals, specifically rodent and insectivores, chigger clusters have been associated with; ear lobes and fringe, intranasal area, anal area and the scrotum of males (Mohr 1947, 1956; Nadchatram, 1970; Traub & Wisseman, 1974; Goff, 1979; Mariana *et al.*, 2011). Chiggers also infest larger mammalian hosts. A study by Cunningham, Phillips, & Welbourn (2001) investigated chigger infestations on Florida black bears in

Florida (United States of America) the study found that clusters of chiggers were distributed primarily over the ventral abdomen and thorax, lower regions of the abdomen and on the proximal medial aspect of the extremities (Cunningham *et al.*, 2001). Dong *et al.* (2008) suggested that the attachment site on the host might be correlated to the thickness of the skin in that specific area of the body and that chiggers often occur in areas where the skin is the thinnest. It has also been suggested by Goff (1979) that there is a correlation between the level of exposure associated with a particular parasitope and the length and structure of the cheliceral blade. Certain parasitopes are more exposed to host-grooming activities than others. When the attachment area is highly exposed, the cheliceral blade will be longer and more serrated (Goff, 1979, 1982). Given this, it is predicted that species occurring in the intranasal cavity will have poorly developed cheliceral blades, with regards to length and serratedness, while species occurring on the perianal parasitope will have well defined cheliceral blades (Goff, 1979, 1982). However, very few studies have tested this theory to date.

It is well documented in the literature that a single host is capable of harbouring multiple species of chiggers, for example thirteen chigger species were collected from the variable spiny rat (*Rattus ruber*) in Papua New Guinea (Goff, 1979, 1982). However on average only four chigger species exploit a given host at one point in time (Goff, 1979; 1982). Co-habitation of a specific parasitope by multiple chiggers has also been widely documented (Mohr, 1956; Goff, 1979; 1982). The intranasal parasitope of the rat, *R. niobe* was recorded to be primarily inhabited by *Ascoschoengastia melanesiana*, however co-inhabitation with two other *Ascoschoengastia* species, *A. accola* and *A. goilala* have been documented (Goff, 1982). Interestingly, the frequency of co-occurrence was not equal for all parasitopes. The intranasal parasitope was less frequently occupied by more than one species of chigger, whereas the aural and perianal parasitopes were exploited by two or more chigger species more readily. The parasitope preference of a given species may differ between different host taxa for example; *Leptotrombidium deliense* is associated with the ears of rats, the belly and inguinal regions of tree-shrews (*Tupaia belangeri*) and the eyelids and eyebrows of monkeys (*Macacus*). Parasitope preference seems to remain constant within each family of hosts, which is primarily determined by the grooming activity of the host (Goff, 1979).

Chiggers are soft bodied, due to lack of sclerotized plates on the idiosoma, and can easily be damaged. This makes it difficult to remove specimens with forceps, as one would do when collecting fleas and other ectoparasites. Another factor that makes the removal of chiggers increasingly difficult is that they attach to the host by inserting their cheliceral blades into the integument of the host and an accessory attachment structure, the stylosome, is produced (Krantz & Walter, 2009). They remain attached to the host for hours after the host has died unlike fleas and ticks that disperse from the host perimortem and shortly after death (Mohr, 1956). Picking them off with forceps usually result in the chelicerae breaking off in the integument of the host which decreases the probability of taxonomic identification. Placing the dead hosts into a chamber with chloroform for a period of time may serve to loosen the attachment and improve results of manual picking (L. Goff, personal communication, 2014). Techniques for the overall collection, clearing and mounting of mites in general are described in *A Manual of Acarology* (Krantz & Walter, 2009). Currently there are only a few existing methods that have been adapted for the removal and mounting of chiggers. These methods include mechanical removal of chiggers with forceps and dissection needles, brushing, washing and boiling the host and collecting chiggers from the broth. The abovementioned methods were not suitable to achieve the aims of the current study, which were to record chigger diversity and abundances associated with rodent and insectivore species in SA and to record preferred attachment site on the host's body. A method for the removal, clearing and mounting of specimens was therefore developed. The current guidelines allows for; total counts per host as well as per parasitope on the host, minimal damage to specimens and by adding a clearing step to the method it improves optical visualisation of specimens. It was also found that the clearing step decreased the time spent separating chiggers from residual hair and tissue. The guidelines described for the removal of chiggers from the host body is newly developed, however the clearing and mounting of specimens is adapted from Krantz & Walter (2009).

### **Current methods used to remove chiggers from the host body**

Various methods have been used to remove chiggers from the body of the host. In particular during an in-depth investigation of the bionomics of chiggers in the Slovak Carpathians, Daniel (1961) collected chiggers from small mammals with the use of forceps and a dissection needle. This method could enable total counts per host and per parasitope

on the host. It is however time-consuming and may inflict damage to chigger specimens which decreases the probability of positive identification of specimens and render them unuseable for taxonomic identification.

Alternatively in *A Manual of Acarology* (Krantz & Walter, 2009), it is proposed that the host is boiled in water where after chiggers can be collected from the broth with a sieve. This method is less time-consuming, but there are multiple shortcomings to this method; total counts cannot be obtained, chiggers tend to become transparent when exposed to heat increasing the possibility of losing them and lastly as they are soft bodied this method could compromise the integrity of the specimens.

Alternatively it is also possible to collect chiggers by placing the dead host on a screen suspended over a dish of water. The chiggers will detach from the host and fall onto the water where they remain on the top of the water held in place by surface tension. The individual specimens can then be collected using an artist's brush and transferred into 70-80% ethanol (Krantz & Walter, 2009; L. Goff, personal communication, 2014). This method is also time-consuming as chiggers tend to stay attached to the host for an extended time after death, unlike other ectoparasites. Furthermore this method is not suitable for determining attachment sites.

Daniel *et al.* (2010) used a three step approach. The animal was first visually examined for chiggers. Thereafter the host was brushed and loose chiggers were removed by using a dissection needle. Finally the animal was washed with water and detergent and chiggers were collected from the water. This technique is the most thorough of the three existing methods, but time-consuming. In addition chiggers are minute and visual inspection of a host with the naked eye will not be sufficient to locate all chiggers on the host. The brushing technique is only effective in removing fully engorged larvae, but is ineffective in the removal of unengorged chiggers that are still firmly attached to the host. Furthermore this method could damage specimens and by washing the host it is likely that some chiggers will be lost in the water. Similar to the method by Krantz & Walter (2009) this method also does not allow for differentiation between parasitopes on the host.

### **Updated guidelines for the removal of chiggers from the host body**

In the proposed method the body of the host was divided into thirteen parasitopes before commencing with the removal of the chiggers (Figure 2.1). The bodies of the hosts were thoroughly examined under a stereomicroscope (Leica MZ12), by working through the hair with a fine-point forcep (size 5). Chiggers were then removed through removal of the superficial skin layers with a scalpel (size 3). This method resulted in minimal damage to specimens. It also ensured that the cheliceral blade and palpi remained undamaged, which is important for taxonomic identification. When mites were located on the ear fringe or in the ear of the host, the entire ear or just the ear tissue containing the chiggers was removed with surgical scissors. Collected specimens were stored in 1 ml plastic tubes containing 70% ethanol. The current method facilitates the removal of several chiggers at one time. It also enabled total counts and the collection and separation of chiggers from specified parasitopes on the host body.

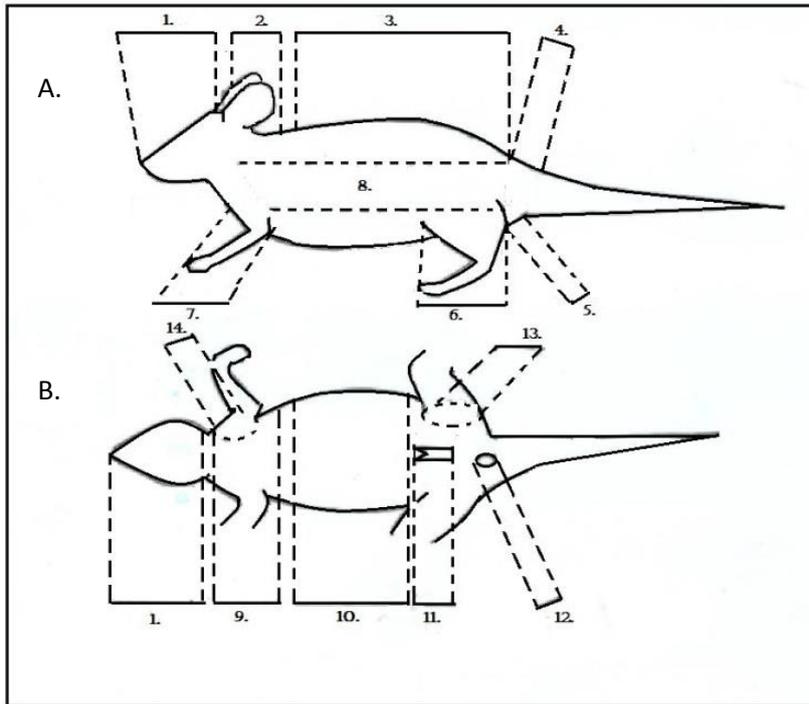


Figure 2.1: Body regions of rodent host (sketch A is the side profile and sketch B is the ventral view of the rodent body) 1: Head, 2: Ear, 3: Back, 4: Tail area, 5 & 12: Anal Area, 6: Hind leg, 7: Front leg, 8: Sides, 9: Chest, 10: Stomach, 11: Genital Area, 13: Fold of hind leg, 14: Fold of front leg.

### Method for clearing of chiggers

The above mentioned method for the removal of chiggers from the host resulted in host tissue and hair being collected with the chiggers. To separate the chiggers from the tissue and hair a clearing step was added. A 10% potassium hydroxide (KOH) solution was used to remove residual tissue and hair fragments. The chiggers were placed in glass embryo containers and submerged in the KOH solution. The specimens were left in the solution for twelve hours or until the tissue was dissolved sufficiently. To neutralize the KOH a 10% acetic acid ( $\text{CH}_3\text{COOH}$ ) solution was added to the solution in the embryo container by using a plastic pipette. After a couple of minutes the pH of the solution was determined using pH indicator paper where after the specimens were removed from the fluid with a plastic pipette and transferred to a plastic petri dish from where the chiggers were counted by making use of a stereomicroscope (Leica MZ12).

### **Preparation of mounting media**

Polyvinyl alcohol (PVA) and Hoyer's medium are commonly used for mounting chiggers (Krantz & Walter, 2009). The current method used Hoyer's medium. Hoyer's medium was chosen due to ease with which remounting can be performed and availability of substances needed to produce the media. Hoyer's medium was prepared using standard techniques (Krantz & Walter, 2009). The medium was prepared in a fume extractor hood, due to toxicity of chloral hydrate. Thirty grams of gum Arabic or gum acacia was added to distilled water and left to soak for 24 hours. After 24 hours 200 grams of chloral hydrate ( $C_2H_3Cl_3O_2$ ) was added to the solution, to prevent bacterial growth on the gum. The solution was left until all the solids were dissolved. Lastly 20 ml of glycerine was added and the mixture was stirred (Krantz & Walter, 2009).

### **Preparation of microscope slides and mounting of specimens**

Counted specimens were transferred to a glass embryo container and 10 ml Hoyer's medium was added. A drop of Hoyer's was placed on a glass microscope slide (size: 76 x 26 x 1 mm) with a small paint brush (prime art gold brush size RT). Chiggers were removed from the embryo container by making use of a handmade micro-spatula. Each specimen was placed in the middle of the drop of Hoyer's on the microscope slide. Specimens were adjusted with the micro-spatula or insect pinning needle. A cover slip (round with 10 mm radius) was placed over the chigger. A heat source (lighter) was held underneath the microscope slide, when the medium started to bubble the heat source was removed and the coverslip was firmly pressed down using forceps. The slides were placed on a hot plate (temperature set between 35 and 40 °C) for 48 hours. Once the slides were sufficiently dried the specimens were ring sealed with clear nail polish or Glyphtal. Slides were labelled on the frosted area with a prime art fine line marker (size 0.2 mm).

## **Chapter 3**

# ***Diversity, ecology and distribution of chiggers parasitizing small mammals in South Africa with a focus on the Cape Floristic Region and South Africa***

### **Introduction**

Parasites make up a large proportion of biodiversity and are omnipresent in the lives of vertebrate animals (Price, 1980). The distribution of parasites within a host population and community is non-random and parasite infestation levels and diversity are influenced by host-, environmental- and parasite-related factors (Nelson *et al.*, 1975; Price, 1980; Shatrov and Kudryashova, 2006; Poulin, 2007; Matthee *et al.*, 2007, 2010; Froeschke *et al.*, 2010; Krasnov *et al.*, 2010; van der Mescht 2012; Froeschke & Matthee, 2014). Several host factors have been shown to play a role in parasite infestation levels and include host density, identity (species), age, body size, sex and reproductive state (Poulin, 1996, 2007, 2013; Krasnov *et al.* 2010; Matthee *et al.*, 2010; Froeschke *et al.*, 2013; Froeschke & Matthee, 2014). More specifically, host density seems to be positively related to parasite infestation levels and species richness as free-living infective stages have a greater chance of coming into contact with hosts if the density is higher compared to lower (Anderson & May, 1978, 1991; May & Anderson, 1978; Morand & Poulin, 1998; Krasnov *et al.*, 2002; Stanko *et al.*, 2002; Altizer *et al.*, 2003). In addition, reproductively active male hosts often sustain higher parasite levels compared to reproductively active females or non-reproductive animals (Daniels & Belosevic, 1994; Zuk & McKean, 1996; Schalk & Forbes, 1997; Klein, 2000; Krasnov *et al.*, 2005; Shatrov and Kudryashova, 2006; Krasnov *et al.*, 2011). This pattern may be due to several factors that include poorer immune response due to circulating hormones (testosterone) (Billingham, 1986; Alexander & Stimson, 1988; Schuurs & Verheul, 1990; Poulin, 1996, 2007; Zuk, 1996; Zuk & McKean, 1996; Hanley *et al.*, 1995; Schalk & Forbes, 1997; Klein, 2000; Rolff, 2002; Schmid-Hempel, 2003; Khokhlova *et al.*, 2004), increased mobility (Mohr, 1961; Tinsley, 1989; Krasnov *et al.*, 2005; Poulin, 2007; Hillegass *et al.*, 2008; Boyer *et al.*, 2010) and reduced time spent on grooming and

allogrooming during the breeding season (Hart, 1991; Klein *et al.*, 1997; Klein & Nelson, 1999; Shatrov and Kudryashova, 2006; Hillegass *et al.*, 2008). However, this pattern is not uniform as studies have also recorded female-biased parasite infestations (Morales-Montor, 2004; Krasnov *et al.*, 2005; Shatrov and Kudryashova, 2006; Patterson *et al.*, 2008). The off-host environment can also influence parasite abundance (and that of their hosts) as free-living stages are more susceptible to adverse environmental conditions that can reduce their survival (Traub & Wisseman, 1974; Sutherst, 2001; Krasnov *et al.*, 2002; Krasnov *et al.*, 2004; Shatrov and Kudryashova, 2006; van der Mescht, 2012; Berkhout *et al.*, 2014). However, the importance of environmental conditions is species-specific and depends on the life-history traits of the parasite taxa that occur on the host (Krasnov *et al.*, 2002; Froeschke *et al.*, 2013; Froeschke & Matthee, 2014). In particular, several studies have shown that the effect of environmental conditions will be more pronounced for parasite taxa that have one or more free-living stages and/or that spend a large proportion of their life cycle off the host (Poulin, 1996; Merino & Potti, 1996; Krasnov *et al.*, 2002; Shatrov and Kudryashova, 2006; Froeschke & Matthee, 2014). Linked to this is the effect of locality on parasite infestations, which is mainly a consequence of environmental conditions that vary spatially and with different land-use practices (Patz *et al.*, 2000; Sutherst, 2001; Bradley & Altizer, 2002; McKinney, 2002; van der Mescht *et al.*, 2013; Froeschke *et al.*, 2013; Froeschke & Matthee, 2014). From this it is evident that there are several factors that can play a role in shaping the species richness and abundances of parasites within a host community.

Mites within the family Trombiculidae are a highly diverse group of arthropods consisting of 3 000 known and described species (Dong *et al.*, 2008; Krantz & Walter, 2009). The vast majority of trombiculid species are known exclusively from the parasitic larval stage (also known as “chigger”) due to difficulties associated with collecting free-living stages (nymphs and adults) in the environment as they are predominantly soil-dwelling (Daniel, 1961; Shatrov and Kudryashova, 2006; Krantz & Walter, 2009). Evidence suggests that chiggers are generalist parasites as the larval stage is capable of parasitizing multiple vertebrate and invertebrate taxa within a landscape (Mohr, 1947, 1956; Lawrence, 1949; Daniel, 1961; Whitaker & Loomis, 1978; Goff, 1979; Dong *et al.*, 2009; Mariana *et al.*, 2011). Moreover, a single host species can harbour multiple chigger species at one time (Goff, 1979). A study by Goff (1979) in Papua New Guinea found that a single chigger species is capable of

exploiting an average of five different host species. It is therefore suggested that chiggers select their hosts through ecological fitting, in other words when the niche utilization of the host and that of the chigger overlap (Brooks *et al.*, 2006; Agosta & Klemens, 2008), rather than phylogenetic relatedness. At a landscape level chiggers exhibit a heterogeneous distribution, forming aggregations in specific patches termed “mite islands” or “mite foci” (Lawrence, 1949; Traub & Wisseman, 1974; Goff *et al.*, 1982; Walter & Proctor, 2004; Scholer *et al.*, 2006). Mite islands or foci are areas within the environment that have specific abiotic- (microclimate, leaf litter and soil composition) and biotic (vegetation structure and cover) characteristics that are favourable for the survival of the larval and post-larval stages (Traub & Wisseman, 1974; Goff, 1982; Clopton & Gold, 1993; Scholer *et al.*, 2006; Diaz, 2010). The location of mite islands within the landscape is determined by species-specific habitat requirements and is associated with various vegetation types. More specifically, mite islands have also been recorded in vegetation transition zones or ecotones, mainly due to the heterogeneity of the landscape and high animal movement and abundance (Mohr, 1947, 1956; Traub & Wisseman, 1974; Goff, 1979; Clopton & Gold, 1993). The climatic requirements of chiggers are species-specific and as a result the occurrence of chiggers on the host varies temporally (Sasa, 1957; Daniel, 1961; Traub & Wisseman, 1974). In the tropics reproduction is incessant and chiggers are prevalent year round (Sasa, 1957; Traub & Wisseman, 1974; Goff, 1982) while only one or two generations are produced annually in the temperate regions (Goff, 1982).

Within SA several studies have investigated factors that shape parasite communities of small mammals, however, most of the studies were performed on ticks, fleas, mesostigmatid mites, lice and helminths (Zumpt, 1961; Matthee *et al.*, 2007, 2010; Viljoen *et al.*, 2011; van der Mescht *et al.*, 2013; Du Toit *et al.*, 2013; Fagir *et al.*, 2014). In addition, several studies have highlighted species- or taxon-specific difference in temporal variation in parasites infestation (Segerman 1995; Horak & Boomker, 1998; Horak *et al.*, 1998; Walker, Keirans & Horak 2000; Matthee *et al.*, 2007; Lutermann, Medger & Horak, 2012; Archer *et al.*, 2014). For example in the Western Cape Province (winter rain fall region of SA) a louse (*Polyplax arvicantis*) and mesostigmatid mite (*Androlaelaps fahrenheitzi*) were most abundant on the rodent, *Rhabdomys pumilio* during the wet-cold winter months (June

and September), while two hard ticks species (*Haemaphysalis elliptica* and *Hyalomma truncatum*) were abundant during the warm-dry spring and summer months (December and February) (Matthee *et al.*, 2007). More importantly, although host-species lists are quite well established for most of the macroparasitic ectoparasites in SA (Zumpt, 1961; Theiler, 1962; Ledger, 1980; Segerman, 1995; Walker, Keirans & Horak, 2000) there are currently only detailed distribution maps available for ticks and fleas (Howell, Walker & Nevill, 1978; Segerman, 1995; Walker *et al.*, 2000). Studies on chiggers associated with vertebrate hosts in SA are sparse (Lawrence, 1949; Zumpt, 1961). The most recent taxonomic study was conducted in the late 1990s (Goff, 1990) and there is a strong bias in the literature towards reptilian and amphibian hosts (Lawrence, 1949, Zumpt, 1961). It is estimated that 11 chigger species occur on small mammals in SA (Lawrence, 1949; Zumpt, 1961). As yet limited studies have been conducted on the ecology and distribution of chiggers associated with small mammal hosts in the country and sub-region. Recent studies on the parasite diversity of ectoparasites of the common mole-rat (*Cryptomys hottentotus hottentotus*) and the Namaqua rock mouse (*Micaelamys namaquensis*) recorded the presence of chiggers however the data were only qualitative and lack species identification (Archer *et al.*, 2014; Fagir *et al.*, 2014).

The present study aims to address this gap by studying the role of host- and environmental factors on the diversity and distribution of chigger species associated with rodent and insectivore species in the Western Cape Province. More specifically, the aims of this study were, firstly to establish whether there is temporal variation in occurrence of chiggers on a generalist rodent species in the winter rainfall region of South Africa. Secondly, to determine if chigger abundance differed between geographic localities, host gender, reproductive state and body size on small mammals trapped at two localities in the Cape Floristic Region. Lastly, to record the distribution and host species association of chiggers associated with small mammals across South Africa.

## Materials and methods

### *Study design*

In the case of the temporal study, adult *R. pumilio* individuals were trapped every three months (February, May, August and November) over a twelve month period in 2009 at a single locality (33° 88' 94.453" S, 18° 81' 89.8" E) in the Cape Floristic Region (CFR), Western Cape Province in South Africa (SA). Following on to this and as part of the ecology study juvenile and adult *R. pumilio* individuals were trapped in addition to adults of all co-occurring rodent and insectivore species during summer (January) of 2013 at two localities, Kanu and Mooiplaas (33° 92' 10.62" S, 18° 74' 59.8"E) in the CFR. One of the localities (Kanu) was the same locality that was used in the earlier temporal study. The month of January falls within the breeding season for *R. pumilio* in SA (Skinner & Chimimba, 2005) and most if not all of the adults that were trapped were reproductively active. Lastly, opportunistic sampling of chiggers was performed as part of a larger study on the parasite diversity of rodents and insectivores within SA. Adult individuals were mainly trapped during spring-summer (October to February) during 2009 - 2012 within five biomes (Fynbos, Succulent Karoo, Nama Karoo, Thicket and Grassland) across SA.

### *Animal handling and parasite removal*

The sampling protocol was standardised and used during each trap session. Sherman-type live traps were set for five to ten trap nights per locality. The traps were set out in trap lines (10 meters apart) and baited with a mixture of peanut butter and oats. Traps were checked twice daily. Following euthanasia each individual was placed in a labelled plastic bag (The study was approved by the Ethical Committee of Stellenbosch University Reference code: SU-ACUM11-00004(P)). Animals were euthanized in the lab with sodium pentobarbitone (200 mg/kg). The euthanized animals were frozen at -80 °C until parasite removal commenced. The frozen animals were thawed and carefully and systematically examined using fine point forceps and a stereomicroscope (Leica MZ12). Host information was recorded and included: sex, reproductive state, weight, total length, tail length and hind foot length. Removal and mounting of all chiggers from the host body followed standardized techniques. Specimens were removed with scalpel and forceps and stored in plastic tubes containing 70% ethanol until mounting commenced. Chiggers were mounted on microscope

slides with Hoyer's medium (as described in Chapter 2). Specimens were identified using taxonomic keys (Lawrence, 1949; Zumpt, 1961). Abundances were recorded for each species. The same protocol was followed during the opportunistic sampling study. However, instead of total counts a subsample of the chiggers that were visible with the eye were removed with forceps and placed into sampling tubes containing 70% alcohol. The same protocol for mounting and identification was followed as described above.

### *Data analyses*

The temporal data included in the current study were unpublished presence-absence data collected during 2009 as part of a separate project on the ectoparasites of *R. pumilio* within the CFR (van der Mescht, 2012). For the current study the temporal data was subjected to Chi-square analyses to determine if a significant difference in chigger occurrence exists in during four sampling periods.

Chigger species abundance data were analysed and the following calculated for all hosts species; mean abundance (mean number of parasites removed divided by total number of hosts sampled; M); variance of abundance (V) and prevalence (proportion of infested hosts divided by the total number of hosts sampled; P), for both localities. Host density (number of hosts divided by number of trapping nights) was calculated for both sampling localities within the CFR.

### *Chigger abundances on R. pumilio*

Number of chigger individuals and species was counted for each host individual and calculated for each population as well as for all trapped mammals. A Generalized Linear Model, using a normal distribution and log-link function, was used to test the effect of host sex, reproductive state, tail length (as a proxy for body size) and locality on total number of chiggers. An interaction between sex and reproductive state was also included in the model. Host body condition index (BCI) was calculated as a regression of weight against the tail length of the specific individual. However it was excluded from the model as it correlated with reproductive state ( $r = 0.58$ ).

### *Species composition on R. pumilio*

For all parasite community analyses, chigger mite prevalence and abundance data were arranged together with host individuals in two separate matrices, while locality, host gender and host reproductive states (reproductive vs non-reproductive) were defined as respective grouping factors. Similarities of parasite communities, based on prevalence and abundance data, between localities, host sex and reproductive states (reproductive vs non-reproductive) were calculated using the Bray-Curtis similarity index (Bray and Curtis, 1957). Chigger species abundance data was normalized and corrected for overdispersion by using fourth root. Differences of mite assemblages between localities, host sex and reproductive states were examined using non-metric multidimensional scaling (MDS) plots and Analyses of Similarity (ANOSIM) (Clarke, 1993). ANOSIM is a multivariate randomisation procedure that tests the null hypothesis that the average rank similarity between objects within a group is the same as the average rank similarity between objects between groups (Rees *et al.*, 2004). The output statistic, R, takes a value of 0 if there is no similarity of community structure attributable to a factor, and 1 if perfect similarity occurs. Similarity Percentage analyses (SIMPER) (Clarke, 1993) were applied to determine which mite species contributed most to the observed results. All parasite community analyses were performed using PRIMER v.6.1.5. for Windows (PRIMER LTD., 2006).

### *Geographic distribution of chiggers*

The data from the opportunistic sampling was only qualitative and therefore no statistical analyses could be conducted. Locality data that was recorded during opportunistic sampling was used to produce geographical distribution maps of chigger species recorded during the current study. Global Positioning Software data was used to construct maps in QGIS 2.4 (2014). In addition, historical locality data was used to produce a comparative map of sampling localities recorded during the current study and previous studies.

## Results

### *Temporal variation in chigger prevalence*

A total of 150 *R. pumilio* individuals were trapped during summer (34), autumn (31), winter (29) and spring (28) of 2009. The occurrence of chiggers on rodents differed significantly ( $p < 0.05$ ) between the four trap sessions. The highest occurrence was recorded in summer (98%) compared to low occurrence recorded during winter and a complete absence during autumn (Figure 3.1).

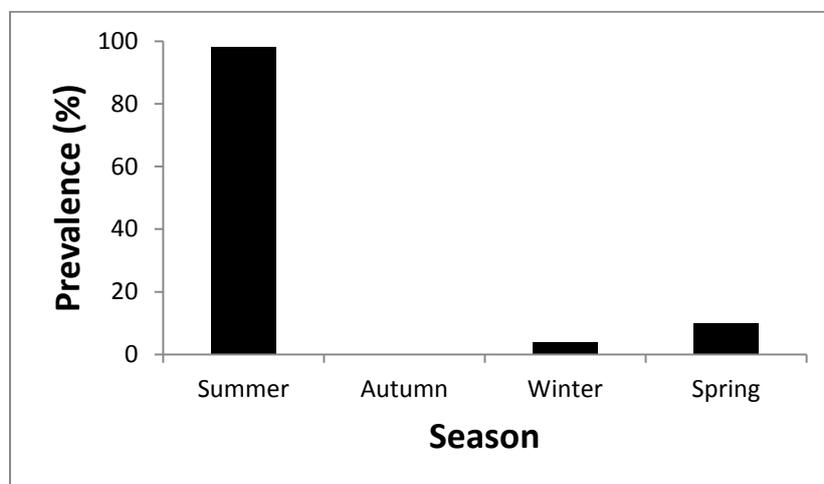


Figure 3.1: Temporal prevalence of chiggers recorded from *Rhabdomys pumilio* ( $n = 150$ ) in the Cape Floristic Region during 2009.

### *Diversity and infestation levels of chiggers on co-occurring small mammals in the CFR*

A total of 137 small mammals (rodents and insectivores) were trapped at two localities in the CFR during January 2013. The individuals that were trapped comprised of 108 *R. pumilio*, 10 *Otomys irroratus*, 12 *Micaelamys namaquensis*, six *Crocidura flavescens* and one *Macroscelides proboscideus*. A total of 31 893 chiggers were removed from all host species collected from the two localities. The total chigger abundance varied between host species (Table 3.1). The highest mean abundance of chiggers was recorded on *R. pumilio* (Kanu:  $289 \pm 262.2$ ; Mooiplaas:  $252 \pm 154.5$ ) compared to the second most abundant host *M.*

*namaquensis* (Kanu:  $34.4 \pm 25.1$ ; Mooiplaas:  $87 \pm 99.3$ ). Similarly, chigger prevalence differed between host species; *R. pumilio* displayed the highest infestation rates for most chigger species (Table 3.1).

A total of nine chigger species were collected from rodent and insectivore species at Kanu and Mooiplaas (Tables 3.1 & 3.2). *Leptotrombidium muridium* was the most prevalent species collected ( $p < 0.05$ ) at both sampling localities within the Fynbos biome. Furthermore *L. muridium* was removed from all hosts species sampled within the Fynbos biome, with the exception of *M. minutoides* (Table 1.3). In the case of the latter, no chigger species were recorded from this rodent. *Gahrliopia (Schöngastiella)* n. sp. was exclusively found on *C. flavescens* (Tables 3.1 & 3.2). Chigger species richness was higher at Kanu (nine species) compared to Mooiplaas (seven species). Species that were exclusive to Kanu were *Shunsennia* spp. and *Gahrliopia (Schöngastiella)* n. sp.

Table 3.1: Descriptive statistics of chigger species removed from various small mammal hosts captured in January in the Cape Floristic Region, South Africa.

Host species	Chigger species	Total	Mean $\pm$ SD	Prevalence (%)
<i>Rhabdomys pumilio</i> (n = 108)	<i>Leptotrombidium muridium</i>	11727	107.12 $\pm$ 94	94
	<i>Schoutedenichia</i> spp.	3311	31.02 $\pm$ 37.33	86
	<i>Neoschongastia</i> spp. A	460	4.32 $\pm$ 8.00	62
	<i>Ascoschongastia</i> spp.	37	0.34 $\pm$ 2.40	1.7
	<i>Schongastia</i> spp.	13	0.11 $\pm$ 0.39	5.5
	<i>Neoschongastia</i> spp. B	3	0.12 $\pm$ 1.07	1.2
	<i>Shunsennia</i> spp.	2	0.04 $\pm$ 0.27	0.9
<i>Otomys irroratus</i> (n = 10)	<i>Leptotrombidium muridium</i>	39	3.90 $\pm$ 9.60	20
	<i>Schongastia</i> spp.	11	1.10 $\pm$ 3.14	20
<i>Micaelamys namaquensis</i> (n = 12)	<i>Schoutedenichia</i> spp.	46	4.83 $\pm$ 6.67	58
	<i>Leptotrombidium muridium</i>	42	7.20 $\pm$ 17.37	50
<i>Crocidura flavescens</i> (n = 6)	<i>Leptotrombidium muridium</i>	28	*	16
	<i>Trombicula</i> spp.	11	*	16
	<i>Gahrliepia (Schöngastiella)</i> n. sp.	4	*	16
	<i>Schoutedenichia</i> spp.	2	*	16
<i>Macroscelides proboscideus</i> (n = 1)	<i>Leptotrombidium muridium</i>	39	*	100
	<i>Schongastia</i> spp.	4	*	100
	<i>Schoutedenichia</i> spp.	1	*	100

\*unable to calculate mean and SD

Table 3.2: Chigger species recorded from small mammal hosts in the Cape Floristic Region.

Family	Subfamily	Genus
<i>Leeuwenhoekiidae</i> (Womersley, 1945)	<i>Leeuwenhoekiinae</i> (Womersley, 1944)	<i>Shunsennia</i> spp.
<i>Trombiculidae</i> (Ewing, 1944)	<i>Trombiculinae</i> (Ewing, 1929)	<i>Ascoshongastia</i> spp.
		<i>Leptotrombidium muridium</i>
		<i>Schongastia</i> spp.
		<i>Trombicula</i> spp.
		<i>Schoutedenichia</i> spp.
		<i>Neoschongastia</i> spp. A
	<i>Neoschongastia</i> spp. B	
	<i>Gahrlepiinae</i> (Womersley, 1952)	<i>Gahrlepiea</i> n. sp.

*Role of locality and host factors on chigger abundance on R. pumilio in the CFR*

A total of 15 553 chiggers, representing seven species, was recorded from 108 *R. pumilio* from the two localities (Table 3.1). Overall, *L. muridium* was the most abundant species ( $107.12 \pm 94$ ) followed by an as yet unknown *Schoutedenichia* species ( $31.02 \pm 37.33$ ). The overall chigger abundance differed between localities, with a larger number recorded at Kanu ( $266.6 \pm 261.4$ ) compared to Mooiplaas ( $244.2 \pm 152.5$ ), though the difference was only marginally significant ( $p = 0.07$ ). Host sex and body size did not affect chigger abundance ( $p > 0.05$  in both cases). However, reproductively active host individuals were characterized by significantly higher chigger abundance ( $337.85 \pm 254.71$ ) compared to non-reproductively active individuals ( $179.66 \pm 148.03$ ) ( $p < 0.001$ ) (Figure 3.2).

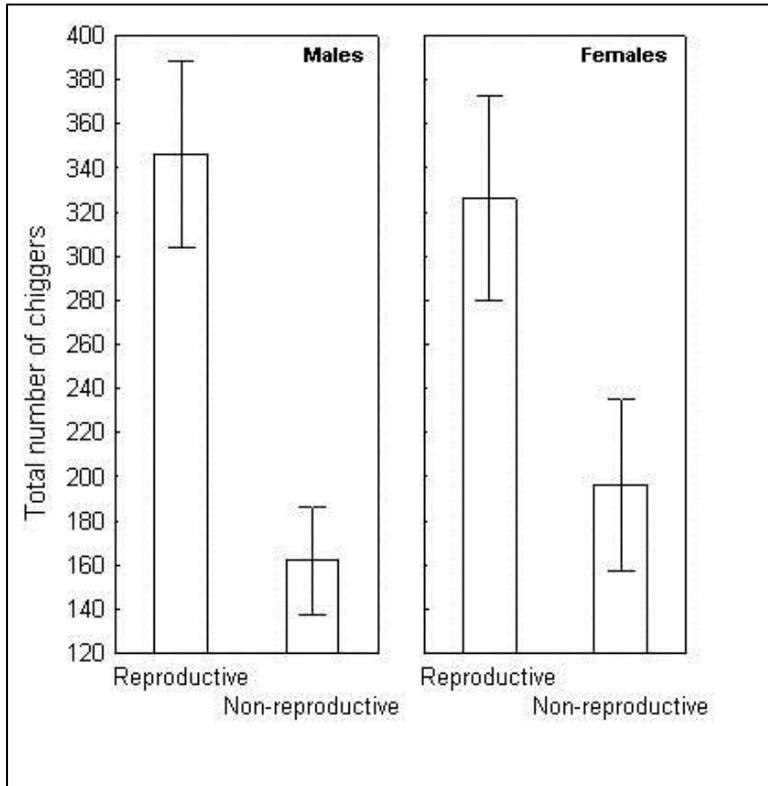


Figure 3.2: Mean abundance ( $\pm$  S.E.) of combined chigger species on reproductively active and non-reproductively active male and female *Rhabdomys pumilio* individuals (n = 108) in the Cape Floristic Region during (January 2013).

*Effect of locality, host sex and reproductive state on chigger species composition of R. pumilio in the CFR*

Visual investigation of MDS plots as well as ANOSIM analyses based on chigger mite prevalence and abundance data did not confirm significant different assemblages between the two localities or between host sex (in all cases  $R \leq 0.066$  and  $p > 0.05$ ). However, MDS plots described (Figure 3.3) and ANOSIM analyses confirmed significant differences in species compositions of chiggers based on host reproductive state (prevalence:  $R = 0.063$ ,  $p = 0.041$ ; abundance  $R = 0.101$ ,  $p = 0.005$ ). SIMPER analyses indicated that 3 species namely; *Neoschoengastia* spp. A (contribution based on prevalence: 39.74%; contribution based on abundance: 23.23%), *Shoutedenichia* spp. (contribution based on prevalence: 26.82%; contribution based on abundance: 33.90%) and *L. muridium* (contribution based on prevalence: 17.52%; contribution based on abundance: 35.78%) contributed most to the observed dissimilarities between reproductive state.

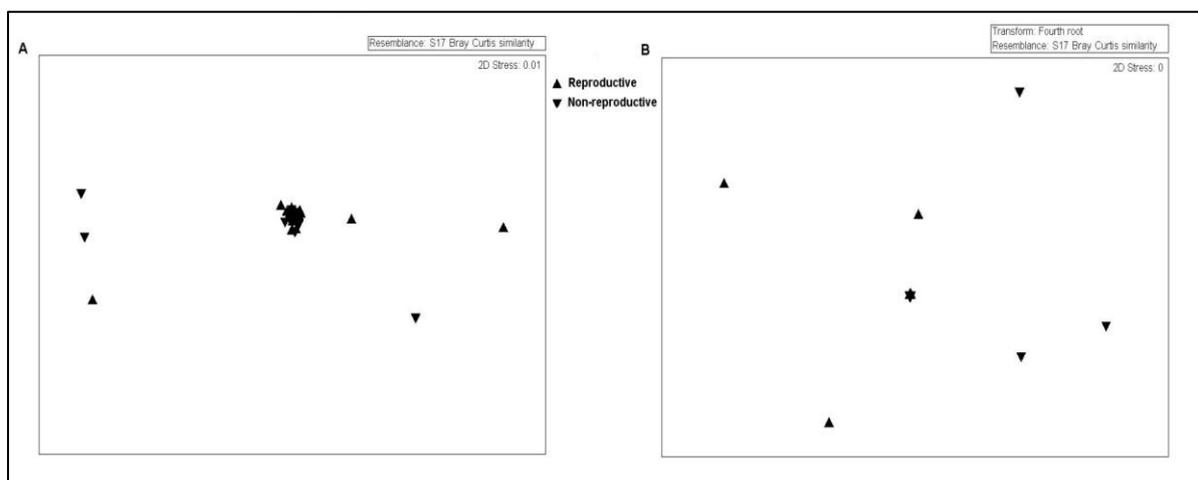


Figure 3.3: Multidimensional scaling plots based on Bray-Curtis similarities of A) mite prevalence and B) mite abundances between reproductive states (reproductive vs non-reproductive) from *Rhabdomys pumilio*.

*Distribution and species associated with small mammals across South Africa*

Several chigger species were widely distributed across various biomes in SA while others exhibited a more restricted distribution (as examples see Figures 3.4, 3.5, 3.6 & 3.7).

Chiggers were opportunistically sampled from three rodent (*R. pumilio*, *O. irroratus*, *M. namaquensis*) and three insectivore species (*C. flavescens*, *M. proboscideus*, *Elephantulus edwardii*) across SA. Three additional chigger species (*Comatacarus* spp., *Microtrombicula* n. sp., *Austracarus* n. sp.), not recorded in the CFR study, were recorded during opportunistic sampling effort across SA (Table 3.3). From the data there were no clear host preference and species seem to occur on multiple host species (Table 3.3).

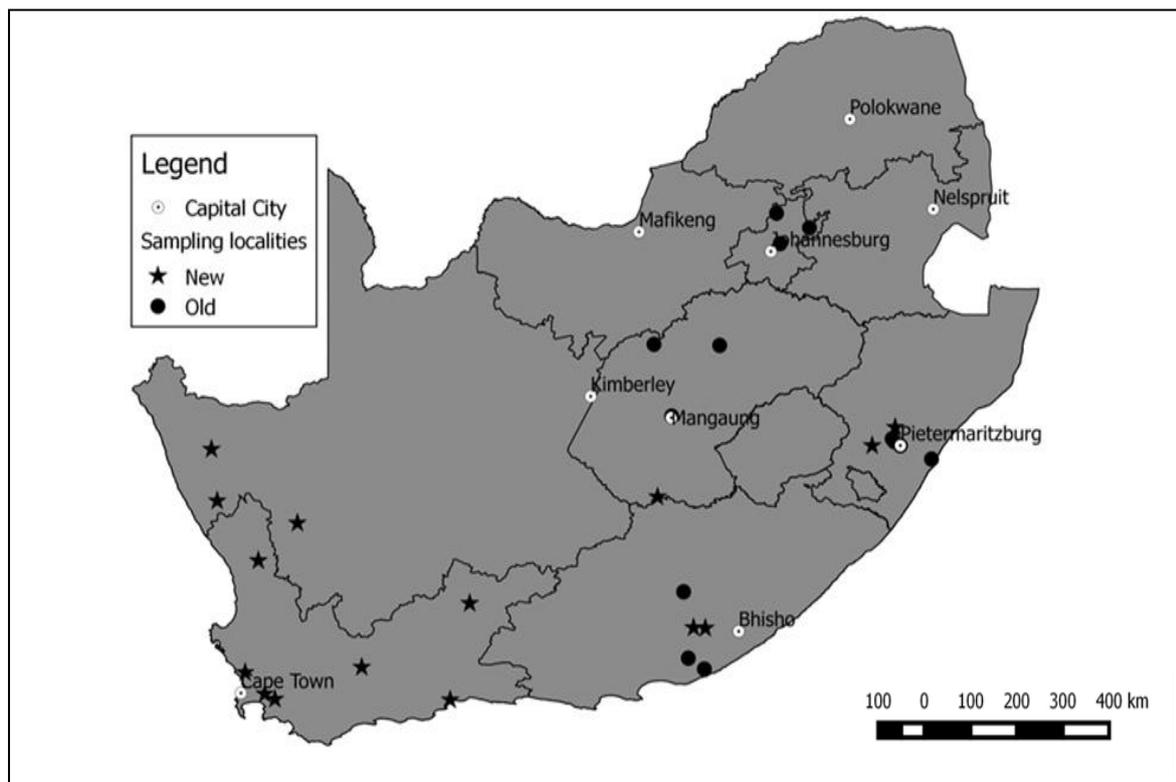


Figure 3.4: Comparative map of historical sampling localities and localities documented during the current study for chigger species associated with small mammals in South Africa.

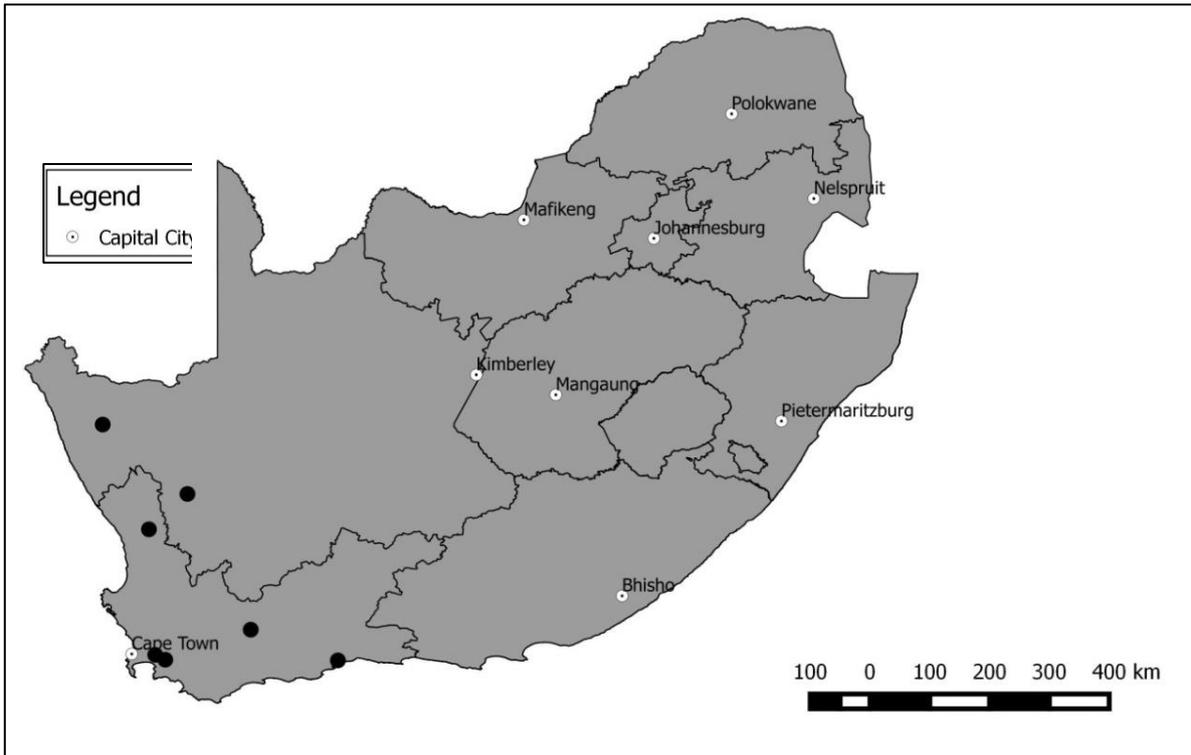


Figure 3.5: Preliminary geographical distribution of *Leptotrombidium muridium* recovered from small mammals in South Africa (2009 - 2013).

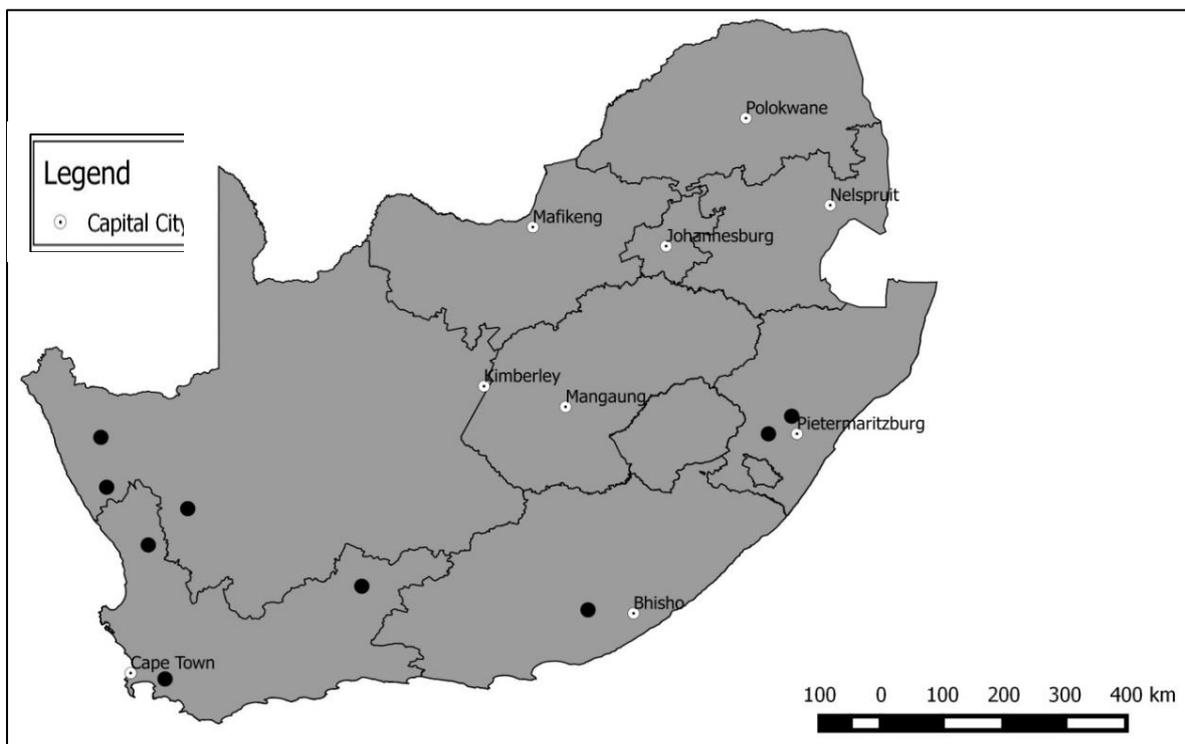


Figure 3.6: Preliminary geographical distribution of *Austracarus* n. sp. recovered from small mammals in South Africa (2009 – 2013).

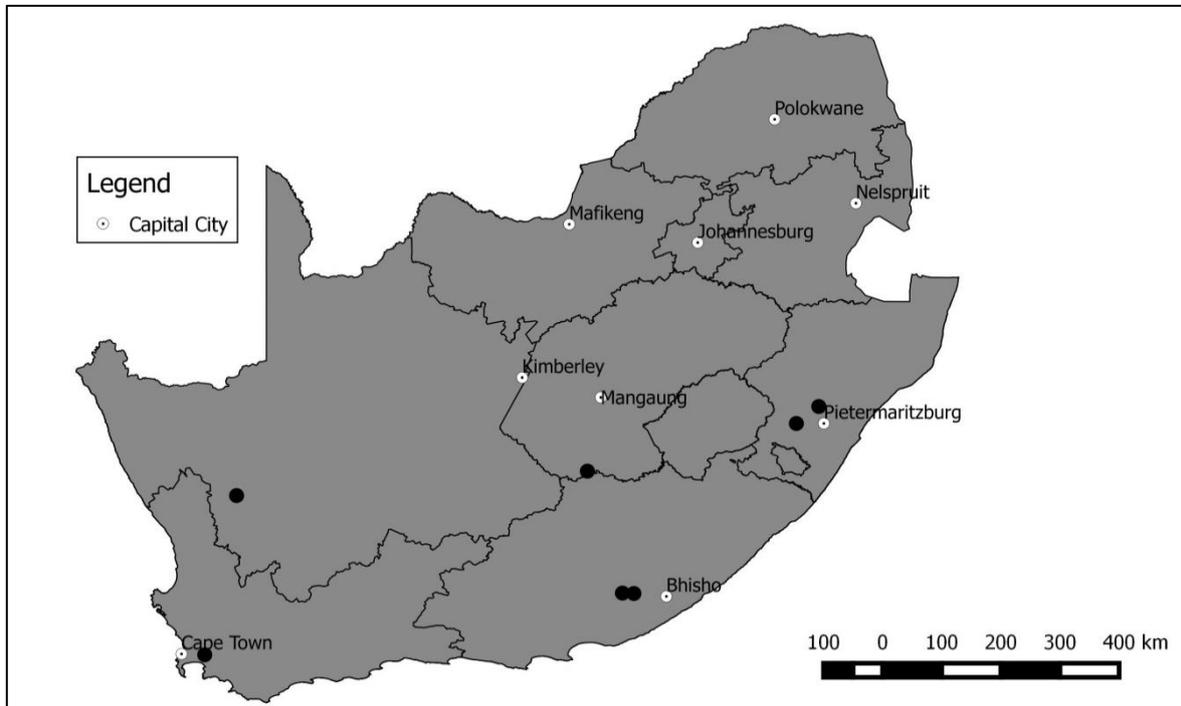


Figure 3.7: Preliminary geographical distribution of *Ascoshongastia* spp. recovered from small mammals in South Africa (2009 – 2013).

Table 3.3: Parasite-host list of chiggers associated with small mammals across South Africa.

Chigger species	Host species	Province
<i>Leptotrombidium muridium</i>	<i>R. pumilio</i>	WCP
	<i>O. irroratus</i>	WCP
	<i>M. namaquensis</i>	WCP, NCP
	<i>C. flavescens</i>	WCP
	<i>E. edwardii</i>	WCP
	<i>M. proboscideus</i>	WCP
<i>Schoutedenichia</i> spp.	<i>R. pumilio</i>	WCP
	<i>O. irroratus</i>	WCP
	<i>C. flavescens</i>	WCP
	<i>M. proboscideus</i>	WCP

<i>Schongastia</i> spp.	<i>R. pumilio</i> <i>O. irroratus</i> <i>E. edwardii</i> <i>M. namaquensis</i> <i>M. proboscideus</i>	WCP, KZN WCP WCP NCP WCP
<i>Ascoschongastia</i> spp.	<i>R. pumilio</i> <i>C. flavescens</i> <i>M. namaquensis</i> <i>O. irroratus</i>	WCP WCP NCP, ECP ECP
<i>Neoschongastia</i> spp. A	<i>R. pumilio</i>	WCP
<i>Neoschongastia</i> spp. B	<i>R. pumilio</i> <i>M. namaquensis</i> <i>E. edwardii</i>	WCP WCP ECP
<i>Trombicula</i> spp.	<i>C. flavescens</i> <i>M. namaquensis</i>	WCP NCP
<i>Microtrombicula</i> n. sp.	<i>Dendromys melanotis</i>	WCP
<i>Comatacarus</i> spp.	<i>M. namaquensis</i>	WCP
<i>Austracarus</i> n. sp.	<i>E. edwardii</i> <i>R. pumilio</i> <i>M. namaquensis</i> <i>E. edwardii</i>	WCP KZN NCP, ECP NCP
<i>Schöngastiella</i> n. sp.	<i>C. flavescens</i> <i>S. campestris</i>	WCP NCP
<i>Shunsennia</i> spp.	<i>R. pumilio</i>	WCP

Key to abbreviations of provinces: WCP: Western Cape Province, KZN: KwaZulu- Natal, NCP: Northern Cape Province, ECP: Eastern Cape Province.

Table 3.4: Updated host-parasite list for all known chiggers (Trombiculidae) associated with mammal hosts in South Africa.

Host species	Chigger species	Province	Reference
<i>Order Rodentia</i>			
<i>Rhabdomys pumilio</i>	<i>L. murudium</i>	WCP	Present study
	<i>Schoutedenichia</i> spp.	WCP	Present study
	<i>Neoschongastia</i> spp. A	WCP, KZN	Present study
	<i>Schongastia</i> spp.	WCP, KZN	Present study
	<i>Austracarus</i> n. sp.	WCP, KZN	Present study
	<i>Ascoshongastia</i> spp.	KZN	Present study
	<i>Leeuwenhoekinae</i> ( <i>Shunsennia</i> ) spp.	WCP	Present study
<i>Otomys irroratus</i>	<i>Ascoshongastia</i> spp.	ECP	Present study
	<i>L. murudium</i>	WCP	Present study
	<i>Schongastia</i> spp.	WCP	Present study
	<i>Schoutedenichia</i> spp.	WCP	Present study
<i>Otomys tugelensis pretoriae</i>	<i>Ascoshongastia otomyia</i>	GP	Lawrence, 1954
	<i>Ascoshongastia africana</i>	GP	Lawrence, 1954
<i>Micaelamys namaquensis</i>	<i>Ascoshongastia</i> spp.	WCP, NCP, ECP	Present study
	<i>Austracarus</i> spp.	WCP	Present study
	<i>L. murudium</i>	WCP, NCP	Present study
	<i>Neoschongastia</i> spp.	NCP	Present study
	<i>Odontacarus</i> spp.	NCP, ECP, ECP	Present study
	<i>Schongastia</i> spp.	WCP	Present study
	<i>Trombicula</i> spp.	WCP	Present study
	<i>Ascoshongastia aethomyia</i>	ECP	Lawrence, 1954
	<i>Ascoshongastia longispina</i>	ECP	Lawrence, 1954
<i>Acomatacarus lawrencei</i> <i>Acomatacarus thalomyia</i>	ECP ECP	Lawrence, 1954 Lawrence, 1954	
<i>Graphiurus murinus</i>	<i>Trombicula claviglicola</i>	Unknown	Lawrence, 1954
<i>Tatera brantsii</i>	<i>Acomatacarus gateri</i>	GP	Lawrence, 1954

<i>Saccostomus campestris campestris</i>	<i>Acomatacarus theileri</i>	ECP	Lawrence, 1954
Order Hyracoidea			
<i>Procavia natalensis</i>	<i>Austracarus procaviae</i>	KZN	Lawrence, 1954
	<i>Hyracarus typicus</i>	KZN	Lawrence, 1954
	<i>Hyracarus longipilosus</i>	KZN	Lawrence, 1954
Order Afrosoricida			
<i>Amblysomus hottentotus longiceps</i>	<i>Leeuwenhoekia womersleyi</i>	KZN	Lawrence, 1954
Order Insectivora			
<i>Crocidura flavescens</i>	<i>Ascoschongastia</i> spp.	WCP	Present Study
	<i>Schöngastiella</i> n. sp.	WCP, ECP	Present Study
	<i>Schongastia</i> spp.	WCP	Present Study
	<i>Trombicula</i> spp.	WCP	Present Study
<i>Elephantulus edwardii</i>	<i>L. muridium</i>	WCP	Present Study
	<i>Schongastia</i> spp.	WCP	Present Study
	<i>Odontacarus</i> spp.	WCP, NCP	Present Study
	<i>Neoschongastia</i> spp. B	NCP	Present Study
<i>Elephantulus myurus jamesoni</i>	<i>Ascoschongastia annulata</i>	GP	Lawrence, 1954
<i>Macrocelidis proboscideus</i>	<i>L. muridium</i>	WCP	Present study
	<i>Schoutedenia</i> spp.	WCP	Present study
	<i>Schongastia</i> spp.	WCP	Present study
Order Chiroptera			

<i>Hipposideros caffer</i>	<i>Acomatacarus polydiscum</i>	KZN	Lawrence, 1954
	<i>Gahrlepiea nanus</i>	KZN	Lawrence, 1954
	<i>Trombicula natalensis</i>	KZN	Lawrence, 1954
<i>Rhinolophus geoffroyi zuluensis</i>	<i>Trombicula minutissimum</i>	KZN	Lawrence, 1954
Order <i>Carnivora</i>			
<i>Suricata suricatta</i>	<i>Trombicula cynictia</i>	FSP	Lawrence, 1954

Key to abbreviations of provinces; WCP: Western Cape Province, KZN: KwaZulu-Natal, ECP: Eastern Cape Province, GP: Gauteng Province, NCP: Northern Cape Province, FSP: Free State Province.

## Discussion

The current study support previous findings in that chigger occurrence on hosts vary temporally, with higher occurrence associated with warm and dry conditions. It is also evident that host species are parasitized by multiple chigger species and that some chigger species occur on multiple host taxa. Host reproductive state seems to influence chigger abundance and species composition on *R. pumilio* however, this pattern needs to be confirmed with larger sample sizes. The geographic distribution of the chiggers was species-specific and ranged between a broad across biome distribution to a narrower within biome distribution.

### *Temporal variation in prevalence of chiggers on a generalist rodent in the CFR*

Two main factors determine the prevalence of chiggers; the ecology of the chigger species (climatic requirements, reproductive cycles, habitat requirements) and the climatic zone in which they occur (Daniel, 1961). The Fynbos biome, where this part of the study was conducted, has a Mediterranean type climate, with hot dry summer months and cold wet winter months. In the present study the occurrence of chiggers on a generalist rodent host, *R. pumilio*, followed a clear seasonal pattern with significantly higher prevalence during the dry warm months and almost complete absence during the cold wet months. This pattern supports previous studies that have recorded reproduction of trombiculid mites to be seasonal in temperate regions (Goff *et al.*, 1982). Previous studies have noted that the temporal pattern does however vary between species with some species prevalent during

spring and summer months whereas other species are prevalent during autumn and early winter (Daniel, 1961; Traub & Wiseman, 1974; Goff, 1982). More specifically, species of the subgenus *Trombiculindus* from Burma are described as xerophilic and are only present during the driest part of the year and completely absent during the wet season (Traub & Wisseman, 1974). In contrast Malayan species of the genus *Leptotrombidium* were predominantly found during wet seasons. Interestingly, when members of the *Leptotrombidium* group emerged during dry months, it was noted that their development and behaviour was abnormal (Traub & Wisseman, 1974). The present study was conducted at a single locality in the winter rainfall region of SA. The species identity of the chigger species are not known, but it does appear that all that chiggers that were present on *R. pumilio* during the study preferred warm and dry conditions in the CFR. It is expected though, that the pattern may be different in other parts, such as the summer rainfall region, of the country and may vary between species.

#### *Host association in the CFR*

The local CFR study concurs with studies that have recorded opportunistic exploitation of multiple host species by chiggers (Traub & Wiseman, 1974; Whitaker & Loomis, 1978; Shatrov and Kudryashova, 2006; Mariana *et al.*, 2011). However, the present study also indicates that even though chiggers are regarded as host generalists they do appear to have a primary host (Mohr, 1947, 1956; Whitaker & Loomis, 1978; Shatrov and Kudryashova, 2006; Dietsch, 2008; Lootvoet *et al.*, 2013). Host preference is strongly influenced by; host population density, co-habitation of a patch by the chigger and the host species, behaviour of the host (e.g. foraging within the area, sociality and home-range size) and the ecology of the specific trombiculid species (Daniel, 1961; Whitaker & Loomis, 1978; Shatrov and Kudryashova, 2006; Dietsch, 2008). The present study corroborates this in that the rodent with the highest chigger abundance and diversity was also the most abundant and generalist species. *Rhabdomys pumilio* was the most abundant small mammal species at the two localities in the CFR. This supports previous studies that have recorded a dominance of *R. pumilio* in natural and transitional vegetation and in agricultural areas in the region (Matthee *et al.*, 2007; Froeschke *et al.*, 2013; Froeschke & Matthee 2014). The mean abundance values of individual chigger species were also the highest on this rodent

compared to other co-occurring rodent and insectivore species. In addition, more chigger species (seven species) were recorded on *R. pumilio* compared to any of the other host species (range of 2-5 species). This pattern support previous studies on parasite taxa with free-living infective stages that recorded a positive relationship between host density, parasite abundance and species richness (Anderson & May, 1978, 1991; May & Anderson, 1978; Arneberg *et al.*, 1998; Morand & Poulin, 1998; Krasnov *et al.*, 2002; Stanko *et al.*, 2002; Altizer *et al.*, 2003). The fact that *R. pumilio* was the most abundant species provides a greater chance for free-living larvae to come into contact with this host. In addition, as mentioned above, the host itself is an opportunist in terms of habitat preference which suggests that it uses a wider diversity of habitats within the landscape compared to host species that are more habitat specific (Schradin & Pillay, 2005; Skinner & Chimimba, 2005). Preference for certain host species have also been recorded in a comparative study on chigger infestations of rats in New Guinea and Luzon where *Trombicula deliensis*, *Walchia disparunguis* and *Neoschongastia rattus* preferred jungle rats (*Rattus ringens*) over Malay rats (*Rattus exulans*) (Mohr, 1947). In addition, a study documenting chigger diversity associated with small mammals in Indiana, USA, found that *Neotrombicula whartoni* exploited 13 different host species, but had an affinity towards three of the hosts; cottontail rabbits (*Lepus sylvaticus*), opossums (*Didelphis virginiana*) and fox squirrels (*Sciurus niger*) (Whitaker & Loomis, 1978).

The three most abundant chigger species recorded on *R. pumilio* were also recorded in high abundance on co-occurring rodent and insectivore species. A study by Lootvoet *et al.* (2013) suggested that the exploitation of alternative hosts by generalist parasites increased when the parasite burden on the primary host was high. This pattern can only be observed if alternative hosts share the same habitat as the primary host species. The two localities in the present study were remnant fragments of natural vegetation within an agricultural matrix and although habitat segregation is observed between *R. pumilio* and some of the co-occurring species in extensive natural habitats (Skinner & Chimimba, 2005) this may not be the case in the current study. The fact that chigger species occurred on multiple hosts may be result of some level of habitat sharing between host species and or due to the presence of chigger species throughout the habitat fragment. Habitat fragments can be considered as islands with no or limited movement beyond it. This is mainly due to the fact that these

fragments provide resources such as cover, food and often water, while the opposite is found in the surrounding agricultural areas (Froeschke *et al.*, 2013; Froeschke & Matthee, 2014)

There were also two chigger species (*Shunsennia* spp. and *Gahrliopia* (*Schöngastiella*) n. spp.) that only occurred on a single host species (*R. pumilio* and *C. flavescens*, respectively). Musk shrews (*C. flavescens*) prefer moist habitats with sufficient vegetation cover. Members of the genus *Gahrliopia* are commonly found clustering on the roof of rodent burrows or seeking refuge within crevices of rocky areas (Traub & Wisseman, 1974). The most adaptable member *Gahrliopia* (*Schöngastiella*) *ligula*, occurs in India where it can be found in almost any habitat type imaginable, ranging from forests, scrub vegetation to herbaceous and cultivated land (Traub & Wisseman, 1974). This species was also present in both humid and arid habitats and at elevations reaching up to 2000 meter above sea level (Traub & Wisseman, 1974). The current results found *Gahrliopia* (*Schöngastiella*) n. sp. in very low abundances that could suggest that the species is not as adaptable as *G. ligula*, but rather exhibit the same scarce distribution as *G. ciliata*. Limited samples of *G. ciliata* exist as they are very rare, even in areas that are known to be optimal for *G. ciliata* (Traub & Wisseman, 1974). Unfortunately no comparable information is available on *Shunsennia* spp. or the subfamily.

In the local CFR study five small mammal species were recorded in the remnant Fynbos fragments. The vegetation in fragments can be regarded as low disturbance vegetation as the fragments occur within an agricultural matrix. Interestingly, the largest number of chigger species was recorded on *R. pumilio* and *C. flavescens*, respectively. Both small mammal species frequently occur in low disturbance vegetation with high vegetation cover. This is in contrast to the other three species that were recorded (Skinner & Chimimba, 2005). Several chiggers are known to proliferate in transitional areas within the landscape (Mohr, 1949; Daniel 1961; Traub & Wisseman, 1974) which may explain the greater number of chigger species on these hosts.

*Chigger infestation levels on small mammals in the CFR*

More than 31 893 chiggers (representing nine species) were removed from all host animals (n = 137) in the local CFR study. The total chigger abundances recorded in the present study is comparable to a study conducted in the Slovak Carpathians by Daniel (1961). In the study 19 000 chiggers were recorded from 16 small mammal species (animals sampled n = 2 148). However, chigger abundances do seem to differ between host species and regions. Collections of chiggers from small mammals in Papua New Guinea recorded a total of 2 774 chiggers (representing 13 species) from 83 *Rattus ruber* specimens, while 2 813 were recorded from 485 *R. niobe* individuals (Goff, 1979; 1982). More recently a study in China collected almost 60 000 chiggers (109 chigger species) from 21 small mammal species (animals sampled n = 3 303) (Dong *et al.* 2008). All four of the latter studies differed from the present study in that the duration and extent (more than a year and in multiple biotopes) and sample size (animals sampled ranged from 2 148 to 3 303) was larger compared to the present study where 137 hosts were trapped in one biotope over a 12-day period. Based on this it is possible that the chigger diversity in the CFR might mimic the high plant diversity that is characteristic of the region. In addition, it is hypothesised that the species richness and abundance of chiggers associated with small mammals in SA will increase with an increase in sampling effort and extent.

*Role of host factors and locality on chigger abundances and species composition on R. pumilio in the CFR*

The life history patterns of ectoparasite taxa are variable and range from a permanent association with the host (sucking lice) to a temporary association (fleas, mites and ticks) (Rödl, 1979; Krasnov & Khoklova, 2000; Krasnov, 2012). The response to host- and environmental factors will therefore depend on the life history of the parasite (Poulin, 1997; Froeschke *et al.*, 2010; Johnson & Thieltges, 2010; Krasnov & Matthee, 2010; Froeschke *et al.*, 2013; van der Mescht *et al.*, 2013; Froeschke & Matthee, 2014). Chiggers can be regarded as temporary parasites as only the larval stage attaches to a host (Lawrence, 1949; Sasa, 1957; Traub & Wisseman, 1974; Goff *et al.*, 1982; Simonova, 1983; Krantz & Walter, 2009). In the present study, higher chigger abundances were recorded on reproductively active male and female hosts. There are various host factors that can facilitate higher parasite infestation levels during the breeding season of rodents and terrestrial vertebrates

in general. During this time both males and females are subjected to higher energy demands as females invest more energy into foetus development and lactation (McLean & Speakman, 1997, 1999, 2000; Christi *et al.*, 2000; Neuhaus, 2003; Speakman, 2008) while males may be more mobile and subjected to aggressive encounters (Barnard *et al.*, 1998; Klein, 2000; Nelson, 2000). Furthermore, reproductively active individuals produce sex hormones, androgens and oestrogens that can either compromise or enhance the animal's immunity (Grossman, 1985; Zuk & Mckean, 1996; Klein, 2000; Krasnov *et al.*, 2005). Testosterone is known to influence behaviour, with scrotal males becoming more territorial and having larger home ranges (Hart, 1997; Madison *et al.*, 1984; Barnard *et al.*, 1998, 2002, 2003; Klein, 2000; Shatrov and Kudryashova, 2006; Schradin, 2008). The hormone is also regarded as an immune suppressor and studies have recorded higher parasite loads in animals with high circulating testosterone levels (Zuk & Mckean, 1996; Klein, 2000). Although oestrogen is regarded as an immune enhancer this advantage may be counteracted by the presence of external stressors such as high densities, lack of breeding sites and infections (David & Jarvis, 1985; Nelson *et al.*, 2002; Schradin & Pillay, 2005; Weil *et al.*, 2012). Rodent density and especially *R. pumilio* has been found to be higher in remnant fragments of natural Fynbos vegetation within an agricultural matrix (van der Mescht, 2012; van der Mescht *et al.*, 2013; Froeschke *et al.*, 2013; Froeschke & Matthee, 2014) and it is quite possible that this is the case in the present study. High rodent densities will facilitate increased contact and subsequent infestation by free-living infective stages (Mohr, 1961; Traub & Wisseman, 1974; Anderson & May, 1978, 1991; May & Anderson, 1978; Morand & Poulin, 1998; Krasnov *et al.*, 2002; Stanko *et al.*, 2002; Altizer *et al.*, 2003). This is supported by previous studies that have recorded significantly higher parasite infestations in *R. pumilio* in these fragments compared to extensive natural areas (van der Mescht *et al.*, 2013; Froeschke *et al.*, 2013; Froeschke & Matthee, 2014). In addition to larger chigger burdens we also recorded higher tick infestations on the reproductively active individuals ( $4.29 \pm 4.24$ ) compared to non-reproductively active individuals ( $1.5 \pm 1.8$ ) in the present study. Another possible explanation for adult-biased exploitation observed in the current study is that of the hypotheses of "well-fed host" versus "poorly-fed host" (Christe *et al.*, 1998; Christe *et al.*, 2003; Hawlena *et al.*, 2005). According to the "well-fed host" hypothesis adult hosts are regarded as "well-fed hosts" as they are larger and represent a better nutritional source than juveniles, who are regarded as "poorly-fed hosts" (Christe *et al.*, 2003; Hawlena *et al.*,

2005). “Well-fed hosts” will also be able to cope better with energy expenditure associated with anti-parasitic behaviour (grooming) than juveniles (Hawlana *et al.*, 2005). Although body size, of *R. pumilio* individuals, was not a significant predictor ( $p > 0.05$ ) for chigger abundance in the present study reproductively active individuals were slightly larger (total length of  $20.89 \pm 3.64$ ) compared to non-reproductively active individuals (total length of  $18.45 \pm 1.54$ ). This pattern is supported in previous studies on *R. pumilio* where significantly higher parasite burdens were recorded on larger compared to smaller animals (Froeschke *et al.*, 2013; Froeschke & Matthee, 2014). It is possible that a combination of factors contributed to a higher chigger abundance on reproductively active individuals in the present study.

The availability and abundance of parasites will influence several parameters such as parasite species richness, diversity and species composition on the host (Schmidt *et al.*, 1999; Klukowski, 2004; Matthee & Krasnov, 2009). For example, high parasite abundances can result in a larger number of parasites species on the host due to a greater chance of obtaining new species (Sasal, 1997; Keesing *et al.*, 2006; Shatrov and Kudryashova, 2006; Sinski *et al.*, 2006). As mentioned above a lower immune status and a slightly larger body size associated with reproductively active animals could have resulted in higher chigger infestation levels in the present study. The latter most probably facilitated the observed difference in species composition (based on chigger prevalence and abundance) between reproductively active and non-reproductive individuals. The fact that the biotope was similar between the two localities could explain why there was no difference in species composition between localities. A biotope or specific habitat type within the environment plays a crucial role in the parasite-host-environment relationship and hosts in a specific biotope will be exploited by the dominant chigger mite species occurring within the biotope (Daniel, 1961). Three chigger species were found to be the most abundant at both study localities; *L. muridium*, *Neoschoengastia* spp. A, *Shoutedenichia* spp. *Leptotrombidium muridium* was the dominant species at both localities followed by *Neoschoengastia* spp. A and *Shoutedenichia* spp. It has also been documented that members of the *Leptotrombidium* genus are predominant in secondary vegetation (or scrub vegetation) and disturbed habitat patches (Traub & Wisseman, 1974). This is in accordance with the results

of the current study indicating that *L. muridium* exhibits generalist habitat requirements and is associated with disturbed habitats. Unfortunately no ecological information is available on the other two abundant species

#### *Geographic distribution of chiggers across South Africa*

Chiggers exhibit a strong association with their physical environment, as the majority of their life cycle takes place off the host within the environment (Sasa, 1957; Daniel, 1961; Goff *et al.*, 1982; Shatrov and Kudryashova, 2006; Dong *et al.*, 2008; Krantz & Walter, 2009). Some species have very specific environmental requirements, for example, *Leptotrombidium scutellare*, which is endemic to Mt Fuji, only occurs along rivers and streams with a specific type of sand (Traub & Wisseman, 1974). However, there are also species that are more ecologically tolerant and they are usually widely distributed within the landscape (Traub & Wisseman, 1974). In contrast, *Schöngastiella ligula*, a dominant chigger species on the Indian subcontinent, is regarded as a highly adaptable species. This species has been recovered in diverse habitat types ranging from humid pristine forests to semi-arid cultivated landscapes, with elevations reaching 2000 m above sea level (Traub & Wisseman, 1974). It is therefore evident that species-specific differences exist in habitat preference which will have knock-on effects for the geographic distribution of species across the landscapes.

It is evident from the geographic distribution data that chiggers of small mammals occur across SA. While the chiggers that were collected at various localities across SA, within the present study, were sub-samples, the collection effort was the first of its kind for SA. The distribution data are thus preliminary as some species might have been under sampled or absent during the sampling time. Irrespective, five of the twelve species (*Schoutedenichia* spp., *Neoschongastia* spp. A, *Microtrombicula* n. sp., *Shunsennia* spp. and *Comatacarus* spp.) were exclusively recorded in the Fynbos biome. This might indicate that these species are specifically adapted to the Fynbos biome. The dominant chigger species within the CFR, *L. muridium*, and four other species (*Schongastia* spp., *Neoschongastia* spp. B, *Gahrlepiea* (*Schöngastiella*) n. sp. and *Trombicula* spp.) were collected in the Fynbos and Succulent

Karoo biomes. The vegetation structure and climate (predominantly winter rainfall) in the Succulent Karoo is more similar to that of Fynbos than to any of the other biomes and it can be argued that low shrub-like vegetation and moist conditions during the colder months are important for the distribution of these species. In the present study *Ascoschongastia* spp. and *Austracarus* n. sp. were the chigger species with the widest distribution as they were recorded across different biomes. *Ascoschongastia* spp. was collected from three different biomes: Fynbos, Succulent Karoo, Grasslands, the ecotone between Albany Thicket and Grasslands and the ecotone between the Savanna and Grassland biome. *Austracarus* n. sp. was recorded in four biomes; Fynbos, Succulent Karoo, Nama Karoo and Grasslands biome. The wide geographical distribution of these two species suggests that they are ecologically tolerant and adaptable. For most of the species sampled it seems that microclimatic characteristics play an important role in determining their distribution within a given landscape. No ecological data is available for the specific chigger species collected during the current study; however it may be possible to make indirect inferences by looking at the ecology and habitat preferences of the host species on which they occur. For example, the chigger species *Neoschongastia* spp. B and *Comatacarus* spp. both occur on *Elephantulus edwardii* and *M. namaquensis*. These two host species frequently occur on rocky outcrops within the landscape (Skinner & Chimimba, 2005; Lancaster & Pillay, 2010). It is therefore possible that the chigger species found on them may have a preference for rocky areas with less dense vegetation cover. Unfortunately no clear pattern could be found for the other chigger species as they occurred on host species that differ in terms of habitat preference. For example, *L. muridium* was recorded on host species that prefer low lying grassy areas and species that occur on higher rocky areas.

In conclusion, the current study provides insight into the diversity and distribution of chigger species on small mammals within SA. It is evident that the chigger diversity is high in the CRF and it is hypothesised that the overall diversity for the country might also show the same pattern. Chiggers collected in the CFR appear to be host species generalists with certain chigger species occurring on both rodents and insectivores. Host quality seems to be important to chiggers as it facilitates high abundance and more similar species composition. The distribution of chiggers across the landscape was species-specific with some taxa

exhibiting a narrower distribution while other species occur across multiple biomes. It is recommended that a wider sampling approach be followed across SA as this will provide a better understanding of regional diversity, biome-specific habitat preference, distribution and ecology of this highly understudied parasite group.

## Chapter 4

### ***Three New Species of Trombiculid Mites (Acari: Trombiculidae) from South African Small Mammals***

\*Prepared for *Systematic Parasitology*

#### **Introduction**

Research on the taxonomy and ecology of the various ectoparasite taxa has mainly focussed on helminths, fleas, lice, mesostigmatid mites and ticks. Relatively little attention has been given to the mites of the Trombiculidae of which only the larval stage (or chigger) is parasitic. Chiggers are highly specious with more than 3000 species described globally, 60 of which occur in South Africa. Lizards and reptiles are common hosts (Lawrence, 1949; Zumpt, 1961), however various studies have demonstrated the importance of small mammals, especially rodents and insectivores as hosts for chiggers (Mohr, 1947, 1956; Daniel, 1961; Traub & Wisseman, 1974; Goff, 1982; Mariana *et al.*, 2011). Eleven chigger species (representing eight genera) have been described from small mammals in SA (Lawrence, 1949; Zumpt, 1961). In particular, the genus *Schöngastiella* (Hirst, 1915) is represented in Africa by three species; *S. caeca* (André, 1951) collected from *Taterillus emini* (Thomas, 1892) and *Mylomys cunninghamei* (Thomas, 1906), *S. durenii* (Jadin and Vercammen-Grandjean, 1952) from *Dasymys incomtus* (Sundevall, 1847) and *S. wansonii* (Wolf and Vercammen-Grandjean, 1952) collected from *Rattus rattus* (Linnaeus, 1758). The genus *Microtrombicula* (Ewing, 1950) was originally proposed as a subgenus of *Trombicula* (Berlese, 1905). Within the genus two groups are recognised namely the *mastomyia* and *ugandea* group. Within SA only two species have been recorded within the genus *Microtrombicula*: *M. cynictia* (Radford, 1942) collected from *Cynictis penicillata* (Cuvier, 1829) and *M. minutissimum* (Oudemans, 1910) collected from *Hipposideros caffer* (Sundevall, 1846). Three species belonging to the genus *Austracarus* have been recorded in SA: *A. polydiscum* (Oudemans, 1910) collected from *Hipposideros caffer* (Sundevall, 1846) and *Cryptomys hottentotus* (Lesson, 1826), *A. procaviae* (Lawrence, 1949) collected from

*Procavia capensis natalensis* (Roberts, 1924) and *A. campestris* (Goff, 1990) collected from *Raphicerus campestris* (Thunberg, 1811). During the present study small mammals were trapped at various localities across South Africa and searched for the presence of chiggers. Three novel species belonging to the three genera were described from small mammals.

## Materials and Methods

From 2009-2013 small mammals (rodents and insectivores) were trapped at various localities across South Africa. Adult individuals were mainly targeted during austral spring-summer (October to February). The sampling protocol was standardised and used during each trap session. Sherman-type live traps were set for five to ten trap nights per locality. Traps were set out in trap lines (10 meters apart) and baited with a mixture of peanut butter and oats. Traps were checked twice daily. Following euthanasia each individual was placed in a labelled plastic bag (The study was approved by the Ethical Committee of Stellenbosch University Reference code: SU-ACUM11-00004(P)). The animals were frozen at -80 °C until parasite removal commenced. The frozen animals were thawed and carefully examined under a stereoscopic microscope for the presence of chiggers. All the chiggers were removed and stored in 70% ethanol in Eppendorf tubes. Specimens were mounted separately on microscope slides using adapted techniques (Krantz & Walter, 2009). Taxonomic keys were used to identify specimens where possible (Lawrence, 1949; Zumpt, 1961). Morphometric measurements were made of 10 selected specimens of each species for identification purposes, it should be noted that there were not ten specimens available for all species. All the measurements are reported in micrometres. Ranges and means are given in brackets. Measurements were made with Zeiss Axioskope Research Microscope and Zeiss ZEN Imaging Software. Figures were first made by hand using the microscope's periscope (Zeiss Axioskope Research Microscope). The sketches were scanned and a digital version was created using Adobe Illustrator CC (2014) software. Terminology of descriptions follows Goff *et al.* (1982).

**Family Trombiculidae Ewing, 1944**

**Subfamily Trombiculinae Ewing, 1929**

**Genus *Schöngastiella* Hirst, 1915**

***Schöngastiella soricina* n. sp.**

*Type-host:* *Crocidura flavescens* (Geoffroy, 1827), *Saccostomus campestris* (Peters, 1846)

*Type-locality:* *C. flavescens*, Stellenbosch, S33° 56.1841', E018° 51.114', Western Cape, South Africa

*S. campestris*, Bethulie, S30° 29.7396', E025° 58.5354', Free State, South Africa

*Type-material:* Holotype and 3 paratypes

*Etymology:* The name was derived from the Latin *sorocinus* meaning shrew-mouse, as this species was collected from both *C. flavescens* and the *S. campestris*

Ten specimens of *Schöngastiella soricina* n. sp. were measured.

**Description of larva (Fig. 4.1)**

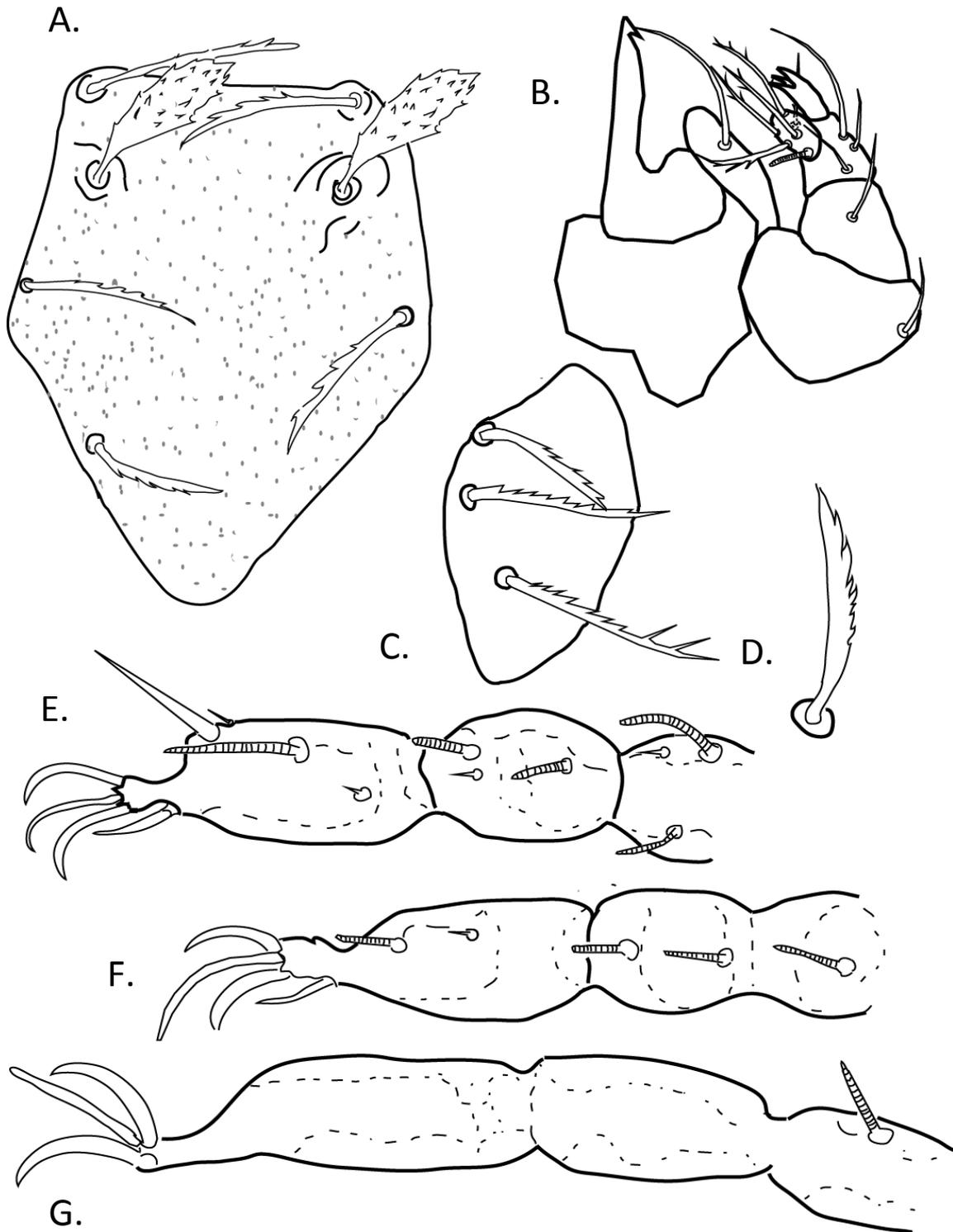
*Diagnosis.* Palpal setal formula N/N/NNN/5B1N, palpal claw 3-pronged. Galeala N. Scutum sub-pentagonal with posterior margin narrowly rounded, width greatest near PPL1. Scutum with stipules. SD 109. Scutal measurements (means followed by ranges of 10 specimens in parentheses): AW 48; PPW1 69; PPW2 40; ASB 18; PSB 91; AL 31; PL1 35; PPL2 26; PPL 61; SEN 35; SB 46. DS 32, arranged 4-6-6-6-4-4-2. VS: 35 – 37. Coxa III trisetose.

*Idiosoma.* Measuring 375 x 275. Ocelli absent. 1 pair of humeral setae measuring 25. Setae on idiosoma dorsal (DS) 32 in number, measuring 22-25, arrangement 4-6-6-6-4-4-2. Ventral idiosomal setae (VS) 35–37 in number; 1 pair anterior sternal setae, measuring 28; 1 pair posterior sternal setae, measuring 25; 30 pre-anal setae, measuring 15-18; 15-17 post-anal setae, measuring 17-21. Total idiosomal setae 42-46 (DS + VS including sternal setae and humeral setae).

*Gnathosoma* (Fig. 4.1, B). Palpal setal formula N/N/BNN/3B1N; palpal claw 3-pronged; galeala N; cheliceral blade measuring 28 (28-35), with single ventral tooth.

*Scutum* (Fig. 4.1, A). Sub-pentagonal with posterior margin narrowly rounded; width greatest near PPL1; anterior margin straight; scutum with stipules; scutal setae ranges from 5 to 6, setae are ciliated; AM lacking, 1 pair AL setae, measuring 28; sensilla narrow stalks and clavate head with situles; two pairs of PL setae. PPL1 > PPL2 > AL. scutal measurements (means followed by ranges of 10 specimens in parentheses): AL 31 (28-38); PPL1 35 (32-37); PPL2 26 (24-29); PPL 61 (58-65); AW 48 (45-54); PPW1 69 (67-73); PPW2 40 (37-49); ASB 18 (17-22); PSB 91 (81-93); SEN 35 (33-35); SB 46 (44-50).

*Legs* (Fig. 4.1, E-G). Segmentation 7/6/6, terminating in a pair of claws and clawlike empodium. Onychotriches absent. IP: 619 Leg I: 219 (206-231); coxa 1B; trochanter; basifemur; genu; 2 genualae; tibia, 2 tibialae and 1 microtibiala; tarsus (45-66 x 17-20), tarsala (19), tarsal-microtarsala (11), subterminala, pretarsala. Leg II: 200 (192-209), coxa 1B; trochanter; basifemur; genu, 1 genuala; tibia, 2 tibialae; tarsus (42-64 x 12-20), tarsala (18), tarsala-microtarsala (2.7); pretarsala. Leg III: 227 (201-246), coxa 3B; trochanter; basifemur; genu, 1 genuala; tibia; tarsus (42-70 x 14-18).



**Figure 4.1** *Schöngastiella soricina* n. sp. Dorsal view of larva. Sketch A-G: A. Scutum. B. Gnathosoma. C. Coxa III. D. Dorsal Seta. E. Leg I. F. Leg II. G. Leg III.

*Remarks*

Scutal shape and scutal setal measurements similar to *S. murphyi* (Nadchadram & Fernandes, 1989). The new species is separable from *S. murphyi* by the palpal setal formula and leg setae. Coxa III with 3 setae.

**Family Trombiculidae Ewing, 1944**

**Subfamily Leeuwenhoekiinae Womersley, 1945**

**Genus *Austracarus* Lawrence, 1949**

***Austracarus aridensis* n. sp.**

*Type-host:* *Elephantulus edwardii* (Smith, 1839), *Micaelamys namaquensis* (Smith, 1834),  
*Rhabdomys pumilio* (Sparrman, 1784)

*Type-locality:* *E. edwardii*, Garies, S30° 33.9336', E017° 59.409', Northern Cape Province,  
South Africa.

*M. namaquensis*, Garies, S30° 33.9336', E017° 59.409', Northern Cape  
Province, South Africa.

Beaufort West, S32° 21.2041', E022° 34.092', Northern Cape, South Africa  
Springbok, S29° 39.9023', E017° 53.157', Northern Cape, South Africa

Fort Beaufort, S32° 46.7947', E026° 37.671', Northern Cape, South Africa

*R. pumilio*, Beaufort West, S32° 21.2041', E022° 34.092', Northern Cape,  
South Africa Springbok, S29° 39.9023', E017° 53.157', Northern Cape, South  
Africa

*Type-material:* Holotype and 4 paratypes

*Etymology:* Species is named after arid conditions of type localities

Ten specimens of *Austracarus aridensis* n. sp. were measured.

**Description of larva (Fig. 4.2)**

*Diagnosis.* Palpal setal formula B/B/BBB/7B; palpal claw 3-pronged. Galeala B. Scutum lightly punctate, anterior margin shallowly biconcave with nose protruding in center;

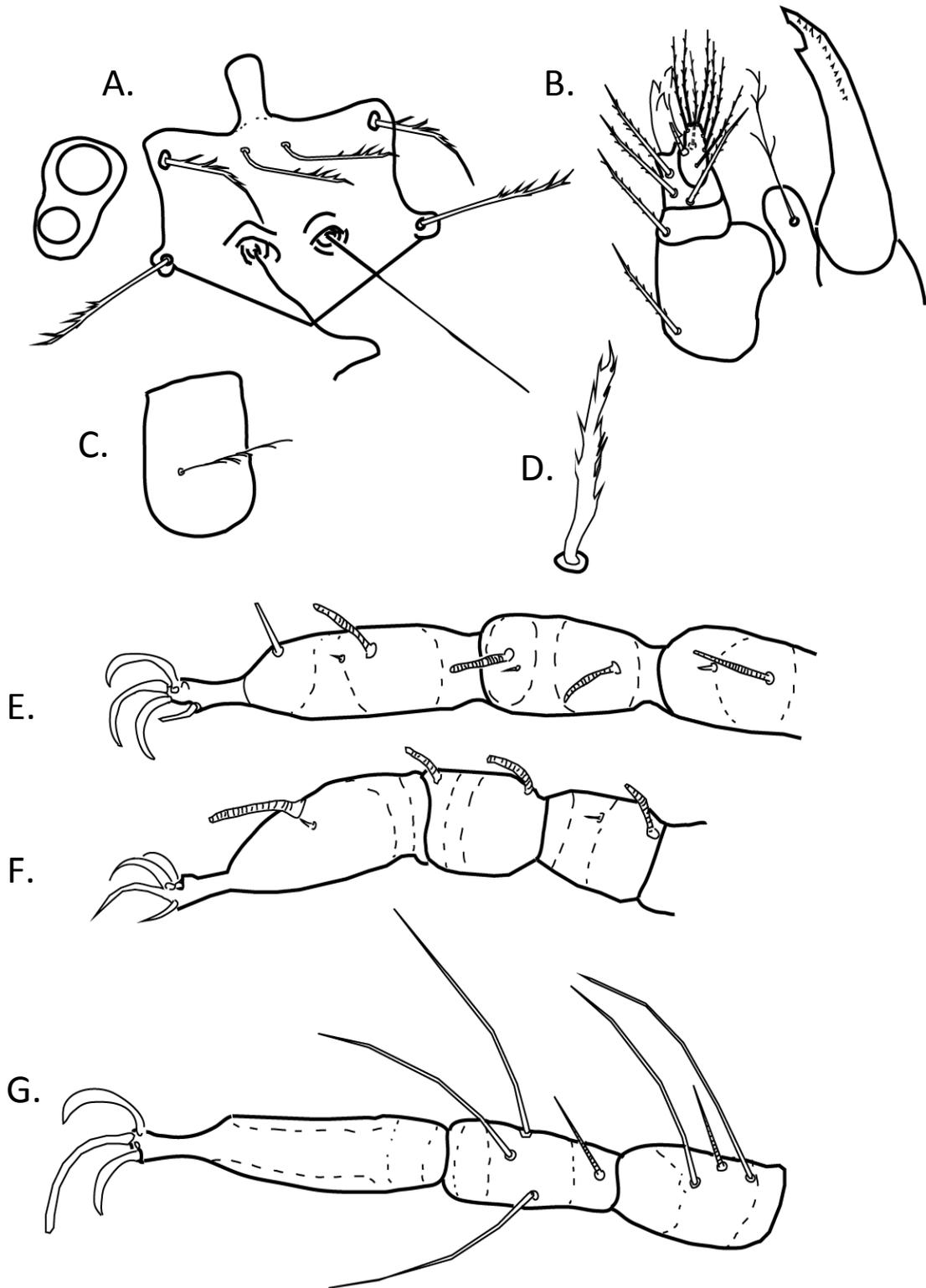
posterior margin acute, width greatest at PL insertion. Scutum finely stipulated. SD 39. Scutal measurements (means followed by ranges of 10 specimens in parentheses):: AW 56 (51-63); PW 71 (68-76); ASB 20 (17 – 24); PSB 19 (18-20); AP 20 (15-23); AM 25 (22-30); AM Base 11 (9-12); AL 29 (23-39); PL 26 (24-32) SEN 56 (57-72); SB 22 (18-25). DS 40, arranged: 6-6-6-8-6-4-6-4-2. VS 40. Coxa III 1B.

*Idiosoma*. Measuring 350 x 221. Ocelli 2/2, on ocular plate. 1 pair humeral setae, measuring 35 (30-41); DS 40, measuring 24-30, arranged: 6-6-6-8-6-4-6-4-2; ventral idiosomal setae (VS) 40; anterior sternal setae absent, posterior sternal setae measuring 30; 19-20 pre-anal setae, measuring 19-23; 20-25 post-anal setae, measuring 32-41; total idiosomal setae 89 (DS + VS including sternal setae and humeral setae).

*Gnathosoma* (Fig. 4.2, B). Palpal setal formula B/B/BBB/7B; palpal claw 3-pronged; galeala B; cheliceral blade measuring 35, with 11 dorsal teeth and a single ventral tooth.

*Scutum* (Fig. 4.2, A). Anterior margin shallowly biconcave; posterior margin acute; width greatest near PL; lightly punctate, nase present; scutal setae 8 in number; setae branched; 1 pair of AM setae, 1 pair of AL setae, sensillae flagelliform nude, 1 pair of PL setae; AM bases level with AL bases; SB level with PL bases; AL > PL > AM; sensillae flagelliform nude; scutal measurements (means followed by ranges of 10 specimens in parentheses):: AW 56 (51-63); PW 71 (68-76); ASB 20 (17–24); PSB 19 (18-20); AP 20 (15-23); AM 25 (22-30); AM Base 11 (9-12); AL 29 (23-39); PL 26 (24-32) SEN 56 (57-72); SB 22 (18-25).

*Legs* (Fig. 4.2, E-G). Segmentation 6/6/6, terminating in a pair of claws and clawlike empodium. Onychotriches absent. IP = 740. Leg I: 265 (258-272); coxa 2B; trochanter 1B; femur 6B; genu 4B; 1 genuala, 1 microgenuala; tibia 9B, 2 tibialae, microtibiala; tarsus (53-79 x 12-22) 4B, tarsala (14), tarsal-microtarsala (5), subterminala, parasubterminala absent, pretarsala. Leg II: 232 (220-260); coxa 1B; trochanter 1B; femur 6B; genu 4B, genuala, microgenuala; tibia 6B, 2 tibialae; tarsus (53-69 x 12-22) 17B, tarsala (16), microtarsala, pretarsala. Leg III: 260 (240-280); coxa 1B; trochanter 1B; femur 3B, 2 mastifemuralae; genu 2B, 2 mastigenualae; tibia 3B, 3 mastitarsalae, tarsus (61-89 x 13-20), 13B, 2 mastitarsalae (37-57), nude.



**Figure 4.2** *Austracarus aridensis* n. sp. Dorsal view of larva. Sketch A-G: A. Scutum. B. Gnathosoma. C. Coxa III. D. Dorsal Seta. E. Leg I. F. Leg II. G. Leg III.

*Remarks*

*Austracarus aridensis* n. sp. can be distinguished from all other members in the genus (*A. procaviae*, *A. campestris*, *A. dendrohyracis*, *A. masonae*, *A. lukoschusi*, *A. wittebolsi* and *A. polydiscum*) with the following characteristics; Palpa setal formula B/B/BBB/7B of this new species differs from that of other known species in the genus (B/N/BNB/7B and B/B/NNB/7B). Number of dorsal 11 and ventral 1 tooth on the cheliceral blade. Legs. Leg II: 1 genuala, 1 microgenuala; tibia 9B; parasubterminala absent. Leg III: femur 3B; genu 2B, 2 mastifemoralae; tibia 3B, 3 mastitibialae; tarsus 13B.

Updated key for members of the genera (adapted from Goff, 1990)

**Key to the Species of *Austracarus***

- |  |  |
|--|--|
| 1. All dorsal idiosomal setae unexpanded                     | 2  |
| Some or all dorsal idiosomal setae expanded                  | 4  |
| 2. Palpal setal formula B/B/BBB/7B                           | <i>aridensis</i> n. sp.                              |
| Palpal setal formula B/B/NNB/7B                              | <i>procaviae</i><br>(Lawrence, 1949)                 |
| 3. Parasubterminala I present; total idiosomal setae 150     | <i>campestris</i><br>(Goff, 1990)                    |
| Parasubterminala I absent; total idiosomal setae 200         | <i>dendrohyracis</i><br>(Vercammen- Grandjean, 1957) |
| 4. AM setae with single accessory branch                     | 5  |
| Am setae lacking accessory branch                            | 6  |
| 5. Galeala N; dorsal idiosomal setae foliate                 | <i>masonae</i><br>(Goff, 1983)                       |
| Galeala B; dorsal idiosomal setae serrate                    | <i>lukoschusi</i><br>(Goff, 1983)                    |
| 6. Posterior dorsal idiosomal and postanal setae cylindrical | <i>wittebolsi</i><br>(Vercammen-Grandjean, 1959)     |
| Posterior dorsal idiosomal and postanal setae dislike        | <i>polydiscum</i>                                    |

(Oudemans, 1910)

**Family Trombiculidae Ewing, 1944**

**Subfamily Trombiculinae Ewing, 1929**

**Genus *Microtrombicula* Ewing, 1950**

***Microtrombicula atlantiensis* n. sp.**

*Type-host:* *Dendromus melanotis* (Smith, 1834)

*Type-locality:* Atlantis, S33° 33.7241', E018° 29.8044', Western Cape Province, South Africa

*Type-material:* 1 Holotypes and 1 paratype

*Etymology:* This species is named after the sampling locality, Atlantis.

Two specimens of *Microtrombicula atlantiensis* n. species were measured.

#### Description of larva (Fig. 4.3)

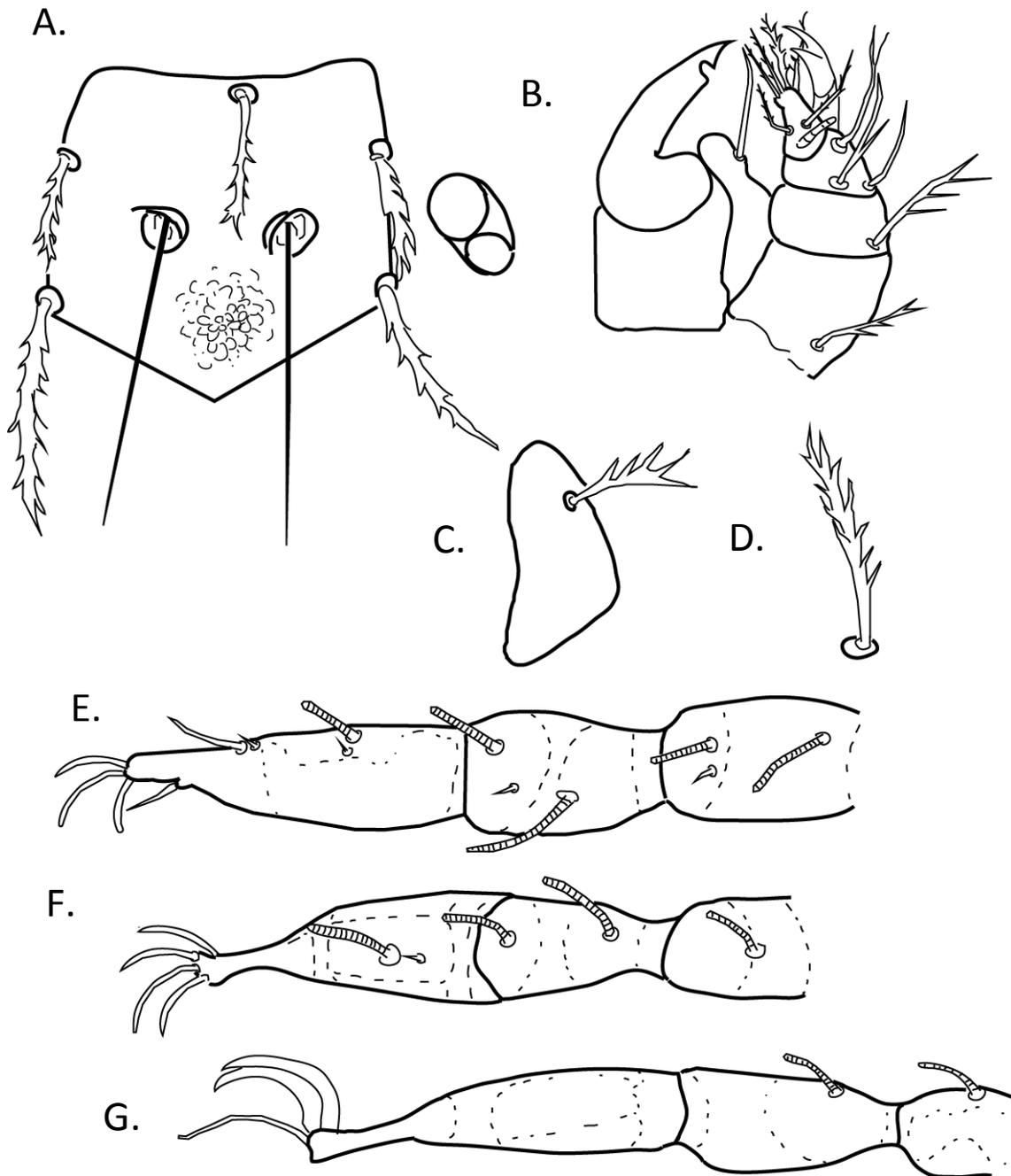
*Diagnosis.* Palpal setal formula, B/B/BNB/6B, palpal claw 2-pronged. Galeala N. Scutum sub-pentagonal with acute posterior margin; width greatest at PL insertion. Scutum unique patterning. SD 23. Scutal measurements (of two specimens): AW 48; PW 48; ASB 14; PSB 9; AP 23; AM 28; AL 24; PL 32; SEN 46; SB 21. DS 42 arrangement unclear. VS 35. Coxa III 1B.

*Idiosoma.* Measuring 295 x 177. Ocelli 2/2, on ocular plate. One pair humeral setae, measuring 34; DS 42, measuring 22-28, arranged unclear; ventral idiosomal setae (VS) 35; anterior sternal setae, measuring 20-22; posterior sternal setae measuring 15-17; pre-anal setae, measuring 16-20, Post-anal setae measuring 18-27; Total idiosomal setae 83 (DS + VS including sternal setae and humeral setae).

*Gnathosoma* (Fig. 4.3, B). Palpal setal formula B/B/BNB/6B; palpal claw 2-pronged; galeala N; cheliceral blade measuring 27, with 1 ventral tooth.

*Scutum* (Fig. 4.3, A). Anterior margin slightly concave; posterior margin acute, width greatest near PL insertion; scutal setae 5 in number; setae branched; single AM setae; 1 pair of AL setae; 1 pair of PL setae; AM posterior to AL bases; SB posterior to PL bases; PL = AL > AM; sensillae flagelliform, nude; scutal measurements (of two specimens): AW 48; PW 48; ASB 14; PSB 9; AP 23; AM 28; AL 24; PL 32; SEN 46; SB 21.

*Legs* (Fig. 4.3, E-G). Segmentation 7/7/7, terminating in a pair of claws and clawlike empodium. Onychotriches absent. IP = 624. Leg I: 227; coxa 1B; trochanter 1B; basifemur 1B; telofemur 5B; genu 4B; genu 4B, 2 genualae, 1 microgenuala; tibia 8B, 2 tibialae, 1 microtibiala; tarsus (62 x 20) 21B, tarsala (12), tarsala-microtarsala (5), 1 microtarsala, subterminala, parasubterminala, pretarsala. Leg II: 190; coxa 1B; trochanter 1B; basifemur 2B; telofemur 3B, genu 3B, 1 genuala; tibia 6B, 2 tibialae; tarsus (56 x 15) 14B, tarsala (13), 1 microtarsala, pretarsala. Leg III: 207; coxa 1B; trochanter 1B; basifemur 2B; telofemur 3B; genu 3B, 1 genuala; tibia 6B, 1 tibiala, tarsus (71 x 14).



**Figure 4.3** *Microtrombicula atlantiensis* n. sp. Dorsal view of larva. Sketch A-G: A. Scutum. B. Gnathosoma. C. Coxa III. D. Dorsal Seta. E. Leg I. F. Leg II. G. Leg III.

#### Remarks

Among species of *Microtrombicula*, *M. atlantiensis* n. sp. was most similar to *M. mastomyia*. *Microtrombicula atlantiensis* n. sp. can easily be distinguished from *M. mastomyia* in having palpal setal formula B/B/BNB/6B (B/B/BBN/4B2N in *M. mastomyia*). Other distinguishing

characteristics include: Scutum: patterning on the scutum, shape of the scutum, lengths of sensilla on scutum, shape and form of SEN. Idiosoma: variations in the number of dorsal setae, idiosoma measurements 295 x 177 (460 x 280 in *M. mastomyia*). Legs: Leg II: tibiala; Leg III: tibiala.

Description of *M. mastomyia* (Radford, 1942). The specimen of *M. mastomyia* was collected in Freetown, Sierra Leone, 1936. The host species was *Mastomys coucha* (Smith, 1834). DS 38 arranged 6-8-6-8-6-4; Idiosoma measurements 0.46 mm x 0.28 mm.

## Chapter 5

### ***Infracommunity dynamics of chiggers (Trombiculidae) on a generalist rodent***

#### **Introduction**

Host individuals are commonly exploited by multiple parasites belonging to different taxonomic groups (Taylor *et al.*, 1998; Poulin, 2007; Pedersen & Fenton, 2007; Matthee *et al.*, 2007, 2010; Rigaud *et al.*, 2014). The parasites that are present on and in a host individual are referred to as an infracommunity of parasites (Poulin, 2007; Krasnov *et al.*, 2010). Although the size of infracommunities may differ between host individuals they are similar in that a community comprises parasite taxa that are dependent on the host for resources (Anderson & May, 1978; Price, 1980; Price *et al.*, 1986; Holt *et al.*, 2003; Shatrov and Kudryashova, 2006; Krasnov *et al.*, 2014). Interspecific species interactions for resources may or may not occur within infracommunities. Communities where interspecific interactions appear to be absent normally have low parasite abundances or irregular co-occurrence of parasite species on the host. These species are characterised by independent niche selection and often display a random distribution in the parasite community (also referred to as isolationist communities) (Rohde, 1979; Bush *et al.*, 1997; Price, 1980; Poulin, 2007; Pilosof *et al.*, 2012). However, interspecific interactions of co-occurring species can result in an overlap of fundamental niches and a possibility of competitive interactions (also referred to as interactive communities) (Poulin, 2007; Pilosof *et al.*, 2012). Competitive interactions commonly result in selective niche segregation and seem to be associated with abundant and prevalent parasite species that co-occur over generations (Bush and Holmes 1986; Stock and Holmes 1988; Poulin 2007). It is through niche segregation that competition is reduced and species are able to co-occur on the host (Poulin, 2007). For example, members of the family Equidae are known to be parasitized by large numbers and a diversity of helminth species (Matthee *et al.*, 2004). Site segregation by helminths is common within equids and examples include Gastrophiliidae that are associated with the stomach and the lining of the stomach and Anoplocephalidae that occur in the caecum and

specifically attaches to the ileocaecal valve (Lichtenfels, 1975). As has been recorded for other parasite-hosts systems the helminth species in equids have a nonrandom distribution pattern and the infracommunity is referred to as structured (Poulin, 2007; Stancampiano *et al.*, 2010). The extent of niche overlap varies between parasite species and narrower niches and co-occurrence of individuals and species have been recorded (Rhode, 1979, 1991; Poulin, 2002). Positive intraspecific encounters and increased mate finding has been suggested as possible mechanisms for restricted niches (Rhode 1979, 1991; Poulin, 2002). Rohde (1991) recorded narrower niches for adult monogeneans in fish during mating compared to juveniles. These mechanisms may be important within species however within guild mechanisms may include avoidance of host defence strategies, morphological adaptations of feeding structure and attachment apparatus and on-host microclimatic conditions (Ramasamy *et al.*, 1985; Poulin, 1998, 2007; Hart, 1990; Piloosof *et al.*, 2002; Piloosof *et al.*, 2012; Krasnov *et al.*, 2014). Spatial segregation of parasites on hosts have been recorded in ecto- and endoparasites of fish and bird hosts (Bush & Holmes 1986; Stock & Holmes, 1987; Cohen *et al.*, 1991; Rosza, 1996) and endoparasites of mammals (Kisielewska, 1970; Lotz & Font, 1985, 1991, 1994; Montgomery & Montgomery, 1989, 1990; Ellis *et al.*, 1999; Behnke *et al.*, 2001; Hillegas *et al.*, 2008). As yet, not much is known with regard to the level of spatial segregation of ectoparasites on mammalian hosts. In a recent study by Piloosof *et al.* (2012) it was recorded that heterogeneric ectoparasite species were not spatially segregated which resulted in similarity in the species composition among body regions on the host. The authors explored various mechanisms that may drive this pattern and concluded that overlap in niche selection in their case may be driven by avoidance of anti-parasitic behaviour of the host (Piloosof *et al.*, 2012). Furthermore, it has been suggested that interactions between different parasite species and taxa are mostly positive when there is less competition for resources and space, e.g. character displacement through evolution of different feeding mechanisms by different parasite taxa to enable them to exploit different resources (Simkova *et al.*, 2002). For example chiggers consume lymph whereas ticks are blood feeders (Krantz & Walter, 2009). Consequently these two species do not compete for the same food resource on the host which potentially lowers competition and increases co-occurrence.

As mentioned above, parasite species often co-occur on the host and the extent of co-occurrence can provide information on the nature of the species association. Species associations are regarded as positive (species aggregate) if the extent of co-occurrence is higher than expected by chance, while a negative species association (species segregate) is observed if the extent of co-occurrence is lower than expected by chance (Krasnov *et al.*, 2005; Tello *et al.*, 2008; Krasnov *et al.*, 2010; Krasnov *et al.* 2011; Pilosof *et al.*, 2012). Numerous studies have investigated parasite community structure in aquatic and terrestrial hosts and it is evident that no clear pattern exists (Krasnov *et al.*, 2005; Poulin 2007; Pilosof *et al.*, 2012). Some studies recorded competitive interactions (Arme & Halton, 1973; Holmes, 1973; Rhode, 1979; Ramansky *et al.*, 1985; Haukisalmi, 1991; O'Callaghan *et al.*, 2006; Lopez-Gomez & Molina-Meyer, 2006; Stancampiano *et al.*, 2010; Anderson *et al.*, 2013; Manica *et al.*, 2013) while other recorded aggregative structure (Bush & Holmes, 1986; Stock & Holmes, 1987; Haukisalmi & Henttonen, 1994; Simkova *et al.*, 2002; Krasnov *et al.*, 2006; 2010; Presley, 2007, 2011; Tello *et al.*, 2008; Pilosof *et al.*, 2012). Although largely unexplored till recently ectoparasite taxa on small mammals seem to follow an aggregative structure (Krasnov *et al.*, 2006; Tello *et al.*, 2008; Krasnov *et al.*, 2011; Pilosof *et al.*, 2012; Sanchez *et al.*, 2013).

To date little is known with regard to the infracommunity dynamics of chiggers (*Trombiculidae*) parasitizing vertebrate and specifically rodent hosts. This may be due to several factors: only the larval stage or "chigger" is parasitic (the rest of the life stages occur in the external environment), the small size of the larvae and taxonomic challenges associated with the identification of chiggers. Chiggers are regarded as host generalists and exploit a wide variety of vertebrate and invertebrate hosts (Goff, 1979; Dietsch, 2008; Arlian, 2009; Krantz & Walter, 2009). It is also not uncommon for a single chigger species to parasitize multiple host taxa within a given environment. For example, *Neotrombicula wartoni* was recorded from 13 different host species in Indiana (USA) (Whitaker & Loomis, 1978). Goff (1979, 1982) also found that chiggers parasitizing small mammals in Papua New Guinea were capable of exploiting an average of five different host species. Furthermore a single host individual is frequently parasitized by multiple chigger species (Mohr, 1952; Daniel, 1961; Goff, 1979, 1982; Dong *et al.*, 2008). More than 58 chigger species were

recorded from a single species of tree-shrew (*Tupaia belangeri*) in China (Dong *et al.*, 2009). Chiggers are also readily recorded in high abundances on host organisms. A total of 4000 chiggers were removed from a single host individual (*Rattus ringens*) and 689 on average per infested rat collected in undisturbed forests in New Guinea (Mohr, 1956). A single chigger rarely attaches to more than one host individual, as such (horizontal) transmission of individuals does not occur within a host population (Krantz & Walter, 2009). The period of attachment to the host varies between species and can range between a few weeks to several months (Daniel, 1961; Traub & Wisseman, 1974). The current study aims to provide a clearer understanding of the infracommunity dynamics of chiggers occurring on a locally abundant and regionally widespread rodent, *Rhabdomys pumilio*. The specific aims of the study were to determine whether: 1) different chigger species prefer certain body regions on the host and whether 2) chigger communities within host individuals are structured and if show if the co-occurrence pattern is aggregated or segregated.

## **Materials and methods**

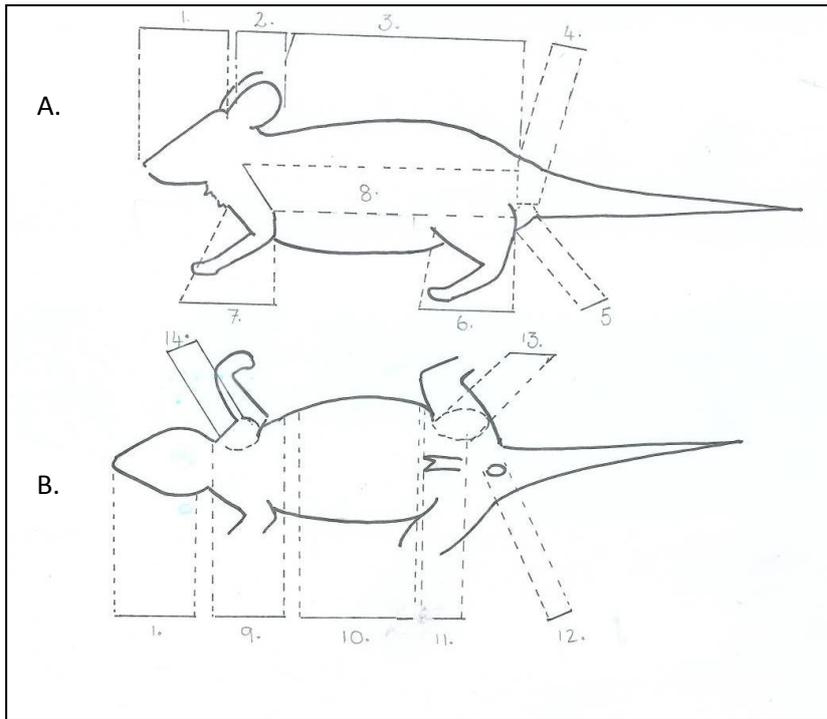
### *Sampling localities*

A total of 108 *Rhabdomys pumilio* individuals were trapped at two sampling localities in the Western Cape Province, South Africa, during January 2013. Seventy three of the animals were captured at Kanu (33° 88' 94.453" S, 18° 81' 89.8" E) and the remaining 35 were trapped at Mooiplaas (33° 92' 10.62" S, 18° 74' 59.8"E). Both localities can be described as remnant fragments of natural lowland fynbos vegetation within an agricultural matrix. However they differ with regards to landscape features. The habitat fragment at Kanu was larger (14 hectares) and more protected (within a shallow valley) compared to Mooiplaas (60 hectares) which was more exposed and had a steep incline.

### *Sampling of rodents and removal of ectoparasites*

A hundred Sherman-type live traps were used at each locality for ten trapping nights. Traps were placed in line transects with 10 meter spacing between traps. The traps were baited

with peanut butter and oats and checked twice daily. Captured animals were taken to the laboratory where they were euthanized using sodium pentobarbital (200 mg/kg). Animals were carefully placed in pre-marked plastic bags and immediately frozen at -80 °C. Prior to examination, animals were thawed and carefully examined under a stereomicroscope. Biological information such as sex, reproductive state (males: scrotal, non-scrotal, moving scrotal and females: non-perforated, perforated and pregnant), weight and measurements were recorded for each animal. The body of the host animal was visually divided into thirteen regions before removal of parasites commenced. The regions were: head, ear, back, tail area, anal area, on hind leg, on front leg, sides, chest, stomach, genital area, fold of hind leg and fold of front leg (Figure 5.1). Specimens were removed with scalpel and forceps and stored in plastic tubes containing 70% ethanol (care was taken to keep chiggers separate that were removed from different parasitopes) until mounting commenced. We recorded the body region from which each individual mite was removed. Chiggers were mounted on microscope slides with Hoyer's medium. Mounted specimens were initially divided into morpho-species groups. Thereafter mites were identified to genus level and if possible to species level using taxonomic keys (Lawrence, 1949; Zumpt, 1961) and with the help of expert taxonomists.



Supplement Figure 5.1: Divisions of body regions of rodent host (sketch A is the side profile and sketch B is the ventral view of the rodent body) 1: Head, 2: Ear, 3: Back, 4: Tail area, 5 & 12: Anal Area, 6: On hind leg, 7: On front leg, 8: Sides, 9: Chest, 10: Stomach, 11: Genital Area, 13: Fold of hind leg, 14: Fold of front leg.

### *Data analyses*

#### *Chigger abundance*

The number of chigger individuals and species was counted for each host individual and calculated for each population as well as for all trapped mammals.

#### *Identification of preferred attachment sites*

Chigger abundance data contained excessive counts of zero, as is the case with many parasite count data (Pilosof *et al.*, 2012). To account for the large number of zeros, we applied zero-inflated models (Zuur *et al.*, 2009) to test for the effect of a body region on chigger number. Separate datasets were generated for each of three most common chigger

species (*Leptotrombidium muridium*, *Shoutedenichia* spp. and *Neoschoengastia* spp. A). For each species we ran two models with mite count as a dependent variable and body region as an independent variable. One model (M1) implemented negative binomial distribution of count component, while another model (M2) implemented Poisson distribution of count component. Because we thoroughly examined the whole body of each animal, both models assumed that false zero have the same probability (i.e. the probability to record a chigger was not related to the body region that was examined). Selection of the best model was done using Akaike Information Criterion (AIC). The coefficient of the best model was selected in each case. The significance of the estimated coefficients for each dummy variable was tested against a reference level which was preselected as the body region with the highest mean count of chiggers, while the significance of the reference level (intercept of the model) was tested against zero (Pilosof *et al.*, 2012). A body region with the highest estimated count of a chigger species was assumed to be the preferred site for this parasite (Pilosof *et al.*, 2012).

#### *Co-occurrence between chigger species in infracommunities*

Null model analyses were carried out on the chigger species associated with *R. pumilio* using the software EcoSim Professional (Gotelli & Ellison, 2013). Only host individuals harboring at least 2 of the three most common chigger species (see above) were included in the analyses. This resulted in a dataset of 95 host individuals. The parasite data for each host individual (infracommunity) were arranged in a presence/absence matrix in which rows represented chigger species and the columns represented body regions. We calculated the C-metric (the average number of checkerboard units that are found for each pair of species: Stone and Roberts, 1990; Gotelli, 2000) to quantify the level of co-occurrence between species in each infracommunity. An observed C-score was calculated for each presence/absence matrix (O, observed index) and compared with the C-scores calculated for 5000 randomly assembled null matrices (E, expected value). The simulated matrices were produced using fixed-equiprobable (FE) algorithm. By not constraining the number of parasite species or taxa that can parasitize a host FE implies that host individuals have equal probability to support a certain number of parasite species. Furthermore FE considers

uninfested hosts as exploitable by parasites, but is uncolonized by chance, rendering the FE algorithm most suitable for analysis of communities of ectoparasites of small mammals (Gotelli & Rohde, 2002; Krasnov *et al.*, 2006). An observed C-score larger than expected by chance ( $O > E$ ) indicates a negative co-occurrence in an infracommunity (i.e. species are segregated), while an observed C-score smaller than expected by chance ( $O < E$ ) indicated that there is a positive co-occurrence and thus species in an infracommunity are aggregated (Gotelli, 2000). To further explore patterns of co-occurrence results across host individuals we calculated the standardized effect size (SES) for each infracommunity matrix. SES measures the number of standard deviations that the observed index is above or below the mean index of simulated matrices (see details in Gotelli and McCabe, 2002) and is calculated by standardizing the difference between the simulated and observed index.

$$SES = (I_{\text{observed}} - I_{\text{simulated}}) / (\sigma_{\text{simulated}})$$

To test the null hypothesis that the average SES across a set of infracommunities was zero we used a one-sample t-tests. Assuming a normal distribution of deviations, approximately 95% of the observed SES values are expected to fall between -2.0 and 2.0.

## Results

A total of 15 553 chiggers, representing seven species were recorded from 108 *R. pumilio* individuals (Table 5.1). Overall, *L. muridium* was the most abundant ( $107.12 \pm 94$ ) and prevalent (94%) species followed by *Schoutedenichia* spp. ( $31.02 \pm 37.33$ ) that was recorded on 85.5% of the hosts and lastly *Neoschongastia* spp. A ( $4.32 \pm 8$ ) recovered from 62% of hosts (Table 5.1).

Table 5.1: Total count, mean abundance ( $\pm$  SD) and prevalence of chigger species recorded from *R. pumilio* trapped at two localities within the Cape Floristic Region, WCP, South Africa.

Chigger species	Total count	Mean abundance $\pm$ SD	Prevalence (%)
<i>Leptotrombidium muridium</i>	11727	107.12 $\pm$ 94.00	94
<i>Schoutedenichia</i> sp.	3311	31.02 $\pm$ 37.33	85.5
<i>Neoschoengastia</i> sp. A	460	4.32 $\pm$ 8.00	62
<i>Ascoschongastia</i> sp.	37	0.34 $\pm$ 2.4	1.7
<i>Schongastia</i> near <i>monticola</i>	13	0.11 $\pm$ 0.39	5.5
<i>Neoschoengastia</i> sp. B	3	0.12 $\pm$ 1.07	1.2
<i>Leeuwenhoekiiinae shunsennia</i>	2	0.04 $\pm$ 0.27	0.9

#### *Identification of preferred attachment sites*

The model that implemented a negative binomial distribution of the count component (M1) was the best model for each of the three species (Table 5.2). In two of the species (*L. muridium* and *Schoutedenichia* spp.) the abundances on the tail region differed significantly from zero and from counts on the remaining body parts (e.g. head and stomach) (Table 5.3, Figure 5.1). In the case of the third species (*Neoschongastia* spp. A) the mean abundance of chiggers was the highest on the tail area, but the value did not differ significantly from zero and there was no significant trend in preferred body region (Table 5.3).

Table 5.2: Akaike Information Criterion (AIC) results indicating the best zero-inflated models for the effect of a body region on counts of three chigger species (*L. muridium*, *Neoschoengastia* spp. A and *Shoutedenichia* spp.).

Species/Model	Df	AIC	AIC weight
<i>L. muridium</i>			
M1	15	5066.64	100
M2	14	15148.08	0
<i>Neoschoengastia</i> spp. A			
M1	15	1433.53	100
M2	14	1751.63	0
<i>Shoutedenichia</i> spp.			
M1	15	2127.92	100
M2	14	4949.15	0

Table 5.3: Zero-inflated model coefficients of chigger count on tail region as compared to intercept for three chigger species.

Mite species	Estimate	Std Error	Z-value	P
<i>L. muridium</i>	3.97	0.15	26.23	<0.001
<i>Neoschoengastia</i> spp. A	0.15	0.33	0.44	0.66
<i>Shoutedenichia</i> spp.	3.16	0.35	8.94	<0.001

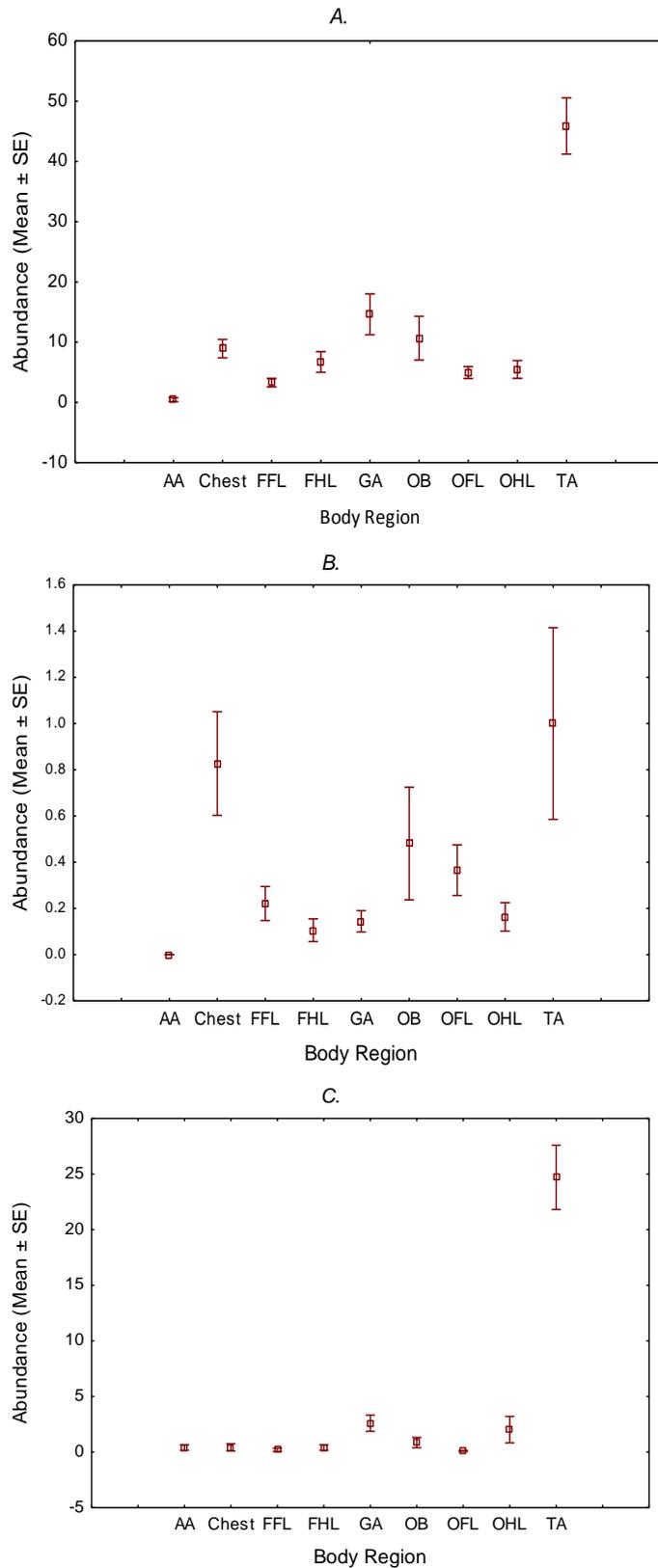


Figure 5.2: Mean abundance of A. *L. muridium*, B. *Neoshoengastia* spp. A and C. *Schoutedenichia* spp. recorded from various body regions of *Rhabdomys pumilio*. AA: anal

area, FFL: fold of front leg, FHL: fold of hind leg, GA: genital area, OB: back, OFL: front leg; OHL: hind leg; TA: tail area.

#### *Establishing the level of co-occurrence between chigger species*

Among the 95 infracommunities of chiggers recorded on *R. pumilio* the observed C-score was never higher than expected by chance. More specifically, observed C-score in 55 of the 95 infracommunities was significantly lower ( $p < 0.05$ ) than expected by chance, in 15 individuals this difference was marginally significant ( $p < 0.09$ ) and for the remaining twenty five individuals the difference between observed and simulated was non-significant ( $p > 0.05$ ). The average SES value for C-score values was  $-2.51 \pm 0.09$  and differed significantly from zero ( $t = -26.48$ ,  $p < 0.001$ ) and from -2 (critical value of SES) ( $t = -5.41$ ,  $p < 0.001$ ).

## **Discussion**

The present study revealed that chiggers have a non-random distribution across the host body with an apparent preference by multiple species for the tail area. Furthermore, the level of co-occurrence between chigger species were in most cases higher than expected by chance which supports previous studies that ectoparasite communities of small mammals are structured and in general aggregative. The various mechanisms that can potentially drive co-occurrence of chigger species will be discussed in more detail below.

#### *Morphological adaptation by chiggers to regions on the host's body*

Parasites are dependant of hosts for resources and over evolutionary time parasites have become adapted to maximize the benefit that they obtain from the host (Price, 1980; Marshall, 1981; Poulin, 1999, 2002; Shatrov and Kudryashova, 2006). The different regions of the host's body differ in terms of skin thickness, hair type, level of exposure and microclimatic conditions (Vansulin and Volkova, 1962; Murray & Nicholls, 1965; Kuris *et al.*, 1980; Marshall, 1981; Sokolov, 1982; Ma, 1983; Pilosof *et al.*, 2012; Krasnov *et al.*, 2014). Resource-type (e.g. blood, lymph and dermal tissue) and morphological adaptation by the

parasite (e.g. feeding and attachment structures) will influence the distribution of the parasite across the host's body (Furman, 1959; Murray *et al.*, 1965; Rust, 1974; Radovsky, 1985; Marshall, 1981; Roubal & Quartararo, 1992; Pedersen & Fenton, 2007). Chiggers attach to the skin of the host by using cheliceral blades. Given the overall small size of chiggers it is not unexpected that their cheliceral blades are small (examples of average cheliceral blade lengths are *Radfordiana nudoseta* 78 µm and *Guntheria scrobiculata* 34 µm) and that they might prefer areas where the skin is the thinnest (Dong *et al.*, 2008). Various studies have found that chiggers of rodents and insectivores readily exploit the ear, intranasal and anal areas (Nadchatram, 1970; Goff, 1979, 1982; Dong *et al.*, 2008, 2009; Mariana *et al.*, 2011). Similarly, a preference for areas where the skin is thinner has also been observed in chiggers on lizards (mite pockets) (Arnold, 1986; Goldberg & Holshuh, 1992; Klukowski, 2004) and bats (wing membrane) (Whitaker & Mumford, 1971; Brennan & Reed, 1975; Spears *et al.*, 1999; Shatrov and Kudryashova, 2006; Pierce & O'Shea, 2007). The present study does not support a preference for thinner skin areas as chiggers were mainly recorded from the tail area of the rodent.

#### *Avoidance of defence strategies of the host*

Grooming and allo-grooming are important defence strategies that are used by host to reduce ectoparasite infestations (Hart, 1990, 1997; Mooring *et al.*, 1996; Hinkel *et al.*, 1998; Mooring *et al.*, 2004; Shatrov and Kudryashova, 2006; Hillegas *et al.*, 2008). As an example, in a controlled study pigeons were experimentally infected with lice, by gluing lice on specific body regions on the birds. Lice that were glued under the wing were more readily removed than those attached to the tail region (Rosza, 1993). When viewing host-parasite relationship in terms of predator-prey dynamics (parasites being the prey), grooming by the host could lead to site segregation or specialization by the parasite towards areas of the body with lower "predation" risk. Cohen *et al.* (1991) isotope-labelled *Amblyceran* lice to enable them to actively track the movements of these lice within the plumage of swans. The study found that lice were capable of orientating themselves and actively migrate towards preferred sites that were less affected by grooming of the host. In the present study it is possible that the hair at the base of the tail is longer and more dense which may provide

more protection to chiggers. Interestingly, a similar preference is displayed by a large mesostigmatid mite *Laelaps giganteus*, that also commonly occur on *R. pumilio*. These mite species also uses cheliceral blades for attachment to the host (Krantz & Walter, 2009).

#### *Facilitation of co-infection through reduced immune response*

Ectoparasites are capable of suppressing the host's immune system which can facilitate colonization by other parasite species (Sheldon & Verhulst, 1996; Wikel, 1999; Combes, 2001; Schmid-Hempel & Egert, 2003; Khokhlova *et al.*, 2004). For example, studies on fleas have indicated that the presence of certain flea species on a host can facilitate the exploitation by other flea species mainly due to a reduction of the host's immune response (Krasnov *et al.*, 2005; Sanchez *et al.*, 2013). In addition, the intensity of a host's anti-parasitic response (immune response or grooming) is limited by the energetic expenditure and time allocated to these actions (grooming). The host will increase anti-parasitic responses in reaction to increased infestation by multiple parasites, however a threshold will be reached when energetic expenses are greater than the reward of removing parasites (Giorgi *et al.*, 2001; Krasnov *et al.*, 2005). Tolerance, by the host, to multi-parasite infestation has also been observed and may be attributed to cross-reactivity between parasite species (Khokhlova *et al.*, 2004; Sanchez *et al.*, 2013). Similarity in salivary components within a taxonomic group can lead to cross-reactivity of a host against closely related parasite species (Sanchez *et al.*, 2013). This can facilitate co-infection by multiple parasite taxa and if combined with immune suppression, of the host, can result in positive species co-occurrence. Upon emergence chiggers are present in great numbers within the environment and they are also restricted to a small area due to limited mobility (Sasa, 1957; Traub & Wisseman, 1974; Goff, 1982; Krantz & Walter, 2009). When an animal moves through the mite foci it will be attacked by multiple (even hundreds) chiggers at once. This could potentially lower the immune response of the host facilitating infestation by even more chigger species. Furthermore, chigger infestations often lead to hair loss, dermatitis and lesions, which could also cause the host's immune system to be compromised (Traub & Wisseman, 1974; Bavaro *et al.*, 2005; Arlain, 2009; Krantz & Walter, 2009; Diaz, 2010; Makajan, 2011). In the present study more than 15 000 chiggers were recorded on the host population. It is therefore quite possible that co-infection between chiggers and other ectoparasite taxa on *R. pumilio* could have reduced the host's immune response.

### *Safety in numbers*

The dilution effect is another theory that is related to the predator-prey relationship. When hundreds of chiggers attach at a specific parasitope some individuals will become dislodged while others will remain attached. Unlike fleas and lice that are capable of avoiding host defences such as grooming by moving actively moving around on the host's body, chiggers are fixed in one place once attachment is achieved (Marshall, 1981; Cohen *et al.*, 1991; Rosza, 1993 Krasnov, 2008; Pulosof *et al.*, 2012). Synchronized infestation by several species in large numbers could dilute the risk of becoming dislodged. Parasites are able to respond to high densities of conspecific and congeneric individuals. A reduction in body size in response to higher densities have been recorded in certain helminth species of vertebrate hosts (Keymer, 1982; Shostak & Scott, 1993; Poulin, 1999). Along the same lines, Goff (1982) found that the level of engorgement of larvae differed with chigger density at a specific parasitope. Chiggers that inhabited areas that were less "crowded" were significantly larger when engorged than those that were attached to more "crowded" parasitopes (Goff, 1982; Kuo *et al.*, 2010). A smaller body size will enable more individuals and species to occur at a preferred parasitope.

To conclude, the different chigger species found displayed a preference for the same body region. This results in an aggregative distribution on the host. Little evidence exists for interspecific interactions and it is therefore suggested that the distribution of chiggers across the host body is most probably driven by a combination of parasite-host interactions.

## **Chapter 6**

### **General Conclusion**

The current study investigated the diversity and ecology of chiggers (Trombiculidae) associated with small mammals across South Africa, with a focus on the Cape Floristic Region. Little information is available on chiggers that are associated with small mammals in South Africa. The majority of existing knowledge is taxonomic or descriptive and biased towards lizard and amphibian hosts. Furthermore, no previous study in the country has performed total counts of chiggers on a host. A lack of clear methodologies for the collection and mounting of specimens could possibly have impeded research on this group. During the current study guidelines were developed for the removal, clearing and mounting of chiggers (Chapter 2). The techniques described will aid in future research on this group that have similar aims (e.g. total counts of chiggers per host).

In Chapter 3 we investigated the diversity, ecology and distribution of chiggers associated with small mammals in South Africa. Currently, limited ecological knowledge exists on chiggers residing in the country. The study revealed that chiggers parasitizing a generalist rodent, *Rhabdomys pumilio*, within the Fynbos biome, exhibited a clear seasonal prevalence. Chiggers were most prevalent during the warm dry months of the year and were absent or recorded in very low prevalences during wet cold months. This is similar to findings in other parts of the world, however temporal variation in chigger abundance is species-specific and chiggers residing in other part of South Africa, with different climatic regimes, can potentially exhibit alternative temporal patterns in occurrence. Trombiculidae are a highly diverse group of temporary parasites that are regarded as host species generalists and habitat specialists. The diversity of chiggers were recorded on several co-occurring small mammals species and it was found that indeed, chigger species occurred on multiple hosts (within and across orders) and that a single host species is often parasitized by multiple chigger species. To date, only eleven species have been described from mammal hosts in South Africa. The study recorded at least three new undescribed chigger species

(*Austracarus* n. sp., *Microtrombicula* n. sp. and *Schöngastiella* n. sp.) (Chapters 3 and 4) and provide evidence that the current species list for small mammals in South Africa is a gross underestimate. Host individuals are not equal in terms of resources provided to parasites. In the present study this theory is supported by the finding that chigger abundances were higher on reproductively active hosts compared to non-reproductively active hosts. Twelve chigger species were recorded at several localities across South Africa. It is evident that the geographic distribution of chiggers is species-specific with some taxa exhibiting a narrower distribution while other species occur across multiple biomes. The underlying mechanisms may be species-specific variation in tolerance to different climatic regimes and microclimatic conditions.

The ecology of chiggers on a generalist rodent host was further researched in Chapter 5. Host individuals are commonly parasitized by multiple parasite taxa. The study recorded a preference for the tail area of the host and this preference is supported by multiple chigger species. This pattern may have facilitated a positive co-occurrence (aggregative) between chigger species on a host. A similar pattern has been recorded for several ectoparasite taxa of small mammals previously.

High species diversity, in addition to species-specific distribution of chiggers on small mammals support the notion that this highly understudied parasite group is in need of well-structured ecology and taxonomy research in South Africa.

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