MITE COMMUNITIES WITHIN *PROTEA* INFRUCTESCENCES IN SOUTH AFRICA

by

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DECLARATION

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GENERAL ABSTRACT

The role of mites as primary vectors of various fungi within *Protea* infructescences was recently confirmed and raised questions about their general diversity and their role within this unique niche. Although mites evidently form an integral part of Fynbos ecosystems and probably play a significant role in *Protea* population dynamics, there is a general void in our knowledge of mite diversity within the Cape Floristic Region. These organisms do not only affect ecological processes within the CFR, but also the economic value of *Protea* exports. This study sets out to describe mite communities within the infructescences of a variety *Protea* species. In the process, the role of various environmental variables and differences in host characteristics affecting these communities are also explored. A total of 24281 mite individuals, comprising of 36 morphospecies in 23 families, were collected from 16 surveyed *Protea* spp. Mite community structure and composition were significantly influenced by plant taxonomy, phenology and infructescence architecture in different *Protea* spp. At a temporal scale, infructescence age and season were influential factors on mite community structure. Collection locality significantly influenced mite communities within the infructescences of a single *Protea* sp. Host architecture had no influence on mite communities within a single host species. Geographic distance had no significant influence on mite community structure within *Protea* infructescences. This implies that factors particular to particular host species determine mite communities. These include factors such as the mode of pollination of the host plant, level of serotiny and plant life form. Numerous newly recorded mite species collected from *Protea* infructescences are also described in this study. An identification key to the Tydeidoidae of South Africa is provided here for the first time. This study forms a baseline dataset for future studies on the biodiversity of mites in this extremely diverse eco-region.

ALGEMENE OPSOMMING

Die rol van myte as primêre vektore van verskeie funguses binne *Protea* vrugtekoppe is onlangs bevestig, en het vrae laat ontstaan oor hulle algemene diversiteit en rol binne hierdie unieke nis. Alhoewel myte duidelik ‘n integrale deel vorm van Fynbos ekosisteme en waarskynlik ‘n belangrike rol speel in *Protea* populasie-dinamika, is daar ‘n algemene leemte in ons kennis van mytdiversiteit binne die Kaapse Floristiese Ryk (KFR). Hierdie organismes affekteer nie slegs ekologiese prosesse binne die KFR nie, maar ook die ekonomiese waarde van *Protea*-uitvoere.

Hierdie studie mik as vertrekpunt om die verkillende myt-gemeenskappe binne die vrugtekoppe van verskeie *Protea* spesies te beskryf. In die proses is die rol van verskillende omgewingsveranderlikes en verskille in gasheer kenmerke wat hierdie gemeenskappe affekteer, ook ondersoek. ‘n Totaal van 24281 myt individue, saamgestel uit 36 morfspesies in 23 families, mytgemeenskappe is beduidende beinvloed deur die taksonomie van die plant, die fenologie en die vrugtekop-argitektuur van verskillende *Protea* spesies. Op ‘n temporale skaal is gevind dat vrugtekop-ouderdom en seisoen beduidende faktore is in die samestelling van mytgemeenskapstruktuur. Versamel-lokaliteit het verder mytgemeenskappe binne die vrugtekoppe mytgemeenskappe binne ‘n enkele gasheerspesie getoon nie. Geografiese afstand het geen beduidende invloed op mytgemeenskapstruktuur binne *Protea* vrugtekoppe getoon nie. Dit faktore in soos die metode van bestuiwing van die gasheer plant, die vlak van saadhoudendheid van die *Protea* koppe en plant-lewensvorm. Verskeie nuwe myt spesies wat uit *Protea* vrugtekoppe versamel is, word ook in hierdie studie beskryf. ‘n Identifikasie-sleutel vir die Tydeidoidae van Suid-Afrika word verder vir die eerste keer hier verskaf. Hierdie studie vorm die basis datastel vir toekomstige studies van die biodiversiteit van myte in hierdie besonder diverse eko-omgewing.

Orbital Consequences

The sun and the earth describe orbital changes which drive climate cycles and modify ranges.

The shape of the land forms a number of places that allow the survival of different races.
When enclaves advance with the ice in retreat some form hybrid zones where two ranges meet.
Such regions are common and yet not very wide so the mixing of genes affects neither side.
They divide up the range in a patchwork of pieces with echoes and glimpses on the nature of species.
A brief rendezvous and the ice comes again

When the glaziers melt so that ranges expand some plants will spread quickly where there's suitable land.
Those insects that eat them will follow this lead
some flying, some walking to establish their breed.
Those that try later meet a resident band,
they must somehow be better to make to make their own stand.
But the mixture will change as more types arrive
And warming conditions allow new species to thrive. Some will move on to fresh places ahead,
Those that remain must adapt, or are dead.
And then the tide turns and the ice comes again.

Each refuge could foster a deviant form,
new neighbors, chance changes and drift from the norm.
When the warm breakout comes, those few in the van disperse from the edge and breed where they can.
Pioneer pockets grow to large populations,
a very good place to strike new variations.
Some may not work well with their parental kind
So stopping the spread of those from behind.
Continental themes provide plenty of chances
to establish new morphs in both retreats and advances.
New species may form when the ice comes again.

So what will you do when the ice comes again?
It could be quite quick, if the ice cores speak plain.
The great ocean currents that warm our green spring
may stop in a season should the salt balance swing.
Great civilizations in north temperate lands
must migrate south to the sun and the sands.
But past pollen and dust tells us these will be drier, wet forests will shrink and population grow higher.
Our forebears hung on near a sea or a cave.
They fished and they painted, they dreamed they were brave
So like Noah and Eric, we must adapt and survive

G. M. Hewitt

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My heavenly Father

I dedicate this work

to my

mother (Amanda Theron) who always believed in me and supported me throughout my studies

and

to my loving grandmother (Athaliah Eichstedt) and aunt (Alice Nieuwoudt), who would have been so proud.

Love you

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CHAPTER 1

GENERAL INTRODUCTION

1. BIODIVERSITY IN THE CAPE FLORISTIC REGION

1.1. The Cape Floristic Region

The Cape Floristic Region (CFR) is confined to the southwestern tip of Africa (between the 31˚ and 34˚30´S latitudes) and comprises an area of only 87,892 km^2 (Cowling *et al*., 2003; Goldblatt, 1997; Goldblatt and Manning, 2002) (Fig. 1). This highly threatened region is regarded as a global conservation priority area due to its unusually high levels of endemism (Goldblatt, 1997; Holmes and Richardson, 1999; Schwilk *et al*., 1997). Of the approximately 9030 vascular plant species that are found in the CFR, 68.7% are endemic (Goldblatt, 1997; Goldblatt and Manning, 2002; Linder, 2003). On a global scale, the CFR rates as one of the most diverse eco-regions, with levels of diversity comparable to that of tropical rainforests (Cowling *et al.*, 1992).

In addition to the high floral diversity, the CFR also houses numerous vertebrates including mammals (Fleming and Nicolson, 2002; Rourke and Wiens, 1977; Wiens *et al*., 1983), birds (Sinclair and Davidson, 1995; Wiens *et al*., 1983), amphibians (Carruthers, 2001), arthropods (including insects (Picker *et al*., 2004), spiders (Visser *et al.*, 1999), scorpions (Leeming, 2003), mites (Lawton *et al*, 1988) and fungi (Lee *et al.*, 2003 and 2005). Many species in these groups are endemic, with 46% of the amphibians, 16% of the reptiles and 13% of fish confined to the CFR

(Cowling *et al*. 2003; Taylor *et al*., 2001). In addition, there is an estimated 42 000 unique fungal species in the CFR, representing 20% of the estimated total number of fungal species in South Africa (Crous *et al.* 2006).

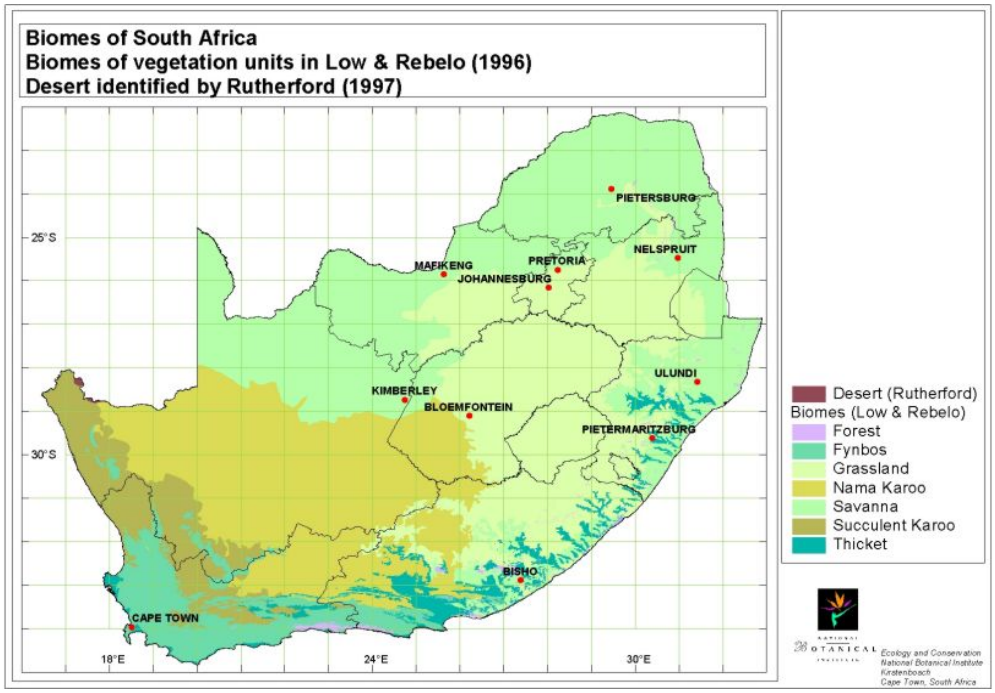

Figure 1: Map of the major biomes of South Africa. The CFR includes the Fynbos, Succulent Karoo and a portion of the Forest biome (South African National Biodiversity Institute, Kirstenbosch).

As a result of this high floral and faunal diversity, the high endemism levels and the high number of rare and endangered species (Viè *et al*., 2009), the CFR is recognized as a reservoir for biodiversity (Holmes and Richardson, 1999; Wright and Samways, 2000). Internationally, the CFR is recognized as an Endemic Bird Area (Scharlemann *et al.*, 2004), one of the Global 200 Ecoregions (Olsen and Dinerstein, 2002), it is on the Centre of Plant Diversity list (Hobohm, 2003) and is a global biodiversity hotspot (Cowling *et al*., 2003; Higgins *et al*., 1997). Most of the CFR biodiversity is confined within Fynbos (including the Renosterveld) (Mucina and Rutherford, 2006).

1.2. Fynbos

Of the eight vegetation types represented in the CFR, the fynbos is the most characteristic (Mucina and Rutherford, 2006). Fynbos, translated as "fine bush", refers to the small-leaved, low-growing, shrubby nature of the plant species that dominate this system. This fire dependant vegetation type is defined based on the co-occurrence of members of any two of the following three plant families Proteaceae, Restionaceae and Ericaceae (O'Brien, 1994). Of these, the Proteaceae is often the structurally dominant member, and included species are considered keystone members of Fynbos communities.

In addition to the biodiversity value of Fynbos, it is of immense economic importance to South Africa. Important economic contributions include ecotourism, pollination of agricultural crops, water supply regulation and beekeeping (Hassen, 2003; Le Maitre *et al.*, 1997; Turpie *et al.*, 2003). In addition, numerous plant species are used for food and medicine (Hassen, 2003; Higgins *et al.*, 1997; Le Maitre *et al.*, 1997; Turpie *et al.*, 2003) and in the building industry (Hassen, 2003; Le Maitre *et al.*, 1997). The flower industry, however, remains the most important generator of income from Fynbos (Hassen, 2003; Higgins *et al.*, 1997; Leonhardt and Criley, 1999; Le Maitre *et al.*, 1997). In this regard, South Africa has established itself as the global leader in the production of protea (including all members of Proteaceae) cut-flowers, with an estimated 3,000 hectares under cultivation (Parvin *et al.*, 2003). This represents 50% of the global protea cut-flower market (Parvin *et al.*, 2003) and generates over 30 million US$ annually (Taylor, 2001; Crous *et al.*, 2004).

1.3. Proteaceae

The Proteaceae is an ancient group of plants (*ca*. 96 million years old), dating back to the Cretaceous (Barker *et al*., 2002, 2004 and 2007; Taylor *et al*., 2001). The family diversified during the Eocene (Barker *et al*., 2007; Itzstein-Davey, 2004), just prior to the break-up of the supercontinent Gondwana in the Mesozoic (Leonhardt and Criley, 1999). This evolutionary history explains the current distribution of the family, with most members confined to the southern Hemisphere. The family is represented by 80 genera and 1,700 species (Barker *et al*., 2007); with fourteen genera and 330 species found in the south-western Cape region of South Africa alone (Bond and Maze, 1999; Goldblatt, 1997; Rebelo, 2001). Proteaceae is even better represented in Australia, including 45 genera and over 800 species. A few members of the Proteaceae are also found in New Guinea, New Caledonia, Central and South America, Madagascar, New Zealand and Asia (Rebelo, 2001). Ninety seven percent of all CFR Proteaceae members are endemic and most of these are confined to the Fynbos (Cowling *et al*, 2003). Speciose South African genera include *Protea*, *Leucospermum*, *Leucadendron* and *Serruria*. Of these, the genus *Protea* is probably the best known internationally, and also includes the national flower of South Africa (*P. cynaroides* (L.)L.)

1.4. Protea

Protea forms the cornerstone of the South African cut-flower industry, comprising up to 30% of flowers being exported (Coetzee and Littlejohn, 2001). As a result, information on the association of *Protea* species with other organisms is very important, especially in terms of possible phytosanitary problems that might lead to major monetary losses. *Protea* is the type genus of the Proteaceae and includes species with diverse growth forms ranging from trees and shrubs, to plants with underground rhizomes and even forms with spherical underground boles and emerging branches

(Rebelo, 2001). The genus *Protea* contains 136 species world-wide, with 117 of these native to the African continent (Leonhardt and Griley, 1999), in which it is the largest member of the Proteaceae (Rourke, 1998). Rebelo (2001) recognized 90 species of *Protea* in South Africa, of which most are confined to the Fynbos. The genus is characterised by 1) involucral bracts surrounding the flower head, 2) hairy, woody fruits, and 3) one free and three fused perianth segments (Fig. 2).

The western Cape *Protea* species have diversified significantly in comparison to the tropical and subtropical species. Morphological adaptations of Cape *Protea* species were aided by selection pressures posed by avian and rodent pollinators (Rourke, 1998). This, combined with alterations made to survive in a fire prone region, resulted in relatively higher generation turnover times (30 – 40 years), and ultimately to rapid diversification of *Protea* species in this region (Rourke, 1998).

Protea inflorescences comprise of many flowers grouped together on a flat involucral receptacle and the flowers are surrounded by large, colourful bracts (Fig. 2). Most *Protea* species are self-incompatible and therefore pollination plays a key role in the reproduction of these plants. The range of *Protea* growth forms and inflorescences morphologies facilitate the utilization of a variety of different pollination syndromes. Rodent-pollinated *Protea* inflorescences generally have a musty smell and are produced at ground level. Bird-pollinated inflorescences are brightly coloured and only slightly odoured to attract birds. Numerous bird-pollinated *Protea* inflorescences also attract many different insect visitors. These inflorescences are typically smaller in size and likely to be pink to cream coloured. Inflorescences of insect-pollinated *Protea* species often also house populations of the *Protea* itch mite (Rebelo, 2001; Fleming and Nicolson, 2003). After seed set, seeds are either stored in seedheads (infructescences) that will accumulate on the plant until their water supply cease or they are released after a certain ripening period (Rebelo, 2001).

Figure 2: *Protea repens* (L.) L. inflorescences (left) and infructescences (right). These represent mini- ecosystems sustaining an immense biodiversity with largely unexplored biotic interactions.

Inside these infructescences a variety of organisms such as insects (Wright and Samways, 1999), fungi and mites (Roets *et al*., 2007) thrive. These infructescences can therefore be viewed as mini-ecosystems with different tropic levels that house numerous arthropods species (Coetzee, 1984; 1986).

2. ARTHROPODS ASSOCIATED WITH SOUTH AFRICAN *PROTEA* SPECIES

2.1. Insects

Numerous studies have explored the relationships between arthropods and Fynbos flora in the form of bio-geographical studies (Terblanche and Hamburg, 2003; Wright and Samways, 2000), monitoring systems and management strategies (Botes *et al*., 2006; Swengel, 2001; Wright and Samways, 1999), assessments of diversity patterns (Giliomee, 2003; Lee *et al*., 2005; Proches and Cowling, 2006), explorations of pollination dynamics (Hargreaves *et al*., 2004; Johnson and Nicolson, 2001; McCall and Primack, 1992; Nicolson, 2002) or studies of evolutionary patterns and speciation (Bernhardt, 2000; Wright and Samways, 1996, 1998). Some studies have specifically focused on the diversity of arthropods associated with *Protea* species. These include studies on ants, bees, beetles and spiders (Coetzee, 1984; Hargreaves *et al*., 2004; Visser *et al*., 1999; Wright and Giliomee, 1992; Wright and Saunderson, 1995). Although arthropod associations with *Protea* have been fairly extensively studied, none of these studies have attempted to compile an extensive diversity assessment of mites.

At present, very little is known about mite diversity in general and even less so with reference to Fynbos. Recent studies by Roets *et al*., (2007; 2009a,b) explored the inter-organismal interactions between ophiostomatoid fungi and *Protea* species, identifying mites as primary and insects as secondary fungal spore vectors within this system. Their results highlighted the importance of mites in ecosystem dynamics, and underscored the void in our knowledge of mite diversity within the CFR. Mites evidently form an integral part of Fynbos ecosystems and probably play a significant role in *Protea* population dynamics.

2.2. Mites

Mites (Acari) are one of the oldest and most diverse groups of Arachnids, which includes an estimated 500,000 species (Krantz and Walter, 2009). They can be found in every habitat type, from tropical forest canopies to marine and freshwater habitats. They are found in the Polar Regions and even in thermal springs with temperatures reaching 50˚C (Krantz and Walter, 2009). Mites are an ecologically diverse group of animals. This is exemplified in the large diversity of feeding guilds that include parasites, predators, fungivores and various decomposers (Proctor and Owens, 2000; Roets *et al*., 2007; Krantz and Walter, 2009). The group is divided into three super-orders: Opilioacariformes, Parasitoformes and Acariformes, with the former two super-orders considered as sister taxa (Domes *et al*., 2007). The Acariformes can be further divided into the Prostigmata, Astigmata, Oribatida and the paraphylectic group, Endeostigmata (Domes *et al*., 2007; Walter *et al*., 1996). There are about 45,000 described species of mites, but this is estimated to represent a mere 5% of the total number of extant species out there (Walter *et al*., 1996).

A recent study by Roets *et al*. (2007) suggested there to be a very large diversity of mites associated with *Protea* infructescences. The study focused on the description of mutualistic associations between certain fungal groups that inhabit these structures and various infructescence-colonizing mites. The fungus is transported between different host plants by the mites and in turn it serves as food source for these mites. To facilitate the transport of symbiotic fungal spores, some of these mites have evolved specialized spore-carrying structures (Roets *et al.*, 2007). The spore-carrying mites are transported between *Protea* plants by pollinating beetles (Roets *et al*., 2009a). Similarly, Childers *et al*. (2003), Van der Geest *et al*. (2000) and Van Doorn (2001) showed that various mites are important vectors of fungal and other plant diseases. It is thus reasonable to assume that mites will influence *Protea* population dynamics by vectoring diseases (Van der Geest *et al*., 2000),

protecting seeds whilst feeding on fungi (Romero and Benson, 2004) or act as predators controlling pests (Pringle and Heunis, 2006). With such diverse ranges of feeding guilds and ecological functions it is further reasonable to assume that they may also have a great diversity within *Protea* infructescences.

3. DESCRIBING BIODIVERSITY

The first step in understanding most ecological processes in any ecosystem is to determine its basic biological components (biodiversity). Biodiversity is defined by Noss and Cooperrider (1994) as the diversity of all living organisms including their genetic variances. This includes interactions between communities, ecosystems, and the ecological and evolutionary processes influencing them. An understanding of biodiversity facilitates the overall interpretation of complexity, stability, productivity and economic value of ecosystems (Bengtsson, 1998; McCann, 2000; Purvis and Hector, 2000; Tilman, 2000). Biodiversity conservation is considered vital in insuring normal ecosystem functioning. Biodiversity loss leads to simplified and unstable ecosystems. The documentation of biodiversity and understanding the processes that create and sustain it is thus of the utmost importance.

Various methods have been introduced by which to describe biological diversity. Usually however, it requires the determination of species richness, density, the identification of keystone species and description of functional groups (Bengtsson, 1998). Of these, species richness has most widely been used to explain biodiversity patterns (Hortel *et al*., 2006). Species richness alone is, however, usually insufficient to explain diversity patterns and needs to be combined with other measurements such as species density, species accumulation and/or rarefaction (Bengtsson, 1998; Gotelli and Colwell, 2001; Petchey and Gaston, 2002; Purvis and Hector, 2000).

3.1. Species richness and diversity

Species richness is defined as the total number of species present in a specific community at a specific time. It is the most generally used indicator of biodiversity (Heltshe and Forrester, 1983; Hortel *et al*., 2006; Mittelbach *et al.*, 2001; Olofsson and Shams, 2007; Whittaker *et al*., 2001). However, to reiterate, using species richness alone as indicator of diversity has shortcomings. For example, species richness is directly influenced by sampling effort, methods used, time factors and scale (Lomolino, 2001; Sobernón and Llorente, 1993). The definition of a species is also under intense debate, making it difficult to precisely determine the number of species within a given area. Another shortcoming of species richness as indicator of species diversity is that it does not take species evenness into account. A better measure for species diversity would thus also take relative abundances of species into account. Simply defined, species diversity is thus the total number of different species in a particular area (species richness) weighted by some measure of abundance (number of individuals or biomass).

3.2. Species density and diversity

Species density refers to the mean number of species per sampled area (Gross *et al*., 2000). Under certain conditions this method is a more precise measure of species diversity than species richness alone, but is less widely used (Whittaker *et al*., 2001). The most common use of species density measurements is to standardize sampling effect (Gross *et al*., 2000). Thus, when two sample sites differ in unit size, one would rather compare species densities than total species richness.

3.3. Species accumulation and diversity

Species accumulation refers to the number of new species added to the overall sample as the number of sampling units or sampling areas increases continuously and is usually represented as a species accumulation curve (Thompson and Withers, 2003). Species accumulation curves are generally used to determine optimal sample size for a given research question. Species accumulation curves are also useful to detect keystone structure in ecosystems (Tews *et al*., 2004) and can provide valuable information on species composition and richness (Thompson and Withers, 2003). Like species richness, however, species accumulation is also directly influenced by sampling intensity and technique (Thomson and Whithers, 2003) and should thus be used with caution (Sobernón and Llorente, 1993). Also, if sampling is partial in time, for example when sampling is conducted only during a single season, it is incongruous to extrapolate any generalizations (Sobernón and Llorente, 1993).

Species diversity alone explains very little about ecosystems structure or processes. Changes in species diversity, can however, be used to identify factors that influence it. Factors that influence species diversity include biotic factors such as spatial heterogeneity and symbiotic interactions such as competition and predation (Stilling, 2002); and abiotic factors such as climate, time and spatial scale, anthropogenic influences and even evolutionary speed (Loreau *et al*., 2001; Tilman, 2000).

4. AIMS OF THE STUDY

The present study sets out to describe the diversity of a little known group of arthropods, the mites (Acari) associated wit the fruiting structures of *Protea* species. In this process, the influence of both biotic and abiotic factors is also described. Chapter 2 deals with determining the influence of host plant characteristics, infructescence phenology and season on mite community structure within the infructescences of numerous *Protea* species. In Chapter 3 the influence of host biogeography on mite community structure is investigated both within a single *Protea* species and between different *Protea* species. Probably because this study constitutes the first attempt to describe mite communities associated with *Protea* species, numerous new species and genera were collected. In Chapter 4 a new genus and eight new species of mites collected from *Protea* infructescences are described. The thesis will conclude with an overview of what is currently known about mite diversity on *Protea* and a discussion of the implications of the results obtained in this study.

5. THESIS STRUCTURE

Chapter 1 gives a general introduction to *Protea* in the Cape Floristic Region and their associated organisms.

Chapter 2 summarizes results of studies into factors that may influence the mite communities associated with *Protea* infructescences including: host taxonomy, plant architecture, infructescence phenology and temporal variations. This chapter is envisaged to result in two possible publications: 1) A MATHEMATICAL METHOD TO DESCRIBE MICROENVIRONMENTAL STRESS WITHIN PLANT FRUITING STRUCTURES. 2) MITE COMMUNITIES WITHIN *PROTEA* INFRUCTESNCES: THE INFLUENCE OF PLANT TAXONOMY, ARCHITECTURE, PHENOLOGY AND SEASON.

Chapter 3 deals with the influence of host intra-species variation and geographic distribution on mite communities associated with the infructescences of *Protea* species The following paper may result from these results: MITE COMMUNITIES WITHIN *PROTEA* INFRUCTESCENCES: THE EFFECT OF HOST INTRA-SPECIES ARCHITECTURAL VARIATION AND HOST BIOGEOGRAPHY.

In **Chapter 4** numerous new mite species that were collected in this study are taxonomically described and evaluated. A paper based o this chapter is currently in the submission process for the journal *International Journal of Acarology*. The paper is entitled: A NEW GENUS AND EIGHT NEW SPECIES OF TYDEIDOIDAE (ACARI: TROMBIDIFORMES) FROM *PROTEA* SPECIES IN SOUTH AFRICA.

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CHAPTER 2

MITE COMMUNITIES WITHIN *PROTEA* INFRUCTESNCES: THE INFLUENCE OF PLANT TAXONOMY, ARCHITECTURE, PHENOLOGY AND SEASON

ABSTRACT

Mites are the primary vectors of various *Protea*-associated fungi e.g. ophiostomatoid fungi and may thus influence the ecology of these plants. Very little is, however, known about the biotic and abiotic factors that influence the association between mites and *Protea*. In this study we investigated factors that may influence mite communities within the infructescences of various *Protea* species collected from across South Africa. The influence of host taxonomic group, plant architecture and various environmental variables were investigated. Mite community structure is significantly influence by a variety of factors, including the taxonomic grouping of *Protea* species, plant life form and modes of pollination. Infructescence architecture, infructescence age and time of year (season) had a significant influence on mite abundance, but not on mite morphospecies richness. Mite communities showed some specificity towards host plants and certain mite morphospecies seemed to be host specific. This study provides baseline data on factors that may influence the association between mites and various *Protea* species. The exact role that these organisms play in the ecology of their hosts, however, still needs further investigation.

Keywords: Acari, *Protea*, environmental conditions, variables, infructescence structure.

1. INTRODUCTION

The Cape Floristic Region (CFR) is confined to the southwestern tip of Africa (between the 31˚ and 34˚30´S latitudes) and comprises an area of only 87,892 km^2 (Cowling *et al*., 2003; Goldblatt, 1997; Goldblatt and Manning, 2002). This highly threatened region is regarded as a global conservation priority area due to its unusually high levels of endemism (Goldblatt, 1997; Linder, 2003). Most of the CFR biodiversity is confined within Fynbos (Mucina and Rutherford, 2006). Of the eight vegetation types represented in the CFR, Fynbos is predominant (Cowling, 1990). Within Fynbos, the Proteaceae is often the structurally dominant members (Richardson *et al*., 1987) and the family is considered to be a keystone member (Littlejohn, 2001).

The type genus *Protea* consists of 136 species globally, with 117 of these native to the African continent (Leonhardt and Griley, 1999). South Africa alone houses 90 species of *Protea* (Rebelo, 2001). It forms the cornerstone of the South African cut-flower industry, representing up to 30% of all exported flowers, and generating an annual income of over US $10 million (Coetzee and Latsky, 1986; Coetzee and Littlejohn, 2001; Crous *et al.*, 2004). Single *Protea* inflorescences (flower heads) comprises of many closely packed flowers surrounded by (mostly) colourful bracts (Rebelo, 2001). These brightly coloured flowerheads attract a variety of pollinators, including numerous bird and insect species (Rebelo, 2001). After seed set, the fruits of serotinous species are retained in a fire-protected woody infructescence (fruiting structure) for several years. During this time these infructescences accommodate a variety of organisms, including insects (e.g. Coetzee, 1984; Wright and Samways, 1999) and fungi (e.g. Lee *et al.*, 2005). Recent studies also revealed that these structures may be home to a large number of mite species (e.g. Roets *et al*., 2007).

Numerous studies have focused on distribution patterns and the ecological role of *Protea* species in the CFR (Rebelo, 2001). Changing ecological conditions, either at a broad scale for example in climate change (Bomhard *et al*., 2005; Midgley *et al*., 2002; Midgley *et al*., 2006; Williams *et al*., 2005), or at local scale for example in root rot in proteas (Turnhill and Crees, 1995), have a significant impact on *Protea* populations and directly and indirectly influence their associated organisms (e.g. arthropods). Arthropods not only play a major role in the pollination of *Protea* (Johnson, 2004), but also pose major threats to their survival and to their monetary value (phytosanitary). Threats posed to *Protea* species caused by arthropods include herbivory (Jonhson and Nicolson, 2001; Wright and Giliomee, 1992) and disease vectoring (directly by vectoring the pathogen or indirectly by vectoring the fungi or mites that carry the pathogens (Roets *et al*., 2007; Van der Geest *et al*., 2000)).

At present, very little is known about mite diversity in general and even less about mite diversity of Fynbos. Recent studies by Roets *et al*. (2007; 2009a, b) explored the interactions between ophiostomatoid fungi and *Protea* species. They identified mites as the primary and insects as secondary fungal spore vectors within this system. Their results highlighted the importance of mites in ecosystem functioning, and underscored the void in our knowledge on mite diversity within the CFR. In contrast, other arthropod groups associated with *Protea* infructescences are fairly well-studied. For example, Wright and Samways (1999) tested a variety of environmental factors, including host-plant variables, on the frequency of occurrence of infructescence-associated insect borer assemblages. They found that it was primarily host-plant characteristics that determined borer frequencies. In their study, Roets *et al*. (2006) concluded that *Protea* infructescence-associated arthropods have higher species richness and abundances in *Protea* species that produces larger infructescences. It was also shown that both seasonal variations and infructescence age influenced

arthropod assemblages (Coetzee *et al*., 1986). It is still unknown whether host-plant characteristics and seasonal changes influence mite assemblages in this unique niche.

Mites are extremely under-studied, despite possibly playing a key role in *Protea* population dynamics. A first step towards determining their ecological role is to understand the underlying factors that influence their community structure and survival. Therefore, the main aim of this study was to document infructescence-associated mite diversity in various *Protea* species. We specifically investigated various factors that may influence mite population dynamics in these *Protea* infructescences. These factors include host-plant relatedness (taxonomy), infructescence architecture (volume and degree of openness), infructescence maturity, temperature and moisture conditions within infructescences and seasonality. The key questions that were asked are: Does a mite community structure vary between *Protea* species according to the taxonomic relatedness of the plants? b) How are mite communities influenced by specific host-plant characteristics (e.g. infructescence structure, plant life form, level of serotiny and mode of pollination)? c) Is there a difference between mite communities in infructescences of different age classes? d) Do mite communities change seasonally?

2. MATERIALS AND METHODS

2.1. The effect of taxonomic similarity between host plants and different host plant characteristics on mite community assemblage structure

A total of 10 infructescences (where possible) of 16 *Protea* species were collected during autumn (April and May) 2009 from various sites across South Africa (Table 1). Species were chosen to represent a wide range of taxonomic groups following the morphological classification system of Rebelo (2001). Infructescences were collected from randomly chosen plants (maximum of 3 infructescences per plant) and stored at 4ºC until further processing in the laboratory. Before extraction of mites, all infructescences were measured (see section 2.2). Infructescences were opened using secateurs and bases were cut into four quarters. Mites were extracted from each quarter by tapping the infructescence base with a hard object over a Petri-dish until no more individuals were observed to fall into the dish. All extracted mite individuals were collected using a fine brush, and placed in Eppendorf tubes containing 80% alcohol. For identification of mite morphospecies, mite specimens were mounted on microscope slides in HPVA medium (Krantz and Walter, 2009) and examined using a Zeiss Axioskop Research microscope. Mite morphospecies were identified to the lowest taxonomic rank possible at the Agricultural Research Centre (ARC), Roodeplaat, Pretoria, South Africa. Reference material was deposited in the National Collection of Arachnida, ARC-Plant Protection Research Institute, Pretoria, South Africa, as well at the Department of Conservation and Entomology Museum, Stellenbosch University, Stellenbosch, South Africa.

Table 1: Sampling sites and taxonomic groupings (according to Rebelo, 2001) of *Protea* species assessed in this study.

Species	Site	Taxonomic Group (Sugarbushes)	Degrees South	Degrees East
P. lanceolata	Albertinia	True	34˚ 04" 58.80'	21˚ 15" 20.52'
P. obtusifolia	Aghulas Nature Reserve	Spoon-bract	34˚ 48" 49.32'	20˚ 01" 15.00'
P. acaulos	Steenbok Park, Bainskloof,	Western Ground	34˚ 06" 05.10'	19˚ 49" 46.08
P. glabra	Pakhuis Pass, Clanwilliam	Shaving-brush	32˚ 08" 05.10'	18˚ 57" 41.64'
P. aurea	Montaque Pass, George	White	33˚ 52" 01.20'	22˚ 25" 54.00'
P. laurifolia	Gifberg (summit), Van Rhynsdorp	Bearded	31˚ 45" 46.38'	18˚ 47" 17.64'
P. repens	Jonkershoek Reserve, Stellenbosch	True	33˚ 58" 40.02'	18˚ 56" 39.36'
P. neriifolia	Jonkershoek Reserve, Stellenbosch	Bearded	33˚ 59" 14.58	18˚ 57" 15.30
P. nitida	Jonkershoek Reserve, Stellenbosch	Shaving-brush	33˚ 59" 48.30	18˚ 56" 26.88'
P. caffra	Groenkloof Reserve, Pretoria	Grassland	25˚ 46" 58.92'	28˚ 11" 56.64'
P. coronata	Riversdal	Bearded	34˚ 04" 58.98'	21˚ 15" 20.46'
P. burchelli	Stellenbosch Mountain, Stellenbosch	Spoon-bract	33˚ 56" 44.58'	13˚ 52" 42.66'
P. susannae	Struisbaai	Spoon-bract	34˚ 45" 02.94'	19˚ 58" 48.60'
P. eximia	Swartberg Pass, Oudsthoorn	Spoon-bract	33˚ 21" 59.10'	22˚ 05" 46.44'
P. lorifolia	Swartberg Pass, Oudsthoorn	Bearded	33˚ 22" 11.22'	22˚ 06" 33.90'
P. punctata	Swartberg Pass, Oudsthoorn	White	33˚ 21" 48.24'	22˚ 03" 50.04'

Species accumulation curves for mite morphospecies associated with each of the 14 *Protea* species for which 10 infructescences could be collected, were calculated using the software programme EstimateS ™ v.7.5.2 (Colwell, 2005, USA) with 50 randomizations of samples. In addition, the combined mite species accumulation curve for all *Protea* hosts was calculated using the same programme and parameters. Estimated species richness was also calculated in Estimates S, using the non-parametric and least biased species richness estimators ICE, Chao2 and Jacknife2 as these

provide the best overall estimates (Hortel *et al*., 2006). This is especially true where a large number of rare species are present in samples (Novotny and Basset, 2000). To test whether mite communities are host taxonomic group specific (Rebelo, 2001), presence-absence data of mite morphospecies were used to construct a dendogram based on Bray-Curtis similarity between mite communities associated with the infructescences of the various *Protea* species using the software programme Primer v.5.2.9 (Clarke, 1993). A log-rank abundance curve was generated and rare species were defined according to the lower quartile definition (Gaston, 1994).

2.1.1. Host plant characteristics

A variety of host plant characteristics that may explain the observed mite assemblages were explored. *Protea* taxonomic groups were considered and categorized according to Rebelo, (2001). Plant life form of each *Protea* species was established based on plant height, and they were grouped into the following classes: ground level (≤1 meters), shrub (> 1 meters, but < 5 meters) and tree (≥ 5 meters). As different mites can potentially be vectored by different pollinators, the influence of the mode of pollination on mite communities of the various *Protea* species was investigated (Roets *et al.*, 2007). Inflorescence colour is generally related to pollination mode (Melendez-Ackermann *et al.*, 1997; Carlson and Holsinger, 2010). *Protea* inflorescence colour was thus used to categorize the *Protea* species into four broad colour groups; pink, silvery pink, cream and brown (Rebelo, 2001). The retainment of infructescences (serotiny) may impact mite community structure due to the longer retainment of their micro-habitats on serotinous plants than on non-serotinous plants. Level of serotiny was categorized into non-serotinous (retainment less or equal to one year) and serotinous (retainment more than one year) based on the fruit retainment period (Rebelo, 2001). The possible influence of soil type was also investigated as it may be a source of infructescence-colonising mites after fires. Soil was divided into three main types: sand (sandstone), lime (limestone) and loam

(loamy soils). Infructescence architecture (volume) was previously shown to affect arthropod assemblages (Roets *et al.*, 2006) and was thus included in this present study. Large, closed infructescences (e.g. those of *P. repens*) are expected to retain moisture better than small, more open infructescences (e.g. infructescences of *P. acaulos*). The degree of infructescence openness may thus influence the stability of temperature and relative humidity (microclimatic stability) within infructescences, which may also affect mite assemblages within this niche (Willmer in Juniper & Southwood, 1986). Infructescence volume and microclimatic stability were calculated as explained in sections 2.1.1.1 and 2.1.1.2.

2.1.1.1. Infructescence volume

The volume of each collected infructescence was calculated using an adapted volume formula (to standardize for different shapes of infructescences) for a cone: $V(cone) = (\frac{1}{3}\pi hb^2) + (ba) + (a^2)$ where h is the height of the infructescence, b is the base diameter of the infructescence (average of two measurements) and a is the diameter at the top of the infructescence (average of two measurements) (Fig. 1). Mean infructescence volumes were statistically compared between the various *Protea* species using an ANOVA on the normally distributed data in Statistica 9 (Statsoft Corporation, USA). A LSD *post hoc* test was performed to evaluate differences between mean volumes of individual *Protea* species Significant differences are reported where $P \leq 0.05$. For analyses of data, infructescence volume (cm^3) was categorized into five size groupings (< 213.271 (extra small); ≥ 213.271, < 426.542 (small); ≥ 426.542, < 639.813 (medium); ≥ 639.813, < 853.084 (large); > 853.084 (extra large)) (see section 2.1.2).

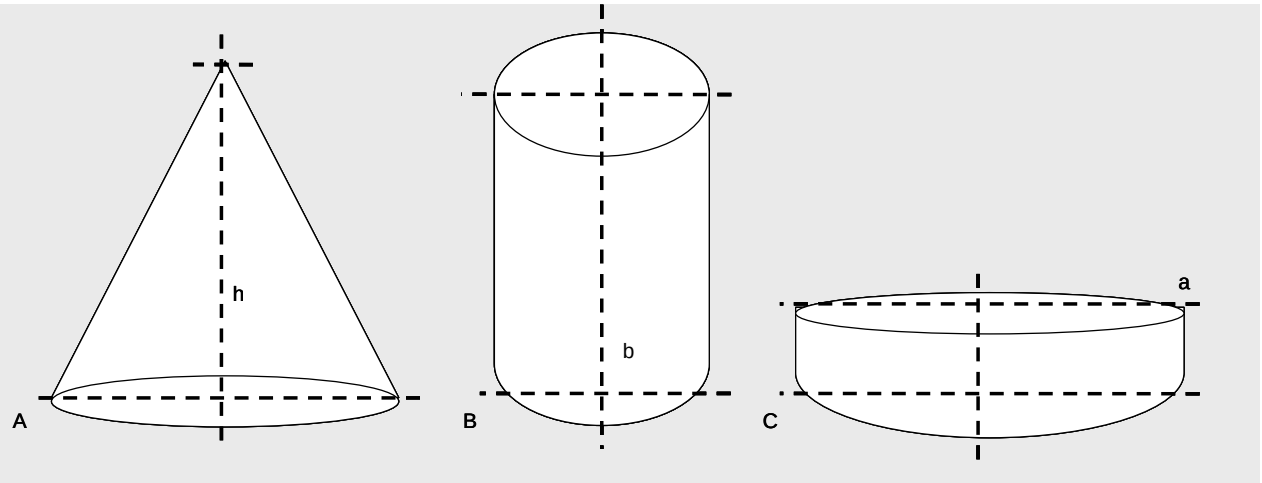

Figure 1: Diagrams of three infructescence shapes and their measurements: A) keel (e.g. infructescence of *P. repens*), B) cylinder (e.g. infructescence of *P. neriifolia*), C) flat cylinder (e.g. infructescence of *P. nitida*) and measurements taken for height (h), base diameter (b) and top diameter (a).

2.1.1.2. Microclimatic stability coefficient

The degree of infructescence openness (Wright and Samways, 1999) was calculated using the same infructescence measurements used for volume calculations. These calculations describe the influence of both the base vs. top diameter ('openness' ratio) and the infructescence height on the microclimatic stability within infructescences (Fig. 2). Thus, an increase in the ratio between the top diameter and bottom diameter will lead to an increase in the openness of the infructescence (resulting in less stable micro-climate within). Similarly, a decrease in height (whilst keeping the top diameter constant) should lead to a decrease in the microclimatic stability within the infructescence (Fig. 2).

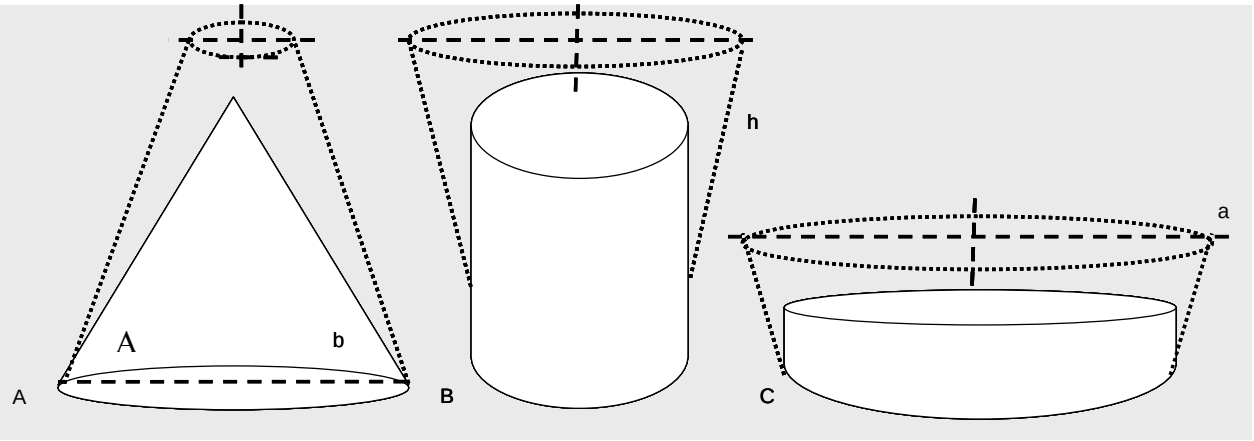

Figure 2: Diagrams depicting three infructescence shapes (solids) and an example of the degree of openness (dashed lines). If the volume of the three shapes are similar (solid shapes), one would expect that shape A will retain moisture better than shape B even though their heights are similar (closed top vs. open top). In this diagram the openness of shape A (dashed lines) is less than shapes B and C. The openness of shapes B and C are similar as the ratios between the top measurements and the base measurements are similar. However, shape C will retain less moisture than shape B as it has a flattened shape.

Both the openness and the height variables were combined in a single formula describing moisture loss of infructescences as follow: Microclimatic stability coefficient (P_i) = [3 $(a/b)^3$] / [2 (h/b) (1+ (a/b) + $(a/b)^2$)] where h is the height of the infructescence, b = the average base diameter (two measurements) and a = the average top diameter (two measurements). In this study volume is considered to be an independent variable as it is not included in the formula. Height (h) and the ratio between the base (b) and top measurements (a) are considered to be dependent variables. Therefore, as these values change, so does the microclimatic stability coefficient (P_i). If the top measurement (a) = 0 then P_i also equals 0 (closed infructescence). The more open the infructescence (greater ratio between a and b) the greater the P_i-value (Figure 3 and 4). We assume that the P_i -value also

indicates the degree of moisture retention within infructescences. Thus, the greater the P_i-value, the greater the surface area exposed to the atmosphere, and therefore the greater the chance of moisture loss under desiccating conditions (Harper & Benton, 1966).

Figure 3: *Protea eximia* infructescence showing measurements used to calculate the microclimatic stability coefficient (P_i).

Mean infructescence microclimatic stability coefficients (P_i) were calculated for 14 *Protea* species and were statistically compared using an ANOVA on the normally distributed data in Statistica 9. A LSD *post hoc* test was performed to evaluate differences between the mean P_i of individual *Protea* species. Significant differences are reported where $P \leq 0.05$. For analysis, infructescence microclimatic stability was categorized into five grouping from most stable to least stable (< 0.588 (closed and most stable); ≥ 0.588, < 1.175; ≥ 1.175, < 1.763; ≥ 1.763, < 2.350; > 2.350 (wide open and least stable)) (see section 2.1.2).

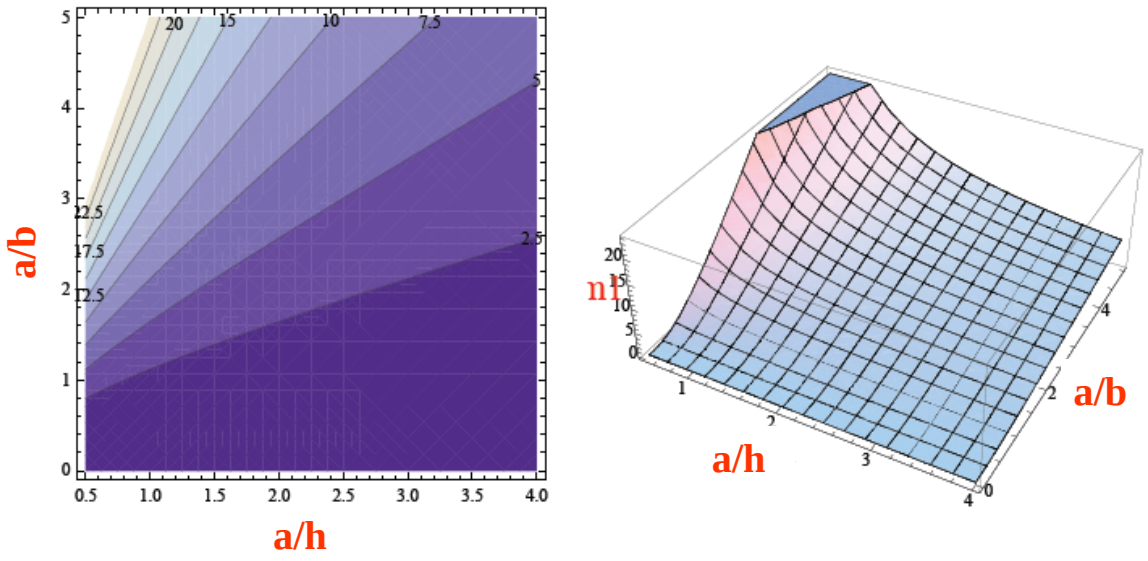

Figure 4: Mathematical model depicting the influence of infructescence height (h) and the ratio between the top diameter (a) and base diameter (b) on the stability coefficient (P_i). The greater the value of the stability coefficient, the greater the expected moisture loss will be.

To verify the role of infructescence morphology on microclimatic stability within infructescences, temperature and humidity fluctuations were recorded in the field using iButtons (Dallas semiconductors, USA). Species were chosen to represent the morphological range of infructescences found within the genus; from closed (*P. repens*) to open and compact (*P. neriifolia*) to open and non-compact (*P. nitida*). iButtons were placed in empty tea bags (for protection) and individually placed inside infructescences of each of the selected *Protea* species (Fig. 5). Chosen *Protea* plants were located close to each other (radius of 1.5 meters) to minimize variance due to site climatic conditions. A control iButton was placed on the shaded side of a stem of one of the plants using an elastic band. Temperature and relative humidity were recorded at 15 minute intervals for a week.

Due to an uncontrolled veld fire during February 2009 the iButtons were destroyed during the second replication of the experiment. In this study, data collected on a daily basis were thus used as separate replicates (n = 7). Mean temperatures were calculated for each of the control and three *Protea* species and were statistically compared using and ANOVA in Statistica 9 after tested for normality. A LSD *post hoc* test was performed to evaluate differences between the mean temperatures of the control and individual *Protea* species. Significant differences are reported where $P \leq 0.05$.

Figure 5: Position of iButtons within the infructescences of *P. repens* (left), *P. neriifolia* (middle) and *P. nitida* (right). Yellow plastic bags were used as markers.

2.1.2. The influence of environmental variables on mite community assemblage structure

To investigate the impact of previously described ecological variables on the mite communities within *Protea* infructescences a Canonical Correspondence Analysis (CCA) was performed using CANOCO version 4. 5 (Ter Braak and Simlauer, 2002). CCA was also preferred as it is able to

effectively accommodate skew data distributions typical of biodiversity data (Palmer, 1993). Variables were tested for significance in describing mite community structures by performing a Monte Carlo permutation test with 499 permutations in CANOCO version 4. 5 (Ter Braak and Simlauer, 2002). A second multivariate analyses technique, analysis of similarity (ANOSIM) was performed in Primer v.5.2.9 (Clarke and Warwick, 2001) to determine which factors significantly explained observed mite community assemblage structure in *Protea* infructescences.

2.1.3. The influence of plant host characteristics and environmental variables on mite species richness and abundance

A generalized linear model with Poisson distribution (identity link function) was used to determine the effect of previously mentioned variables and specific host *Protea* species on mite species richness and abundance (McCulloch *et al.*, 2008) using the software programme SAS/STAT Software (SAS Institute Inc., USA). The same model was used to calculate pair-wise differences between the 14 *Protea* species in terms of their mite richness and abundance. Significant differences under this model are reported where $P \leq 0.05$.

2.2. The influence of infructescence age on mite assemblages

Two *Protea* species were selected for intensive sampling in order to test the influence of infructescence age on mite community composition. *Protea nitida* was excluded from this experiment as it does not retain its infructescences for much longer than one year. Infructescences of *P. repens* and *P. neriifolia* were chosen as these species share distribution ranges and often grow in close proximity to each other. Twenty five infructescences of the last three flowering seasons were collected for each of the two *Protea* species. Sampling sites where these species were found

growing sympatrically included Gordon's Bay (S 34˚ 10" 28.96'; E 18˚ 50" 06.36'), Franschoek Pass (S 33˚ 54" 20.94'; E 19˚ 09" 27.36') and Jonkershoek Nature Reserve (S 33˚ 59" 14.58; E 18˚ 57" 15.30). Sampling was conducted in March, 2009. Infructescences were stored in a refrigerator at 4˚C until extraction of mites as previously described. We tested the influence of infructescence age on both the mite species richness and abundance, using a generalized linear model with Poisson distributions and identity link function computed in the software programme SAS/STAT Software.

2.3. The influence of season on mite assemblages within infructescences

Twenty five infructescences of *P. repens P. neriifolia* and *P. nitida* were collected from above-mentioned sites on a seasonal basis. Infructescences from the most recent flowering season were collected every third month starting during August 2008 and ending in June 2009. Each fieldwork session resulted in the collection of 300 infructescences, which were treated similar to those mentioned above. We tested for the influence of season on both the mite species richness and abundance, using a generalized linear model with Poisson distributions and identity link function computed in the software programme SAS/STAT Software.

3. RESULTS

3.1. The effect of taxonomic similarity between host plants and different host plant characteristics on mite assemblages

A total of 23 mite morphospecies (666 individuals), representing 14 families, were collected from the 16 *Protea* species sampled (n = 140 infructescences) (Appendix, 1). Most of the mites collected from *Protea* infructescences belonged to the family Uropodidae and comprised 22 % of all individual mites collected (Fig. 6). The Orbatidae and Ghlycyphagidae represented the second and third largest families, respectively (Fig. 6). Individuals of Tarsonemidae and Tydeidae were also fairly abundant compared to other families.

The mite families Uropodidae, Ghlycyphagidae and Tarsonemidae (Fig. 6) were represented by a single morphospecies (Fig. 7). The Orbatidae were represented by three morphospecies, although 90% of individuals were of a single morphospecies (Fig. 6 and Fig. 7). The family Tydeidae included four morphospecies, of which 60% of collected individuals comprised of the morphospecies *Tydeidae sp.1* (Fig. 6 and Fig. 7).

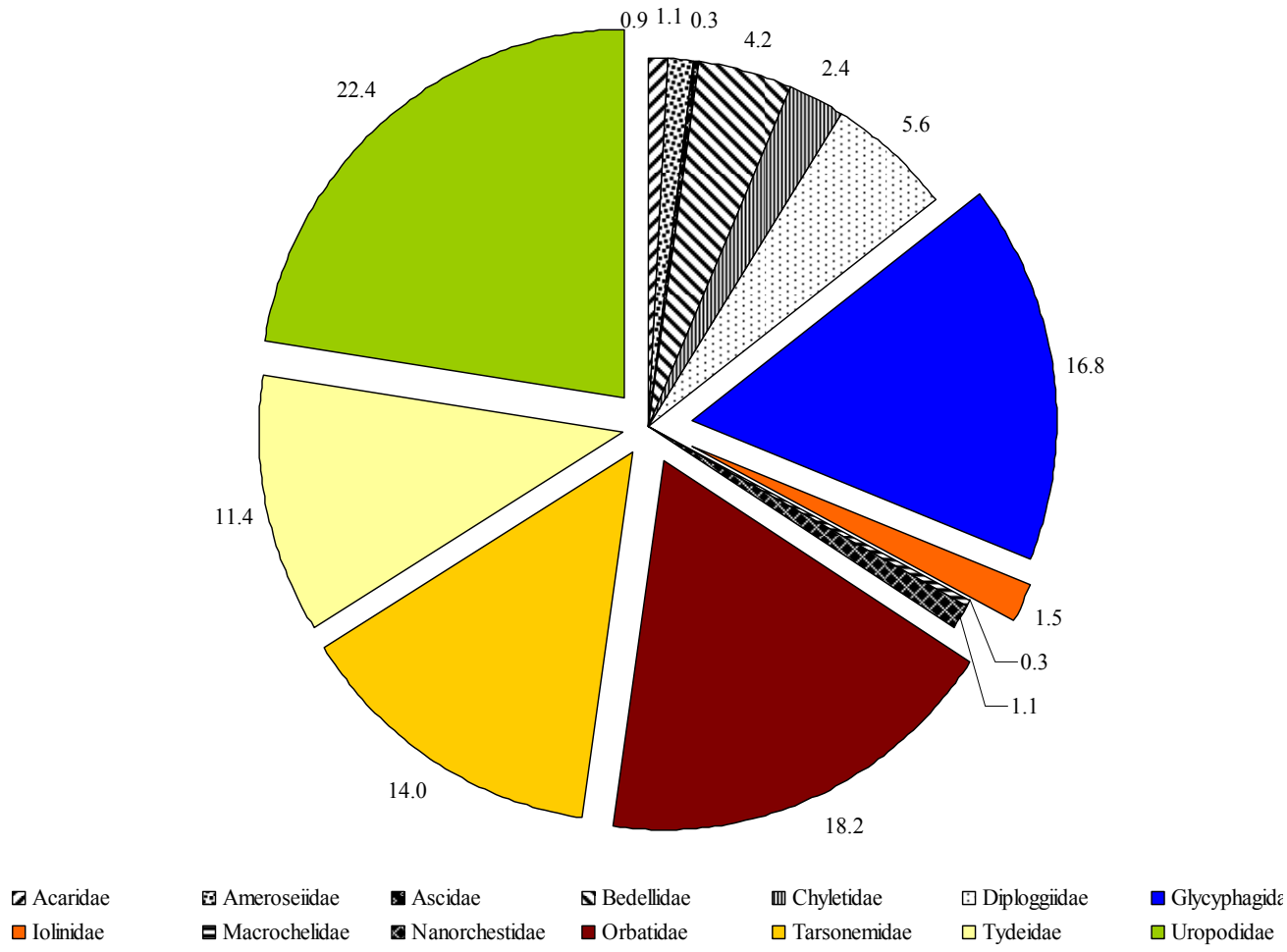

Figure 6: Proportion of collected mite individuals grouped according to family (as a percentage) collected from the infructescences of 16 *Protea* species (n = 10 infructescences for all proteas, except for *P. glabra* and *P. coronata* that had n = 5 and n = 3, respectively).

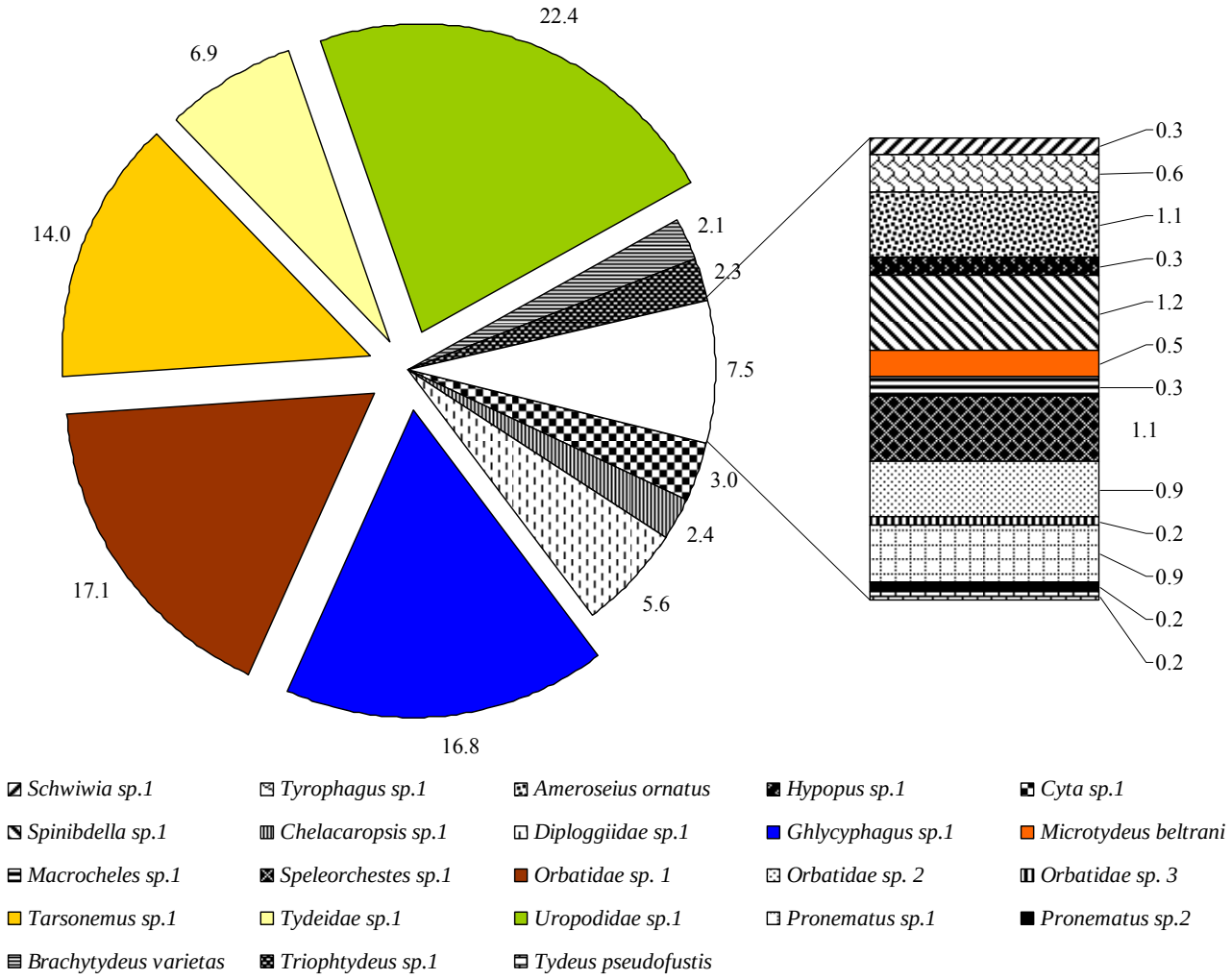

Figure 7: Proportion of collected mite individuals grouped according to morphospecies (as percentage) collected from the infructescences of 16 *Protea* species (n = 10 infructescences for all proteas except for *P. glabra* and *P. coronata* that had n = 5 and n = 3, respectively).

According to the quartile definition of Gaston (1994), 17 of the 23 species were categorized as abundant (major category, left-hand side of the dashed line) with the rest being regarded as rare (minor) morphospecies (Fig. 8, Appendix 2). The first four highest ranking morphospecies had very high abundances relative to other species collected. Their numbers ranged between 90 to 150 individuals per *Protea* species.

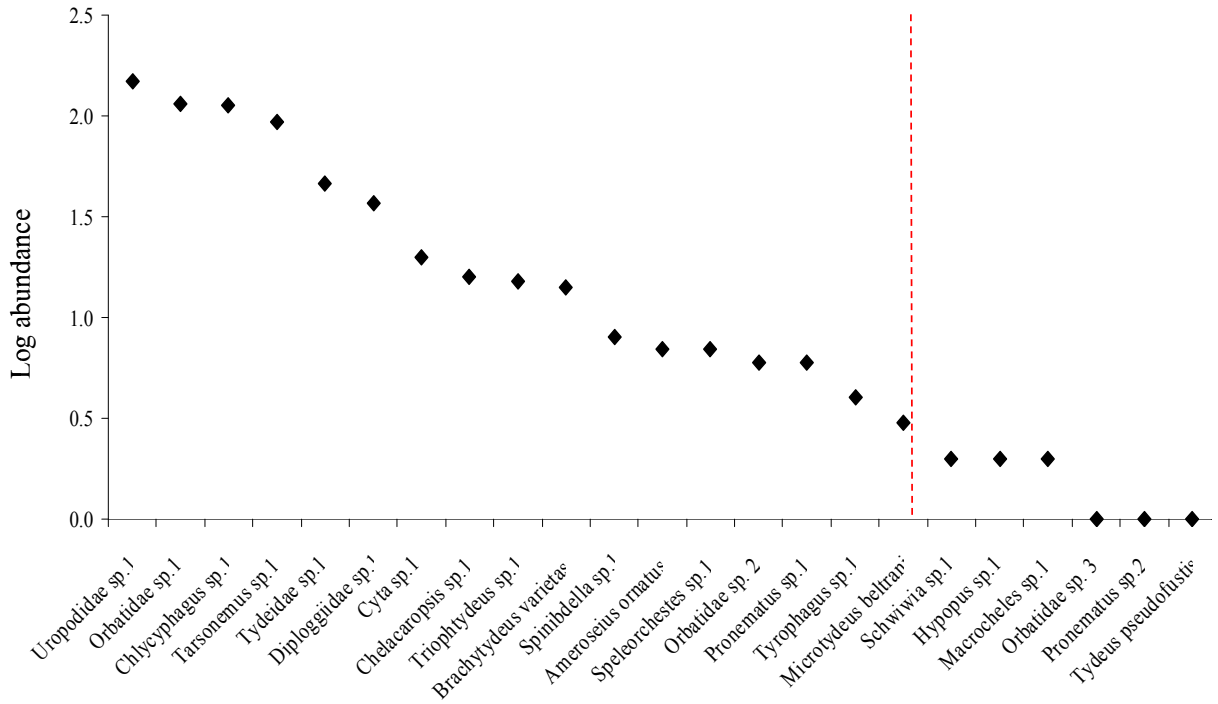

Figure 8: Rank log-abundance relationship for 666 mite morphospecies collected from the infructescences of 16 *Protea* species. The dashed line indicates those species that are regarded as rare under the quartile definition.

Estimated species richness indicators are summarised in Table 2 for 14 *Protea* species (n = 10). *Protea obtusifolia* and *P. neriifolia* had the highest overall estimated morphospecies richness, whereas *P. punctata* and *P. aurea* had the lowest (Table 2). The overall morphospecies richness estimations (ICE = 24, Chao = 24, Jackknife2 = 27) indicate a general under-representative sample size (observed species = 22) (Table 2). Observed mite morphospecies numbers for *P. nitida*, *P. lorifolia*, *P. laurifolia* and *P. punctata* are very similar to the estimated species numbers, indicating a good representation of the mite species richness sampled within these four *Protea* species (Table 2).

Table 2: Estimated mite morphospecies richness for 14 *Protea* species (n = 10 infructescences) calculated from a total of 657 collected individuals.

Species	**Observed number of species**	**Total abundance**	**ICE***	**Chao2** (± SD)**	**Jackknife2*****
Overall	22	656	24.33	23.49 (2.22)	26.98
P. caffra	6	10	12	7.80 (2.63)	11.38
P. nitida	5	83	5	5 (0.18)	4.29
P. burchelli	7	27	11.23	7.90 (1.66)	10.68
P. eximia	4	13	6.56	4.45 (1.19)	6.69
P. obtusifolia	9	63	11.37	11.70 (4.06)	14.1
P. susannae	3	5	6	3.45 (1.19)	5.69
P. lorifolia	5	21	5.44	5 (0.15)	5.28
P. laurifolia	5	206	5.39	5 (0.40)	6.7
P. neriifolia	8	170	10.66	10.70 (4.04)	13.1
P. lanceolata	4	11	4	4 (0.61)	1.87
P. repens	6	21	9.25	7.35 (2.37)	10.39
P. punctata	2	4	3	2 (0.23)	2.99
P. aurea	2	2	2.9	2.90 (1.85)	5.4
P. acaulos	6	20	9.33	7.35 (2.37)	10.39

* Incidence-based coverage estimator, **Second order Chao estimator, *** Second order Jackknife estimator

When combining mite collection data for all *Protea* species in an accumulation curve, an asymptote is reached (Fig. 9). This indicates that sampling effort was sufficient to determine overall mite morphospecies richness on the collected *Protea* species. When sampling for individual *Protea* species was considered, accumulation curves of most proteas did not reach asymptotes (Fig. 10). Similar to that found with the calculated species estimators, species accumulation curves for most host *Protea* species indicated a continuous increase in numbers after ten infructescences, except for *P. nitida*, *P. lorifolia*, *P. laurifolia* and *P. lanceolata*.

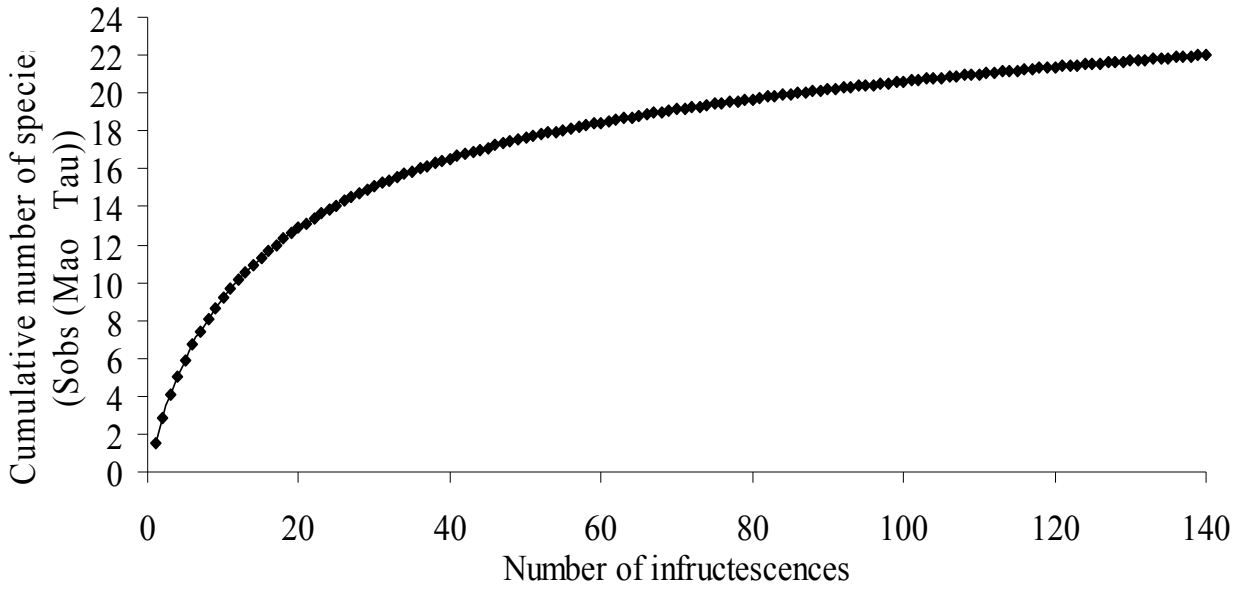

Figure 9: Accumulation curve for all mite morphospecies collected from the infructescences of 14 *Protea* species combined (n = 10 infructescences per *Protea* species).

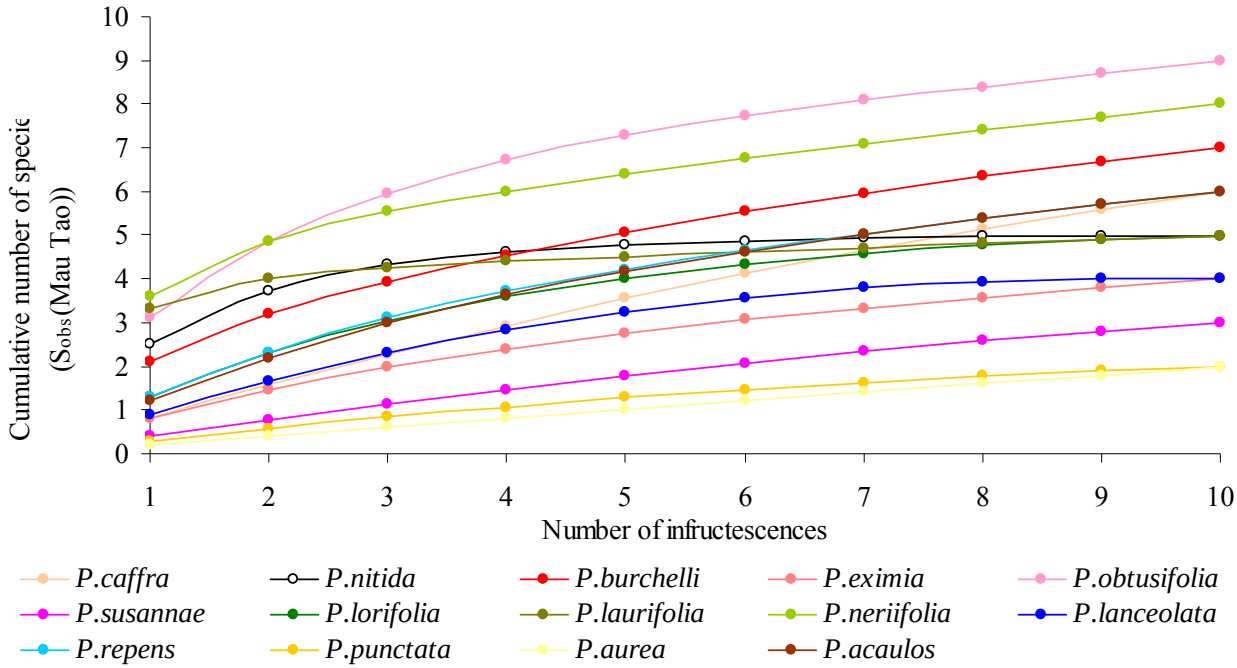

Figure 10: Accumulation curves for mite morphospecies collected from the infructescences of 14 individual *Protea* species (n = 10 infructescences per *Protea* species). Colour codes represent different *Protea* species within taxonomic groups (cream = grassland sugarbush, white = shaving-brush sugarbush, red to pink = spoon-bract sugarbush, greens = bearded sugarbush, blue = true sugarbush, yellow = white sugarbush and brown = western ground sugarbush).

Figure 11 depicts a dendogram of *Protea* species based on the Bray-Curtis similarity analysis (see Appendix 3) of mite assemblage data. In general, similarity between *Protea* species in terms of their mite communities is low, with the highest pair-wise similarities ranging between 40 to 60%. There is no obvious pattern in terms of the morphological grouping of *Protea* species and their mite community structure (Fig. 11). *Protea obtusifolia*, *P. laurifolia*, *P. caffra* and *P. aurea* branched of at the base, with less than 20% similarity in mite assemblages to the other *Protea* species (Fig. 11). *Protea lorifolia* and *P. lanceolata* showed the highest similarity between *Protea* species (62%). The

taxonomically distantly related *P. repens*, *P. nitida* and *P. neriifolia* grouped together (Fig. 11). Interestingly, they shared the same sampling site (Table 1).

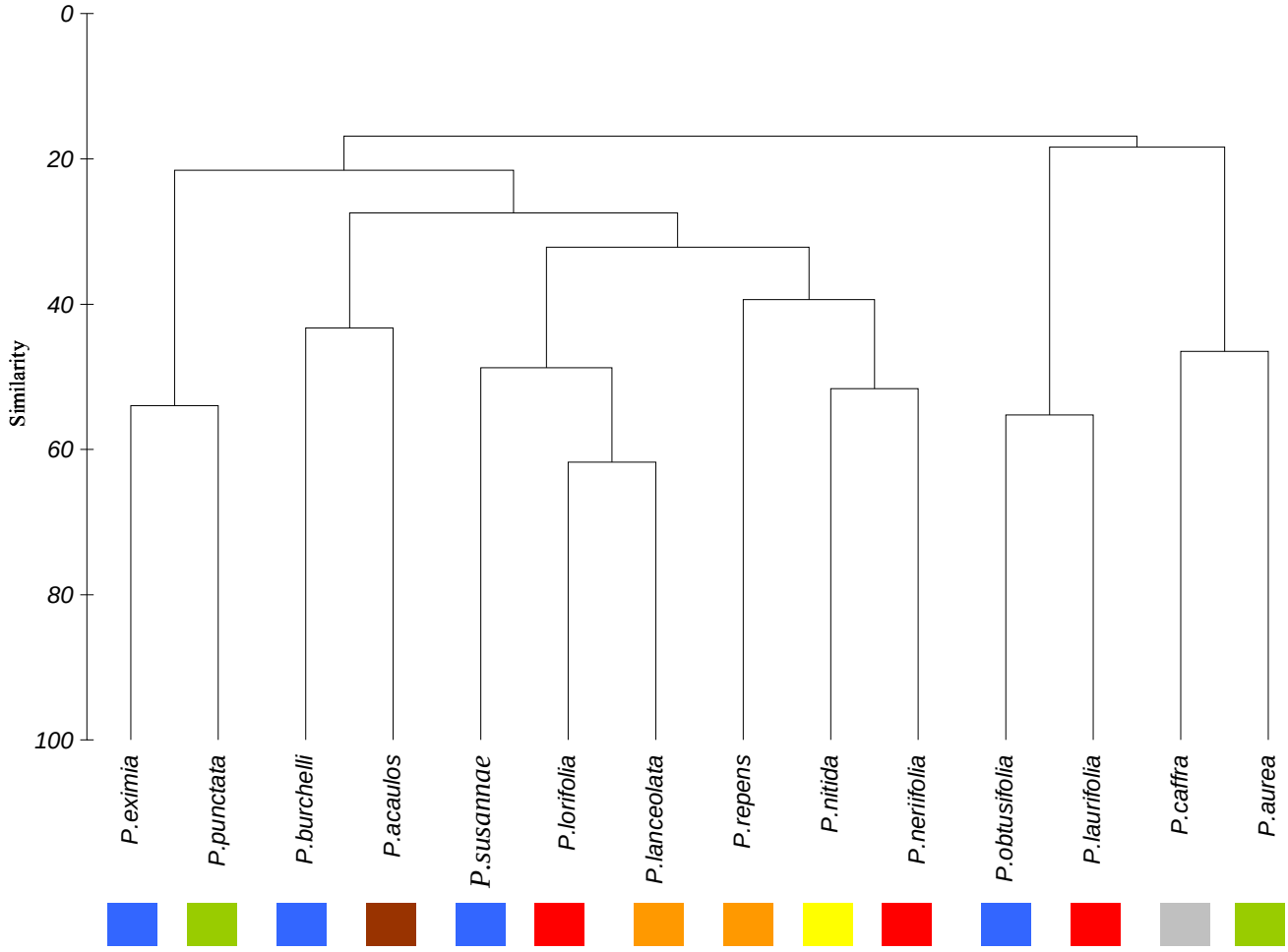

Figure 11: Dendogram showing the results of a cluster analysis for 14 *Protea* species based on mite assemblage data. *Protea* taxonomic groups are indicated by different colours (grey = grassland sugarbush, blue = spoon-bract sugarbush, green = white sugarbush, yellow = shaving-brush sugarbush, orange = true sugarbush, red = bearded sugarbush, brown = western ground sugarbush).

3.1.1. Host plant characteristics

3.1.1.1. Infructescences volume

There were significant differences in the mean volume (d.f. = 9, F = 487.0573, P = 0.035152) between infructescences of most *Protea* species collected (Fig. 12). *Protea lorifolia*, *P. susannae* and *P. eximia* had the largest infructescences, while *P. lanceolata* had the smallest (Fig. 12). There were significant difference in infructescence volume between the constituent *Protea* taxa of certain taxonomic groups, such as the spoon bract sugarbushes, shaving-brush sugarbushes and true sugarbushes (Fig 12).

3.1.1.2. Microclimatic stability coefficient

There were significant differences in the microclimatic stability coefficients (d.f. = 9, F = 28859.17, P = 0.004568) of infructescences of most *Protea* species collected (Fig. 13). *Protea nitida*, *P. susannae* and *P. acaulos* had the highest P_i values, while *P. caffra* and *P. repens* had the lowest P_i values making the latter two species the most climatic stable. (Fig. 13). There were also significant differences in the P_i values within certain *Protea* taxonomic groups, for example the spoon bract sugarbushes, shaving-brush sugarbushes, true sugarbushes and white sugarbushes (Fig 13).

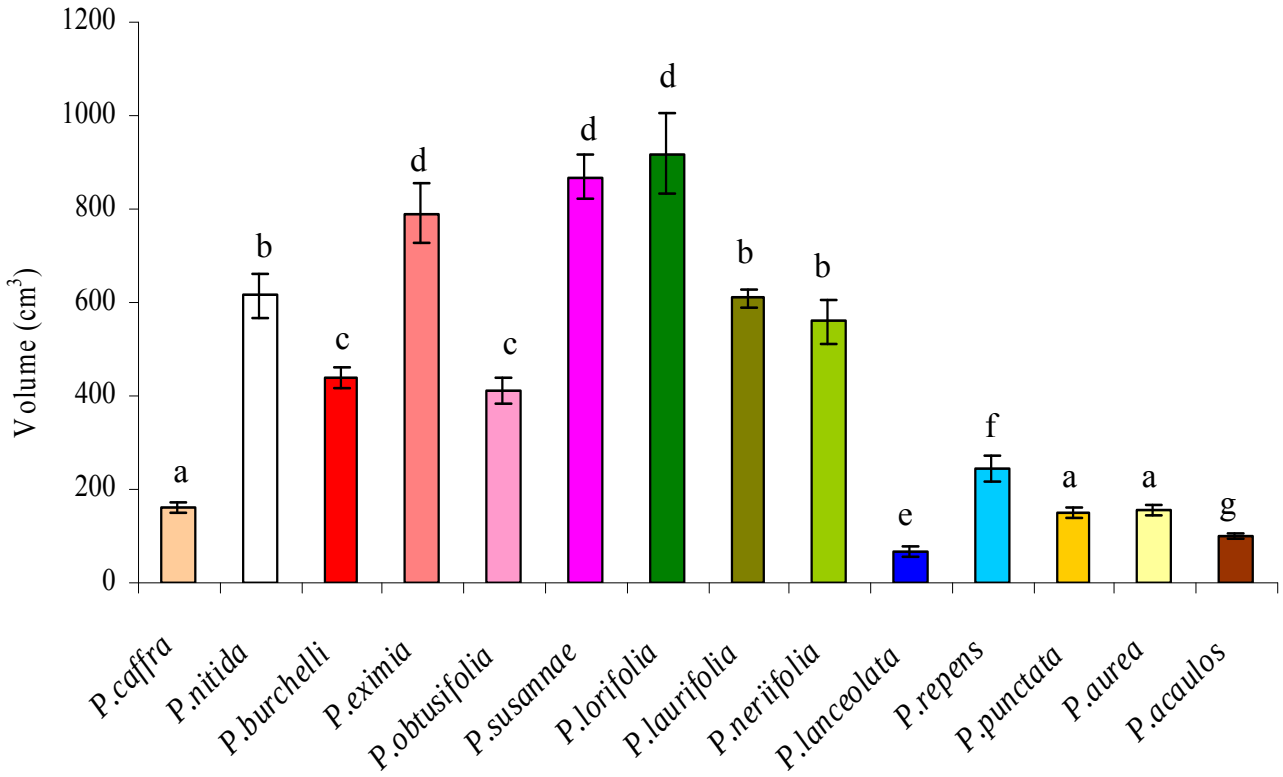

Figure 12: Comparisons between mean infructescence volumes (± SE) of 14 *Protea* species Significant differences are indicated by different letters. Colour codes for sugarbush morphological groups are as follows: cream = grassland sugarbushes, white = shaving-brush sugarbushes, red to pink = spoon-bract sugarbushes, greens = bearded sugarbushes, blue = true sugarbushes, yellow = white sugarbushes and brown = western ground sugarbushes.

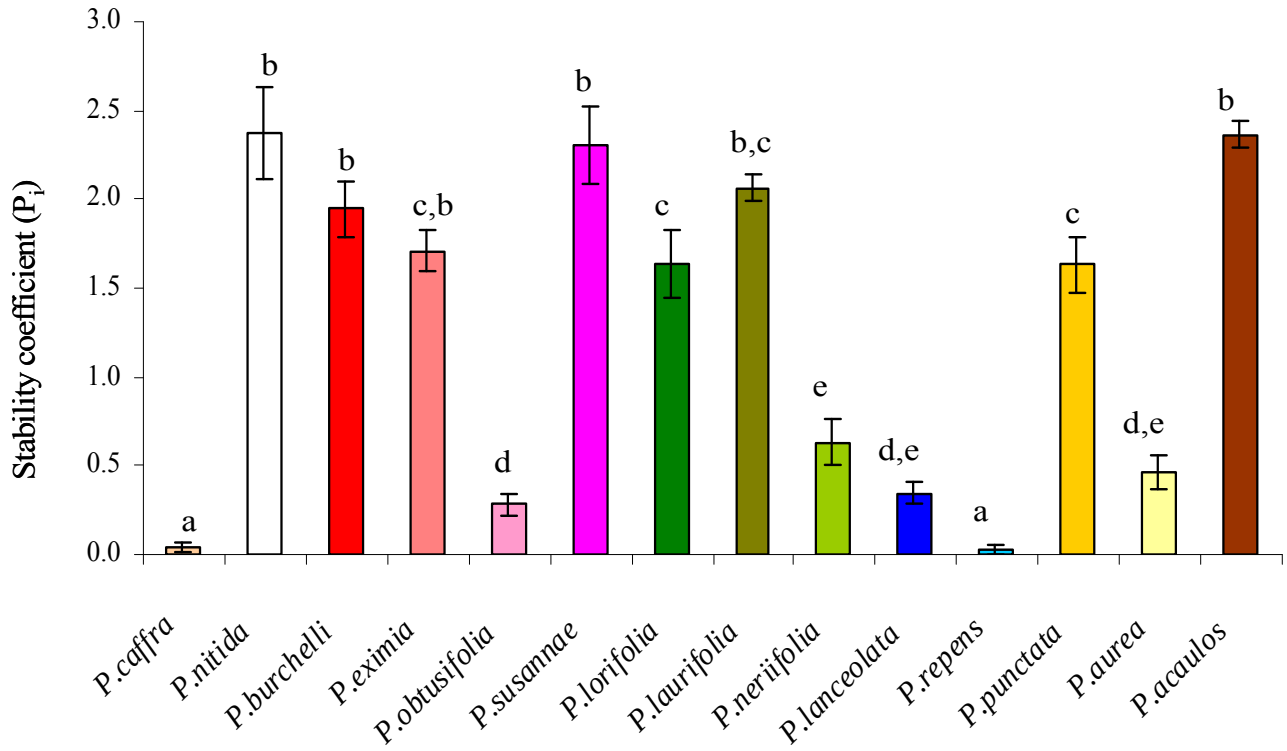

Figure 13: Mean microclimatic stability coefficient (P_i) comparisons (± SE) between 14 *Protea* species. Significant differences in P_i are indicated by different letters. Colour codes for sugarbush morphological groups as follows: cream = grassland sugarbushes, white = shaving-brush sugarbushes, red to pink = spoon-bract sugarbushes, greens = bearded sugarbushes, blue = true sugarbushes, yellow = white sugarbushes and brown = western ground sugarbushes.

Recordings of temperature and humidity fluctuations within the infructescences of *P. repens* (smallest measured P_i value), *P. nitida* (largest P_i value) and *P. neriifolia* (intermediate P_i value) in the field were compared and are presented in Table 3. There were significant differences in the mean maximum temperatures reached within the infructescences of the three *Protea* species (d. f. = 3, F = 3.08, P = 0.047). Maximum temperatures within *Protea* infructescences were always higher than the ambient air temperature, with *P. nitida* being an average of *ca*. 7ºC warmer than the ambient temperature (d. f. = 2, F = 24.43, P < 0.01). Temperatures within the infructescences of *P. nitida* were always the highest of the three *Protea* species (Table 3). The infructescences of *P. repens* rarely warmed to over 40°C, while the infructescences of *P. neriifolia* and *P. nitida* regularly reached this temperature (Table 3). Absolute minimum temperatures within infructescences varied very little compared to fluctuations in the minimum ambient air temperatures (Table 3).

There were significant differences between the mean minimum relative humidity within the infructescences of the three *Protea* species studied (d. f. = 3, F = 10.03, P < 0.001). Generally, relative humidity levels within *P. neriifolia* and *P. nitida* infructescences followed that of the ambient air humidity. The infructescences of *P. repens* had the highest mean relative humidity (Table 3). Furthermore, the relative humidity was above 50% for all but five and a half hours over the seven day measuring period. In contrast, over the same seven day period the relative humidity within the infructescences of *P. neriifolia* and *P. nitida* were below 50% for 45 and 75 hours, respectively (Table 3). Absolute minimum humidity levels within *P. neriifolia* and *P. nitida* infructescences varied very little compared to the minimum ambient humidity.

Table 3: Temperature and relative humidity recorded over a 7 day period within the infructescences of three sympatric *Protea* species.

Temperature (°C)				
	Control	*P. repens*	*P. neriifolia*	*P. nitida*
Absolute minimum	13.16	13.16	13.10	13.19
Absolute maximum	38.66	40.65	41.09	45.67
Mean	22.74	24.25	24.33	24.18
Mean maximum (SE)	32.24 (1.89)[a]	33.37 (2.09)[a]	35.89 (1.65)[ab]	39.54 (1.71)[b]
Mean difference in maximum from control (SE)	0	1.14 (0.28)[a]	3.67 (0.47)[b]	7.31 (0.94)[c]
Events over 35°C	23	64	79	80
Time over 35°C (hrs)	5.75	16	19.75	20
Events over 40°C	0	9	23	32
Time over 40°C (hrs)	0	2.25	5.75	8
Events below 14°C	16	9	8	15
Time below 14°C (hrs)	4	2.25	2	3.75
Relative Humidity (%)				
Absolute minimum	16.42	42.55	17.31	16.99
Absolute maximum	96.12	83.36	82.01	85.01
Mean	54.05	66.05	50.75	57.99
Mean minimum (SE)	32.34 (4.90)[a]	55.24 (3.25)[b]	28.82 (4.04)[a]	29.36 (3.55)[a]
Mean difference in minimum from control (SE)	-	22.90[a]	-3.42[b]	-2.98[b]
Events over 80%	40	40	8	60
Time over (hrs)	10	10	2	15
Events over 90%	16	0	0	0
Time over (hrs)	4	0	0	0
Events below 50%	259	22	299	183
Time below (hrs)	64.75	5.5	74.75	45.75
Events below 30%	88	0	95	65
Time below (hrs)	22	0	23.75	16.25

Significant difference are indicated by differences in superscript letters

3.1.2. The influence of host plant characteristics and environmental variables on mite community assemblage structure

Numerous variables were tested for significant influence on mite community structure within the infructescences of 14 *Protea* species (Table 4). In general, variables with the greatest influence on mite assemblages included flower colour, soil, and level of serotiny and to some extent, taxonomic group (Table 4).

Significant variables were plotted (as vectors) in a Canonical Correspondence analysis (CCA) biplot with *Protea* species (Fig. 14). *Protea susannae*, *P. lorifolia*, *P. eximia*, *P. punctata*, *P. lanceolata*, *P. caffra*, *P. repens*, *P. neriifolia* and *P. nitida* are clumped together in the right-bottom corner of the graph and shared more similar mite communities than *P. acaulos*, *P. burchelli*, *P. aurea*, *P. obtusifolia* and *P. laurifolia* (Fig. 14). There is a strong interaction between the mite community structure of *P. acaulos* and its morphological group (Fig. 14). *Protea obtusifolia* and *P. laurifolia* group together based on the influence of flower colour on mite community structure (Fig. 14).

Testing the influence of numerous environmental variables on mite community structure in 14 *Protea* species using ANOSIM also identified various significant factors (Table 5). Significant variables included flower colour, level of serotiny, plant life form and plant taxonomy (Table 5). These are the same variables that were found to be significant in the CCA analysis, with the exception of soil type, which was not found to significantly influence mite communities in the ANOSIM analyses (Table 4, Table 5).

Table 4: Monte Carlo permutation test (CCA) showing the influences of the tested variables on mite assemblages in *Protea* infructescences. P values in bold typeface indicate factors that have significant influences on mite assemblages.

Environmental and host characteristics	**N**	**F**	**P**
Silvery pink	499	5.13	**0.002**
Western ground	499	4.45	**0.002**
Spoon bract	499	3.02	**0.002**
Sand	499	2.78	**0.002**
Grassland	499	2.17	0.082
White	499	2.02	0.132
True	499	1.99	**0.028**
Volume	499	1.96	0.074
Insects	499	1.69	0.068
Non-serotinous	499	2.19	**0.008**
Pink	499	1.64	0.146
Tree	499	1.41	0.138
Openness	499	1.58	0.104

Table 5: ANOSIM Global R values for tested variables and their P levels based on mite community assemblage structures. P values in bold typeface indicate factors that have significant influences on mite assemblages.

Environmental and host characteristics	**Nr of Permutations**	**Global R**	**P**
Volume (V)	999	-0.0310	0.0566
Microhabitat (P_i)	999	-0.0540	0.0634
Flower colour	999	0.1300	**0.0161**
Serotiny	999	0.0026	**0.0415**
Life form	999	0.0560	**0.0341**
Taxonomy	999	0.0500	**0.0368**
Soil class	999	-0.0720	0.0659

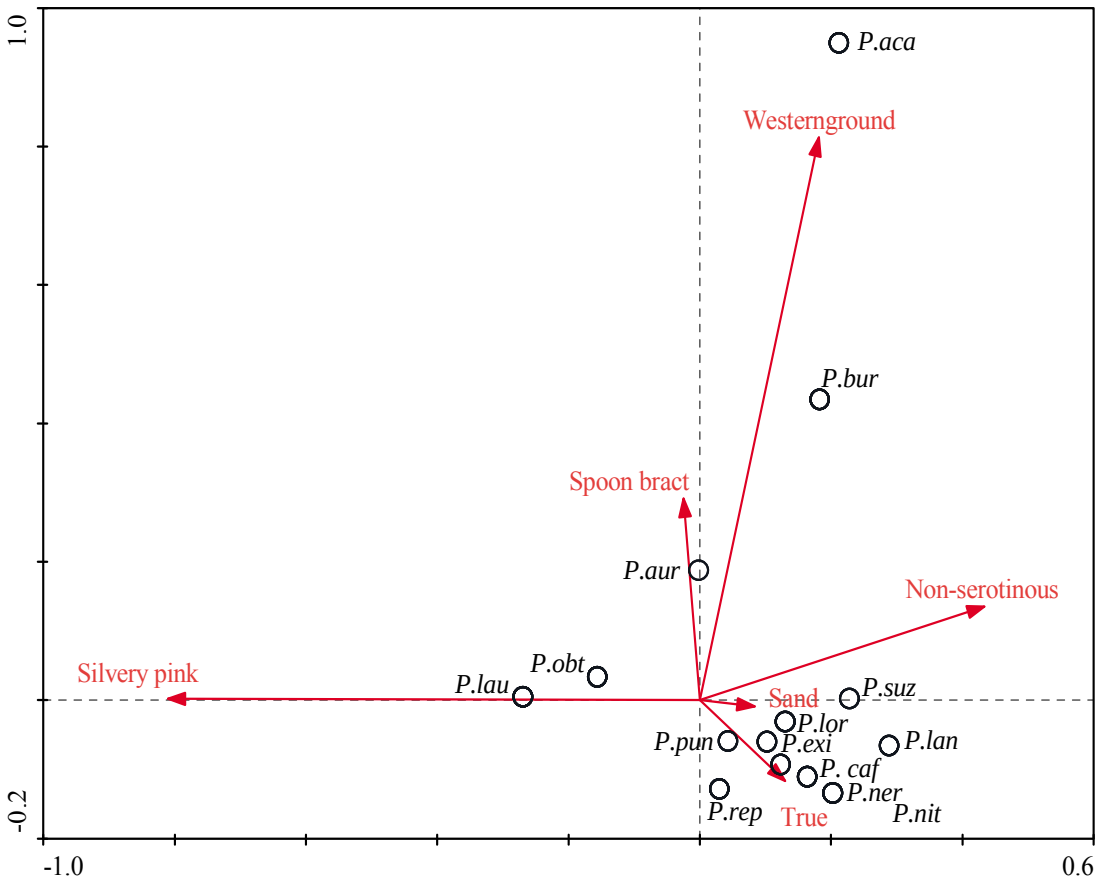

Figure 14: Canonical correspondence analysis (CCA) biplot for host plant characteristics and 14 *Protea* species (Eigen values: CCA1 = 0.731; CCA2 = 0.584). The angle between arrows indicates the correlation between these variables, with smaller angles indicating higher correlation. *P. aca* = *Protea acaulos*, *P. bur* = *P. burchelli*, *P. aur* = *P. aurea*, *P. lau* = *P. laurifolia*, *P. suz* = *P. susannae*, *P. pun* = *P. punctata*, *P. nit* = *P. nitida*, *P. obt* = *P. obtusifolia*, *P. rep* = *P. repens*, *P. caf* = *P. caffra*, *P. ner* = *P. neriifolia*, *P. lor* = *P. lorifolia*, *P. exi* = *P. eximia*, *P. lan* = *P. lanceolata*.

3.1.3. The influence of host plant characteristics and environmental variables on mite species richness and abundance

Results from generalized linear model analyses with Poisson distribution on mite morphospecies richness and abundance relative to various variables tested are summarised in Table 6. Also presented in the table are the relative mite morphospecies density and density of individuals (mite abundances) according to standardised infructescence size (mite abundance and morphospecies richness when infructescence size (i.e. sample size) is standardised). Numerous variables were significant in explaining both mite morphospecies richness and abundance. Infructescence volume, the host plant species, flower colour, plant life form and plant taxonomy and, in the case of abundance, level of serotiny, all influenced mite numbers (Table 6). After standardization for *Protea* infructescence size, relative morphospecies density were significant for all tested variables except for microclimatic stability (Table 6). All variables were found to have a significant influence on mite abundance when infructescence size was standardised.

Table 7 summarises the results of a generalized linear model with Poisson distribution with pair-wise comparisons of the host plants and mite morphospecies richness and abundance. Species richness indicated that 52% of pair-wise comparisons were significant, while 68% of comparisons for abundance between *Protea* species were significant (Table 7). Table 8 summarises the results of a generalized linear model with Poisson distribution with pair-wise comparisons between the host plants and mite morphospecies density and density of individuals. About 76% of pair-wise comparisons between *Protea* species in terms of their mite morphospecies density were significant. For density of individuals (abundance), 88% of pair-wise comparisons were significant (Table 8).

Table 6: A Generalized linear model with Poisson distribution, indicating the influence of eight environmental variables on species richness and abundance of mites found in infructescences of 14 *Protea* species. P values in bold typeface indicate factors that have significant influences on mite assemblages.

	Morphospecies Richness			**Abundance**		
	d.f.	Wald stat.	*P*	d.f.	Wald stat.	P
Intercept	1	3.08	0.0790	1	156.18	**<.0001**
Volume (V)	1	6.88	**0.0090**	1	53.63	**<.0001**
Microhabitat (P_i)	1	0.61	0.4360	1	0.37	0.5430
Host plant	13	86.41	**<.0001**	3	46.08	**<.0001**
Flower colour	2	10.87	**0.004**	2	83.38	**<.0001**
Serotiny	1	0.1	0.7510	1	6.94	**0.0080**
Life form	1	8.09	**0.0040**	1	99.36	**<.0001**
Taxonomy	3	23.04	**<.0001**	3	179.16	**<.0001**
Soil class	1	0.05	0.8290	1	0.86	0.3550

	Morphospecies density			**Density of individuals**		
	d.f.	Wald stat.	P	d.f.	Wald stat.	P
Intercept	1	1323.05	**<.0001**	1	3225.81	**<.0001**
Volume (V)	1	148.43	**<.0001**	1	36.4	**<.0001**
Microhabitat (P_i)	1	1.88	0.1710	1	12	**<.0001**
Host plant	13	326.89	**<.0001**	13	874.37	**<.0001**
Flower colour	2	32.76	**<.0001**	2	154.66	**<.0001**
Serotiny	1	33.17	**<.0001**	1	16.38	**<.0001**
Life form	1	19.7	**<.0001**	1	158.09	**<.0001**
Taxonomy	3	78.85	**<.0001**	3	421.07	**<.0001**
Soil class	1	30.2	**<.0001**	1	30.68	**<.0001**

Table 7: GLZ with Poisson distribution indicating pair-wise comparisons between *Protea* species according to mite morphospecies richness (bottom of diagonal) and abundance (top of diagonal). The mean morphospecies richness and abundance per infructescence for each host plant is also given.

	P. caf	*P. nit*	*P. bur*	*P. exi*	*P. obt*	*P. suz*	*P. lor*	*P. lau*	*P. ner*	*P. lan*	*P. rep*	*P. pun*	*P. aur*	*P. aca*
Mean species richness	0.6	0.5	0.7	0.4	0.9	0.3	0.5	0.5	0.8	0.4	0.6	0.3	0.2	0.6
Mean abundance	1	8.3	2.7	1.3	6.3	0.5	2.1	20.6	17	1.1	2.1	0.4	0.2	2
		39.97	9.68	0.39	27.52	1.6	3.73	87.29	75.81	0.05	3.73	2.4	4.32	3.2
P. caf		**(<.0001)**	**(0.0019)**	(0.5328)	**(<.0001)**	(0.2057)	(0.0535)	**(<.0001)**	**(<.0001)**	(0.8273)	(0.0535)	(0.1214)	**(0.0377)**	(0.0735)
	7.87		21.89	38.63	3.67	37.22	31.65	48.89	28.67	39.67	31.65	35.09	27.11	32.64
P. nit	**(0.0050)**		**(<.0001)**	**(<.0001)**	(0.0555)	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**
	5.4	0.35		6.92	8.91	14.33	1.9	96.65	75.93	8.72	1.9	14.86	14.11	2.33
P. bur	**(0.0202)**	(0.5558)		**(0.0085)**	**(0.0028)**	**(0.0002)**	(0.1682)	**(<.0001)**	**(<.0001)**	**(0.0032)**	(0.1682)	**(0.0001)**	**(0.0002)**	(0.1265)
	0	7.87	5.4		24.99	3.3	1.85	93.35	79.82	0.17	1.85	4.25	6.07	1.46
P. exi	(1.000)	**(0.0050)**	**(0.0202)**		**(<.0001)**	(0.0694)	(0.1742)	**(<.0001)**	**(<.0001)**	(0.6834)	(0.1742)	**(0.0393)**	**(0.0317)**	(0.2266)
	11.67	0.64	1.9	11.67		28.5	17.14	70.7	48.1	26.75	17.14	27.5	22.39	18.1
P. obt	**(0.0006)**	(0.4236)	(0.1682)	**(0.0006)**		**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**
	1.28	11.58	9.24	1.28	14.86		8.32	67.5	60.4	2.14	8.32	0.11	1.2	7.69
P. suz	(0.2577)	**(0.0007)**	**(0.0024)**	(0.2577)	**(0.0001)**		**(0.0039)**	**(<.0001)**	**(<.0001)**	(0.1438)	**(0.0039)**	(0.7394)	(0.2734)	**(0.0056)**
	1.17	3.66	1.85	1.17	6.92	4.25		99.36	81.74	3.02	0	8.24	10.1	0.02
P. lor	(0.2799)	(0.0558)	(0.1742)	(0.2799)	**(0.0085)**	**(0.0393)**		**(<.0001)**	**(<.0001)**	(0.0823)	(1.000)	**(0.0024)**	**(0.0015)**	(0.8759)
	12.93	1.1	2.62	12.93	0.06	15.89	8.09		3.44	89.65	99.36	60.96	42.55	99.15
P. lau	**(0.0003)**	(0.2951)	(0.1054)	**(0.0003)**	(0.8026)	**(<.0001)**	**(0.0044)**		(0.0638)	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**
	14.81	1.96	3.85	14.81	0.37	17.38	9.91	0.13		77.45	81.74	54.94	39.02	81.96
P. ner	**(0.0001)**	(0.1613)	(0.0497)	**(0.0001)**	(0.5417)	**(<.0001)**	**(0.0016)**	(0.7181)		**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**
	0.06	6.91	4.52	0.06	10.67	1.82	0.72	11.94	13.84		3.02	3	4.92	2.54
P. lan	(0.8085)	**(0.0086)**	**(0.0334)**	(0.8085)	**(0.0011)**	(0.1772)	(0.3964)	**(0.0006)**	**(0.0002)**		(0.0823)	(0.0832)	(0.0266)	(0.1112)
	1.17	3.66	1.85	1.17	6.92	4.25	0	8.09	9.91	0.72		9.24	10.1	0.02
P. rep	(0.2799)	(0.0558)	(0.1742)	(0.2799)	**(0.0085)**	**(0.0393)**	(1.000)	**(0.0044)**	**(0.0016)**	(0.3964)		**(0.0024)**	**(0.0015)**	(0.8759)
	1.28	11.58	9.24	1.28	14.86	0	4.25	15.89	17.38	1.82	4.25		0.64	8.63
P. pun	(0.2577)	**(0.0007)**	**(0.0024)**	(0.2577)	**(0.0001)**	(1.000)	**(0.0393)**	**(<.0001)**	**(<.0001)**	(0.1772)	**(0.0393)**		(0.4235)	**(0.0033)**
	30.7	11.81	2.39	30.7	14.11	0.64	6.07	14.82	15.83	3.7	6.07	0.64		9.64
P. aur	(0.0795)	**(0.0006)**	(0.1220)	(0.0795)	**(0.0002)**	(0.4235)	**(0.0137)**	**(0.0001)**	**(<.0001)**	(0.0544)	**(0.0137)**	(0.4235)		**(0.0019)**
	0.79	4.37	10.1	0.79	7.79	3.62	0.04	9.01	10.86	0.43	0.04	3.62	5.5	
P. aca	(0.3744)	**(0.0366)**	**(0.0015)**	(0.3744)	**(0.0052)**	(0.0571)	(0.8415)	**(0.0027)**	**(0.0010)**	(0.5141)	(0.8415)	(0.0571)	**(0.0190)**	

Value = Wald statistic (Probability), P values in bold typeface indicate factors that have significant influences on mite assemblages.

Table 8: GLZ with Poisson distribution indicating pair-wise comparisons between *Protea* species according to mite morphospecies density (bottom of diagonal) and density of individual mites (top of diagonal). The mean morphospecies richness and abundance per infructescence for each host plant is also given.

	P. caf	*P. nit*	*P. bur*	*P. exi*	*P. obt*	*P. suz*	*P. lor*	*P. lau*	*P. ner*	*P. lan*	*P. rep*	*P. pun*	*P. aur*	*P. aca*
Mean species richness	0.6	0.5	0.7	0.4	0.9	0.3	0.5	0.5	0.8	0.4	0.6	0.3	0.2	0.6
Mean abundance	1	8.3	2.7	1.3	6.3	0.5	2.1	20.6	17	1.1	2.1	0.4	0.2	2
P. caf		18.44 **(<.0001)**	0.26 (0.6090)	21.99 **(<.0001)**	28.65 **(<.0001)**	32.79 **(<.0001)**	20.18 **(<.0001)**	148.51 **(<.0001)**	128.29 **(<.0001)**	37.44 **(<.0001)**	5.07 **(0.0244)**	16.9 **(<.0001)**	30.83 **(<.0001)**	73.21 **(<.0001)**
P. nit	2.3 (0.1294)		14.63 **(0.0001)**	59.8 **(<.0001)**	1.36 (0.2436)	54.66 **(<.0001)**	58.4 **(<.0001)**	89.01 **(<.0001)**	69.5 **(<.0001)**	4.13 **(0.0422)**	4.58 **(0.0324)**	55.39 **(<.0001)**	62.49 **(<.0001)**	24.02 **(<.0001)**
P. bur	0.11 (0.7402)	1.42 (0.2339)		25.86 **(<.0001)**	24.07 **(<.0001)**	35.4 **(<.0001)**	24.02 **(<.0001)**	142.94 **(<.0001)**	122.4 **(<.0001)**	32.38 **(<.0001)**	3.06 (0.0810)	20.61 **(<.0001)**	34.39 **(<.0001)**	67.2 **(<.0001)**
P. exi	21 **(<.0001)**	11.71 **(0.0006)**	18.86 **(<.0001)**		70.75 **(<.0001)**	7.5 **(0.0062)**	0.08 (0.7813)	155.41 **(<.0001)**	143.43 **(<.0001)**	79.04 **(<.0001)**	40.38 **(<.0001)**	0.6 (0.4396)	2.48 (0.1150)	107.45 **(<.0001)**
P. obt	7.79 **(0.0287)**	13.02 **(0.0003)**	6.28 **(0.0122)**	36.39 **(<.0001)**		60.14 **(<.0001)**	69.68 **(<.0001)**	72.06 **(<.0001)**	53.93 **(<.0001)**	0.76 (0.3832)	10.73 **(0.0011)**	67.14 **(<.0001)**	70.89 **(<.0001)**	14.39 **(0.0001)**
P. suz	25.86 **(<.0001)**	18.81 **(<.0001)**	24.33 **(<.0001)**	3.43 (0.0641)	35.91 **(<.0001)**		8.68 **(0.0032)**	97.7 **(<.0001)**	92.71 **(<.0001)**	64.14 **(<.0001)**	44.23 **(<.0001)**	10.9 **(0.001)**	1.94 (0.1638)	77.18 **(<.0001)**
P. lor	19.7 **(<.0001)**	10.47 **(0.0012)**	17.55 **(<.0001)**	0.06 (0.8034)	35.4 **(<.0001)**	4.23 **(0.0397)**		158.9 **(<.0001)**	145.5 **(<.0001)**	78.25 **(<.0001)**	38.6 **(<.0001)**	0.25 (0.6191)	3.36 (0.0666)	107.8 **(<.0001)**
P. lau	0 (0.9996)	2.3 (0.1293)	0.11 (0.7398)	2.1 **(<.0001)**	4.79 **(0.0287)**	25.87 **(<.0001)**	19.7 **(<.0001)**		1.65 (0.1985)	59.92 **(<.0001)**	120.31 **(<.0001)**	161.87 **(<.0001)**	131.38 **(<.0001)**	25.11 **(<.0001)**
P. ner	1.45 (0.2278)	7.19 **(0.0073)**	2.35 (0.1253)	29.23 **(<.0001)**	1 (0.3176)	31.4 **(<.0001)**	28.05 **(<.0001)**	1.45 (0.2280)		43.11 **(<.0001)**	99.49 **(<.0001)**	148.19 **(<.0001)**	123.12 **(<.0001)**	14.16 **(0.0002)**
P. lan	37.44 **(<.0001)**	51.7 **(<.0001)**	40.6 **(<.0001)**	68.73 **(<.0001)**	17.86 **(<.0001)**	54.33 **(<.0001)**	69.09 **(<.0001)**	37.43 **(<.0001)**	26.22 **(<.0001)**		16.89 **(<.0001)**	76.15 **(<.0001)**	77.12 **(<.0001)**	8.64 **(0.0032)**
P. rep	0.26 (0.6095)	4.05 **(0.0441)**	0.71 (0.4002)	24.4 **(<.0001)**	2.86 (0.0910)	28.21 **(<.0001)**	23.14 **(<.0001)**	0.26 (0.6098)	0.49 (0.4849)	32.61 **(<.0001)**		35.1 **(<.0001)**	46.93 **(<.0001)**	46.48 **(<.0001)**
P. pun	8.68 **(0.0032)**	2.27 (0.1318)	6.98 **(0.0082)**	4.49 **(0.0342)**	23.5 **(<.0001)**	11.94 **(0.0005)**	3.59 **(0.0582)**	8.68 **(0.0032)**	16.12 **(<.0001)**	63.38 **(<.0001)**	11.6 **(0.0007)**		5.22 **(0.0223)**	107.57 **(<.0001)**
P. aur	21.94 (<.0001)	12.66 **(0.0004)**	19.81 **(<.0001)**	0.04 (0.8478)	37.04 **(<.0001)**	2.85 (0.0914)	0.19 (0.6598)	21.94 **(<.0001)**	30.04 **(<.0001)**	68.24 **(<.0001)**	25.3 **(<.0001)**	5.22 **(0.0223)**		97.83 **(<.0001)**
P. aca	31.5 **(<.0001)**	45.41 **(<.0001)**	34.53 **(<.0001)**	64.25 **(<.0001)**	13.43 **(0.0002)**	51.91 **(<.0001)**	64.39 **(<.0001)**	31.5 **(<.0001)**	20.99 **(<.0001)**	0.36 (0.5508)	26.93 **(<.0001)**	57.39 **(<.0001)**	63.94 **(<.0001)**	

Value = Wald statistic (Probability), P values in bold typeface indicate factors that have significant influences on mite assemblages.

3.2. The influence of infructescence age on mite assemblages

No significant differences were found between infructescence age and mite morphospecies richness in *P. repens* and *P. neriifolia* using the generalized linear model analyses with Poisson distribution and the identity link function (d.f. = 5, F = 7.10, P = 0.21). In contrast, results of the application of this model to mite abundance data revealed significant differences between the age classes (d.f. = 5, F = 2710.29, P < 0.0001). Results from pair-wise comparisons between mite abundances of the two *Protea* species and the different age classes are presented in Figure 15.

Within *Protea* species there were significant differences in mite abundance between different *P. neriifolia* age classes (d.f. = 1, F = 250.1, 1298.0, 997.0, P = 0.001). There is a significant decrease in mite abundance within the infructescences of *P. neriifolia* over time. There is also a significant decrease in mite abundance within the infructescences of *P. repens* at three years old as compared to infructescences of one- and two years old (d.f. = 1, F = 0.24, P = 0.6278) (Fig. 15).

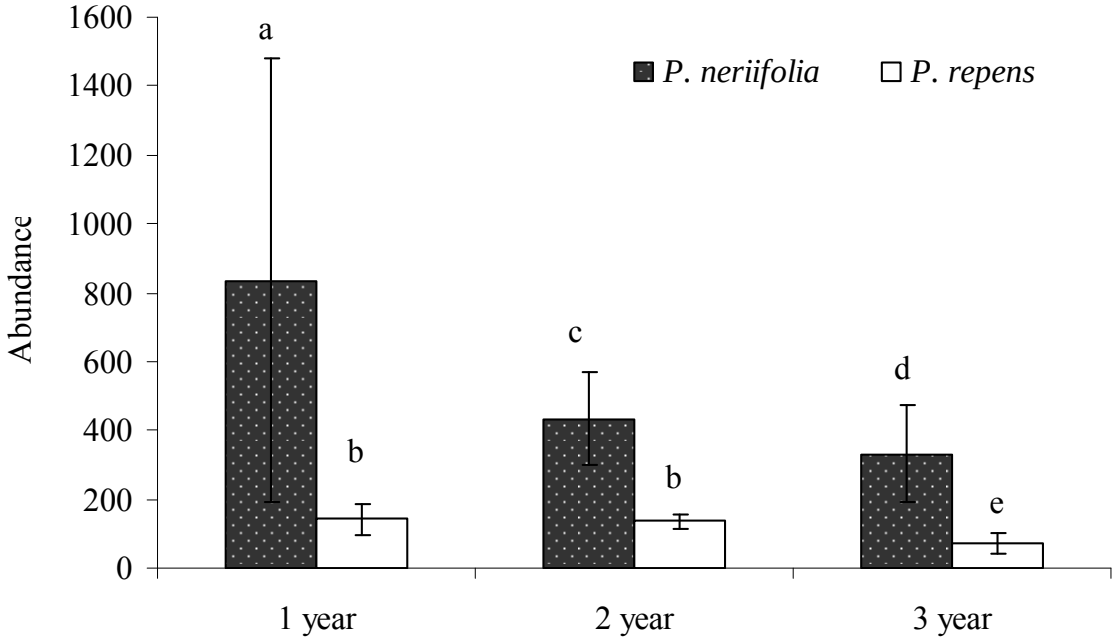

Figure 15: Average abundance (± SE) of mites collected from the infructescences of *P. neriifolia* and *P. repens* between three infructescence age-classes collected in autumn. Different letters indicate significant differences.

3.3. The influence of season on mite assemblages within infructescences

The generalized linear model analyses of mite morphospecies richness in relation to season showed that there were no significant differences between the different seasons in *P. nitida*, *P. repens* or *P. neriifolia* (d.f. = 11, F = 10.45, P = 0.49). In contrast, the linear model analysis again showed that mite abundance differed significantly between seasons in all three species (d.f. = 11, F = 5226.7, P < 0.001). Results of pair-wise comparisons between mite abundances in the infructescences of the *Protea* species in different seasons are summarized in Figure 16. Generally, the highest numbers of mites were collected during winter (June), while the lowest numbers were recorded during spring (September).

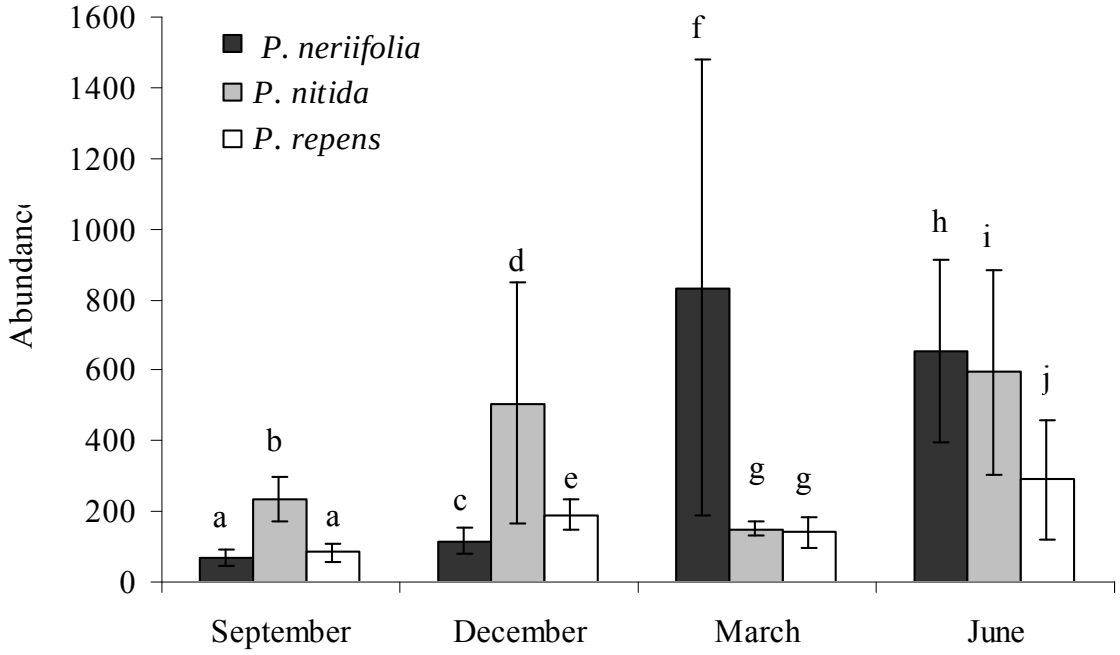

Figure 16: The average abundance (± SE) of mites collected per season from the infructescences (*ca*. one year old) of *P. neriifolia*, *P. nitida* and *P. repens.* Different letters indicate significant differences.

Figure 17 depicts absolute mite morphospecies numbers collected from the infructescences of 14 *Protea* species during different seasons. The Orbatidae was most abundant during the warmer months (spring and summer) and decreased in numbers during the colder months (autumn and winter). The Ghlycyphagidae increased in numbers from the warmer to the colder months (Fig. 17). The Eupodidae stayed abundant throughout most of the year (except during autumn). The abundance of Erythridae also stayed fairly constant throughout the year (Fig. 17). The Uropodidae, Tarsonemidae and Tydeidae all had their highest abundance during autumn (Fig. 17).

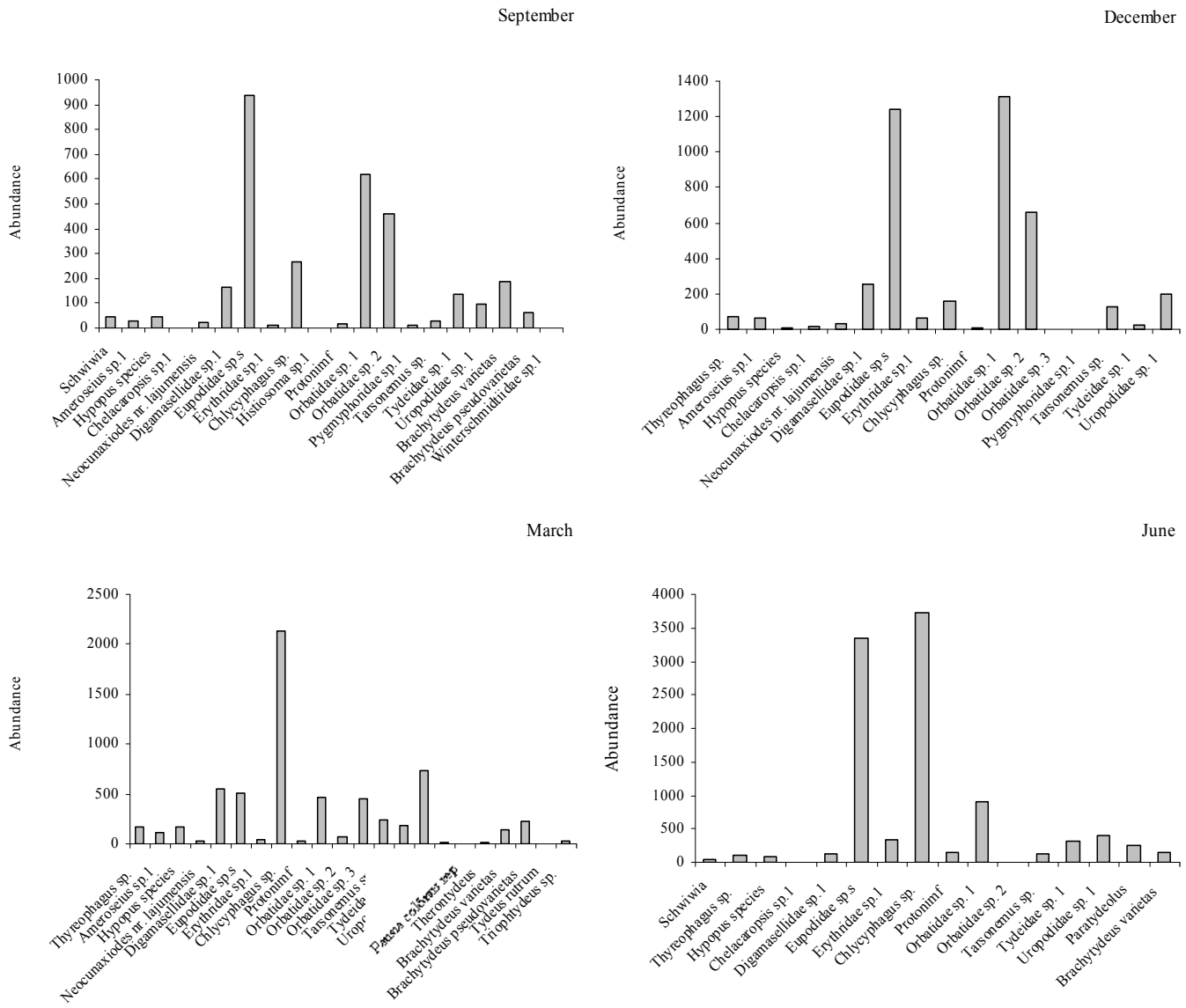

Figure 17: Absolute morphospecies richness and abundance of mites collected per season (September = spring, December = summer, March = autumn, June = winter) from the infructescences of three *Protea* species, collected in the Cape Winelands region.

4. DISCUSSION

In this study 23 mite morphospecies were collected from a total of 140 *Protea* infructescences during a single season. This suggests relatively high mite richness in *Protea* infructescences compared to a study by Wright and Samways, (1999) where they found only nine borer species within a total of 1000 infructescences over four seasons. The most abundant mites belong to the families Uropodidae, Orbatidae, Ghlycyphagidae, Tarsonemidae and Tydeidae. All of these mite families are known to be phoretically associated with other organisms (i.e. they are dispersed via vectors such as other arthropods or animals (Krantz and Walter, 2009)). This suggests that the mode of transport by mites may be a determinant factor shaping the mite communities in this niche. The Uropodidae and the Tarsonemidae species that were collected are known to feed on fungi (Krantz and Walters, 2009; Roets *et al.*, 2007) and are also closely associated with the dominant fungi found in *Protea* infructescences (ophiostomatoid fungi, Roets *et al.,* 2005). They have been shown to act as the primary vectors of these fungi between *Protea* infructescences (Roets *et al.*, 2007, 2009b) and are known to have a mutualistic association with *Ophiostoma* species (Roets *et al.,* 2007). Their high numbers within infructescences thus probably relates to this close association with the dominant fungi in *Protea* infructescences. Similarly, the high numbers of Orbatidae that are primarily saprophores and fungivores, indicates a possible close link between these mites and ophiostomatoid fungi. The high numbers of Orbatidae mites in *P. nitida* and *P. neriifolia* (tree-like plants) compared to the low numbers (a single specimen) of this family in *P. acaulos* (a *ca*. 30 cm high prostrate shrub) is interesting given that these mites are largely associated with soils (Krantz and Walter, 2009). The Tydeidae (considered to be mostly fungivores) and the Iolinidae (both in the superfamily Tydeoidae) all probably represent undescribed species and/or newly recorded genera/species in South Africa. These will be taxonomically evaluated in a later chapter (Chapter 3). According to the rank log-abundance curve, approximately six of 23 mite morphospecies found in

Protea infructescences were rare species. This suggests that a large proportion of mites associated with the infructescences of *Protea* species may only have loose associations with these plants, and should probably be considered as tourists in this instance. More intensive surveys are needed to corroborate this.

Combing mite collection data from 14 *Protea* species in an accumulation curve indicated that sampling was adequate to estimate mite numbers on *Protea* in general. Accumulation curves for separate *Protea* species, however, indicated different levels of adequacy in sampling effort. This empathizes that, in order to make accurate descriptions the number of mites associated with a specific *Protea* species, adequate sampling sizes for each species should be determined separately.

4.1. The effect of taxonomic similarity between host plants on mite community assemblages

Protea is a very diverse genus and has a diverse set of vectors for pollination. As insects and birds are known to vector mites between infructescences and different *Protea* species have different insect visitors, it would be expected that different *Protea* species will host different mite communities. Host plant taxonomy showed a significant influence on mite assemblages within *Protea* species infructescences. However, analyses of mite community structure between the various *Protea* species showed no general pattern of similarity for specific *Protea* taxonomic groups. Also, when considering mite community structure similarities between *Protea* species and the phylogenetic reconstruction of *Protea* (Valente *et al.*, 2010), no congruency was found. This may be explained by the spatial scale at which the study was done; with Cavendder-Bares *et al*. (2006) concluding that a broader taxonomic scale will enhance phylogenetic clustering and Lewinsohn *et al*. (2005) emphasized the importance of beta-diversity in community assemblages. In addition, temporal scale

as well as species turnover-effects may explain mite community assemblage structures in relation to their host plant, as these may influence tropic levels (Leibold *et al.*, 1997).

Interestingly, the taxonomically distantly related *P. repens*, *P. neriifolia* and *P. nitida* grouped together. These three *Protea* species are sympatric over most of their distribution ranges (Rebelo, 2001). Infructescences of these three species were also collected from the same locality in this study. Mite communities within the infructescences of *Protea* species may thus be influenced more by: site effects e.g. moisture availability (Janzen and Schoener, 1968), congenicity of host plants, such as in the study by Leather (1986) on different genera within the family Rosaceae, or by geographic distance (Strong and Levin, 1979) than by plant taxonomic similarity. The influence of site and geographic distance on mite communities associated with *Protea* is explored in more detail in Chapter 2.

4.2. Host plant characteristics and environmental factors influencing mite communities

Results indicated that closed infructescences such as those of *P. repens* have both higher relative humidity levels (moisture content) and more stable temperatures than open infructescences such as those of *P. neriifolia* and *P. nitida*. Similar findings of microclimates and architecture were found in other plant organs. Flowers with a closed shaped have more stabile microclimatic conditions throughout the day and night than flowers with a wider, more open shape (Willmer in Juniper and Southwood, 1986). According to a temperature and humidity study done on Tarsonemidae mites by Jones and Brown (1983), the infructescences of *P. repens*, *P. neriifolia* and even *P. nitida* can support mite reproduction, though, of the three, the infructescences of *P. repens* provides the most ideal niche in which to maintain these mite communities.

It was shown that infructescence architecture (volume and microclimatic stability coefficient (P_i)) has no influence on mite community assemblages. Thus, plants with similar sized infructescences do not necessarily share similar mite communities. However, infructescence volume does influence the abundance and richness of mites within infructescences, even if infructescence size is controlled for. Similarly, microclimatic stability has a significant influence on mite abundance when controlling for infructescence volume. This indicates that larger and more climatically stable infructescences can house more mite species and individuals, probably due to additional resources becoming available with an increase in size (i.e. an increase or differentiation in the assemblages of other organisms within infructescences with increase in infructescence size). This agrees with the recent results of insects and spider assemblages found to be associated with *Protea* infructescences (Roets *et al.*, 2006).

Host plant life form had a significant influence on mite community assemblages as well as on the abundance of mites within infructescences. This might be that different height forms of host plants influence the colonization process of these infructescences. Similarly, Haysom and Coulson (1998) found plant height to influence insect abundance, with a positive trend towards taller host plants. The relationship between mite abundance and mite morphospecies is higher in *Protea* species with the intermediate life forms (shrubs). According to Rebelo (2001) the chosen shrub *Protea* species in this study in general have thread-like pollen presenters with mostly pink inflorescences. Different plant life forms therefore may also play a role in the attraction of pollinators visiting their flowers (Klinkhamer *et al.*, 1989), having an effect on mite vectors. Raghu *et al.* (2004) argued that the influence of plant life form on the behaviour (presence / absence) of a given taxon may even operate at the individual species level. Other factors that may lead to the influence of plant life form on mite community structure may be pollinator (vector) preferences towards host plants and host plant

community structure as well as specific site variables (Janzen and Schroener, 1968; Klinkhamer et al., 1989), which were not investigated in this study.

As the majority of mite morphospecies found are phoretic and associated with insects and birds (Krantz and Walter, 2009), mite vectors may be influenced by flower colour. Inflorescence colour does show to have a significant influence on mite assemblages of different *Protea* species. This indicates that mite communities may be influenced by the mode of pollination of the various *Protea* species. This, in turn, suggests that mites may possibly use different vectors to move between different plant species. However, most mites do not show general host specificity towards *Protea* species, therefore they may, in addition, not be vector specific, and mite community structures within infructescences depends on flower pollinator movements. Previous studies indicated that insect visitations are influenced by flower colour, even though it was less important than flower shape and the time of the year (McCall and Primack, 1992), or the presence of hummingbirds (Melendez-Ackerman *et al.*, 1997). In general, flower visiting insects associated with *Protea* species are generalists. This may explain why mite morphospecies are not generally host specific (Coetzee and Gilliomee, 1985).

Mite community structure within infructescences was also influenced by the level of serotiny of the host plants. Serotinous species house more mite species and individuals than non-serotinous species. This was also found to influence communities of insects associated with *Protea* species (Roets *et al*., 2006). The fact that ophiostomatoid fungi are also only found in serotinous species (Roets *et al.*, 2007) may explain why mites associated with this fungus are also more abundant in these *Protea* species. Roets *et al.* (2007) also found that not only do these mites vector ophiostomatoid fungi, but they can sustain themselves on cultures of this fungus, utilizing it as a food source. Also, in serotinous species, the specific niche (infructescence) persists for a longer time and thus gives mites

more time to colonise them than when infructescences are shed after a short time. Levels of serotiny (the duration of time for which the niche is available) may also play a role in the colonizing effect of the infructescences, as highly serotinous infructescences provide a more complex niche of varying ages (Gillipsie and Roderick, 2002, Roets *et al.*, 2005). It is clear that a variety of factors play a role in mite community structure and in addition these variables may also interact with each other (Hurts *et al.*, 1980).

4.3. The influence of infructescence age on mite assemblages

Infructescence age classes did not play a significant role in the mite morphospecies richness. Different age levels did, however, influence the numbers of mites found within infructescences. According to the colonizing effect hypothesis (Gillipsie and Roderick, 2002) older islands (infructescences) are expected to have higher mite richness, because they have had a longer colonization period than the younger islands (infructescences). This was confirmed to be the case for other arthropods associated with *Protea* infructescences (Roets *et al.*, 2006). Older infructescences create more feeding opportunities by either excluding lesser feeding guilds or by augmenting others such as predators (Roets *et al.*, 2006). Interestingly, the same was not found for mites in this system. There is no significant difference between mite morphospecies richness from the one year old to the third year old infructescences, but with a negative trend in mite abundances. Similar results were found in the study of Boggs and Gilbert (1987) on *Lantana* flower-dwelling mites, with younger flower heads having higher mite abundances. This may be a result of changes in infructescence volume and microclimatic stability as, over time, the exposed infructescences become smaller and more open as these are susceptible to damage and weathering. It may also be related to the colonization stability process (Gillipsie and Roderick, 2002), whereby older areas become more stable over time and various tourist groups are out-competed.

4.4. The influence of season on mite assemblages within infructescences

Mite community assemblages change during the different seasons of the year. Roets *et al.* (2006) showed that season does play a significant role in insect abundance and richness, with peaks during the winter months. These seasonal changes of mite assemblages may be ascribed to different climatic conditions during the different seasons affecting the moisture availability and temperatures within infructescences (Lombardero *et al*., 2003; Roets *et al*., 2005). The Uropodidae and Tarsonemidae are associated with ophiostomatoid fungi (Roets *et al*., 2007) and their abundances peak during the same season as the abundance levels of these fungi (Roets *et al*., 2005). The Tydeidae are also associated with fungi and may even be associated with the dominant ophiostomatoid fungi.

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CHAPTER 3

MITE COMMUNITIES WITHIN *PROTEA* INFRUCTESCENCES: THE EFFECT OF HOST INTRA-SPECIES ARCHITECTURAL VARIATION AND HOST BIOGEOGRAPHY

ABSTRACT

This study investigated the influence of host intra-species architectural variation and host geographic distribution on associations between infructescence-colonising mites and *Protea* species. Mite communities were compared between different *P. repens* populations in addition to different *Protea* species from across South Africa. In addition, infructescence size and microclimatic stability were investigated as factors potentially influencing mite communities of *P. repens* from different localities. *Protea repens* individuals from different localities had significantly different infructescence volumes. Infructescence volume and host locality were found to significantly influence mite communities, however, neither had a significant influence on morphospecies density nor density of individuals. These data indicate that host locality influences infructescence size, with bigger infructescences housing higher population sizes and mite morphospecies numbers due to increased habitat space. Geographic distance between *P. repens* localities and other *Protea* species did not play a significant role in determining mite communities. Instead, results suggest that a combination of host species and locality characteristics determine mite communities within *Protea* infructescences.

Keywords: Acari, pollination vectors, range sharing

1. INTRODUCTION

The Cape Floristic Region (CFR) is exceptionally rich in plant species, with *ca*. 68.7% of the approximately 9030 vascular plant species endemic to the region (Goldblatt and Manning, 2002; Higgins *et al.*, 1997). The genus *Protea* is one of the more notable plant genera in the CFR and includes the national flower of South Africa (*P. cynaroides* (*L.*) *L.*). It also forms the cornerstone of the South African cut-flower industry with up to 30% of all flowers exported from the country belonging to this genus (Coetzee and Littlejohn, 2001). This generates an estimated annual income of over US $10 million (Coetzee and Latsky, 1986; Coetzee and Littlejohn, 2001; Crous *et al.*, 2004). *Protea* species are not only economically important, but they are also keystone members of the veld types in which they grow (Littlejohn, 2001). For example, numerous pollinators (both vertebrates and invertebrates) and other organisms rely on *Protea* species for their survival.

Protea population dynamics are influenced by numerous biotic factors, including invasive plant species (Blancafort and Gomez, 2005; Yelenik *et al*., 2004), pollination syndromes (Flemming and Nicolson, 2002 and 2003; Mustart *et al*., 1995), diseases (Crous *et al*., 2000, Swart *et al*., 2000, Taylor and Crous, 2000; Crous *et al*., 2004) and insect pests (Moran, 1983; Wright, 2003; Wright and Giliomee, 1992). Studies on the diversity of arthropods associated with *Protea* species have largely focussed on insects, as these are known to cause major economic problems (Coetzee, 1986; Wright and Giliomee, 1992; Coetzee *et al*., 1997; Wright and Samways, 1999; 2000). Mites, however, represent an often-overlooked biotic element that may affect *Protea* population dynamics. Their influence may include vectoring of diseases (Van der Geest *et al*., 2000), pests control (Faroni *et al*., 2000; Pratt *et al*., 2003) or even mutualistic relationships (e.g. Romero and Benson, 2004). In addition to these ecological effects on *Protea*, mites are also of phytosanitary importance in the cut-

flower industry (Coetzee *et al.*, 1986). Therefore, it is of vital importance to document the diversity, and understand the ecology of these organisms.

Protea life forms range from procumbent woody sub-shrubs to large shrubs and even small trees. Flowers are small and inconspicuous, but clustered together in large, often showy, inflorescences (flower heads) surrounded by colourful involucral bracts. In many species these involucral bracts close after flowering to form protective cone-shaped infructescences within which the seeds are stored above-ground (serotiny) (Rebelo, 2001). Infructescences typically only reopen after a fire to release stored seeds into the open niches created by the fire, and the smoke from the fire triggers seed germination. *Protea* infructescences also provide safe, warm and fairly moist environments within which a diversity of associated organisms can thrive. The infructescences of *Protea* species can thus be considered as miniature ecosystems (Zwölfer 1979) that house different food chains and trophic levels. Mites seem to be a particularly well-represented constituent of the *Protea* infructescence fauna (Chapter 2) and probably forms one of the basal trophic levels.

Results from previous studies (Chapter 2) showed that mite community structure within *Protea* infructescences are significantly influenced by various biotic and abiotic factors, including *Protea* taxonomic group, plant life form and modes of pollination. Infructescence architecture, infructescence age and time of year (season) had a significant influence on mite abundance between different *Protea* species, but not on mite morphospecies richness. Although not specifically tested for in that study, it was also suggested that host geographic distribution might play a role in determining mite communities within *Protea* infructescences. The present study focuses on the effects of intra-host architectural variation and host geographic distribution on the association between infructescence-colonising mites and *Protea* species.

2. MATERIALS AND METHODS

2.1. Factors that influence mite community assemblages within the infructescences of a single Protea species

The influence of various factors on the community assemblage structure of mites within the infructescences of a single *Protea* species was determined using *P. repens* as model host. This species was chosen, as it has one of the widest distribution ranges of all Cape *Protea* species (Rebelo, 2001). Infructescences of various ages were collected from ten *P. repens* populations during autumn 2009 (April and May) from randomly chosen plants. No more than three infructescences were collected from a single plant. Sample sites (Table 1, Fig.1) where located across the entire Cape Floristic Region of South Africa. Samples were stored at 4ºC until further processing.

Mites were extracted from infructescences as previously described (Chapter 2). They were then mounted onto microscope slides in HPVA medium (Krantz and Walter, 2009) and examined using a Zeiss Axioskop Research light microscope. Mounted mites were grouped based on morphospecies and identified to the lowest taxonomic rank possible at the Agricultural Research Centre (ARC), Roodeplaat, Pretoria, South Africa. Reference material is kept at the National Collection of Arachnida, ARC Plant Protection Research Institute, Pretoria, South Africa, as well at the Department of Conservation and Entomology Museum, Stellenbosch University, Stellenbosch, South Africa.

Species richness was estimated using the non-parametric and least biased species richness estimators ICE, Chao2 and Jacknife2 (Hortel *et al.*, 2006). Species accumulation curves calculated from 50

times randomization of samples was plotted for each individual *P. repens* population using Estimate S, Version 7.5.2 (Colwell, 2005). In addition, the species accumulation curve for all sites combined was also calculated using the same software.

To test similarity of mite communities between populations of *P. repens*, presence-absence data of mites were used to construct a dendogram based on Bray-Curtis similarity between mite communities in Primer v.5.2.9 (Clarke, 1993). Data were fourth-root transformed to enhance the weight of uncommon species by stabilizing variance in the samples (Downing, 1979).

Table 1: *Protea repens* sampling sites used in this study.

Site	**Degrees South**	**Degrees East**
Mitchells Pass, Ceres	33˚ 23" 19.08'	19˚ 17" 17.64'
Franschoek Pass, Franschoek	33˚ 55" 13.86'	19˚ 09" 40.74'
Garcia Pass, Riversdale	33˚ 56" 57.54'	21˚ 16" 04.56'
Gordon's Bay	34˚ 10" 20.11'	18˚ 50" 30.67'
Jonkershoek Reserve, Stellenbosch	33˚ 58" 40.02'	18˚ 56" 39.36'
Nieuwoudtville	31˚ 22" 14.46'	19˚ 04" 24.06'
Riviersonderend	34˚ 60" 05.04'	19˚ 49" 46.14'
Struisbaai	34˚ 45" 39.30'	20˚ 00" 00.60'
Swartberg Pass	33˚ 22" 11.22'	22˚ 06" 33.90'
Uniondale Road, George	33˚ 49" 34.44'	22˚ 23" 46.14'

Figure 1: Map of South Africa indicating sampling sites of various *Protea* species and populations of *P. repens* used in this study.

2.1.1. Intra-specific host plant characteristics

The influence of infructescence volume and microhabitat stability on mite assemblages was determined for each infructescence as described in Chapter 2. Mean infructescence volumes and stability coefficients (P_i) were statistically compared between the various *P. repens* populations using an ANOVA on the normally distributed data in Statistica 9 (Statsoft Corporation, USA). A LSD *post hoc* test was performed to evaluate differences between mean volumes of individual *P. repens* populations. Significant differences are reported where $P \leq 0.05$.

To test the impact of these factors on the structure of mite communities within *P. repens* populations a Canonical Correspondence Analysis (CCA) was performed using CANOCO version 4. 5 (Ter Braak and Simlauer, 2002). This method is preferred as it accommodates skew data distributions (Palmer, 1993). To test whether the host plant characteristics had a significant influence on mite communities a Monte Carlo permutation test with 499 permutations was performed. A second multivariate analyses technique, analysis of similarity (ANOSIM) was performed in Primer v.5.2.9 (Clarke and Warwick, 2001) using data from individual infructescences.

2.1.2. Factors that influence mite richness and abundance within the infructescences of P. repens

A generalized linear model with Poisson distribution (with identity link function) in the software programme SAS/STAT Software (SAS Institute Inc., USA) was used to determine the effects of infructescence volume, microclimatic stability and different sites on mite species richness and abundance (McCulloch *et al.*, 2008). Additionally, the influence of these same variables were tested on mite morphospecies density and individual density (species richness and abundance when

controlling for differences in infructescence size) using a generalized linear model with Poisson distribution and logit link function.

2.1.3. The effect of geographic distance on mite assemblages between different P. repens populations

A geographic distance matrix was constructed (km) for distances between all *P. repens* populations using the Geographical Distance Matrix Generator v. 1.2.3 (Ersts, 2010). A dissimilarity matrix was constructed based on mite communities found within the infructescences of *P. repens* at the different sites using Primer v.5.2.9 (Clarke, 1993). The two variables were combined in a regression analysis using Statistica 9 (Statsoft Corporation, USA). Pearson product-moment coefficient for data distributions was calculated in SAS/STAT Software (SAS Institute Inc., USA).

2.2. The combined influence of host taxonomy and host geographic distribution on mite assemblages

Ten infructescences of 14 *Protea* species were collected from various sites across South Africa (see Chapter 2) and mites were extracted from these. This data was combined with mite assemblage data collected in the present study for mites associated with the infructescences of *P. repens* from various populations. Patterns of association were investigated to determine if mite communities were similar due to taxonomic similarity of the hosts or due to the geographic location of the host plants (Fig. 1).

To identify trends between host plant taxonomy and mite assemblages, presence-absence data were used to construct a dendogram based on Bray-Curtis similarity of the mites associated with the infructescences of various *Protea* species (Chapter 2) combined with data collected in the present study using Primer v.5.2.9 (Clarke, 1993). To enhance the weight of uncommon species data were fourth-root transformed (Downing, 1979).

A geographic distance matrix was constructed for distances (km) between all *P. repens* populations combined with distances between sampling localities of other *Protea* species using the Geographical Distance Matrix Generator v. 1.2.3 (Ersts, 2010). A dissimilarity matrix was constructed based on mite communities found within the infructescences of *P. repens* at the different sites and mite communities from the different *Protea* species using Primer v.5.2.9 (Clarke, 1993). A regression analysis was performed in Statistica 9 (Statsoft Corporation, USA) with Pearson's product-moment coefficient calculated in SAS/STAT Software (SAS Institute Inc., USA).

3. RESULTS

3.1. Factors that influence mite community assemblages within the infructescences of a single Protea species

A total of 14 mite morphospecies (335 individuals) representing 12 families were collected from the 10 *P. repens* populations sampled (Appendix 4). Most of the collected mite individuals belonged to the family Uropodidae, representing 29 % of all the mite individuals collected from these infructescences (Fig. 1). The Tarsonemidae and Tydeidae were the second and third largest families, respectively (Fig. 1). Individuals of the Iolinidae were also abundant relative to other families. The Orbatidae and Ghlycyphagidae were scarce in comparison to previous results (Chapter 2) where these two families were rather commonly found on various *Protea* hosts. Two morphospecies of the family Tydeidae were extracted from *P. repens* infructescences, with 68% of the collected individuals belonging to morphospecies *Tydeidae sp.1* (Fig.1 and Fig. 2). Similarly, in the family Iolinidae, the species *Microtydeus beltrani* represented 85% of sampled individuals. The families Uropodidae and Tarsonemidae were represented by single morphospecies that were very abundant within the infructescences of *P. repens*.

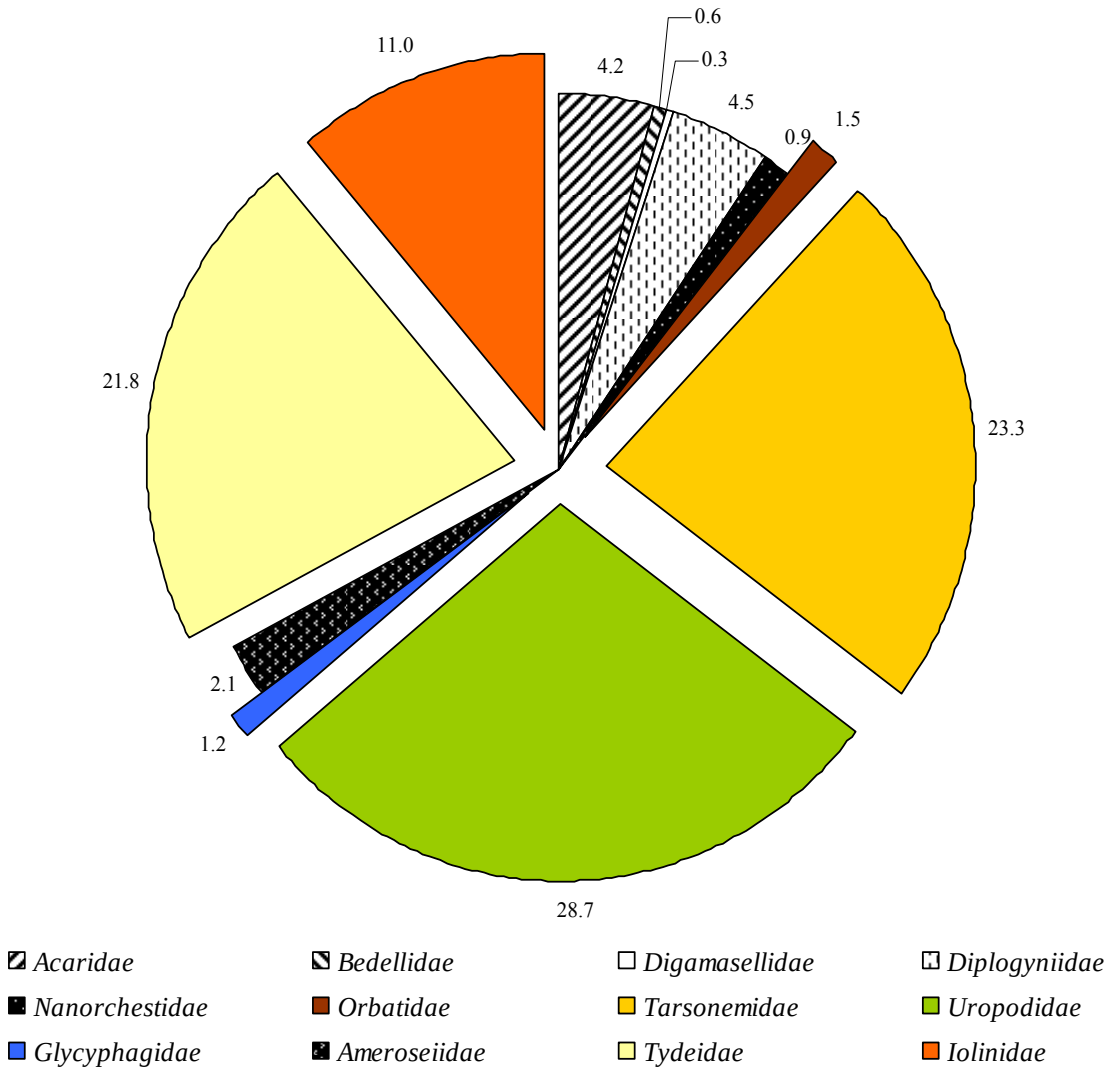

Figure 2: Proportion of collected mite individuals grouped according to family (as a percentage) collected from the infructescences of ten *P. repens* sites (n = 10 infructescences per site).

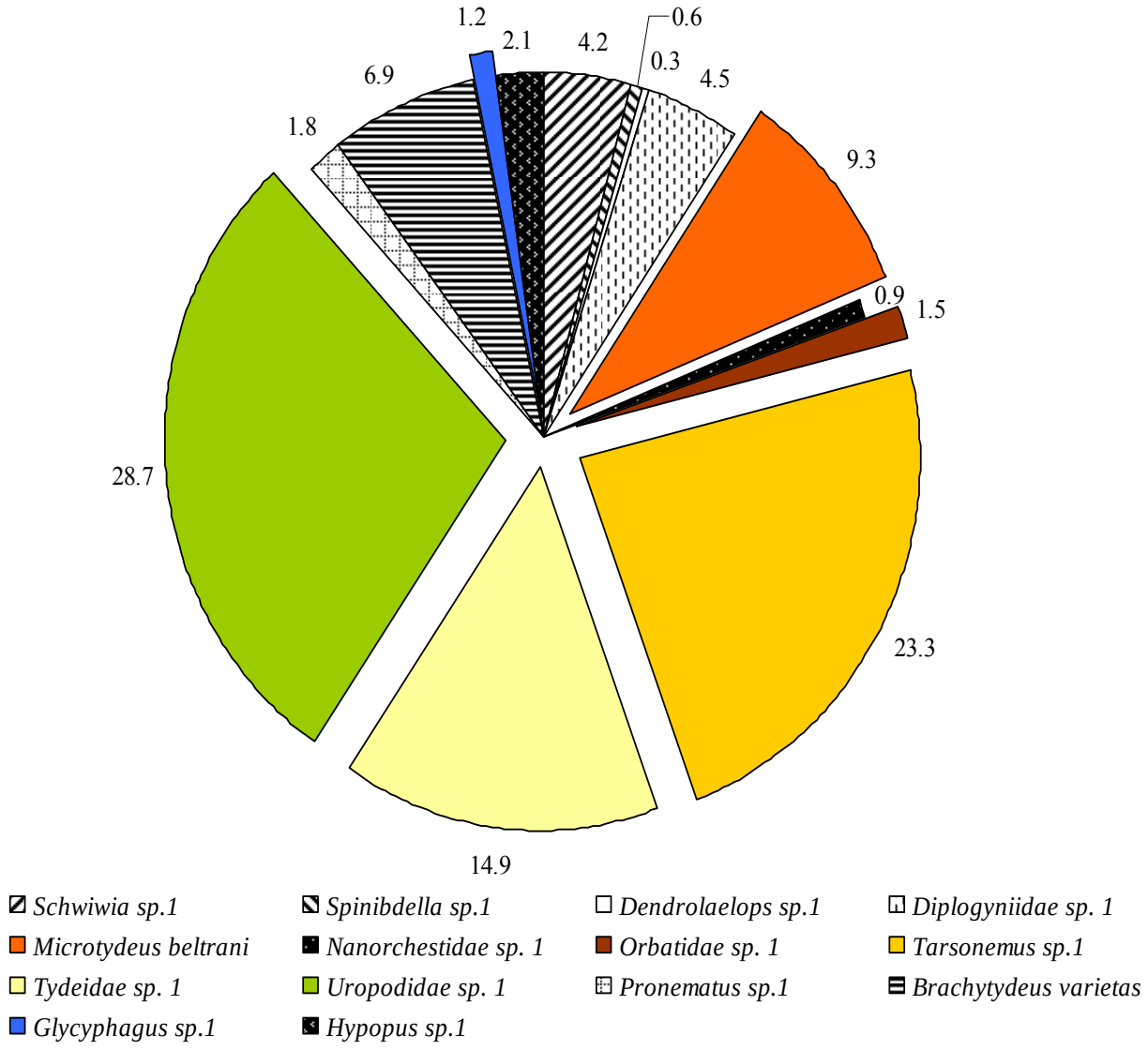

Figure 3: Proportion of collected mite individuals grouped according to species (as a percentage) collected from the infructescences of ten *P. repens* sites (n = 10 infructescences per site).

Estimated species richness indicators are summarised in Table 2. The Ceres locality had the highest overall estimated morphospecies richness, while individuals from George showed the lowest mite morphospecies richness (Table 2). The overall morphospecies richness estimations (ICE = 13 species, Chao 2 = 13, Jackknife 2 = 14) indicated a slight under-representative sample size (observed species = 13) (Table 2). The observed mite morphospecies numbers from *P. repens* infructescences collected at Nieuwoudtville, Struisbaai, George, Riverdale, Franschoek and Gordon's Bay compared well to the estimated species numbers, confirming adequate sampling efforts from these populations (Table 2).

Table 2: Estimated mite morphospecies richness collected from 10 different *P. repens* sites (n = 10 infructescences per site) calculated from 335 collected individuals.

Sites	**Observed number of species**	**Total abundance**	**ICE***	**Chao2 ** (± SD)**	**Jackknife2*****
All sites	13	335	13.36	13 (0.25)	14
Nieuwoudtville	4	35	4	4 (0.21)	2.58
Ceres	9	31	11.61	9.68 (1.31)	11.97
Riviersonderend	4	7	10.5	5.35 (2.37)	8.39
Struisbaai	4	13	4.72	4 (0.15)	4.28
George	2	32	2	2 (0.15)	2
Swartberg	5	26	7.55	5.45 (0.19)	7.69
Riverdale	4	11	4.58	4 (0.15)	4.28
Jonkershoek	5	21	6.57	5.45 (0.19)	7.69
Franschoek	4	56	4	4 (0.16)	4
Gordon's Bay	5	103	5	5 (0.25)	4.29

* Incidence-based coverage estimator, **Second order Chao estimator, *** Second order Jackknife estimator

When combining mite collection data for all *P. repens* sites in a species accumulation curve an asymptote was reached (Fig. 3). This indicated that sampling effort was sufficient to determine overall mite morphospecies richness on *P. repens* in general. Similarly, when considering sampling effort for individual *P. repens* sites, accumulation curves mostly also reached asymptotes (Fig. 4).

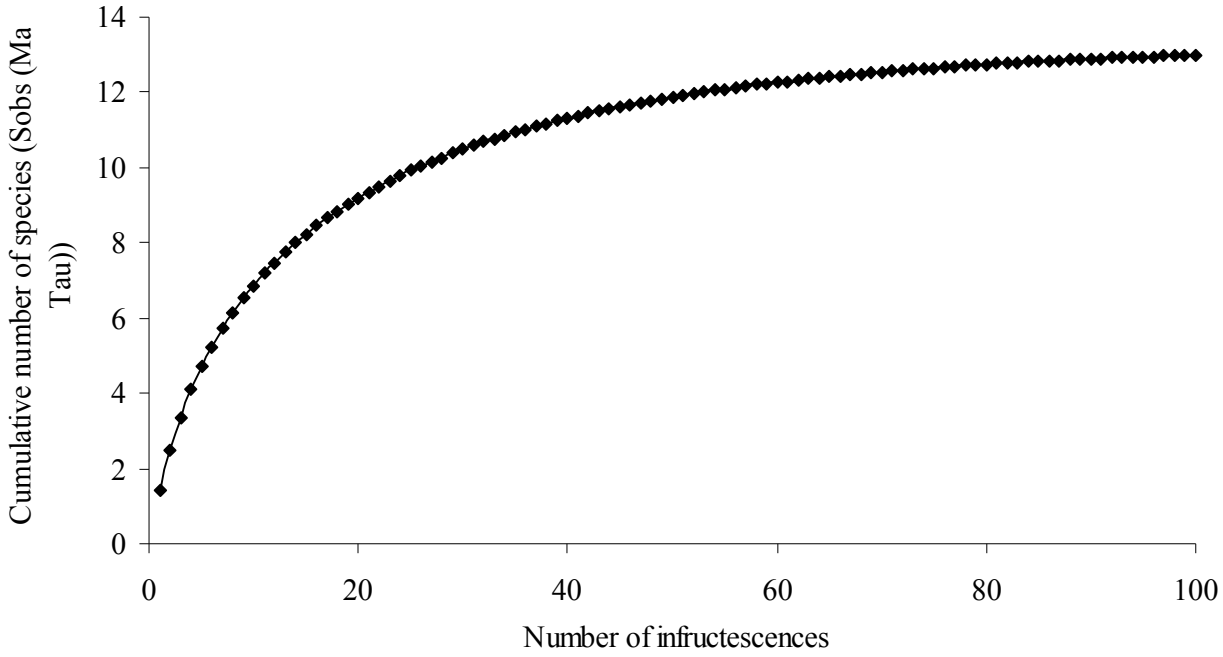

Figure 4: A combined accumulation curve for all mite morphospecies collected from the infructescences of ten *P. repens* populations (n = 10 infructescences per site).

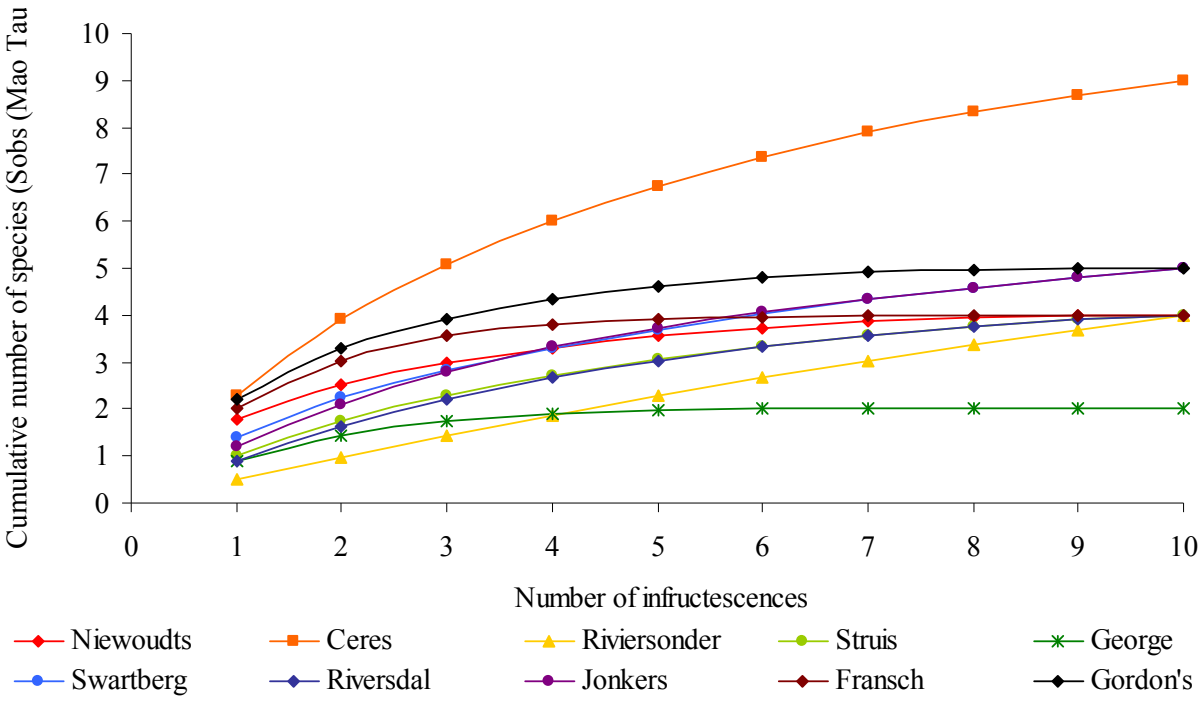

Figure 5: Accumulation curves for mite morphospecies collected from the infructescences of different *P. repens* populations (n = 10 infructescences per site). Franch = Franschoek, Goerge = George, Riviersonder = Riversonderend, Struis = Struisbaai, Gordon's = Gordon's Bay, Niewoudts = Niewoudtville, Jonkers = Jonkershoek.

Figure 5 depicts a dendogram based on the Bray-Curtis similarity analysis of mite assemblage data obtained from the different *P. repens* populations. In general, *P. repens* populations show fairly high levels of similarity in terms of their mite communities. The highest pair-wise similarities ranged between 50 and 60%. The Franschoek and Gordon's Bay populations branched of at the base with less than 20% similarity in mite assemblages to all other populations (Fig. 5). Three population clusters, the Struisbaai, Riviersonderend and Riversdal populations; the Ceres, Nieuwoudtville and Jonkershoek populations; and the George and Swartberg branch at a similarity of *ca.* 60% (Fig. 5). In some instances, populations that are in close proximity seemed to group together (e.g. the branch

with populations from George and the Swartberg and the branch containing populations from Riviersonderend, Riversdal and Struisbaai). In other instances, populations that were geographically distant grouped together with high similarity (e.g. the Nieuwoudtville and Jonkershoek populations).

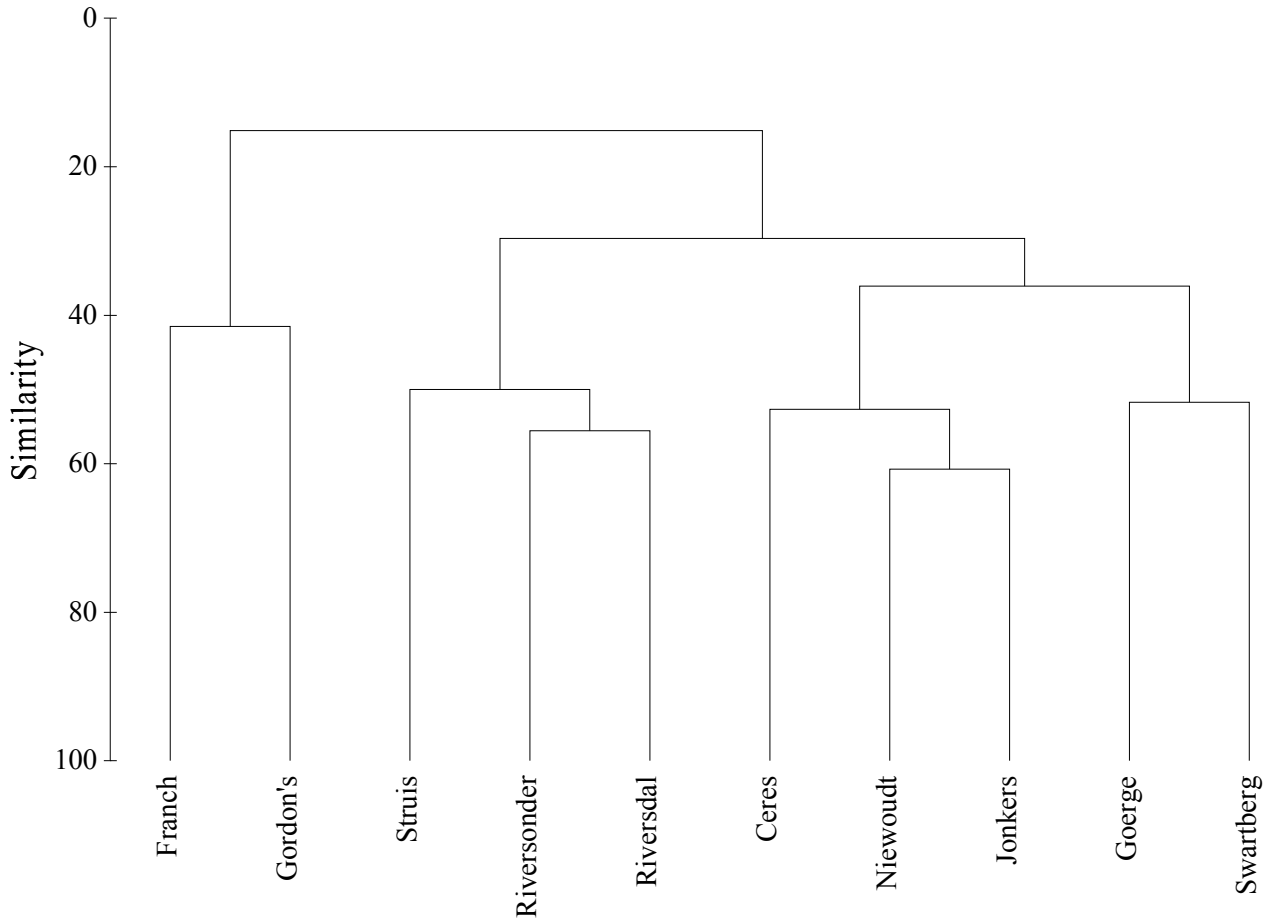

Figure 6: Dendogram depicting the results of a cluster analysis of 10 *P. repens* populations based on mite assemblage data collected from 10 infructescences from each population. Franch = Franschoek, Goerge = George, Riviersonder = Riversonderend, Struis = Struisbaai, Gordon's = Gordon's Bay, Nieuwoudt = Niewoudtville, Jonkers = Jonkershoek.

3.1.1. Intra-specific host plant characteristics

There were significant differences between the mean volumes (d.f. = 9, F = 5.869, P = <0.001) of infructescences from most *P. repens* populations collected (Fig. 6). Individuals from Riverdale, Franschoek and Riviersonderend had the largest infructescences, while individuals from the Swartberg, George and Nieuwoudtville had the smallest infructescences (Fig. 6). There were no significant differences detected for the microclimatic stability coefficients (d.f. = 9, F = 1.007, P = 0.440) between any of the *P. repens* populations.

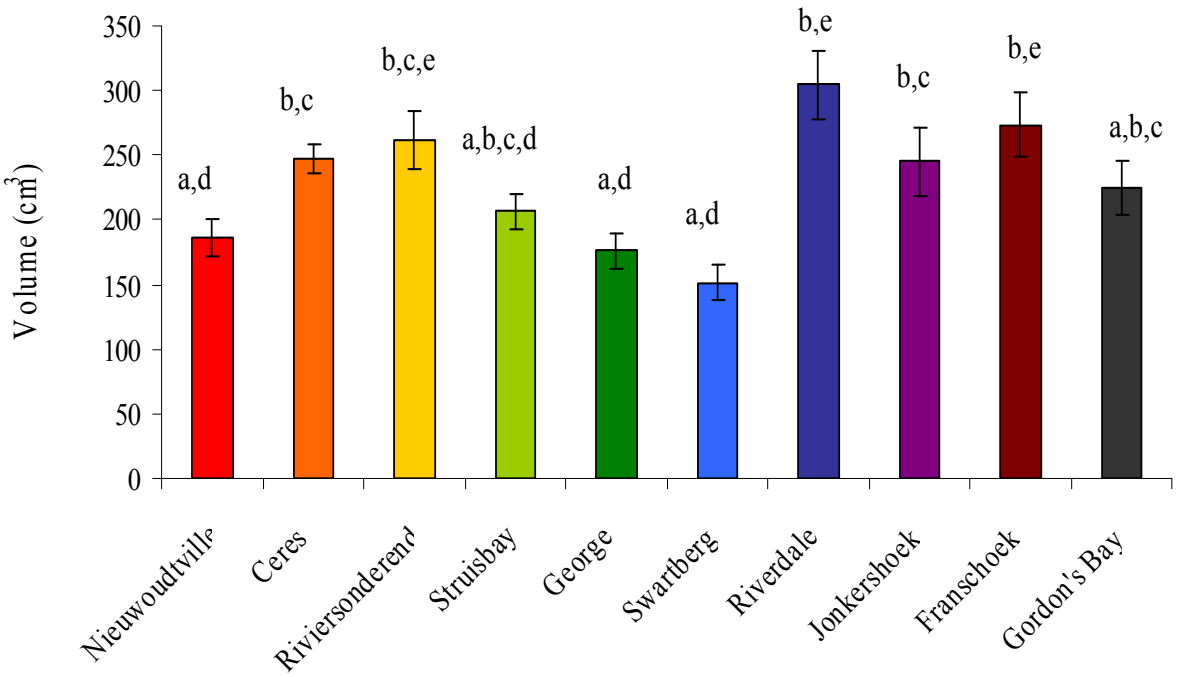

Figure 7: Mean volume (± SE) of the infructescences of ten *P. repens* populations (n = 10 infructescences per site). Significant differences in volume are indicated by different letters.

Infructescences architectural variables were tested to establish their influence on mite community structure in ten populations of *P. repens* using a CCA Monte Carlo permutation test. Infructescences volume had a significant influence on mite community structure within the infructescences of *P. repens* (Table 3), while microclimatic stability had no influence on assemblages of mites (Table 3).

Table 3: Summary of Monte Carlo permutation tests (CCA) that show the influence of tested plant architectural variables on mite assemblages in the infructescences of their *P. repens* hosts. P values in bold typeface indicate factors that had significant influences on mite assemblages.

Architectural variables	**N**	**F**	**P**
Infructescence Volume	499	2.31	**0.024**
Microclimatic Stability coefficient (P_i)	499	0.69	0.684

The architectural variables were plotted (as vectors) in a Canonical Correspondence analysis (CCA) biplot containing the *P. repens* sites (Fig. 7). Numerous *P. repens* populations clustered together based on mite community structure when volume and microclimatic stability were included in the analyses. Populations from Riviersonderend and Struisbaai; Gordon's Bay and Ceres; Nieuwoudtville and George; and Franschoek and Jonkershoek clustered very close to one another, respectively, while the Swartberg and Riversdal populations did not group near any other populations (Fig. 7).

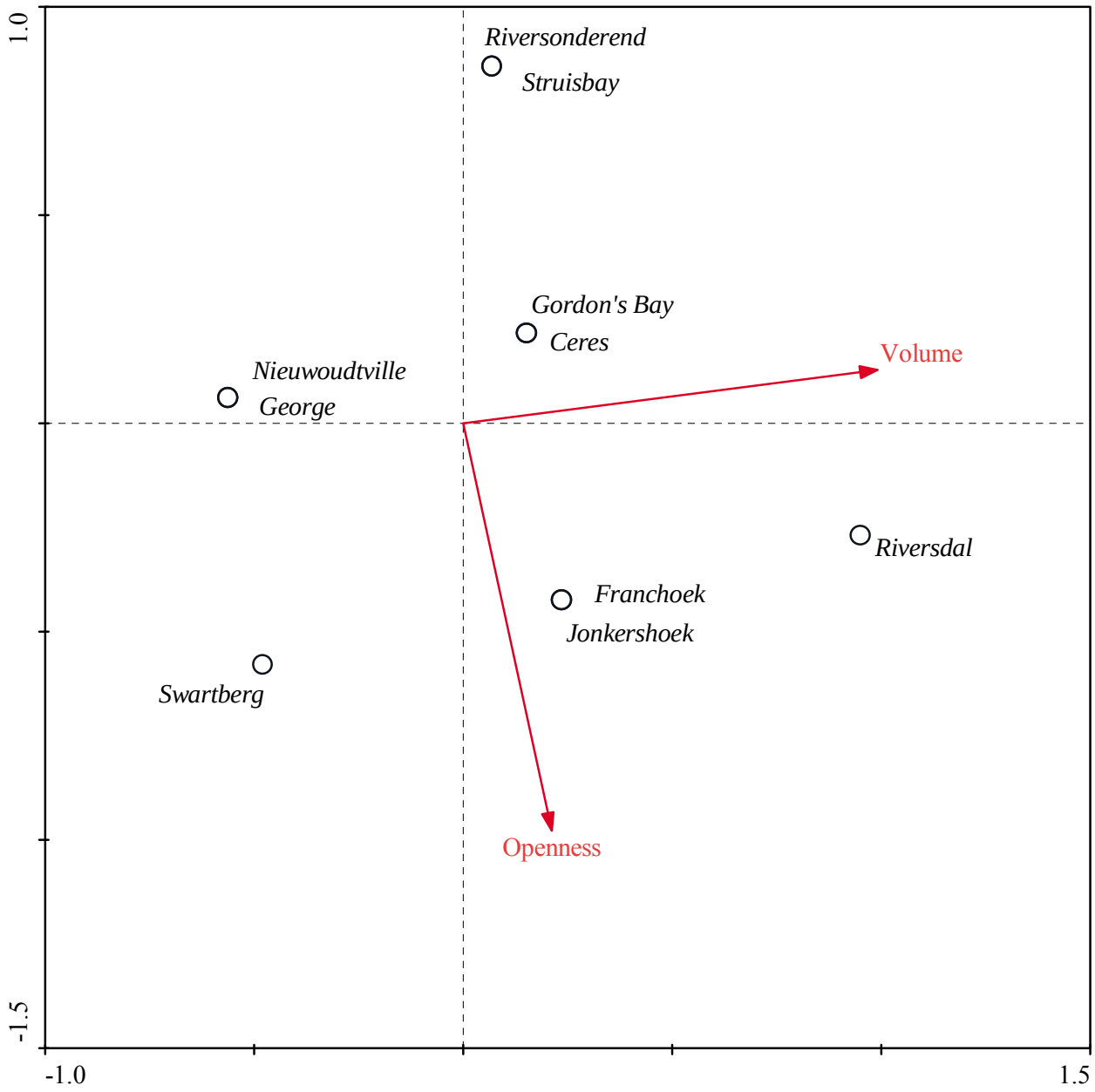

Figure 8: Canonical correspondence analysis (CCA) biplot for host plant characteristics and 10 *P. repens* collection sites (Eigen values: CCA1 = 0.392; CCA2 = 0.116). Openness = microclimatic stability coefficient, Volume = infructescence volume.

Testing for the influence of host plant architectural variables on mite community structure within *P. repens* using ANOSIM yielded similar results to the CCA analysis (Table 4). Again, infructescence volume had a significant influence on mite community structures whilst microclimate had no influence. Different sites had a highly significant influence on mite assemblages (Table 4).

Table 4: Summary of ANOSIM Global R values for the influence of tested variables on mite community structure, along with their P levels, based on mite community assemblages in the infructescences of their *P. repens* hosts. P values in bold typeface indicate factors that have significant influences on mite assemblages.

Variables	**No. of permutations**	**Global R**	**P**
Volume (V)	999	0.012	**0.0299**
Microhabitat (P_i)	999	-0.038	0.0753
Site	999	0.278	**0.001**

3.1.2. Factors that influence mite richness and abundance within the infructescences of P. repens.

Results from generalized linear model analyses with Poisson distribution on the influence of various tested variables on mite morphospecies richness and abundance for *P. repens* are summarised in Table 5. Also presented in the table are the mite morphospecies density and density of mite individuals. Collection locality for *P. repens* had a significant influence on both mite morphospecies richness and abundance, while infructescence volume only had a significant effect on mite abundance (Table 5). After standardization for *Protea* infructescence size, none of the variables showed significant differences for either morphospecies- or individual density.

Table 5: A Generalized linear model with Poisson distribution, indicating the influence of host architectural variables and collection sites on morphospecies richness and abundance of mites collected from the infructescences of 10 *P. repens* populations.

	Species Richness			**Abundance**		
	d.f.	Wald stat.	P	d.f.	Wald stat.	P
Intercept						
Volume (V)	1	0.51	0.4744	1	21.21	**<.0001**
Microhabitat (P_i)	1	0.06	0.8064	1	0.23	0.6296
Site	9	31.59	**0.0002**	9	157.04	**<.0001**

	Species density			**Individual density**		
	d.f.	Wald stat.	P	d.f.	Wald stat.	P
Intercept						
Volume (V)	1	0	0.9922	1	0	0.9803
Microhabitat (P_i)	1	0	0.9977	1	0	0.9959
Site	9	0	1	9	0	1

3.1.3. The effect of geographic distance on mite assemblages between different P. repens populations

Figure 8 depicts the relationship between dissimilarity in mite community structures of *P. repens* populations and the geographic distance (km) between collection sites. There was a slight positive, though non-significant, correlation between geographic distance and dissimilarity in *P. repens* mite communities. Thus, as the distance between *P. repens* populations increased, the dissimilarity between mite community assemblages did not vary significantly (Fig. 8).

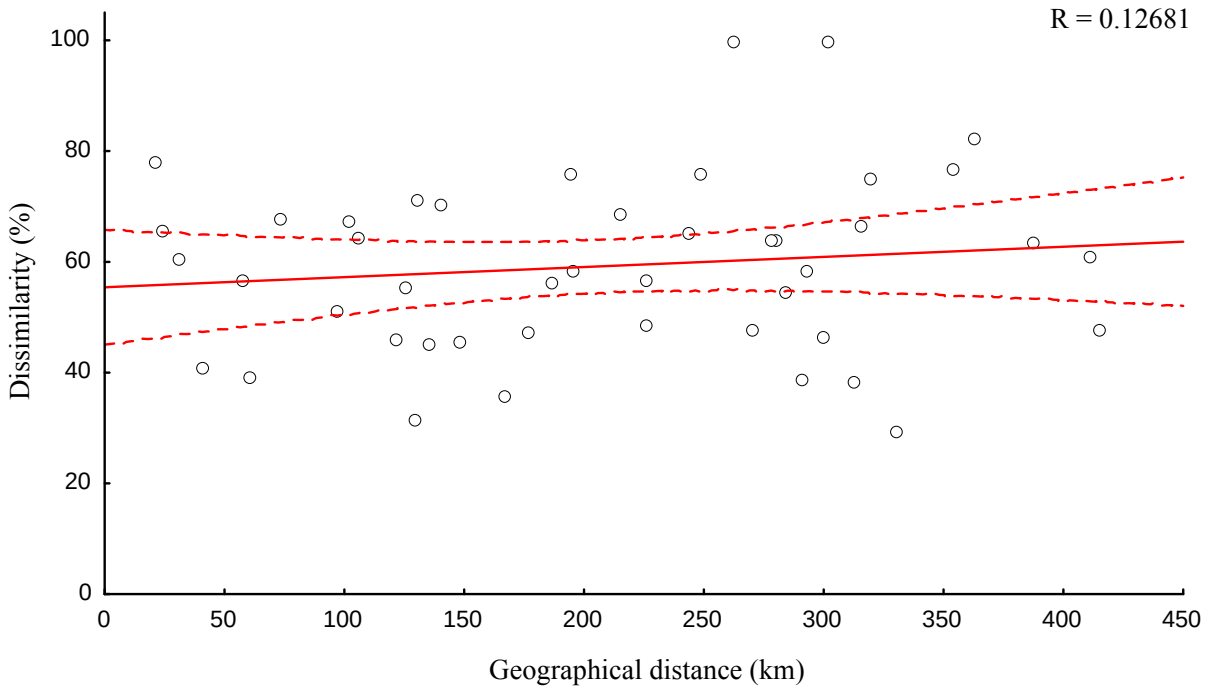

Figure 9: Linear regression for *P. repens* populations from a variety of different inter-site geographic distances (km) and their mite community structures (Pearson coefficient P = 0.406).

3.2. The combined influence of host taxonomy and host geographic distribution on mite assemblages

The inter- and intra-taxonomic relationship between 14 *Protea* species based on their mite community structures is summarized in figure 10. A clear grouping of mite communities from *P. repens* collected from different sites was found. A notable exception is the grouping of *P. repens* from Franschoek with *Protea* species from other taxonomic groups and other geographical regions. In general, grouping of other *Protea* species was similar to what was previously found (Chapter 2).

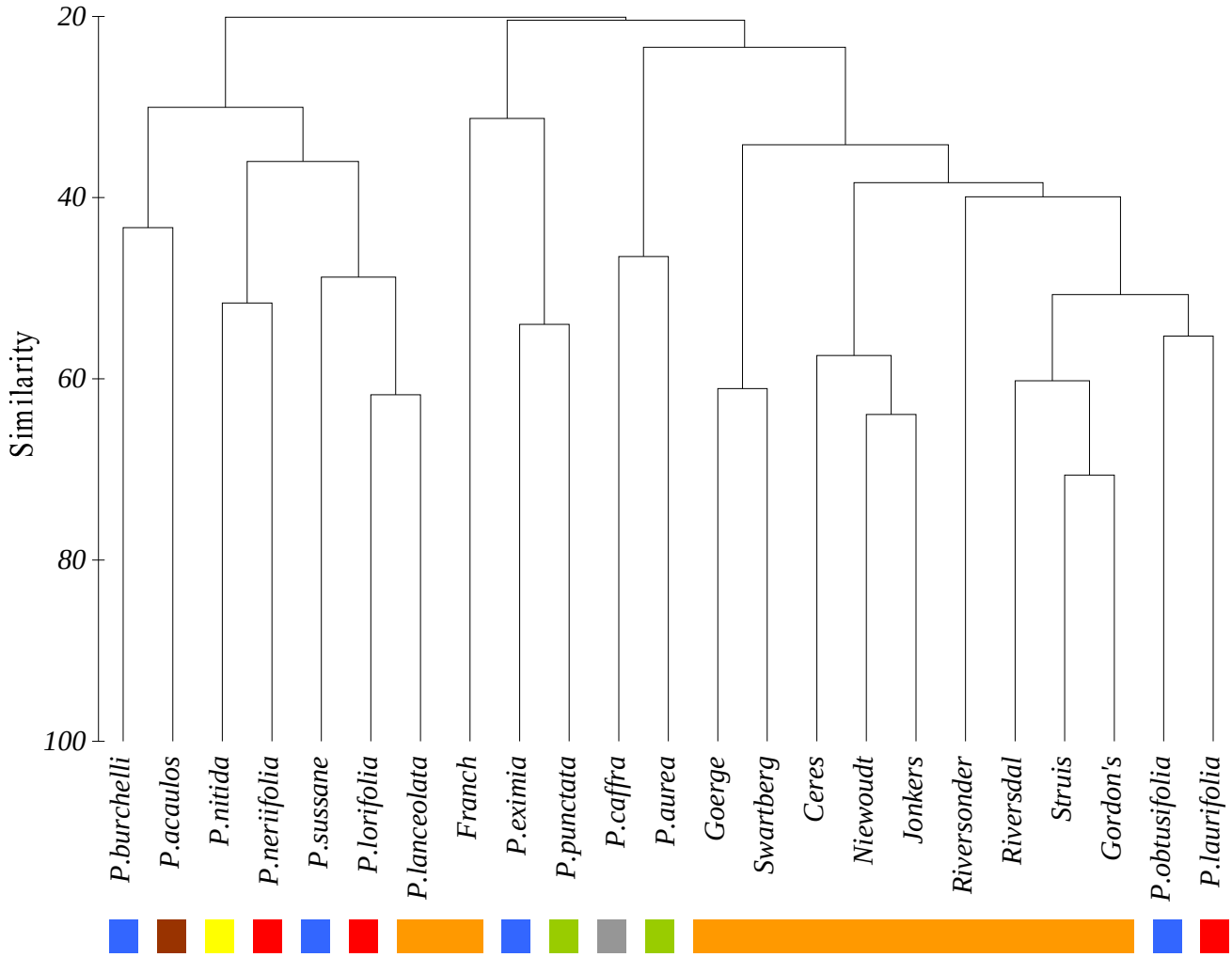

Figure 10: Dendogram showing the results of a cluster analysis for 14 *Protea* species and ten *P. repens* populations based on mite assemblage data (n = 10 infructescences per *Protea* species and site). Different *Protea* taxonomic groups are indicated by different colours (grey = grassland sugarbush, blue = spoon-bract sugarbush, green = white sugarbush, yellow = shaving-brush sugarbush, orange = true sugarbush, red = bearded sugarbush, brown = western ground sugarbush).

Similar as with results obtained for *P. repens* from different sites, geographic distance between collection sites had no influence on mite communities (Fig. 9). Thus, with an increase in geographic distance between *Protea* collection sites (up to 1600 km), dissimilarity between mite community structures did not generally increase.

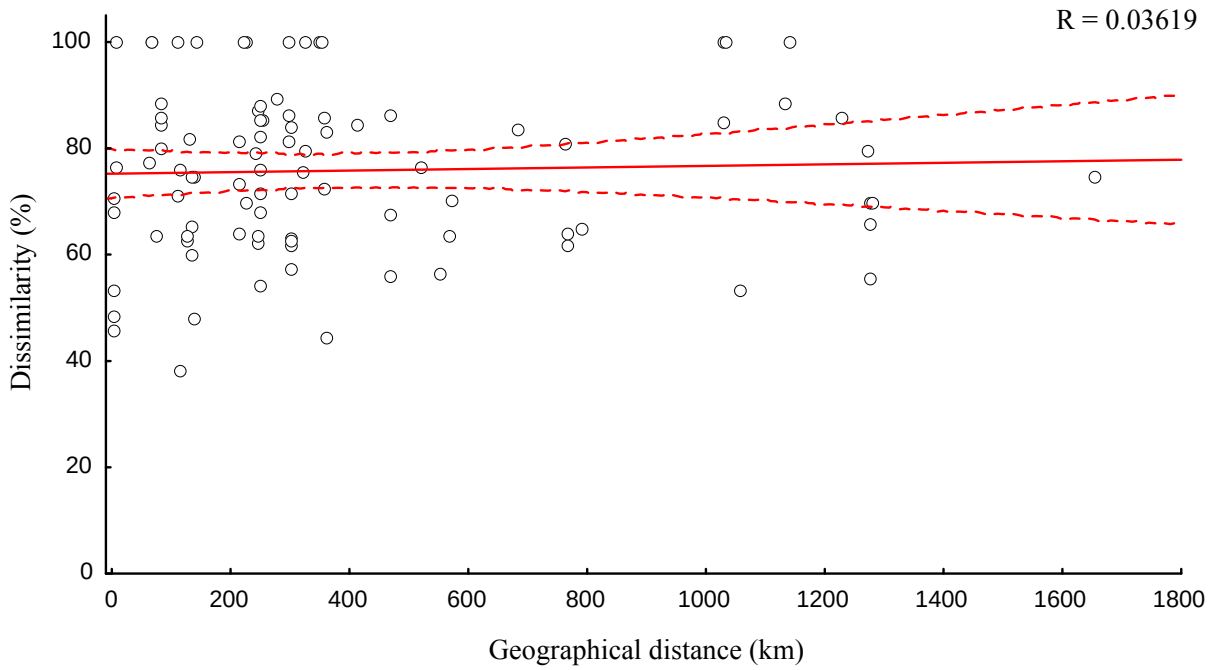

Figure 11: Linear regression for *Protea* species collected from sites of various distances (km) and dissimilarity in their mite community structures (Pearson coefficient P = 0.733).

4. DISCUSSION

In this study 14 mite morphospecies (335 individuals) were collected from 100 *P. repens* infructescences during a single season (autumn). Generally, *P. repens* infructescences showed high mite morphospecies richness in comparison to e.g. insect borers and other arthropod groups from this niche (Wright and Samways, 1999). When compared to previous results (Chapter 2), the Orbatidae and Ghlycyphagidae were far less abundant in this study. This may be ascribed to the influence of differences in infructescence climatic conditions between *P. repens* and other *Protea* species and/or differences in host plant characteristics.

The Uropodidae, Tarsonemidae and Tydeidae represented the most abundant mite morphospecies from *P. repens* infructescences. All of these are phoretically associated with other organisms (Krantz and Walter, 2009). The morphospecies of Uropodidae and the Tarsonemidae collected from *P. repens* are known to feed on fungi (Krantz and Walters, 2009; Roets *et al.*, 2007) and are closely associated with the dominant fungi found in *Protea* infructescences (ophiostomatoid fungi, Roets *et al.*, 2005). The have been identified as primary vectors of ophiostomatoid fungi between *P. repens* infructescences (Roets *et al.*, 2007). The association between the fungi and the mites is mutualistic as the mites feed and reproduce on a diet consisting solely of their phoretic fungi. This close association with the dominant fungi in *P. repens* infructescences thus explains the high proportion of these mites collected from the infructescences of this species. Similar to the Tydeidae and the Iolinidae morphospecies previously collected (Chapter 2); species collected in this study probably represent undescribed species and/or newly recorded genera in Africa. These will be taxonomically assessed in a later study (Chapter 4).

4.1. The effect of host plant characteristics on mite assemblages between different populations of Protea repens

It was shown that infructescence volume had a significant influence on mite community structure within a single species (*P. repens*). This is in contrast to results of previous studies (Chapter 2) in which volume was not found to significantly affect mite community structure between different *Protea* species. Different factors may thus influence community structures of mites associated with hosts at different taxonomic levels.

When only considering the effect of host plant characteristics on the abundance and morphospecies richness of mites associated with *P. repens* it was found that the size of infructescences plays a significant role in the abundance of mites. However, these effects disappear when standardizing for differences in the volume of infructescences. Specifically, infructescence size had no influence on mite density. Thus, larger infructescences of *P. repens* house more mite individuals simply because these are larger (more space to occupy). This contrasts to what was found for the abundance and richness of mites associated with different *Protea* species (Chapter 2). In that study mite morphospecies richness, morphospecies density, abundance and density of individuals were all significantly influenced by infructescence size. This may indicate that differences between host species have a larger role to play in the abundance of infructescence colonising mites than the actual size of infructescences. The observed patterns (Chapter 2) may thus have been mainly caused by differences in host plant characters other than infructescence size.

Similar to what was found for comparisons between mite communities between different *Protea* species, microclimatic stability did not influence community assemblages within *P. repens*. For *P. repens* this is probably due to the lack of significant differences between the microclimatic stability

of different infructescences between populations of a single species. However, the lack of significant differences in mite community structures between different *Protea* species in relation to microclimatic differences is more difficult to explain as there were marked differences between these for the different *Protea* species (Chapter 2). The lack of significant differences in mite community structure in these species is probably due to the overall mediocre variance in their microclimates (see Chapter 2). Thus, mites are probably adapted to, and can thrive in a range of different microclimatic conditions experienced in *Protea* infructescences. For instance, Hodkinson *et al.*, (1996) investigated the influence of warmer summer temperatures on arctic soil fauna, including mites, and concluded that these can easily survive temperature fluctuations outside the norm. Similarly, Fields (1992) showed that mites could survive a range of different temperatures, even if there were also changes in humidity levels (Jones and Brown, 1983).

4.2. The influence of host species, site differences and geographic distance on mite communities between different populations of P. repens and other Protea species

In the present study it was found that locality had a significant influence on the community structure of mites within *P. repens* infructescences. Thus, different localities housed different mite communities. However, even though numerous sites that were in close proximity clustered together, this pattern was independent of the specific geographic distances between the collection sites. Other factors that influence these sites are thus important to explain the observed patterns. The George and Swartberg and the Franschoek and Gordon's Bay populations are in relative close proximity to one another. Populations from Struisbaai, Riviersonderend and Riversdal, on the other hand, may group together due to their association with the lowland limestone Fynbos in the Bredarsdorp-Riversdale Centre (BRC) (Willis *et al.*, 1996). The clustering of the Ceres-Nieuwoudtville-Jonkershoek populations and other groupings found in this study are more difficult to explain but may be due to

the restrictions in the movement of pollinators (mite vectors) and food availability (distribution of fungal species). These factors need further investigation. The lack of significance in regression analyses of mite dissimilarity and geographic distance may indicate that there is movement of vectors between populations, sometimes over great distances. Although the travel ranges of *Protea* visitors/pollinators differ considerably, these results suggest that these ranges probably overlap, promoting the dissemination of mite species across the entire distribution range of specific *Protea* species.

Results of the cluster analyses with combined data from the different *P. repens* populations and other *Protea* species indicated that host species played a large role in mite community assemblages within infructescences. Most *P. repens* sites clustered together indicating that the mite communities within their infructescences are very similar as when compared to those of other *Protea* species. In addition, geographic distance had no role in explaining the observed patterns. Thus, it seems as if mite communities are particular to specific *Protea* species mostly due to characters of the hosts themselves. The influence of host chemistry may also play an important yet unexplored role in determining mite assemblages (Jones and Lawton, 1991).

Together with previous studies on mites associated with *Protea* (Chapter 2), this study forms a basis for future studies on this system. These studies should lead to the stimulation of more studies on mites in the Cape Floristic Region and to a better understanding of the factors that influence mite numbers in the region. Ultimately, these studies should indicate whether the CFR houses a mite biodiversity equally impressive to its exceptionally rich flora.

Acknowledgements

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CHAPTER 4

A NEW GENUS AND EIGHT NEW SPECIES OF TYDEIDOIDAE (ACARI: TROMBIDIFORMES) FROM *PROTEA* SPECIES IN SOUTH AFRICA

ABSTRACT

Seven new Tydeoidae species (*Brachytydeus rutrus*, *Brachytydeus varitas*, *Brachytydeus pseudovaritas*, *Paratydeaolus athaliahea*, *Pausia colonus*, *Therontydeus proteacapensis* and *Tydeus pseudofustis* are described from the infructescences of various South African *Protea* species. An unusual member of the family Triophtydeidae was assigned to the monotypic genus *Therontydeus*. To the best of our knowledge, the genera *Microtydeus*, *Paratydaeolus* and *Pausia* are here recorded from Africa for the first time. The collection of the South African *Microtydeus* emphasized a need for the revision of the genus. An identification key for all known Tydeoidae mites present in the infructescence of African *Protea* species is provided.

Keywords: Acari, taxonomy, Tydeoidae.

1. INTRODUCTION

A study by Roets *et al*., (2007) suggested there to be a very large diversity of mites associated with *Protea* infructescences. Their study focused on the description of a mutualistic association between ophiostomatoid fungi that inhabit these structures and various mite species. In Chapter 2 mite communities in a variety of *Protea* species where investigated and numerous new discoveries were made, specifically in one superfamily of mites, the Tydeoidae.

The Tydeoidea includes four families: Ereynetidae, Iolinidae, Triophtydeidea (Edbakerellidae) and Tydeidae (Donczyk, 2006, Krantz and Walter, 2009) and *ca*. 620 described species (Andre and Fian, 2000). The Ereynetidae includes *ca*. 180 known species, and is distinguished by the presence of an ereynetal organ on tibia I (Krantz and Walter, 2009). This family prefers humid to moist conditions and includes various feeding guilds, ranging from predators to parasites. The Iolinidae includes *ca*. 125 known species and is defined by the absence of genital papilla, the absence of empodia on legs I and the gain of direct copulation (Krantz and Walter, 2009). The free-living Iolinidae can occur in soil, on foliage or in association with, or dependant on, insects. The family Triophtydeidea includes *ca*. 40 species and is defined by the presence of three sets of prodorsal eyespots (Krantz and Walter, 2009). This newly derived group includes soil, plant and cortical living species, but little is known about the details of their feeding behaviour. The cosmopolitan Tydeidae is the largest family in the Tydeoidea and includes *ca*. 340 known species characterized by the variation and combinations of setal characters (Krantz and Walter, 2009). Tydeid mites include a wide range of feeding guilds such as fungivores, phytophores, predators and even scavengers. The Krantz and Walters (2009) Manual of Acarology, makes very little reference to South African examples, and none to mites from the Cape Floristic Region.

Mite diversity in general is still extremely understudied in South Africa, and almost no research has been done on this topic in the Fynbos of the Cape Floristic Region (CFR). Roets *et al*., (2007, 2009) recently explored the inter-organismal interactions between ophiostomatoid fungi and *Protea* species, identifying mites as primary, and insects as secondary, fungal spore vectors in this system. Their results highlighted the importance of mites in ecosystem functioning, and underscored the void in our knowledge of mite diversity in the CFR. Mites evidently form an integral part of Fynbos ecosystems and probably play a significant role in *Protea* populations. The genus *Protea* forms the cornerstone of the South African cut-flower industry, and comprised *ca.* 30% of all flowers exported from South Africa in 1998 (Coetzee and Littlejohn, 2001). The association of *Protea* species with other organisms is thus very important, especially in terms of phytosanitary problems that may lead to major monetary losses.

Protea flowers are borne on woody involucral receptacles to form colourful capitulum-type flowerheads (inflorescences) (Rebelo, 2001). Some *Protea* species retain their seeds inside these flowerheads after flowering (serotiny). These fruiting structures (infructescences) are fire-safe and only release their seeds after fires, which frequent their natural habitat (Rebelo, 2001). These infructescences offer a sheltered and moist environment in which fungi (Roets *et al*. 2007) and arthropods (Coetzee, 1984), including numerous mite species (Chapter 2, Chapter 3), thrive.

During surveys of mites associated with infructescences of South African *Protea* species, several members of the Tydeoidea were retrieved (Chapter 2, Chapter 3). Here we describe eight new species in six genera, one of which is also proposed as a new genus. A key to the Tydeoidea associated with *Protea* infructescences is also provided.

2. MATERIALS AND METHODS

Between September 2007 and April 2009 mites were collected from the infructescences of various *Protea* species in the Western Cape and Gauteng Provinces of South Africa. Mite extraction was accomplished by cutting open the infructescence and shaking them out onto a Petri dish (Chapter 2). Mites shaken loose in this way were collected with a camel hair brush with the aid of a Nikon SMZ645 light microscope and stored in Eppendorf tubes filled with 80% alcohol. These mites were later mounted onto microscope slides in HPVA medium (Krantz and Walter, 2009) and examined using a Zeiss Axioskop Research microscope equipped with a drawing tube. Measurements were done using an Olympus soft imaging system.

Setal notation follows that of Kethley (1990). Measurements are report in micrometers (µm) and are displayed as follows: first measurement signifies the mean, followed by the range in brackets and that of the holotype in square brackets. Leg setal counts include solenidia. Type material was deposited in the National Collection of Arachnida, ARC-Plant Protection Research Institute, Pretoria, South Africa, while some paratypes were sent to the Mite Collection of the British Museum, London, England.

3. TAXONOMY

Family TYDEIDAE Kramer, 1877

Subfamily TYDEINAE Andre, 1980

Genus *Brachytydeus* Thor, 1931

Brachytydeus Thor, 1931, p 102.

TYPE SPECIES: *Tydeus cruciatus* Koch *sensu* André, 2005.

DIAGNOSIS : This genus is defined as follows: Prodorsum recurved; opisthosoma with 10 pairs of setae (ps included); poriodotaxy: three (im sometimes posterior to setae e1); genital organotaxy - adults: no eugenital setae in female but male has four pairs, six pairs of genital setae are present and four pairs of aggenital setae in both adults; coxa I with coxal organ; chaetotaxy of leg segments: 8(ω)-6(ω)-5-5, tibiae 3 + k-2-2-2, genua 3-2-1-1, femora 3-3-2-1, trochantera 1-0-1-0, epimeral formula adults: 3-1-4-2; solenidiotaxy: two; femur IV entire; palp chaetotaxy: 6(ω)-2-2.

3.1. *Brachytydeus rutrus* Theron and Ueckermann, **n. sp.**

Type material

South Africa: Western Cape Province: holotype male, *P. repens*, Gordon's Bay, collected by N. Theron.

Diagnosis

Adults – This species can be recognized by all dorsal setae being short and acute, except for setae f1-2 and h1 which are leaf-like and setae sci which are more than twice the length of the other dorsal setae and smooth; dorsal striae transverse between setae d1; Cheliceral stylets longer than half the length of palptarsus; empodial claws absent. Male (1). Dimensions of holotype: length of idiosoma (including gnathosoma) 340 [Holotype], width 186; legs: I 153, II 128, III 159, IV 171; setae: vi 14,

ve 14, sci 29, sce 14, c1 13, c2 14, d1 13, d2 13, e1 12, e2 11, f1 13, f2 13, h1 12, ps3 13; Cheliceral stylets 17; palptarsus 28.

Dorsum (Figure 1a): all 13 pairs of dorsal setae (Figure 1f) simple and acute, except f1-2 and h1 which are short and leaf-like (Figure 1e), others equal to sub-equal in length with sci (Figure 1g) the longest. Prodorsum with four pairs of setae, opisthosoma nine pairs and three pairs of cupules. Striae with small tubercles, longitudinal on prodorsum and transverse medially on opisthosoma, transverse between setae d1.

Venter (Figure 1b): epimeral formula 3-1-4-2. Genital area with four pairs of aggenital and six pairs of genital setae. Only one pair of anal setae (ps) present. Cupule ih lateral to anal opening.
Gnathosoma: palp chaetotaxy (tibiotarsus to femur): 6(ω)-2-2. Setae pζ and perhaps v slightly forked distally, setae ba and solenidion ω minute.

Legs (Figure 1c and 1d): chaetotaxy of leg segments: tarsi 8(ω)-6(ω)-5-5, tibiae 4-2-2-2, genua 3-2-1-1, femora 3-3-2-1, trochantera 1-0-1-0. All tarsi terminate in two claws and a hairy empodium. Empodial claws absent.

Remarks: This species is unique in that all dorsal setae are short, smooth and acute, except for setae f1-2 and h1, which is leaf-like and striae dorsal between d1 transverse.

Etymology – The species is derived from the Latin word *rutrum* referring to the caudally leaf-like setae.

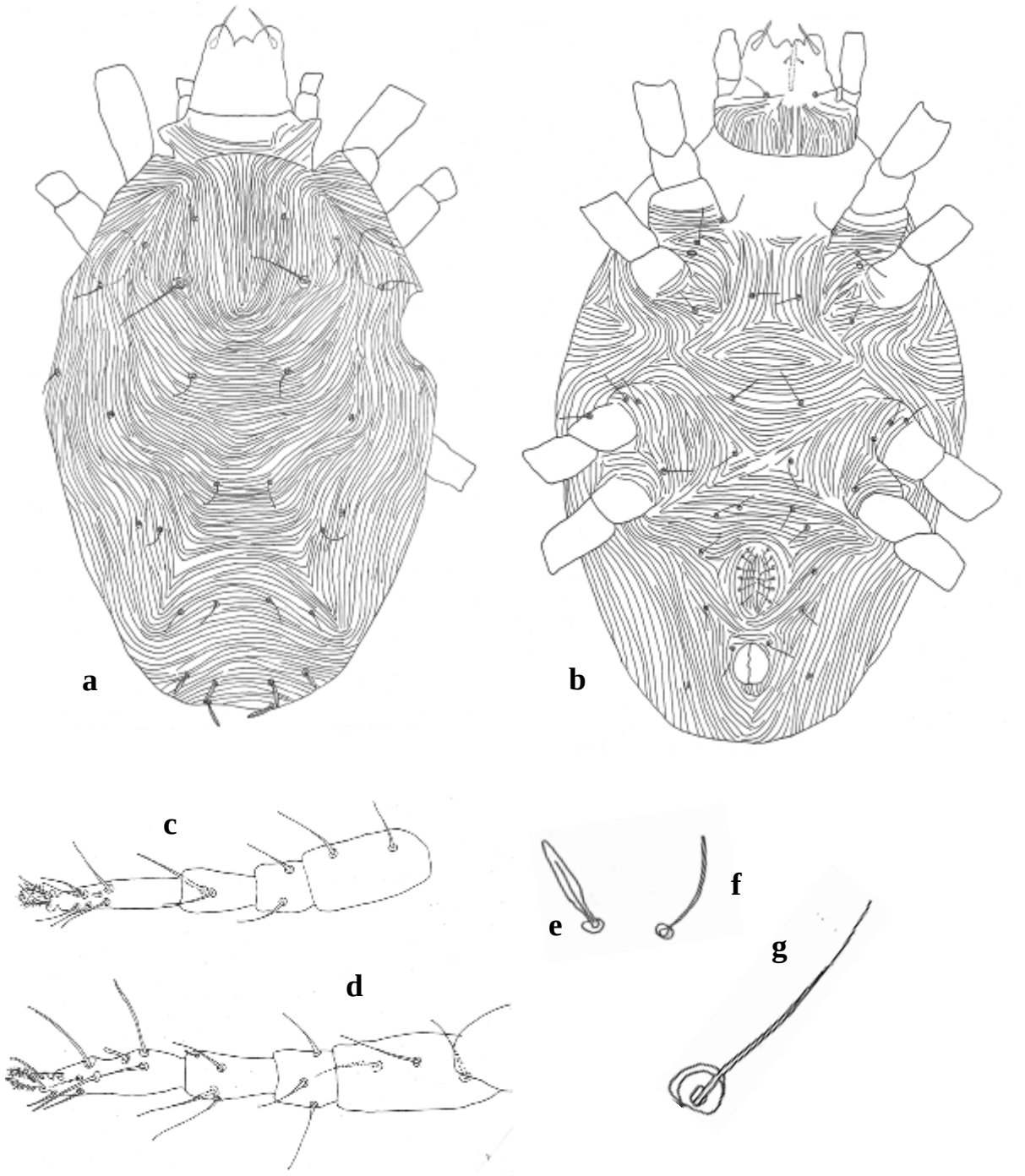

Figure 1: *Brachytydeus rutrus* Theron and Ueckermann, **n. sp.** body characteristics a) dorsum, b) venter, c) leg II, d) leg I, e) seta f2, f) seta c1, g) sci

3.2. *Brachytydeus varitas* Theron and Ueckermann, **n. sp.**

Type material

South Africa: Western Cape Province: holotype female, *P. neriifolia*, Gordon's Bay; six paratype females, *P. repens*, Jonkershoek, Riversdal, Nieuwoudtville; *P. neriifolia*, Gordon's Bay; one paratype male, *P. neriifolia*, Franschoek; three paratype tritonymphs, *P. nitida*, Gordon's Bay, *P. repens*, Nieuwoudtville; one paratype deutonymph, *P. nitida*, Gordon's Bay, and one paratype protonymph, *P. nitida*, Gordon's Bay; collected by N. Theron.

Diagnosis

Adults – The following combination of characters distinguish this new species: All dorsal setae serrate, except for sci which is the longest and smooth, setae become progressively longer towards posterior; dorsal striae longitudinal between setae d1; Cheliceral stylets almost half length of palptarsus; empodial claws present. Female (1). Dimensions: length of idiosoma (including gnathosoma) 301.7 (283–316) [317], width 157.3 (135-167) [159]; legs: I 195.9 (177–218) [209], II 142.6 (125–165) [154], III 159.7 (148–177) [160], IV 177.8 (161–199) [181]; setae: vi 15.7 (14–19) [19], ve 15.6 (14–19) [16], sci 33.1 (31–36) [36], sce 19.1 (17–23) [23], c1 15.7 (14–17) [17], c2 18.1 (15–21) [21], d1 16.7 (14–19) [19], d2 17.7 (16–21) [21], e1 19.7 (18–21) [21], e2 22.7 (20–27) [27], f1 24.2 (17–30) [29], f2 25 (20–32) [28], h 25.3 (20–31) [30], ps 11.5 (9-13) [12]; Cheliceral stylets 14.7 (14–16) [14]; palptarsus 25.5 (24–27) [26].

Dorsum (Figure 2a): all 13 pairs of dorsal setae, except for sci, serrate (Figure 2e). Setae sci (Figure 2h) longest, slender and smooth along entire length, others equal to sub-equal in length. Prodorsum with four pairs of setae, opisthosoma nine pairs (excluding ps) and two pairs of cupules, ip

apparently absent. Striae with small tubercles, longitudinal on prodorsum and transverse medially on opisthosoma, longitudinal between setae d1.

Legs (Figures 2c and 2d): chaetotaxy of leg segments: tarsi 8(ω)-6-5-5, tibiae 4 (φ)-2-2-2, genua 3-2-1-1, femora 3-3-2-1, trochantera 1-0-1-0. Femur IV entire. All tarsi terminate in two claws (Figure 2f) and a hairy empodium. Empodial claws present. Coxa I with coxal organ.

Venter (Figure 2b and Figure 2d): epimeral formula 3-1-4-2. Genital area has four pairs of aggenital and six genital setae and no eugenital setae. Only one pair of anal setae (ps) present. Cupule ih lateral to anal opening.

Gnathosoma: palp (Figure 2e) chaetotaxy (tibiotarsus to femur): 6(ω)-2-2. Setae p ζ and perhaps v slightly forked distally, setae ba and solenidion ω minute.

Male (n=1). Dimensions: length of idiosoma (including gnathosoma) 288, width 155; legs: I 182, II 129, III 147, IV 146; setae: vi 12, ve 14, sci 29, sce 16, c1 14, c2 16, d1 14, d2 15, e1 16, e2 18, f1 20, f2 21, h 23, ps 11; cheliceral stylets - ; palptarsus - . Similar to female but differs in that the genital area has four pairs of aggenital and six genital and eugenital setae. Coxa I with coxal organ.

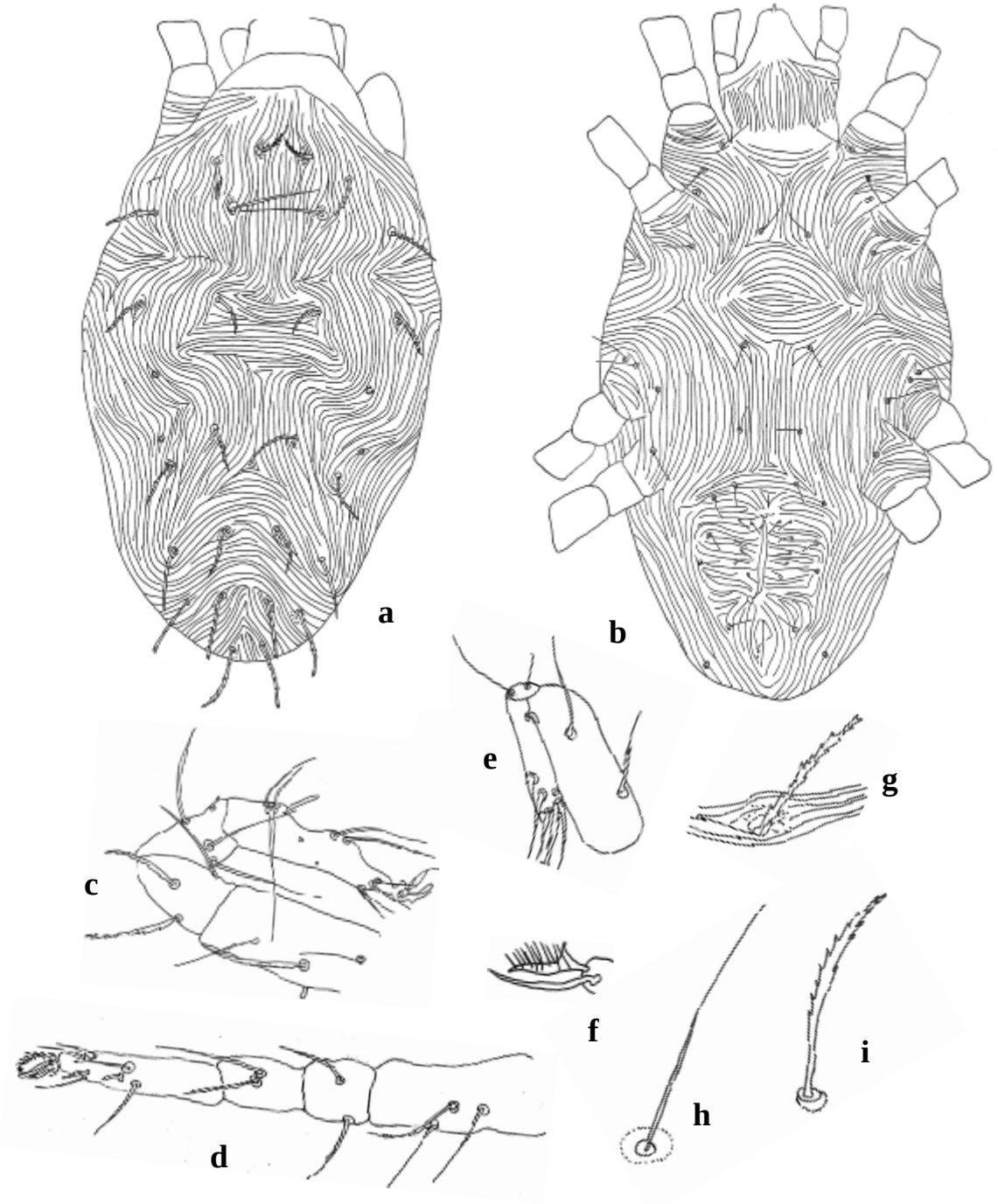

Figure 2: *Brachytydeus varitas* Theron and Ueckermann **n. sp.** body characteristics a) dorsum, b) venter, c) leg I, d) leg II, e) palptarsus, f) tarsus claws, g) seta, h) sci, i) seta f2

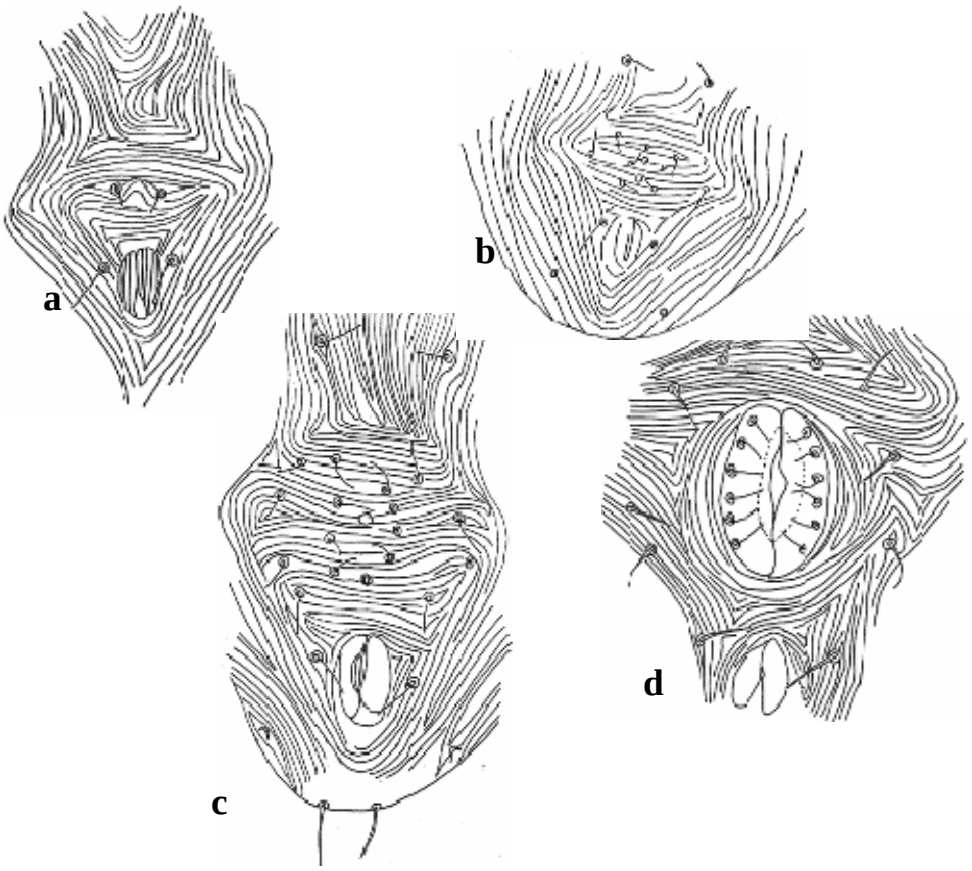

Figure 3: *Brachytydeus varitas* Theron and Ueckermann, **n. sp**. genitalia in different life stages a) protonymph, b) deutonymph, c) tritonymph, d) adult

Tritonymph (n=3). Dimensions: length of idiosoma (including gnathosoma) 243– 249,width 122–135; legs: I 161–167, II 115–131, III 131–135, IV 132–142; setae: vi 10–14, ve 9–12, sci 28–29, sce 14–15, c1 11–14, c2 14–15, d1 13, d2 14–16, e1 13–15, e2 16–18, f1 17–18, f2 18–20, h 11–19, ps 8–9; Cheliceral stylets 6–12; palptarsus 18–22.

Venter (Figure 3c): epimeral formula 3-1-4-2. Tritonymph differs from adults by lacking the progenital aperture, represented by two pores, presence of four pairs of aggenital, four pairs of genital and no eugenital setae, and one pair of anal setae.

Deutonymph n=1). Dimensions: length of idiosoma (including gnathosoma) 200, width 109; legs: I 128, II 94, III 106, IV 96; setae: vi 9, ve 10, sci 25, sce 15, c1 10, c2 14, d1 12, d2 13, e1 14, e2 16, f1 15, f2 16, h 17, ps 10; Cheliceral stylets 12; palptarsus 18.

Venter (Figure 3b): epimeral formula 3-1-3-0. Deutonymph can be defined as having two pairs of aggenital, two pair of genital, one pair of anal setae, and two progenital pores. Coxa I with coxal organ.

Protonymph (n=1). Dimensions: length of idiosoma (including gnathosoma) 176, width 100; legs: I 105, II 79, III 85, IV 79; setae: vi 9, ve 7, sci 22, sce 11, c1 8, c2 10, d1 10, d2 11, e1 11, e2 13, f1 12, f2 11, h 11, ps 8; Cheliceral stylets 9; palptarsus 16.

Venter (Figure 3a): epimeral formula 3-1-3-0. Protonymph can be distinguished by the presence of only two pairs of aggenital setae, one pair of progenital pores, and one pair of anal setae. Coxa with coxal organ.

Remarks: *B. varitas* is closely related to *B. monticola* (Ueckermann & Meyer, 1979) but differs from the latter in that the dorsal setae are more tapered distally, not curved, stronger serrate and dorsum without reticulated patches.

Etymology – The name of the species is derived from Latin *varietas* meaning "variety" and refers to its variety of *Protea* hosts.

3.3. *Brachytydeus pseudovaritas* Theron and Ueckermann, **n. sp.**

Type material

South Africa: Western Cape Province: holotype female, *P. neriifolia*, Jonkershoek; five paratype females, *P. neriifolia*, Franschoek, Gordon's Bay; *P. nitida*, Jonkershoek, Gordon's Bay; collected by N. Theron.

Diagnosis

Adults – This species can be recognized by having all dorsal setae short and serrate, except for sci which is about twice the length of the other dorsal setae and smooth; dorsal striae longitudinal between setae d1; Cheliceral stylets, almost half the length of the palptarsus; empodial claws absent. Female (1). Dimensions: length of idiosoma (including gnathosoma) 277.3 (259–308) [265], width 156.7 (147-171) [149]; legs: I 154.5 (149–167) [156], II 110.7 (102–120) [119], III 118.7 (109–136) [122], IV 135.8 (127–153) [140]; setae: vi 11.3 (10–13) [12], ve 13.8 (13–15) [14], sci 29 (27–33) [27], sce 14 (13–15) [14], c1 11.7 (10–13) [112], c2 13.8 (13–15) [15], d1 12.8 (12–14) [13], d2 13.5 (13–15) [13], e1 15.7 (14–17) [14], e2 17.2 (16–18) [18], f1 17 (15–18) [17], f2 16 (14–19) [15], h 15.7 (14–19) [15], ps 7.8 (6-9) [9]; Cheliceral stylets 13.4 (11–15) [15]; palptarsus 23.2 (22–25) [25].

Dorsum (Figure 4a): all 13 pairs of dorsal setae, except sci, plumose (Figure 4e). Setae sci (Figure 4h) longest, slender and smooth along entire length, others short, equal to sub-equal in length. Prodorsum with four pairs of setae and with granular eye patches between ve and sce, opisthosoma nine pairs and two pairs of cupules, ip apparently absent. Striae with small tubercles, longitudinal on prodorsum and transverse medially on opisthosoma, longitudinal between setae d1.

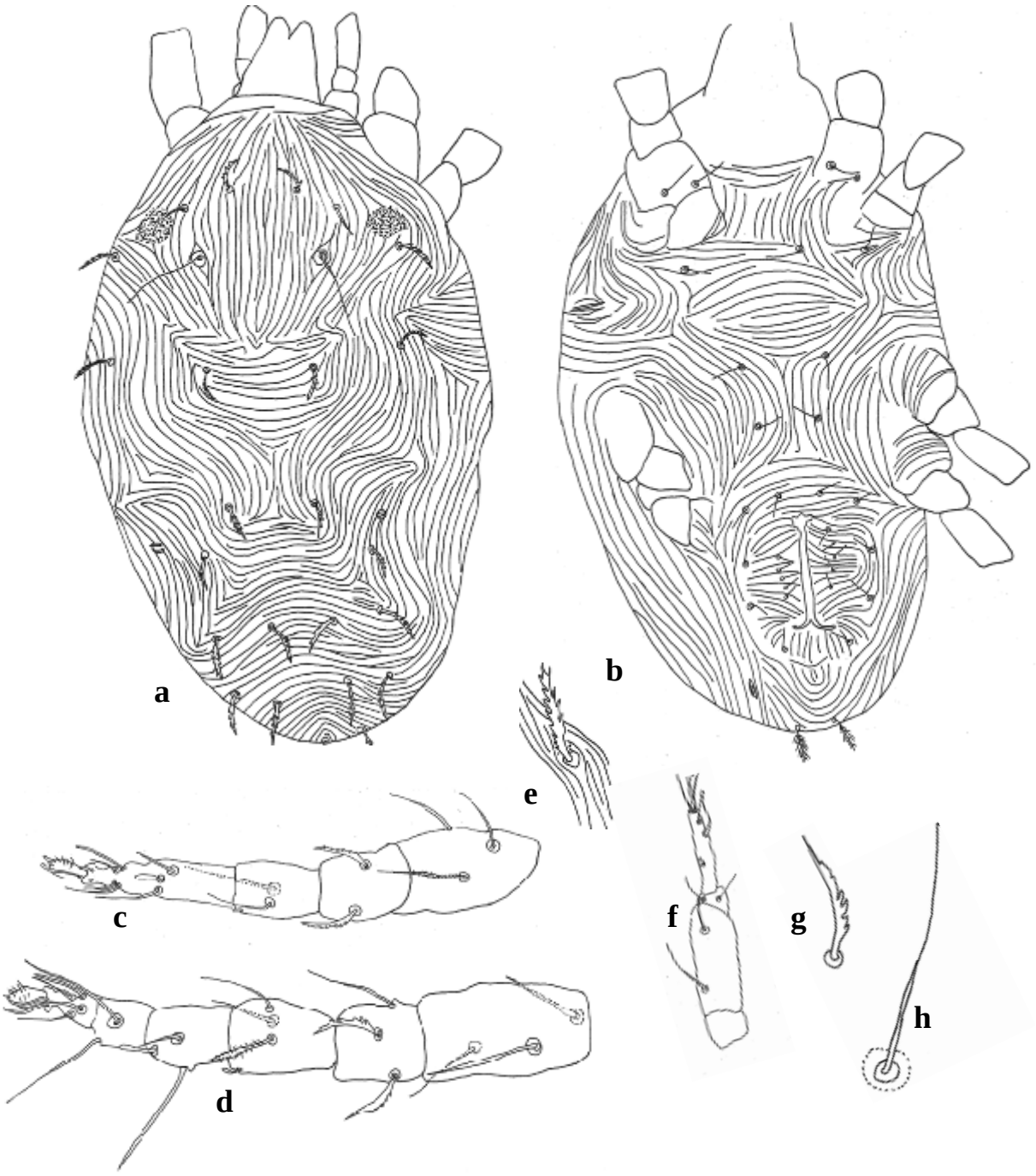

Figure 4: *Brachytydeus pseudovaritas* Theron and Ueckermann, **n. sp.** body characteristics a) dorsum, b) venter, c) leg II, d) leg I, e) seta, f) palptarsus, g) seta e1, h) sci

Venter (Figure 3b): epimeral formula 3-1-4-2. Genital area with four pairs of aggenital and five genital setae, eugenital setae absent. Only one pair of anal setae (ps) present. Cupule ih lateral to anal opening.

Gnathosoma: palp (Figure 3f) chaetotaxy (tibiotarsus to femur): 6(ω)-2-2. Setae pζ and v slightly forked distally, setae ba and solenidion ω minute.

Legs (Figure 3c and 3d): chaetotaxy of leg segments: tarsi 8(ω)-6-5-5, tibiae 4 (φ)-2-2-2, genua 3-2-1-1, femora 3-3-2-1, trochantera 1-0-1-0. Femur IV entire. All tarsi terminate in two claws and a hairy empodium. Empodial claws absent.

Remarks: This species closely resembles *B. varietas* n. sp., but differs from the latter in that the dorsal setae are shorter, it has five genital setae and empodial claws are absent.

Etymology – The species name *pseudovaritas* implies strong similarities to *B. varietas*, but shorter setae.

Subfamily TYDEINAE André, 1980

Genus *Tydeus* Koch, 1836

Tydeus Koch, 1836 *sensu* Baker, 1968

Lorryia Oudemans, 1925 *sensu* Baker, 1968

Paralorryia Baker, 1965

Tydulous Baker, 1965

Venilia Kuzentzov 1979

TYPE SPECIES: *Tydeus spathuatus* Oudemans, 1928 sensu André (2005).

DIAGNOSIS : This genus is defined as follows: Prodorsum recurved; opisthosoma with 10 pairs of setae (ps included); poriodotaxy: three; genital organotaxy - adults: no eugenital setae in female, but male with four pairs, four or six pairs of genital setae are present and four pairs of aggenital setae in both adults; coxa I with coxal organ; chaetotaxy of leg segments: 8(ω)-6(ω)-5-5, tibiae 4-2-2-2, genua 3-2-1-1, femora 3-2-1-1, trochantera 1-0-1-0, epimeral formula adults: 3-1-4-2; solenidiotaxy: two; femur IV entire; palp chaetotaxy: 6(ω)-2-2.

3.4. *Tydeus pseudofustis* Theron and Ueckermann, **n. sp.**

Type material

South Africa: Western Cape Province: holotype male, *P. punctata*, Swartberg, collected by N. Theron.

Diagnosis

Adults – The following combination of characters distinguish this new species: Most dorsal setae short, smooth and club-shaped, except for sci which is about twice as long as other setae; striae longitudinal between d1; Cheliceral stylets half the length of palptarsus; empodial claws absent. Male (1). Dimensions of holotype: length of idiosoma (including gnathosoma) 314 [Holotype], width 169; legs: I 177, II 153, III 160, IV 193; setae: vi 19, ve 18, sci 38, sce 20, c1 19, c2 20, d1 18, d2 19, e1 19, e2 18, f1 19, f2 21, h1 19, ps3 18; Cheliceral stylets 17; palptarsus 30.

Dorsum (Figure 5a): all 13 pairs of dorsal setae, except for sci, club-shaped (Figure 5f). Setae sci longest, slender and smooth along entire length, others equal to sub-equal in length and also smooth. Prodorsum with four pairs of setae, opisthosoma with nine pairs and three pairs of cupules, ip apparently absent. Striae with small tubercles, longitudinal between setae d1.

Venter (Figure 5b): epimeral formula 3-1-4-2. Genital area with four aggenital setae and six pairs of genital setae. Only one pair of anal setae (ps) present. Cupule ih lateral to anal opening.

Gnathosoma: palp (Figure 5e) chaetotaxy (tibiotarsus to femur): 6(ω)-2-2. Setae pζ and perhaps v slightly forked distally, setae ba and solenidion ω minute.

Legs (Figure 5c and 5d): chaetotaxy of leg segments: tarsi 8(ω)-6(ω)-5-5, tibiae 4-2-2-2, genua 3-2-1-1, femora 3-3-1-1, trochantera 1-0-1-0. All tarsi terminate in a hairy empodium. Empodial claws absent.

Remarks: This species resembles *T. fustis* (Meyer and Ueckermann, 1988), but differs in that dorsal striae between d1 are longitudinal and not transverse as in latter and in that empodial claws are absent in the new species.

Etymology – This species name *pseudofustis* refers to the false resemblance to *T. fustis* Meyer & Ueckermann.

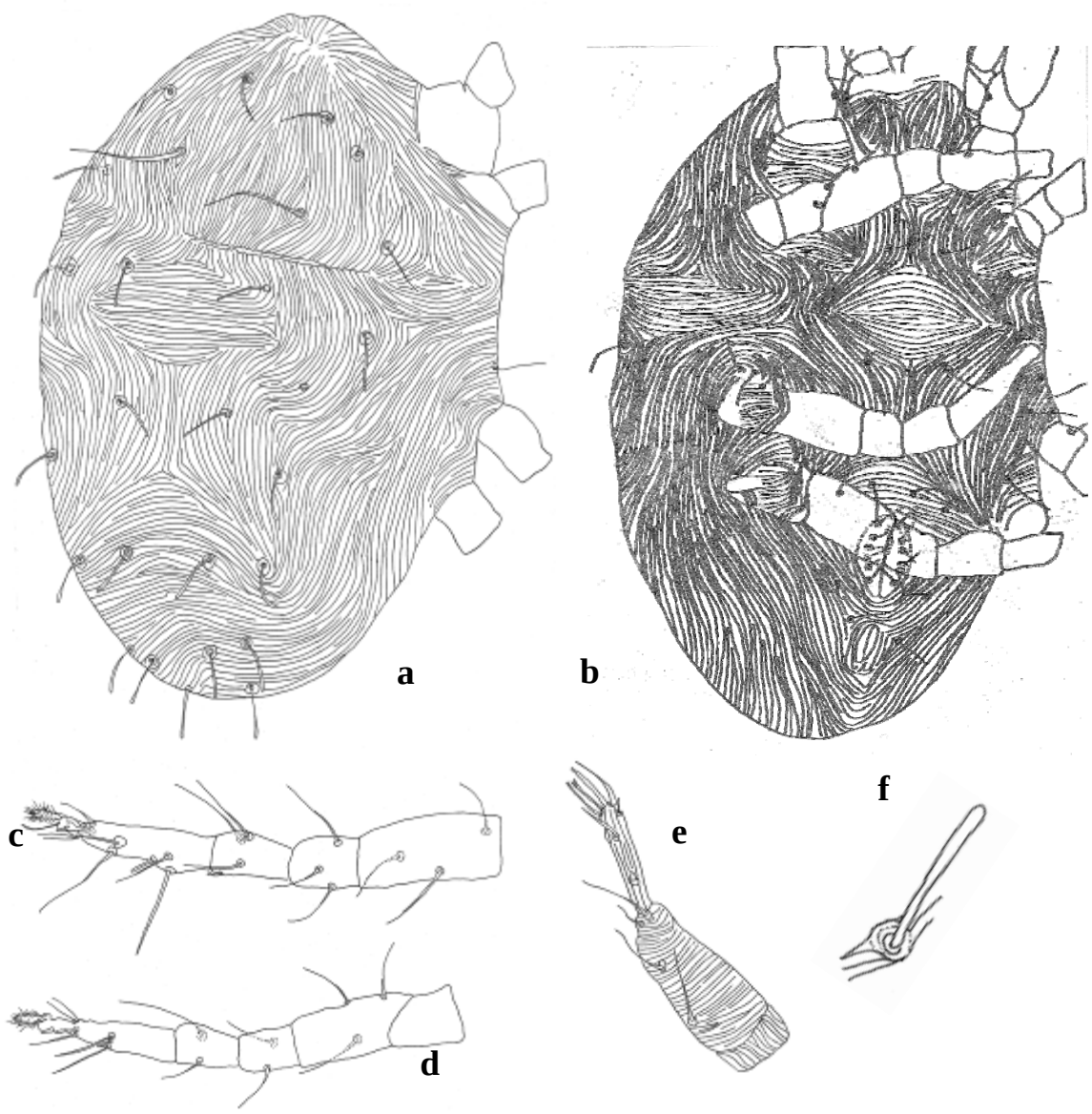

Figure 5: *Tydeus pseudofustis* Theron and Ueckermann, **n. sp.** body characteristics a) dorsum, b) venter, c) leg II, d) leg I, e) palp tarsus, f) seta f1

Family IOLINIDAE Pritchard, 1956

Subfamily TYDAEOLINAE André, 1980.

Genus *Microtydeus* Thor, 1931 *sensu* Baker, 1965

Microtydeus Thor, 1931

TYPE SPECIES : *Microtydeus constans* Thor, 1931

DIAGNOSIS : This genus is defined as follows: Prodorsum procurved; opisthosoma with 10 pairs of setae (ps included); poriodotaxy: four; genital organotaxy - adults: two pairs of genital and three pairs of aggenital setae, eugenital setae absent in females and presence of eugenital setae not yet known in males; coxa I with coxal organ; chaetotaxy of leg segments: tarsi 11(ω)-8(ω)-7-7, tibiae 5(φ)-2-2-2, genua 4-4-1-1, femora 6-4-3-2, trochantera 1-1-1-0, epimeral formula adults: 3-1-4-3; solenidiotaxy: three; femur IV entire; palp chaetotaxy: 6(ω)-2-2.

3.5. *Microtydeus beltrani* Baker, 1944

Microtydeus beltrani Baker, 1944: 159; Baker, 1965: 111

Type material

South Africa: Western Cape Province: holotype female, *P. nitida*; Jonkershoek, four paratype females, *P. repens*, Gordon's Bay, George, Swartberg; *P. lanceolata*, Albertinia; collected by N. Theron.

Diagnosis

Adults – This species can be recognized in having all dorsal setae short and smooth, except for sci which is pilose and the longest, some of the caudal setae are also longer. Most dorso-central setae are clearly shorter than the distance to the setae next behind. Female (1). Dimensions of holotype followed (in parentheses) by variations in measurements of paratypes: length of idiosoma (including

gnathosoma) 185 (170–201) [196], width 85.6 (70-112) [88]; legs: I 83.8 (78–89) [88], II 63.8 (61–66) [65], III 64.4 (57–70) [68], IV 70.4 (64–79) [72]; setae: vi 5.6 (4–7) [6], ve 10.4 (9–11) [11], sci 25.6 (23–27) [25], sce 12.2 (11–13) [13], c1 6.6 (6–7) [7], c2 10.8 (10–13) [13], d 6.8 (6–7) [7], e 7.8 (7–8) [8], f1 9 (8–10) [10], f2 13.8 (13–15) [15], h1 10.6 (9–12) [12], h2 18.8 (18–20) [18], ps1 19.6 (9–10) [10], ps2 13.6 (12–15) [12], ps3 5 (4–6) [4]; Cheliceral stylets 4 [-]; palptarsus 10.8 (10–11) [11].

Dorsum (Figure 6a): all 15 pairs of dorsal setae (ps included), except sci, simple. Setae sci (Figure 6f) longest and pilose along entire length, others equal to sub-equal in length except for setae f1 to h2 which are longer and smooth. Prodorsum with four pairs of setae, opisthosoma with eleven pairs of setae including three ps and three pairs of cupules. Striae with small tubercles, longitudinal on prodorsum and transverse medially and irregular longitudinal laterally on opisthosoma.

Venter (Figure 6b): epimeral formula 3-1-4-3. Genital area with three pairs of aggenital and two genital setae, eugenital setae absent. One pairs of anal setae (ps) present. Cupule ih lateral to anal opening.

Gnathosoma: palp (Figure 6e) chaetotaxy (tibiotarsus to femur): 6(ω)-2-2. Setae pζ and perhaps v slightly forked distally, setae ba and solenidion ω minute.

Legs (Figures 6c and 6d): chaetotaxy of leg segments: tarsi 11(ω)-8(ω)-7-7, tibiae 5(φ)-2-2-2, genua 4-4-1-1, femora 6-4-3-2, trochantera 1-1-1-0. Femur IV entire. All tarsi terminate in two claws and an empodium. Empodial claws absent Coxa I with coxal organ.

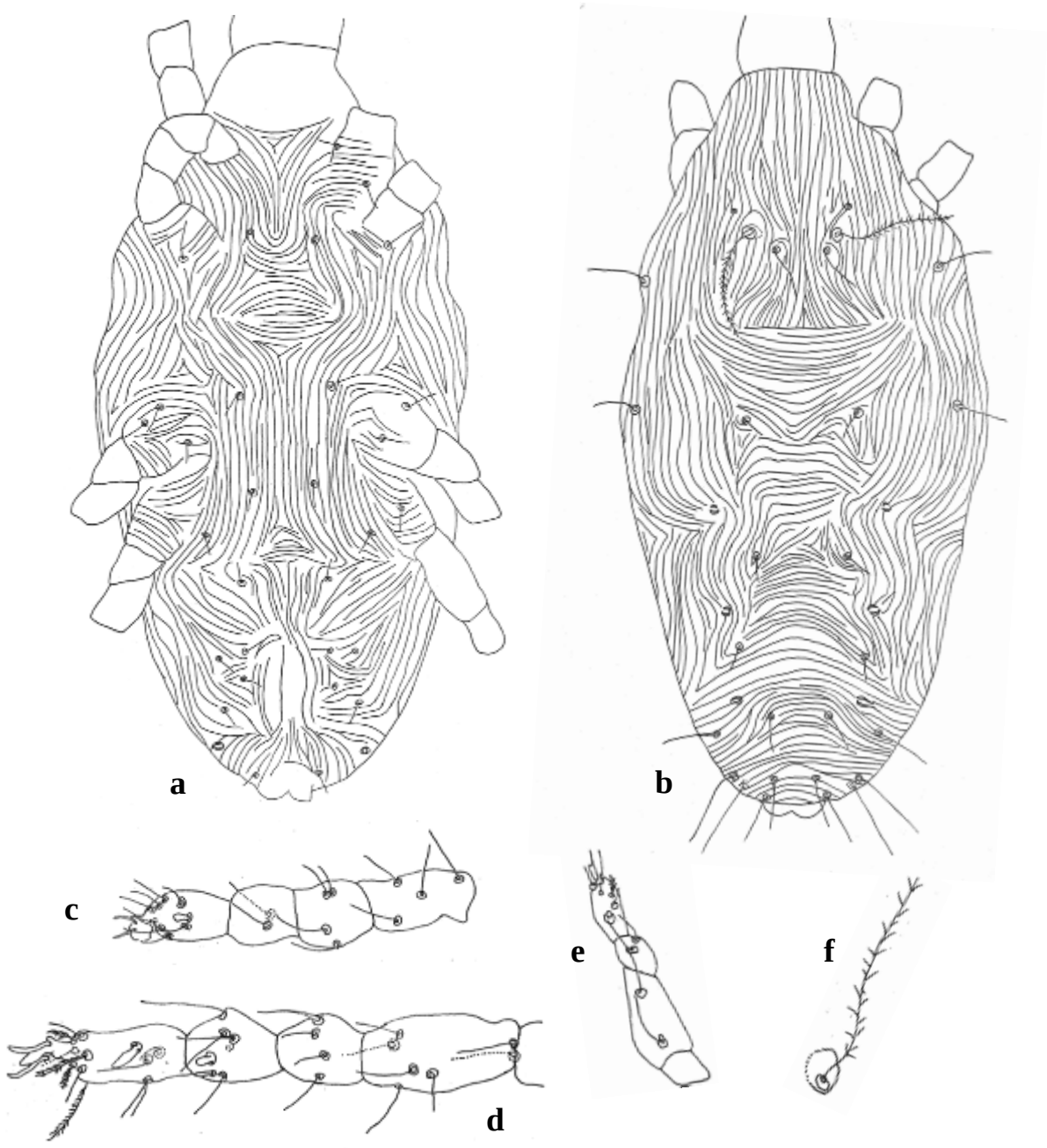

Figure 6: *Microtydeus beltrani* Baker, body characteristics a) dorsum, b) venter, c) leg II, d) leg I, e) palptarsus, f) sci

Remarks: Unfortunately the species originally described [*M. rectangulus* (Berlese, 1910), *M. subtilis* (Koch, 1838), *M. fenilis* (Canestrini, 1886) and *M. similis* (Canestrini, 1886)], including the type species, *M. constans* Thor (1931) were either not well kept or destroyed during the two world wars. Baker (1944) described a new species *M. beltrani* and Baker (1965) indicated variations in the chaetotaxy of tarsus and tibia I and palp tarsus, which was used to distinguish between the latter and *M. bellus* Livshitz & Kuznetzov, 1973. However, Andre (1980) revised this genus based on the latter two species and *M. subteraneus* Wood, 1965 and in this revision no mention was made of any variation in the chaetotaxy of these segments in the adults. The variations indicated by Baker (1965) correspond with those of the deuto- and protonymph. This immediately necessitates a re-examination of these three closely related species as the descriptions of these species also lack detail, and André (1980) has not pointed out any differences between above mentioned species. Fan & Li (1992) described *M. hylinus* which can only be distinguished from the above-mentioned three species by setae f1 reaching to bases of setae h1. Therefore, until *M. subterraneus* Wood, *M. beltrani* Baker and *M. bellus* Livshitz & Kuznetzov is re-examined, the South African species will be considered *M. beltrani* Baker.

Genus *Paratydeaolus* André, 1980

Paratydeaolus André, 1980

Coccotydeus Wood, 1965.

TYPE SPECIES : *Paratydaeolus lukoschusi* André, 1980

DIAGNOSIS : This genus is defined as follows: Prodorsum procurved; opisthosoma with 10 pairs of setae (ps included); poriodotaxy: four; genital organotaxy - adults: eugenital setae absent in female, still unknown in male, both adults with three pairs of genital and four pairs of aggenital setae; coxa I with coxal organ; chaetotaxy of leg segments: tarsi 12(ω)-8(ω)-7-7, tibiae 5-2-2-2,

genua 4-4-1-0, femora 6-3-3-2, trochantera 1-1-1-0, epimeral formula adults: 3-1-4-3; solenidiotaxy: three; femur IV entire; palp chaetotaxy: 5(ω)-2-2.

3.6. *Paratydaeolus athaliahea* Theron and Ueckermann, **n. sp.**

Type material

South Africa: Western Cape Province: holotype female, *P. neriifolia*, Gordon's Bay; three paratype females, *P. neriifolia*, *P. nitida*, Gordon's Bay; collected by N. Theron.

Diagnosis

Adults – The following combination of characters distinguish this new species: Length of body more than twice length of leg I.; all dorsal setae smooth, except for sci; setae ve and sci same distance apart; setae sci situated between the levels of setae vi and ve; setae sci almost twice length of sce. Female (1). Dimensions: length of idiosoma (including gnathosoma) 248 (230–275) [275], width 112.3 (102–127) [127]; legs: I 109.5 (102–119) [119], II 84 (72–95) [95], III 99.5 (90–114) [114], IV 106.5 (80–129) [129]; setae: vi 6.3 (5–7) [7], ve 14.8 (14–15) [15], sci 28.3 (27–29) [29], sci width 6.5 (6–7) [7], sce 16.5 (16–17) [16], c1 10.5 (10–11) [11], c2 15, d 10.3 (10–11) [11], e 12 (11–15) [15], f1 12.5 (12–14) [14], f2 16.5 (16–17) [16], h1 13 (12–15) [15], h2 18.5 (17–20) [17], ps1 13.3 (12–15) [15], ps2 16 (15–17) [16], ps3 8; Cheliceral stylets 7.3 (6–10) [10]; palptarsus 13.3 (12–14) [14].

Dorsum (Figure 7a): all 14 pairs of dorsal setae, except sci, short, smooth and acute (Figure 7f). Setae sci (Figure 7g) longest and club-shaped, others equal to sub-equal in length. Prodorsum with four pairs of setae, opisthosoma with ten pairs of setae and three pairs of cupules. Striae with small tubercles, longitudinal on prodorsum and transverse medially on opisthosoma.

Venter (Figure 7b): epimeral formula 3-1-4-3. Genital area with four pairs of aggenital and three pairs of genital setae. One pair of anal setae (ps) present. Cupule ih lateral to anal opening.

Gnathosoma: palp (Figure 7e) chaetotaxy (tibiotarsus to femur): 5(ω)-2-2. Setae pζ and perhaps v slightly forked distally, setae ba and solenidion ω minute.

Legs (Figure 7c and 7d): chaetotaxy of leg segments: tarsi 12(ω)-8(ω)-7-7, tibiae 5(φ)-2-2-2, genua 4-4-1-1, femora 6-3-3-2, trochantera 1-1-1-0. All tarsi terminate in two claws and a hairy empodium.

Remarks: Members of this genus are closely related and differ mostly in the lengths of the dorsal setae, shape of setae sci, dorsal setae smooth or pilose, distances between setae ve and sci, setae sci as long as or longer than sce; striae between vi longitudinal or transverse and sci situate in line with either ve or vi or between these two setae. This new species is very closely related to the northern England *P. expressus* (Kuznetzov) and apparently only differs in that the length of the body (248) is more than twice the length of leg I (110) opposes to leg I (80) longer than half the length of body (150). Until specimens of *P. expressus* can be obtained for examination we have decided to consider the South African species as new.

Etymology – The species is named in memory of the collectors' grandmother, Athaliah Eichstedt.

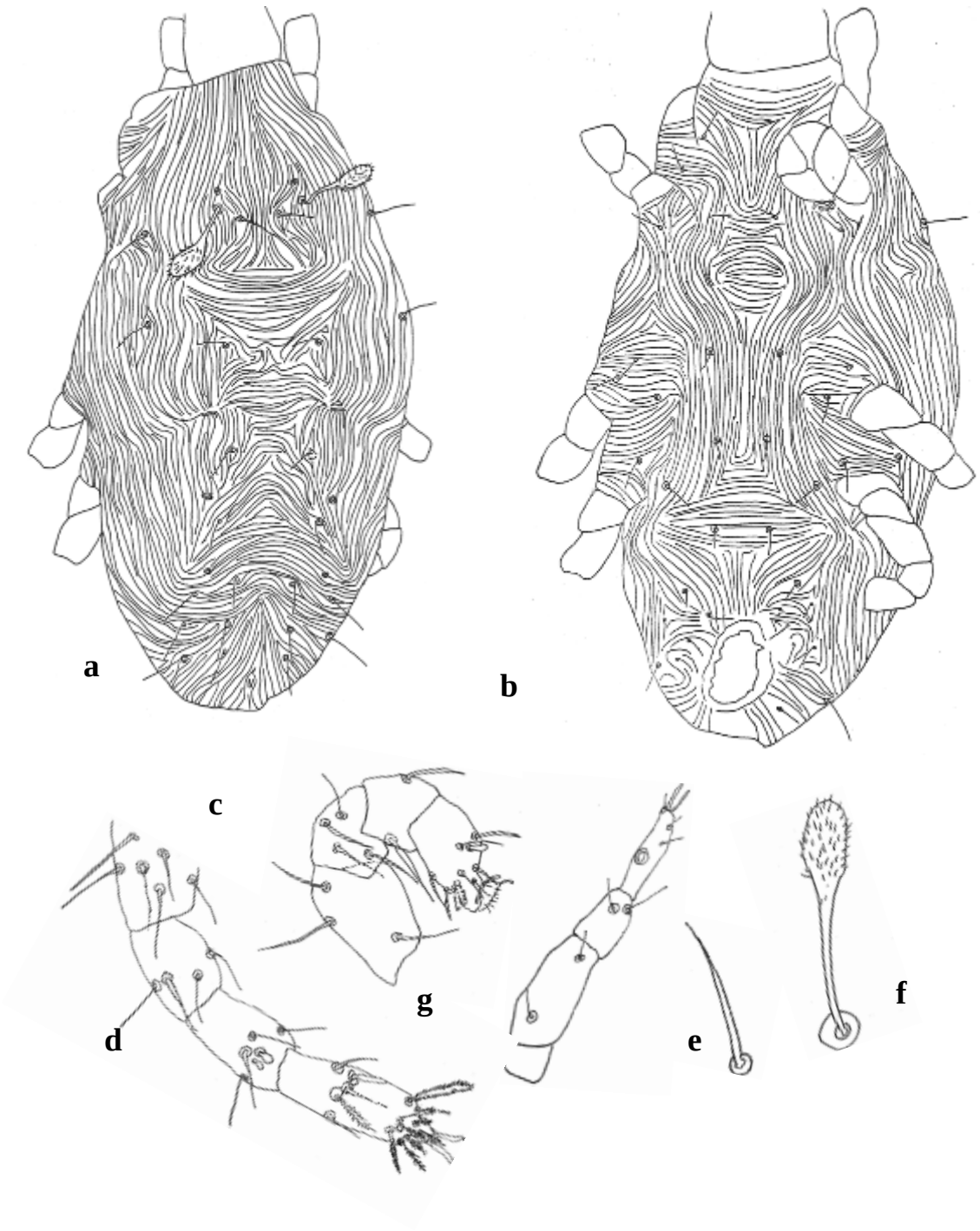

Figure 7: *Paratydaeolus athaliahea* Theron and Ueckermann, **n. sp.** body characteristics a) dorsum, b) venter, c) leg II, d) leg I, e), palp, f) seta f2, g) sci

Family TRIOPHTYEIDAE Andre, 2004b

Subfamily EDBAKERELLINAE Andre, 2004b.

Genus *Therontydeus* Theron and Ueckermann, n. gen.

Therontydeus proteacapensis **n. sp.**

DIAGNOSIS : Male **–** prodorsum recurved with four pairs of setae, including a pair of trichobothria similar in shape and length; opisthosoma with eight pairs of setae and four pairs of slit-like pores or cupules, namely ia halfway between setae c1 and d, im halfway between d and e, and ip anterior to setae f1, and a fourth pore (ih) anterolateral to setae ps3, posteroventral; genital area with six pairs of genital setae (g), five pairs of aggenital setae (ag), six pairs of eugenital setae (eu) and three pairs of anal setae (ps); epimeral formula (coxal plus ventral setae): 3-1-3-3; leg chaetotaxy (with solenidia in parentheses): tarsi 10(ω)-6(ω)-5-5, tibiae 5 (φ,ε)-3(φ)-2-2, genua 4-1-2-2, femora 5-4-2-(1+1), trochantera 1-1-1-0. Femur IV divided.

Remarks: This genus resembles *Pseudotriophtydeus* Andre but varies from the latter in the setal formulae of the genua, 4-1-2-2 opposed to 4-2-2-3 in the latter and palp, 5(ω)-2-2 instead of 6(ω)-2-2.

Etymology – The genus is named after the French family name Theron meaning hunter or untamed.

3.7. *Therontydeus proteacapensis* Theron and Ueckermann, **n. sp.**

Type material

South Africa: Western Cape Province: holotype male, *P. neriifolia*, Jonkershoek; one paratype male, *P. neriifolia*, Jonkershoek; collected by N. Theron.

Diagnosis

Adults - The characters of this species comply with those defining the genus. Male (1).: length of idiosoma (including gnathosoma) 198.5 (194-203) [203], width 84 (82-86) [86]; legs: I 107.5 (106–109) [109], II 78.5 (78–79) [78], III 84.5 (82–87) [87], IV 101 (100–102) [100]; setae: vi 7, ve 7.5 (7–8) [7], sci 9, sce 8.5 (8–9) [9], c1 8.5 (8–9) [9], c2 10 (9–11) [11], d 8.5 (8–9) [8], e 9, f1 9, f2 11.5 (11–12) [12], h1 8 (7–9) [9], h2 10.5 (10–11) [10], ps1 9, ps2 8.5 (8–9) [9], Cheliceral stylets 8 (7–9) [9]; palptarsus 12.

Dorsum (Figure 8a): all 14 pairs of dorsal setae (including ps1-2) feathery (Figures 8f and 8g). Setae f2 longest, others equal to sub-equal in length. Prodorsum with four pairs of setae including a pair of trichobothria, opisthosoma with eight pairs of setae and four pairs of cupules. Striae longitudinal on prodorsum and transverse medially on opisthosoma.

Venter (Figure 8b): epimeral formula 3-1-3-3. Genital area with five pairs of aggenital, six pairs of genital and eugenital setae. Two pairs of anal setae (ps) present. Cupule ih lateral to anal opening.

Gnathosoma: palp (Figure 8e) chaetotaxy (tibiotarsus to femur): 5(ω)-2-2. Setae pζ and perhaps v slightly forked distally, setae ba and solenidion ω minute.

Legs (Figure- 8c and 8d): chaetotaxy of leg segments: tarsi 10(ω)-6(ω)-5-5, tibiae 5(φε)-3(φ)-2-2, genua 4-1-2-2, femora 5-4-2-(1+1), trochantera 1-1-1-0. Femur IV divided. All tarsi terminate in two claws and a hairy empodium.

Etymology – The species name refers to *Protea*, the host plant genus of the species, and its location in the Western Cape Province, South Africa.

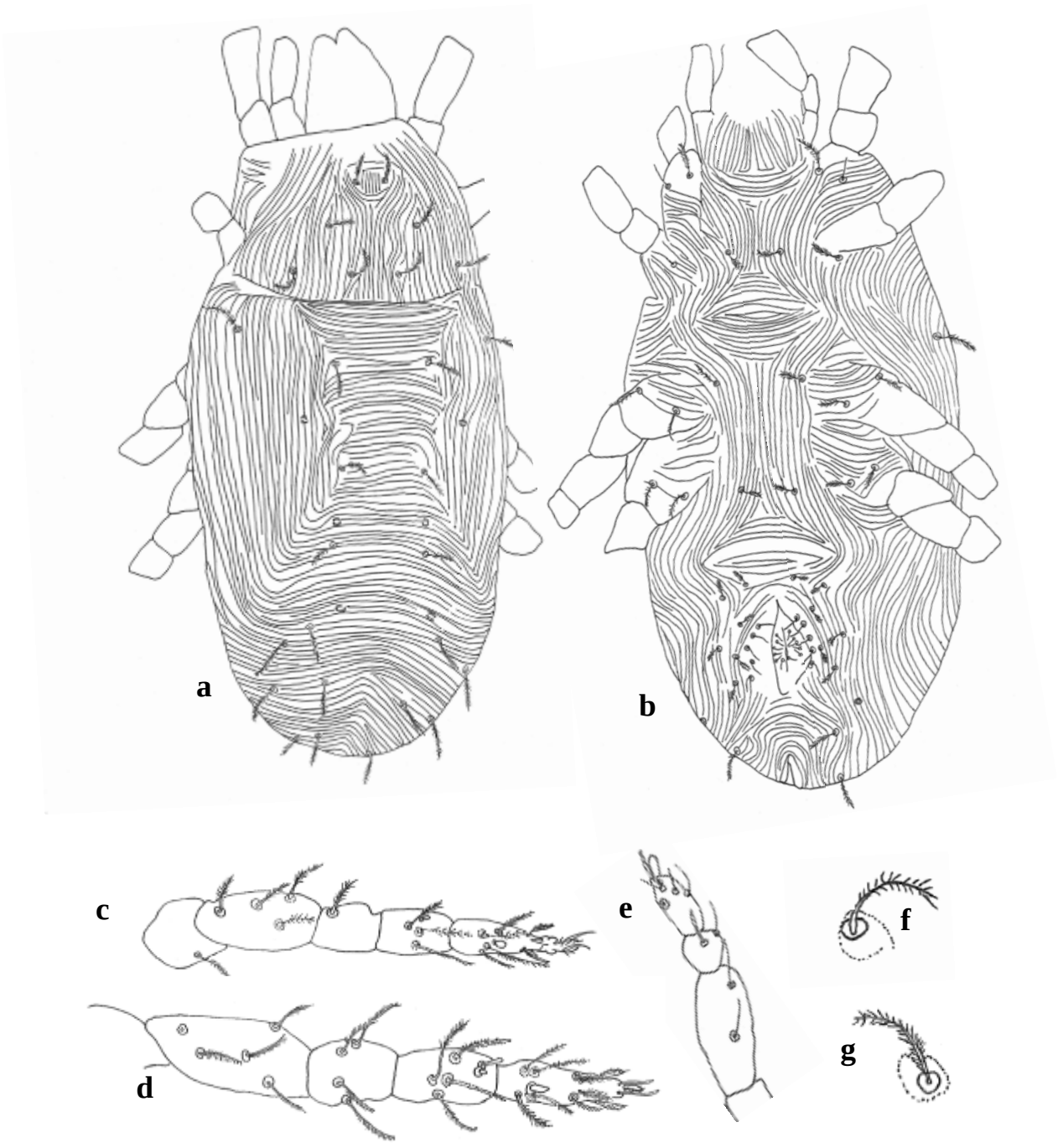

Figure 8: *Therontydeus proteacapensis* Theron and Ueckermann, **n. sp.** body characteristics a) dorsum, b) venter, c) leg II, d) leg I, e) palptarsus, f) sce, g) sci

Family IOLINIDAE Pritchard, 1956

Subfamily PRONEMATINAE Andre, 1980

Genus *Pausia* Kuznetzov and Livshits, 1972

Pausia Kusnetzov and Livshits, 1972, p 1739.

TYPE SPECIES : *Pausia taurica* Kuzentzov, 1972.

DIAGNOSIS : This genus is defined as follows: tarsus I with ordinary empodium, lacking claws, opisthosoma with 10 pairs of setae (ps 1-2 included); poriodotaxy: two; genital organotaxy - adults: four pairs of genital and no paragenital setae and one pair anal setae; chaetotaxy of leg segments: tarsi 8(ω)-6(ω)-6-5, tibiae 4(ω)-2-2-2, genua 3-3-2-1, femora 3-3-2-(1-1), trochantera 1-1-1-0, epimeral formula adults: 3-1-4-1; solenidiotaxy: three; femur IV divided into telo- and basifemur; palp chaetotaxy: 5(ω)-1-2.

Remarks: This genus resembles *Naudea* Meyer and Rodrigues but varies from the latter in that the opisthosoma bear 10 pairs of setae, instead of 9 and the chaetotaxy of the tarsi, 8-6-6-5 in *Pausia*, while 8-7-7-7 in *Naudea*.

3.8. *Pausia colonus* Theron and Ueckermann, **n. sp.**

Type material

South Africa: Western Cape Province: holotype female, *P. repens*, Gordon's Bay, collected by N. Theron.

Diagnosis

Adults – This species is characterized by its short dorsal setae with most dorso-central shorter than half the distance to the setae next behind. Female (1). Dimensions: length of idiosoma (including

gnathosoma) 312, width 139; legs: I 164, II 117, III 134, IV 141; setae: vi 15, ve 12, sci 29, sce 21, c1 11, c2 15, d 12, e 14, f1 12, f2 26, h1 13, h2 31, ps1 18, ps2 27, ps3 9; Cheliceral stylets 14; palptarsus 19.

Dorsum (Figure 9a): all 15 pairs of dorsal setae (including ps setae), except for sci, feathery (Figure 9f and 9g). Setae sci (Figure 9h) longest and sparsely pilose, others equal to sub-equal in length. Prodorsum with four pairs of setae, opisthosoma with ten pairs and two pairs of cupules, ip apparently absent. Striae longitudinal on prodorsum and transverse medially on opisthosoma.

Venter (Figure 9b): epimeral formula 3-1-4-1. Genital area with four pairs of genital setae, aggenital and eugenital setae absent. Three pairs of anal setae (ps) present.

Gnathosoma: palp (Figure 9e) chaetotaxy (tibiotarsus to femur): 5(ω)-1-2. Setae pζ and perhaps v slightly forked distally, setae ba and solenidion ω minute.

Legs (Figure 9c and 9d): chaetotaxy of leg segments: tarsi 8(ω)-6-6-5, tibiae 4(φ)-2-2-2, genua 3-3-2-1, femora 3-3-2-(1-1), trochantera 1-1-1-0. Femur IV divided. All tarsi terminate in two claws and hairy empodium, except leg I which is without claws.

Etymology – Latin word Colōnus means settler, and reflects that it is the first record of the genus in South Africa.

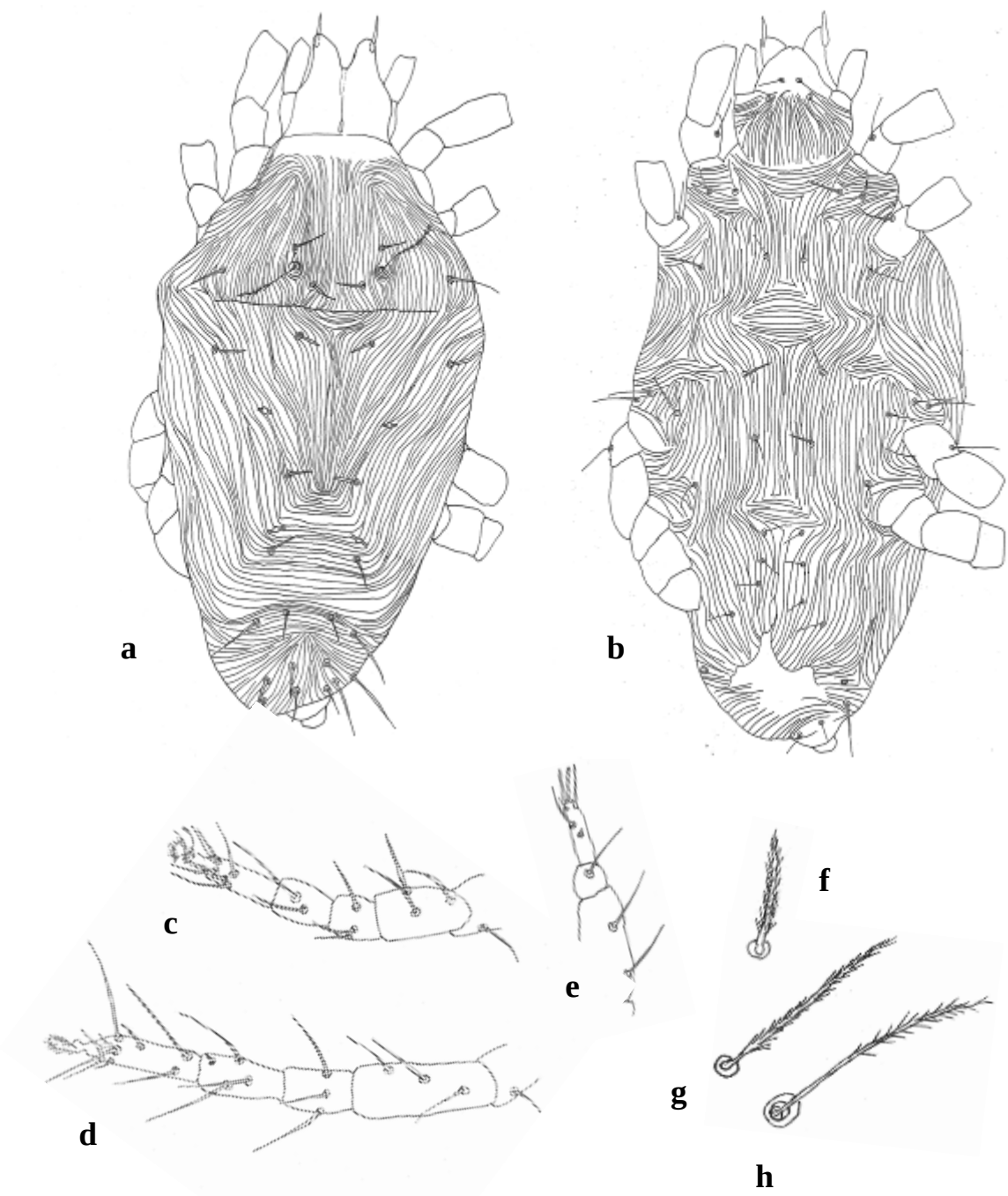

Figure 9: *Pausia colonus* Theron and Ueckermann, **n. sp.** body characteristics a), dorsum, b) venter, c) leg II, d) leg I, e) palp, f) seta c2, g) seta f2, h) sci

4. KEY TO TYDOIDIDAE SPECIES ON SOUTH AFRICAN *PROTEA* SPECIES

1.Tarsus I with 12 setae; femur IV entire; body length more than twice length of leg I ……..…..

……..…..……...*Paratydaeolus athaliahea* n. sp.

– Tarsus I with 11 or less setae ……...…….……..2

2. Tarsus I with 11 setae……..... *Microtydeus beltrani* Baker

– Tarsus I with 10 setae or less……...3

3. Tarsus I with 10 setae; genua, 4-1-2-2..... *Therontydeus proteacapensis* n. gen. & n. sp.

– Tarsus I with 8 setae...……...……...……..4

4.Tarsus I without claws; most dorso-central setae shorter than half distances to setae next behind…….…….……. *Pausia colonus* n. sp.

– Tarsus I with claws…….……...5

5. Femora 3-3-2-1……...……...6

– Femora 3-2-1-1; most dorsal setae short, smooth and club-shaped; striae longitudinal between d1……..…… *Tydeus pseudofustis* n. sp.

6. Empodial claws present; dorsal setae tapered and clearly serrated, not curved……..……

……..……..…..*Brachytydeus varitas* n. sp.

– Empodial claws absent…….……7

7. All dorsal setae plumose, except for sci…….....……..….. *Brachytydeus pseudovaritas* n. sp.

– All dorsal setae smooth, with f1-2 and h1 leaf-like…….…....*Brachytydeus rutrus* n. sp.

5. DISCUSSION

Mite diversity in South African *Protea* infructescences is greatly unexplored, and in this study represented a variety of new species. The known distribution ranges of *Microtydeus*, *Paratydaeolus* and *Pausia* were also expanded to, for the first time, include the African continent.

The lack of distinguishable variations between the descriptions of *Microtydeus* species caused difficulties in this study, and reinforces the urgent need for a re-examination of this genus. This study also generated a base upon which to build an identification key to *Protea* mites in South Africa.

Acknowledgements

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CHAPTER 5

CONCLUDING REMARKS

1. The importance of investigating mite communities associated with Protea species

Globally, the Cape Floristic Region rates as one of the most diverse eco-regions, with levels of diversity comparable to that of tropical rainforests (Cowling *et al.*, 1992). The CFR is also unique in that it is the only region that houses three highly threatened vegetation types, the Fynbos, Renosterveld and Succulent Karoo (Mucina and Rutherford, 2006). The genus *Protea* (Proteaceae), which includes many species, is confined to the Fynbos (Cowling *et al*, 2003) and forms the cornerstone of the South African cut-flower industry (Coetzee and Littlejohn, 2001; Crous *et al.*, 2004). The inflorescences of *Protea* have evolved an array of different shapes and colours and attract diverse pollinators such as birds, insects and rodents. These inflorescences not only provide pollen and nectar, but after forming fruiting structures (infructescences) they also provide an important niche for a multitude of organisms to flourish in.

Recent studies indicated that *Protea* infructescences house a number of insects (Coetzee, 1984), spiders (Roets *et al.*, 2006), and fungi (Lee *et al*., 2005). It was also noted that numerous mites can be found thriving within infructescences (Roets *et al*., 2007). Studies on these mites have largely focussed on their role as primary vectors of ophiostomatoid fungi (Roets *et al*. 2007, 2009). However, these discoveries raised questions about the diversity and ecology of mites associated with *Protea* infructescences in general (Roets *et al*., 2007, 2009). These studies thus emphasized that there is

insufficient knowledge of mite diversity associated with *Protea* species and also within the Cape Floristic Region in general.

As *Protea*-associated mites are also of considerable phytosanitary concern (Coetzee, 1986), studies on these animals should aid their control in the cut-flower industry. The economic importance of mites within *Protea* infructescences would largely depend on their specific function within this system. The Tetranycidae, for example, is ranked according to Moran (1983), as the Acari family with the highest pest status on South African cultivated plants. Other mites, such as the Phytoseiids, are predatory and may be used as bio-control agents against pests such as these spider mites (Tetranycidae) (McMurtry and Croft, 1997).

Mites unmistakably form an integral part of normal ecosystem functioning within Fynbos ecosystems and most probably play a significant role in *Protea* population dynamics. It is reasonable to assume that mites have a significant influence on *Protea* ecology, as they thrive within the structures that form the propagules for the next generation of plants. Within these structures they may assume various roles - from protecting seeds from e.g. insects (mites as predators and parasites) to vectoring fungi, some of which may be pathogenic to their hosts. Mites may thus impact on the biodiversity of the Fynbos as a whole and consequently also the CFR. From the results of the current research we now know a great deal more about the Acari inhabitating the infructescences of *Protea* species. Not only do we have more information on the mite communities, we also have a greater understanding of the ecological factors influencing mite population dynamics. These results lead to a better understanding of the patterns and processes that could influence species richness and abundance of mites across the CFR.

2. Significant outcomes of the study

The present study confirmed that host plant architecture plays a significant role in mite community assemblages within this unique and very complex niche. Host plant characteristics such as plant life form, inflorescences colour and taxonomy may play a significant role in the availability of specific mite vectors (e.g. insects and birds). This in turn, will influence mite community structures. Infructescence age and seasonality also had a significant influence on mite abundance and this correlates well with previous studies on insects and fungi from this niche. The geographical distance between *Protea* species and between populations of a single species did not significantly influence mite community structure. Rather, the influence of host plant species themselves (life form, level of serotiny etc.) seemed to be the most important factors that determine mite community assemblages. The distribution size of the *Protea* hosts may also influence mite diversity. It is expected that species with wide distributional ranges have more resources such as food, habitat range and dispersal vectors than species with narrow distribution ranges, taking into consideration the higher heterogeneity of a larger area compared to a smaller area (Gotelli, 2001). It is also expected that wide ranging species will host more generalized mite species, while narrow ranged species will have more specialized mite species according to the meta-population dynamic models and Brown's hypothesis (Hanski and Gyllenberg, 1997; Swihart *et al*., 2003). These factors have not been investigated in the present study, but will undoubtedly be an interesting field for future study. As *Protea* species have a variety of different pollinators (and thus potential mite vectors) including birds, insect, rodents and wind (Rebelo, 2001), multiple factors may influence the distribution of mite species. This may impact both on the survival of mites within infructescences, and the restrictions placed on the movement of mites between plants.

Many new mite species were described in this study, while the known distribution ranges of the genera *Microtydeus, Paratydaeolus* and *Pausia* were significantly expanded to now also include the African

continent. Given that we only extensively sampled 14 *Protea* species, it is likely that many more new species may be identified from this niche once host sampling is expanded. Results from the present study sets a solid platform from which future mite diversity studies can be initiated in the CFR. For this reason, it is deemed appropriate to here also provide a summary of all mite species currently known to be associated with *Protea* in South Africa (Appendix 5).

3. Conservation and management implications

To maintain greater stability within an ecosystem it is important to conserve both biodiversity and ecosystem processes (McCann, 2000; Loreau *et al*., 2001). As mites affect *Protea* pollination (Smith *et al.*, 1992), seeds protection against seed predators (Romero and Benson, 2004) and dispersal of fungal propagules (Roets *et al.*, 2007), they are central to the functioning of this ecosystem. Mites are also likely to operate as ecosystem engineers, where they impact upon various different tropic levels (Fournier *et al.*, 2003). It is thus of the utmost importance to maintain their presence within *Protea* infructescences. Therefore, veld management objectives should also consider possible variables that may influence mite diversity and their continued existence (e.g. fire management and flower harvesting practices). Also, when management objectives are formulated, host plant community structure as well as pollinators (mite vectors) should be considered in order to conserve all processes within these *Protea*-mite-fungi systems. In addition, one cannot extrapolated results from single *Protea* species to generalize impacts on other *Protea* species as all taxa seem to have different mite communities. Importantly, small range or endangered *Protea* species may thus house potentially rare and/or host specific mite species that may be of special conservation concern.

4. Limitations to the study

This study sets out to investigate some ecological variables and host plant characteristics that influence mite communities within *Protea* infructescences. Clearly, not all factors that may influence mite populations could be dealt with in a study with a limited scope such as this. Also, very little is known about mite diversity in the Fynbos and therefore there were no previous studies available for strategic guidance. As such this study forms a baseline for future studies on mite diversity within the region. Difficulties with the identification of specimens were apparent, as mite taxonomy in many parts of South Africa is virtually unknown and completely understudied. I attempted to start filling this void with the description of many new taxa. Numerous other taxa in this study probably also represent undescribed species and/or genera and these will have to be evaluated in future studies. Most importantly, a general lack of information on the feeding habits of the mite species collected in this study hampered any conclusions to be drawn on the specific roles they play within this unusual ecosystem. Unraveling these interactions may prove to be a fruitful field for future studies.

5. Future research opportunities

It is clear that different factors influence mite community structure at different levels and this should be considered when specific research questions are formulated. From this study it is clear that different factors influence both inter- and intra-host species mite diversity levels. Further environmental factors and host plant characteristics that could influence mite communities within infructescences (e.g. host plant distribution ranges and plant community structure, different macroclimatic conditions, and the role of different mite vectors) should be studied. Elucidating the influence of such factors will aid predictions of mite numbers associated with *Protea* species, and aid our understanding of the distribution of various other mite groups in South Africa.

The lack of easily distinguishable taxonomic characters to distinguish various species of *Microtydeus* caused difficulties in this study, and reinforced the urgent need for a taxonomic revision of this genus. With the knowledge gained in this study our understanding of the Fynbos biome will progress and would lead to an enhanced perceptive on how to manage these systems more effectively. This study will enable us to bring together current knowledge of mite-*Protea* interactions and assist in formulating recommendations to management plans for, not only, reserve managers and conservancies, but *Protea* growers and the cut-flower industry as well.

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APPENDIX 1: Mite morphospecies and abundance collected from the infructescences of 14 *Protea* species (n = 10infructesncences). *P. caf = P. caffra, P. nit = P. nitida, P. bur = P. burchelli, P. exi = P. eximia, P. obt = P. obtusifolia, P. suz = P. susannae, P. lor = P. lorifolia, P. lau = P. laurifolia, P. ner = P. neriifolia, P. lan = P. lanceolata, P. rep = P. repens, P. pun = P. punctata, P. aur = P. aurea, P. aca = P. acaulos.*

Family	**Species**	***P.caf***	***P.nit***	***P.bur***	***P.exi***	***P.obt***	***P.suz***	***P.lor***	***P.lau***	***P.ner***	***P.lan***	***P.rep***	***P.pun***	***P.aur***	***P.aca***
Acaridae	*Schwiwia sp.1*	-	-	-	1	-	-	-	-	-	-	1	-	-	-
Acaridae	*Tyrophagus sp.1*	-	-	4	-	-	-	-	-	-	-	-	-	-	-
Ameroseiidae	*Ameroseius ornatus*	-	-	-	-	5	-	-	-	1	-	-	-	-	-
Ascidae	*Hypopus sp.1*	-	-	-	-	-	-	-	-	1	-	1	-	-	-
Bedellidae	*Cyta sp.1*	-	-	-	-	2	-	-	18	-	-	-	-	-	-
Bedellidae	*Spinibdella sp.1*	-	-	1	-	5	-	-	-	-	-	-	-	-	2
Chyletidae	*Chelacaropsis sp.1*	4	8	-	-	4	-	-	-	-	-	-	-	-	-
Diploggiidae	*Diploggiidae sp.1*	2	-	-	-	1	-	-	-	31	-	3	-	-	-
Glycyphagidae	*Ghlycyphagus sp.1*	-	43	-	-	-	1	3	-	55	4	2	-	-	2
Iolinidae	*Microtydeus beltrani*	-	-	-	-	-	-	-	-	-	3	0	-	-	-
Macrochelidae	*Macrocheles sp.1*	-	-	-	-	2	-	-	-	-	-	-	-	-	-
Nanorchestidae	*Speleorchestes sp.1*	-	-	-	-	-	-	-	1	-	-	-	-	-	6
Orbatidae	*Orbatidae sp. 1*	-	20	12	9	-	3	9	-	56	2	-	2	-	-
Orbatidae	*Orbatidae sp. 2*	-	-	-	-	-	-	3	-	-	2	-	-	-	1
Orbatidae	*Orbatidae sp. 3*	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Tarsonemidae	*Tarsonemus sp.1*	-	-	-	2	6	-	-	84	-	-	-	1	-	-
Tydeidae	*Tydeidae sp.1*	-	5	1	1	16	-	-	14	5	-	-	-	-	-
Uropodidae	*Uropodidae sp.1*	1	-	1	-	22	-	5	89	20	-	8	-	1	-
Iolinidae	*Pronematus sp.1*	1	-	1	-	-	1	-	-	1	-	-	-	1	1
Iolinidae	*Pronematus sp.2*	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Tydeidae	*Brachytydeus varietas*	1	7	-	-	-	-	-	-	-	-	6	-	-	-
Tydeidae	*Triophtydeus sp.*	-	-	7	-	-	-	-	-	-	-	-	-	-	8
Tydeidae	*Tydeus pseudofustis*	-	-	-	-	-	-	-	-	-	-	-	1	-	-

APPENDIX 2: Log-rank abundance for mite morphospecies collected from the infructescences of 14 *Protea* species

Species	**Abundance**	**Rank**	**Log abundance**
Uropodidae sp.1	149	1	2.2
Orbatidae sp.1	114	2	2.1
Chlycyphagus sp.1	112	3	2.0
Tarsonemus sp.1	93	4	2.0
Tydeidae sp.1	46	5	1.7
Diploggiidae sp.1	37	6	1.6
Cyta sp.1	20	7	1.3
Chelacaropsis sp.1	16	8	1.2
Triophtydeus sp.1	15	9	1.2
Brachytydeus varietas	14	10	1.1
Spinibdella sp.1	8	11	0.9
Ameroseius ornatus	7	12	0.8
Speleorchestes sp.1	7	12	0.8
Orbatidae sp. 2	6	14	0.8
Pronematus sp.1	6	14	0.8
Tyrophagus sp.1	4	16	0.6
Microtydeus beltrani	3	17	0.5
Schwiwia sp.1	2	18	0.3
Hypopus sp.1	2	18	0.3
Macrocheles sp.1	2	18	0.3
Orbatidae sp. 3	1	21	0.0
Pronematus sp.2	1	21	0.0
Tydeus pseudofustis	1	21	0.0
Total number of individuals =	666		22.4
Cut-off point of 25% = 5.6			

APPENDIX 3: Bray-Curtis similarity matrix of mite assemblages from the infructescences (n = 10) of various *Protea* species Species codes: *P. caf = P. caffra, P. nit = P. nitida, P. bur = P. burchelli, P. exi = P. eximia, P. obt = P. obtusifolia, P. suz = P. susannae, P. lor = P. lorifolia, P. lau = P. laurifolia, P. ner = P. neriifolia, P. lan = P. lanceolata, P. rep = P. repens, P. pun = P. punctata, P. aur = P. aurea, P. aca = P. acaulos.*

	P.caf	*P.nit*	*P.bur*	*P.exi*	*P.obt*	*P.suz*	*P.lor*	*P.lau*	*P.ner*	*P.lan*	*P.rep*	*P.pun*	*P.aur*	*P.aca*
P.caf														
P.nit	30.023													
P.bur	25.017	32.339												
P.exi	0.000	37.944	38.191											
P.obtu	34.075	25.394	29.607	23.851										
P.suz	20.162	36.202	36.468	31.954	0.000									
P.lor	14.856	37.312	35.964	29.405	14.736	45.522								
P.lau	11.302	14.538	23.228	27.341	55.252	0.000	16.659							
P.ner	30.327	51.622	44.004	28.239	40.073	37.376	42.686	28.291						
P.lan	0.000	35.693	16.409	23.713	0.000	51.971	61.738	0.000	26.651					
P.rep	44.432	31.968	13.878	15.781	25.314	18.070	36.745	17.849	46.771	18.493				
P.pun	0.000	18.774	18.914	53.985	12.030	36.561	23.669	14.003	13.500	28.663	0.000			
P.aur	46.493	0.000	35.131	0.000	12.957	37.622	22.575	15.275	24.348	0.000	20.508	0.000		
P.aca	14.056	13.905	43.287	0.000	11.293	36.559	30.228	10.685	19.853	34.384	15.467	0.000	20.779	

APPENDIX 4: Mite morphospecies and abundance collected from the infructescences of 10 *Protea repens* populations (n = 10infructesncences). Niew = Niewoudtville, Riversonder = Riviersonderend, Struis = Struisbay, Swart = Swartberg, Jonkers = Jonkershoek, Fransch = Franschoek, Gordon = Gordon's Bay.

Family	Species	Niew	Ceres	Riversonder	Struis	George	Swart	Riversdal	Jonkers	Franch	Gordon
Acaridae	*Schwiwia sp.1*	-	-	1	-	-	-	-	1	12	-
Ascidae	*Hypopus sp.1*	-	-	-	-	-	-	-	1	6	-
Bedellidae	*Spinibdella sp.1*	-	-	-	-	-	-	-	-	-	2
Digamasellidae	*Dendrolaelops sp.1*	-	-	-	-	-	1	-	-	-	-
Diplogyniidae	*Diplogyniidae sp. 1*	3	2	-	3	-	1	-	3	-	3
Glycyphagidae	*Glycyphagus sp.1*	-	1	1	-	-	-	-	2	-	-
Iolinidae	*Microtydeus beltrani*	-	-	-	-	24	7	-	-	-	-
Iolinidae	*Pronematus sp.1*	-	1	-	-	-	-	-	-	5	-
Nanorchestidae	*Nanorchestidae sp. 1*	-	3	-	-	-	-	-	-	-	-
Orbatidae	*Orbatidae sp. 1*	3	2	-	-	-	-	-	-	-	-
Tarsonemidae	*Tarsonemus sp.1*	-	8	-	2	-	4	1	-	19	44
Tydeidae	*Tydeidae sp. 1*	-	1	2	2	-	0	5	-	14	26
Tydeidae	*Brachytydeus varietas*	12	3	-	-	-	-	2	6	-	-
Uropodidae	*Uropodidae sp. 1*	17	10	3	6	8	13	3	8	-	28

APPENDIX 5: Updated list of South African *Protea* associated mites. Infructescence associated mites = Collected by N. Theron during this study.

	Mite			
***Protea* host**	**Family**	**Species**	**Location**	**Ref.**
Protea caffra	Anystidae	*Anystis baccarum*	Natal	ARC Collection
		Amblyseus citri	Natal	ARC Collection
		Raphienathus sp.1	Natal	ARC Collection
		Auothranium sp.1	Natal	ARC Collection
	Bedellidae	*Spinibdella sp.1*	Natal	ARC Collection
		Histiostoma sp.1	Natal	ARC Collection
	Tydeidae	*Tydeus grabouwii*	Natal	ARC Collection
		Aceria proteae	Pretoria, Magalies berg	ARC Collection
		Tenuipalpus acritus	Natal	ARC Collection
		Typhlodromusia saevus	Rustenberg	ARC Collection
		Rainbowia sp.1	Natal	ARC Collection
		Acarophenat	Natal	ARC Collection
	Macrochelidae	*Macrocheles*	Natal	ARC Collection
	Ascidae	*Proctolaelaps van den bergi*	Natal	ARC Collection
		Lasioseius sp.	Natal	ARC Collection
	Chyletidae	*Chelacaropsis sp.1*	Pretoria	N. Theron
	Orbatidae	*Orbatidae sp. 3*	Pretoria	N. Theron
	Eopodidae	*Eopodus sp. 1*	Pretoria	N. Theron
	Uropodidae	*Uropodidae sp. 1*	Pretoria	N. Theron
	Chyletidae	*Chelacaropsis sp.2*	Pretoria	N. Theron
	Tydeidae	*Brachytydeus varietas*	Pretoria	N. Theron
	Diploggiidae	Diploggiidae *sp. 1*	Pretoria	N. Theron
Protea glabra	Ghlycyphagidae	*Chlyciphagus sp.1*	Clanwilliam	N. Theron
	Ameroseiidae	*Ameroseius ornatus*	Clanwilliam	N. Theron
Protea nitida	Eriophygidae	*Aceria proteae*	Cederberge	ARC Collection
	Orbatidae	*Orbatidae sp. 1*	Gordon's Bay, Franschoek, Jonkershoek	N. Theron
	Orbatidae	*Orbatidae sp. 2*	Gordon's Bay, Franschoek, Jonkershoek	N. Theron
	Orbatidae	*Orbatidae sp. 3*	Gordon's Bay, Franschoek, Jonkershoek	N. Theron
	Iolinidae	*Microtydeus beltrani*	Jonkershoek	N. Theron
	Iolinidae	*Paratydeolus athaliahea*	Gordon's Bay	N. Theron
	Tydeidae	*Brachytydeus pseudovaritas*	Gordon's Bay, Jonkershoek	N. Theron

	Tydeidae	*Brachytydeus varietas*	Gordon's Bay	N. Theron
	Ghlycyphagidae	*Clycyphagus sp.1*	Franschoek, Gordon's Bay	N. Theron
	Chyletidae	*Chelacaropsis sp.1*	Franschoek	N. Theron
	Ascidae	*Gamasellodes sp.1*	Gordon'sBay	N. Theron
	Erythraeidae	*Erythraeidae sp. 1*	Gordon'sBay	N. Theron
Protea burchelli	Iolinidae	*Pronematus sp.1*	Stellenbosch Berg	N. Theron
	Acaridae	*Tyrophagus sp.1*	Stellenbosch Berg	N. Theron
	Tydeidae	*Triophtydeus sp.1*	Stellenbosch Berg	N.Theron
	Bedellidae	*Spinibdella sp.1*	Stellenbosch Berg	N. Theron
	Uropodidae	*Uropodidae sp. 1*	Stellenbosch Berg	N. Theron
	Orbatidae	*Orbatidae sp. 1*	Stellenbosch Berg	N.Theron
	Tydeidae	*Tydeidae sp. 1*	Stellenbosch Berg	N. Theron
Protea eximia	Orbatidae	*Orbatidae sp. 1*	Swartberg	N. Theron
	Acaridae	*Schwiwiasp. 1*	Swartberg	N. Theron
	Tarsonemidae	*Tarsonemus sp.1*	Swartberg	N. Theron
	Tydeidae	*Tydeidae sp. 1*	Swartberg	N. Theron
Protea obtusifolia	Eupodidae	*Eupodus sp.1*	Aguals	N. Theron
	Chyletidae	*Chelacaropsis sp.1*	Aguals	N. Theron
	Ameroseiidae	*Ameroseius ornatus*	Aguals	N. Theron
	Orbatidae	*Orbatidae sp. 1*	Aguals	N. Theron
	Macrochelidae	*Macrocheles sp.1*	Aguals	N. Theron
	Tarsonemidae	*Tarsonemus sp.1*	Aguals	N. Theron
	Uropodidae	*Uropodidae sp. 1*	Aguals	N.Theron
	Diploggiidae	*Diploggiidae sp. 1*	Aguals	N. Theron
	Bedellidae	*Cyta sp.1*	Aguals	N. Theron
	Bedellidae	*Spinibdella sp.1*	Aguals	N. Theron
	Tydeidae	*Tydeidae sp. 1*	Aguals	N. Theron
Protea susannae	Phytosiidae	*Typhlodrumus latus*	Kirstenbosch	ARC Collection
	Ghlycyphagidae	*Chlycyphagus sp.1*	Struis Bay	N. Theron
	Orbatidae	*Orbatidae sp. 1*	Struis Bay	N. Theron
	Iolinidae	*Pronematus sp.1*	Struis Bay	N. Theron
Protea lorifolia	Tetranychidae	*Oliganychus caffeae*	Oudtshoorn	ARC Collection
	Orbatidae	*Orbatidae sp. 1*	Swartberg	N. Theron
	Orbatidae	*Orbatidae sp. 2*	Swartberg	N. Theron
	Uropodidae	*Uropodidae sp. 1*	Swartberg	N. Theron
	Ghlycyphagidae	*Chlycyphagus sp.*	Swartberg	N. Theron
	Iolinidae	*Pronematus sp.2*	Swartberg	N. Theron
Protea laurifolia	Ascidae	*Proctolaelaps sp. 1*	Du Toitskloof	ARC Collection

	Eriophygidae	*Aceria proteae*	Rawsonville, Kirstenbosch	ARC Collection
	Bedellidae	*Cyta sp.1*	Gifberg	N. Theron
	Nanorchestidae	*Speleorchestes sp1*	Gifberg	N. Theron
	Tarsonemidae	*Tarsonemus sp.1*	Gifberg	N. Theron
	Tydeidae	*Tydeidae sp. 1*	Gifberg	N. Theron
	Uropodidae	*Uropodidae sp. 1*	Gifberg	N. Theron
Protea coronata	Orbatidae	*Orbatidae sp. 1*	Riviersonderend	N. Theron
	Tydeidae	*Tydeidae sp. 1*	Riviersonderend	N. Theron
	Uropodidae	*Uropodidae sp. 1*	Riviersonderend	N. Theron
Protea neriifolia	Tetranychidae	*Tetranychus telarius*	Roodeplaat	ARC Collection
	Eriophygidae	*Aceria proteae*	Stellenbosch	ARC Collection
	Ascidae	*Garmania van der bergi*	Sir Lowry's Pass	ARC Collection
	Ascidae	*Proctolaelaps roodeplaatensis*	Roodeplaat	ARC Collection
	Orbatidae	*Orbatidae sp. 1*	Gordon's Bay, Franschoek, Jonkershoek	N. Theron
	Orbatidae	*Orbatidae sp. 2*	Gordon's Bay, Franschoek, Jonkershoek	N. Theron
	Orbatidae	*Orbatidae sp. 3*	Gordon's Bay, Franschoek, Jonkershoek	N. Theron
	Iolinidae	*Triophtydeus sp.1*	Gordon's Bay	N. Theron
	Iolinidae	*Paratydeolus athaliahea*	Gordon's Bay	N. Theron
	Triophtyeidae	*Therontydeus proteocapensis*	Jonkershoek	N. Theron
	Tydeidae	*Brachytydeus pseudovaritas*	Gordon's Bay, Franschoek, Jonkershoek	N. Theron
	Tydeidae	*Brachytydeus varietas*	Gordon's Bay, Franschoek	N. Theron
	Eupodidae	*Eupodus sp. 1*	Franschoek	N. Theron
	Ameroseiidae	*Ameroseius ornatus*	Franschoek	N. Theron
	Ameroseiidae	*Ameroseiidae sp. 1*	Gordon's Bay	N. Theron
	Uropodidae	*Uropodidae sp. 1*	Gordon's Bay	N. Theron
	Ghlycyphagidae	*Chlycyphagus sp.1*	Jonkershoek	N. Theron
	Glycyphagidae	*Chlycyphagus sp.2*	Jonkershoek, Franschoek, Gordon's Bay	N. Theron
	Tarsonemidae	*Tarsonemus sp.1*	Gordon's Bay	N. Theron
	Tarsonemidae	*Tarsonemus sp.2*	Franschoek	N. Theron
	Chyletidae	*Chelacaropsis sp.1*	Gordon's Bay, Franschoek, Jonkershoek	N. Theron

	Pygmephoidae	*Pygmephoidae sp. 1*	Jonkershoek, Gordon's Bay	N. Theron
	Diploggiidae	*Diploggiidae sp. 1*	Jonkershoek, Gordon's Bay	N. Theron
	Ascidae	*Hypopus sp. 1*	Gordon's Bay	N. Theron
	Erythraeidae	*Erythraeidae sp. 1*	Jonkershoek	N. Theron
	Phytosiidae	*Meyerius immutatus*	Franschoek	N. Theron
	Cunaxidae	*Neocunaxoides zuluensis*	Franschoek, Jonkershoek	N. Theron
Protea lanceolata	Orbatidae	*Orbatidae sp. 1*	Albertinia	N. Theron
	Orbatidae	*Orbatidae sp. 2*	Albertinia	N. Theron
	Iolinidae	*Microtydeus beltrani*	Albertinia	N. Theron
	Ghlycyphagidae	*Chlycyphagus sp.1*	Albertinia	N. Theron
Protea repens	Eriophygidae	*Aceria proteae*	Citrusdal, Stellenbosch	ARC Collection
	Anystidae	*Anystis baccarum*	Bianskloof, Betty's bay	ARC Collection
	Erythraeidae	*Abrocophus sp.1*	Bianskloof	ARC Collection
	Acaridae	*Iryreophagus sp.1*	Stellenbosch	ARC Collection
	Orbatidae	*Orbatidae sp. 1*	Gordon's Bay, Franschoek, Jonkershoek	N. Theron
	Orbatidae	*Orbatidae sp. 2*	Gordon's Bay, Franschoek, Jonkershoek	N. Theron
	Orbatidae	*Orbatidae sp. 3*	Gordon's Bay, Franschoek, Jonkershoek	N. Theron
	Iolinidae	*Microtydeus beltrani*	Gordon's Bay, Swartberg, George	N. Theron
	Iolinidae	*Triophtydeus sp.1*	Franschoek	N. Theron
	Tydeidae	*Brachytydeus varietas*	Jonkershoek, Riversdal, Niewoudtsville	N. Theron
	Eupodidae	*Eupodus sp. 1*	Franschoek	N. Theron
	Eupodidae	*Eupodus sp. 2*	Gordon's Bay	N. Theron
	Uropodidae	*Uropodidae sp. 1*	Riversdal, Gordon's Bay, Niewoudtsville, Swartberg, George	N. Theron
	Ghlycyphagidae	*Chlycyphagus sp.*	Gordon's Bay, J onkershoek, Ceres, Riviersonderend	N. Theron
	Tarsonemidae	*Tarsonemus sp.*	Struis Bay,	N. Theron

			Ceres, Riversdal	
	Acaridae	*Tyrophagus sp.*	Gordon's Bay	N. Theron
	Acaridae	*Schwiwia sp. 1*	Gordon'sBay, Franschoek	N. Theron
	Digamasellidae	*Dendrolaelops sp. 1*	Swartberg	N. Theron
	Diploggiidae	*Diploggiidae sp. 1*	Gordon's Bay, Ceres, Struis Bay, Nieuwoudtville	N. Theron
	Ascidae	*Hypopus sp. 1*	Franschoek	N. Theron
	Cunaxidae	*Neocunaxoides zuluensis*	Gordon's Bay, Franschoek, Jonkershoek	N. Theron
	Iolinidae	*Pausia sp. 1*	Gordon's Bay	N. Theron
Protea punctata	Tydeidae	*Tydeus pseudofustis*	Swartberg	N. Theron
	Orbatidae	*Orbatidae sp. 1*	Swartberg	N. Theron
	Tarsonemidae	*Tarsonemus sp.1*	Swartberg	N. Theron
Protea aurea	Ascidae	*Proctolaelaps sp.1*	George	N. Theron
	Tydeidae	*Tydeidae sp. 1*	George	N. Theron
	Uropodidae	*Uropodidae sp. 1*	George	N. Theron
	Iolinidae	*Pronematus sp.1*	George	N. Theron
Protea acaulos	Tydeidae	*Triophtydeus sp.1*	Bainskloof	N. Theron
	Bdellidae	*Spinibdella sp.1*	Bainskloof	N. Theron
	Ghlycyphagidae	*Chlycyphagus sp.1*	Bainskloof	N. Theron
	Nanorchestidae	*Speleorchestes sp.1*	Bainskloof	N. Theron
	Orbatidae	*Orbatidae sp. 2*	Bainskloof	N. Theron
	Iolinidae	*Pronematus sp.1*	Bainskloof	N. Theron