MITE (ACARI) ECOLOGY WITHIN *PROTEA* COMMUNITIES IN THE CAPE FLORISTIC REGION, SOUTH AFRICA

NATALIE THERON-DE BRUIN

Dissertation presented for the degree of

Doctor of Philosophy

in the

Faculty of Agrisciences

at

Stellenbosch University

Promoter: Doctor F. Roets,

Co-promoter: Professor L.L. Dreyer

March 2018

DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author hereof (save to the extent explicitly otherwise stated), that reproduction and publication therefore by the Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

.....

Natalie Theron-de Bruin

March 2018

GENERAL ABSTRACT

Protea is a key component in the Fynbos Biome of the globally recognised Cape Floristic Region biodiversity hotspot, not only because of its own diversity, but also for its role in the maintenance of numerous other organisms such as birds, insects, fungi and mites. *Protea* is also internationally widely cultivated for its very showy inflorescences and, therefore, has great monetary value. Some of the organisms associated with these plants are destructive, leading to reduced horticultural and floricultural value. However, they are also involved in intricate associations with *Protea* species in natural ecosystems, which we still understand very poorly. Mites, for example, have an international reputation to negatively impact crops, but some taxa may be good indicators of sound management practices within cultivated systems. Their role in natural systems is even less well-understood. In this dissertation I explore the role of mites within *Protea* populations in both natural and cultivated systems, focussing on assemblages from inflorescences, infructescences and soil. *Protea* inflorescences and infructescences provide a niche for a unique assemblage of mites that have associations with a group of arthropod-associated fungi, the ophiostomatoid fungi. The mites feed on the fungi and carry their spores to new inflorescences as phoretic partners of *Protea*-pollinating beetles. As it was shown that some of the fungi have a panmictic population genetic structure over as much as 1000 km, it was assumed that organisms other than beetles must be responsible for this extremely long-range dispersal. Here I present the first concrete evidence of the ability of birds to vector spore-carrying mites to new *Protea* trees. I also provide evidence for a newly discovered mite-fungus mutualism within ornithophilous *Protea neriifolia* inflorescences between a *Glycyphagus sp.* mite and various species within the ophiostomatoid genus *Sporothrix*. New mite-mite commensalisms between the *Proctolaelaps vandenbergi* flower mite and the *Glycyphagus sp.* mite was also discovered and documented.

In this intriguing system the *Glycyphagus sp.* mites have a mutualistic association with species in the fungal genus *Sporothrix*. These small mites are phoretic on the larger *P. vandenbergi* mites that, in turn, are phoretic on *Protea* pollinating birds, explaining genetic evidence for the long distance dispersal of the fungi.

It is well-known that flower-associated mites such as *Proctolaelaps kirmsei* are nectar and pollen thieves of hummingbird pollinated plants in America. These mites reduce nectar and pollen rewards for pollinators, which influences pollinator visitation patterns and decreases available pollen for dispersal, thereby negatively influencing seed-set and plant population dynamics. This phenomenon has, however, not been investigated in similar systems in other parts of the world. I, therefore, set out to determine the possible role of *P. vandenbergi* flower mites, the most abundant flower mite within *Protea* inflorescences, as pollen and nectar thieves and as secondary pollinators of *P. neriifolia*. I provide the first evidence that *P. vandenbergi* feeds on nectar and pollen and that its reproduction is strongly linked to pollen availability. Nectar consumption rates of *P. vandenbergi* likely have little effect on total nectar availability for pollinators, but they can significantly reduce available pollen in inflorescences and may ultimately negatively influence seed set. This is exacerbated by the fact that I could show that they do not contribute to *Protea* pollination.

There is rising global concern about the negative impact of land transformation on natural ecosystems. With the increase in land transformation for agriculture, natural flora is replaced by intensively managed exotic crops. This has devastating effects on biodiversity and ecosystem services. Ecologically more friendly management systems are thus urgently required. One proposed such system is the production of native plants as crops, as these can provide known niche space for native organisms including beneficial ones, which may reduce required management inputs. *Protea* is of high ecological significance and economic value as

it is harvested for export within both natural and cultivated systems in South Africa. Although mites associated with these plants can be beneficial, they are usually regarded as pests and/or organisms that pose significant phytosanitary risks. I, therefore, investigated the impact of *Protea repens* cultivation on the mite assemblages associated with inflorescences, infructescences (the crop products where the presence of mites pose agricultural risks) and the rhizosphere (where most of the agriculturally beneficial mite species would reside). I show that this indigenous crop may well be able to maintain a large native mite biodiversity component in all three of these niches. However, essential environmental services such as the maintenance of sound soil ecology may be hindered even with very low management intensity. Results also indicated that current intensive pest management strategies do not effectively control mites associated with inflorescences. Continued improvement of post-harvest pest management practices, as difficult as these are for sensitive and fresh produce, are urgently needed. Less reliance on intensive management systems during the production phases of *Protea* inflorescences would also help preserve some natural ecological processes, such as the ones discovered and described in this dissertation.

ALGEMENE OPSOMMING

Protea is ’n sleutelkomponent in die Fynbos Bioom van die wêreldwyd erkende Kaapse Floristiese Streek biodiversiteit sentrum, nie net as gevolg van die genus se eie diversiteit nie, maar ook vir sy rol in die behoud van verskeie organismes soos voëls, insekte, fungi en myte. *Protea* word ook internasionaal wyd gekweek vir hul baie aanskoulike bloeiwyses en is daarom van groot monetêre belang. Sommige van die organismes wat met hierdie plante geassosieer word, is destruktief, wat lei tot verminderde hortologiese en snyblom waarde. Hulle is egter ook betrokke in komplekse assosiasies met *Protea* spesies in hul natuurlike ekosisteme, wat ons steeds baie swak verstaan. Myte, byvoorbeeld, het ’n internasionale reputasie daarvoor dat hulle gewasse negatief beïnvloed, maar sommige taksa mag goeie aanduiders wees van gesonde bestuurspraktyke binne gekultiveerde sisteme. Hulle rol in natuurlike sisteme word nog swakker verstaan. In hierdie dissertasie verken ek die rol van myte binne *Protea* populasies in beide natuurlike en gekultiveerde sisteme, en fokus op groeperings vanuit bloeiwyses, saadkeëls en die grond. *Protea* bloeiwyses en saadkeëls bied ’n nis vir ’n unieke versameling myte wat assosiasies het met ’n groep fungi wat weer met geleedpotiges geassosieer word, naamlik die ophiostomatoide fungi. Die myte voed op die fungi en dra hul spore na nuwe bloeiwyses as foretiese maats van *Protea*-bestuiwende kewers. Aangesien dit getoon is dat sommige fungi ’n panmiktiese populasie genetiese struktuur oor meer as 1000 km het, is dit aangeneem dat ander organismes as kewers verantwoordelik moes wees vir hierdie geweldige langafstand verspreiding. Hier bied ek die eerste konkrete bewyse van die vermoë van voëls om as vektore van spoordraende myte na nuwe *Protea* bome op te tree. Ek verskaf ook bewyse vir ’n nuut ontdekte myt-fungus mutualisme binne voëlbestuifde *Protea neriifolia* bloeiwyses tussen ‘n *Glycyphagus sp.* myt en verskeie *Sporothrix* spp. fungi. Nuwe myt-myt kommensialismes tussen die *Proctolaelaps vandenbergi* blommyte en die

Glycyphagus sp. myte is ook ontdek en gedokumenteer. In hierdie interessante sisteem het die *Glycyphagus sp.* myte 'n mutualistiese assosiasie met die *Sporothrix* spp. fungi. Hierdie klein myte is foreties op die groter *P. vandenbergi* myte wat op hulle beurt weer foreties is op *Protea*-bestuiwende voëls, wat die genetiese bewyse van langafstand vervoer van die fungi verduidelik.

Dis is goed bekend dat blomgeassosieerde myte soos *Proctolaelaps kirmsei* nektar en stuifmeel diewe van kolibrie bestuifde plante in Amerika is. Hierdie myte verminder nektar en stuifmeel belonings vir bestuiwers, wat bestuiwer besoekpatrone beïnvloed en die hoeveelheid beskikbare stuifmeel en nektar verminder. Dit beïnvloed saad-vorming en plant populasiedinamika negatief. Hierdie fenomeen is egter nog nooit in eenderse sisteme in ander dele van die wêreld ondersoek nie. Ek het daarom ten doel gehad om die moontlike rol van *P. vandenbergi* blommyte, die mees volop blommyt binne *Protea* bloeiwyses, as stuifmeel en nektardiewe en as sekondêre bestuiwers van *P. neriifolia* te ondersoek. Ek verskaf die eerste bewyse dat *P. vandenbergi* op nektar en stuifmeel voed en dat sy reproduksie sterk gekoppel is aan die beskikbaarheid van stuifmeel. Tempo van nektarinname het waarskynlik min effek op die totale beskikbaarheid vir bestuiwers, maar hulle kan die hoeveelheid beskikbare stuifmeel in die bloeiwyse beduidend verminder, en mag so uiteindelik saadvorming negatief beïnvloed. Dit word vererger deur die feit dat ek kon wys dat hulle nie bydra tot Protea bestuiwing nie.

Daar is toenemende globale kommer oor die negatiewe impak van landtransformasie op natuurlike ekosisteme. Met die toename in landtransformasie vir landbou, word natuurlike flora verplaas deur intensief beheerde uitheemse gewasse. Dit het verwoestende effekte op biodiversiteit en ekosisteem dienste. Ekologies vriendeliker bestuursisteme word dus dringend benodig. Een voorgestelde sodanige sisteem is die produksie van natuurlike plante as

gewasse, aangesien hulle natuurlike nisspasies vir inheemse organinsmes, insluitend voordeliges, kan bied, wat die bestuursinsette wat benodig word mag verminder. Protea is van groot ekologiese en ekonomiese belang aangesien dit geoes word vir uitvoere in beide natuurlike en aangeplante sisteme in Suid Afrika. Alhoewel myte wat met hierdie plante geassosieer word voordelig kan wees, word hulle gewoonlik as peste en/of organisme wat ’n fitosanitêre risiko dra beskou. Ek het daarom die impak van *Protea repens* aanplanting op myt samestellings wat met bloeiwyses, saadkeëls (die gewasprodukte waar die aanwesigheid van myte landboukundige gevare inhou) en die risosfeer (waar meeste van die landboukundig voordelige myte aangetref word) geassosieer word ondersoek. Ek wys dat hierdie inheemse gewas wel in staat mag wees om ’n groot natuurlike myt biodiversiteit in al drie hierdie nisse te onderhou. Essensiële omgewingsdienste soos die voorsiening van gesonde grond-ekologie mag egter verhinder word deur self lae bestuursintensiteit. Resultate het ook aangetoon dat die huidige intensiewe pesbestrydings strategieë nie myte wat met bloeiwyses geassosieer word doeltreffend bestry nie. Volgehoue verbetering van na-oes pesbeheer praktyke word dringend benodig, ongeag hoe moeilik hulle toepassing is vir sensitiewe en vars produkte. ’n Verminderde afhanklikheid van hierdie intensiewe bestuursisteme tydens die produksie fases van Protea bloeiwyses sal ook help om sommige natuurlike ekosisteem prosesse te bewaar, soos dié wat in hierdie dissertasie ontdek en beskryf is.

Words of Wisdom

"That which does not kill us, makes us stronger."

Friedrich Nietzsche

"Never give up on a dream because of the time it will take to accomplish it.

The time will pass anyway."

Earl Nightingale

ACKNOWLEDGEMENTS

I would like to thank the following people:

My promoters, Doctor F. Roets and Professor L.L. Dreyer for their guidance and support throughout this study, especially towards the end.

Prof. Ueckermann for introducing me to the world of acarology - I have so much more to learn!

Dr. Lizel Hugo-Coetzee at the National Museum in Bloemfontein and Davina Saccaggi at the Plant Health Diagnostic Services (PHDS), Department of Agriculture, Forestry and Fisheries (DAFF) for assistance with mite identifications.

Prof. Martin Kidd for assistance with and reviewing of statistical analysis.

Dr. Anina Coetzee and Dr. Janneke Aylward for their willingness to share their expertise during field- and laboratory work.

Monean Jacobs at the Department of Conservation Ecology and Entomology for her encouragement and sympathetic ear throughout my studies and for being a life-long friend and role model I can look up to.

I would like to acknowledge the following institutions:

The Departments of Conservation Ecology and Entomology and Botany and Zoology for providing the necessary facilities to carry out this research.

The National Research Foundation and the NRF/DST Center of Excellence in Tree Health Biotechnology (CTHB) for financial support, as well as the Harry Crossly Foundation without which this study would not have been possible.

The Directorate of Western Cape Nature Conservation Board for issuing the necessary collection permits and granting reserve access.

A special thanks to:

My husband, Leon, who supported me throughout the preparation of this manuscript.

My parents, for their financial support throughout my years at university. Extra special thanks to my mother for her encouragement, trust and for believing in me, and without whom I would not have been where I am today.

My heavenly Father for giving me the strength to go on when I felt all hope was gone, the patience for those days when I needed it the most, and above all, the ability to do all the things I possibly can think of doing and more.

I dedicate this work

to my

Mother,
Amanda Theron

Ek was nie die maklikste kind om groot te kon maak nie, maar ma het altyd die beste gedoen wat ma kon, al het ek nie altyd verstaan of daarvan gehou nie. Woorde kan ook nie regtig beskryf hoe dankbaar ek is vir ma nie en vir ALLES wat ma vir my gedoen het en nogsteeds doen nie.

Ma is my superhero
Lief vir mamma

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 2 – Birds mediate a fungus-mite mutualism

CHAPTER 3 - Mites steal *Protea* pollen

CHAPTER 4 - Cultivation impacts on mite assemblages associated with *Protea repens*

LIST OF FIGURES

CHAPTER 2 - Birds mediate a fungus-mite mutualism

CHAPTER 4 - Cultivation impacts on mite assemblages associated with *Protea repens*

CHAPTER 1

GENERAL INTRODUCTION

THE IMPORTANCE OF MITES

Mites (Acari) are miniature, spiderlike creatures, ubiquitous within almost all habitats on earth, from Antarctica (Pugh 1997), the summit of volcanoes (Schatz 1997) to deep-sea hyper thermal vents (Bartsch 1994). Their role in ecosystems can be immense, but they are often ignored in general biodiversity surveys due to their small body size, their sheer numbers and difficulties with their identification. To date, about 5000 mite species have been described (Halliday et al. 2000), representing a very small fraction of the estimated 1 000 000 species in existence (Krantz & Walter 2009). Their diverse feeding habits range from fungivores (Mitchell & Parkinson 1976, Roets et al. 2007, 2009, Theron-de Bruin et al. 2017), herbivores (Hislop & Jeppson 1976, Krantz & Linquist 1979, Hallman et al. 2016), predators (Siepel & de Ruiter-Dijkman 1993, Zhang & Sanderson 1997, De Moraes et al. 2002), saprophages (sessile), detritivores (mobile) (Walter & Proctor 1999, Krantz & Walter 2009) to parasites (Krantz & Walter 2009).

Due to the diversity and ubiquitous distribution of mites, their high abundance, their sensitivity to change and low mobility, they can be used as bio-indictors (Carignan & Villard 2002, Duelli & Obrist 2003, Gerlach et al. 2013). Mites have, for example, been used as indicators of changes in bio-diversity (Oliver & Beattie 1992), indicators of restoration success (Więcek et al. 2013), land-use monitoring (Gulvik 2007) and estimations of environmental toxicity (Huguier et al. 2014). Mites are not only useful in ecological studies,

but can also be beneficial to agriculture. A variety of mites are used as bio-control agents against various crop pests in greenhouses (Zhang et al. 2003), on agricultural crops in the field (Van Houton et al. 1995, McMurtry et al. 1997) and on floricultural crops (Hessein & Parrella 1990). However, mites can also be detrimental to the health of humans and other invertebrates such as the *Sarcoptes scabiei* L. mite that causes scabies, a parasitic infection that leads to pruritic (itchy) lesions, which can also become secondarily infected (Walton & Currie 2007, Currier 2011). In addition, *S. scabiei* can cause mange on animals such as dogs and pigs (George et al. 1992, Walton & Currie 2007). Various other mite species also cause mange of domestic animals such as *Demodex canis* Leydig on dogs (Lacey et al. 2009), *Notoedres cati* Hering on cats (Sivajothi et al. 2015), *Psoroptes ovis* Hering on sheep (Van den Broek & Huntley 2003) and *Chorioptes bovis* Hering on dairy cattle (Rehbein et al. 2005). Other than these disease-causing parasites, some mites may be associated with animals and humans without causing negative effects. For example, two species of *Demotex*, *D. folliculorum* Simon and *D. brevis* Akbulatova, are very common on humans and feed on epithelial cells and sebum within hair follicles (Lacey et al. 2009). These ectoparasites are asymptomatic and most humans are only carriers. They may, however, be multi-factorial and cause pathogenic problems when present in high abundance, but this has not yet been proven (Lacey et al. 2009, Rather & Hassen 2014).

Mites may regulate ecosystems in numerous ways, but we are only starting to become aware of the multitude of roles that they play in various ecological processes. They interact in some way with nearly all other life-forms on earth. Given the diversity of mites, numerous opportunities exist for research on the importance of mites to other organisms in ecosystems. Clearly one cannot consider all known interactions between mites and other organisms within the restrictive bounds of a dissertation. As separate data chapters in this dissertation introduces the topics of interest for that particular study, I will highlight only a few well-

studied interactions between mites and other arthropods, vertebrates and plants by means of general introduction. Thereafter I will introduce my major study area, the Cape Floristic Region (CFR) and my focal study organisms (*Protea* plants and their associated organisms) in more detail. I end this section with an outline of the objectives of the different studies reported on in this dessirtation.

Mite-arthropod interactions

The internationally best-known mite-arthropod interactions may be that between the verroa mite (*Verroa destructor* Anderson & Trueman) and honey bees (De Jong et al. 1982, Smith & Oliver 1986, Sammataro et al. 2000, Martin et al. 2012). These mites feed by sucking the hemolymph from their hosts. They consequently infect their host with diseases such as honey bee RNA viruses, for example the deformed wing virus (DWV), which is associated with honey bee colony collapse disorder (CCD) worldwide (Martin et al. 2012). As honey bees are important pollinators of both wild plants and agricultural crops, these colony collapses can significantly influence normal ecosystem processes and crop yields. Therefore, various studies have focused on ways to control the spread of the verroa and other destructive honey bee mites, including *Acarapis woodi* Rennie and *Tropilaelaps clareae* Delfinado & Baker (De Jong et al. 1982, Martin et al. 2012).

Mites also form associations with ants (Formicidae) and other social insects. Examples of animals involved in such interactions include *Forcellinia* mites and ants (Uppstrom 2010, Uppstrom & Klompen 2011) and termites (Isoptera) and *Cosmoglyphus* mites (Hunter & Rosario 1988, Eickwort 1990, Wang et al. 2002). These interactions are not always well-understood and can be very complex. Ito & Takatu (1994), for example, found that an oribatid mite, *Aribates javensis* Aoki, Takatu & Ito seems to be an obligate myrmecophile within

Myrmecina ant nests. It is kept alive and groomed by its ant hosts, while they are likely microbivorous within the nest. The ants only feed on the dead mites, improving egg-laying by isolated workers (ants separated from the queen and the brood). These ants will feed on the living mites only if they are starving, which may help sustain ant colonies during long, dry seasons (Ito & Takatu 1994, Ito 2013).

Mites can be parasites of numerous insects. For example, the moth ear mites, *Dicrocheles phalaenodectes* Treat and *D. scedastes* Treat (Hunter & Rosario 1988) and the Mexican bean beetle (*Epilachne varivestis* Mulsant) mite *Coccipolipus epilachnae* Smiley, all feed on the hymolymph of their hosts (Schroder 1979). Water mites (Hydrachnida) are parasitic on aquatic insects including Hemiptera, Diptera and Odonata (Smith & Oliver 1976, 1986, Zawal 2004, 2006, Zawal et al. 2017). Mites such as *Arrenurus* spp. are also common parasites of mosquitoes, including species that can transmit malaria (Simmons & Hutchinson 2016). These mites can reduce flight mobility and inhibit growth and reproduction of their hosts, making them valuable bio-control agents of some malaria vectors (Werblow et al. 2015).

Mites are unable to fly. For dispersal over short distances they can crawl to suitable niches in close vicinity (Roets et al. 2009). For longer range dispersal some mites use wind (*e.g.* the coconut mite *Aceria guerreronis* Keifer and the wheat curl mite *Aceria tosichella* Keifer (Melo et al. 2014, Umina et al. 2015)) or even ballooning with silken strands (*e.g.* the spider mite *Tetranychus urticae* Koch (Tehri 2014)). These types of dispersal, however, offer very little control over which substrates mites would end up on. Therefore, a commonly adopted long-distance dispersal mechanism for mites is to use other animals as vectors in a process called phoresy (Houck & O'Conner 1991, Krantz & Walter 2009). This is very often the mode of dispersal for mites that live in temporally or spatially disjunct niches (Hunter & Rosario 1988). *Macrocheles saceri* Costa mites, for example, use dung beetles as vectors to

fresh dung piles where they feed on nematodes, fly eggs and larvae (Krantz 1998, Niogret et al. 2006). In the process of phoresy, the mites neither feed nor develop further and they are generally considered harmless to their vector organisms.

A particularly well-studied system is the association between certain mites and bark beetles that infest trees. Bark and ambrosia beetles (Coleoptera: Curculionidae, Scolytinae and Platypodinae) infest trees by boring galleries into the inner bark and phloem where they lie their eggs. At the same time, these beetles inoculate their tunnels with various fungal species, carried on their exoskeletons, which they use as additional or main food source. These beetles also vector various mites, which, in turn, transport a different fungal species, that they rely on for nutrition, within specialized structures known as sporothecae (Levieux et al. 1989, Klepzig et al. 2001a, b). The best-studied bark beetle-mite-fungus interaction system is one responsible for extensive damage to pine trees in the United States of America (USA). It is caused by the southern pine beetles, *Dendroctonus frontalis* Zimmermann, that vector the fungi *Entomocorticium sp.*A and *Ceratocystiopsis ranaculosus* Perry and Bridges within their mycangia. These fungi serve as food source for the beetles. In addition to these beneficial fungi, the blue stain fungus *Ophiostoma minus* (Hedgcock) H. & P. Sydow is also often present in the galleries of the beetle. This fungus is an antagonist and outcompetes *Entomocorticium sp.*A, causing a decrease in larval development and growth and inhibiting egg production, leading to a decline in beetle populations (Paine et al. 1997, Klepzig et al. 2001b, Six & Wingfield 2011). *Tarsonemus* mites that are phoretic on these beetles (Lombardero et al. 2003) carry the spores of *O. minus*, which they need for nutrition, causing a negative feedback system that can help to regulate beetle numbers (Lombardero et al. 2000, Klepzig et al. a, b). These mite-insect associations may be very complex (Six & Wingfield 2011, Hofstetter et al. 2014) and the system collapses when either one of the interactions are negatively influenced.

Mite-plant associations

Many mites are considered notorious pests of agricultural crops across the world. For example, the two-spotted spider mite, *Tetranychus urticae* Koch, is a cosmopolitan pest species that cause great monetary losses through yield losses (in some cases 100% loss) of agricultural crops (Childers et al. 2003, Attia et al. 2013, Van Leeuwen et al. 2014). It can infest *ca.* 1200 plant species of which *ca.* 150 are economically important (Tehri 2014, Van Leeuwen et al. 2014). *Tetranychus urticae* feeds directly on the plant, puncturing plant cells and emptying the contents. In 2008, 62% (€372 million) of the entire acaricide market was invested in controlling *T. urticae* alone (Van Leeuwen et al. 2014). Other major phytophagous mite pests includes the papaya pests, *Polyphagotarsonemus latus* Banks (Tarosnemidae) and numerous *Amblyseius* spp. (Phytoseiidae) and *Tetranychus* spp. (Tetranychidae) that inhibit stem growth and destroys terminal buds, causing severe reduction in fruit formation (Collier et al. 2004). In addition to causing losses in agricultural and greenhouse systems (Zhang 2003), various mite species within the natural environment can also hamper the fitness of their natural hosts. Flower associated mites, for example, are mostly pollen and nectar thieves consuming pollen and nectar from their host plant and competing with pollinators for nectar rewards. In the process they negatively influence seed set (Dobkin 1985, Heyneman et al. 1991, Colwell & Naeem 1994).

Mites may not necessarily cause sufficient damage to crops to be considered a significant problem in agriculture. The presence of mites alone on cut flowers, for example, is considered a phytosanitary problem and various expensive post-harvest control methods are needed to rid cut-flowers from possible infestations (Myburgh et al. 1973, Hansen & Hara 1994, Coetzee et al. 2007, Da Silva 2003). Preventing the introduction of any new mite species into a country is of great importance as it is the best form of pest control (Hallman 1998). New invasions can

be devastating, such as was the case of the cassava green mite, *Mononychellus tanajoa* Bondar (Tetranychidae). It was introduced into East Africa from Brazil in 1971, where after it spread to 27 countries within less than 20 years, decreasing yields of this staple crop by up to 80% in some areas (Yaninek & Herren 1988).

Mites may also interact positively with plants. For example, it has recently been discovered that mites may assist with fertilization of mosses by transporting sperm (Cronberg et al. 2006, Cronberg 2012). To show this, male and female plants of the moss *Bryum argenteum* Hedw. where separated in dishes where sperm movement via water was not possible. Sporophyte development (resulting from fertilization) was compared between plants that were separated with and without the presence of mites (*Scutovertex minutus* Koch and *S. sculptus* Michael) and springtails (*Isotoma caerulea* Bourlet), respectively (Cronberg et al. 2006). Fertilization was possible only when these organisms were present. In addition, it was found that these mites preferred to visit fertile plants above sterile plants, indicating that this association was due to active visitation/attraction and not due to random/passive movements of the organisms.

Mites also positively interact with plants in more subtle ways. For example, many plants produce specialised structures on leaves, called acarodomatia, that seem to only serve the purpose of providing shelter for leaf-associated mites (Dicke & Sabelis 1987, O'Dowd & Wilson 1989, 1991, Walter 1996, Norton et al. 2001). These domatia often contain mixtures of predatory and microbivorous mites (O'Dowd & Wilson 1989, 1991, Walter & O'Dowd 1992, Walter 1996). The predatory mites may serve the plant by controlling pests on the plants (O'Dowd & Wilson 1989, 1991, Walter & O'Dowd 1992, Walter 1996), while the microbivorous mites feed on fungal hyphae on leaf surfaces and may help control infection by pathogenic fungi (O'Dowd & Wilson 1989, 1991). *Orthotydeus lambi* Baker, a tydeid mite for example, associated with leaf domatia on the riverbank grape, *Vitis riparia* Mchx., feeds

on hyphae of the pathogenic grape powdery mildew fungus, *Uncinula necator* (Schw.) Burr (Norton et al. 2000). They significantly increased in numbers when leaf domatia were present on the host plant, and subsequently significantly reduce infestation by *U. necator* (Norton et al. 2000). In return, the domatia provide protection against mite predators (O'Dowd & Willson 1991, Norton et al. 2001).

The most significant positive interaction between mites and plants likely involves the soil mites and their role as detritivores (Moore & Walter 1988). These soil-associated mites (largely Oribatida) play a vital role in the breakdown of organic waste and the release of nutrients for uptake by plants. For example, the Oribatida mite, *Scheloribates moestus* Banks, not only drastically improves microbial respiration within litter, but also improves enzyme activities and increase dissolved C and N for plant uptake, which enhances mineralisation and improves oxidative and hydrolytic activities (Wickings & Grandy 2010). Tydeid mites can regulate nematode feeding on litter bacteria, resulting in an increase of decomposition of soil litter (Santos et al. 1981). Some Oribatida mites inhabit trees where they decompose and mineralize litter and assist in nutrient availability to epiphytes (Behan-Pelletier & Walter 2000). In addition, soil mites such as the Oribatida can act as valuable indicators of ecosystem recovery after catastrophic events. This is because mites are among the first to colonise areas in the primary successional stage (Skubala & Gulvik 2005). On mine heaps Oribatid mites are one of the fist organisms to colonize the soil and start ecological restoration processes (Wanner & Dunger 2002). High numbers of Oribatida mites are present in young soils of receding glaciers, with increase in species richness and abundance as these soils age, proving their value as indicators in this system (Hågvar et al. 2009).

Mite-vertebrate interactions

The Acari includes the notorious ticks, which are obligate hematophagous parasites at some stage during their life-cycle (Krantz & Walter 2009). Ticks (and some other mites) regularly feed on a variety of hosts (Radford 1950, Jongejan & Uilenberg 2005) including reptiles (Bochkov et al. 1991, Burridge & Simmons 2003, Mendoza-Roldan et al. 2017), amphibians (Quinzio & Goldberg 2015, Jacinto-Maldonado et al. 2016) and mammals (including humans). In the feeding process, ticks may transmit some of the most notorious diseases that affect vertebrates (De La Fuente et al. 2008). For example, Lyme decease is caused by the bacterium *Borrelia burgdorferi* ss, which is transmitted through the bite of *Ixodes scapularis* Say ticks. Similarly, the bacterium *B. hermsii* causes tick-borne relapsing fever that is vectored by *Ornithodoros hermsi* Weeler ticks (Schwan & Pieman 2002). African tick bite fever is caused by a bacterium *Rickettsia africae* Kelly, which is transmitted through the bite of *Amblyomma* spp. ticks such as *A. variegatum* Fabricius and *A. hebraeum* Koch (Jensenius et al. 2003). These diseases can also be transmitted to humans via other wild and domestic animals such as birds, dogs or rats (Comstedt et al. 2006). In addition to ticks, scabies and mange mites, mites can further hamper the quality of human life by releasing allergens that can lead to health problems such as asthma (Arlian et al. 2001).

Just as in mite-arthropod interactions, mite-vertebrate interactions include phoresy. A well-known phoretic mite-vertebrate association is that between flower mites and hummingbirds (Dobkin1984, Colwell 1973, Colwell & Naeem 1994, Maloof & Inouye 2000, Irwin et al. 2001, Lara & Ornelas 2002a, b). The mites climb onto the beak and into the nostrils of the hummingbirds when these visit flowers of their host plants for nectar and pollen (Colwell 1973, 1995, Proctor & Owens 2000). The mites use the birds as vectors to the next flower that these birds visit. These flower mites (*Proctolaelaps*, *Rhinoseius* and *Tropicoseius* spp.) can

only reproduce when feeding on nectar and pollen of their host plants and usually do not contribute to pollination, rendering them pollen and nectar thieves (Dobkin 1984, 1990, Lara & Ornelas 2001, 2002 a, b, Colwell & Naeem 1994, Paciorek et al. 1995).

STUDY AREA AND FOCAL PLANT HOST

The Cape Floristic Region (CFR) represents one of only six floral kingdoms worldwide. It comprises a mere 87 892 km^2 in area and is confined to the southwestern tip of Africa (Cowling et al. 2003, Goldblatt 1997, Goldblatt & Manning 2002). This is a highly threatened region with unusually high levels of endemism and regarded as a global conservation priority area (Goldblatt 1997, Holmes & Richardson 1999). With diversity levels comparable to that of tropical rainforests, the CFR is rated as one of the most diverse eco-regions in the world (Cowling et al. 1992, Meyrs et al. 2000). Fynbos is the most characteristic vegetation type found within the CFR and predominantly consist of members of the Ericaceae, Restionaceae and Proteaceae (Mucina & Rutherford 2006). Ninety seven percent of all CFR Proteaceae members are endemic and most are confined to Fynbos (Cowling et al. 2003). Fynbos not only enhances the biodiversity of the region, but acts as an economic entity, providing revenue from ecotourism, helping with water supply regulation, providing ample foraging for beekeeping and its pollination service of agricultural crops and acts as basis for the thriving South African cut-flower industry (Reinten et al. 2011; Hassen 2003, Le Maitre et al. 1997, Turpie et al. 2003).

Worldwide, the Proteaceae includes 1700 species of which 330 species are confined to the CFR (Barker et al. 2007, Rebelo 2001). The type genus *Protea* L. includes 136 species in Africa. In South Africa *Protea* plants range from small, cryptic, low growing shrubs (*P. amplexicaulis* (Salisb.) R.Br.) to large, conspicuous trees (*P. nitida* Mill) that grow from

coastal plains to snow covered mountain tops (Rourke 1998, Rebelo 2001). *Protea* form terminal capitula (inflorescences) with strong, usually colourful involucral bracts that surround the flowers (Rourke 1998). Most *Protea* species also form woody structures that contain their hairy fruits (infructescences) (Collins & Rebelo 1987, Rebelo 2001). The great variety in *Protea* growth forms and inflorescence morphologies facilitate the utilization of multiple different pollinators.

Protea pollination and pollinators

Protea are hermaphroditic (both sexes on the same plant and flower) and protandrous (anthers (♂) maturing before the pistil (♀)). *Protea* species are generally self-compatible (Van der Walt & Littlejohn 1996, Steenhuisen et al. 2012, Steenhuisen & Johnson 2012a, b) through autogamy (flower receives it own pollen) or geitonogamy (flower receives pollen from another flowers within the same inflorescences). A *Protea* flower comprises of four tepals (perianth segments) that are variously fused. Stamens are lacking (fused to the tepals), and the four anthers are borne at the tip of each tepal. The gynoecium comprises of a single carple that forms a small, unilocular ovary, a massive (thick and long) style with a stigma at the tip, often enlarged to form a pollen presenter. The true stigma essentially comprises of a groove that remains closed during the male phase of the flower, and only opens and becomes receptive after the flower's own pollen has been shed. At anthesis the anthers deposit pollen onto the pollen presenter and, as the style elongates, the pollen presenter is released from its position between the anthers to be exposed to the outside world (Collin & Rebelo 1987). It thus picks up its own pollen on the pollen presenter, and offers it for pick-up by pollinators. During this time the stigmatic groove remains closed, and the flower is in the male phase (Van der Walt & Littlejohn 1996b, Ramsey & Vaughton 1991). About three days after anthesis, when most own pollen has been removed, the flower enters the female phase by

opening its stigmatic groove, which now becomes receptive for pollen. Flowers start opening and maturing from the outside of the inflorescence to the inside. The fact that flowers display a distinct male and female phase decreases the extent of self-pollination through autogamy (Steenhuisen & Johnson 2012a, b).

After pollination, the inflorescence closes up and the involucral bracts harden, forming a fruiting structure known as the infructescence. Seed development can take up to 9 months (Van Staden 1978, Wright 1994), but seed set (fertile seeds) is generally low, ranging between 2%-30% (Rebelo & Rourke, 1986, Mustart et al. 1995). Seeds are either dropped to the ground at maturity or stored in closed infructescence on the plant for prolonged periods. In some species, for example *P. repens* L. and *P. neriifolia* R.Br., infructescences will accumulate on the plant year after year (serotiny) until their water supply ceases (mostly when the parent is destroyed in a fire) (Rebelo 2001). The infructescences then open and release their seeds.

Depending on the species, *Protea* flowers can be pollinated by rodents, insects and/or birds (Collins & Rebelo 1987, Rebelo 2001). Some *Protea* species have geoflorous (inflorescences carried close to the ground) morphologies and are mainly pollinated by rodents and shrews (Wiens 1978, Wiens et al. 1983, Rebelo & Breytenbach 1987, Fleming & Nicolson 2002, Biccard & Midgley 2009, Zoeller et al. 2016). The inflorescences of *Protea humiflora* Andrews, for example, are morphologically specialized for rodent pollination in that they are strong-smelling, cryptic and bowl-shaped (Fleming & Nicolson 2002). Mammal-pollinated *Protea* species and their pollinators are not thought to have co-evolved, because of the brief flowering period and limited plant distributions, rendering the nectar source limited and unreliable (Rourke and Wiens 1977, Wiens et al. 1983, Rebelo & Breytenbach 1987). In contrast, the flowers of other Proteaceae, particularly the Australian genus *Banksia*, are often

important dietary sources for their non-flying mammal pollinators (Wiens et al. 1979, Turner 1982, Collins & Rebelo 1987, Carthew 1993, Wooller et al. 1993). A recent study showed that some, mostly carnivorous, mammals such as genets and mongoose also feed on *Protea* pollen and nectar and may assist with pollination (Steenhuisen et al. 2015).

Insect-pollinated *Protea* species produce inflorescences that are mostly small with yellow, pink or cream coloured involucral bracts, have low nectar quantities and have sour, sweet or spicy odours (Rebelo 2001). Some species have more showy inflorescences and may be pollinated by insects and birds. For example, Coetzee and Giliomee (1985) showed that *P. repens* inflorescences are visited by many insects that play a key role in pollinating these flowers, despite often also being visited by nectivorous birds. The most notable visitor group found during their study was small beetle species, especially of the family Chrysomelidae, which constituted 70% of all insects encountered. Gideon et al. (1980) also found an abundance of small beetles of the genus *Chirodica* (Chrysomelidae) in *P. repens* inflorescences. Similarly, Roets et al. (2006) and Sasa & Samways (2015) found that beetles were the most abundant arthropod taxa associated with the *Protea* species they studied. Chafer beetles (Cetoniini) and monkey beetles (Hopliini) are also known to feed on *Protea* pollen and nectar (Johnson & Nicolson 2001) and are the main pollinators of various *Protea* species (Coetzee & Giliomee 1985, Rebelo 2001, Steenhuisen et al. 2012).

Bird-pollinated *Protea* species produce inflorescences that are generally brightly coloured with no odour, have elongated pollen presenters and abundant nectar (Vogts 1984, Rebelo 2001). Cape sugarbirds (Promeropidae) and the orange breasted sunbird (Nectariniidae) are the most prominent nectivorous Fynbos bird species and have a close association with *Protea* plants (Skead 1967, Collins & Rebelo 1987, Calf et al. 2003). The breeding season of the Cape sugarbird coincides with the peak flowering period of *Protea neriifolia* (bird-pollinated)

and *P. repens* (Broekhuysen 1963, Winterbottom 1962). When the birds visit *Protea* inflorescences for nectar (Calf et al. 2003), they force their heads in between the floral parts (Collins 1983, Rebelo et al. 1984, Collins & Rebelo 1987, Rebelo 1987), triggering some closed flowers to open, and deposit pollen on the heads and chests of the birds (Gideon et al. 1980, Hargreaves et al. 2004). Even though some *Protea* inflorescences are exclusively visited by birds (Collins & Rebelo 1987), most bird-pollinated *Protea* species also attract insects as additional pollinators.

Other Protea-associated organisms

Apart from insects associated with pollination, numerous studies have documented the diversity of arthropods associated with other *Protea* plant parts such as foliage and infructescences (Gess 1968, Coetzee 1989, Wright 1990a, b, Wright & Giliomee 1992, Wright & Samways 1999, 2000, Fleming & Nicolson 2003, Tjørve et al. 2005, Roets et al. 2006, Sasa & Samways 2015). Many of these cause major damage and limit *Protea* agricultural production (Myburgh et al. 1973, Wright & Saunderson 1995, Coetzee & Giliomee 1987, Coetzee et al. 2007). For example, borer insects, including *Genuchus hottentotus* F., destroy inflorescence buds, consume seeds, damage flowers and even cause discolouration of inflorescences (Coetzee & Giliomee 1987). Despite this, they form natural components of a normally functioning Fynbos ecosystem.

Various fungi are also found within *Protea* inflorescences and infructescences (Marais & Wingfield 1994, 2001, Lee et al. 2005, Roets et al. 2005, 2006, 2013). The most dominant *Protea*-associated fungal species in the CFR include *Knoxdaviesia proteae* Wingfield, Van Wyk & Marasas, *K. capensis* Wingfield & Van Wyk, *Sporothix phasma* Roets, De Beer Wingfield and *S. splendens* Marais & Wingfield (ophiostomatoid fungi). It is unclear how

these fungi influence their *Protea* hosts, but many other fungal species can cause diseases (Knox-Davies et al. 1986, Crous et al. 2004, 2011, Coetzee et al 2007). *Protea* root rot, for example, is caused by *Phytophthora cinnamomi* Rands (Von Broemsen & Brits 1986), fusarium wilt is caused by *Fusarium oxysporum* Schlect. (Swart et al. 1999) and *Botryosphaeria proteae* Wakef. is an important stem canker pathogen (Swart et al. 2000).

The association of fungi and other micro-organisms with various mite species on *Protea* hosts are of special interest in this study. The mite *Aceria proteae* Meyer is thought to be the carrier of the devastating Witches broom disease, which is caused by a phytoplasma (Myburgh et al. 1973, Coetzee et al. 1985, Wieczorek & Wright 2003). Recently, while investigating fungal diversity within *Protea* infructescences, Roets et al. (2009, 2011) discovered that mites where the primary vectors of *Protea*-associated ophiostomatoid fungi, and not insects as previously believed. These mites can use the fungi as food source (Roets et al. 2007, Theron-de Bruin et al. 2017), demonstrating an unusual mutualism between some *Protea* mites and *Protea* ophiostomatoid fungi (Roets et al. 2007, 2009, 2011). These mites were shown to use various *Protea*-pollinating beetles as vectors between *Protea* inflorescences and, in their part, the mites disperse their fungal mutualists (Roets et al. 2009). Dispersal of the fungus can occur over vast distances, >200 km for *Knoxdaviesia* (Aylward et al. 2015) and >1000 km for *Sporothrix* (Ngubane 2017) as was shown by population genetic studies. This fungal-mite mutualism prompted an investigation into mite communities within *Protea* infructescences of various *Protea* species (Theron 2011) during which numerous new mite species were discovered and described (Theron et al. 2012). Results highlighted our very meagre knowledge of mites associated with *Protea* spp. and with Fynbos plants in general. Also, apart from the initial studies on mite-*Protea*-fungus interactions and their general diversity, almost nothing is known about the ecology of the mites associated with this iconic plant genus.

Agricultural activities and land transformation have detrimental impacts on natural ecosystems, ranging from soil erosion and enrichment (McLaughlin & Mineau 1995), contamination of water to loss of non-targeted species due to pesticides (Wauchope 1978, Zhang et al. 2007). This has led to massive decreases in biodiversity (Tilman et al. 2001, Swift et al. 2004, Clergue et al. 2005, Tscharntke et al. 2005). Such losses result in the disruption and/or damage of ecological process and to reductions in ecosystem services such as pollination, pest management, improvement of soil quality and structure and hydrology (Zhang et al. 2007, Swinton et al. 2009, Tilman 1999, Power 2010). There are various agricultural practices and management strategies that may alleviate these impacts, such as intercropping or crop rotation, which create heterogeneity within the landscape (Liebman & Dyck 1993, Khan et al. 1997, Smith & McSorley 2000). Another form of agriculture (agroecological farming) has started to consider farming with the integration of ecological principles (Tilman 1999, Tomich et al. 2011, Tscharntke et al. 2012, Wezel et al. 2014). In addition to agroecological farming, the cultivation of indigenous crops is becoming more popular. It is seen as an effort to alleviate overexploitation and to assist with conservation of plant species (Schippmann et al. 2002). In the Western Cape Province agroecological farming of members of the Proteaceae has become common practice (Reinten & Coetzee 2002, Coetzee et al. 2007).

Floriculture of indigenous South African crops has become a popular option for farmers and currently comprises *ca*. 900 ha (Gerber & Hoffmann 2014). The South African floricultural industry provides livelihoods to over 7500 people, of which 1500 are permanent positions and 400 seasonal (Gerber & Hoffmann 2014). The South African floricultural market was worth $ 38 649 million in 2002 (Matthee et al. 2006), with companies such as Mutiflora Johannesburg

reporting a turnover of €18 million (Reinten et al. 2011). *Protea* is a well-known and favoured cut-flower, and extensively cultivated for the global floriculture industry (Brits et al. 1983, Coetzee et al. 2007, Reinten & Coetzee 2002). It is also cultivated in many other countries including Australia, Chile, Ecuador, France, Hawaii, New Zealand and Portugal (Gerber & Hoffmann 2014). *Protea* inflorescences and infructescences are still also commercially harvested within natural populations (Myburgh et al. 1973, Coetzee et al. 2007). *Protea repens* and *P. neriifolia* represent two of the very few non-hybrid taxa that are extensively cultivated (Coetzee et al. 2007). Mainly hybrid cultivars are planted for cultivation, and require that virgin land (natural system) needs to be ploughed up for these plantations. Conradie & Knoesen (2010) indicated that 41% of producers, who planned to expand their flower production, would do so by ploughing up virgin land. *Protea* are cultivated within well-drained, acidic soils containing less than 20% clay. These soils, depending on the depth, would either be tilled and ridged or limed and fertilised or, in the case of rocky soils or steep slopes, no preparation occurs. 75% of producers make use of irrigation and fertilisation and 79% use pesticides (Conradie & Knoesen 2010). Very little is known about the ecological impacts of such indigenous crop cultivation practices on the local bio-diversity and ecosystem functioning in the CFR.

THE PRESENT STUDY

The current study focuses on three major components of mite diversity and ecology in the CFR and their possible role within ecosystems dominated by *Protea* species. The first component builds on previous knowledge on mite-fungus symbioses on *Protea* by investigating possible additional mite-fungus mutualisms and their dispersal via avian vectors. The second component investigates the possible role of mites in *Protea* pollination, an aspect never before considered in the floristically hyper-diverse CFR. The third component

investigates the impact of agricultural production of indigenous crops on mite assemblages associated with *Protea* species in a bid to understand the impact of such practices on native biodiversity and ecosystem processes.

In Chapter 2, the fungus-mite mutualism discovered within the unique *Protea* infructescences niche (Roets et al. 2007, 2011) is investigated in more detail. It was shown that some mites (*Trichouropoda sp.*) and fungi (*Sporothrix spp*.) have a mutualistic association and that these mites are phoretic on *Protea*-pollinating beetles for dispersal to uncolonized inflorescences (Roets et al. 2009). However, genetic panmixia was discovered within populations of these fungal species over vast distances (Aylward et al. 2014), suggesting long-distance dispersal via organisms that can cover larger distances than insects, whose movement would be confined due to mountains and other areas of unsuitable habitat. In Chapter 2 I, therefore, investigate the possible role of *Protea*-pollinating birds as vectors of mites that carry the fungal spores. The manuscript prepared from this chapter was recently accepted for publication in the journal *Microbial Ecology* (doi.org/10.1007/s00248-017-1093-9), co-authored by Prof. Leanne L. Dreyer, Prof. Edward A. Ueckermann, Prof. Michael J. Wingfield and Dr. Francois Roets. The format of the references in this chapter, therefore, differs from that presented in the other chapters.

Flower-associated mites such as those associated with hummingbirds in the Americas are known to be nectar and pollen thieves of hummingbird-pollinated host plants (Colwell 1995, Lara & Ornelas 2001, 2002a, b). The removal of nectar and pollen by these mites can lead to a reduction in nectar rewards for pollinators, and suitable pollen for fertilization, ultimately influencing host plant population dynamics (Colwell 1995, Paciorek et al. 1995, Hargreaves et al. 2009). In some cases the presence of these flower mites may assist with secondary pollination as the mites can pollinate allogamous protandrous plants (Lara & Ornelas 2001,

2002a). A similar situation may exist in *Protea* species that are predominantly pollinated by birds in the CFR. The inflorescences of these *Protea* species often swarm with thousands of individuals of the flower mite *Proctolaelaps vandenbergi* (Ryke 1954, 1964). It is presumed that they feed on nectar and pollen, but this has never been tested. Given their high abundance within inflorescences, it is reasonable to assume that they may influence *Protea* fertilization by either aiding pollination or by depleting nectar and pollen sources available to the usual pollinators. In Chapter 3 I investigate the possible impact of mites on *Protea* pollination and seed set by testing their role as secondary pollinators and by quantifying their consumption of *Protea* pollen and nectar.

Transformation of natural areas into agricultural lands has enormous effects on biodiversity, ecological processes and environmental services. The cultivation of indigenous crops is becoming more popular and lucrative, and various native species are now cultivated within South Africa. However, very few studies have investigated the influence of indigenous crop cultivation on natural ecosystems. In Chapter 4 I investigate the influence of cultivation of indigenous *Protea* species on mite assemblages associated with inflorescences, infructescences and soils. Results will indicate if native production systems provide suitable niches for maintenance of native biodiversity and associated ecological processes. Results also provide an indication of the presence of mite communities within inflorescences intended for the export market, which has obvious phytosanitary importance.

I conclude with Chapter 5 which provides a summary of my main results. I further highlight the significance of these results, and provide some suggestions for future research.

References

1. Arlian LG, Platts-Mills TA (2001) The biology of dust mites and the remediation of mite allergens in allergic disease. Journal of Allergy and Clinical Immunology 107:406–413
2. Attia S, Grissa KL, Lognay G, Bitume E, Hance T, Mailleux AC (2013) A review of the major biological approaches to control the worldwide pest *Tetranychus urticae* (Acari: Tetranychidae with special reference to natural pesticides. Journal of Pest Science 86:361–386
3. Aylward J, Dreyer LL, Steenkamp ET, Wingfield MJ, Roets F (2014) Panmixia defines the genetic diversity of a unique arthropod-dispersed fungus specific to *Protea* flowers. Ecology and Evolution 4:3444–3455
4. Aylward J, Dreyer LL, Steenkamp ET, Wingfield MJ, Roets F (2015) Long-distance dispersal and recolonization of a fire-destroyed niche by a mite-associated fungus. Fungal biology 119:245–256
5. Barker NP, Weston PH, Rutschmann F, Sauquet H (2007) Molecular dating of the 'Gondwanan' plant family Proteaceae is only partially congruent with the timing of the break-up of Gondwana. Journal of Biogeography 34:2012–2027
6. Bartsch I (1994) Halacarid mites (Acari) from hydrothermal deep-sea sites: new records. Cahiers de Biologie marine 35:479–490
7. Behan-Pelletier VM, Walter DE (2000) Biodiversity of oribatid mites (Acari: Oribatida) in tree canopies and litter. In: Coleman DC, Hendrix PF (eds) Invertebrates as webmasters in ecosystems, CABI Publishing, Wallingford, UK. pp.187–202
8. Biccard A, Midgley JJ (2009) Rodent pollination in *Protea nana*. South African Journal of Botany 75:720–725
9. Bochkov AV, Galloway TD, Mertins JW, Mironov SV, Fain A (1999) Phylogeny and host-parasite relationships of the mite family Harpirhynchidae (Acari, Prostigmata). *Acarina*. Русский акарологический журнал 7:69–87

10. Brits GJ, Jacobs G, Vogts MM (1983) Domestication of fynbos Proteaceae as a floricultural crop. Bothalia 14:641–646
11. Broekhuysen GJ (1963) The breeding biology of the orange-breasted sunbird *Anthobaphes vzolacea* (Linnaeus). Ostrich 34:187–234
12. Burridge MJ, Simmons LA (2003) Exotic ticks introduced into the United States on imported reptiles from 1962 to 2001 and their potential roles in international dissemination of diseases. Veterinary Parasitology 113:289–320
13. Calf KM, Downs CT, Cherry MI (2003) Foraging and territorial behaviour of male Cape and Gurney's sugarbirds (*Promerops cafer* and *P. gurneyi*). African Zoology 38:297–304
14. Carignan V, Villard MA (2002) Selecting indicator species to monitor ecological integrity: a review. Environmental monitoring and assessment 78:45–61
15. Carthew SM (1993) An assessment of pollinator visitation to *Banksia spinulosa*. Austral Ecology 18:257–268
16. Childers CC, French JV, Rodrigues JCV (2003) *Brevipalpus californicus*, *B. obovatus*, *B. phoenicis*, and *B. lewisi* (Acari: Tenuipalpidae): a review of their biology, feeding injury and economic importance. Experimental and Applied Acarology 30:5–28
17. Clergue B, Amiaud B, Pervanchon F, Lasserre-Joulin F, Plantureux S (2005) Biodiversity: function and assessment in agricultural areas: A review. Agronomy for sustainable development 25:1–15
18. Coetzee JH (1989) Arthropod communities of Proteaceae with special emphasis on plant-insect interactions. Doctoral dissertation, Stellenbosch University, Stellenbosch
19. Coetzee JH, Giliomee JH (1985) Insects in association with the inflorescence of *Protea repens* (L.) (Proteaceae) and their role in pollination. Journal of the Entomological Society of southern Africa 48:303–314
20. Coetzee JH, Giliomee JH (1987a) Borers and other inhabitants of the inflorescences and infructescences of Protea repens in the Western Cape. Phytophylactica, 19:1–6

21. Coetzee JH, Giliomee JH (1987b) Seed predation and survival in the infructescences of *Protea repens* (Proteaceae). South African Journal of Botany 53:61–64
22. Coetzee JH, Littlejohn GM, Janick, J (2007) *Protea*: a floricultural crop from the Cape Floristic Kingdom. Scripta Horticulturae 5:77–112
23. Collier KF, de Lima JO, Albuquerque GS (2004) Predacious mites in papaya (*Carica papaya* L.) orchards: in search of a biological control agent of phytophagous mite pests. Neotropical Entomology 33:799–803
24. Collins BG (1983) A first approximation of the energetics of cape sugarbirds (*Promerops cafer*) and orange-breasted sunbirds (*Nectarinia violacea*). South African Journal of Zoology 18:363–369
25. Collins BG, Rebelo T (1987) Pollination biology of the Proteaceae in Australia and southern Africa. Australian Journal of Ecology 12:387–421
26. Colwell RK (1973) Competition and coexistence in a simple tropical community. The American Naturalist 107:737–760
27. Colwell RK (1995) Effects of nectar consumption by the hummingbird flower mite *Proctolaelaps kirmsei* on nectar availability in *Hamelia patens*. Biotropica 27:206–217.
28. Colwell RK, Naeem S (1994) Life-history patterns of hummingbird flower mites in relation to host phenology and morphology. In: Houck MA (ed) Mites: Ecological and evolutionary analyses of life pattern. Springer US. pp.23–44
29. Comstedt P, Bergström S, Olsen B, Garpmo U, Marjavaara L, Mejlon H, Barbour AG, Bunikis J (2006) Migratory passerine birds as reservoirs of Lyme borreliosis in Europe. Emerging infectious diseases, 12:1087
30. Conradie B, Knoesen H (2010) A survey of the cultivation and wild harvesting of fynbos flowers in South Africa (No. 1). Report. pp1–19

31. Cowling, R. M., Holmes, P. M. & Rebelo, A. G. (1992) Plant diversity and endemism. In: Cowling RM (ed) The ecology of fynbos: nutrients, fire and diversity. Oxford University Press, Cape Town. pp.62–112
32. Cowling RM, Pressey RL, Rouget M, Lombard AT (2003) A conservation plan for a global biodiversity hotspot - the Cape Floristic Region, South Africa. Biological Conservation 112:191–216
33. Cronberg N (2012) Animal-mediated fertilization in bryophytes–parallel or precursor to insect pollination in angiosperms? Lindbergia 35:76–85
34. Cronberg N, Natcheva R, Hedlund K (2006) Microarthropods mediate sperm transfer in mosses. Science 313:1255–1255
35. Crous PW, Denman S, Taylor JE, Swart LZ, Palm ME (2004) Cultivation and diseases of Proteaceae: *Leucadendron*, *Leucospermum* and *Protea*. Centraal bureau voor Schimmelcultures CBS-KNAW Biodiversity Centre: Utrecht, The Netherlands
36. Crous PW, Summerell BA, Swart L, Denman S, Taylor JE, Bezuidenhout CM, Palm ME, Marincowitz S, Groenewald JZ (2011) Fungal pathogens of Proteaceae. Persoonia: Molecular Phylogeny and Evolution of Fungi 27:20–45
37. Currier RW, Walton SF, Currie BJ (2011) Scabies in animals and humans: history, evolutionary perspectives, and modern clinical management. Annals of the New York Academy of Sciences, 1230:50–60
38. Da Silva JAT (2003) The cut flower: postharvest considerations. Journal of Biological Sciences 3:406–442
39. De Jong DD, Morse RA, Eickwort GC (1982) Mite pests of honey bees. Annual Review of Entomology 27:229–252
40. De la Fuente J, Estrada-Pena A, Venzal JM, Kocan KM, Sonenshine DE (2008) Overview: Ticks as vectors of pathogens that cause disease in humans and animals. Frontiers in Bioscience13:6938–6946

41. De Moraes GJ, Zacarias MS (2002) Use of predatory mites for the control of eriophyid mites. In: Fernando LCP, De Moraes GJ, Wickramananda IR (eds) Proceedings of the international workshop on coconut mite. Coconut Research Institute, Lunuwila, Sri Lanka. pp.78–88
42. Dicke M, Sabelis MW (1987) How plants obtain predatory mites as bodyguards. Netherlands journal of zoology 38:148–165
43. Dobkin DS (1984) Flowering patterns of long-lived *Heliconia* inflorescences: implications for visiting and resident nectarivores. Oecologia 64:245–254
44. Dobkin DS (1985) Heterogeneity of tropical floral microclimates and the response of hummingbird flower mites. Ecology 66:536–543
45. Dobkin DS (1990) Distribution patterns of hummingbird flower mites (Gamasida: Ascidae) in relation to floral availability on *Heliconia* inflorescences. Behavioral Ecology 1:131–139
46. Duelli P, Obrist MK (2003) Biodiversity indicators: the choice of values and measures. Agriculture, ecosystems & environment 98:87–98
47. Eickwort GC (1990) Associations of mites with social insects. Annual review of entomology 35:469–488
48. Fleming P, Nicolson S (2002) How important is the relationship between Protea humiflora (Proteaceae) and its non-flying mammal pollinators?. Oecologia 132:361–368
49. Fleming PA, Nicolson SW (2003) Arthropod fauna of mammal-pollinated *Protea humiflora*: ants as an attractant for insectivore pollinators?. African Entomology 11:9–14
50. Gerber AI, Hoffman EW (2014) International Protea Association and current global Proteaceae production: achievements and challenges. In: XI International Protea Research Symposium 1031:17–28
51. Gerlach J, Samways M, Pryke J (2013) Terrestrial invertebrates as bioindicators: an overview of available taxonomic groups. Journal of insect Conservation 17:831–850
52. Gess FW (1968) Insects found on *Proteas*. Veld & Flora 54:29–33

53. George JBD, Otobo S, Ogunleye J, Adediminiyi B (1992) Louse and mite infestation in domestic animals in northern Nigeria. Tropical animal health and production, 24:121–124
54. Gideon N, Mostert DP, Siegfried WR (1980) Protea nectar and satellite fauna in relation to the food requirements and pollinating role of the Cape Sugarbird. South African Journal of Science 76:409–412
55. Goldblatt P (1997) Floristic diversity in the Cape flora of South Africa. Biodiversity and Conservation 6:359–377
56. Goldblatt P, Manning JC (2002) Plant diversity of the Cape region of southern Africa. Annals of the Missouri Botanical Garden 89:281–302
57. Gulvik M (2007) Mites (Acari) as indicators of soil biodiversity and land use monitoring: a review. Polish Journal of Ecology 55:415–440
58. Hågvar S, Solhøy T, Mong CE (2009) Primary succession of soil mites (Acari) in a Norwegian glacier foreland, with emphasis on oribatid species. Arctic, Antarctic, and Alpine Research 41:219–227
59. Halliday RB, O'Connor BM, Baker AS (2000) Global Diversity of Mites. In: Raven PH, Williams T (eds) Nature and human society: the quest for a sustainable world: proceedings of the 1997 Forum on Biodiversity. National Academies Press, Washington, DC, USA. pp.192–212
60. Hallman GJ (1998) Phytosanitary measures to prevent the introduction of invasive species. In: Nentwig W (ed) Biological Invasions. Ecological Studies (Analysis and Synthesis), vol 193. Springer, Berlin, Heidelberg. pp.367–384
61. Hallman GJ, Zhang D, Arthur V (2016) Generic phytosanitary irradiation dose for phytophagous mites (Sarcoptiformes: Acaridae; Trombidiformes: Eriophyidae, Tarsonemidae, Tenuipalpidae, Tetranychidae). The Florida Entomologist 99:202–205
62. Hansen JD, Hara AH (1994) A review of postharvest disinfestation of cut flowers and foliage with special reference to tropicals. Postharvest Biology and Technology 4:193–212

63. Hargreaves AL, Johnson SD, Nol E (2004) Do floral syndromes predict specialization in plant pollination systems? An experimental test in an “ornithophilous” African *Protea*. Oecologia 140:295–301

64. Hargreaves AL, Harder LD, Johnson SD (2009) Consumptive emasculation: the ecological and evolutionary consequences of pollen theft. Biological Reviews 84:259–276

65. Hassan RM (2003) Measuring asset values and flow benefits of non-traded products and ecosystems services of forest and woodland resources in South Africa. Environment, Development and Sustainability 5:403–418

66. Hessein N, Parrella M (1990) Predatory mites help control thrips on floriculture crops. California Agriculture 44:19–21

67. Heyneman A, Colwell RK, Naeem S, Dobkin DS, Hallet B (1991) Host Plant Discrimination: Experiments with Hummingbird Flower Mites. In: Price PW, Lewinsohn TG, Fernandes W, Benson WW (eds) Plant-Animal Interactions: Evolutionary ecology in tropical and temperate regions. John Wiley & Sons, Inc, New York. pp.455–485

68. Hislop RG, Jeppson LR (1976) Morphology of the mouthparts of several species of phytophagous mites. Annals of the Entomological Society of America 69:1125–1135

69. Hofstetter RW, Moser JC, Blomquist S (2014) Mites associated with bark beetles and their hyperphoretic ophiostomatoid fungi. In: Seifert KA, De Beer ZW, Wingfield MJ (eds) The Ophiostomatoid Fungi: Expanding Frontiers, CBS Biodiversity Series 12, CBS-KNAW Biodiversity Centre, Utrecht, The Netherlands. pp.65–176

70. Holmes PM, Richardson DM (1999) Protocols for restoration based on recruitment dynamics, community structure, and ecosystem function: perspectives from South African fynbos. Restoration Ecology 7:215–230

71. Houck MA, OConnor BM (1991) Ecological and evolutionary significance of phoresy in the Astigmata. Annual review of entomology 36:611–636

72. Huguier P, Manier N, Owojori OJ, Bauda P, Pandard P, Römbke J (2015) The use of soil mites in ecotoxicology: a review. Ecotoxicology 24:1–18
73. Hunter PE, Rosario RMT (1988) Associations of Mesostigmata with other arthropods. Annual review of entomology 33:393–417
74. Irwin RE, Brody AK, Waser NM (2001) The impact of floral larceny on individuals, populations, and communities. Oecologia 129:161–168
75. Ito F (2013) Evaluation of the benefits of a myrmecophilous oribatid mite, *Aribates javensis*, to a myrmicine ant, *Myrmecina sp.* Experimental and applied acarology 61:79–85
76. Ito F, Takaku G (1994) Obligate myrmecophily in an oribatid mite. Naturwissenschaften, 81:180–182
77. Jacinto-Maldonado M, Paredes-León R, Salgado-Maldonado G, García A, Suzán G (2016) New records of amphibians parasitized by chiggers in Los Tuxtlas Biosphere Reserve, Mexico, and taxonomic notes on *Hannemania mexicana* (Acariformes: Prostigmata: Leeuwenhoekiidae). Systematic and Applied Acarology 21:13–20
78. Jensenius M, Fournier PE, Kelly P, Myrvang B, Raoult D (2003) African tick bite fever. The Lancet infectious diseases 3:557–564
79. Johnson SA, Nicolson SW (2001) Pollen digestion by flower-feeding Scarabaeidae: protea beetles (Cetoniini) and monkey beetles (Hopliini). Journal of Insect Physiology 47:725–733
80. Jongejan F, Uilenberg G (2004) The global importance of ticks. Parasitology 129:3–14.
81. Khan ZR, Ampong-Nyarko K, Chiliswa P, Hassanali A, Kimani S, Lwande W, Overholt WA, Picketta JA, Smart LE, Woodcock CM (1997) Intercropping increases parasitism of pests. Nature 388:631–632
82. Klepzig KD, Moser JC, Lombardero FJ, Ayres MP (2001a) Symbiosis and competition: complex interactions among beetles, fungi and mites. Symbiosis 30:83–96
83. Klepzig KD, Moser JC, Lombardero MJ, Ayres MP, Hofstetter RW, Walkinshaw CJ (2001b) Mutualism and Antagonism: Ecological Interactions Among Bark Beetles, Mites and Fungi.

In: Jeger MJ, Spence NJ (eds) Biotic Interactions in Plant-Pathogen Associations, CABI Publishing, New York. pp.237–267

84. Knox-Davies PS, Van Wyk PS, Marasas WFO (1986) Diseases of proteas and their control in the South-Western Cape. In: I International Protea Research Symposium 185:189–200
85. Krantz GW (1998) Review Reflections on the biology, morphology and ecology of the Macrochelidae. Experimental & applied acarology 22:125–137
86. Krantz GW, Lindquist EE (1979) Evolution of phytophagous mites (Acari). Annual Review of Entomology 24:121–158
87. Krantz G, Walter D (eds) (2009) A manual of Acarology. 3rd edn. Texas Tech University Press, Lubbock, Texas, USA
88. Lacey N, Kavanagh K, Tseng SC (2009) Under the lash: Demodex mites in human diseases. The biochemist 31:2–6
89. Lara C, Ornelas JF (2001) Nectar 'theft'by hummingbird flower mites and its consequences for seed set in *Moussonia deppeana*. Functional Ecology 15:78–84
90. Lara C, Ornelas JF (2002a) Effects of nectar theft by flower mites on hummingbird behaviour and the reproductive success of their host plant, *Moussonia deppeana* (Gesneriaceae). Oikos 96:470–480
91. Lara C, Ornelas JF (2002b) Flower mites and nectar production in six hummingbird-pollinated plants with contrasting flower longevities. Canadian Journal of Botany 80:1216–1229
92. Lee S, Roets F, Crous PW (2005) Biodiversity of saprobic microfungi associated with the infructescences of *Protea* species in South Africa. Fungal Diversity 19:69–78
93. Le Maitre D, Gelderblom C, Maphasa L, Yssel S, Van den Belt M, Manuel T (1997) Communicating the value of fynbos: results of a survey of stakeholders. Ecological Economics 22:105–121.

94. Liebman M, Dyck E (1993) Crop rotation and intercropping strategies for weed management. Ecological applications 3:92–122

95. Levieux J, Lieutier F, Moser JC, Perry TJ (1989) Transportation of phytopathogenic fungi by the bark beetle *Ips sexdentatus* Boerner and associated mites. Journal of applied Entomology 108:1–11

96. Lombardero MJ, Ayres MP, Hofstetter RW, Moser JC, Lepzig KD (2003) Strong indirect interactions of Tarsonemus mites (Acarina: Tarsonemidae) and *Dendroctonus frontalis* (Coleoptera: Scolytidae). Oikos 102:243–252

97. Lombardero MJ, Klepzig KD, Moser JC, Ayres MP (2000) Biology, demography and community interactions of Tarsonemus (Acarina: Tarsonemidae) mites phoretic on *Dendroctonus frontalis* (Coleoptera: Scolytidae). Agricultural and Forest Entomology 2:193–202

98. Maloof JE, Inouye DW (2000) Are nectar robbers cheaters or mutualists? Ecology 81:2651–2661

99. Marais GJ, Wingfield MJ (1994) Fungi associated with infructescences of *Protea* species in South Africa, including a new species of *Ophiostoma*. Mycological Research 98:369–374

100. Marais GJ, Wingfield MJ (2001) *Ophiostoma africanum* sp. nov., and a key to ophiostomatoid species from *Protea* infructescences. Mycological Research 105:240–246

101. Martin SJ, Highfield AC, Brettell L, Villalobos EM, Budge GE, Powell M, Nikaido S, Schroeder DC (2012) Global honey bee viral landscape altered by a parasitic mite. Science 336:1304–1306

102. Matthee M, Naudé W, Viviers W (2006) Challenges for the floriculture industry in a developing country: a South African perspective. Development Southern Africa 23:511–528

103. McLaughlin A, Mineau P (1995) The impact of agricultural practices on biodiversity. Agriculture, Ecosystems & Environment 55:201–212

104. McMurtry JA, Croft BA (1997) Life-styles of phytoseiid mites and their roles in biological control. Annual review of entomology 42:291–321

105. Melo JWS, Lima DB, Sabelis MW, Pallini A, Gondim MGC (2014) Behaviour of coconut mites preceding take-off to passive aerial dispersal. Experimental and Applied Acarology 64:429–443

106. Mendoza-Roldan JA, Barros-Battesti DM, Bassini-Silva R, Jacinavicius FC, Nieri-Bastos FA, Franco FL, Marcili A (2017) A New Species of Pit Mite (Trombidiformes: Harpirhynchidae) from the South American Rattlesnake (Viperidae): Morphological and Molecular Analysis. Entomology, Ornithology and Herpetology 6: DOI: 10.4172/2161-0983.1000201

107. Mitchell MJ, Parkinson D (1976) Fungal feeding or Oribatid mites (Acari: Cryptostigmata) in an aspen woodland soil. Ecology 57:302–312

108. Moore JC, Walter DE, Hunt HW (1988) Arthropod regulation of micro-and mesobiota in below-ground detrital food webs. Annual review of entomology 33:419–435

109. Mucina L, Rutherford MC (2006) The vegetation of South Africa, Lesotho and Swaziland. Strelitzia 19, South African National Biodiversity Institute, Pretoria

110. Mustart PJ, Cowling RM, Wright MG (1995) Clustering of fertile seeds in infructescences of serotinous Protea species: an anti-predation mechanism?. African Journal of Ecology 33:224–229

111. Myburgh LC, Rust DJ, Starke LC (1973) Pests of protea cut-flowers. Journal of the Entomological Society of Southern Africa 36:251–255

112. Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GA, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403:853–858

113. Ngubane NP (2017) Population genetics of the *Sporothrix splendens* complex from *Protea* L. in South Africa. Masters dissertation, Stellenbosch University, Stellenbosch

114. Niogret J, Lumaret JP, Bertrand M (2006) Semiochemicals mediating host-finding behaviour in the phoretic association between *Macrocheles saceri* (Acari: Mesostigmata) and *Scarabaeus* species (Coleoptera: Scarabaeidae). Chemoecology, 16:129–134

115. Norton AP, English-Loeb G, Belden E (2001) Host plant manipulation of natural enemies: leaf domatia protect beneficial mites from insect predators. Oecologia 126:535–542

116. Norton AP, English-Loeb G, Gadoury D, Seem RC (2000) Mycophagous mites and foliar pathogens: leaf domatia mediate tritrophic interactions in grapes. Ecology 81:490–499

117. O'Dowd DJ, Willson, M.F (1989) Leaf domatia and mites on Australasian plants: ecological and evolutionary implications. Biological Journal of the Linnean Society 37:191–236

118. O'Dowd DJ, Willson MF (1991) Associations between mites and leaf domatia. Trends in Ecology & Evolution 6:179–182

119. Oliver I, Beattie AJ (1993) A possible method for the rapid assessment of biodiversity. Conservation biology 7:562–568

120. Paciorek CJ, Moyer BR, Levin RA, Halpern SL (1995) Pollen consumption by the hummingbird flower mite *Proctolaelaps kirmsei* and possible fitness effects on *Hamelia patens*. Biotropica 27:258–262

121. Paine TD, Raffa KF, Harrington TC (1997) Interactions among scolytid bark beetles, their associated fungi, and live host conifers. Annual review of entomology 42:179–206

122. Power AG (2010) Ecosystem services and agriculture: tradeoffs and synergies. Philosophical Transactions of the Royal Society of London B: Biological Sciences 365:2959–2971

123. Proctor H, Owens I (2000) Mites and birds: diversity, parasitism and co-evolution. Trends in ecology & evolution 15:358–364

124. Pugh PJA (1997) Acarine colonisation of Antarctica and the islands of the Southern Ocean: the role of zoohoria. Polar Record 33:113–122

125. Quinzio S, Goldberg J (2015) Intradermal infections by chigger mites (Hannemania spp.) in the Andean frog *Telmatobius atacamensis* (Anura, Telmatobiidae). Salamandra 51:1–7

126. Radford CD (1950) The mites (Acarina) parasitic on mammals, birds and reptiles. Parasitology 40:366–394

127. Rather PA, Hassan I (2014) Human demodex mite: the versatile mite of dermatological importance. Indian journal of dermatology 59:60–66

128. Ramsey M, Vaughton G (1991) Self-incompatibility, protandry, pollen production and pollen longevity in *Banksia menziesii*. Australian Journal of Botany 39:497–504

129. Rebelo AG (1987) Bird pollination in the Cape Flora. A preliminary synthesis of pollination biology in the Cape flora. South African National Scientific Programmes Report 141:83–108

130. Rebelo AG, Breytenbach GJ (1987) Mammal pollination in the Cape flora. A preliminary synthesis of pollination biology in the Cape Flora. In: Rebelo (ed) South African national scientific programmes report no. CSIR. 141:109–125

131. Rebelo AG, Rourke JP (1986) Seed germination and seed set in southern African Proteaceae: ecological determinants and horticultural problems. Acta Horticulturae 185:75–88

132. Rebelo AG, Siegfried WR, Crowe AA (1984) Avian pollinators and the pollination syndromes of selected mountain fynbos plants. South African Journal of Botany 3:285–296

133. Rebelo T (2001) Proteas: A field guide to the Proteas of Southern Africa. Fernwood Press, Vlaeberg, South Africa

134. Rehbein S, Winter R, Visser M, Maciel AE, Marley SE (2005) Chorioptic mange in dairy cattle: treatment with eprinomectin pour-on. Parasitology research 98:21–25

135. Reinten EY, Coetzee JH (2002) Commercialization of South African indigenous crops: aspects of research and cultivation of products. In: Janick J, Whipkey A (eds) Trends in new crops and new uses, ASHS Press, Alexandria, VA.

136. Reinten EY, Coetzee JH, Van Wyk BE (2011) The potential of South African indigenous plants for the international cut flower trade. South African Journal of Botany 77:934–946

137. Roets F, Crous PW, Wingfield MJ, Dreyer LL (2009) Mite-mediated hyperphoretic dispersal of Ophiostoma spp. from the infructescences of South African Protea *spp*. Environmental entomology 38:143–152

138. Roets F, Dreyer LL, Crous PW (2005) Seasonal trends in colonisation of Protea infructescences by *Gondwanamyces* and *Ophiostoma* spp. South African Journal of Botany 71:307–311

139. Roets F, Dreyer LL, Geertsema H, Crous PW (2006) Arthropod communities in Proteaceae infructescences: seasonal variation and the influence of infructescence phenology. African Entomology 14:257–265

140. Roets F, Wingfield MJ, Crous PW, Dreyer LL (2007) Discovery of fungus-mite mutualism in a unique niche. Environmental Entomology 36:1226–1237

141. Roets F, Wingfield MJ, Crous PW, Dreyer LL (2013) Taxonomy and ecology of ophiostomatoid fungi associated with *Protea* infructescences. Ophiostomatoid fungi: expanding frontiers. Utrecht: CBS Biodiversity Series. pp.177–187

142. Roets F, Wingfield MJ, Wingfield BD, Dreyer LL (2011) Mites are the most common vectors of the fungus *Gondwanamyces proteae* in *Protea* infructescences. Fungal biology 115:343–350

143. Rourke JP (1998) A review of the systematics and phylogeny of the African Proteaceae. Australian Systematic Botany 11:267–285

144. Rourke J, Wiens D (1977) Convergent floral evolution in South African and Australian Proteaceae and its possible bearing on pollination by nonflying mammals. Annals of the Missouri Botanical Garden 64:1-17

145. Ryke PJA (1954) Two New Predatory Mites (Acarina; Phytoseiinae) from Proteas in the Western Province. Journal of the Entomological Society of Southern Africa 17:241–245

146. Ryke PAJ (1964) Acarina associated with *Protea* flowers in the Cape Province. Journal of the Entomological Society of Southern Africa 26:337–354

147. Sammataro D, Gerson U, Needham G (2000) Parasitic mites of honey bees: life history, implications, and impact. Annual review of entomology 45:519–548

148. Santos PF, Phillips J, Whitford WG (1981) The role of mites and nematodes in early stages of buried litter decomposition in a desert. Ecology 62:664–669

149. Sasa A, Samways MJ (2015) Arthropod assemblages associated with wild and cultivated indigenous proteas in the Grabouw area, Cape Floristic Region. African Entomology 23:19–36

150. Schatz H (1998) Oribatid mites of the Galapagos Islands: Faunistics, ecology and speciation. Series Entomologica 55:499–530

151. Schroder RF (1979) Host Specificity Tests of *Coccipolipus epilachnae*, a Mite Parasitic on the Mexican Bean Beetle. Environmental Entomology 8:46–47

152. Schippmann U, Leaman DJ, Cunningham AB (2002) Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues. In: Biodiversity and the ecosystem approach in agriculture, forestry and fisheries. Satellite event on the occasion of the Ninth Regular Session of the Commission on Genetic Resources for Food and Agriculture. Rome. pp.1-21

153. Schwan TG, Piesman J (2002) Vector interactions and molecular adaptations of Lyme disease and relapsing fever spirochetes associated with transmission by ticks. Emerging infectious diseases 8:115–121

154. Siepel H, de Ruiter-Dijkman EM (1993) Feeding guilds of oribatid mites based on their carbohydrase activities. Soil Biology and Biochemistry 25:1491–1497

155. Simmons TW, Hutchinson ML (2016) A Critical Review of All Known Published Records for Water Mite (Acari: Hydrachnidiae) and Mosquito (Diptera: Culicidae) Parasitic Associations From 1975 to Present. Journal of medical entomology 53:737–752

156. Sivajothi S, Reddy, BS, Rayulu VC, Sreedevi C (2015) *Notoedres cati* in cats and its management. Journal of Parasitic Diseases 39:303–305

157. Six DL, Wingfield MJ (2011) The role of phytopathogenicity in bark beetle–fungus symbioses: a challenge to the classic paradigm. Annual review of entomology 56:255–27

158. Skead CJ (1967) The sunbirds of southern Africa, also the sugarbirds, the white-eyes and the spotted creeper. Cape and Transvaal Printer, Cape Town, South Africa

159. Skubala P, Gulvik M (2005) Pioneer oribatid mite communities (Acari, Oribatida) in newly exposed natural (glacier foreland) and anthropogenic (post-industrial dump) habitats. Polish Journal of Ecology 53:395–407

160. Smith HA, McSorley R (2000) Intercropping and pest management: a review of major concepts. American Entomologist 46:154–161

161. Smith IM, Oliver DR (1976) The parasitic associations of larval water mites with imaginal aquatic insects, especially Chironomidae. The Canadian Entomologist 108:1427–1442

162. Smith IM, Oliver DR (1986) Review of parasitic associations of larval water mites (Acari: Parasitengona: Hydrachnida) with insect hosts. The Canadian Entomologist 118:407–472

163. Steenhuisen SL, Balmer A, Zoeller K, Kuhn N, Midgley J, Hansen D, Johnson SD (2015) Carnivorous mammals feed on nectar of *Protea* species (Proteaceae) in South Africa and likely contribute to their pollination. African Journal of Ecology 53:602–605

164. Steenhuisen SL, Johnson SD (2012a) Evidence for autonomous selfing in grassland *Protea* species (Proteaceae). Botanical Journal of the Linnean Society 169:433–446

165. Steenhuisen SL, Johnson SD (2012b) Evidence for beetle pollination in the African grassland sugarbushes (*Protea*: Proteaceae). Plant systematics and evolution 298:857–869

166. Steenhuisen SL, Van der Bank H, Johnson SD (2012) The relative contributions of insect and bird pollinators to outcrossing in an African Protea (Proteaceae). American journal of botany 99:1104–1111

167. Swart L, Crous PW, Petrini O, Taylor JE (2000) Fungal endophytes of Proteaceae, with particular emphasis on *Botryosphaeria proteae*. Mycoscience 41:123–127

168. Swart L, Denman S, Lamprecht SC, Crous PW (1999) Fusarium wilt: A new disease of cultivated *Protea* in Southern Africa. Australasian plant pathology 28:156–161

169. Swift MJ, Izac AM, Van Noordwijk M (2004) Biodiversity and ecosystem services in agricultural landscapes—are we asking the right questions?. Agriculture, Ecosystems & Environment 104:113–134

170. Swinton SM, Lupi F, Robertson GP, Hamilton SK (2007) Ecosystem services and agriculture: cultivating agricultural ecosystems for diverse benefits. Ecological Economics 64:245–252

171. Tehri K (2014) A review on reproductive strategies in two spotted spider mite, *Tetranychus Urticae* Koch 1836 (Acari: Tetranychidae). Journal of Entomology and Zoology Studies 2:35–39

172. Theron N (2011) Mite communities within Protea infructescences in South Africa. Masters dissertation, Stellenbosch University, Stellenbosch

173. Theron-de Bruin N, Dreyer LL, Ueckermann EA, Wingfield MJ, Roets F (2017) Birds Mediate a Fungus-Mite Mutualism. Microbial Ecology https://doi.org/10.1007/s00248-017-1093-9

174. Theron N, Roets F, Dreyer LL, Esler KJ, Ueckermann EA (2012) A new genus and eight new species of Tydeoidea (Acari: Trombidiformes) from *Protea* species in South Africa. International Journal of Acarology 38:257–273

175. Tilman D (1999) Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. Proceedings of the National Academy of Sciences 96:5995–6000

176. Tilman D, Fargione J, Wolff B, D'Antonio C, Dobson A, Howarth R, Schindler D, Schlesinger WH, Simberloff D, Swackhamer D (2001) Forecasting agriculturally driven global environmental change. Science 292:281–284

177. Tjørve KMC, Geertsema, GH, Underhill LG (2005) Do sugarbirds feed on arthropods inside or outside Protea inflorescences?. Emu-Austral Ornithology 105:293–297

178. Tomich TP, Brodt S, Ferris H, Galt R, Horwath WR, Kebreab E, Leveau JH, Liptzin D, Lubell M, Merel P, Michelmore R (2011) Agroecology: a review from a global-change perspective. Annual Review of Environment and Resources 36:193–222

179. Tscharntke T, Clough Y, Wanger TC, Jackson L, Motzke I, Perfecto I, Vandermeer J, Whitbread A (2012) Global food security, biodiversity conservation and the future of agricultural intensification. Biological conservation 151:53–59

180. Tscharntke T, Klein AM, Kruess A, Steffan-Dewenter I, Thies C (2005) Landscape perspectives on agricultural intensification and biodiversity–ecosystem service management. Ecology letters 8:857–874

181. Turner V (1984) Banksia pollen as a source of protein in the diet of two Australian marsupials *Cercartetus nanus* and *Tarsipes rostratus*. Oikos 43:53–61

182. Turpie JK, Heydenrych BJ, Lamberth SJ (2003) Economic value of terrestrial and marine biodiversity in the Cape Floristic Region: implications for defining effective and socially optimal conservation strategies. Biological conservation 112:233–251

183. Umina PA, Schiffer M, Parker P, Hoffmann AA (2016) Distribution and influence of grazing on wheat curl mites (*Aceria tosichella* Keifer) within a wheat field. Journal of Applied Entomology 140:426–433

184. Uppstrom KA (2010) Mites (Acari) associated with the ants (Formicidae) of Ohio and the harvester ant, *Messor pergandei*, of Arizona. Doctoral dissertation, The Ohio State University, Ohio, US

185. Uppstrom KA, Klompen H (2011) Mites (Acari) associated with the desert seed harvester ant, *Messor pergandei* (Mayr). Psyche: A Journal of Entomology 2011:1–7

186. Van den Broek AH, Huntley JF (2003) Sheep scab: the disease, pathogenesis and control. Journal of comparative pathology 128:79–91

187. Van der Walt ID, Littlejohn GM (1996a) Pollen morphology, male hybrid fertility and pollen tube pathways in Protea. South African Journal of Botany 62:236–246

188. Van der Walt ID, Littlejohn GM (1996b) Stigma receptivity of two *Protea* cultivars in relation to the development of hybridization techniques. South African Journal of Botany 62:258–262

189. Van Houten YM, Rijn PC, Tanigoshi LK, Stratum P, Bruin J (1995) Preselection of predatory mites to improve year-round biological control of western flower thrips in greenhouse crops. Entomologia experimentalis et applicata 74:225–234

190. Van Leeuwen T, Tirry L, Yamamoto A, Nauen R, Dermauw W (2014) The economic importance of acaricides in the control of phytophagous mites and an update on recent acaricide mode of action research. Pesticide biochemistry and physiology 121:12–21

191. Van Staden J (1978) Seed viability in *Protea neriifolia*. I. The effects of time of harvesting on seed viability. Agroplantae 10:65–67

192. Vogts M (1984) Research into the South African Proteaceae: In the beginning. Veld & Flora 70:101

193. Von Broembsen SL, Brits GJ (1985) Control of Phytophthora root rot of proteas in South Africa. In: I International Protea Research Symposium 185:201-208

194. Walter DE (1996) Living on leaves: mites, tomenta, and leaf domatia. Annual review of entomology 41:101–114

195. Walter DE, O'Dowd DJ (1992) Leaves with domatia have more mites. Ecology 73:1514–1518

196. Walter DE, Proctor HC (1999) Mites: ecology, evolution and behaviour. UNSW Press, Sydney

197. Walton SF, Currie BJ (2007) Problems in diagnosing scabies, a global disease in human and animal populations. Clinical microbiology reviews 20:268–279

198. Wang C, Powell JE, O'Connor BM (2002) Mites and nematodes associated with three subterranean termite species (Isoptera: Rhinotermitidae). Florida Entomologist 85:499–506

199. Wanner M, Dunger W (2002) Primary immigration and succession of soil organisms on reclaimed opencast coal mining areas in eastern Germany. European Journal of Soil Biology 38:137–143

200. Wauchope RD (1978) The pesticide content of surface water draining from agricultural fields - a review. Journal of environmental quality 7:459–472

201. Werblow A, Martin P, Dörge DD, Koch LK, Mehlhorn H, Melaun C, Klimpel S (2015) Hyperparasitism of mosquitoes by water mite larvae. Parasitology research 114:2757–2765

202. Wezel A, Casagrande M, Celette F, Vian JF, Ferrer A, Peigné J (2014) Agroecological practices for sustainable agriculture. A review. Agronomy for sustainable development 34:1–20

203. Wickings K, Grandy AS (2011) The oribatid mite *Scheloribates moestus* (Acari: Oribatida) alters litter chemistry and nutrient cycling during decomposition. Soil Biology and Biochemistry 43:351–358

204. Więcek M, Martin P, Lipinski A (2013) Water mites as potential long-term bioindicators in formerly drained and rewetted raised bogs. Ecological indicators 34:332–335

205. Wieczorek A, Wright M (2003) PCR detection of phytoplasma from Witchesbroom decease on *Protea* spp. (Proteaceae) and associated arthropods. In: VI International Protea Research Symposium 602:161–166

206. Wiens D (1978) Rodent pollination in southern African *Protea* spp. Nature 276:71–73

207. Wiens D, Renfree M, Wooller RO (1979) Pollen loads of honey possums (*Tarsipes spenserae*) and nonflying mammal pollination in southwestern Australia. Annals of the Missouri Botanical Garden 66:830–838

208. Wiens D, Rourke JP, Casper BB, Rickart EA, LaPine TR, Peterson CJ, Channing A (1983) Nonflying mammal pollination of southern African proteas: a non-coevolved system. Annals of the Missouri Botanical Garden 276:1–31

209. Winterbottom JM (1962) Breeding season of Long-tailed Sugarbird Promerops cafer (L.). Ostrich 33:77

210. Wright JH (1990a) Guild composition and seasonal distribution of insects on *Protea magnifica* and *P. laurifolia* (Proteaceae). African Zoology 25:245–249

211. Wright MG (1990b) The insect communities, herbivory, seed predation and pollination of *Protea magnifica* and *Protea laurifolia*. Doctoral dissertation, Stellenbosch University, Stellenbosch

212. Wright MG (1994) Seed production by *Protea laurifolia* (Proteaceae) after insect versus bird pollination: a quality difference?. Suid-Afrikaanse Tydskrif vir Wetenskap 90:199–199

213. Wright MG, Giliomee JH (1992) Insect herbivory and putative defence mechanisms of *Protea magnifica* and *P. laurifolia* (Proteaceae). African Journal of Ecology 30:157–168

214. Wright MG, Samways MJ (1999) Plant characteristics determine insect borer assemblages on *Protea* species in the Cape Fynbos, and importance for conservation management. Biodiversity and Conservation 8:1089–1100

215. Wright MG, Samways MJ (2000) Biogeography and species richness of endophagous insects associated with Proteaceae in South Africa. African Journal of Ecology 38:16–22

216. Wright MG, Saunderson MD (1995) Protea plant protection: from the African context to the international arena. In: III International Protea Research Symposium 387:129–140

217. Wooller RD, Richardson KC, Collins BG (1993) The relationship between nectar supply and the rate of capture of a nectar-dependent small marsupial *Tarsipes rostratus*. Journal of Zoology 229:651–658

218. Yaninek JS, Herren HR (1988) Introduction and spread of the cassava green mite, *Mononychellus tanajoa* (Bondar)(Acari: Tetranychidae), an exotic pest in Africa and the

search for appropriate control methods: a review. Bulletin of Entomological Research 78:1–13

219. Zawal A (2004) Parasitizing of dragonflies by water mite larvae of the genus *Arrenurus* in the neighbourhood of Barlinek (NW Poland). Zoologica Poloniae 49:37–45

220. Zawal A (2006) Phoresy and parasitism: water mite larvae of the genus *Arrenurus* (Acari: Hydrachnidia) on Odonata from Lake Binowskie (NW Poland). Biological letters 43:257–276

221. Zawal A, Therry L, Stoks R, Michoński G (2017) New records of host-parasite relationships between Coenagrion scitulum (Rambur, 1842) (Odonata) and water mite larvae (Hydrachnidia) in core and edge host populations. Acta parasitologica 62:38–45

222. Zhang ZQ (2003) Mites of greenhouses: identification, biology and control. CABI Publishing, Cambrdige, UK

223. Zhang W, Ricketts TH, Kremen C, Carney K, Swinton SM (2007) Ecosystem services and dis-services to agriculture. Ecological economics 64:253–260

224. Zhang ZQ, Sanderson JP (1997) Patterns, mechanisms and spatial scale of aggregation in generalist and specialist predatory mites (Acari: Phytoseiidae). Experimental and Applied Acarology 21:393–404

225. Zoeller KC, Steenhuisen SL, Johnson SD, Midgley JJ (2016) New evidence for mammal pollination of *Protea* species (Proteaceae) based on remote-camera analysis. Australian journal of botany 64:1–7

CHAPTER 2

BIRDS MEDIATE A FUNGUS-MITE MUTUALISM

Acknowledgements

We thank the Department of Science and Technology/National Research Foundation Centre of Excellence in Tree Health Biotechnology and the Harry Crossley Foundation for financial support for this study as well as the Western Cape Nature Conservation board for issuing the necessary colleting permits. We extend our gratitude to Dr. Phoebe Barnard (South African National Biodiversity Institute) and Dr. Anina Coetzee (Stellenbosch University and South African National Biodiversity Institute) for leading avian sampling in the field as well as Carina Wessels and Leon De Bruin for fieldwork assistance; Drs. Kenneth Oberlander and Janneke Aylward for assistance with DNA extractions and ITS sequencing; Carina Wessels, Alan Lee and David Packer for the use of their photographs; and ringers Alan Lee (Blue Hill Nature Reserve, Uniondale), Francis Hannay (Helderberg Nature Reserve, Somerset West) and Michael and Valerie Ford (Fernkloof Nature Reserve, Hermanus) for additional mite samples collected from birds.

Abstract

Mutualisms between ophiostomatoid fungi and arthropods have been well documented. These fungi commonly aid arthropod nutrition and, in turn, are transported to new niches by these arthropods. The inflorescences of *Protea* trees provide a niche for a unique assemblage of ophiostomatoid fungi. Here, mites feed on *Sporothrix* fungi and vector the spores to new niches. *Protea*-pollinating beetles transport the spore-carrying mites between *Protea* trees. However, many *Protea* species are primarily pollinated by birds that potentially play a central role in the *Protea*-*Sporothrix*-mite system. To investigate the role of birds in the movement of mites and/or fungal spores, mites were collected from *Protea* inflorescences and cape sugarbirds, screened for *Sporothrix* fungal spores and tested for their ability to feed and reproduce on the fungal associates. Two mite species where abundant in both *Protea* inflorescences and on cape sugarbirds and regularly carried *Sporothrix* fungal spores. One of these mite species readily fed and reproduced on its transported fungal partner. For dispersal, this mite (a *Glycyphagus sp*.) attached to a larger mite species (*Proctolaelaps vandenbergi*) which, in turn, were carried by the birds to new inflorescences. The results of this study provide compelling evidence for a new mite-fungus mutualism, new mite-mite commensalisms, and the first evidence of birds transporting mites with *Sporothrix* fungal spores to colonise new *Protea* trees.

Introduction

Animal-fungal mutualisms are associations between fungi and faunal hosts where both parties benefit from their interaction (*e.g.* attine ants, fungus-growing termites and ambrosia beetles) [1]. Many fungi that are not freely mobile via water and air currents, or that associate with highly disjunct and ephemeral niches rely on their associated faunal hosts for transport to new localities, and in turn, often offers nutritional benefits to their phoretic faunal partners. [2-7]. Disruptions in these mutualisms, such as reduction in abundance (or extinctions) of one of the interacting partners, or changes in resource quality and/or quantity, can cause additional species extinctions (coextinctions) or reduction of ecological fitness of interacting partners [8, 9]. Understanding the role of all interacting partners in multipartite symbioses in the maintenance of biodiversity and ecological function is of major importance for assessing ecological threats for conservation management [10-12].

The ophiostomatoid fungi [13] include well known tree pathogens in genera such as *Ceratocystis*, *Ophiostoma* and *Sporothrix* [14, 15]. The group represents a polyphyletic assemblage of fungi that share morphologically convergent traits, such as the production of sticky spores, for dispersal via arthropods [2-4]. Best-known vectors include bark- and ambrosia beetles (Coleoptera: Curculionidae, Scolytinae and Platypodinae) that often obtain additional nutrition from their mutualistic fungal partners when feeding on inoculated vascular tissues [16-19]. Mites, phoretic on the beetles, commonly also transport ophiostomatoid fungi [17, 18, 20-23] with some having evolved specialized spore-carrying structures known as sporothecae [24]. These associations are often mutualistic because the mites obtain complete nutrition from their fungal partners [25-27].

Members of two ophiostomatoid fungal genera, *Sporothrix* and *Knoxdaviesia*, live in a very unusual niche. Here, they are the dominant saprobic fungi within the inflorescences and infructescences of *Protea* trees in Africa [28]. *Protea*-associated mites such as *Proctolaelaps vandenbergi*, *Tarsonemus sp*.A and a *Trichouropoda sp.* act as primary vectors of fungal species including *S. phasma, S. splendens* and *K. proteae* [29-31]. The association between the *Trichouropoda* mite and the *Sporothrix* fungi from *Protea* trees is mutualistic because the mites can use the fungi as only nutritional source to complete an entire life cycle [29].

Mites disperse the fungi by crawling between infructescences and inflorescences on individual *Protea* trees [30]. For longer distance dispersal, the mites are vectored by *Protea*-associated Cetoniidae beetles (e.g. *Genuchus hottentottus* and *Trichostetha facicularis*) [29, 30]. It was recently demonstrated that *Knoxdaviesia* fungal populations distantly separated from each other are in near genetic panmixia; suggesting a prevalence of long distance dispersal in the *Protea* system [32-35]. However, the ubiquitous distribution of *Sporothrix* and *Knoxdaviesia* fungi within the inflorescences and infructescences of host *Protea* species [29, 36] and the lack of population genetic differentiation of populations separated by more than 200 km, is difficult to explain based purely on dispersal via beetles [34]. This is because the mountainous nature of the region where these *Protea* trees are found would impede free movement of insects over very long distances and these beetles are encountered within structures in low frequencies [37-39]. To explain the observed lack of population differentiation of the fungi, [34] hypothesised that birds could possibly be involved in the long-distance dispersal of these unusual *Protea*-infecting mite-associated fungi.

Insects such as *Genuchus* and *Trichostetha* beetles involved in carrying mites, that in turn vector ophiostomatoid fungi, are important pollinators of many *Protea* species [37]. It is thus interesting that most *Protea* hosts of ophiostomatoid fungi are primarily pollinated by

nectarivorous birds [37, 40-42]. Dominant avian *Protea*-visitors in the biologically diverse Cape Floristic Region of South Africa are the endemic orange-breasted sunbird (*Nectarinia violacea*) and cape sugarbird (*Promerops cafér*) with the latter species being the primary pollinator [43, 44]. These birds are capable of flying vast distances (more than 160 km have been recorded for *Promerops cafér*) in search of suitable habitats [45, 46], where they predominantly feed on *Protea* nectar [47, 48]. Any phoretic organisms present on these birds would consequently spread over these same distances.

While no previous study has considered the role of birds as vectors of *Protea*-associated mites, numerous observations of *P. vandenbergi* mites on especially the cape sugarbird have been made (T. Rebelo pers. com., www.ispotnature.org, www.proteaatlas.org.za). *Proctolaelaps vandenbergi* is known to attain very high numbers (over 60,000 individuals) within the inflorescences of bird-pollinated *Protea* species where they likely feed on pollen and nectar [49, 50]. This mite species has been implicated in the transport of the ophiostomatoid fungus *S. phasma* [30] and it is possible that it utilises the fungus as an additional food source. If this mite (or any other *Protea*-associated mite) can regularly spread *Sporothrix* fungal species via birds, the ubiquitous distribution of *Sporothrix* in *Protea* and the near panmictic population structure of ecologically similar mite-associated fungi from this niche could be explained.

In this study, we consider whether birds play a role in the complex and intriguing fungus-mite symbiotic interactions found in the *Protea* system. We hypothesise that *Protea*-pollinating birds carry *Protea*-associated mites, that in turn, carry spores of the same fungal species (*Sporothrix*) that are present in *Protea* inflorescences. We further hypothesise that mites that vector *Sporothrix* fungal species can utilise these fungi as a food source indicating a possible mutualistic association. Results of this study may shed light on the possible

cascading effects of ecosystem disruptions on multipartite mutualisms on the maintenance of normal ecosystem functioning.

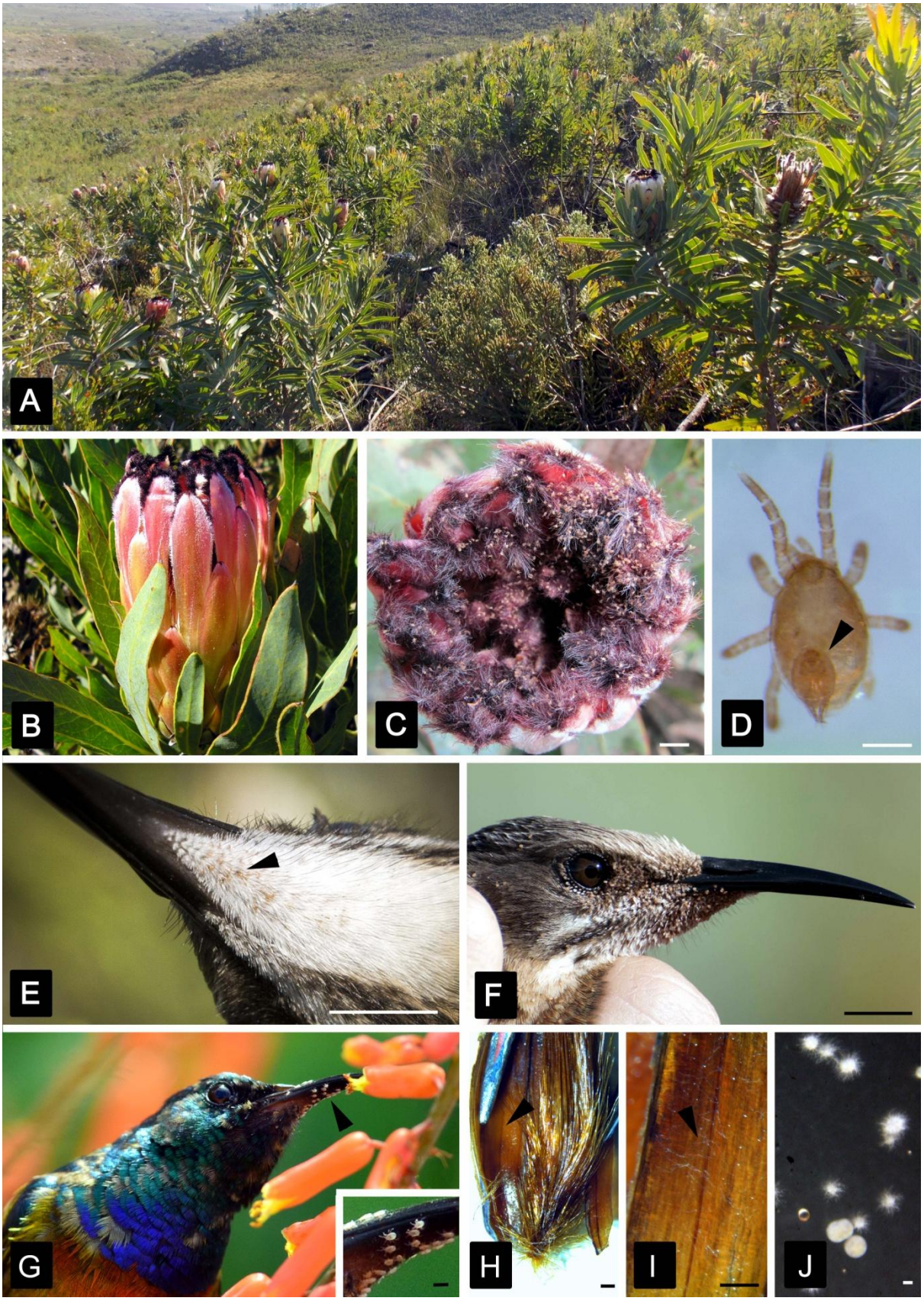

Figure 1: A) *Protea neriifolia* population (foreground) in the Jonkershoek Nature Reserve, Western Cape Province, South Africa. B) *Protea neriifolia* inflorescence. C) Mites accumulating at the top of an inflorescence in anticipation of flower-visitors. D) Hypopus of a *Glycyphagus* mite (arrow) attached to *Proctolaelaps vandenbergi* mite from a *P. neriifolia* inflorescences. E) *Proctolaelaps vandenbergi* mites visible under the beak of a cape sugarbird (photo by Carina Wessels). F) Cape sugarbird covered with *Proctolaelaps vandenbergi* mites (photo by Alan Lee). G) Orange-breasted sunbird with *Proctolaelaps* mites on its beak (Insert to g) Same, with beak area enlarged (photo by David Parker). H) *Protea neriifolia* fruit surrounded by perianth forming a nectar well (arrow). I) Close-up of same perianth in region of nectar well showing fine whitish fungal hyphae (arrow), later identified as *Sporothrix phasma*. J) *Sporothrix phasma* fungal colonies (white, fluffy) and two colonies of an unidentified yeast (lower left) originating from mites allowed to crawl on the surface of petri-dishes after 7 days.

Methods

Mites associated with Protea neriifolia *inflorescences*

Mites associated with the inflorescences of *Protea neriifolia*, one of the most wide-spread bird pollinated *Protea* species in the Western Cape Province (**Fig. 1a, b**) were surveyed. This *Protea* provides the niche for two ophiostomatoid fungi, *K. capensis* and *S. phasma* [31] and three mites (*Trichouropoda sp.*, *Tarsonemus sp.*A and *P. vandenbergi)* that are known vectors of ophiostomatoid fungi [29, 30]. Twenty inflorescences at early to mid flowering stage (where 30 - 50% of the individual flowers within the inflorescences were open and when birds actively visit for nectar) were sampled during October 2014 in Jonkershoek Nature Reserve (33˚59’24.5”S, 18˚57’25.2”E), Stellenbosch, stems submerged in a water filled

bucket to keep them fresh and transported to the laboratory. Inflorescences were placed in separate water-filled glass containers to maintain freshness for extended periods. After two days, mites that accumulated at the tops of flowers in anticipation of arriving flower visitors (**Fig. 1c**) were collected from each inflorescence by patting a 5 cm long by 1 cm wide strip of adhesive tape (Sellotape, Henkel limited, UK) across the top of the inflorescence for 40 seconds. This method did not collect all mites present, but gave some indication of relative abundance of each species per inflorescence. The adhesive strips were mounted on clear transparent cellophane sheets to trap mites between the adhesive tape and the sheet and kept at 4˚C. All mites collected from inflorescences were sorted into morpho-species and identified to the lowest taxonomic rank possible. Phoretic associations between mites were also documented. The numbers of each mite species collected per inflorescence were counted and median abundance compared using a Kruskal-Wallis ANOVA in Statistica 13, Statistica 13 (StafSoft Inc, Tulsa, OK, USA) for the non-parametrically distributed data (as determined by a Shapiro-Wilk test in Statistica). Significant differences are reported at $P \leq 0.05$.

Mites phoretic on cape sugarbirds

Sites for bird captures were selected based primarily on the presence of substantial populations of *P. neriifolia* that were frequented by bird visitors. The main *Protea*-visiting species *Promerops cafér* (cape sugarbird) was selected because they occur in fairly high numbers in *Protea* populations, they have a relatively large body size making handling easier and they are highly active [51]. Mist nests (ECOTONE, 15mm x15mm netting) with a total span of 21m x 2m were set up in three areas of natural CFR vegetation (Franschoek Pass (33˚55’10.2”S, 19˚09’42.0”E), Jonkershoek Nature reserve and Du Toits Kloof Pass (33˚41’45.2”S, 19˚05’14.2”E) in the Western Cape Province, South Africa from April to June 2014. Mist nets were set up early in the morning (08:00 am - 11:00 am) because this is a time

of peak activity for this bird species [52]. Birds were removed from nets as soon as possible after capture. Non-target bird species were very rarely caught and were immediately released. Collected sugarbirds were placed into small cotton bags, weighed and measured in accordance with guidelines of SAFRING (South African Bird Ringing Authority) by ringer no. 1600 (A. Heystek) and thereafter scanned for the presence of mites. Because the beak and breast areas of these birds make most contact with *Protea* flowers when probing inflorescences during feeding [53, 54], these areas were targeted for the removal of mites. Mites were collected from the birds using adhesive tape strips, 10 cm long and 1 cm wide, that were repeatedly dabbed over the target areas of the bird (one strip per bird) and then adhered to a clean transparent sheet as described for mite collection from inflorescences. The sheets were placed within a cooler box and transported to the laboratory where it was stored at 4˚C. Importantly, this method did not capture all mites present on birds even in the targeted areas, because mites are agile and were able to escape between the feathers. In order to minimise stress on the birds, handling time was also kept to a minimum, which further hampered exhaustive mite collection. In addition to our own collections, a few random collections of mites (using the adhesive tape method), received from SAFRING ringers that were active in other areas of the CFR, were also added.

All samples were stored at 4ºC until further analyses could be conducted in the laboratory within 12 hours of collection. All mites collected from birds were sorted into morpho-species under sterile conditions (and using tools that were flame-sterilised between handling of individual mites), all individuals were placed in separate sterile eppindorf tubes and were then identified to the lowest taxonomic rank. The abundance of the different mite species sampled from birds was compared using a Mann-Whitney U test in Statistica for the non-normally distributed data.

Fungal isolation from mites and young inflorescences

Twenty individuals of each mite species encountered within each of five randomly collected *P. neriifolia* inflorescences (at the mid flowering stage) from Du Toits Kloof Pass during June 2014 were used to determine the presence of *Protea*-associated *Sporothrix* fungi. For each inflorescence, mites were collected by shaking the inflorescence over a Petri-dish under sterile conditions, after which 20 mite individuals of each mite species were taken from the Petri dish and placed individually into micro-tubes filled with 100 µl sterile distilled water using a sterile needle and with gloved hands. The needle was sterilised between each individual mite using a flame. Tubes were vortexed (VX-200 Lab Vortexer, Labnet International, Inc., Edison, NJ, USA) for 1 min to loosen and displace fungal spores.

A sub-set of mites collected from birds using the adhesive tape method were also screened for the presence of *Sporothrix* fungi. Seven sugarbirds were caught at Du Toits Kloof Pass during a single day in August 2015 using methods described above. For the collection of the mites from these birds, care was taken to minimise possible contamination with *Sporothrix* fungi from external sources such as soil and plant material adhering to hands. Precautionary measures included reducing collecting time to 30 seconds, wearing sterile gloves and sticking the adhesive tape strips onto sterilised clear plastic sheets (wiped clean using 70% ethanol). In the laboratory, ten mite individuals per species per bird (where possible), were individually removed using fine tweezers (sterilized between handling of each individual mite) and placed in separate micro-tubes filled with 100 µl distilled water that were again vortexed for 1 min.

The content of all tubes containing individual mites from inflorescences and birds were individually plated onto selective medium for *Sporothrix* fungi prepared from Malt Extract Agar (MEA, Merck, Wadeville, South Africa) containing 0.1g/L Cycloheximide and 0.05 g/L

Streptomycin [29]. Plates were monitored daily for two weeks and all fungal colonies that resembled *Sporothrix* fungi were counted. Up to five colonies per plate were selected at random and purified as representatives of the *Sporothrix* species present on mite individuals. The percentage of mites that carried spores of *Sporothrix* fungi and the number of colony forming units of *Sporothrix* fungi isolated per mite individual from birds were compared using a Mann-Whitney U tests in Statistica. The percentage of mites that carried spores of *Sporothrix* fungi and the number of colony forming units of *Sporothrix* fungi isolated per mite individual from each mite species collected from inflorescences were compared using generalized linear mixed models (GLMM) using R software (R Development Core Team 2013) and the *lme4* package [55]. Data on counts of colony forming units was fitted to a Poisson curve and percentage data was fitted to a binomial curve (with Laplace approximations). For analyses of fungi from mites from infructescences, the structure from which the mites were collected were included as random variable. These models followed the formulas: glmer (cbind (number of mites carrying spores, number of mites not carrying spores) ~ mite species + (1|infructescence), family = "binomial") for data on the percentage of mites that carried fungal spores and glmer (number of colony forming units ~ mite species + (1|infructescence), family = "poisson") for counts data. These models were tested against models that only contained the random variable and in both cases models including mite species identity were significantly better as judged by the Akaike Information Criterion using the *anova* function (for percentage data: AIC = 87.3 vs. AIC = 174.998, X^2 (2) = 91. 616; $p < 0.001$; for counts data: AIC = 3511.4 vs. AIC = 5205.6, X^2 (2) = 1698.2; $p < 0.001$). In addition, Tukey *post-hoc* tests in the R package *multcomp* were used to determine the pairwise differences in colony forming units and percentages of mites associated with *Sporohrix* fungi between the different mite species [55].

To determine whether mites could transfer *Sporothrix* fungal spores to uninhabited material, ten living mites per species collected from inflorescences and birds were placed on Petri dishes containing *Sporothrix* selective media. This was replicated 10 times for each mite species. These plates were monitored for the presence of fungal colonies that were subsequently purified.

Sexual fruiting structures (ascomata) of *Sporothrix* fungi are not usually encountered in inflorescences, as these form only after flower fertilization and initiation of infructescence formation [36]. We consequently determined the site of first growth of these fungi in their asexual conidial-producing state in young inflorescences (only *ca.* 50% of individual florets open). Inflorescences were dissected and individual flowers were scanned for hyphal growth using a dissection microscope. We assumed that the area in the inflorescence in which we encountered *Sporothrix* fungi early in its development would represent the site of inoculation. Observed hyphae were collected by lifting individual mycelial strands with a sterile needle and plating these onto selective media as described above. All fungal cultures obtained from all mite individuals and inflorescences were grouped according to morpho-type based on colony growth form, texture and colour. Three to five individuals of each morpho-type were selected for further identification using DNA sequence comparisons.

Fungal identification

Fungal DNA was extracted using a modified CTAB procedure following the methods of [32]. The internally transcribed spacer regions I and II (including 5.8S) of the rDNA of selected strains where amplified using primers ITS1F and ITS4 [56, 57]. Amplification reaction mixtures comprised 1 µl DNA template, 9 µl distilled water, 2.5 µl $MgCl_2$ (2.5 mM), 0.25 µl (10 mM) of each primer and 12 µl KAPA Taq ReadyMix (Kapa Biosystems, Inc. Boston,

USA). Negative controls were included. PCR products were amplified using a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA) programmed for an initial denaturation step for 3 min at 95°C, followed by 40 cycles of 94°C for 30 s, 50°C for 1 min, 72°C for 50 s, and a final elongation step at 72°C for 7 min. Amplified PCR products were purified and sequenced at the Stellenbosch University Central Analytical Facility, Stellenbosch, South Africa. Species identities were established by performing BLAST (Basic Local Alignment Search Tool) searches on the GenBank data base (http://www.ncbi.nlm.nih.gov) using BIOEDIT, Version7.2.5.0 and manually corrected ITS sequence data [58].

Fungi as a food source for mites

To study the interaction between collected mite species and *Sporothrix* fungi, feeding and reproduction of mites that had been confirmed to carry *Sporothrix* fungal spores were tested on the various fungi following the methods described by [29]. Mites were collected in *P. neriifolia* inflorescences from Du Toits Kloof Pass in November 2015 and tested on a diet of *S. phasma* and *S. splendens*. Ten individuals of each mite species were placed on MEA plates (without antibiotic supplementation) that contained three-week-old cultures of either *S. splendens* or *S. phasma*, respectively. Mites on plates containing only MEA served as controls. Mites were prevented from escaping the plates by applying a thick layer of petroleum jelly on the inside of the lid, which formed a seal between the base and lid of the Petri dish, by sealing plates with Para film (Parafilm M®, Bemis Company, Inc.), and by floating plates in large trays containing water with a few drops of added detergent. The experiment was replicated five times with plates kept in the dark at 25˚C for 40 days. Thereafter the numbers of living mites (including adults and immatures) on each plate were counted. Differences in mite numbers between the different treatments per mite species were statistically compared using a t-test [59] in Statistica 13 for the normally distributed data.

Results

Mites associated with Protea neriifolia *inflorescences*

Three mite species, *Proctolaelaps vandenbergi*, *Tarsonemus sp.*A and a heteromorphic deutonymphs (hypopodes) of a *Glycyphagus sp.*, were associated with the top surface of *P. neriifolia* inflorescences at the stage when these structures are pollinated. *Proctolaelaps vandenbergi* and the *Tarsonemus* mites were the same species implicated in the dispersal of ophiostomatoid fungi from *Protea* infructescences by [29, 36]. The *Glycyphagus* mite was previously recorded from the infructescences of various *Protea* species [60]. Mites differed in their abundance on these inflorescences ($H(2) = 38.048$, $P < 0.0001$), with *Proctolaelaps vandenbergi* significantly more abundant than either the *Tarsonemus* or *Glycyphagus* ($Z = 5.993$, $P < 0.0001$ and $Z = 4.246$, $P < 0.0001$, respectively) (**Table 1**). More than a thousand individuals of *P. vandenbergi* mites were commonly collected from a single inflorescence. The other two mite species were collected in very similar numbers ($Z = 1.747$, $P = 0.242$). Interestingly, a phoretic association was commonly observed between the *Proctolaelaps vandenbergi* and the smaller *Tarsonemus* and *Glycyphagus* mites (**Table 1**, **Fig. 1d**). In some cases, both the *Tarsonemus* and the *Glycyphagus* mites were found carried on a single *Proctolaelaps vandenbergi* individual.

Table 1: Number of mites collected from the top of *Protea neriifolia* inflorescences.

Mite species	n[a]	min (25%) median (75%) max	n[b]	% with phoretic mite partner
P. vandenbergi	19808	17(417)706.5(1142.5)3697	50	0.25[#]
Glycyphagus	582	1(4.5)13(25.5)245	42	7.22[*]
Tarsonemus	224	0(1.5)2.5(9.5)99	13	5.8[*]

Notes: na Total number of individuals collected from 20 inflorescences; nb Total number of individuals with a phoretic partner; # Percentage of individuals associated with *Glycyphagus* and/or *Tarsonemus*; * Percentage of individuals associated with *Proctolaelaps vandenbergi.*

Mites phoretic on cape sugarbirds

A total of 54 cape sugarbirds were captured from which 549 *Protea*-associated mites were removed. Only the *Protea*-associated *Proctolaelaps vandenbergi* (431 individuals) and hypopodes of the *Glycyphagus sp.* (55 individuals) were collected on these birds (**Table 2**). Overall, *P. vandenbergi* was significantly more abundant on the birds than the *Glycyphagus sp.* ($U = 636.500$, $Z = 5.044$, $P < 0.001$). All *Glycyphagus* mite individuals collected from birds were phoretic on *P. vandenbergi* mites with no individuals collected separately.

Table 2: Cape sugarbird sampling areas with total number of birds, *Proctolaelaps vandenbergi*[1] and *Glycyphagus*[2] mites collected.

Locality	GPS co-ordinates	Number of birds	Total number of mites collected from birds
Vermont	34˚24'38.5"S 19˚09'19.1"E	11	7[1]
Helderberg	34˚03'55.3"S 18˚52'26.3"E	4	3[1]
Port Elizabeth	33˚35'23.9"S 23˚24'15.9"E	19	155[1], 2[2]
Franschoek	33˚55'10.2"S 19˚09'42.0"E	4	15[1], 4[2]
Jonkershoek	33˚59'24.5"S 18˚57'25.2"E	6	43[1], 13[2]
Du Toits Kloof	33˚41'45.2"S 19˚05'14.2"E	10	208[1], 32[2]

Mites were collected from both the beak and breast areas of the birds with the mites most commonly encountered on the undersides of the beaks (**Fig. 1e**). Photographic evidence

suggested that when infestation levels increase, individual birds can carry more than 1000 mites (**Fig. 1f**), which can cover the entire head and body of a bird. In addition, photographic evidence suggested that the orange-breasted sunbird (*Anthobaphes violacea*) can also vector these mites as demonstrated by a photograph taken at Kirstenbosch National Botanic Garden, Cape Town, South Africa during the main flowering season of the numerous *Protea* spp. in the vicinity (**Fig. 1g**).

Table 3: Results of GLMM models, including summary statistics of effects included in the final models, testing for the effects of mite species on number of individuals that were associated with *Sporothrix* fungi (Model 1) and number of colony forming units of *Sporothrix* fungi isolated per mite individual, for mites collected from the infructescences of *Protea neriifolia*.

	Model 1				Model 2			
	Estimate	Standard error	z-value	P	Estimate	Standard error	z-value	P
Fixed Parts								
Intercept	-1.8925	0.4263	-4.439	< **0.001**	0.90204	0.90204	1.68	0.093
Proctolaelaps vandenbergi	3.2687	0.4315	7.575	< **0.001**	1.33566	0.05616	23.78	< **0.001**
Tarsonemus spp.	0.4373	0.3835	1.140	0.254	-1.43759	0.11406	-12.60	< **0.001**
Random Parts								
N (group)	5				5			
Variance	0.4629				1.423			
Standard Deviation	0.6804				1.193			
Observations	14				300			
Summary								
AIC	87.3				3511.4			
BIC	89.9				3526.2			
loglink	-39.7				-1751.7			
Deviance	79.3				3503.4			
Degrees of freedom for residuals	10				296			

Fungal isolation from mites and young inflorescences

Eighty-three percent of all the *Proctolaelaps vandenbergi* mite individuals collected from inflorescences were associated with fungi that morphologically resembled *Sporothrix* spp. This is significantly more than *Glycyphagus* ($Z = 10.479$, $P < 0.001$) and *Tarsonemus* ($Z = 12.601$, $P < 0.001$) (**Fig. 2**; **Table 3**). Isolations from *Proctolaelaps vandenbergi* mites resulted in significantly greater numbers of colony forming units of *Sporothrix* fungi compared to the *Glycyphagus* ($Z = 23.78$, $P < 0.001$) and *Tarsonemus* ($Z = 26.24$, $P < 0.001$) mites (**Fig. 2**, **Table 3**). *Glycyphagus* mites carried significantly larger numbers of *Sporothrix* spores than *Tarsonemus* mites ($Z = 12.60$, $P < 0.001$; **Fig. 2**). DNA sequence-based identification confirmed that all isolates belonged to the genus *Sporothrix* (**Table 4**). *Sporothrix phasma* was the dominant fungal species present and was collected from all three mite species (**Table 4**). However, *S. splendens*, a species not thought to be associated with this host [61], was also regularly isolated from the collected mites (Table 4). Hyphae of both *S. splendens* and *S. phasma* were commonly observed in the nectar-well formed between the ovaries and the surrounding perianths in open florets *i.e.* florets where the petals no longer covered the pollen presenter (**Fig. 1h, i**). These fungi were never observed in any other area of the individual florets or on florets that were still closed. These same areas often contained the exuviae of *Glycyphagus* mite hypopodes and in many cases also adult *P. vandenbergi* mite individuals as well as the larvae, nymphs and adults of *Glycyphagus* mites. Only a few *Tarsonemus* mites were observed during this period in this part of the floret. The only other arthropods observed on florets during this young stage of the inflorescence development were a few individuals of Thysanoptera, Psocoptera and the bright orange larvae of a small Diptera species.

Table 4: Fungal species isolated from mites that were collected from young *P. neriifolia* inflorescences and cape sugarbirds. The frequency (as percentage) of mites from which the *Sporothrix* fungi could be isolated are also provided.

Fungal species	Vector mite	Frequency of association	Representative Culture and GenBank accession number	Accession of closest match on GenBank	Similarity (Gaps)
S. phasma	*P. vandenbergi*	72%	P8 (MF490797)	DQ316216	100% (0)
	Glycyphagus	66%			
	Tarsonemus	73%			
S. splendens	*P. vandenbergi*	28%	P7 (MF490798)	DQ316205	100% (0)
	Glycyphagus	34%			
	Tarsonemus	28%			

Twenty-one percent of *P. vandenbergi* mite individuals and 20% of *Glycyphagus* mite individuals collected from birds were associated with *Sporothrix* fungi (U= 0, Z = 0, P = 1.000). However, isolations from *P. vandenbergi* mites resulted in greater numbers of colony forming units of *Sporothrix* fungi in total, compared to *Glycyphagus* mites, although this difference was not significant (U = 343.00, Z = 0.132, P = 0.925). Both *S. phasma* and *S. splendens* were isolated from the mites collected from birds.

When mites were placed on *Sporothrix*-selective media and allowed to crawl over the surfaces, all plates contained colonies of *Sporothrix* fungi (**Fig. 1j**). The numbers of colony forming units per plate could not be reliably counted because mites initially transferred many spores and they also transferred spores between developing colonies as they moved around on the plates. All plates were dominated by *S. phasma* with some also containing *S. splendens*.

Sporothrix *as food source for mites*

All *P. vandenbergi* and *Tarsonemus* mites that were allowed to feed on *S. phasma* or *S. splendens* had died after 40 days and they were never observed to feed on these colonies. All three mite species placed on the control plates were also dead after 40 days and these plates

often contained contaminant fungi transferred by the mites. *Glycyphagus* mites placed on colonies of *S. phasma* or *S. splendens* were observed to feed on these fungi and their numbers increased substantially over 40 days. Populations of *Glycyphagus* mites increased from 10 individuals to an average of 372.2 (± 38) individuals on colonies of *S. phasma* over this time period. Colonies on *S. splendens* had significantly larger population sizes of *Glycyphagus* mites than when these mites fed on *S. phasma* after the same time period ($t = -10.5019$, $P < 0.0001$) with an average of 3527.2 (± 298) individuals counted per plate.

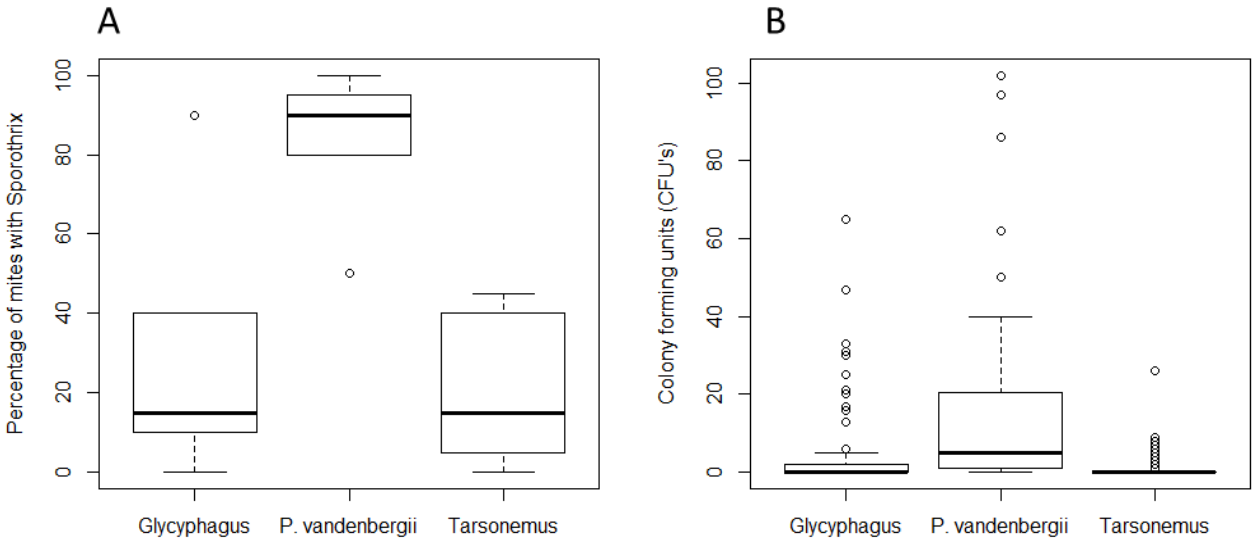

Figure 2: (a) Median percentage of mites (box indicates 25%-75% data range, whiskers indicate 1.5 times the interquartile range, dots represent outliers) collected from *P. neriifolia* inflorescences from which *Sporothrix* fungi could be isolated. (b) Median number of colony forming units (CFU's) of *Sporothrix* fungi originating from mites collected from inflorescences (box indicates 25%-75% data range, whiskers indicate 1.5 times the interquartile range, dots represent outliers).

Discussion

Results of this study show for the first time that various *Protea*-associated mites are phoretic on birds. But more importantly, in terms of complex symbiotic patterns, these mites, vectored by birds were shown to carry fungi that live in a specific association with *Protea* inflorescences that are pollinated by these birds. The mites, in turn, transfer the fungi to the lower parts of the developing inflorescences, where the fungi grow and provide a food source for the mites. While it has previously been shown that mites vector and are engaged in 'agriculture" with *Sporothrix* fungi in *Protea* fruiting structures, this is the first evidence of a mite-fungus-bird symbiosis.

Proctolaelaps vandenbergi and the *Tarsonemus* mites collected from inflorescences and birds are well-known associates of *Protea* trees [30, 61] and transmit *Sporothrix* fungi from fruiting structures via beetles [29, 31]. Here we show for the first time that *Glycyphagus* mites are also involved in these mite-fungi symbioses. Strong evidence is provided that, other than for the aforementioned species that have a commensal relationship with the fungi, *Glycyphagus* mites have a mutualistic association with *Sporothrix* fungi [62]. This is the second mutualism between mites and *Sporothrix* fungi discovered in *Protea*, the other involving *Trichouropoda* mites from fruiting structures dispersed by *Genuchus* beetles [30]. Fungus-mite-insect interactions are well-known for ophiostomatoid fungi associated with conifer-infesting bark beetles [27, 63], but they are less known in other environments such as the one studied here. Sporotrichosis disease caused by *Sporothrix schenckii* [64] can infect numerous distantly related animals such as armadillos, cats, dogs, dolphins, fish, horses, insects, parrots and rodents and be transmitted to humans [65]. *Sporothrix*-mite symbioses could be a common phenomenon and may well be relevant to the control and the spread of socially and economic important species such as the human pathogens *S. schenckii* and *S. brasiliensis* [66].

Glycyphagus mites are not known to be phoretic on *Protea*-associated beetles [29, 30]. Rather than direct transport by birds, the *Glycyphagus* mites were transported secondarily by the larger *P. vandenbergi* mites. Mite-mite hyperphoresy is a rare phenomenon [27, 67, 68] and mostly observed between the Uropodidae and Macrochelidae. In the present study, we document what is to the best of our knowledge, the first case of members of the Glycyphagidae as hyperphoretic on members of the Ascidae. It is also the first record of mite-mite hyperphoresy involving the Chordata and birds in particular. To the best of our knowledge, the only threat these mites, more specifically *Procotlaelaps vandenbergi*, potentially pose to the birds is to directly compete with birds for resources such as nectar [59].

Other than the beetle-mediated mite-fungus mutualism between *Trichouropoda* mites and *Sporothrix* fungi that commences only after the formation of *Protea* fruiting structures [29, 30], the bird-mediated mite-fungus mutualism between *Glycyphagus* mites and *Sporothrix* fungi starts long before the formation of *Protea* fruiting structures and is continuous throughout the *Protea* flowering season. *Sporothrix* occupies nectar wells as soon as the first florets of very young *Protea* inflorescences open. The presence of exuviae of *Glycyphagus* mite hypopodes (specialised inert deutonymph stages) where their sole role is survival during phoresy [6, 69] in nectar wells indicates that these are amongst the earliest visitors to *Protea* florets. When hypopodes reach a new habitat (e.g. after reaching a *Protea* inflorescence) and find a suitable location (e.g. a nectar well) they moult, transfer *Sporothrix* fungal spores and begin to feed. *Proctolaelaps vandenbergi* mites are also expected to visit these sites early in the development of inflorescences, as they likely feed on pollen and nectar [7, 70]. Mites will continuously feed on cultivated *Sporothrix* fungi and/or nectar and pollen, and reproduce rapidly within developing inflorescences until maturity. Thereafter, spore-laden mites congregate in very large numbers at the apices of mature inflorescences in anticipation of arriving vectors in the form of *Protea*-pollinating birds such as cape sugarbirds and sunbirds.

This fungus-mite-bird symbiosis will result in a very rapid colonisation and spread of *Sporothrix* fungi throughout the *Protea* flowering season.

Mites disperse over short distances using branches, dispersing *Sporothrix* fungal spores from infructescences to developing inflorescences on the same plant [30]. However, *P. vandenbergi*, the *Tarsonemus* and the *Trichouropoda* mites utilise *Genuchus* beetles for transport over longer distances from old *Protea* infructescences to young inflorescences [29, 30]. *Proctolaelaps vandenbergi* and the *Tarsonemus* mites also use *Protea*-pollinating *Trichostetha* beetles for dispersal between inflorescences over longer distances [29, 30]. Therefore, *Protea*-associated *Sporothrix* fungi engage in multiple symbiotic interactions to ensure dispersal and dominance within this fire-ephemeral niche during all phenological stages of the trees [63]. For example, the fungi have mutualistic associations with *Glycyphagus* mites during the flowering stage and *Trichouropoda* mites during the non-flowering stage of *Protea* trees, and commensal associations with *P. vandenbergi* and *Tarsonemus sp.*A mites during both stages of plant development. All of these mites are transported over long distances either directly, or indirectly via hyperphoresy on *P. vandenbergi* mites, on *Protea*-associated beetles and/or birds. Unlike *Protea*-associated beetles, cape sugarbirds disperse over hundreds of kilometres in search of flowering *Protea* populations for food [51, 71] and this likely explains the lack of genetic structure between distant populations of ecologically similar fungi from this niche as recently described by [32, 34]. If we consider that these birds can carry hundreds of mites between distant *Protea* populations, and that the vast majority of these mites carry fungal spores, then a single long-distance dispersal event by the bird could lead to the dispersal of thousands of fungal spores. Therefore, sporadic dispersal of only a few bird individuals between various *Protea* populations will lead to continuous genetic intermixing of fungal populations (panmixia) over the entire distribution range of the bird species.

Although a considerable proportion of the dispersal ecology of two *Protea*-associated *Sporothrix* fungal species has been clarified in this study, many questions remain. For example, in addition to the dominant *S. phasma*, we provide the first confirmed report of *S. splendens* on *P. neriifolia* trees since the formal description of the fungus more than twenty years ago [72]. *Sporothrix splendens* is dominant within *P. repens* inflorescences, a species that often occurs sympatrically with *P. neriifolia*, but does not host *S. phasma* [73]. Cape sugarbirds and sunbirds are known to visit both of these hosts [74] and could easily transfer spore-laden mites, also known from both hosts [61, 75], between them. However, the low numbers of *S. splendens* fungal isolates found on *P. neriifolia* trees indicates that it is not the preferred host. The growth of *S. splendens* on media prepared from *P. neriifolia* is also significantly more rapid than when it is grown on material prepared from its preferred *P. repens* host [61]. Differential competitive abilities between different fungal species due to differences in host chemistry may therefore be an additional complicating factor in determining host range and dispersal ecology of *Protea*-associated *Sporothrix* fungi and should be explored in future studies.

Symbiotic interactions may lead to the coevolution of the interacting partners and multiple dependencies on other mutualisms [76] as in the case of the attine ants, their cultivated fungi and their bacteria [77, 78]. The mutualistic interactions between the ants, which act as protectors and transporters of the fungal cultivar they feed on, and the bacterium which protects the fungal cultivar against pathogens, are all depended on the successful cultivation of the fungus [77]. Resent work also suggests a role for bacteria in the release of nutrients from plant material collected by the ants which may prove to enhance the growth of the fungi [79]. Therefore, the mutualism between the fungus and the ant may be dependent on the mutualism between the bacteria and the fungus. A similar symbiotic relationship has been found within the beetle-fungus mutualism. The southern pine beetle and its fungal cultivar is

threatened by an antagonistic fungal species that can outcompete the fungal cultivar and interfere with beetle development [80]. The success of this beetle-fungus mutualism is strengthened by a bacterium that produces antibiotics against the antagonistic fungal species, assisting the successful cultivation of the fungal cultivar [80]. The mutualism between the fungus and the beetle may therefore also depend on a mutualism between the fungus and the bacterium. In these examples, mutualisms between all organisms are strongly interdependent and the entire system would collapse if one of the interacting partners are removed. This could have large consequences for forest ecosystems that are dependent on the ecological functions performed by these multipartite symbioses. This contrasts with the fungus-mite-bird symbioses described here as the mutualistic association between the birds and the plants do not dependent on the interaction between the mites and the fungi. Also, the larger *Proctolaelaps* mites that transport the fungus-carrying *Glycyphagus* mites do not seem to benefit from these associations. However, species that rely heavily on interactions with other organisms for reproduction or survival (such as the fungi and/or mites in the *Protea* system), often have higher partner diversity (revised by [12]). This would decrease the chances of coextinction with the removal of a single interacting partner, as also suggested by simulated network models [*e.g.* 12, 81].

Networks of interacting species can behave unpredictably with anthropogenic interference, and the effect of changes in interaction networks on ecosystem function and evolutionary processes, remains unclear [10]. The loss of birds in the *Protea*-system my, for example, lead to disruptions in the extremely long-distance dispersal processes that are characteristic for the fungi in this niche and disrupt normal evolutionary processes [33-35]. Importantly, loss of interacting partners in networks and subsequently ecosystem function do not only depend on species extinctions (*e.g.* loss of pollinators, fungi or mites in the *Protea* system), but could also be realised by ecological mismatches driven by environmental change [10]. For example,

changes in flowering and/or fungal growth and sporulation times due to climate change or other factors, could lead to mismatches between the timing of sporulation and the availability of fungal vectors. Alternatively, environmental change could change the nature of the interactions between interacting partners from mutualistic or commensialistic (*e.g.* fungi-plant or fungi-mite interaction), to antagonistic due to changing cost: benefit ratios [9]. The conservation of networks of interacting species should therefore be a focus for biodiversity conservation management [11].

This study has shown that *Protea*-associated birds such as the cape sugarbird carry *Protea*-associated mites such as *Proctolaelaps vandenbergi* and a *Glycyphagus sp.* In addition, these birds act as tertiary vectors for ophiostomatoid fungi such as *Sporothrix phasma* and *S. splendens*. A new mutualistic interaction between *Glycyphagus* mites and these *Sporothrix* fungi was recorded and the hyperphoretic behaviour of *Glycyphagus* mites on *Proctolaelaps* mites was revealed. The exact nature of the mutualism between the fungi and the mites needs further exploration. For example, it is possible that the fungi may, in addition to being a food source for the mites, also protect mites from other antagonistic organisms such as contaminating fungi. Inter-fungal competition studies and the influence on mite survival should be conducted to clarify these potential interactions. This study has also provided clear evidence for the very early colonisation of *Protea* inflorescences with *Sporothrix* fungi via mites. The impact of the fungi on *Protea* ecology is, however, not currently known. It is possible that this early occupation of this niche by the fungi and their mutualistic mites may well influence seed viability and/or the behaviour of potential pollinators which could impact *Protea* populations.

References

1. Dighton J, White JF (eds) (2017) The fungal community: its organization and role in the ecosystem. 4th edn. CRC Press, Taylor & Francis Group, London, United Kingdom.
2. Malloch D, Blackwell M (1992) Dispersal of fungal diaspores. In: Christensen M (ed) The fungal community: its organization and role in the ecosystem. Marcel Dekker Inc, New York. pp.147–171
3. Malloch D, Blackwell M (1993) Dispersal biology of ophiostomatoid fungi. In: Wingfield MJ, Seifert KA, Webber JF (eds) Ceratocystis and Ophiostoma: taxonomy, ecology and pathogenicity. American Phytopathogical Society Press, St. Paul, Minnesota, USA. pp.195–205
4. Cassar S, Blackwell M (1996) Convergent origins of ambrosia fungi. Mycologia 88:596–601
5. Houck MA, OConnor BM (1991) Ecological and evolutionary significance of phoresy in the Astigmata. Annual review of entomology 36:611–636
6. Binns ES (1982) Phoresy as migration-some functional aspects of phoresy in mites. Biological Reviews 57:571–620
7. Krantz G, Walter D (eds) (2009) A manual of Acarology. 3rd edn. Texas Tech University Press, Lubbock, Texas, USA
8. Aslan CE, Zavaleta ES, Tershy B, Croll D (2013) Mutualism Disruption Threatens Global Plant Biodiversity: A Systematic Review. PLoS ONE 8:e66993
9. Hoek TA, Axelrod K, Biancalani T, Yurtsev EA, Liu J, Gore J (2016) Resource availability modulates the cooperative and competitive nature of a microbial cross-feeding mutualism. PLoS Biol 14:e1002540
10. Kiers ET, Palmer TM, Ives AR, Bruno JF, Bronstein JL (2010) Mutualisms in a changing world: an evolutionary perspective. Ecology Letters 13:1459–1474

11. Tylianakis JM, Laliberté E, Nielsen A, Bascompte J (2010) Conservation of species interaction networks. Biological Conservation 143:2270–2279
12. Fricke EC, Tewksbury JJ, Wandrag EM, Rogers HS (2017) Mutualistic strategies minimize coextinction in plant–disperser networks. Proceedings of the Royal Society B 284:20162302
13. Wingfield MJ, Seifert KA, Webber J (1993) *Ceratocystis* and *Ophiostoma*: Taxonomy, Ecology and Pathogenicity. APS Press, St. Paul, Minnesota, USA
14. Seifert KA, De Beer ZW, Wingfield MJ (2013) The Ophiostomatoid Fungi: Expanding Frontiers. CBS Biodiversity Series 12. CBS-KNAW Biodiversity Centre: Utrecht, The Netherlands
15. de Beer Z., Duong TA, Wingfield MJ (2016) The divorce of *Sporothrix* and *Ophiostoma*: solution to a problematic relationship. Studies in Mycology 83:165–191
16. Paine TD, Raffa KF, Harrington TC (1997) Interactions among scolytid bark beetles, their associated fungi, and live host conifers. Annual Review of Entomology 42:179–206
17. Klepzig KD, Moser JC, Lombardero FJ, Hofstetter RW, Ayres MP (2001) Symbiosis and competition: complex interactions among beetles, fungi, and mites. Symbiosis 30:83–96
18. Klepzig KD, Moser JC, Lombardero MJ, Ayres MP, Hofstetter RW, Walkinshaw CJ (2001b) Mutualism and Antagonism: Ecological Interactions Among Bark Beetles, Mites and Fungi. In: Jeger MJ, Spence NJ (eds), Biotic Interactions in Plant-Pathogen Associations, CAB International. pp.237–267
19. Bleiker KP, Six DL (2007) Dietary benefits of fungal associates to an eruptive herbivore: potential implications of multiple associates on host population dynamics. Environmental Entomology 36:1384–1396

20. Moser JC, Bridges JR (1986) *Tarsonemus* (Acarina: Tarsonemidae) mites phoretic on the southern pine beetle (Coleoptera: Scolytidae): attachment sites and numbers of bluestain (Ascomycetes: Ophiostomataceae) ascospores carried. Proceedings of the Entomological Society of Washington 88:297–299
21. Levieux J, Lieutier F, Moser JC, Perry TJ (1989) Transportation of phytopathogenic fungi by the bark beetle *Ips sexdentatus* Boerner and associated mites. Journal of Applied Entomology 108:1–11
22. Moser JC, Konrad H, Blomquist SR, Kirisits T (2010) Do mites phoretic on elm bark beetles contribute to the transmission of Dutch elm disease?. Naturwissenschaften 97:219–227
23. Hofstetter RW, Moser JC, Blomquist S (2014) Mites associated with bark beetles and their hyperphoretic ophiostomatoid fungi. In: Seifert KA, De Beer ZW, Wingfield MJ (eds) The Ophiostomatoid Fungi: Expanding Frontiers, CBS Biodiversity Series 12, CBS-KNAW Biodiversity Centre, Utrecht, The Netherlands.pp.65–176
24. Moser JC (1985) Use of sporothecae by phoretic *Tarsonemus* mites to transport ascospores of coniferous bluestain fungi. Transactions of the British Mycological Society 84:750–753
25. Bridges JR, Moser JC (1983) Role of two phoretic mites in transmission of bluestain fungus, *Ceratocystis minor*. Ecological Entomology 8:9–12
26. Lombardero MJ, Ayres MP, Hofstetter RW, Moser JC, Lepzig KD (2003) Strong indirect interactions of *Tarsonemus* mites (Acarina: Tarsonemidae) and *Dendroctonus frontalis* (Coleoptera: Scolytidae). Oikos 102:243–252
27. Hofstetter RW, Moser JC (2014) The role of mites in insect-fungus associations. Annual Review of Entomology 59:537–557
28. Lee S, Roets F, Crous PW (2005) Biodiversity of saprobic microfungi associated with the infructescences of *Protea* species in South Africa. Fungal Diversity 19:69–78

29. Roets F, Wingfield MJ, Crous PW, Dreyer LL (2007) Discovery of fungus-mite mutualism in a unique niche. Environmental Entomology 36:1226–1237
30. Roets F, Crous PW, Wingfield MJ, Dreyer LL (2009) Mite-mediated hyperphoretic dispersal of *Ophiostoma* spp. from the infructescences of South African *Protea* spp. Environmental Entomology 38:143–152
31. Roets F, Wingfield MJ, Wingfield BD, Dreyer LL (2011) Mites are the most common vectors of the fungus *Gondwanamyces proteae* in *Protea* infructescences. Fungal Biology 115:343–350
32. Aylward J, Dreyer LL, Steenkamp ET, Wingfield MJ, Roets F (2014a) Development of polymorphic microsatellite markers for the genetic characterisation of *Knoxdaviesia proteae* (Ascomycota: *Microascales*) using ISSR-PCR and pyrosequencing. Mycological Progress 13:439–444
33. Aylward J, Dreyer LL, Steenkamp ET, Wingfield MJ, Roets F (2014b) Panmixia defines the genetic diversity of a unique arthropod-dispersed fungus specific to *Protea* flowers. Ecology and Evolution 4:3444–3455
34. Aylward J, Dreyer LL, Steenkamp ET, Wingfield MJ, Roets F (2015) Long-distance dispersal and recolonization of a fire-destroyed niche by a mite-associated fungus. Fungal biology 119:245–256
35. Aylward J, Dreyer LL, Laas T, Smit L, Roets F (2017) *Knoxdaviesia capensis*: dispersal ecology and population genetics of a flower-associated fungus. Fungal Ecology 26:28–36
36. Roets F, Dreyer LL, Crous PW (2005) Seasonal trends in colonisation of *Protea* infructescences by *Gondwanamyces* and *Ophiostoma* spp. South African Journal of Botany 71:307–311

37. Coetzee JH, Giliomee JH (1985) Insects in association with the inflorescence of *Protea repens* L. (Proteaceae) and their role in pollination. Journal of the Entomological Society of Southern Africa 48:303–314
38. Coetzee JH, Latsky LM (1985) Faunal list of *Protea repens*. In: International Protea Research Symposium 185:241–246
39. Wright MG, Samways MJ (1999) Plant characteristics determine insect borer assemblages on *Protea* species in the Cape Fynbos, and importance for conservation management. Biodiversity and Conservation 8:1089–1100
40. Wright MG, Visser D, Coetzee JH, Giliomee JH (1991) Insect and bird pollination of *Protea* species in the Western Cape - further data. South African Journal of Science 87:214–215
41. Rebelo T (2001) SASOL Proteas: A Field Guide to the Proteas of South Africa. 2nd edn, Fernwood Press (Pty) Ltd., Vlaeberg, Cape Town, South Africa
42. Schmid B, Nottebrock H, Esler KJ, Pagel J, Pauw A, Böhning-Gaese K, Schurr FM, Schleuning M (2015) Reward quality predicts effect of bird-pollinators on the reproduction of African *Protea* shrubs. Perspectives in Plant Ecology, Evolution and Systematics 17:209–217
43. Siegfried WR, Crowe TM (1983) Distribution and species diversity of birds and plants in Fynbos vegetation of Mediterranean climate-zone, South Africa. In: Kruger FJ, Mitchell DT, Jarvis JUM (eds) Mediterranean-Type Ecosystems: the role of nutrients. Ecological studies 43, Springer, Berlin Heidelberg, New York. pp.403–416
44. Latimer AM, Silander Jr JA, Rebelo AG, Midgley GF (2009) Experimental biogeography: the role of environmental gradients in high geographic diversity in Cape Proteaceae. Oecologia 160:151–162
45. Fraser M W (1997) Cape Sugarbird *Promerops cafer*. In: Harrison JA, Allan DG, Underhill LG, Herremans M, Tree AJ, Parker V, Brown CJ (eds) The atlas of southern

African birds. Vol. 2: Passerines, pp 484-485 BirdLife South Africa, Johannesburg, South Africa

46. Mackay B (2014) The effect of urbanisation and climate on the frequency of ecological stress indicators in the Fynbos endemic nectarivore, the cape sugarbird. Masters dissertation, University of Cape Town, Cape Town
47. Mostert DP, Siegfried WR, Louw GN (1980) *Protea* nectar and satellite fauna in relation to the food requirements and pollinating role of the cape sugarbird. South African Journal of Science 76:409–412
48. Nicolson SW, Flemming PA (2003) Nectar as food for birds: the physiological consequences of drinking dilute sugar solutions. Plant Systematics and Evolution 238:139–153
49. Myburg AC, Rust DJ, Starke LC (1973) Pests of Protea cut flowers. Journal of the Entomological Society of Southern Africa 36:251–255
50. Coetzee JH, Rust DJ, Latsky LM (1985) Mites (Acari) on proteas. In: International Protea Research Symposium 185:247–252
51. Calf KM, Downs CT, Cherry MI (2003) Foraging and territorial behaviour of male Cape and Gurney's sugarbirds (*Promerops cafer* and *P. gurneyi*). African Zoology 38:297–304
52. Collins BG (1983) A first approximation of the energetics of cape sugarbirds (*Promerops cafer*) and orange-breasted sunbirds (*Nectarinia violacea*). South African Journal of Zoology 18:363–369
53. Broekhuysen GJ (1959) The biology of the cape sugarbird *Promerops cafer* (L.). Ostrich 30:180–221
54. Westerkamp C (1990) Bird-Flowers: Hovering versus perching exploitation. Botanica Acta 103:366–371

55. Bates D, Sarkar D (2008) The lme4 Package, 2006. URL http://cran. r-project. org. Accessed 1 June 2017
56. White TJ, Bruns T, Lee SJWT, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. New York Academic Press, New York, U.S.A. pp.315–322
57. Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Molecular Ecology 2:113–118
58. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: Nucleic acids symposium series 41:95–98
59. De Winter JC (2013) Using the Student's t-test with extremely small sample sizes. Practical Assessment, Research and Evaluation 18:1–12
60. Colwell RK (1995) Effects of nectar consumption by the hummingbird flower mite *Proctolaelaps kirmsei* on nectar availability in *Hamelia patens*. Biotropica 27:206-217
61. Theron N (2011) Mite communities within *Protea* infructescences in South Africa. Masters dissertation, Stellenbosch University, Stellenbosch
62. Roets F, Theron N, Wingfield MJ, Dreyer LL (2012) Biotic and abiotic constraints that facilitate host exclusivity of *Gondwanamyces* and *Ophiostoma* on *Protea*. Fungal biology 116:49–61
63. Klepzig KD, Six DL (2004) Bark beetle-fungal symbiosis: context dependency in complex associations. Symbiosis 37:189–205
64. Hektoen L, Perkins CF (1900) Refractory subcutaneous abscesses caused by *Sporothrix schenckii*. A new pathogenic fungus. The Journal of experimental medicine 5:77–89

65. De Lima Barros MB, de Almeida Paes R, Schubach AO (2011) *Sporothrix schenckii* and Sporotrichosis. Clinical microbiology reviews 24:633–654
66. Rodrigues AM, De Hoog S, De Camargo ZP (2013) Emergence of pathogenicity in the *Sporothrix schenckii* complex. Medical Mycology 51:405–412
67. Bajerlein D, Błoszyk J (2003) Two cases of hyperphoresy in mesostigmatic mites (Acari: Gamasida: Uropodidae, Macrochelidae). Biological Letters 40:135136
68. Chmielewski W (1977) Results of observations on associations of mites with insects (Acari-Insecta). Bulletin Entomologique de Pologne 47:59–7
69. Knülle W (2003) Interaction between genetic and inductive factors controlling the expression of dispersal and dormancy morphs in dimorphic astigmatic mites. Evolution 57:828–838
70. Cutraro JL, Ercelawn AY, LeBrun EG, Lonsdorf EW, Norton HA, McKone MJ (1998) Importance of pollen and nectar in flower choice by hummingbird flower mites, *Proctolaelaps kirmsei* (Mesostigmata: Ascidae). International Journal of Acarology 24:345–351
71. Hockey PAR, Dean WRJ, Ryan PG (2005) Roberts' birds of Southern Africa, Trustees of the John Voelcker Bird Book Fund, Cape Town, South Africa
72. Marais GJ, Wingfield MJ (1994) Fungi associated with infructescences of *Protea* species in South Africa, including a new species of *Ophiostoma*. Mycological Research 98:369–374
73. Roets F, Wingfield MJ, Crous PW, Dreyer LL (2013) Taxonomy and ecology of ophiostomatoid fungi associated with *Protea* infructescences. In: Seifert KA, De Beer ZW, Wingfield MJ (eds) The Ophiostomatoid Fungi: Expanding Frontiers. In: CBS Biodiversity Series 12. pp.177–187

74. Hargreaves AL, Johnson SD, Nol E (2004) Do floral syndromes predict specialization in plant pollination systems?. An experimental test in an “ornithophilous” African *Protea*. Oecologia 140:295–301

75. Theron N, Roets F, Dreyer LL, Esler KJ, Ueckermann EA (2012) A new genus and eight new species of Tydeoidea (Acari: Trombidiformes) from *Protea* species in South Africa. International Journal of Acarology 38:257–273

76. Farrell BD, Sequeira AS, O'Meara BC, Normark BB, Chung JH, Jordal BH (2001) The evolution of agriculture in beetles (Curculionidae: Scolytinae and Platypodinae). Evolution 55:2011–2027

77. Currie CR, Wong B, Stuart AE, Schultz TR, Rehner SA, Mueller UG, Sung GH, Spatafora JW, Straus NA (2003) Ancient tripartite coevolution in the attine ant-microbe symbiosis. Science 299:386–388

78. Currie CR, Poulsen M, Mendenhall J, Boomsma JJ, Billen J (2006) Coevolved crypts and exocrine glands support mutualistic bacteria in fungus-growing ants. Science 311:81–83

79. Aylward FO, Burnum KE, Scott JJ, Suen G, Tringe SG, Adams SM, Barry KW, Nicora CD, Piehowski PD, Purvine SO, Starrett GJ, Goodwin LA, Smith RD, Lipton MS Currie CR (2012) Metagenomic and metaproteomic insights into bacterial communities in leaf-cutter ant fungus gardens. The ISME Journal 6:1688-1701

80. Scott JJ, Oh DC, Yuceer MC, Klepzig KD, Clardy J, Currie CR (2008) Bacterial protection of beetle-fungus mutualism. Science 322:63–63

81. Rohr RP, Saavedra S, Bascompte J (2014) On the structural stability of mutualistic systems. Science 345:1253497

CHAPTER 3

MITES STEAL *PROTEA* POLLEN

Acknowledgements

We would like to thank the DST/NRF Centre of Excellence in Tree Health Biotechnology and the Harry Crossley Foundation for financial support throughout the study as well as the Western Cape Nature Conservation board for issuing colleting permits. We would like to extend our gratitude towards Professor Martin Kidd at the Centre for Statistical Consultation, Stellenbosch University for assistance with statistical analyses. A special thank you to Avril Powrie (Olive Glen Mountain Farm) for access to private land and Dr Caroli De Waal and students for laboratory assistance.

Abstract

Flower-associated mites are well-known nectar and pollen thieves of hummingbird-pollinated plants in the Americas. They use the birds as vectors between flowers and, for some plant species, may act as secondary pollinators. However, they can influence pollinator visitation patterns and often reduce nectar and pollen availability, thereby negatively influencing seed-set. For African ornithophilous *Protea* trees, the hummingbird-pollination niche is largely filled by sugarbirds and sunbirds. These birds also vector flower mites, but the role of these mites in *Protea* pollination is unknown. We investigated the role of *Proctolaelaps vandenbergi* flower mites as secondary pollinators and/or pollen and nectar thieves of ornithophilous *Protea neriifolia* trees in South Africa. Field-based mite and pollinator exclusion experiments indicated that *P. vandenbergi* mites played a non-significant role as secondary pollinators of *P. neriifolia*. Feeding experiments showed that *P. vandenbergi* regularly consumed pollen and nectar and often reproduced when pollen is available. Quantification of nectar consumption rates showed that *P. vandenbergi* likely has little effect on total nectar availability due to the mass production of nectar in *P. neriifolia* inflorescences. In contrast, *Proctolaelaps vandenbergi* mites consumed significant quantities of *P. neriifolia* pollen, with more than 50% of total available pollen consumed when mite numbers peak. Pollen consumption by these mites may decrease *Protea* male fitness by reducing the available pollen for dispersal and ultimately impact *Protea* population dynamics.

Introduction

Numerous flowering plant species rely on animals for pollination and, in turn, provide nectar and pollen rewards for this service. However, flowers often also host organisms that exploit these resources without providing pollination services (*e.g.* mites and ants), which are considered nectar and pollen robbers or thieves (Colwell 1973, Inouye 1980, Maloof & Inouye 2000, Guerra et al. 2010). Their actions can have negative ecological and evolutionary consequences (Hargreaves et al. 2009, Irwin et al. 2010) as nectar and pollen robbers often affect host population dynamics (Irwin et al. 2001, Hargreaves et al. 2010).

A particularly well-studied multipartite, pollinator/robber system involves the associations between hummingbirds, their host plants and flower mites (Acari: Mesostigmata: Melicharidae) (*e.g.* Colwell 1973, Colwell & Naeem 1994). In this system, flowers that are adapted to hummingbird pollination are often exploited by flower mites (Maloof & Inouye 2000, Irwin et al. 2001, Lara & Ornelas 2001a, b) that disperse to new flowers by travelling on the beaks or within the nostrils of the birds (Colwell 1973, 1995, Proctor & Owens 2000). These mites consume large quantities of pollen and nectar (Colwell 1973, 1995, Paciorek et al. 1995), can decrease the quantity of male gametes available for dispersal, and may decrease female reproductive success (Irwin et al. 2001, Burkle et al. 2007, Maloof & Inouye 2000). However, hummingbird-associated flower mites may also act as secondary pollinators, at least of self-compatible, non-autogamous species (Dobkin 1984, 1987, 1990, Lara & Ornelas 2002a, b).

Although flower mite-bird-plant interactions are well-studied in the Americas, to the best of our knowledge, similar systems have received no attention in the rest of the world, despite the near global distribution of these mite genera (Halliday et al. 1998, Krantz & Walter 2009,

Eliaderani et al. 2013). In South Africa, for example, certain members of the plant genus *Protea* L. (Proteaceae) are primarily pollinated by sugarbirds (Promeropidae) and sunbirds (Nectariniidae) that feed on the copious amounts of nectar produced (Gideon et al. 1980, Nicolson & Flemming 2003). The infructescences and inflorescences of *Protea* species house numerous mite species (Ryke 1964, Roets et al. 2007, 2009, Theron 2011, Theron et al. 2012, Theron-de Bruin et al. 2017). The flower mite *Proctolaelaps vandenbergi* Ryke (Melicharidae) often attain particularly high numbers (upwards of 60 000 per infructescence have been reported) in *Protea* (Myburgh et al. 1973). Even though studies by Roets et al. (2007, 2009) indicated that a variety of insects can vector these mites, the *Protea*-pollinating birds are likely their main vectors (Theron-De Bruin et al. 2017).

Numerous mites from *Protea* inflorescences appear to be mainly fungivorous (Roets et al. 2007, 2013, Theron-de Bruin et al. 2017). However, like other flower-associated members of the genus, *P. vandenbergi* likely feeds principally on nectar and pollen (Krantz & Walter 2009, Colwell & Naeem 1994, Dobkin 1984, Royce & Krantz 1989, Paciorek et al. 1995, Krantz & Lindquist 1979). Many species within this genus can complete their entire life cycle (under the right microclimatic conditions) on ornithophilous host plants and are phoretic on bird pollinators (Heyneman et al. 1991, Krantz & Walter 2009). Of special interest is the presence of *Proctolaelaps* mites within the *Protea* system. In the hummingbird system (Colwell 1979, 1995, Colwell & Naeem 1994, Dobkin 1984) they are nectar and pollen thieves (Colwell 1995, Paciorek et al. 1995) that may influence host plant reproduction (Colwell 1995, Paciorek et al. 1995, Hargreaves et al. 2009), which suggest that they may have similar effects in the *Protea* system.

The role that *P. vandenbergi* and other flower-associated mites may play in *Protea* pollination is currently unknown. Seed-set for *Protea* is generally low with infructescences containing

between 1-30 % fertile seeds (Rebelo & Rourke 1986, Collins & Rebelo 1987). This low seed set may be caused by various factors, including a shortage of pollinators, low or inadequate pollen transfers, resource shortages and resource allocation to other plant parts (Rebelo & Rourke 1986, Littlejohn 2001). Some of these aspects may, in part, be explained by the consumption of pollen and/or nectar by flower mites as was found in the hummingbird system (Colwell 1995, Paciorek et al. 1995). However, as was suggested for the hummingbird system, flower mites may act as secondary pollinators of *Protea*. This can either be through direct transfer of pollen from one plant to the next via phoresy on birds (Theron-de Bruin et al. 2017), or indirectly when moving around within inflorescences. *Protea* flowers are protandrous (anthers (♂) mature before the pistil (♀)) and have a modified style with pollen attached laterally (Collins & Rebelo 1987, Van der Walt & Littlejohn 1996a, b). Stigmas become receptive for pollen (opening of a narrow split) after *ca*. 48 hours (Ramsey & Vaughton 1991). The maturation of sexually active flowers progress from the outer ring of the inflorescence towards the centre. This difference in maturing-time prevents self-pollination to a certain extent, however, as *Protea* species are generally self-compatible (Van der Walt & Littlejohn 1996a, Steenhuisen et al. 2012, Steenhuisen & Johnson 2012, Nottebrock 2016), the transfer of pollen from another flower within the same inflorescence may lead to fertilization. Therefore, as *Proctolaelaps* mites move around within inflorescences, they may deposit pollen inside mature stigmatic grooves and enhance fertilization (Kaufmane & Rumpunen 2002). This kind of self-fertilization can lead to inbreeding depression that can lead to reduced flowering and survival of later generations (Charlesworth & Willis 2009, Robertson et al. 2011, Forrest et al. 2011).

In this study we investigated the role of flower mites on a bird-pollinated *Protea* species and compare it to the hummingbird system. We hypothesise that *Protea*-associated flower mites act as secondary pollinators within *Protea* inflorescences. We further hypothesise that, as in

the hummingbird system, flower mites consume copious amounts of nectar and pollen, potentially hampering *Protea* pollinators.

Methods

Protea flower mites as secondary pollinators of Protea neriifolia

Protea neriifolia (**Fig.1A**) is a widely distributed tree species in the Cape Floristic Region of South Africa, globally recognised as one of the 'hottest' biodiversity hotspots (Cowling et al. 2003, Myers et al. 2000, Goldblatt 1997, Holmes & Richardson 1999). It often dominates fynbos plant communities (Cambell & Van der Meulen 1980, Van Wilgen & McDonald 1992, Rebelo 2001) and is widely cultivated for the flower export market (Leonhardt & Criley 1999, Littlejohn 2001). It produces large and colourful inflorescences throughout most of the year (February to November) (Coetzee et al. 2007, Rebelo 2001) and it is primarily pollinated by birds (*Promerops cafér* Linnaeus and *Anthobaphes violacea* Linnaeus), although insects such as beetles may also play a minor role (Wright et al. 1991, Wright & Saunderson 1995). This species also houses particularly large numbers of inflorescence-associated mites such as *Proctolaelaps vandenbergi* (Roets et al. 2009, 2013, Theron et al. 2012) that use the pollinators as vectors to new inflorescences (Theron-de Bruin et al. 2017). *Proctolaelaps vandenbergi* mites are large, and the smaller inflorescence-associated mite species use them as intermediate vectors (an interesting case of hyperphoresy) when travelling between inflorescences, rather than to adhere to the birds themselves (Theron-de Bruin et al. 2017). We determined whether mites play a role in the pollination of *P. neriifolia* by determining whether they can carry pollen grains and by conducting field-based pollinator exclusion experiments.

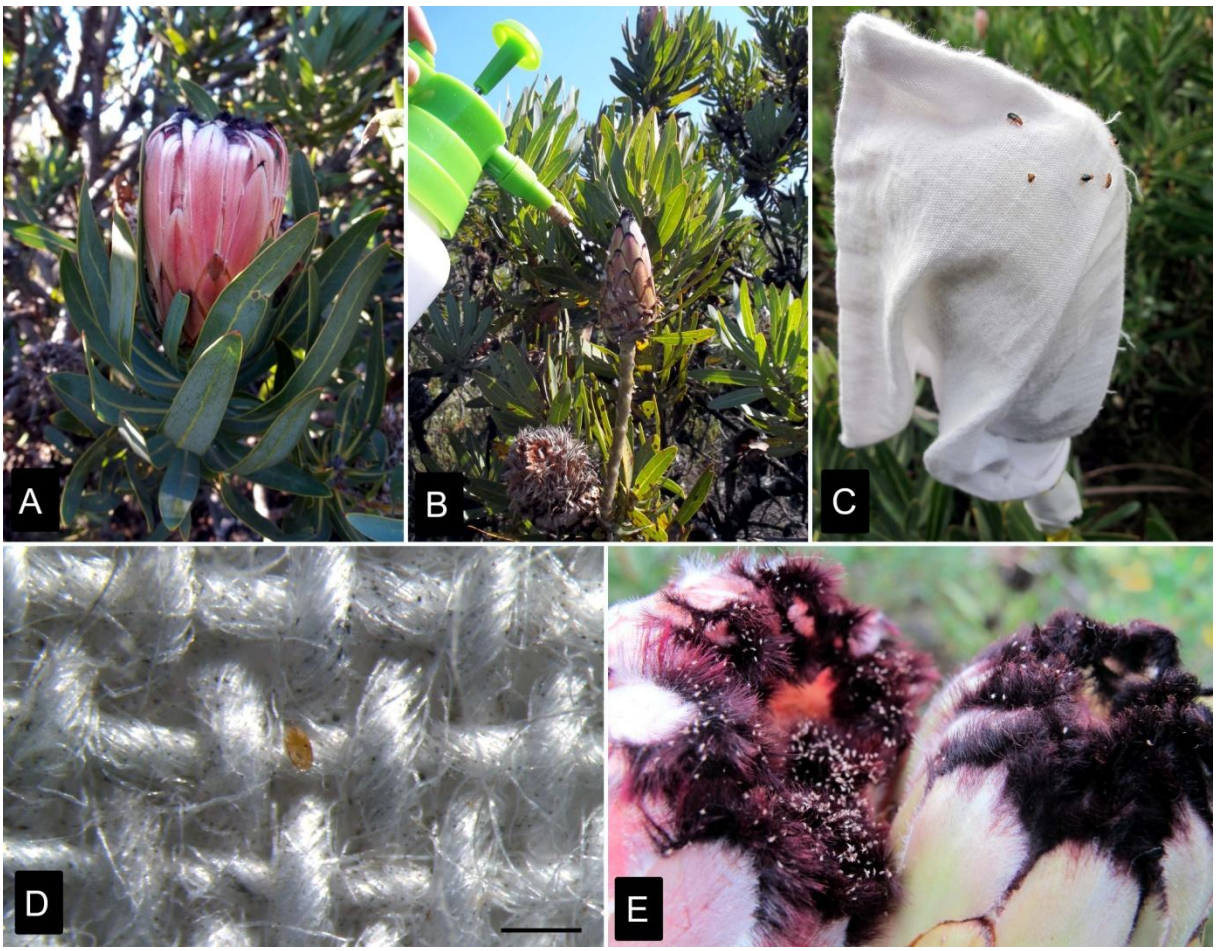

Figure 1: A*) P. neriifolia* inflorescence. B) Applying EKO-spray to experimental *P. neriifolia* bud. C) *P. neriifolia* inflorescence covered by material bag to exclude insect and bird visitors such as the Chrysomelidae beetles depicted. D) Close-up of very small *Tarsonemus sp.*1 mite on material bag. Scale bar = 0.04 mm. E) Transfer of *P. vandenbergi* mites to uncolonized *P. neriifolia* inflorescence.

Mites as Protea *pollen carriers*

During October 2014, 20 *P. neriifolia* inflorescences at mid flowering stage (*ca*. 40-60% of flowers open) were collected from Jonkershoek Nature Reserve, Stellenbosch (33˚59’24.5”S, 18˚57’25.2”E). In the laboratory, inflorescences were individually placed in water-filled vases and re-visited after two days when flower-associated mites started to accumulate at the top of the inflorescences in anticipation of pollinators to act as vectors (Theron-de Bruin et al. 2017).

Mites were collected from these structures following methods in Theron-de Bruin et al. (2017). Broadly, this entailed collecting mites for 40 seconds from the top of inflorescences using adhesive tape strips. A hundred, randomly chosen mites per adhesive strip (*i.e.* per inflorescence) were examined for the presence of pollen. Mites were only counted as positive for carrying pollen when pollen grains were clearly stuck to their integument (Dobkin 1984). Data were recorded as presence/absence only as, when present, pollen grains were often innumerable. The percentage of mites that carried pollen per mite species was compared using a Kruskal-Wallis ANOVA in Statistica 13 (StafSoft Inc, Tulsa, OK, USA). Significant differences are reported when $P \leq 0.05$.

Pollinator exclusion experiment

Exclusion experiments were conducted in three natural *Protea neriifolia* populations (Du Toits Kloof Pass (33˚41'45.2"S 19˚05'14.2"E), Jonkershoek Nature Reserve (33˚59'24.5"S, 18˚57'25.2"E) and Franschoek Pass (33˚55'10.2"S, 19˚09'42.0"E)) during March 2014 in the Western Cape Province, South Africa. At each site, 90 *P. neriifolia* inflorescences in the budding stage (before visitation by arthropods) were treated with SK ECO oil spray (Makhro-Agro, SA (Pty) Ltd), an environmental friendly acaricide and insecticide to eliminate all arthropods. SK ECO oil spray (diluted 1:100 water) was applied using a plastic gardening spray bottle until thoroughly drenched (**Fig.1B**). The top 15 cm of leaves on the stem under each bud were removed to create a smooth stem and the bud was enclosed in cotton voile muslin fabric bags (Neal & Anderson, 2004) to prevent arthropods and birds from visiting them (**Fig.1C**). This material was fine enough to exclude larger arthropods including *Proctolaelaps* mites, but potentially not very small mites such as a *Tarsonemus sp.* (**Fig.1D**). Each bag was sealed around the stem using durable adhesive tape (duct tape - Sellotape,

Henkel limited, UK). These sites were revisited 6-8 weeks later when the inflorescences had opened.

The first treatment involved the permanent removal of 25 bags per site to allow flower visitor access to the inflorescences from this stage onwards (positive control). The second treatment involved the introduction of mites to 25 pre-treated inflorescences. Untreated inflorescences in full flower (all flowers within inflorescences open) that contained high abundances of mites on their surface waiting for vectors were collected from neighbouring plants. The bags surrounding 25 treated inflorescences were carefully re-opened, and mites from these untreated inflorescences were allowed to move freely across to the treated inflorescence (**Fig.1E**). To minimise accidental transferring of pollen to treated inflorescences, untreated inflorescences were brought into contact with treated inflorescences such that the longest bracts of the untreated inflorescence were at least 1 cm below the rim of the open untreated inflorescence. Mites, presumably carrying *Protea*-pollen, were allowed to self-disperse from untreated inflorescences to the treated inflorescences for a period of two minutes, where after the treated inflorescences were closed in their bags again. We thus did not standardise for number of mites per transfer, but for mite transfer time. For a negative control and to eliminate any arthropod interference (to judge levels of autogamy), bags were removed from 25 inflorescences, SK ECO oil was re-applied and the inflorescences were closed off again. For a control of treatment effect, 25 inflorescences at the same flowering stage as the bagged inflorescences were initially marked, but never enclosed in a bag at any stage. After seed set in March 2015 (Van Staden 1978, Wright 1994), the treated infructescences and controls were collected from each site. Only 20 infructescences were chosen for data collection, as a number of infructescences were damaged by baboons and/or arthropods and were therefore excluded from analyses.

Each individual seeds contained within each infructescence was cut open with a scalpel to establish percentage seed set per infructescence. Fertile seeds displayed clear white cotyledons when cut horizontally, while infertile seeds looked woody with a small hollow centre (www.proteaatlas.org.za Rebelo 2006). In addition, infructescences were examined for any signs of pre-dispersal seed predation by, for example, boring insects. Seed set was calculated as the mean percentage of fertile seeds per intact infructescence (Nottebrock et al. 2013).

As *Protea* species are protandrous, it was necessary to establish the number of stigmas that were available to receive pollen at the initial mite transfer stages when the bags were opened. Assuming that only this proportion of potential flowers could be pollinated by the transferred mites (and the pollen they carried), and that *P. neriifolia* is self-compatible (Coetzee et al. 2007), this would give an upper limit for the percentage of seeds produced as a direct result of the added mites (as large numbers of (then closed) flowers would be excluded from the final data set). Therefore, twenty inflorescences at the same flowering stage as that of the experimental inflorescences were collected from the same study sites. They were dissected and individual flowers were separated into open (open stigmatic groove) and closed flowers using a dissecting microscope. Seed set results in final analyses for the treatment where mites were added were, therefore, adjusted by subtracting the mean number of flowers with closed stigmatic grooves from the total number of flowers within inflorescences. Seed set was statistically compared between the treatments and sites using a general linear model with a Games-Howell post hoc test (calculated in R (R Development Core Team 2013)).

Pollen and nectar availability in Protea neriifolia *inflorescences*

Total pollen and nectar availability was calculated for three flowering stages of *P. neriifolia*, established using percentage of flowers at anthesis or later: stage 1 *ca*. 30%, stage 2 *ca*. 60 % and stage 3 *ca*. 100%. Twenty inflorescences per stage were collected during July 2017 in Stettynskloof pass (33°47'48.7"S 19°19'14.4"E), Rawsonville and transported to the laboratory in water filled buckets to keep them fresh. The average pollen load on the pollen presenter per flower (0.431 µg) was calculated from the total amount of pollen removed from ten randomly selected pollen presenters (using a scalpel blade) from each of 20 *P. neriifolia* inflorescences. In addition, the total number of flowers in each of the collected inflorescences where counted. These data were used to determine the total amount of pollen available for each infructescence at each of the flowering stages.

Flowers within inflorescences mature from the outside inwards. We therefore calculated the average daily rate of opening of flowers within *P. neriifolia* inflorescences to estimate the total mass of pollen that becomes available for mites to feed on per day. Ten inflorescences (each from a different *P. neriifolia* individual) at flowering stage 1 (*ca*. 30% flowers open) were collected from Jonkershoek Nature Reserve and kept in vases in a temperature-controlled growth chamber at 24°C at a 12/12h light/dark cycle. The number of open flowers was counted daily for 5 days and the average (± standard error) number of newly opened flowers per inflorescence per day calculated.

The volume of nectar available in each of the above-mentioned inflorescences was established by first removing the top half of flowers by cutting horizontally through inflorescences using

pruning shears. The inflorescence was then placed inside a clean, re-sealable plastic bag and sealed around the exerted stem. The bagged inflorescences were swung in a circular motion for 15 seconds at a constant speed to produce enough centrifugal force to expel nectar from them (Armstrong & Paton 1990). Nectar that collected at the bottom of the bag was collected with a pipette, filtered and quantified (µl) using measuring beakers and pipettes. This method only captures about 70% of the total volume of nectar produced (Armstrong & Paton 1990). All collected nectar was stored at 4˚C in a sterilized container for later use in feeding studies.

Statistical analyses were conducted in Statistica 13 (StafSoft Inc, Tulsa, OK, USA) after testing for normality using Shapiro-Wilk tests and Levene's Test for Homogeneity of Variances. Pollen mass and nectar volumes were compared between stages using ANOVA with LSD post hoc tests on BoxCox transformed data (Osborne 2010). Significant differences are reported when $P \leq 0.05$.

Numbers of Proctolaelaps *mites present*

The tops of inflorescences that were removed for the quantification of nectar (where *P. vandenbergi* typically gather), were used to establish the numbers of *Proctolaelaps* mites at each flowering stage. These flower parts were placed in separate containers for each inflorescence and then frozen for 2 days to kill the mites. The material was dried in an oven at 30˚C for one day, and then shaken by hand for 1 minute to loosen dead and dry mites from the plant material. Material was sieved to separate mites from larger plant material where after mites could easily be counted using a dissecting microscope. As with the data on pollen mass and nectar volumes, data on *P. vandenbergi* numbers where compared between the three flowering stages using ANOVA with LSD post hoc tests on BoxCox transformed data (Osborne 2010). Significant differences are reported when $P \leq 0.05$. In addition, we included

data from a previous study on mites associated with *P. neriifolia* inflorescences (Theron-de Bruin et al. 2017) for comparative purposes. In that study, mites were sampled from the top surface of inflorescences collected during October 2014 in Jonkershoek Nature Reserve (33°59′ 24.5″ S, 18° 57′ 25.2″ E), Stellenbosch, when 30–50% of flowers within the inflorescences were open. These data were included as it represented a different collection site and a different season (spring as opposed to winter), both of which may affect mite numbers within inflorescences. Importantly, immature stages of *P. vandenbergi* are not phoretic. Therefore, due to the collection method used, data from Theron-de Bruin et al. (2017) only included mature mites that were awaiting pollinators for transport to new inflorescences.

Pollen and nectar as a food source for Proctolaelaps *mites*

Feeding and reproduction of mites were tested on a diet of pollen, nectar and a combination of the two. *Proctolaelaps vandenbergii* mites were collected from *P. neriifolia* inflorescences from Stettynskloof Pass in July 2017 and placed in artificial feeding chambers (n = 5, fully grown females) (Krantz & Walter 2009) using a fine paintbrush. Feeding chambers consisted of 100 µl Eppendorf tubes (20 replicates per treatment) containing: 1) 5 µl nectar with pollen free pollen presenter, 2) 5 µl water with pollen free pollen presenter, 3) 5 µl nectar with pollen laden pollen presenter and 4) 5 µl water with pollen laden pollen presenter. Tubes were kept in the dark at room temperature for 10 days after which numbers of mites (including eggs and larvae) in each tube were counted. Data were used to calculate and compare survival rate (as a percentage) of adults and the numbers of eggs, larvae and adults in each tube after six days and the population growth (as a percentage) after ten days. Consumed pollen resources could be enumerated by determining the percentage of pollen removed (visual scoring) from each pollen presenter after 10 days and calculating its weight as a proportion of the mean of 0.431 µg available per pollen presenter. Due to the actions of mites within the tubes

containing pollen on pollen presenters, it was not possible to determine the amount of nectar consumed in all experimental units. However, fluid consumed in the treatments that contained only nectar or water and no pollen could be determined by pipetting. Mite survival rates (percentage of surviving mites that were initially placed in tubes), total numbers of individuals in tubes (eggs, larvae and adults), population growth (percentage increase in number of living individuals per tube including adults, larvae and eggs) and pollen and fluid consumption were statistically compared between treatments using ANOVA with LSD post hoc tests (where necessary).

Results

Pollen and nectar as a food source for Proctolaelaps *mites*

Three mite species were collected from the tops of *Protea neriifolia* inflorescences at the mid flowering stage. These included *Proctolaelaps vandenbergi*, a *Tarsonemus* species and the hypopus of a *Glycyphagus* species. These same three mite species were reported from the inflorescences of *P. neriifolia* in a previous study (Theron-de Bruin et al. 2017). Very few individuals of the *Tarsonemus* and *Glycyphagus* mites carried *Protea* pollen grains (**Fig.2**). Significantly more *P. vandenbergi* mites carried *Protea* pollen, even though numbers were still fairly low (median = 12%).

In the pollinator exclusion experiments, an average of 44% of flowers were receptive to pollen (open stigmatic groove) at the time of mite transfer. Seed set results for this treatment were, therefore, adjusted to reflect this before statistical analyses were conducted. Both site ($F = 11.68$, $P < 0.001$) and treatment ($F = 60.91$, $P < 0.001$) had a significant influence on seed set ($F = 12.119$, $P < 0.001$, Appendix 1). Inflorescences that were kept closed throughout the

experimental period failed to produce any viable seeds, indicating that this species is not autogamous. Inflorescences to which mites were added also mostly failed to produce viable seeds (**Fig.3**). Only 7 of these inflorescences contained viable seeds, but seed set was always extremely low (max < 2%). Seed set was higher for the re-opened inflorescences, but only significantly so at Du Toits Kloof (**Fig.3**). When considering inflorescences that were left completely untreated, Jonkershoek had significantly higher seed set than either Du Toits Kloof or Franschoek that were, in turn, statistically similar (**Fig.3**). This control group always had higher seed set compared to the re-opened treatments, but only significantly so at the Jonkershoek site (**Fig.3**).

Mites as competitors for pollen and nectar

Pollen and nectar availability

Based on calculations of mean pollen mass per intact pollen presenter (0.431 ± 0.112 µg) and total number of pollen presenters in *P. neriifolia* inflorescences, there would be an continuous increase in available pollen mass from flowering stage 1 to stage 3 (assuming no removal) with an average of *ca*. 40 µg pollen at stage 1, *ca*. 80 µg pollen at stage 2 and *ca*. 133 µg of pollen available when all flowers have opened at stage 3 (**Fig.4**).

Nectar production per flower would be continuous over extended periods and could therefore not be quantified per flower. In addition, due to lack of inflorescences that were void of pollen and nectar consumers, nectar availability reported here is likely underestimated. Nectar availability (as measured from field-collected inflorescences) differed between the different stages ($F = 2.99$, $P = 0.058$) with the highest amounts of nectar available during stage 2 (mean of 2060.75 µl) when *ca*. 60% of flowers were open (**Fig.5**). Nectar availability was

statistically similar at stage 1 (mean of 1385.75 µl) and stage 3 (mean of 1102.25 µl) (P = 0.485), and stages 1 and 2 (**Fig.5**) (P = 0.01). Nectar availability decreased significantly from stage 2 to stage 3 (P = 0.021).

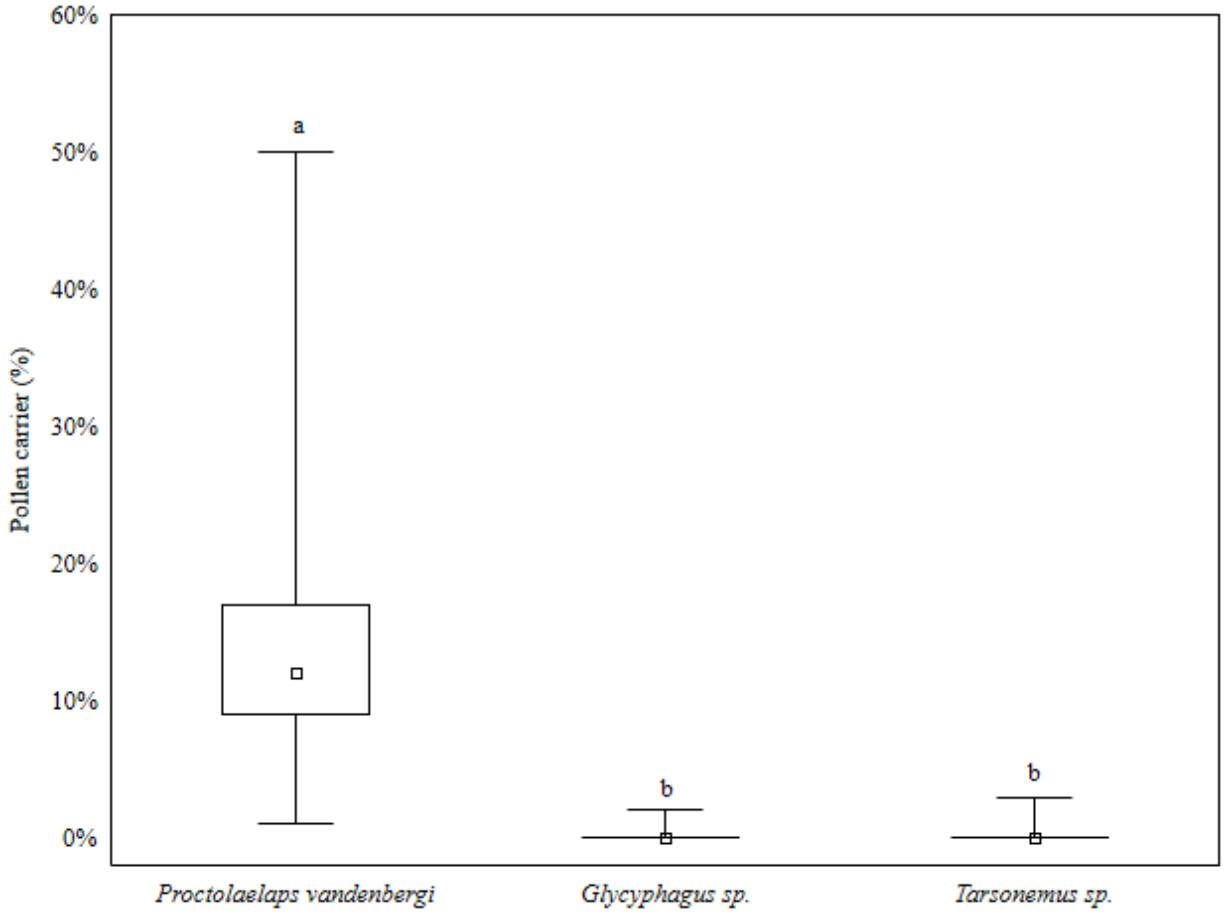

Figure 2: The percentage of mites (n = 100 individuals per mite species per inflorescence) collected from *P. neriifolia* inflorescences (n = 20) that carried *Protea* pollen (H(2) = 46.84, P < 0.001). Significantly more *P. vandenbergi* mites carried *Protea* pollen than *Glycyphagus* mites (Z = 5.18, P < 0.001) or *Tarsonemus* mites (Z = 5.52, P < 0.001).

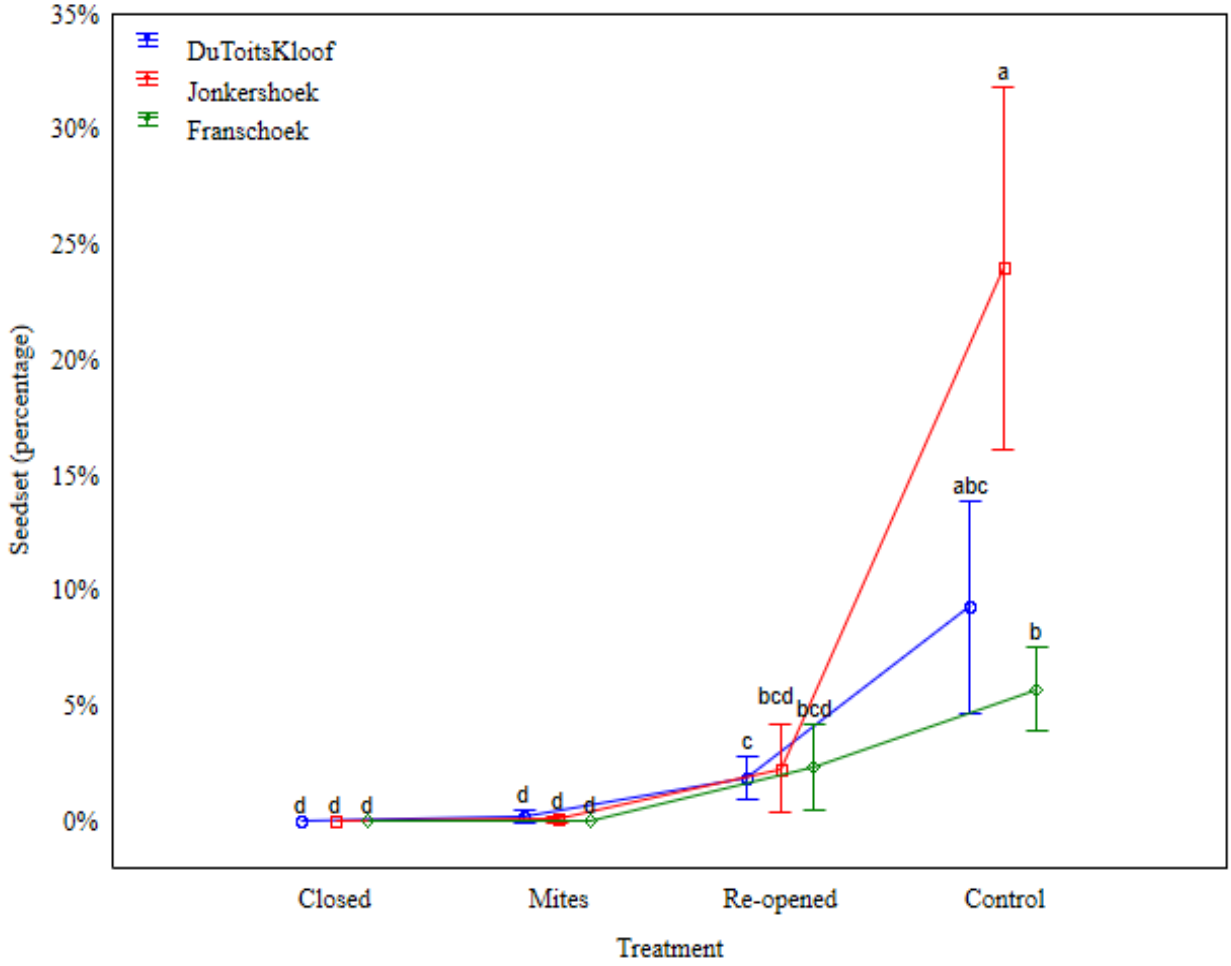

Figure 3: *P. neriifolia* seed set between three treatments and a control at three sites within the Western Cape, South Africa.

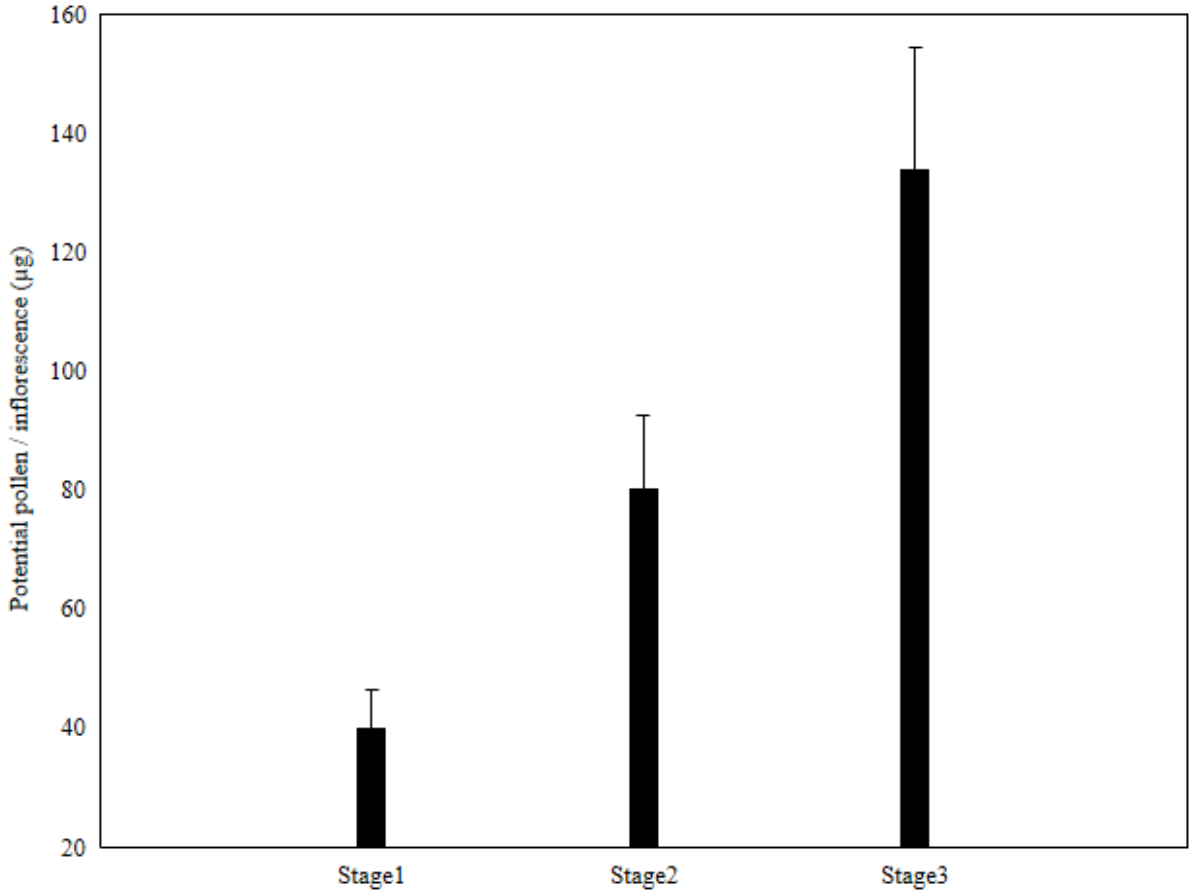

Figure 4: The average mass (± SE) of potential pollen (µg) available inside inflorescences of *P. neriifolia* at three flowering stages (stage 1 = 30% flowers open, stage 2 = 60% flowers open and stage 3 = 100% flowers open).

Flowers within *P. neriifolia* inflorescences that were kept at 24°C in the temperature-controlled chamber opened at a rate of 8.43 ± 3.66 flowers/day/inflorescence. The total mass of pollen that became exposed per day in an inflorescence was therefore calculated as 8.43 flowers × 0.431 µg pollen per flower = *ca*. 3.63 µg pollen per day. This represents a minimum limit for pollen exposure rate, as pollinators often forcibly open flowers to obtain nectar (Collins & Rebelo 1987). This value therefore represent pollen exposure rate in the absence of pollinators.

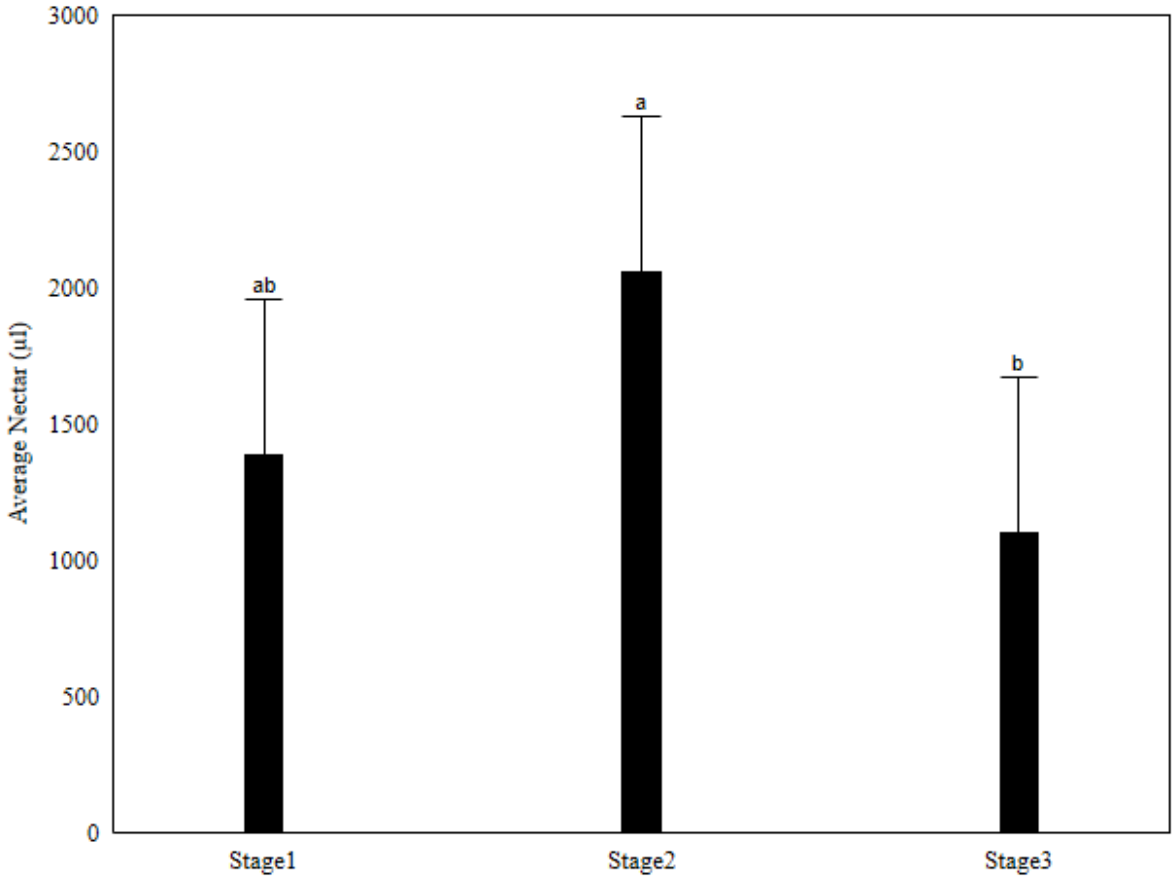

Figure 5: The average (± 95% confidence intervals) amount of nectar (µl) available inside field-collected *P. neriifolia* inflorescences during three flowering stages (stage 1 = 30% flowers open, stage 2 = 60% flowers open and stage 3 = 100% flowers open).

Numbers of Proctolaelaps *mites present*

Proctolaelaps vandenbergi abundance differed significantly between all three stages ($F = 12.982$, $P < 0.001$) with the highest abundance during stage 2 (mean of 178 individuals) (**Figs.6**, **7**). Mite abundance increased significantly from stage 1 (mean of 71 individuals) to stage 2 ($P < 0.001$) followed by a significant decrease from stage 2 to stage 3 (mean of 92 individuals) ($P = 0.009$) (**Figs.6**, **7**). However, stage 3 inflorescences contained significantly more mites than inflorescences at stage 1 ($P = 0.021$) (**Figs.6**, **7**).

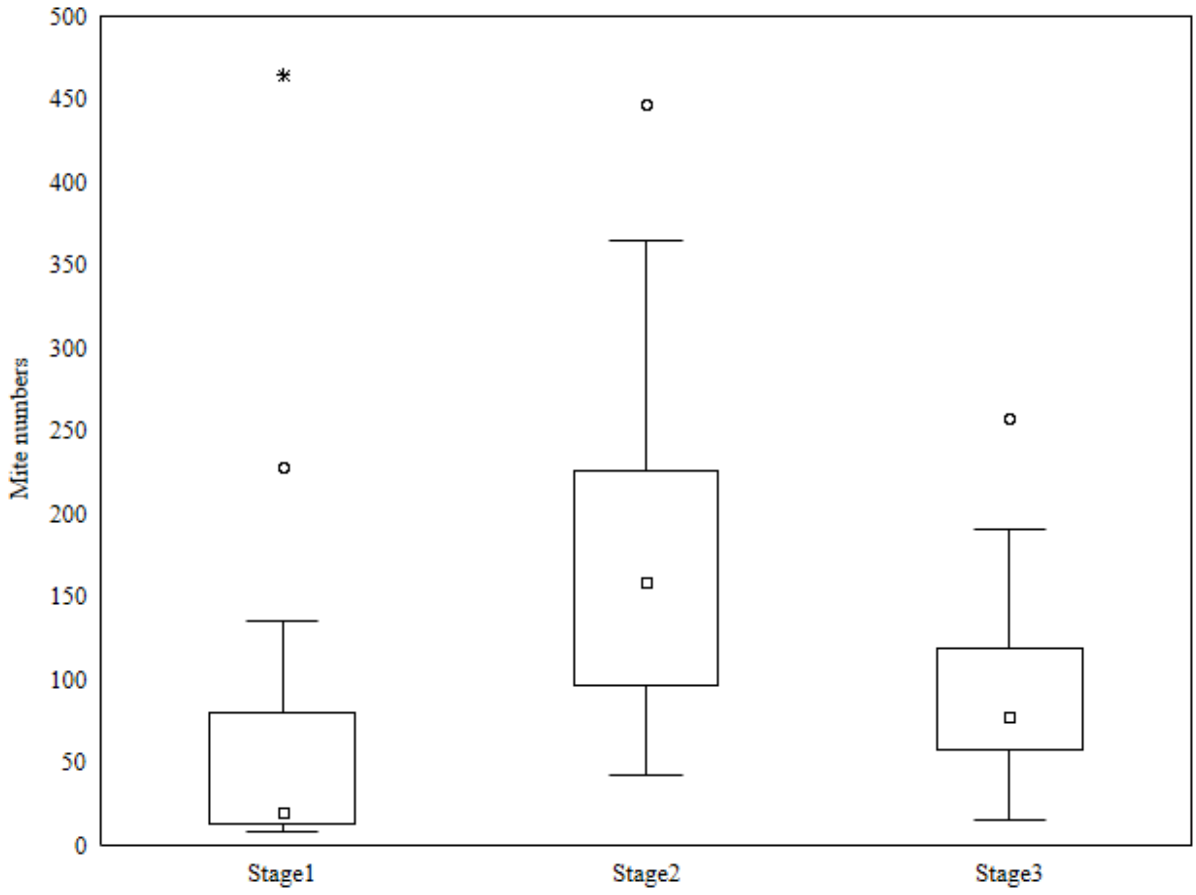

Figure 6: Box plot for the abundance of *Proctolaelaps vandenbergi* mites collected from *P. neriifolia* inflorescences at three flowering stages (stage 1 = 30% flowers open, stage 2 = 60% flowers open and stage 3 = 100% flowers open).

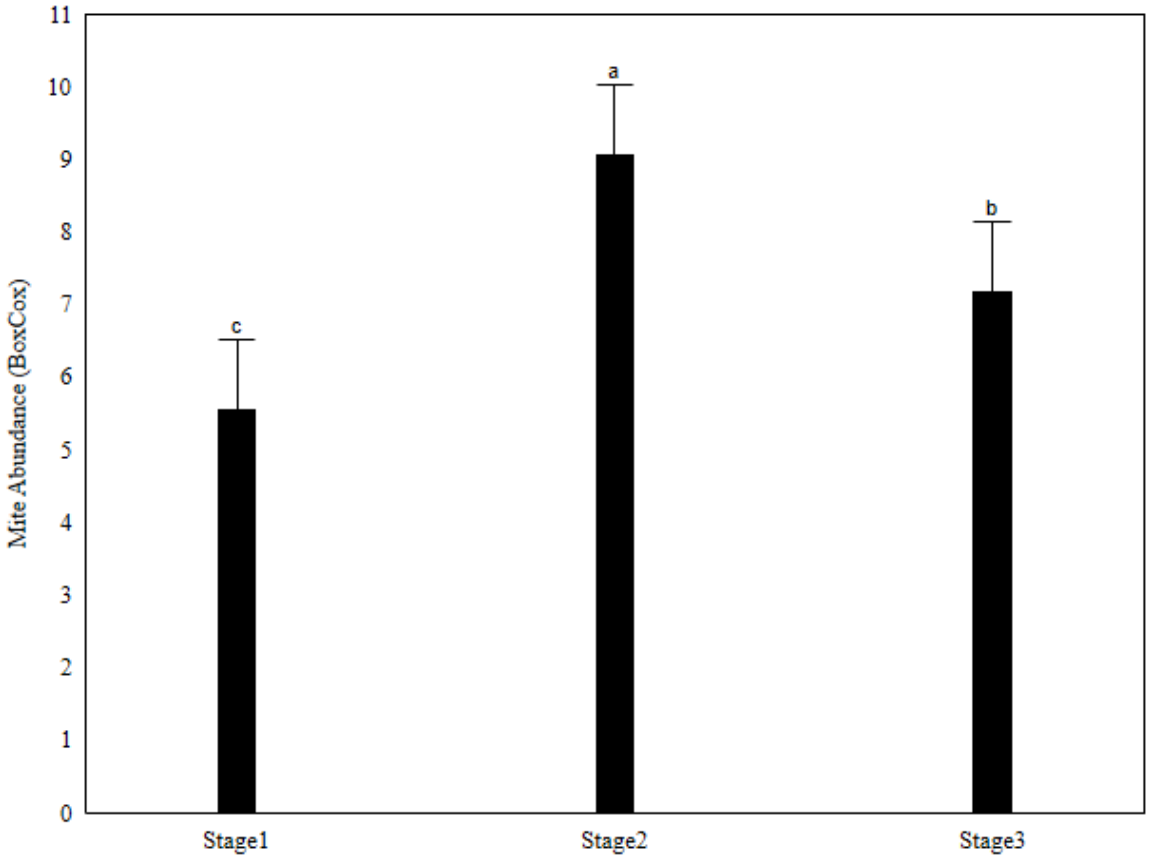

Figure 7: Abundance of *Proctolaelaps vandenbergi* mites (BoxCox transformed with ± 95% confidence intervals) collected from *P. neriifolia* inflorescences at three flowering stages (stage 1 = 30% flowers open, stage 2 = 60% flowers open and stage 3 = 100% flowers open).

Pollen and nectar as a food source for Proctolaelaps *mites*

All *P. vandenbergi* mites that fed on the control diet consisting only of water died after 4 days even when ingesting water (**Table 1**). Mites in other treatments were observed to regularly ingest pollen and nectar and many of these survived for at least 6 days (**Table 1**). The survival rates of mites that fed only on nectar were similar to those feeding on a combination of pollen and nectar. However, mites that only fed on pollen had significantly higher survival rates compared to those feeding on nectar and on a combination of nectar and pollen. Eggs and larvae were observed from day 4 onwards, but only in treatments that contained pollen. Significantly more larvae were found within the treatment that only contained pollen as a food

source compared to the treatment that contained both pollen and nectar (**Table 1**). The mass of pollen consumed by mites was significantly higher for treatments where mites fed only on pollen than those that were provided with both pollen and nectar (**Table 1**).

Table 1: Summary of the feeding study analyses represented as the mean (standard deviation). Survival (%) and egg, larvae and adult numbers were calculated for day six (D6). Growth rate (%), pollen consumption (µg) and fluid consumption (µl) were calculated at day ten (D10).

	Water	Nectar	Pollen	Nectar & Pollen	F-value	p-value
Survival$^{(D6)}$	0	27 (31.3) ^{b}	50 (31.5)^{a}	13 (32)^{b}	6.996	**p<0.01**
Eggs$^{(D6)}$	0	n.a	0.4 (0.8)^{a}	1.2 (1.6)^{a}	4.053	p=0.05
Larvae $^{(D6)}$	0	n.a	2.8 (1.9)^{a}	1 (2.1)^{b}	7.377	**p<0.01**
Adults$^{(D6)}$	0	1.4 (1.6)^{b}	2.5 (1.6)^{a}	0.7 (1.6)^{b}	6.996	**p<0.01**
Growth Rate$^{(D10)}$	0	n.a	63 (44.6)^{a}	45 (52.3)^{a}	1.372	p=0.25
Pollen consumed$^{(D10)}$	n.a	n.a	0.3 (0.1)^{a}	0.1 (0.1)^{b}	21.852	**p<0.01**
Fluid consumed$^{(D10)}$	2.78 (1.1)	2.35 (1.2)	n.a	n.a	1.407	p=0.24

Superscripts indicates significant difference ($P < 0.05$) between treatments

Initial pollen = 0.431 µg, Initial fluid = 5 µl

It was not possible to precisely determine the amount of pollen and nectar consumed per mite individual over the experimental period of 10 days as numerous individuals died (presumably of old age and/or malnutrition) and in some cases larvae were produced that also consumed resources. However, for mites that were fed only nectar, and where no larvae were produced, all available nectar was consumed within 10 days in some replicates. This indicated that 5 mature mites are capable of consuming 5 µl of nectar within 10 days (= 0.1 µl nectar consumed per mite per day). For nectar consumption at stage 1 (30% of open flowers = 1385.75 µl available), 71 mites may consume up to 7.1 µl nectar per day. At stage 2 (60% open flowers = 2060.75 µl available) there was an average of 178 mites per inflorescence that could consume *ca*. 17.8 µl nectar per day. The study of Theron-de Bruin et al. (2017) reported the collection of a median of 706.5 adult *P. vandenbergi* mites per inflorescence at mid-

flowering stage (30–50% of open flowers) from the Jonkershoek Nature Reserve in spring. This is expected to only represent a small portion of the total number of mites in these inflorescences, as not all mites that gathered at the top of inflorescences could be collected, and all immature individuals within inflorescences were discounted. This number of adult mites would be able to consume at least 70.65 µl nectar per day.

For mites that were fed both pollen and nectar, and where no larvae were produced, mites could consume up to *ca*. 0.10 µg of pollen over the 10 days (= 0.002 µg of pollen consumed per mite on average per day). When mites were fed pollen only, most tubes contained larvae after 10 days. For those that did not, maximum pollen consumption was *ca*. 0.14 µg after ten days (= 0.0028 µg of pollen consumed per mite on average). These values represent minimum values, as all mites in these tubes were dead by day 6. By using these values, it was possible to calculate predicted consumption rates for pollen and nectar by mites in *P. neriifolia* inflorescences. For pollen consumption at stage 1 (30% of open flowers), when there is a mean number of 71 mites in inflorescences, mites can consume *ca*. 0.142 µg – 0.199 µg of pollen per day (= 3.91–5.48% of daily available pollen). At stage 2 (60% open flowers) there was an average of 178 mites per inflorescence. These may be capable of consuming 0.356 µg – 0.498 µg of pollen per day (= 9.8–13.72% of daily available pollen). Using data from the study of Theron-de Bruin et al. (2017), the adults collected in that study may be capable of consuming 1.43 µg – 1.98 µg of pollen per day (= 39.39–54.55% of daily available pollen).

Discussion

In this study we show that *Proctolaelaps vandenbergi* flower mites do not significantly contribute to pollination of *Protea neriifolia*. In contrast, mites readily fed and reproduced on a diet consisting only of *P. neriifolia* nectar and pollen. Consumption of nectar likely has little effect on *Protea* pollination, as we have shown that *P. neriifolia* produces vast volumes of nectar for its avian pollinators. However, pollen consumption by mites can be quite severe. The reduction in pollen availability for pollinators may lead to a decrease in male fitness and ultimately influence *Protea* seed-set and population dynamics.

No viable seeds formed within inflorescences that acted as negative controls, indicating that that *P. neriifolia* is non-autogamous. When mites were added, very few viable seeds formed, demonstrating that pollen transfer by mites is possible, but very limited. It was not possible to determine whether successful pollination in these cases resulted from cross-pollination (*i.e.* from pollen carried by mites in the initial transfer between inflorescences) or from self-pollination (via the transfer of pollen from anthers and receptive stigmas within the inflorescence) when mites moved between flowers while feeding on pollen and nectar. If seed set resulted from the latter, the reduction in out-crossing could lead to inbreeding depression that is known to cause decreased fitness and future reproductive success in the Proteaceae (Johnson & Nilsson 1999, Eckert 2000, Robertson et al. 2011). This very low successful seed set excludes *P. vandenbergi* as secondary pollinators of *P. neriifolia*, unlike in some hummingbird-pollinated systems (Dobkin 1984, Lara & Orneals 2001, Kaufmane & Rumpunen 2002).

Protea generally have low seed set (2%–30%) (Rebelo & Rourke 1986). Seed set for *P. neriifolia* in previous studies varied between 1.5%–6.4%, with 5 to 18 seeds per

infructescence (*e.g.* Collins & Rebelo 1987, Maze & Bond 1996). In the present study, natural seed set of *P. neriifolia* varied between 5 and 25%, depending on the study site. Low seed set therefore seems to be the norm for *Protea* species and for *P. neriifolia*, but reasons for this are generally unclear. Various proposed reasons include a shortage in viable pollen (inadequate pollen transfer, vector shortage or unsuitable pollen), resource limitations, predation, a lack of space within the inflorescence or genetic polymorphism (Wiens 1984, Rebelo & Rourke 1986, Collins & Rebelo 1987, Ayre & Whelan 1989). In the present study, we suggest that pollen consumption by mites may be a contributing factor to the low seed set in *P. neriifolia*. At the mid flowering stage, the mites are capable of consuming up to 2% of available pollen. At particular sites, and perhaps during warmer time-periods, mite numbers can be very high (*e.g.* Theron-de Bruin et al. 2017) and could easily consume more than 50% of available pollen. This is high in comparison to pollen robbing by some hummingbird-associated flower mites. Paciorek et al. (1995), for example, found that *Proctolaelaps kirmsei* Fain, Hyland, & Aitken can consume on average 5.4% and 16% of *Hamelia patens* Jacq. pollen (which is believe to be an over estimation). Velázquez & Ornelas (2010) found decreases of 69% in available pollen in *Moussonia deppeana* Schlecht. & Cham., 36% in *Lobelia laxiflora* H.B.K. and 63% in *L. cardinalis* L. flowers after 24 hours of consumption by the hummingbird flower mites *Tropicoseius sp.* nov. and *T. chiriquensis* Baker & Yunker. This reduction in pollen availability negatively affects male fitness. *Hamelia patens*, for example, is self-incompatible and mites did not assist in pollination (Paciorek et al. 1995). Similarly, it is expected that *P. vandenbergi* mites negatively influence male fitness in *P. neriifolia* by reducing the amount of available pollen for transfer by birds and insects (Hargreaves et al. 2009).

Proctolaelaps vandenbergi mites regularly consumed nectar in our study. However, even when mite numbers were very high (Theron-de Bruin et al. 2017), daily nectar consumption

by mites remained less than 6.5% of the total available nectar. In addition, nectar production is expected to be continuous throughout the flowering season, diminishing the impact of nectar robbing by these mites. This contrasts with results from studies on nectar consumption by flower mites associated with hummingbirds. Colwell (1995) showed that *Proctolaelaps kirmsei* mites consumed on average 40% of available nectar within *Hamelia patens*. Lara and Orneals (2001) found that flower mites removed 50% of nectar from *Moussonia deppeana* flowers. Da Cruz et al. (2007) found that flower mites from *Heliconia laneana* Barr. & *H. spathocircinata* Aristig. reduced nectar by between 33% and 49% and consequently led to the decrease of nectar sugars within nectar due to continuous nectar production to compensate for nectar robbery. A *Proctolaelaps sp.* was also found to decrease nectar availability by 22% for pollinators of *Neoregelia johannis* Carrière flowers (Guerra et al. 2010).

From the feeding experiments it was evident that *P. vandenbergi* mites could survive and reproduce on a diet consisting of *P. neriifolia* pollen and nectar only. Members of this genus have diverse ecologies and can feed on various arrays of substances including fungi, pollen and other mites (Krantz & Walter 2009). A previous study indicated that this mite does not appear to feed on *P. neriifolia* flower-associated fungi (Theron-de Bruin et al. 2017). It is unknown whether *P. vandenbergi* is also predaceous on other arthropods, but as *Protea* flowers are not consistently available throughout the flowering season, they may switch to a more predaceous life-style when they live within *Protea* infructescences during the non-flowering stages (Roets et al. 2007, 2009, 2011, 2013, Theron 2011, Theron et al. 2012, Theron-de Bruin et al. 2017). However, as far as we know, predatory behaviour has not been documented for other flower-associated *Proctolaelaps* species.

Both adults and immature *P. vandenbergi* individuals fed on pollen and nectar in experimental units. Interestingly, mites reproduced only when *Protea* pollen was available

within experimental units, even though they could survive for prolonged periods when feeding on *Protea* nectar only (compared to when offered water only). Mites, therefore, seem to be able to differentiate between suitable breeding sites (those containing *Protea* pollen) and non-suitable breeding sites (areas without pollen), even when some resources are available (*Protea* nectar). Pollen provides high quantities of nutrients such as amino acids that are scarce in nectar (Stanley & Linskens 1974). Amino acids would be particularly important for egg development in female mites and for growing juveniles (Gilbert 1972, Royce & Krantz 1989, Chmielewski 1999). The ability to survive only on nectar may be an adaption to use this nearly continuous source of carbohydrates at the end of the flowering stage of inflorescences, when all available pollen is depleted, and mites await the last few visits by pollinators to transport them to uncolonized inflorescences (Roets et al. 2009, Theron-de Bruin et al. 2017).

Previous feeding studies that used flower-associated mites (including *Proctolaelaps kirmsei*) in preference experiments indicated that these mites could distinguish between and show preference towards their host plants (Heyneman et al. 1991, Cutraro et al. 1998). These flower mites are therefore very host specific (loyal) as only *ca*. 1 in 200 individuals were found on another host (Heyneman et al. 1991). We expect that this monophagous habit persists in species that are associated with flowering plants that flower throughout the year. As *P. neriifolia* does not flower throughout the year, *P. vandenbergi* mites need additional host/s species to survive, except if they switch diet to other sources as mentioned above. However, *P. vandenbergi* mites are associated with numerous *Protea* species (Theron 2011) and may therefore be a sequential specialist in that they specialise on the genus *Protea*, but switch host species according to the availability of flowering inflorescences (Colwell 1973). More feeding and survey studies are needed to corroborate this.

Nectar thieves are generally considered to have negative impacts on their hosts. However, a study of the effect of *Tropicoseius* flower mites on *Moussonia deppeana* (Lara & Ornelas 2001, 2002a) showed the opposite. The authors found that the nectar and pollen robbing flower mites aided outcrossing in this species by influencing the behaviour of hummingbird visitors (Lara & Ornelas 2002a). It was found that when mites were absent, hummingbird visitation were less frequent, but lasted longer. In the presence of mites, hummingbird visitations were more frequent, but had shorter durations. This had positive consequences for seed production (Lara & Ornelas 2002a). A similar situation may exist in the *Protea* system. When birds perch on *Protea* inflorescences or probe them for nectar, *P. vandenbergi* mites swarm to the top to climb on birds for transport (pers. obsv.). When the mites are particularly numerous, they may irritate the bird to such an extent that it remains on the inflorescences for shorter periods of time. This would therefore decrease visitation times, but increase the frequency of visits, which could ultimately improve prospects for outcrossing and increased fitness (Lara & Ornelas 2001).

Proctolaelaps vandenbergi mites feed and reproduce on *Protea* pollen and are pollen and nectar thieves. They have the potential to drastically decrease pollen availability within inflorescences and therefore pose a significant risk to *Protea* reproduction, at least at certain sites and/or during certain times of the year. The reasons for these large differences in mite numbers are unclear, but may be important considerations under future predicted climate chance scenarios and accompanying shifts in flowering phenology. These mites offer very little in terms of secondary pollination of *Protea* plants, and may even reduce fitness if successful pollination is due to selfing. The impact of mites on avian visitation duration and frequency should be investigated further in future studies to determine possible trade-offs between pollen robbing and outcrossing success.

References

1. Armstrong DP, Paton DC (1990) Methods for measuring amounts of energy available from *Banksia* inflorescences. Austral Ecology 15:291–297
2. Ayre DJ, Whelan RJ (1989) Factors controlling fruit set in hermaphroditic plants: studies with the Australian Proteaceae. Trends in Ecology & Evolution 4:267–272
3. Burkle LA, Irwin RE, Newman DA (2007) Predicting the effects of nectar robbing on plant reproduction: implications of pollen limitation and plant mating system. American Journal of Botany 94:1935–1943
4. Campbell BM, Van der Meulen F (1980) Patterns of plant species diversity in fynbos vegetation, South Africa. Vegetatio 43:43–47
5. Charlesworth D, Willis JH (2009) The genetics of inbreeding depression. Nature Reviews Genetics 10:783–796
6. Chmielewski W (1999) Pollen as a medium for rearing plant-feeding acarid mites (Acari: Acaridae). In: Bruin J, Van der Geest L PS, Sabelis MW (eds) Ecology and Evolution of the Acari: Proceedings of the 3rd Symposium of the European Association of Acarologists 1–5 July 1996, Amsterdam, The Netherlands. Kluwer Academic Publishers, Springer Netherlands. pp.419–422
7. Coetzee JH, Littlejohn GM, Janick J (2007) *Protea*: a floricultural crop from the Cape Floristic Kingdom. Scripta Horticulturae 5:77–112
8. Collins BG, Rebelo T (1987) Pollination biology of the Proteaceae in Australia and southern Africa. Australian Journal of Ecology 12:387–421
9. Colwell RK (1973) Competition and coexistence in a simple tropical community. The American Naturalist 107:737–760
10. Colwell RK (1979) The geographical ecology of hummingbird flower mites in relation to their host plants and carriers. Recent advances in acarology 2:461–468

11. Colwell RK (1995) Effects of nectar consumption by the hummingbird flower mite *Proctolaelaps kirmsei* on nectar availability in *Hamelia patens*. Biotropica 27:206–217
12. Colwell RK, Naeem S (1994) Life-history patterns of hummingbird flower mites in relation to host phenology and morphology. In: Houck MA (ed) Mites: Ecological and evolutionary analyses of life pattern. Springer US. pp.23–44
13. Cowling RM, Pressey RL, Rouget M, Lombard AT (2003) A conservation plan for a global biodiversity hotspot - the Cape Floristic Region, South Africa. Biological Conservation 112:191–216
14. Cutraro JL, Ercelawn AY, LeBrun EG, Lonsdorf EW, Norton HA, McKone MJ (1998) Importance of pollen and nectar in flower choice by hummingbird flower mites, *Proctolaelaps kirmsei* (Mesostigmata: Ascidae). International Journal of Acarology 24:345–351
15. Da Cruz DD, Righetti De Abreu VH, Van Sluys M (2007) The effect of hummingbird flower mites on nectar availability of two sympatric *Heliconia* species in a Brazilian Atlantic forest. Annals of botany 100:581–588
16. Dobkin DS (1984) Flowering patterns of long-lived *Heliconia* inflorescences: implications for visiting and resident nectarivores. Oecologia 64:245–254
17. Dobkin DS (1987) Synchronous flower abscission in plants pollinated by hermit hummingbirds and the evolution of one-day flowers. Biotropica 19:90–93
18. Dobkin DS (1990) Distribution patterns of hummingbird flower mites (Gamasida: Ascidae) in relation to floral availability on *Heliconia* inflorescences. Behavioral Ecology 1:131–139
19. Eckert CG (2000) Contributions of autogamy and geitonogamy to self-fertilization in a mass-flowering, clonal plant. Ecology 81:532–542
20. Eliaderani F, Nemati A, Kocheili F (2013) Some mesostigmatid mites from Iran with their world distribution. Journal of Crop Protection 2:127–138

21. Forrest NC, Ottewell KM, Whelan RJ, Ayre DJ (2011) Tests for inbreeding and outbreeding depression and estimation of population differentiation in the bird-pollinated shrub *Grevillea mucronulata*. Annals of botany 108:185–195
22. Gideon N, Mostert DP, Siegfried WR (1980) *Protea* nectar and satellite fauna in relation to the food requirements and pollinating role of the Cape Sugarbird. South African Journal of Science 76:409–412
23. Gilbert LE (1972) Pollen feeding and reproductive biology of *Heliconius* butterflies. Proceedings of the National Academy of Sciences 69:1403–1407
24. Goldblatt P (1997) Floristic diversity in the Cape flora of South Africa. Biodiversity and Conservation 6:359–377
25. Guerra TJ, Romero GQ, Benson WW (2010) Flower mites decrease nectar availability in the rain-forest bromeliad *Neoregelia johannis*. Journal of Tropical Ecology 26:373–379
26. Halliday RB, Walter DE, Lindquist EE (1998) Revision of the Australian *Ascidae* (Acarina: Mesostigmata). Invertebrate Systematics 12:1–54
27. Hargreaves AL, Harder LD, Johnson SD (2009) Consumptive emasculation: the ecological and evolutionary consequences of pollen theft. Biological Reviews 84:259–276
28. Hargreaves AL, Harder LD, Johnson SD (2010) Native pollen thieves reduce the reproductive success of a hermaphroditic plant, *Aloe maculata*. Ecology 91:1693–1703
29. Heyneman A, Colwell RK, Naeem S, Dobkin DS, Hallet B (1991) Host Plant Discrimination: Experiments with Hummingbird Flower Mites. In: Price PW, Lewinsohn TG, Fernandes W, Benson WW (eds) Plant-Animal Interactions: Evolutionary ecology in tropical and temperate regions. John Wiley & Sons, Inc, New York. pp.455–485
30. Holmes PM, Richardson DM (1999) Protocols for restoration based on recruitment dynamics, community structure, and ecosystem function: perspectives from South African fynbos. Restoration Ecology 7:215–230
31. Inouye DW (1980) The terminology of floral larceny. Ecology 61:1251–1253

32. Irwin RE, Brody AK, Waser NM (2001) The impact of floral larceny on individuals, populations, and communities. Oecologia, 129:161–168
33. Irwin RE, Bronstein JL, Manson JS, Richardson L (2010) Nectar robbing: ecological and evolutionary perspectives. Annual Review of Ecology, Evolution, and Systematics 41:271–292
34. Johnson SD, Nilsson, LA (1999) Pollen carryover, geitonogamy, and the evolution of deceptive pollination systems in orchids. Ecology 80:2607–2619
35. Kaufmane E, Rumpunen K (2002) Pollination, pollen tube growth and fertilization in *Chaenomeles japonica* (Japanese quince). Scientia Horticulturae 94:257–271
36. Krantz GW, Lindquist EE (1979) Evolution of phytophagous mites (Acari). Annual Review of Entomology 24:121–158
37. Krantz G, Walter D (eds) (2009) A manual of Acarology. 3rd edn. Texas Tech University Press, Lubbock, Texas, USA
38. Lara C, Ornelas JF (2001) Nectar 'theft' by hummingbird flower mites and its consequences for seed set in *Moussonia deppeana*. Functional Ecology 15:78–84
39. Lara C, Ornelas JF (2002a) Effects of nectar theft by flower mites on hummingbird behaviour and the reproductive success of their host plant, *Moussonia deppeana* (Gesneriaceae). Oikos 96:470–480
40. Lara C, Ornelas JF (2002b) Flower mites and nectar production in six hummingbird-pollinated plants with contrasting flower longevities. Canadian Journal of Botany 80:1216–1229
41. Leonhardt KW, Criley RA (1999) Proteaceae Floral Crops: cultivar development and underexploited uses. In: Janick J (ed) Perspectives on new crops and new uses. ASHS Press, USA. pp.410–430
42. Littlejohn GM (2001) Genetic resource conservation in Proteaceae: limitations and challenges. ISHS Acta Horticulture 545:21–28

43. Maze KE, Bond WJ (1996) Are *Protea* populations seed limited? Implications for wildflower harvesting in Cape Fynbos. Austral Ecology 21:96-105
44. Maloof JE, Inouye DW (2000) Are nectar robbers cheaters or mutualists?. Ecology 81:2651–2661
45. Myburgh LC, Rust DJ, Starke LC (1973) Pests of protea cut-flowers. Journal of the Entomological Society of Southern Africa 36:251–255
46. Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GA, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403:853–858
47. Neal PR, Anderson GJ (2004) Does the 'old bag' make a good 'wind bag'?: comparison of four fabrics commonly used as exclusion bags in studies of pollination and reproductive biology. Annals of Botany 93:603–607
48. Nicolson SW, Fleming PA (2003) Nectar as food for birds: the physiological consequences of drinking dilute sugar solutions. Plant Systematics and Evolution 238:139–153
49. Nottebrock H (2016) Spatial and functional determinants of long-term fecundity in serotinous shrub communities. Doctoral dissertation, University of Hohenheim
50. Nottebrock H, Esler KJ, Schurr FM (2013) Effects of intra-specific and community density on the lifetime fecundity of long-lived shrubs. Perspectives in plant ecology, evolution and systematics 15:150–161
51. Osborne JW (2010) Improving your data transformations: Applying the Box-Cox transformation. Practical Assessment, Research & Evaluation 15:1–9
52. Paciorek CJ, Moyer BR, Levin RA, Halpern SL (1995) Pollen consumption by the hummingbird flower mite *Proctolaelaps kirmsei* and possible fitness effects on *Hamelia patens*. Biotropica 27:258–262
53. Proctor H, Owens I (2000) Mites and birds: diversity, parasitism and co-evolution. Trends in ecology & evolution 15:358–364

54. Ramsey M, Vaughton G (1991) Self-incompatibility, protandry, pollen production and pollen longevity in *Banksia menziesii*. Australian Journal of Botany 39:497–504

55. Rebelo AG (2006) Protea atlas project. http://protea.worldonline.co.za/default.htm (accessed 9 October 2017)

56. Rebelo AG, Rourke JP (1986) Seed germination and seed set in southern African Proteaceae: ecological determinants and horticultural problems. Acta Horticulturae 185:75–88

57. Rebelo T (2001) Proteas: A field guide to the Proteas of Southern Africa. Fernwood Press, Vlaeberg, South Africa

58. Robertson AW, Kelly D, Ladley JJ (2011) Futile selfing in the trees *Fuchsia excorticata* (Onagraceae) and *Sophora microphylla* (Fabaceae): inbreeding depression over 11 years. International Journal of Plant Sciences 172:191–198

59. Roets F, Crous PW, Wingfield MJ, Dreyer LL (2009) Mite-mediated hyperphoretic dispersal of *Ophiostma* spp. from the infructescences of South African *Protea* spp. Environmental Entomology 38:143–152

60. Roets F, Wingfield MJ, Crous PW, Dreyer LL (2007) Discovery of fungus-mite mutualism in a unique niche. Environmental Entomology 36:1–12

61. Roets F, Wingfield MJ, Crous PW, Dreyer LL (2013) Taxonomy and ecology of ophiostomatoid fungi associated with *Protea* infructescences. In: Seifert KA, De Beer ZW, Wingfield MJ (eds) The Ophiostomatoid Fungi: Expanding Frontiers. In: CBS Biodiversity Series 12. pp.177–187

62. Roets F, Wingfield MJ, Wingfield BD, Dreyer LL (2011) Mites are the most common vectors of the fungus *Gondwanamyces proteae* in *Protea* infructescences. Fungal biology 115:343–350

63. Royce LA, Krantz GW (1989) Observations on pollen processing by *Pneumolaelaps longanalis* (Acari: Laelapidae), a mite associate of bumblebees. Experimental and Applied Acarology 7:161–165

64. Ryke PAJ (1964) Acarina associated with *Protea* flowers in the Cape Province. Journal of the Entomological Society of Southern Africa, 26:337–354

65. Stanley RG, Linskens HF (eds) (1974) Pollen: biology, chemistry, management. Springer-Verslag, Berlin, New York

66. Steenhuisen SL, Johnson SD (2012) Evidence for autonomous selfing in grassland *Protea* species (Proteaceae). Botanical Journal of the Linnean Society 169:433–446

67. Steenhuisen SL, Van der Bank H, Johnson SD (2012) The relative contributions of insect and bird pollinators to outcrossing in an African *Protea* (Proteaceae). American journal of botany 99:1104–1111

68. Theron N (2011) Mite communities within *Protea* infructescences in South Africa. Masters dissertation, Stellenbosch University, Stellenbosch.

69. Theron N, Roets F, Dreyer LL, Esler KJ, Ueckermann EA (2012) A new genus and eight new species of *Tydeoidea* (Acari: Trombidiformes) from *Protea* species in South Africa. International Journal of Acarology 38:257–273

70. Theron-de Bruin N, Dreyer LL, Ueckermann EA, Wingfield MJ, Roets F (2017) Birds Mediate a Fungus-Mite Mutualism. Microbial Ecology https://doi.org/10.1007/s00248-017-1093-9

71. Van der Walt ID, Littlejohn GM (1996a) Pollen morphology, male hybrid fertility and pollen tube pathways in Protea. South African Journal of Botany 62:236–246

72. Van der Walt ID, Littlejohn GM (1996b) Stigma receptivity of two *Protea* cultivars in relation to the development of hybridization techniques. South African Journal of Botany 62:258–262

73. Van Staden J (1978) Seed viability in *Protea neriifolia*. I. The effects of time of harvesting on seed viability. Agroplantae 10:65–67

74. Van Wilgen BW, McDonald DJ (1992) The Swartboskloof experimental site. In: Van Wilgen BW, Richardson DM, Kruger FJ, Hensbergen HJ van (eds), Fire in South African mountain fynbos, Springer-Verlag Berlin Heidelberg. pp.1–20
75. Velázquez T, Ornelas JF (2010) Pollen consumption by flower mites in three hummingbird-pollinated plant species. Experimental and Applied Acarology 50:97–105
76. Wiens D (1984) Ovule survivorship, brood size, life history, breeding systems, and reproductive success in plants. Oecologia 64:47–53
77. Wright MG (1994). Seed production by *Protea laurifolia* (Proteaceae) after insect versus bird pollination: a quality difference?. Suid-Afrikaanse Tydskrif vir Wetenskap 90:199
78. Wright MG, Saunderson MD (1995) Protea plant protection: from the African context to the international arena. Acta Horticulturae 387:129–140
79. Wright MG, Visser D, Coetzee JH, Giliomee JH (1991) Insect and bird pollination of *Protea* species in the western Cape: Further data. Suid-Afrikaanse Tydskrif vir Wetenskap 87:214–215

CHAPTER 4

EFFECTS OF CULTIVATION OF AN INDIGENOUS CROP ON ASSOCIATED MITE ASSEMBLAGES

Acknowledgements

We thank the Department of Science and Technology/National Research Foundation Centre of Excellence in Tree Health Biotechnology and the Harry Crossley Foundation for financial support for this study as well as the Western Cape Nature Conservation board for issuing the necessary colleting permits. We would also like to extend our gratitude towards Prof. M. Kidd at the Centre for Statistical Consultation, Stellenbosch University for assistance with statistical analyses, Ms. D Saccaggi at the Plant Health Diagnostic Services (PHDS), Department of Agriculture, Forestry and Fisheries (DAFF) for the assistance with mite identification and Dr L. Hugo-Coetzee at the National Museum, for Oribatida mite identification. A special thanks to B. Moelich (Boesmanzicht), P. De Villers (Tamarak), R. Bailey (Flower Valley) and Alwyn and Alte Naude (Ben Lomond), Barry and Maggie Gibson (Heuningklip) and R. Middelmann (Honingklip) for allowing us to do fieldwork on their farms.

Abstract

Transformation of natural ecosystems for agriculture has devastating impacts on biodiversity. Exotic crops replace native vegetation and are intensively managed, which affects normal ecosystem functioning and decreases ecosystem services provided by native biodiversity. The cultivation of native crops may mediate some of these impacts, as they are often less intensively managed and native plants provide familiar niches for native organisms. *Protea* (Proteaceae), an internationally cultivated floricultural crop with high economic value and ecological importance, is harvested for export markets within both natural and cultivated systems in South Africa. A multitude of organisms are intimately involved in *Protea* ecology and other ecosystem processes, but many of these taxa (*e.g.* mites) are also considered pests and/or pose significant phytosanitary risks. Here we evaluate the impact of cultivation on the diversity of mites associated with *Protea repens* inflorescences, infructescences and the rhizosphere from natural and cultivated sites in the Cape Floristic Region of South Africa. Natural sites generally harboured richer and more abundant mite communities than cultivated sites, although this was only evident for mites associated with the rhizosphere or when *Protea* crops were intensively managed. Mite community assemblages differed between the different management types, localities and niches. More severe management actions had little effect on mite assemblages from infructescences, likely due to their long-distance dispersal via *Protea* pollinators. However, mite assemblages associated with the rhizosphere were severely impacted in all cultivated areas. These results indicate that cultivated native crops can house substantial native mite biodiversity, but important ecological processes performed by *e.g.* soil-dwelling mites may be hampered. It also shows that management strategies for pests are not effective in controlling mites associated with inflorescences, which may pose phytosanitary risks.

Introduction

Worldwide, human population growth continuously places more pressure on the natural environment by conversion of natural areas into other land use types such as urbanisation, mining and agriculture (Hooke et al. 2012). Today, about 47% of the earth's land surface has already been modified for agriculture and forestry, which undeniably has massive negative impacts on native biodiversity and ecosystem services around the world (Hooke et al. 2012). In general, agricultural crops are exotic and planted as monocultures. Therefore, in addition to replacing natural flora, it leaves very little habitat alternatives within the landscape for native fauna. This, combined by the overuse of pesticides, lead to a reduction in biodiversity within the landscape, and ultimately loss in ecological services (Kremen et al. 2002, Tscharntke et al. 2005).

Agricultural systems are intensively managed to keep it in a low successional state to control problem organisms such as weeds and pests, and has increased nutrient inputs in the form of fertilizers. This increase in nutrients, introduction of pests and diseases and water pollution can have detrimental effects on biodiversity (Swinton et al. 2007, Zhang et al. 2007, Power 2010). These negative effects on biodiversity have been documented for almost all taxa, including minute organisms such as mites (Andrén 1994, Perfecto et al. 1997, Witt & Samways 2004, Bedano et al. 2006). However, more sustainable farming practises that consider ecological principles can assist in groundwater recharge, increase pollination services, balance soil fertility and increase carbon sequestration (Swinton et al. 2007, Zhang et al. 2007, Power 2010). Incorporating ecological principals and/or planting native crops in native ranges often decreases the need for intensive management as native pests may be controlled by native predators and parasitoids (Tomich et al. 2011, Wezel et al. 2014, Sasa & Samways 2015). This, and the fact that native host plants are planted, also means that there is

still some familiar niches that the native biota can occupy, especially when these hosts are perennials (Gurr et al. 2003, Joubert et al. 2009).

Mites (Acari) are small, spider-like creatures closely related to other Arachnids. They are a diverse group of organisms with over 55 000 recognised species (estimated actual numbers of up to 1 000 000) (Krantz & Walter 2009) in six orders and 125 super families (Walter & Proctor 1999, Krantz & Walter 2009). Mites inhabit a fast variety of environments and are generally niche specialists, such as the communities associated with forest canopies, deep-sea vents, human skin and serotinous fruits (Walter & Proctor 1999, Krantz & Walter 2009, Roets et al. 2007, Theron et al. 2012). With such a diverse range of occupied habitats and niche specialisation, mites evolved to have a large variety of feeding habits (guilds) including fungivorous, nematophagous, phytophagous, predatory, parasitic and pollen- and nectarivorous (Walter et al. 1986, Roets et al. 2007, Krantz & Walter 2009, Martin et al. 2012, Theron-de Bruin et al. 2017). A large number of mite taxa are saprophytes, detritivores and microbivores that feed on dead and decaying organic material from plants, animals and microbes (Krantz & Walter 2009). These taxa are important decomposers within the environment and assist with soil enrichment and microstructure, and may even assist with nutrient provision to epiphytes within tree canopies (Behan-Pelletier & Walter 2000, Krantz & Walter 2009). In addition, various mites may also provide other ecological services such as predation of phytophagous mite and other invertebrate pests (Dicke & Sabelis 1988, Herren & Neuenschwander 1991, McMurtry & Croft 1997). Some Mesostigmata mites are, for example, extensively used as bio-control agents in agricultural systems (McMurtry et al. 2013). They also have great potential to serve as bio-indicators of environmental change (Beaulieu & Weeks 2007). In fact, the vast number of environments occupied, large number of different feeding guilds and their niche specificity and sensitivity, make mites the ideal taxon to use as bio-indicators (Gulvik 2007). For example, Oribatida are one of the most

diverse and abundant suborders of mites in soils, they have diverse feeding habits, have a long lifespan and low mobility. Obviously these traits make them very good bio-indicators for monitoring environmental change (Norton 1990, Behan-Pelletier 1999, Gulvik 2007).

Protea L. (Proteaceae) plants are extensively planted globally for the floricultural industry, with numerous hybrids produced for both the South African and international markets (Gerber & Hoffman 2014). *Protea* flowers develop into fruiting structures that are mostly retained on the plant until a fire event, after which seeds are released into the nutrient rich post-fire environment (Bond et al. 1984, Coetzee & Giliomee 1985, Rebelo 2001). Both flower (inflorescences) and fruit structures (infructescences) are commercially harvested in cultivated and natural populations (Coetzee et al. 2007). These often contain potential pest species (Myburgh et al. 1973, Myburgh & Rust 1975, Coetzee & Giliomee 1987, Wright 2002, Coetzee 1985, Wright & Saunderson 1995) that usually cause physical damage to infructescences, seeds and leaves, while others may transmit disease (Myburgh 1973, Coetzee & Latsky 1985, Wieczorek & Wright 2003). Within the cut-flower industry, the mere presence of arthropods is a phytosanitary problem that is often difficult to control (Hansen & Hara 1994, Reinten & Coetzee 2002, Reinten et al. 2011).

In addition to the high economic value of *Protea*, it is also of considerable ecological importance as numerous organisms utilize these plants for shelter, food and movement across the landscape. To date, studies have documented fungi (Marais & Wingfield 1994, Lee et al. 2005, Roets et al. 2006, 2007, 2012, 2013), insects (Coetzee & Giliomee 1985, 1987, Wright & Samways 1999, 2000, Zachariades & Midgley 1999), spiders (Coetzee et al. 1990, Zachariades & Midgley 1999, Roets et al. 2011) and mites associated with the inflorescences, infructescences and foliage of *Protea* plants (Roets et al. 2007, 2009, Theron-de Bruin et al. 2017). The few studies that investigated the *Protea*-rhizosphere niche mostly only targeted

bacteria (Stafford et al. 2005, Lamont & Pérez-Fernandez 2016), leaving the biotic composition of this niche poorly studied.

The recent discovery of the complex *Protea*-fungal-mite-bird symbioses (Theron-de Bruin et al. 2017) and the possible impact of mites on *Protea* pollination (Theron-de Bruin et al. 2017, Chapter 3) highlight the importance of investigating the diversity of mites and the factors that influence their communities within the *Protea* system. As a dominant taxon, *Protea* plays a vital role in normal ecological processes in natural systems. However, it is unclear how this role changes under cultivation. An estimated 75% of producers make use of chemical fertilizers, with 79% using pesticides (Conradie & Knoesen 2010). Despite this, cultivated Proteaceae can provide habitats for indigenous arthropods associated with inflorescences, infructescences and leaves and therefore add to the biodiversity value of these production landscapes (Sasa & Samways 2015). However, a study by Conradie & Knoesen (2010) indicated that though most *Protea* producers are aware of the biodiversity guidelines, they lack information regarding integrated pest management (IPM) practices that would promote the protection of beneficial organisms.

In the present study we assess the impact of agricultural practices on mite assemblages from *P. repens* (L.) L. inflorescences, infructescences and their rhizosphere. We hypothesise that cultivated plants would provide habitats for numerous native mite taxa, but mite assemblages would differ between natural and commercially grown *P. repens* populations in all of these niches.

Methods

Study area and design

We identified three study localities in the Western Cape Province of South Africa where natural and cultivated populations of *P. repens* **(Fig.1A)** occur in close proximity (**Fig.2**). At each locality, a natural site within a protected area (**Fig.1B**) (Piketberg, Tamarak farm (32°48'16.3"S 18°38'11.0"E), Kleinmond, Heuningklip farm (34°19'44.9"S 19°04'10.4"E) and Gansbaai, Flower valley farm (34°33'11.2"S 19°28'01.9"E)) and a nearby site where *P. repens* was cultivated (Piketberg, Boesmanzight farm (**Fig.1C**) (32°47'31.1"S 18°40'18.3"E), Kleinmond, Honingklip farm (**Fig.1D**) (34°17'27.5"S 19°08'03.5"E) and Gansbaai, Ben Lomond farm (**Fig.1E**) (34°32'44.9"S 19°30'44.4"E)) were selected no further than *ca.* 4-6 km apart (**Table 1**). At each site, 20 inflorescences (**Fig.1F**) at mid flowering stage (30-50% of individual flowers within inflorescences open), 20 infructescences (**Fig.1G**) (*ca.* 6-12 months old) and 10 soil samples from the rhizosphere were collected during August to November 2013. Initially we also collected 50 mature leaves per plant (n = 10) for assessing foliar mite communities, but mites were largely absent from leaves and leaves were therefore excluded from further study.

Inflorescences and infructescences were collected from randomly chosen plants (1 structure per plant that was *ca.* 10 m apart) in each population. Soil samples (250 ml, taken from the O horizon - excluding the O_i layer (leaf litter) (Sayer 2006)) (**Fig.1H**) were collected from the rhizosphere of 10 randomly chosen mature individual plants (10 years and older). Soil and plant structures were individually placed in brown paper bags and stored at 4ºC until further processing within a week after collection.

Figure 1: A) *Protea repens* mature plant with inflorescences and infructescences. B) *P. repens* in its natural environment. C) Cultivated *P. repens* biotope at Piketberg, D) Cultivated *P. repens* biotope at Kleinmond. E) Cultivated *P. repens* biotope at Gansbaai. F) Close-up of mature *P. repens* inflorescence. G) Close-up of *P. repens* infructescence. H) Soil surface above *P. repens* rhizosphere covered with litter.

Table 1: Sampling sites of *P. repens* populations assessed in this study, with indication of natural or commercial status and management intensity.

Locality	Status	Farming Practice
Piketberg, Tamarak Farm	Natural	n.a
Piketberg, Boesmanzight Farm	Cultivation	Direct pesticide control and intensive management
Kleinmond, Heuningklip Farm	Natural	n.a
Kleinmond, Honingklip Farm	Cultivation	Indirect pesticides from surrounding crops with less intensive management
Gansbaai, Flower Valley Farm	Natural	n.a
Gansbaai, Ben Lomond Farm	Cultivation	Indirect pesticides from surrounding crops with no management as the site will be rehabilitated

Collection of mites from inflorescences and infructescences followed methods described in Theron et al. (2012). Briefly, secateurs were used to open the structures by cutting them in half, whereafter the arthropods were shaken out onto a Petri-dish from where all mite individuals were collected with fine tweezers and stored in 70% ethanol until sorting. Soil-associated mites were extracted using Berlese funnels (Krantz & Walter 2009) with ethylene glycol (anti-freeze: AutoZone Chemicals, South Africa) as preservative, because ethanol evaporated too fast. After four days of extraction, 70% ethanol was added to the anti-freeze (1:1 ratio) and samples were stored at 4ºC until sorting of individuals.

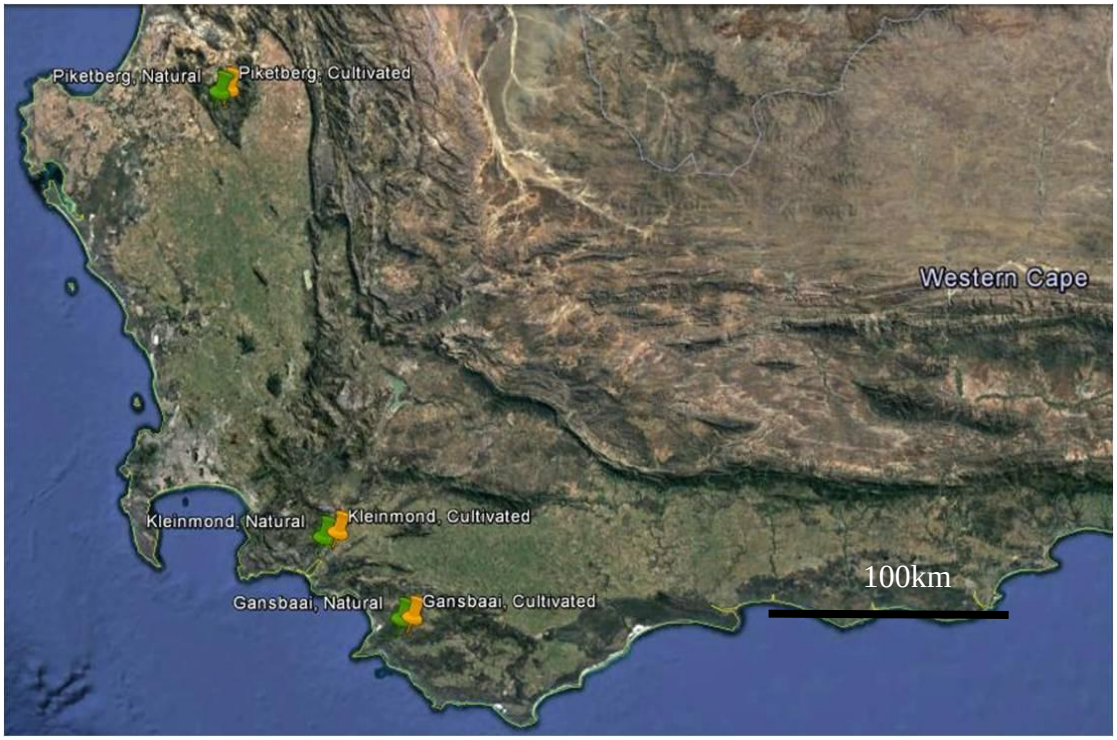

Figure 2: Map of the Western Cape Province of South Africa indicating three sites in protected areas (green) and three sites where *Protea repens* is commercially cultivated (yellow) that were used for the assessment of associated mites in the present study.

Mites from collected samples were sorted according to the morphospecies concept (Mayr 1996, Oliver & Beattie 1993, Hackman et al. 2017 (useful for biodiversity assessment)) and counted, where after representatives of mite morphospecies were mounted in HPVA medium (Krantz & Walter 2009) on microscope slides and examined using a Zeiss Axioskop Research microscope. Mites were identified to the lowest taxonomic rank possible using appropriate guides (Krantz & Walter 2009) and with the help of expert mite taxonomists (D. Saccaggi and Dr. Hugo-Coetzee). Reference material was deposited in the National Collection of Arachnida, ARC-Plant Protection Research Institute, Pretoria, South Africa, as well as in the Department of Conservation and Entomology Museum, Stellenbosch University, Stellenbosch, South Africa.

Statistical analyses

Mite communities were compared between the two biotopes (natural and cultivated), the three niche types (inflorescences, infructescences and soil) and the three sample localities (Piketberg, Kleinmond and Gansbaai). Diversity measures evaluated included: 1) alpha-diversity (α), including comparisons of mite morphospecies richness and abundance, 2) beta-diversity 1 (β1), as the changeover in mite community assemblage composition within a particular locality, biotope or niche (*i.e.* a measure of beta diversity within a sample type), and 3) beta-diversity 2 (β2), as comparisons in mite community assemblage composition between different localities, biotopes or niches (*i.e.* a measure of beta-diversity between different sample types) (Pryke et al. 2013).

Species richness was estimated using ICE, Chao2 and Jacknife2 (**Table 2**) in EstimateS TM v.7.5.2 (Colwell 2005, USA) for mite assemblages from each niche within each locality and biotope using 9999 randomizations of samples. These non-parametric and least biased species richness estimators provide the best overall estimates (Hortel et al. 2006). Generalized Linear Models (GLM) performed in Statistica 13 (StafSoft Inc, Tulsa, OK, USA) was used to test factor influence (locality, niche biotope) on alpha-diversity (species richness and mite abundance). Data sets where tested for normality using Shapiro-Wilk tests and Levene's Test for Homogeneity of Variances and hereafter, BoxCox transformed (Osborne 2010). For significant factors, a Games-Howell post hoc test was performed (calculated in R software (R Development Core Team 2013)). For β1, presence-absences data was used to calculate Jaccard similarity measures, which were used to evaluate the changeover in mite community structure within different localities, biotopes and niches (and the interactions between these factors) using permutational analyses of dispersion (PERMDISP) and 9999 permutations in PRIMER 6 (PRIMER-E 2008) (Anderson 2006, Pryke et al. 2013). For β2, Bray-Curtis

similarity measures using square root transformed abundance data (Anderson 2001)) were calculated to compare mite community assemblage structure between factors and their interactions using permutational analysis of variance (PERMANOVA) with 9999 permutations in PRIMER 6 (PRIMER-E 2008). Significant differences within and between factors are reported when $P \leq 0.05$. Community assemblage data were further explored and visualised using principal coordinate analysis (PCO) and canonical analysis of principal coordinates (CAP) (Anderson & Willis 2003).

Results

Overall, 4395 individuals from *ca.* 82 morphospecies (Mayr 1996) of mites were collected. Species estimates indicated that sampling was adequate to assess mite diversity in our samples (**Table 2**). All factors tested had a significant influence on mite species richness and abundance, except for locality (**Table 3**). Mite richness and abundance was highest in soils, then in infructescences, and both mite richness and abundance were the lowest in inflorescences (**Table 3**). However, all factors significantly interacted (**Table 3**, **Fig.3**). Piketberg stood out as particularly significant in terms of having much lower mite species richness and abundance in the cultivated biotope vs. the natural biotope (**Fig.3**). Mite species richness and abundance were higher within all natural niches compared to cultivated niches, but these differences were small in the cultivation site at Kleinmond (**Table 2**, **Fig.3**). Mite numbers in the infructescences and inflorescences changed only marginally between the two biotopes at this locality (**Fig.3**). At the Gansbaai locality, mite numbers were always lower in all niches when plants were in cultivation, but never significantly so (**Fig.3**).

Table 2: Observed and estimated species richness of mites associated with *Protea repens* from three different sites (Piketberg, Kleinmond and Gansbaai), two biotopes (natural and cultivated) and three niche types (inflorescences, infructescences and soil) in the Western Cape Province, South Africa.

Samples	Observed species	Total abundance	ICE*	Chao2** (±SD)	Jackknife2***
Inflorescences					
Natural all	11	606	12.26	11.66 (1.29)	12.05
Cultivated all	10	63	11.34	10.49 (1.28)	12.95
Piketberg natural	6	66	6.33	6 (0.53)	7.85
Piketberg cultivated	3	5	6.67	3.95 (2.02)	6.7
Kleinmond natural	7	495	10.29	11.28 (6.85)	11.7
Kleinmond cultivated	9	27	13.29	16.6 (11.1)	15.55
Gansbaai natural	6	45	6.4	6 (0.24)	7
Gansbaai cultivated	6	31	7.32	6.95 (2.12)	9.7
Infructescences					
Natural all	16	422	19.47	18.21 (3.34)	19.95
Cultivated all	15	290	16.79	16.47 (2.55)	19.87
Piketberg natural	11	135	11.93	11.95 (1.79)	12.99
Piketberg cultivated	5	49	6.48	6.71 (3.25)	7.55
Kleinmond natural	9	122	10.49	9.95 (2.16)	12.7
Kleinmond cultivated	7	150	7	7 (0.4)	6.15
Gansbaai natural	9	165	9.37	9 (0.54)	10.85
Gansbaai cultivated	9	91	10.88	9.63 (1.25)	10.14
Soil					
Soil natural all	52	2573	55.3	54.26 (2.48)	58.09
Soil cultivated all	25	442	40.69	54.24 (27.71)	44.1
Piketberg natural	19	688	19.84	19.45 (1.19)	21.69
Piketberg cultivated	12	182	13.14	12.45 (1.19)	14.69
Kleinmond natural	18	440	20.71	19.8 (2.63)	23.38
Kleinmond cultivated	16	131	24.39	23.35 (7.5)	25.77
Gansbaai natural	24	1444	24.99	24.13 (0.45)	23.13
Gansbaai cultivated	11	129	15.23	14.6 (4.8)	16.38

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

PERMDISP analyses indicated that the magnitude of changeover in mite assemblage composition differed within different niches and biotopes, but not for localities when overall assemblages were considered (**Table 3**). When considering niche, β1 was similar between inflorescences and infructescences, but these were significantly higher than for soil communities (**Table 3**). Cultivated areas had significantly higher β1 than natural areas when considering overall assemblages (**Table 3**). However, all factors significantly interacted (**Table 3**, **Figs.4**, **5**). When considering the interaction between niche and locality, Piketberg generally had higher β1 for infructescences and soil than the other localities, but inflorescences were similar (**Fig.4a**, **5**). This was largely due to significant higher β1 in the cultivated area at Kleinmond (**Fig.4b**) that had significant positive impacts on the mite assemblage turnover in inflorescences and soil (**Fig.5**). In general, however, mite assemblage turnover within inflorescences and infructescences increased due to cultivation and soil associated β1 diversity (**Fig.4c**). When investigating the interaction of all three factors, there is a general trend for less change in β1 diversity in inflorescences and infructescences from cultivated and natural sites (except at Kleinmond), with soil communities particularly significantly affected at Piketberg and Kleinmond (**Fig.5**).

Table 3: A summary of the effect niche type, locality type and biotope on alpha- and beta-diversity (within and between factors) have on mite assemblages associated with *P. repens*.

Variables	Df	x^2	P	Post hoc
Richness				
Niche type	2	130.97	**0.000**	S > Ifr > Ifl
Locality	2	1.61	0.202	KM = GB = PB
Biotope	1	53.83	**0.000**	N > C
Niche* Locality* Biotope	4	3.76	**0.005**	Fig.3
Abundance				
Niche type	2	104.82	**0.000**	S > Ifr > Ifl
Locality	2	2	0.137	KM = GB = PB
Biotope	1	52.3	**0.000**	N > C
Niche* Locality* Biotope	4	4.34	**0.002**	Fig.3
Variables- PERMDISP	Df	F	P	Post hoc
Beta-diversity 1 (β1)				
Niche type	2	4.98	**0.0115**	Ifr = Ifl > S
Locality	2	0.40	0.7084	KM = GB = PB
Biotope	1	16.93	**0.0001**	C > N
Niche*Locality	8	1.91	0.0973	Fig.4
Locality*Biotope	5	3.71	**0.0075**	Fig.4
Biotope*Niche	4	7.62	**0.0001**	Fig.4
Niche* Locality* Biotope	14	4.35	**0.0001**	Fig.5
Variables- PERMANOVA	Df	Pseudo F	P	Post hoc
Beta-diversity 2 (β2)				
Niche type	2	27.48	**0.0001**	All differ
Locality	2	5.32	**0.0001**	All differ
Biotope	1	10.47	**0.0001**	Both differ
Niche*Locality	4	4.71	**0.0001**	Table 3
Locality*Biotope	2	4.67	**0.0001**	Table 3
Biotope*Niche	1	7.51	**0.0001**	Table 3

Factors include niche types (soil = S, infructescences = Ifr and inflorescences = Ifl), locality (Piketberg = PB, Kleinmond = KM, Gansbaai = GB) and biotope (natural = N, cultivated = C).

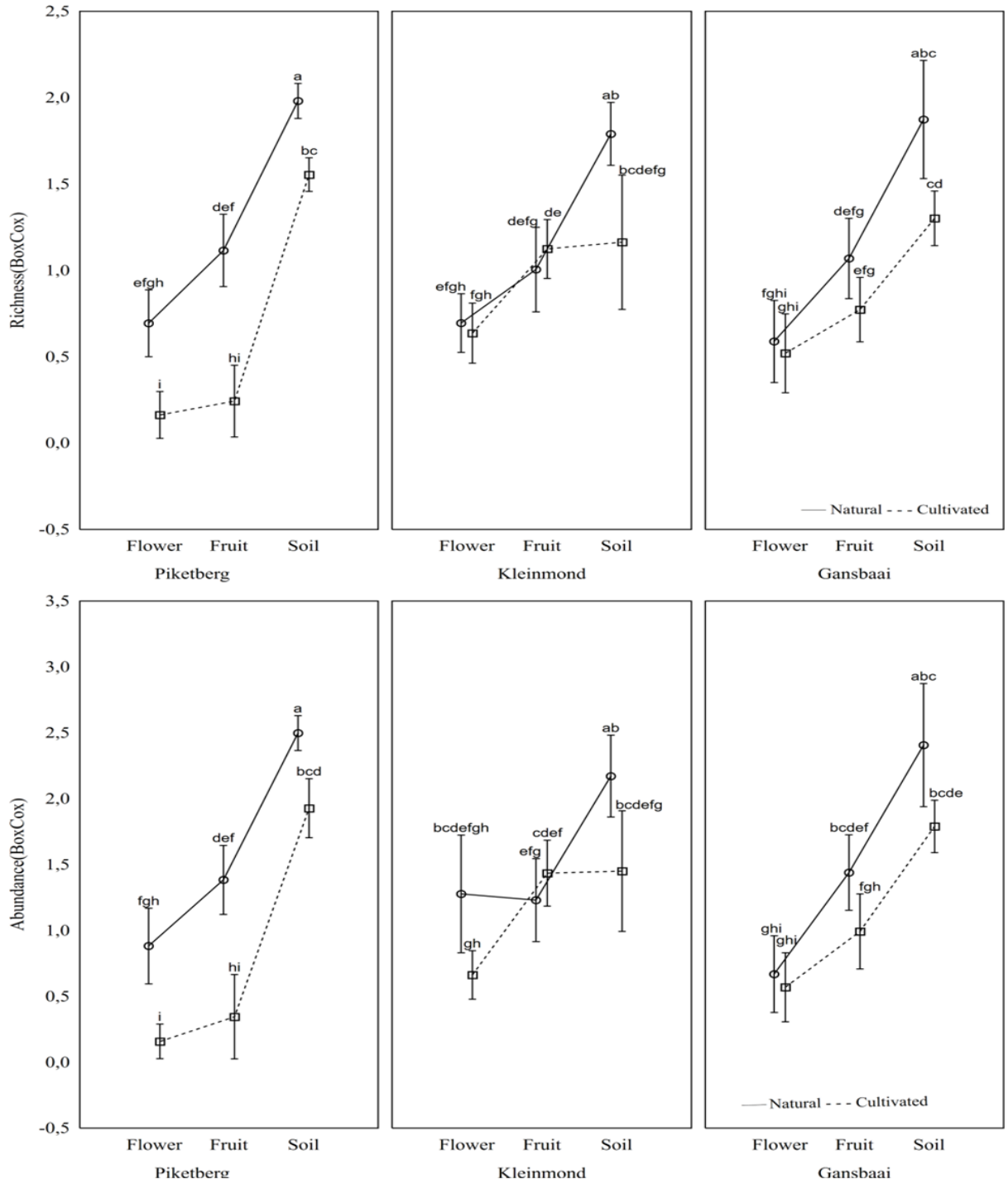

Figure 3: Comparisons between mean BoxCox transformed (± 95%) species richness (top) and abundance (bottom) (alpha-diversity (α)) between localities (Piketberg, Kleinmond, Gansbaai), biotopes (natural, cultivated) and niche types (Soil, Inflorescences = Flower, Inflorescences = Fruit). Different letters above bars indicate significantly different means ($P < 0.05$) (see Appendix 2 for Post hoc test results).

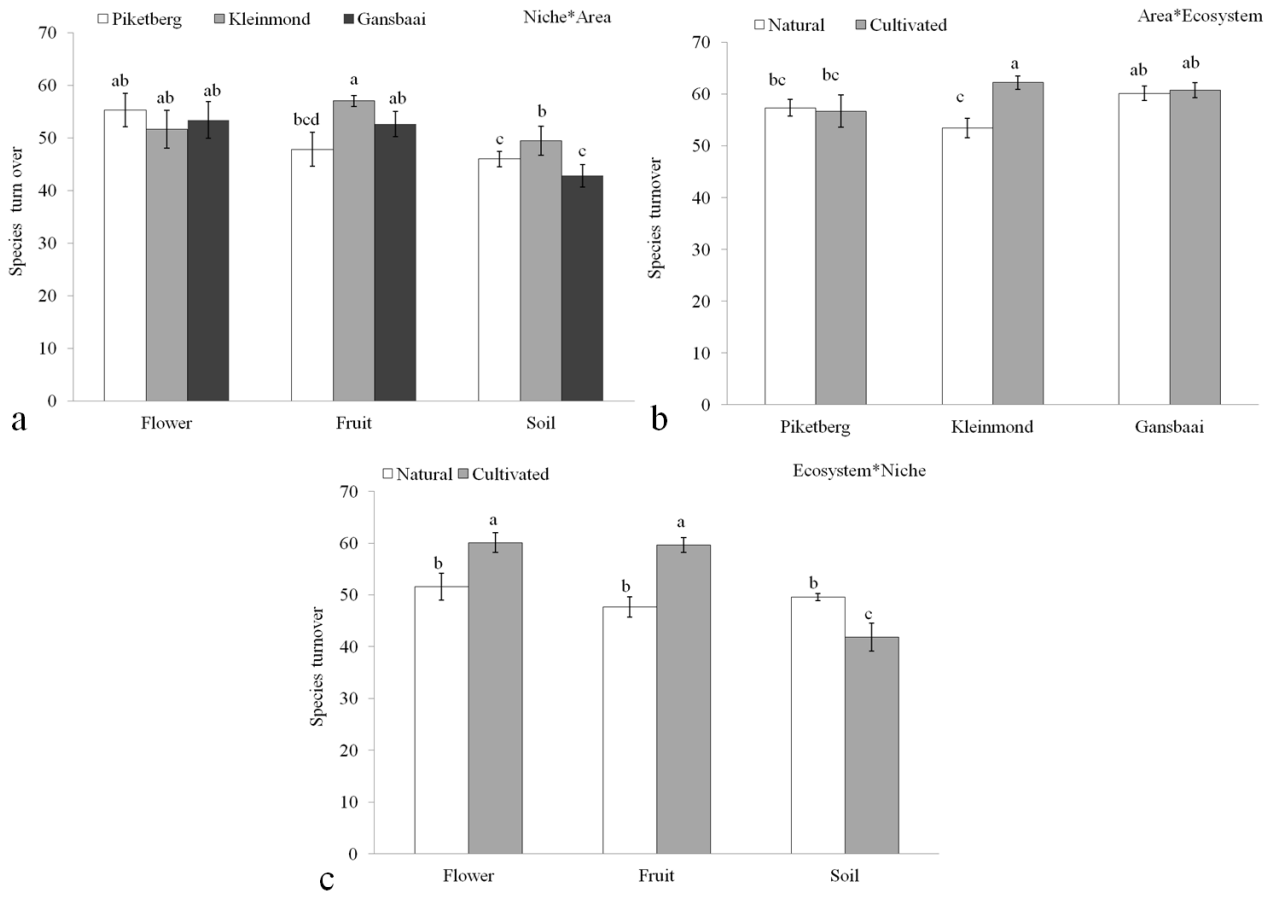

Figure 4: Between site comparisons for beta-diversity (β1) for the interaction: (a) locality*niche type, (b) locality*biotope type and (c) biotope*niche type. Mean (± SE). Letters above bars indicate significantly different means ($P < 0.05$). Inflorescences = Flower, Infructescences = Fruit.

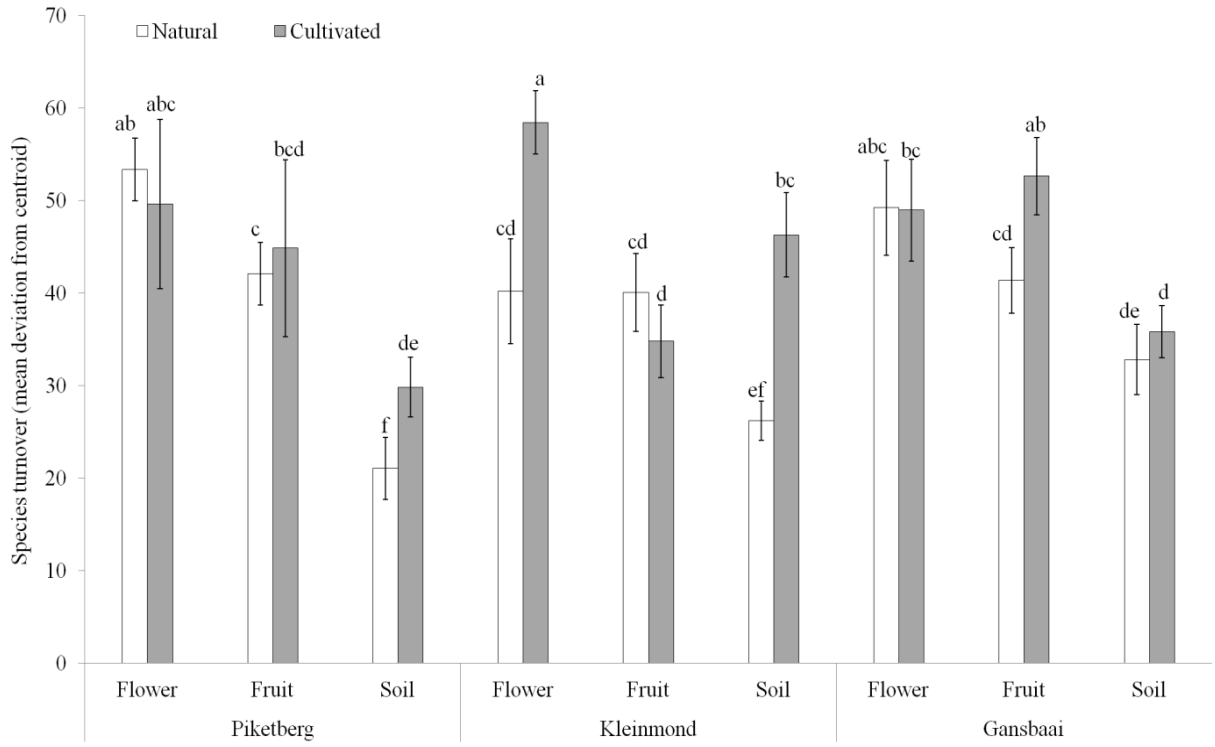

Figure 5: Pairwise comparisons of mite community assemblage composition (β1) for interactions within localities (Piketberg, Kleinmond, Gansbaai) and biotopes (natural = white, cultivated = grey) and niche types (Soil, Inflorescences = Flowers, Infructescences = Fruit). Comparisons of distance from the centroids (Mean, ± SE) within factors are presented. Letters above bars indicate significantly different means ($P < 0.05$).

PERMANOVA analyses indicated that mite assemblage composition was significantly different between nearly all factors tested (**Tables 3**, **4**). For the PCO, the main axis explained *ca.* 36% of the variation and is strongly associated with the separation of soil assemblages with those of inflorescences and infructescences (**Fig.6a**). The PCO2 axis explained 12% of the assemblage variations and separated inflorescences and infructescences (**Fig.6a**). Communities from natural and cultivated biotopes also separated, but did not form clusters, indicating that niche and locality had the largest influence on mite assemblages (**Fig.6b**). Communities from soil formed a more tightly grouped unit than assemblages from inflorescences or from infructescences, indicating overall less within-niche turnover (β1 diversity).

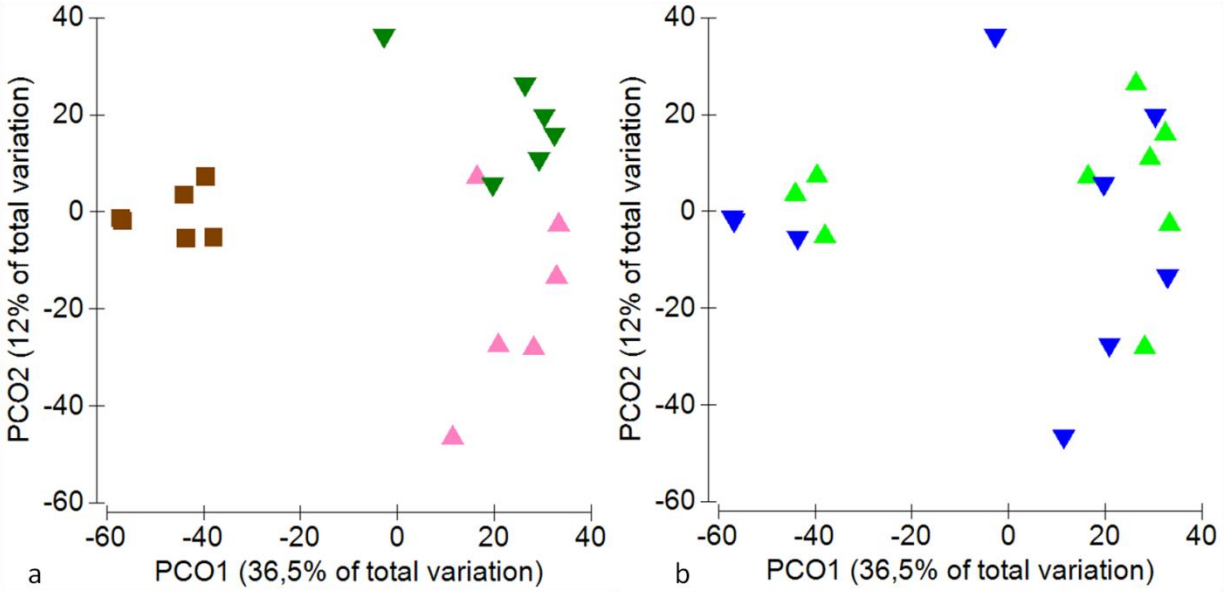

Figure 6: PCO mite assemblages with niche type in 'a' indicated by symbols (Inflorescences = pink triangle, Infructescences = green diamond and Soil = brown circle) and biotope in 'b' indicated by symbols (Natural = green and Cultivated = blue).

Similar to the PCO, CAP analyses with niche as main effect illustrated that soil mite assemblages were very dissimilar to those of inflorescences and infructescences, which also separated out (**Fig.7**). Main mite taxa that contributed to this result were the Oribatid mite *Anellozetes neonominatus* Mahunka and *Stigmaeidae sp.*1 for soil, the *Tarsonemidae sp.*1 and *Glycyphagidae sp.*1 for inflorescences, *Tydeidae sp.*1 and *Trichouropoda sp.*1 for inflorescences, and *Proctolaelaps vandenbergi* Ryke that was associated with both inflorescences and infructescences (**Fig.7**). In addition to dissimilarities in mite assemblages due to niche type, a second CAP analyses indicated some separation of communities based on location (Piketberg, Kleinmond and Gansbaai) strengthening previous results (**Fig.8**). Main mite taxa that contributed to this result were *Cunaxidae sp.*1 and *Cunaxidae sp.*2 from Kleinmond, the *Anystidae sp.*1 from Gansbaai and *Proctolaelaps vandenbergi* that was associated with both Gansbaai and Piketberg (**Fig.8**).

Table 4: Pairwise comparisons of mite community assemblage composition (β2) for interactions between localities (Piketberg, Kleinmond, Gansbaai), biotopes (natural, cultivated) and niche types (Soil, Inflorescences = Flowers, Infructescences = Fruit) calculated using PERMANOVAs. P < 0.05*, P < 0.01**, P < 0.001***

	Piketberg			Kleinmond			Gansbaai		
	Flower	Fruit	Soil	Flower	Fruit	Soil	Flower	Fruit	Soil
Piketberg									
Flower		1.91*	3.89***	1.91**			1.15		
Fruit			4.09***		2.52***			2.00**	
Soil						2.68***			3.00***
Kleinmond									
Flower					3.12***	3.52***	2.05***		
Fruit						4.41***		3.23***	
Soil									2.29***
Gansbaai									
Flower								2.54***	4.24***
Fruit									4.65***
Soil									

	Natural			Cultivated		
	Piketberg	Kleinmond	Gansbaai	Piketberg	Kleinmond	Gansbaai
Natural						
Piketberg		2.62***	2.33***	2.38***		
Kleinmond			2.68***		3.36***	
Gansbaai						1.91**
Cultivated						
Piketberg					1.92**	1.08
Kleinmond						2.52***
Gansbaai						

	Natural			Cultivated		
	Flower	Fruit	Soil	Flower	Fruit	Soil
Natural						
Flower		3.54***	5.08***	1.69*		
Fruit			6.24***		3.30***	
Soil						4.13***
Cultivated						
Flower					1.94**	4.30***
Fruit						4.37***
Soil						

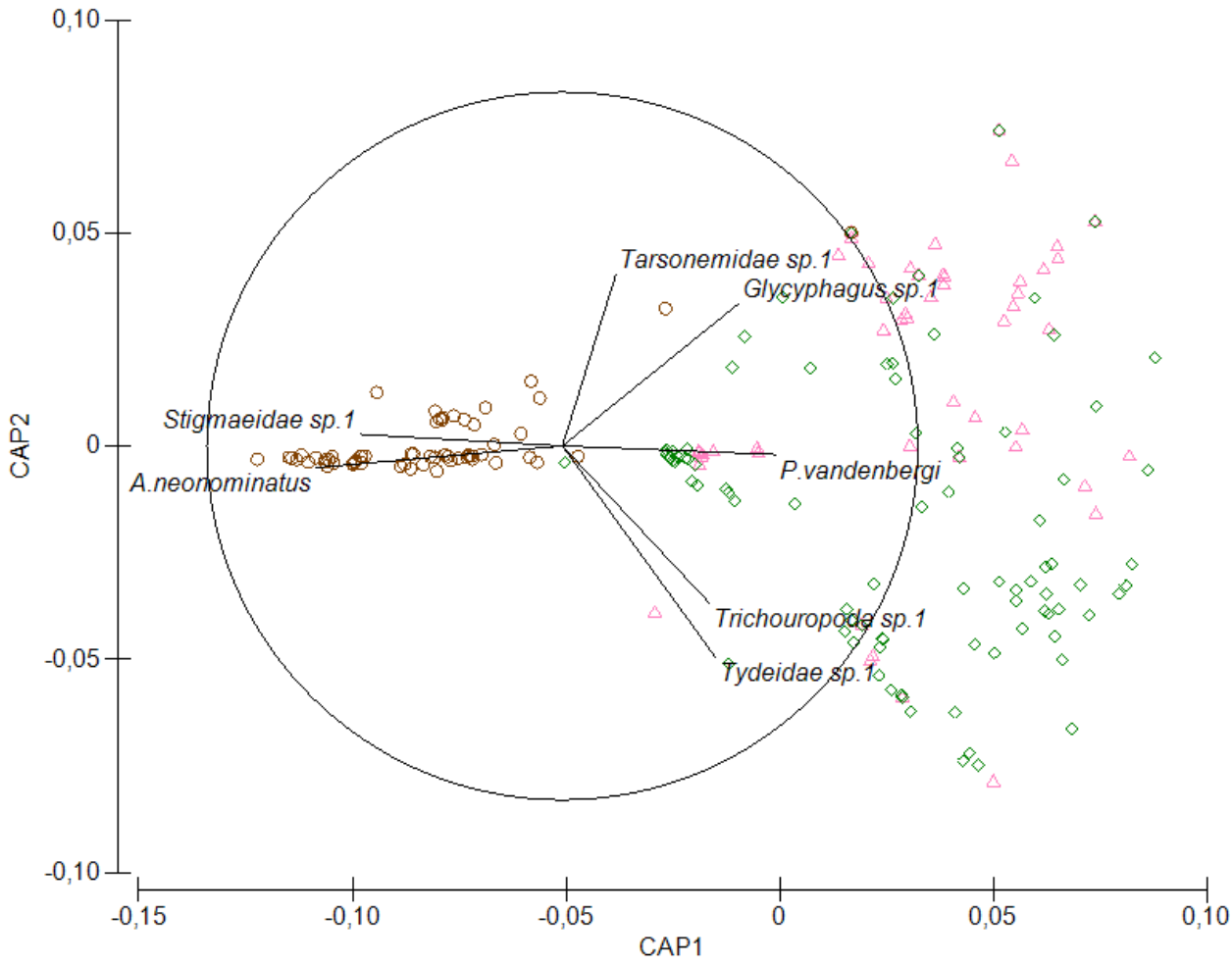

Figure 7: Canonical analysis of principal coordinates ordination (CAP) of the mite assemblages for three niche types (β2). Inflorescences = pink triangle, Infructescences green diamond and Soil = brown circle.

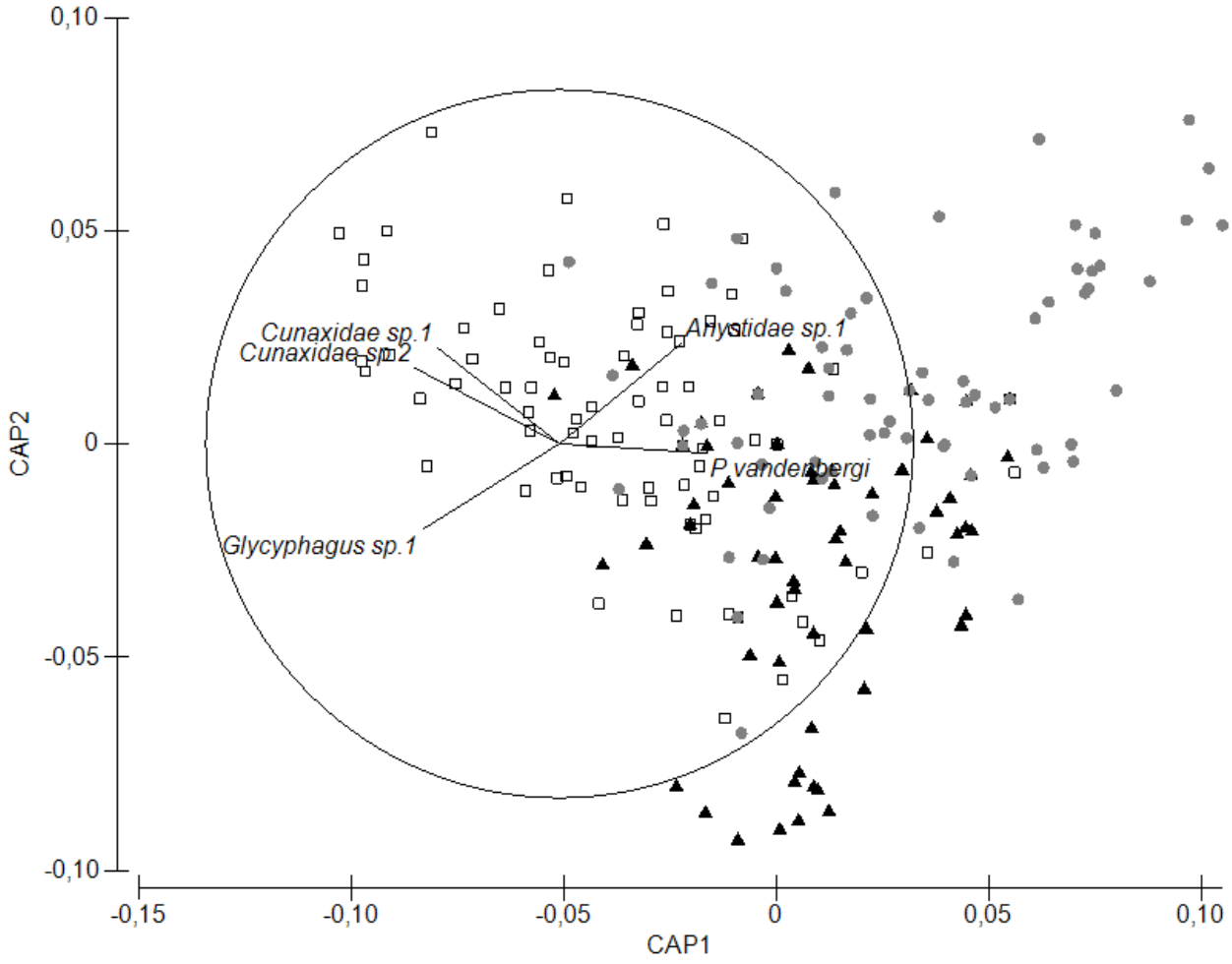

Figure 8: Canonical analysis of principal coordinates ordination (CAP) of the mite assemblages for three area types (β2). Piketberg = black triangle, Kleinmond = open square, Gansbaai = grey circle.

Discussion

There is a rich assemblage of mites associated with *Protea repens* in natural populations. All niches investigated differed in terms of their mite assemblage composition, with those from soil substantially different from mite assemblages associated with inflorescences and infructescences. In cultivated *Protea* stands, species numbers and abundance of mites reduced, but the magnitude of this reduction depended strongly on the intensity of management and potential exposure to pesticides. The assemblage structure of mites also

changed within all niches associated with these plants under cultivation. The changes were, however, the strongest for the particularly rich soil-associated assemblages (Appendix 3).

At Piketberg cultivation practises were most intense, with various pesticides and fertilizers applied to plants and the soil. At this site there is also limited diversity within the landscape, such that there were no natural stepping-stones or corridors in the form of other plants (Tscharntke et al. 2005). Despite this, the alpha-diversity in the natural area at this site was still comparable to the other natural sites sampled. Therefore, these changes in natural site conditions (higher elevation and drier climate in this case) may not have a large influence on mite alpha-diversity associated with *Protea* within the CFR. Interestingly, mite numbers within inflorescences at this site, even though reduced in comparison to those from the natural site, was not significantly different from the numbers of mites within the inflorescences from both natural and cultivated populations at other sites sampled. This indicates that, even though there is an intensive spraying regime, it is not sufficient to reduce mites associated with inflorescences to lower than expected levels in general. Most mites associated with inflorescences of *Protea* are likely phoretic on pollinators such as insects and birds (Roets et al. 2009, Theron-de Bruin et al. 2017). These pollinators are known to disperse the mites between *P. repens* stands over vast distances (>200 km based on population genetics of associated fungal species for some mites, Aylward et al. 2015) and could easily continuously introduce at least some mites (*e.g. Proctolaelaps vandenbergi* and *Glycyphagus sp.* 1) when these inflorescences are open, irrespective of spraying regime. Intense spraying of these plants therefore seems to have little effect on the mites from inflorescences.

Protea cultivation at Kleinmond was less intense in the sense that *Protea* pests are not directly controlled via spraying of insecticides. However, this site is surrounded by other crop species, including fruit trees that are regularly sprayed. Here wind can carry spray mists to the

neighbouring *Protea* stands, where these chemicals could affect mite assemblages outside the target areas. Even so, mite numbers on aboveground plant parts did not differ between the plants that are under cultivation and those from the nearby natural area. Even though not significantly so, the belowground mite numbers were the most negatively influenced, likely due to weed-control and other management practices. At Gansbaai, there was no contact with chemical sprays and the site was left for natural regeneration. Here, mite alpha-diversity was similar to the natural site, but soil-associated mites still tended to have the greatest reduction in numbers compared to other niches. This reduction in soil-mite assemblages likely has large negative effects on ecosystem services provided (Bedano et al. 2006).

The difference in mite assemblages between different niche types and areas were driven by a few prominent mite species. In terms of inflorescences, for example, *Proctolaelaps vandenbergi* were abundant within natural and cultivated systems. They are *Protea* pollen and nectar thieves (Theron-de Bruin et al. 2017, Chapter 3) and can vector fungi via a phoretic association with insects and birds (Roets et al. 2009, Theron-de Bruin et al. 2017). *Glycyphagus sp.*1 and *Tarsonemus sp.*1 are also mainly inflorescence-associated. *Glycyphagus* hypopi are phoretic on *Proctolaelaps vandenbergi* between *Protea* inflorescences and feed and reproduce on *Protea*-associated *Sporothrix* Hektoen & Perkins fungi that are very common within inflorescences (Theron-de Bruin et al. 2017). Similarly, *Tarsonemus sp.*1 mites are also phoretic on *Proctolaelaps vandenbergi*, but as far as we know, cannot feed on *Protea- Sporothrix* fungi (Theron-de Bruin et al. 2017). *Tarsonemus* mites in general can be either fungivorous, phytophagous, algivorous or polyphagous, but their food source in *Protea* inflorescences remains unknown (Krantz & Walter 2009). Both *Tydeidae sp.*1 and *Trichouropoda sp.*1 were mainly associated with infructescences from natural *Protea* populations. Neither of these mite taxa seem to physically harm the *Protea*

inflorescences in terms of floricultural quality, but their abundance within inflorescences may pose phytosanitary risks.

*Trichouropoda sp.*1 mites characterised *Protea* infructescences where they also feed and reproduce on *Protea*-*Sporothrix* fungi (Roets et al. 2007). These mites use *Protea*-pollinating insects such as beetles for dispersal and can therefore colonise *Protea* populations over large distances. The *Tydeidae sp.*1 mites were also commonly found within *Protea* infructescences, but their feeding habits and dispersal mechanisms are still unknown (Krantz & Walter 2009, Theron et al. 2012). As these structures are not usually commercially used in an unprocessed state, these mites may not be of any agricultural concern. However, as some inflorescence-associated mites such as *P. vandenbergi*, *Tarsonemus sp.*1 and *Glycyphagus sp.*1 are also found within these structures, albeit in much reduced numbers, *Protea* infructescences may be a reservoir for re-infestations of inflorescences by these species during the following flowering season.

The oribatid mites *Anellozetes neonominatus* and *Stigmaeidae sp.*1 characterised the *Protea* rhizosphere. Oribatida mites are generally soil associated and usually fungivorous or detritivores (Maraun et al. 2007, Krantz & Walter 2009, Theron 2011). These mites pose no major threats to agriculture and have traditionally been used as bio-indicators of soil health (Behan-Pelletier et al. 1999, Chandler et al. 2000, Krantz & Walter 2009). Similarly, Stigmaeidae mites are recognised as important predators of phytophagous pests and may be crucial in the control of some soil associated *Protea* pests (Nelson et al. 1973, Childers et al. 2001, Gerson et al. 2008). The reduction in numbers of these beneficial soil organisms at all cultivated sites may therefore be an indication of less than optimal soil conditions (Giller et al. 1997, Tsiafouli et al. 2015) and management practices should aim to reduce the impact on this

ecosystem service (Tilman 1999, Tomich et al. 2011, Tscharntke et al. 2012, Wezel et al. 2014).

In addition to the above-mentioned mite taxa that drove differences in mite assemblage structure between different niches, some mites were site specific. For example, *Anystidae sp.*1 mites were only found within infructescences in the natural biotope at Gansbaai. These macropredators are used in the control of various global pests such as *Anystis wallacei* Otto that pray on the lucerne flea (*Sminthurus viridis* L.) and the red-legged earth mite (*Halotydeus destructor* Tucker) in New Zealand (Bell & Willough 2003). *Anystis baccarum* L. feed on cattle tick (*Boophilus microplus* Can.) larvae in Indonesia and Australia (Holm & Wallace 1989) and the apple rust mite (*Aculus schlechtendali* Nalepa) in Ireland (Cuthbertson et al. 2003). Both *Cunaxidae sp.*1 and *Cunaxidae sp.*2 were associated only with infructescences at the cultivated Kleinmond site. These mites prey on a variety of arthropods and nematodes, but do not discriminate between pests and non-pests and therefore do not qualify as a good bio-control agent (Walter & Kaplan 1991). In addition, *Tydeidae sp.*2 and *Paratydeidae sp.*2 were only found in soils of the natural biotope at Piketberg. These, and numerous other similar examples, indicate that there is a high turnover of mite species assemblages between and within different sites and niches at these sites, as was indicated by PERMDISP and PERMANOVA analyses, despite the fact that all collections of mites in this study were from a single plant species.

Given the high number of mite species and their apparent sensitivity to ecosystem change detected in this study, mites, especially soil-associated taxa, would make good indicators for *Protea* cultivation system health, habitat quality and management intensity (Carignan & Villard 2002, Duelli & Obrist 2003, Gerlach et al. 2013). Indeed, various mite groups, especially Oribatida mites (Jamshidian et al. 2015, N'Dri et al. 2016), are regularly used as

bio-indicators (O'Neill et al. 2010), while others are useful for the biological control of pests. However, in terms of *Protea*, the feeding habits of the associated mites would first need to be determined before they could be considered as viable control options (Beaulieu & Weeks 2007). Most importantly, though mites can be important indicators of ecosystem health, they can be very difficult to correctly identify without the help of trained experts (Gerlach et al. 2013). This became evident in this study with the Oribatida, where numerous morphospecies were subsequently found to contain more than one species after identification by experts. This taxonomic hurdle needs urgent attention, not only in South Africa, but worldwide, if significant progress into the understanding of the ecological role of mites is to be made.

To conclude, results of this study indicate that cultivated indigenous plant species may be suitable to host natural biodiversity to some level, but that this depends strongly on cultivation practices. In addition, control of mite numbers within inflorescences and infructescences within cultivated systems, no matter what the level of management, does not seem to be effective. In contrast, these practices seem to affect soil biota negatively even with minimal management of these systems. Reliance on post-harvest treatments of inflorescences intended for export markets will therefore remain essential. A variety of post-harvesting treatments are currently available (Jamieson et al. 2009), but they are still inadequate to rid fresh plant material from mites without damaging the inflorescences and infructescences (Coetzee et al. 2007). Therefore, future studies are required to investigate improved treatments or to develop new post-harvest treatments.

References

1. Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecology 26:32–46
2. Anderson MJ (2006) Distance-based tests for homogeneity of multivariate dispersions. Biometrics 62:245–253
3. Anderson MJ, Willis TJ (2003) Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology 84:511–525
4. Andrén H (1994) Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. Oikos 71:355–366
5. Aylward J, Dreyer LL, Steenkamp ET, Wingfield MJ, Roets F (2015) Long-distance dispersal and recolonization of a fire-destroyed niche by a mite-associated fungus. Fungal biology 119:245–256
6. Beaulieu F, Weeks AR (2007) Free-living mesostigmatic mites in Australia: their roles in biological control and bioindication. Australian Journal of Experimental Agriculture 47:460–478
7. Bedano JC, Cantú MP, Doucet ME (2006) Influence of three different land management practices on soil mite (Arachnida: Acari) densities in relation to a natural soil. Applied Soil Ecology 32:293–304
8. Behan-Pelletier VM (1999) Oribatid mite biodiversity in agroecosystems: role for bioindication. Agriculture, Ecosystems & Environment 74:411–423
9. Behan-Pelletier VM, Walter DE (2000) Biodiversity of oribatid mites (Acari: Oribatida) in tree canopies and litter. In: Coleman DC, Hendrix PF (eds) Invertebrates as webmasters in ecosystems, CABI Publishing, Wallingford, UK. pp.187–202
10. Bell NL, Willoughby BE (2003) A review of the role of predatory mites in the biological control of lucerne flea, *Sminthurus viridis* (L.) (Collembola: Sminthuridae) and their potential use in New Zealand. New Zealand Journal of Agricultural Research 46:141–146

11. Bond WJ, Volk J, Viviers M (1984) Variation in seedling recruitment of Cape Proteaceae after fire. The Journal of Ecology 1:209–21.
12. Carignan V, Villard MA (2002) Selecting indicator species to monitor ecological integrity: a review. Environmental monitoring and assessment 78:45–61
13. Chandler D, Davidson G, Pell JK, Ball BV, Shaw K, Sunderland KD (2000) Fungal biocontrol of Acari. Biocontrol Science and Technology 10:357–384
14. Childers CC, Villanueva R, Aguilar H, Chewning R, Michaud JP (2001) Comparative residual toxicities of pesticides to the predator *Agistemus industani* (Acari: Stigmaeidae) on citrus in Florida. Experimental and Applied Acarology 25:461–474
15. Coetzee JH, Dippenaar-Schoeman A.S, Berg A (1990) Spider assemblages on five species of proteaceous plants in the fynbos biome of South Africa. Phytophylactica 22:443–447
16. Coetzee JH, Giliomee JH (1985) Insects in association with the inflorescence of *Protea repens* (L.)(Proteaceae) and their role in pollination. Journal of the Entomological Society of southern Africa 48:303–314
17. Coetzee JH, Giliomee JH (1987) Borers and other inhabitants of the inflorescences and infructescences of Protea repens in the Western Cape. Phytophylactica 19:1–6
18. Coetzee JH, Latsky LM (1985) Faunal list of *Protea repens*. In: I International Protea Research Symposium 185:241–246
19. Coetzee JH, Littlejohn GM, Janick, J (2007) *Protea*: a floricultural crop from the Cape Floristic Kingdom. Scripta Horticulturae 5:77–112
20. Colwell RK (2005) EstimateS: Statistical estimation of species richness and shared species from samples. Version 7.5. Persistent URL < purl.oclc.org/estimates>
21. Conradie B, Knoesen H (2010) A survey of the cultivation and wild harvesting of fynbos flowers in South Africa (No. 1). Report. pp.1–19
22. Cuthbertson AGS, Bell AC, Murchie AK (2003) Impact of the predatory mite *Anystis baccarum* (Prostigmata: Anystidae) on apple rust mite *Aculus schlechtendali* (Prostigmata:

Eriophyidae) populations in Northern Ireland Bramley orchards. Annals of applied biology 142:107–114

23. Dicke M, Sabelis MW, De Jong M (1988) Analysis of prey preference in phytoseiid mites by using an olfactometer, predation models and electrophoresis. Experimental and Applied Acarology 5:225–241
24. Duelli P, Obrist MK (2003) Biodiversity indicators: the choice of values and measures. Agriculture, ecosystems & environment 98:87–98
25. Gerber AI, Hoffman EW (2014) International Protea Association and currant global Proteaceae production: Achievements and challenges. Acta Horticulturae 1031:17–28
26. Gerlach J, Samways M, Pryke J (2013) Terrestrial invertebrates as bioindicators: an overview of available taxonomic groups. Journal of insect Conservation 17:831–850
27. Gerson U, Smiley RL, Ochoa R (2008) Mites (Acari) for pest control. John Wiley & Sons. New York. pp.667–756
28. Giller KE, Beare MH, Lavelle P, Izac AM, Swift MJ (1997) Agricultural intensification, soil biodiversity and agroecosystem function. Applied soil ecology 6:3–16
29. Gulvik M (2007) Mites (Acari) as indicators of soil biodiversity and land use monitoring: a review. Polish Journal of Ecology 55:415–440
30. Gurr GM, Wratten SD, Luna JM (2003) Multi-function agricultural biodiversity: pest management and other benefits. Basic and Applied Ecology 4:107–116
31. Hackman KO, Gong P, Venevsky S (2017) A rapid assessment of landscape biodiversity using diversity profiles of arthropod morphospecies. Landscape Ecology 32:209–223
32. Hansen JD, Hara AH (1994) A review of postharvest disinfestation of cut flowers and foliage with special reference to tropicals. Postharvest Biology and Technology 4:193–212
33. Herren HR, Neuenschwander P (1991) Biological control of cassava pests in Africa. Annual Review of Entomology 36:257–283

34. Holm E, Wallace MMH (1989) Distribution of some anystid mites (Acari: Anystidae) in Australia and Indonesia and their role as possible predators of the cattle tick, *Boophilis microplus* (Acari: Ixodidae). Experimental and Applied Acarology 6:77–83
35. Hooke RL, Martín-Duque JF, Pedraza J (2012) Land transformation by humans: a review. GSA today 22:4–10
36. Hortel J, Borges PV, Gaspar C (2006) Evaluating the performance of species richness estimators: sensitivity to sample grain size. Journal of Animal Ecology 75:274–287
37. Jamieson LE, Meier X, Page B, Zulhendri F, Page-Weir N, Brash D, McDonald RM, Stanley J, Woolf AB (2009) A review of postharvest disinfestation technologies for selected fruits and vegetables. Sustainable Farming Funding Project, Horticulture New Zealand Inc. pp.1–11
38. Jamshidian MK, Saboori A, Akrami MA, Van Straalen NM (2015) Oribatid mite communities in contaminated soils nearby a lead and zinc smelting plant in Zanjan, Iran. Systematic and Applied Acarology 20:251–262
39. Joubert L, Esler KJ, Privett SDJ (2009) The effect of ploughing and augmenting natural vegetation with commercial fynbos species on the biodiversity of Overberg Sandstone fynbos on the Agulhas Plain, South Africa. South African Journal of Botany 75:526–531
40. Krantz G, Walter D (eds) (2009) A manual of Acarology. 3rd Edition. Texas Tech University Press, Lubbock, Texas, USA
41. Kremen C, Williams NM, Thorp RW (2002) Crop pollination from native bees at risk from agricultural intensification. Proceedings of the National Academy of Sciences 99:16812–16816
42. Lamont BB, Pérez-Fernández M (2016) Total growth and root-cluster production by legumes and proteas depends on rhizobacterial strain, host species and nitrogen level. Annals of botany 118:725–732
43. Lee S, Roets F, Crous PW (2005) Biodiversity of saprobic microfungi associated with the infructescences of *Protea* species in South Africa. Fungal Diversity 19:69–78

44. Marais GJ, Wingfield MJ (1994) Fungi associated with infructescences of *Protea* species in South Africa, including a new species of *Ophiostoma*. Mycological Research 98:369–374
45. Maraun M, Schatz H, Scheu S (2007) Awesome or ordinary? Global diversity patterns of oribatid mites. Ecography 30:209–216
46. Martin SJ, Highfield AC, Brettell L, Villalobos EM, Budge GE, Powell M, Nikaido S, Schroeder DC (2012) Global honey bee viral landscape altered by a parasitic mite. Science 336:1304–1306
47. Mayr E (1996) What is a species, and what is not?. Philosophy of science 63:262–277
48. McMurtry JA, Croft BA (1997) Life-styles of phytoseiid mites and their roles in biological control. Annual review of entomology 42:291–321
49. McMurtry JA, De Moraes GJ, Sourassou NF (2013) Revision of the lifestyles of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. Systematics and Applied Acarology 18:297–320
50. Myburg AC, Rust DJ (1975) A survey of pests of the Proteaceae in the Western and Southern Cape Province. Journal of the Entomological Society of southern Africa 38:55–60
51. Myburgh LC, Rust DJ, Starke LC (1973) Pests of protea cut-flowers. Journal of the Entomological Society of Southern Africa 36:251–255
52. N'Dri JK, Hance T, André HM, Lagerlöf J, Tondoh JE (2016) Microarthropod use as bioindicators of the environmental state: case of soil mites (Acari) from Côte d'Ivoire. Journal of Animal &Plant Sciences 29:4622–4637
53. Nelson EE, Croft BA, Howitt AJ, Jones AL (1973) Toxicity of Apple Orchard Pesticides to *Agistemus fleschneri*. Environmental entomology 2:219–222
54. Norton RA (1990) Acarina: Oribatida In: Wiley J, Dindal DL (ed) Soil biology guide. John Wiley & Sons, Toronto. pp.779–803
55. Oliver I, Beattie AJ (1993) A possible method for the rapid assessment of biodiversity. Conservation biology 7:562–568

56. O'Neill KP, Godwin HW, Jiménez-Esquilín AE, Battigelli JP (2010) Reducing the dimensionality of soil microinvertebrate community datasets using Indicator Species Analysis: Implications for ecosystem monitoring and soil management. Soil Biology and Biochemistry 42:145–154
57. Osborne JW (2010) Improving your data transformations: Applying the Box-Cox transformation. Practical Assessment, Research & Evaluation 15:1–9
58. Perfecto I, Vandermeer J, Hanson P, Cartín V (1997) Arthropod biodiversity loss and the transformation of a tropical agro-ecosystem. Biodiversity and conservation 6:935–945
59. Power AG (2010) Ecosystem services and agriculture: tradeoffs and synergies. Philosophical Transactions of the Royal Society of London B: Biological Sciences 365:2959–2971
60. Pryke JS, Roets F, Samways MJ (2013) Importance of habitat heterogeneity in remnant patches for conserving dung beetles. Biodiversity Conservation 22:2857–2873
61. Rebelo T (2001) SASOL Proteas: A Field Guide to the Proteas of South Africa. 2nd Edition, Fernwood Press (Pty) Ltd., Vlaeberg, Cape Town, South Africa
62. Reinten E, Coetzee JH (2002) Commercialization of South African indigenous crops: aspects of research and cultivation of products. In: Janick J, Whipkey A (eds) Trends in new crops and new uses, ASHS Press, Alexandria, VA.
63. Reinten EY, Coetzee JH, Van Wyk BE (2011) The potential of South African indigenous plants for the international cut flower trade. South African Journal of Botany 77:934–946
64. Roets F, Crous PW, Wingfield MJ, Dreyer LL (2009) Mite-mediated hyperphoretic dispersal of *Ophiostma* spp. from the infructescences of South African *Protea* spp. Environmental Entomology 38:143–152
65. Roets F, De Beer ZW, Dreyer LL, Zipfel R, Crous PW, Wingfield MJ (2006) Multi-gene phylogeny for *Ophiostoma* spp. reveals two new species from *Protea* infructescences. Studies in Mycology 55:199–212

66. Roets F, Theron N, Wingfield MJ, Dreyer LL (2012) Biotic and abiotic constraints that facilitate host exclusivity of *Gondwanamyces* and *Ophiostoma* on *Protea*. Fungal biology 116:49–61

67. Roets F, Wingfield MJ, Crous PW, Dreyer LL (2007) Discovery of fungus-mite mutualism in a unique niche. Environmental Entomology 36:1226–1237

68. Roets F, Wingfield MJ, Crous PW, Dreyer LL (2013) Taxonomy and ecology of ophiostomatoid fungi associated with *Protea* infructescences. In: Seifert KA, De Beer ZW, Wingfield MJ (eds) The Ophiostomatoid Fungi: Expanding Frontiers. In: CBS Biodiversity Series 12. pp.177–187

69. Roets F, Wingfield MJ, Wingfield BD, Dreyer LL (2011) Mites are the most common vectors of the fungus *Gondwanamyces proteae* in *Protea* infructescences. Fungal Biology 115:343–350

70. Sasa A, Samways MJ (2015) Arthropod assemblages associated with wild and cultivated indigenous proteas in the Grabouw area, Cape Floristic Region. African Entomology 23:19–36.

71. Sayer EJ (2006). Using experimental manipulation to assess the roles of leaf litter in the functioning of forest ecosystems. Biological reviews 81:1–31

72. Stafford WH, Baker GC, Brown SA, Burton SG, Cowan DA (2005) Bacterial diversity in the rhizosphere of Proteaceae species. Environmental Microbiology 7:1755–1768

73. Swinton SM, Lupi F, Robertson GP, Hamilton SK (2007) Ecosystem services and agriculture: cultivating agricultural ecosystems for diverse benefits. Ecological Economics 64:245-252

74. Theron N (2011) Mite communities within *Protea* infructescences in South Africa, Master dissertation, Stellenbosch University, Stellenbosch

75. Theron-de Bruin N, Dreyer LL, Ueckermann EA, Wingfield MJ, Roets F (2017) Birds Mediate a Fungus-Mite Mutualism. Microbial Ecology https://doi.org/10.1007/s00248-017-1093-9

76. Theron N, Roets F, Dreyer LL, Esler KJ, Ueckermann EA (2012) A new genus and eight new species of *Tydeoidea* (Acari: Trombidiformes) from *Protea* species in South Africa. International Journal of Acarology 38:257–273

77. Tilman D (1999) Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. Proceedings of the National Academy of Sciences 96:5995–6000

78. Tomich TP, Brodt S, Ferris H, Galt R, Horwath WR, Kebreab E, Leveau JH, Liptzin D, Lubell M, Merel P, Michelmore R (2011) Agroecology: a review from a global-change perspective. Annual Review of Environment and Resources 36:193–222

79. Tscharntke T, Clough Y, Wanger TC, Jackson L, Motzke I, Perfecto I, Vandermeer J, Whitbread A (2012) Global food security, biodiversity conservation and the future of agricultural intensification. Biological conservation 151:53–59

80. Tscharntke T, Klein AM, Kruess A, Steffan-Dewenter I, Thies C (2005) Landscape perspectives on agricultural intensification and biodiversity–ecosystem service management. Ecology letters 8:857–874

81. Tsiafouli MA, Thébault E, Sgardelis SP, Ruiter PC, Putten WH, Birkhofer K, Hemerik L, Vries FT, Bardgett RD, Brady MV, Bjornlund L (2015) Intensive agriculture reduces soil biodiversity across Europe. Global change biology 21:973–985

82. Walter DE, Hudgens RA, Freckman DW (1986) Consumption of nematodes by fungivorous mites, *Tyrophagus* spp. (Acarina: Astigmata: Acaridae). Oecologia 70:357–361

83. Walter DE, Kaplan DT (1991) Observations on *Coleoscirus simplex* (Acarina: Prostigmata), a predatory mite that colonizes greenhouse cultures of rootknot nematode (*Meloidogyne* spp.), and a review of feeding behavior in the Cunaxidae. Experimental and Applied Acarology 12:47–59

84. Walter DE, Proctor HC (1999) Mites: ecology, evolution and behaviour. UNSW Press, Sydney

85. Wezel A, Casagrande M, Celette F, Vian JF, Ferrer A, Peigné J (2014) Agroecological practices for sustainable agriculture. A review. Agronomy for sustainable development 34:1–20

86. Wieczorek A, Wright M (2003) PCR detection of phytoplasma from Witchesbroom decease on *Protea* spp. (Proteaceae) and associated arthropods. In: VI International Protea Research Symposium 602:161–166

87. Witt ABR, Samways MJ (2004) Influence of agricultural land transformation and pest management practices on the arthropod diversity of a biodiversity hotspot, the Cape Floristic Region, South Africa. African Entomology 12:89–95

88. Wright M (2002) Insect pests of Proteaceae: assessment of predictions of new pests internationally, and management implications. In: VI International Protea Research Symposium 602:167–171

89. Wright MG, Samways MJ (1999) Plant characteristics determine insect borer assemblages on *Protea* species in the Cape Fynbos, and importance for conservation management. Biodiversity and Conservation 8:1089–1100

90. Wright MG, Samways MJ (2000) Biogeography and species richness of endophagous insects associated with Proteaceae in South Africa. African Journal of Ecology 38:16–22

91. Wright MG, Saunderson MD (1995) *Protea* plant protection: from the African context to the international arena. In: III International Protea Research Symposium 387:129–140

92. Zachariades C, Midgley JJ (1999) Extrafloral nectaries of South African Proteaceae attract insects but do not reduce herbivory. African Entomology 7:67–76

93. Zhang W, Ricketts TH, Kremen C, Carney K, Swinton, SM (2007) Ecosystem services and dis-services to agriculture. Ecological economics 64:253–260

CHAPTER 5

CONCLUDING REMARKS

To understand the ecology of mites within the *Protea* system a little better, I investigated their role as pollinators, antagonists to pollination, fungal vectors, fungal mutualists, commensialists of other mites, commensialists of birds and evaluated mite community change as a factor of agricultural disturbance. This dissertation provides the first evidence that Cape sugarbirds are vectors of the *Protea*-associated flower mites, *Proctolaelaps vandenbergi* Ryke. In addition, *Protea*-associated *Glycyphagus* and *Tarsonemus* mites have tailored their own hyperphoretic relationship within this system. The smaller *Glycyphagus* and *Tarsonemus sp.*1 mites climb onto the opisthosoma (lower backs) of the much larger *P. vandenbergi*. The *P. vandenbergi* mites accumulate in their thousands at the top of open inflorescences, awaiting pollinators for dispersal. As a bird inserts its beak into the inflorescence to reach for nectar, the *P. vandenbergi* mites climb onto the beak and breast area of these birds and are dispersed to the next suitable host. Here the *P. vandenbergi* mites will disembark with their phoretic *Glycyphagus* partners. The *Glycyphagus* mites will inoculate these new inflorescences with their mutualistic fungal partners.

My research provides proof for the very early colonization of *Protea* inflorescences by *Sporothrix* spp. fungi at a stage when all the tissues inside the inflorescence are still alive. Exactly what the effect of these fungi are on their *Protea* hosts remain unknown. As the fungi were first described form *Protea* infructescences that mostly consist of dead flowering material, it was initially assumed that they are saprobes and do not play a large role in *Protea* ecology (Roets et al. 2012). However, the presence of these fungi at the onset of the flowering

stage may mean that they could have some negative effects on *Protea* seed formation, especially if it can be shown that they can infect living plant tissues. This may be another reason for the very low seed set of *Protea* in general (Collins & Rebelo 1987) and needs to be evaluated in future studies. Even if these fungi only use the nectar sugars within these plants as nutritional source, their presence and metabolic actions may change the quality of the nectar, which could have unknown impacts on pollinators. It has been shown that certain yeast species colonise *Protea* nectar in a few *Protea* species, and cause fermentation that helps attract their pollinators (De Vega et al. 2009, Steenhuisen et al. 2010). The metabolic actions of the *Sporothrix* species and their possible role in *Protea* pollination should receive focussed attention in future studies.

I also investigated the role of mites in the movement of materials and energy through the living community by considering them as potential secondary pollinators and investigating their feeding habits within inflorescences. I discovered that flower mites are *Protea* pollen and nectar thieves, and that the abundant *P. vandenbergi* consumes staggering amounts of pollen in a plant genus that is already known to have very low seed-set. The 'pollination limitation hypothesis' states that low seed-set within *Protea* is due to a shortage of pollinators or a shortage of viable pollen (Rebelo & Rourke 1986). By removing these large amounts of *Protea* pollen from the system, there is an obvious decrease in the availability of viable pollen for pollination, lending support to this hypothesis as explanation for the observed low seed-set. If this is indeed the case, then contrasting seed-set between closely related plant species that differ in the extent of colonization by this mite species should be evident in natural populations. Although untested in *Protea*, some evidence for this is found for the Proteaceae genera *Leucadendron* and *Aulax* for which seed-set ranges between 87-100% have been confirmed (Collins & Rebelo 1987). There are numerous obvious compounding factors, but future studies should investigate this hypothesis in greater detail and ideally between closely

related sister species pairs. Further research would also be necessary to explore the feeding habits of *P. vandenbergi* mites on other Proteaceae genera and species in the field and in the laboratory. Additionally, even though mites do not pollinate *Protea* and consume large quantities of pollen, they could still improve *Protea* fitness by interfering with pollinators. Their mere presence in high numbers may increase the rate of visitation by pollinators such as the Cape sugarbird, potentially increasing fitness by forcing more out-crossing (Lara & Ornelas 2001, 2002).

Ecological processes, environmental services and natural biodiversity are diminished due to urban, industrial and agricultural encroachment. In an attempt to lessen impacts, indigenous crop cultivation has become more popular and profitable, with various native crop species cultivated within South Africa. There is, however, very limited understanding of how these systems impact natural fauna and even less so for the more inconspicuous taxa such as mites. To add to our current meagre knowledge, I investigated the distribution and assemblages of mites across a changing landscape within inflorescences, infructescences and the rhizosphere of *Protea repens*. I presented evidence that agroecological management may provide appropriate niches to support native mite biodiversity and their associated ecological processes. I also indicated that present pest management strategies using pesticides, no matter how intense, do not eliminate inflorescences- and infructescence-associated mites, as these are most probably constantly re-introduced by pollinating birds and insects. Future research should explore new pre-harvesting pest management options such as bio-control agents against harmful mites and insects and controlling bird and insect visitation to inflorescences using shade nets or mesh (some trials for this has been started at the Piketberg site). However, in terms of the most beneficial solution for the maintenance of biodiversity and reductions in monetary loss, focus should probably shift from in-field control strategies to post-harvest control strategies, at least in terms of the mites. However, the assemblages that benefit

agroecosystems the most, *i.e.* the soil associated mite biota, seem to be most negatively impacted by current management strategies. Unlike *Protea* inflorescences and infructescences, soil-associated mites are less mobile and therefore much slower to re-colonise and establish after disturbance events. Augmentation of soil-mite communities may be explored as an option in future studies for more rapid establishment of normal ecosystem functioning of rehabilitations sites. As agricultural land transformation, either for intensely managed exotic crops or indigenous flora is inevitable, more sustainable ways will need to be explored to assist with the preservation of natural fauna, including mites and more specifically those associated with the rhizosphere.

References

1. Collins BG, Rebelo T (1987) Pollination biology of the Proteaceae in Australia and southern Africa. Australian Journal of Ecology 12:387–421
2. De Vega C, Herrera CM, Johnson SD (2009) Yeasts in floral nectar of some South African plants: quantification and associations with pollinator type and sugar concentration. South African Journal of Botany 75:798–806
3. Lara C, Ornelas JF (2001) Nectar 'theft'by hummingbird flower mites and its consequences for seed set in *Moussonia deppeana*. Functional Ecology 15:78–84
4. Lara C, Ornelas JF (2002) Effects of nectar theft by flower mites on hummingbird behaviour and the reproductive success of their host plant, *Moussonia deppeana* (Gesneriaceae). Oikos 96:470–480
5. Rebelo AG, Rourke JP (1986) Seed germination and seed set in southern African Proteaceae: ecological determinants and horticultural problems. Acta Horticulturae 185:75–88
6. Roets F, Theron N, Wingfield MJ, Dreyer LL (2012) Biotic and abiotic constraints that facilitate host exclusivity of *Gondwanamyces* and *Ophiostoma* on *Protea*. Fungal biology, 116:49–61
7. Steenhuisen SL, Raguso RA, Jürgens A, Johnson SD (2010) Variation in scent emission among floral parts and inflorescence developmental stages in beetle-pollinated Protea species (Proteaceae). South African Journal of Botany 76:779–787

Appendix 1: P-values for *P. neriifolia* seed set compared between three treatments and a control at three sites within the Western Cape, South Africa. Sites are presented here as Du Toits Kloof (DTK), Jonkershoek (JH) and Franschoek (FH).

	Area	Treatment	1	2	3	4	5	6	7	8	9	10	11	12
1	DTK	Closed		0,692634	**0,014241**	**0,016664**	1,000000	0,807456	0,386082	**0,000194**	1,000000	1,000000	0,286104	**0,000109**
2	DTK	Mites	0,692634		**0,048431**	**0,020883**	0,693348	0,998176	0,541024	**0,000220**	0,692634	0,692747	0,416503	**0,000181**
3	DTK	Re-opened	**0,014241**	**0,048431**		0,102364	**0,014242**	**0,024790**	1,000000	**0,000541**	**0,014241**	**0,014241**	0,999997	**0,018834**
4	DTK	Control	**0,016664**	**0,020883**	0,102364		**0,016664**	**0,018536**	0,187393	0,071573	**0,016664**	**0,016664**	0,182718	0,926305
5	JH	Closed	1,000000	0,693348	**0,014242**	**0,016664**		0,809483	0,386093	**0,000194**	1,000000	1,000000	0,286113	**0,000109**
6	JH	Mites	0,807456	0,998176	**0,024790**	**0,018536**	0,809483		0,455207	**0,000206**	0,807456	0,807778	0,342242	**0,000139**
7	JH	Re-opened	0,386082	0,541024	1,000000	0,187393	0,386093	0,455207		**0,000678**	0,386082	0,386084	1,000000	0,254248
8	JH	Control	**0,000194**	**0,000220**	**0,000541**	0,071573	**0,000194**	**0,000206**	**0,000678**		**0,000194**	**0,000194**	**0,000693**	**0,004947**
9	FH	Closed	1,000000	0,692634	**0,014241**	**0,016664**	1,000000	0,807456	0,386082	**0,000194**		1,000000	0,286104	**0,000109**
10	FH	Mites	1,000000	0,692747	**0,014241**	**0,016664**	1,000000	0,807778	0,386084	**0,000194**	1,000000		0,286106	**0,000109**
11	FH	Re-opened	0,286104	0,416503	0,999997	0,182718	0,286113	0,342242	1,000000	**0,000693**	0,286104	0,286106		0,220679
12	FH	Control	**0,000109**	**0,000181**	**0,018834**	0,926305	**0,000109**	**0,000139**	0,254248	**0,004947**	**0,000109**	**0,000109**	0,220679	

Appendix 2: LSD Post Hoc test with mite species richness (BoxCox) comparison between two ecosystems types (Natural, Cultivated), three niche types (Flower, Fruits, Soils) between three areas (*PB* Piketberg, *KM* Kleinmond, *GB* Gansbaai).

Ecosystem			Natural									Cultivated								
	Niche		Flower			Fruit			Soil			Flower			Fruit			Soil		
		Area	PB	KM	GB	PB	KM	GB	PB	KM	GB	PB	KM	GB	PB	KM	GB	PB	KM	GB
N	Fl	PB		1.000	1.000	0.211	0.809	0.484	**0.000**	**0.000**	**0.001**	**0.004**	1.000	0.998	0.130	0.091	1.000	**0.000**	0.616	**0.002**
N	Fl	KM	1.000		1.000	0.151	0.756	0.407	**0.000**	**0.000**	**0.001**	**0.001**	1.000	0.997	0.085	0.051	1.000	**0.000**	0.584	**0.001**
N	Fl	GB	1.000	1.000		0.093	0,509	0.240	**0.000**	**0.000**	**0.000**	0.162	1.000	1.000	0.685	**0.042**	0.997	**0.000**	0.396	**0.001**
N	Fr	PB	0.211	0.151	0.093		1.000	1.000	**0.000**	**0.002**	**0.040**	**0.000**	0.059	**0.024**	**0.000**	1.000	0.508	**0,036**	1.000	0.981
N	Fr	KM	0.809	0.756	0.509	1.000		1.000	**0.000**	**0,001**	**0.016**	**0.000**	0.502	0.237	**0.002**	1.000	0.976	**0.016**	1.000	0.755
N	Fr	GB	0.484	0.407	0.240	1.000	1.000		**0.000**	**0.001**	**0.029**	**0.000**	0.204	0.083	**0.000**	1.000	0.812	**0,033**	1.000	0.932
N	S	PB	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**		0.796	1.000	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.041**	**0.000**
N	S	KM	**0.000**	**0.000**	**0.000**	**0.002**	**0.001**	**0.001**	0.796		1.000	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**	0.511	0.211	**0.017**
N	S	GB	**0.001**	**0.001**	**0.000**	**0.040**	**0.016**	**0.029**	1.000	1.000		**0.000**	**0.000**	**0.000**	**0.000**	0.036	0.001	0.808	0.252	0.176
C	Fl	PB	**0.004**	**0.001**	0.162	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**		**0.007**	0.350	1.000	**0.000**	**0.000**	**0.000**	**0.010**	**0.000**
C	Fl	KM	1.000	1.000	1.000	0.059	0.502	0.204	**0.000**	**0.000**	**0.000**	**0.007**		1.000	0.233	**0.015**	0.999	**0.000**	0.425	**0.000**
C	Fl	GB	0.998	0.997	1.000	0.024	0.237	0.083	**0.000**	**0.000**	**0.000**	0.350	1.000		0.902	**0.009**	0.931	**0.000**	0.237	**0.000**
C	Fr	PB	0.130	0.085	0.685	0.000	0.002	0.000	**0.000**	**0.000**	**0.000**	1.000	0.233	0.902		**0.000**	**0.028**	**0.000**	**0.020**	**0.000**
C	Fr	KM	0.091	0.051	**0.042**	1.000	1,000	1.000	**0.000**	**0.001**	**0.036**	**0.000**	**0.015**	**0.009**	**0.000**		0.295	**0.007**	1.000	0.961
C	Fr	GB	1.000	1.000	0.997	0.508	0.976	0.812	**0.000**	**0.000**	**0.001**	**0.000**	0.999	0.931	**0.028**	0,295		**0,000**	0,823	**0,007**
C	S	PB	**0.000**	**0.000**	**0.000**	**0.036**	**0.016**	**0.033**	**0.000**	0.511	0.808	**0.000**	**0.000**	**0.000**	**0.000**	**0.007**	**0.000**		0,722	0,274
C	S	KM	0.616	0.584	0.396	1.000	1.000	1.000	**0.041**	0.211	0.252	**0.010**	0.425	0.237	**0.020**	1.000	0.823	0.722		1,000
C	S	GB	**0.002**	**0.001**	**0.001**	0.981	0.755	0.932	**0.000**	**0.017**	0.176	**0.000**	**0.000**	**0.000**	**0.000**	0.961	**0.007**	0.274	1,000	

Appendix 3: List of some soil-associated Oribatida mite species collected during this study.

Family	Genus and species	Niche	Biotope	Location
Aleurodamaeidae	*Aleurodamaeus woasi*	Soil	Natural	Piketberg
Brachychthoniidae	*Liochthonius sp.*	Soil	Natural	Kleinmond
Ceratozetoidea larvae	*Anellozetes?*	Soil	Cultivated	Kleinmond
Cosmochthoniidae	*Phyllozetes sp.*	Soil	Natural	Kleinmond
Eremulidae	*Austroeremulus sp.*	Soil	Natural	Kleinmond
Gymnodamaeidae	*Adrodamaeus johanni*	Soil	Cultivated	Kleinmond
Gymnodamaeidae	*Adrodamaeus johanni*	Soil	Natural	Kleinmond
Gymnodamaeidae	*Adrodamaeus johanni*	Soil	Natural	Piketberg
Haplozetidae	*Afroleius minor*	Soil	Natural	Piketberg
Haplozetidae	*Afroleius sp.*	Soil	Natural	Piketberg
Humerobatidae	*Africoribates depilatus*	Soil	Natural	Piketberg
Humerobatidae	*Anellozetes auriculatus*	Soil	Natural	Kleinmond
Humerobatidae	*Anellozetes neonominatus*	Soil	Cultivated	Kleinmond
Humerobatidae	*Anellozetes neonominatus*	Soil	Natural	Kleinmond
Humerobatidae	*Anellozetes neonominatus*	Soil	Natural	Piketberg
Humerobatidae	*Anellozetes sp.*	Soil	Natural	Kleinmond
Licnodamaeidae	*Pedrocortesella Africana*	Soil	Natural	Piketberg
Lohmannioidea/Nothroidea	Nymph	Soil	Natural	Kleinmond
Oppiidae	*Brachioppiella (Brachioppiella) sp.*	Soil	Natural	Kleinmond
Oppiidae	*Brachioppiella (Gressittoppia) sp.*	Soil	Natural	Kleinmond
Oppiidae	*Graptoppia sp.*	Soil	Natural	Piketberg
Oppiidae	*Graptoppia sp.*	Soil	Natural	Kleinmond
Oppiidae	*Multioppia wilsoni*	Soil	Natural	Kleinmond
Oppiidae	*Oppiella nova*	Soil	Cultivated	Kleinmond
Oppiidae	*Oppiella nova*	Soil	Natural	Kleinmond
Oribatulidae	*Capilloppia smithersi*	Soil	Natural	Kleinmond
Oribatulidae	*Oribatula (Zygoribatula) gracilata*	Fruit	Cultivated	Piketberg
Oribatulidae	*Oribatula (Zygoribatula) gracilata*	Soil	Natural	Piketberg
Parapirnodidae	*Gerloubia sp.*	Soil	Cultivated	Kleinmond
Parapirnodidae	*Gerloubia sp.*	Soil	Natural	Kleinmond
Scheloribatidae	*Scheloribates parvus*	Soil	Natural	Kleinmond
Scheloribatidae	*Scheloribates sp.*	Soil	Natural	Piketberg
Scheloribatidae	*Topobates heterodactylus*	Soil	Cultivated	Kleinmond
Scheloribatidae	*Topobates heterodactylus*	Soil	Natural	Kleinmond
Tectocepheidae	*Tectocepheus velatus*	Soil	Cultivated	Kleinmond
Tectocepheidae	*Tectocepheus velatus*	Soil	Natural	Kleinmond
Trizetoidea	*Suctobelbella sp.1*	Soil	Natural	Kleinmond
Trizetoidea	*Suctobelbella sp.2*	Soil	Natural	Kleinmond
Zetomotrichidae	*Demisalto (Saltatrichus) magnus*	Soil	Cultivated	Kleinmond
Zetomotrichidae	*Demisalto (Saltatrichus) magnus*	Soil	Natural	Piketberg