Studies in leaf domatia - mite mutualism in South Africa

A thesis submitted in fulfilment of the requirements of the degree of Doctor of Philosophy in Botany

RHODES UNIVERSITY

by

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March 2018

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Abstract

Plants have various traits which allow them to cope and resist their enemies including both insects and fungi . In some cases such traits allow plants to build mutualistic relationships with natural enemies of plant pests. This is the case in many dicotyledonous plants which produce leaf domatia. Leaf domatia are plant cavities usually found in the axils of major veins in the abaxial side of leaves. They are usually associated with mites and often mediate mutualistic relationships with predacious mites. Mites use leaf domatia primarily for shelter, to reproduce, and to develop. In turn, plants benefit from having predaceous mites on their leaves, because mites act as plant "bodyguards" and offer defence against pathogens and small arthropod herbivores. This phenomenon has been well documented all over the world, but Africa remains disproportionally understudied.

The aim of this study was to fill the gap that exists in our knowledge of the extent of the distribution of leaf domatia-mite mutualisms and generate a better understanding of the diversity of mites found within leaf domatia from an African perspective. This was done by surveying plant species that bear leaf domatia from different vegetation types in South Africa. The plants with leaf domatia were examined for the presence of mites in order to determine patterns of mite abundance and diversity and, in so doing, address the following questions:

- Does each tree species host have a specific mite or mite assemblage?
- Do some mites prefer certain types of leaf domatia?
- Do mites prefer a specific place in the tree canopy and does the microclimate in the tree canopy affect the distribution of mites?
- Do different vegetation sites and types differ in their mite diversity and species composition?
- Does mite abundance and diversity vary with seasons? Do coffee plantations have a different suite of mites than the adjacent forest?

The anatomical structures of leaf domatia from six selected plant species(*Coffea arabica*, *Gardenia thunbergia*, *Rothmannia capensis*, *Rothmannia globosa* (Rubiaceae), *Ocotea bullata* (Lauraceae) and *Tecoma capensis* (Bignoniaceae) with different types of leaf domatia were also studied. The results from this study suggested that the key futures which distinguish domatia are the presence of an extra layer of tissue in the lower epidermis, a thick cuticle, cuticular folds, the presence of trichomes and an invagination. This study provides a better understating of the structure of leaf domatia.

Leaf domatia bearing plants are widely distributed in South Africa, and species and vegetation-specific associations were assessed. Over 250 plant specimens with leaf domatia were collected and examined and more than 60 different mite species were found in association with the sampled plant species. The majority of mites found within the domatia of these tree species were predaceous and included mites from Stigmatidae, Tydeidae and Phytoseiidae. Furthermore, 15 new species were collected, suggesting that mites are understudied in South Africa. This study showed that the different vegetation types sampled did not differ markedly in terms of their mite biota and that similar mites were found across the region, and the association between leaf domatia and mites was found to be opportunistic and that mites had no preference for any particular domatia types. No host specificity relationship was observed between plants and mites.

The assessment of mites associated with *Coffea arabica* showed that indigenous mites are able to colonise and establish a beneficial mutualism on exotic species. This is important as it ascertains that economically important plants that are cultivated outside their area of natural distribution can still benefit from this mutualism.

This study also found that mite abundance and diversity in plants with leaf domatia were influenced by factors such as temperature, relative humidity and rainfall. Mite communities found in association with domatia changed as the year progressed and over the seasons. The seasonal fluctuations varied between the sampled plant species. In addition, this study found that mites were sensitive to extreme environmental conditions, and thus, mites preferred leaves found in the lower parts of the tree canopy and avoided exposed leaves.

This study provides a better understanding of the distribution of domatia bearing plants in South Africa and their associated mites and contributes to our knowledge of the biodiversity of mites in

the region. Furthermore, this study also adds to our understanding of the leaf domatia - mite mutualism in Africa. The applied example looking at the plant-mite mutualism in *Coffea arabica* highlights the importance of this mutualism in commercial plants.

Declaration

This thesis is the result of the author's original work except where acknowledged or specifically stated in the text. It has not been submitted for any other degree for examination at any other university or academic institution outside of Rhodes University (Grahamstown, South Africa).

S. Situngu

Publications

Situngu, S., Barker, N. 2016. Position, position, position: Mites occupying leaf domatia are not uniformly distributed in the tree canopy. *South African Journal of Botany* 108: 23–28. http//dx.doi.org/10.1016/j.sajb.2016.09.012

Acknowledgements

I am grateful for the sustained support of many people who made this thesis possible. To my supervisor, Professor Nigel Barker, it would be impossible to overstate my gratitude. Thank you for your endless support, for sharing your knowledge and expertise and for guiding me to the successful completion of this thesis. To my co-supervisor, Professor Susi Vetter, I am very thankful for your guidance, motivation and endless advice.

I would also like to thank my funders, the Centre of Excellence in Tree Health and Biotechnology (CTHB) and the GreenMatter Fellowship, for making my studies a reality and for offering career development opportunities.

I wish to thank Professor Eddie Ueckermanne from the Agricultural Research Institute (ARC) Biosystematics Division and Ms Natalie Theron for your assistance with mite identification. This study would not have been possible without the assistance of my field trip colleagues; Duncan Stodart, Aviwe Matiwane, Costance Mothwa, Alistair Barker and Chantal Taylor. Thank you for driving and making our field trips fun.

Thank you to the landowners and private reserve managers for giving me permission to conduct my study on their land and allowing me to collect samples. I would also like to thank Tony Dold from the Grahamstown Herbarium for helping with identification of numerous plant specimens collected.

Lastly, a big thank you to my partner, Azwihangwisi Mufamadi, and to my family for your endless support and words of encouragement. You are all an inspiration and a blessing in my life.

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Chapter 1: General Introduction

Plant leaf surfaces are extremely diverse in their anatomy, morphology, and physiological properties. This variability makes leaves an important environment for microbes and allows plants to interact with a large range of organisms (Pereira et al., 2002; O’Connell, 2010a; Sudo and Osakabe, 2011). A variety of plants interact both directly and indirectly with organisms and these interactions may assist in building mutualistic relationships with natural enemies of plant pests (Romero and Benson, 2005). A mutualism is a relationship between two organisms in which both individuals gain greater fitness benefits from the association than when they occur alone (Bronstein, 1998). In many dicotyledonous plants such interactions exist between plants with leaf domatia and mites.

This chapter provides a literature review of the current understanding of the leaf domatia and mite mutualism and points out research gaps that still exist in the knowledge of this association. This discussion leads to project aims and objectives and a breakdown of the various chapters that address each of the aims set out in the project.

1. What are leaf domatia?

Leaf domatia are plant-produced cavities usually found in the axils of major veins on the abaxial side of leaves (Figure 1; Pemberton and Turner, 1989; O’Dowd and Willson, 1991a; Walter and O’Dowd, 1992a; Walter, 1996; Norton et al., 2000; Romero and Benson, 2005). The Latin term ‘domatium’ means ‘little house’ (Nakamura et al., 1992). Leaf domatia are often referred to as acaro-domatia, because they are usually inhabited by mites and facilitate a protective mutualism between mites and the host plant (O’Dowd and Pemberton, 1998; Norton et al., 2000; Romero and Benson, 2005). These structures are not induced by their inhabitants and thus differ from galls (O’Dowd and Willson, 1991; Walter, 1996; O’Connell et al., 2015). These structures are always produced by plants irrespective of the density of their associated partner. However, O’Connell et al. (2010b) and Monks et al. (2007) suggest that investment in leaf domatia may be limited by the availability of carbon and that plants have the ability to regulate domatia formation during leaf development (O’Connell et al., 2015).

Figure 2: *The location of leaf domatia on a leaf of Rothmannia globosa (Rubiaceae). The domatia are always found where the middle vein and the secondary veins join on the underside of leaves. Arrows are pointing to Tydiedae mites associated with domatia.*

The protective mutualism between mites and plants with leaf domatia was first proposed by the Swedish biologist, Alex Lundström, in 1887. In his hypothesis he suggested that leaf domatia provide mites with shelter and in turn mites decrease the amount of herbivore and pathogen loads on leaf surfaces. As a result, plants benefit from an increased overall fitness due to a decrease in the damage caused by plant enemies (O'Dowd and Willson, 1991; Norton et al., 2000). Lundström suggested that this mutualism was analogous to that found between some plants and ants. However, it is not clear whether or not plants with domatia reward mites, as commonly seen in plant-ant mutualism. Leaves with domatia lack extra-floral nectaries inside domatia to provide a reward to mites. Also, mites do not induce the formation of domatia, but they are rather formed by plants even when they are not inhabited by mites (O'Dowd and Willson, 1991). Nonetheless, Tilney et al. (2012) observed channel-like structures made up of thick cuticular folds with electron dense micro-fibrils in the domatia of *Plectroniella armata* (K.Schum). They believe these structures to be an indication of some form of communication between this plant and their inhabitants and that these folds facilitate translocation of compounds and metabolites (Tilney et al., 2012).This is the only study that observed structures inside domatia that are thought to facilitate communication between plants and mites.

2. Types of leaf domatia

Pemberton and Turner (1989) described four types of domatia. These are pits or pouches (which are invaginations of the leaf surfaces that extend to the mesophyll), a dense tuft of hairs, pocket cavities which extend beneath expanded veins and in some cases a combination of these types (Figure 2). Furthermore, leaf domatia in some species may be found in the form of rolled vein margins or raised domes, but these are less common (Romero and Benson, 2005). The morphological structure of leaf domatia varies with environmental conditions, and in their developmental forms both between and within individuals of the same species (Nishida et al., 2006; Richards and Coley, 2012). The size and density of domatia on leaves may also vary depending on relative rainfall at the site where the plant occurs. Richards and Coley (2012) found that *Psychotria horizontalis* (Smith) produced smaller domatia with smaller domatium openings in dry forests and bigger domatia in wet forests. They suggest that this might be an adaptive trait relative to the environmental conditions at each site. Producing small domatia with small openings may also be a strategy to reduce airflow inside domatia and thus reduce water loss in dry conditions.

3. The distribution of plants with leaf domatia

Our analysis of species cited in literature on leaf domatia reveals that these structures are commonly found throughout the angiosperm order including the basal angiosperm clade such as the magnoliids (Table 1, Figure 4). The magnoliid clade is one of the most basal sister groups in the angiosperm tree that is neither eudicotyledonous nor monocotyledonous, and is considered to retain the characteristics of the more primitive angiosperm (Hedges and Kumar, 2009). Within the magnoliids, species of Annonaceae (*Annonia muricata L.*) and Lauraceae (*Ocotea bullata* (Burch.) E. Meyer in Drege) are known to bear leaf domatia.

Within angiosperms, these structures are common in eudicotyledonous woody plants, including trees, shrubs, and vines. Approximately 28% of 290 dicotyledonous families have species with leaf domatia (Pemberton and Turner, 1989; O'Dowd and Willson, 1991; O'Dowd and Pemberton, 1998; Nishida et al., 2006). Among the eudicots, leaf domatia are particularly abundant in the Rubiaceae and Vitaceae (Figure 3); 32% and 11% out of 291 representative species cited in literature from 58 plant families belong to the Rubiaceae and Vitaceae, respectively. Gregory (1990) also reported the occurrence of leaf domatia in 780 species in the Rubiaceae family. In contrast, these structures are absent in monocotyledons.

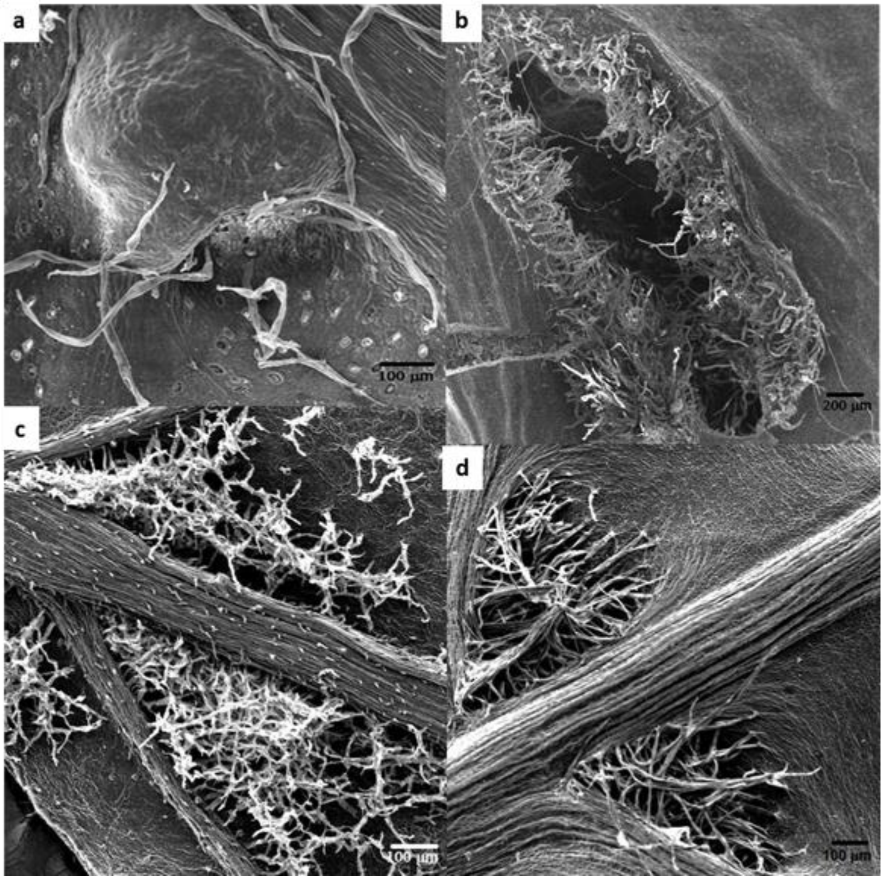

Figure 3: *The different domatia types found in woody species, (a) a raised dome found in Rhoicissus digitata(Vitaceae) , (b) a pit-type domatia from Ocotea bullata (Lauraceae), (c) a hair-tuft (Tecoma capensis, Bignoniaceae) and (d) is a combination of a pit with hairs (Gardenia thunbergia, Rubiaceae).*

Plant species that produce leaf domatia are widely distributed geographically and have been recorded from many regions of world, including Australia, Japan, New Zealand, Southern Africa, West Africa and the United States (Table 1) (O'Dowd and Willson, 1991; Rozario, 1995). Domatia bearing species are most abundant in tropical and subtropical regions, and their distribution ranges in altitude from lowlands to mountain forests. They seem to be restricted to relatively humid areas and their frequency decreases with increasing aridity (O'Dowd and Willson, 1991). Their relative frequency is high in temperate forests of the Northern Hemisphere (O'Dowd and Pemberton, 1994; O'Dowd and Willson 1997). In north Asia approximately half of all woody species found in broadleaf deciduous forests have leaf domatia (O'Dowd and Pemberton, 1998).

However, literature suggests that these structures are also common in subtropical and temperate forest of the Southern Hemisphere as more than half of the species cited in the literature on leaf domatia come from New Zealand and Australia (O'Dowd and Willson, 1989; Walter and O'Dowd, 1992a; 1995; Sampson and Mclean, 2014). In addition, this study surveyed species with domatia from South Africa (Table 1). It is important to note, however, that these trends on the distribution of domatia-bearing species also reveal some sampling bias and domatia-bearing plants appear to be most common in regions where more research has been conducted (Table 1). Research in Africa is still in its early stages, and many more leaf domatia trees may still be found.

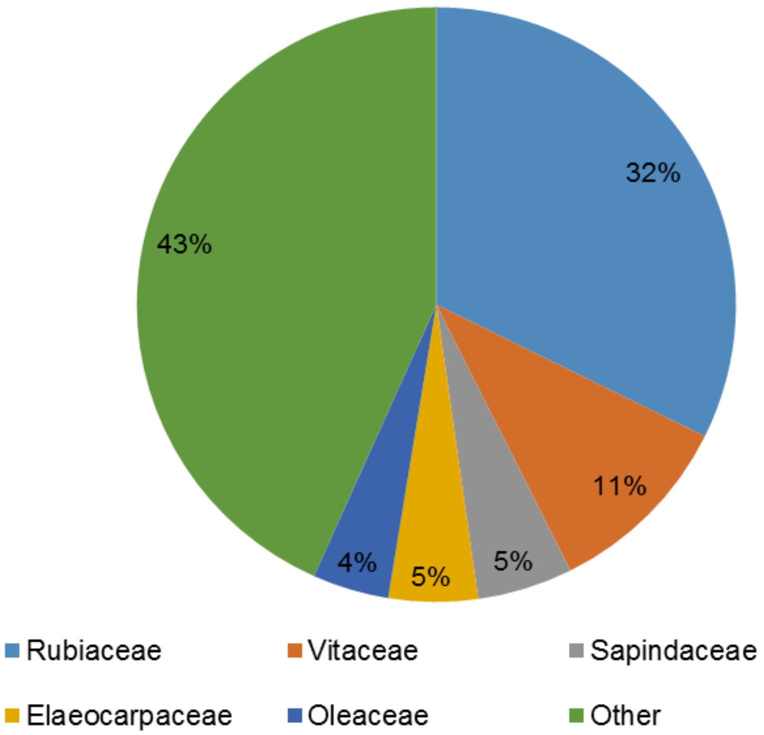

Figure 4: *Proportion of species that possess leaf domatia from different plant families. Out of 291 representatives from 58 families, the Rubiaceae and the Vitaceae had the most species with leaf domatia. For literature sources see Table 1.*

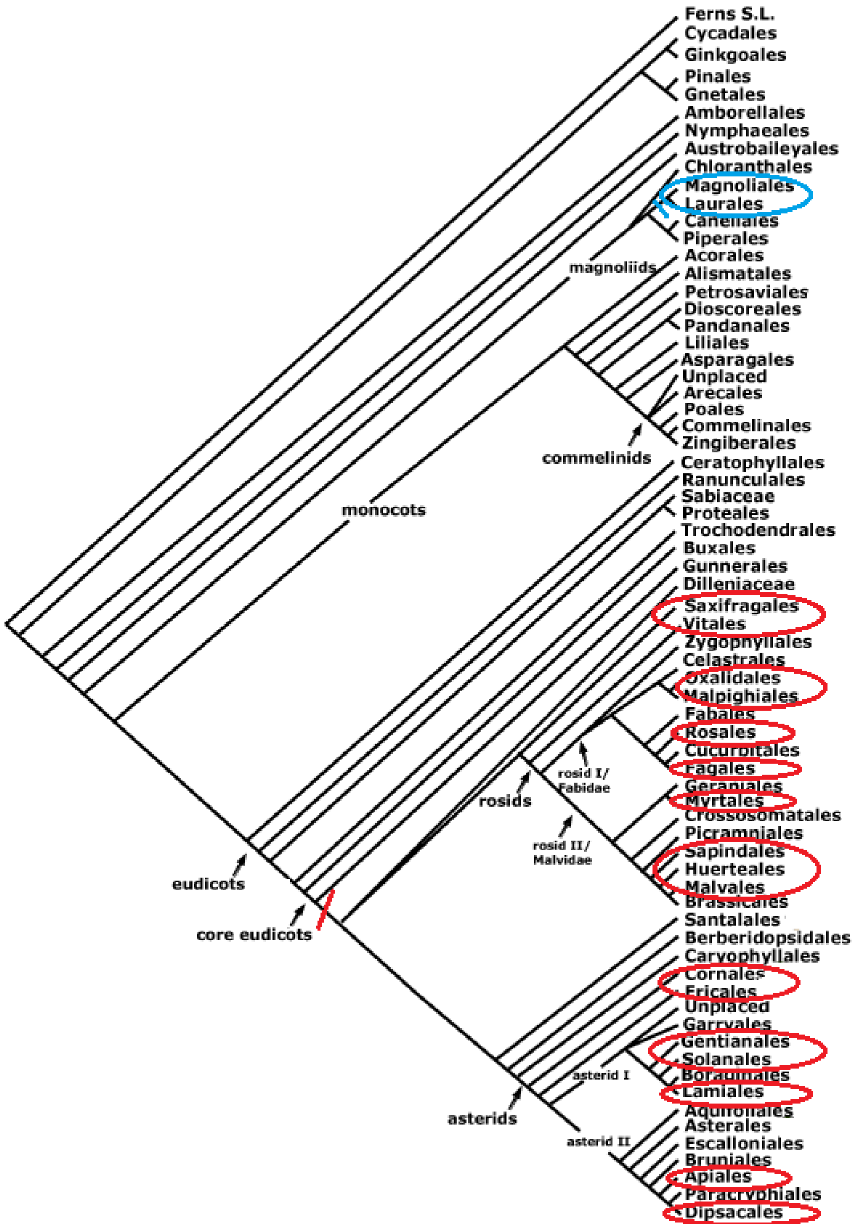

Figure 5: *Angiosperm plant phylogeny showing the presence of leaf domatia at the ordinal level. Leaf domatia are common in core eudicots families (Red circles, but are also present in the magnoliids (blue circles). Tree adapted and modified from Stevens, P. F. (2001 onwards).*

Table 1: *A comprehensive list of plant species that bear leaf domatia from different geographical regions of the world synthesised from literature on the leaf domatia - mite mutualism. Plant species listed below were obtained from literature obtained via an extensive search on Google Scholar and Scopus using the keywords leaf domatia mite- mutualism, leaf domatia, leaf domatia bearing plants and plant-mite association.*

Family	**Species name**	**Country/Region**	**Reference**
Acanthaceae	*Mackaya bella* Harv.	South Africa	This study
Actinidiaceae	*Actinidia polygama* (Siebold & Zucc.) Maxim.	Korea	O'Dowd and Pemberton, (1998)
Adoxaceae	*Sambucus nigra* L.	Italy	Duso et al., (2004)
Adoxaceae	*Viburnum erosum var. punctatum* Thunb.	Japan	Duso et al., (2010); Sudo and Osakabe, (2011)
Adoxaceae	*Viburnum odoratissimum* Ker Gawl.	India, Japan	Pemberton and Turner, (1989)
Adoxaceae	*Viburnum sargentii* Koehne	Korea	O'Dowd and Pemberton, (1998)
Adoxaceae	*Viburnum tinus* L.	Australia	Grostal and O'Dowd, (1994); Rowles and O'Dowd, (2009);
Alangiaceae	*Alangium salviifolium* L.f. Wangerin	Bangladesh	Rozario (1995)
Anacardiaceae	*Euroschinus falcata* (Hook & Benth.)	Australia	Walter and O'Dowd, (1992a)
Anacardiaceae	*Pleiogynium timoriense* (A. DC.) Leenh.	Australia	O'Dowd and Willson, (1989)
Anacardiaceae	*Schinus terebinthifolius* Raddi	Brazil	Pemberton and Turner, (1989)
Annonaceae	*Annona muricata* L.	USA	Pemberton and Turner, (1989); Romero and Benson, (2005)
Annonaceae	*Annona reticulata* L.	Bangladesh, Brazil	Rozario, (1995)
Atherospermataceae	*Doryphora sassafras* Endl.	Australia	Walter and O'Dowd, (1992a)
Betulaceae	*Betula costata* Trautv.	Korea	O'Dowd and Pemberton, (1998)
Betulaceae	*Carpinus betulus* L.	Italy	Duso et al., (2004)

Betulaceae	*Carpinus cordata* Blume	Korea	O'Dowd and Pemberton, (1998)
Betulaceae	*Carpinus laxiflora* (Siebold & Zucc.) Blume	Korea	O'Dowd and Pemberton, (1998)
Betulaceae	*Corylus avellana* L.	Italy	Duso et al., (2004)
Betulaceae	*Corylus sieboldiana* Blume	Korea	O'Dowd and Pemberton, (1998)
Bignoniaceae	*Distictis buccinatoria* (DC.) A.H. Gentry	Mexico	Pemberton and Turner, (1989)
Bignoniaceae	*Dolichandrone stipulata* (Wall.) Benth. ex C.B.Clarke	Bangladesh	Rozario, (1995)
Bignoniaceae	*Tabebuia impetiginosa* (Mart. ex DC.) standl.	Brazil	Romero and Benson, (2005)
Bignoniaceae	*Tabebuia rosa-alba* (Ridl.) Sandwith	USA	Pemberton and Turner, (1989)
Caprifoliaceae	*Abelia grandiflora* Villarreal	China	Pemberton and Turner, (1989)
Caprifoliaceae	*Viburnum tinus* L.	Australia, France	Grostal and O'Dowd, (1994); Parolin et al., (2011) & (2014a); Bresch et al., (2015)
Cardiopteridaceae	*Citronella moorei* (F.Muell. ex Benth.) R.A.Howard	Australia	Walter and O'Dowd, (1995)
Celastraceae	*Perottetia sandwicensis* A.Gray	Hawaii	Pemberton and Turner, (1989)
Cannabaceae	*Celtis africana* Burm.f.	South Africa	This study
Combretaceae	*Terminalia argentea* Mart.	Brazil	Romero and Benson, (2005)
Combretaceae	*Terminalia catappa* L.	East indies	Pemberton and Turner, (1989) ; Rozario, (1995)
Combretaceae	*Combretum apiculatum* Sond.	South Africa	This study
Combretaceae	*Combretum kraussii* Hochst.	South Africa	This study
Combretaceae	*Combretum sp.* Loefl.	South Africa	This study
Cornaceae	*Alangium platanifolium* (Siebold &Zucc.) Harms	Korea	O'Dowd and Pemberton, (1998)
Cornaceae	*Cornus capitata* Wall.	Himalaya	Pemberton and Turner, (1989)

Cornaceae	*Cornus controversa* Hensl.	Korea	Pemberton and Turner, (1989)
Cunoniaceae	*Ackama rosifolia* (A.Cunn.)	New Zealand	Sampson and Mclean, (2014)
Cunoniaceae	*Caldcluvia australiensis* (Schltr.) Hoogland	Australia	O'Dowd and Willson, (1989)
Cunoniaceae	*Caldcluvia paniculosa* (F.Muell.) Hoogland	Australia	Walter and O'Dowd, (1992a)
Cunoniaceae	*Callicoma serratifolia* Andrews	Australia	Walter and O'Dowd, (1992a)
Cunoniaceae	*Ceratopetalum apetalum* D.Don	Australia	Walter and O'Dowd, (1994)
Dipterocarpaceae	*Hopea odorata* Roxb.	Bangladesh	Rozario, (1995)
Dipterocarpaceae	*Shorea robusta* Gaertn.	Bangladesh	Rozario, (1995)
Ebenaceae	*Diospyros glandulosa* Lace	South Africa	This study
Ehretiaceae	*Ehretia acuminata* R.Br.	Australia	Walter and O'Dowd, (1992a)
Ehretiaceae	*Ehretia rigida* (Thunb.) Druce	South Africa	This study
Elaeocarpaceae	*Elaeocarpus angustifolius* Blume	Australia	Walter and O'Dowd, (1995)
Elaeocarpaceae	*Elaeocarpus dentatus* (J.R.Forst. & G.Forst.) Vahl	New Zealand	Sampson and Mclean (2014)
Elaeocarpaceae	*Elaeocarpus hookerianus* Raoul	New Zealand	Sampson and Mclean (2014)
Elaeocarpaceae	*Elaeocarpus grandiflorus* Sm.	Australia	Walter and O'Dowd, (1992a)
Elaeocarpaceae	*Elaeocarpus holopetalus* F.Muell.	Australia	Walter and O'Dowd, (1992a)
Elaeocarpaceae	*Elaeocarpus japonicus* Siebold	China, Japan	Pemberton and Turner, (1989)
Elaeocarpaceae	*Elaeocarpus longiflorens* C.T.White	New Zealand	O'Dowd and Willson, (1989); (1990)
Elaeocarpaceae	*Elaeocarpus coorangooloo* J.F.Bailey & C.T.White	Australia	O'Dowd and Willson, (1989)
Elaeocarpaceae	*Elaeocarpus obovatus* G.Don.	Australia	Walter and O'Dowd, (1992a); (1995)
Elaeocarpaceae	*Elaeocarpus reticulatus* Sm.	Australia, Canada	O'Dowd and Willson, (1989); Walter and O'Dowd, (1992a)
Elaeocarpaceae	*Sloanea australis* Benth & F.Muell.	Australia	O'Dowd and Willson, (1989)

Elaeocarpaceae	*Sloanea langii* F.Muell.	Australia	O'Dowd and Willson, (1989); Walter and O'Dowd, (1995)
Elaeocarpaceae	*Sloanea woollsii* F.Muell.	Australia	O'Dowd and Willson, (1989); Walter and O'Dowd, (1995)
Ericaceae	*Lyonia ovalifolia* (Walll.) Drude	Japan	Sudo and Osakabe, (2011)
Escalloniaceae	*Carpodetus serratus* J.R.Forst & G.Fost.	New Zealand	Sampson and Mclean (2014)
Escalloniaceae	*Polyosma cunninghamii* Benn.	Australia	Walter and O'Dowd, (1996)
Euphorbiaceae	*Acalypha glabrata* Thunb.	South Africa	This study
Euphorbiaceae	*Bischofia javanica* Blume	Bangladesh, New Guinea	O'Dowd and Willson, (1989); Rozario, (1995)
Euphorbiaceae	*Flueggea verrucosa* (Thunb.) G.L. Webster	South Africa	This study
Euphorbiaceae	*Euphobia sp.* L.	South Africa	This study
Euphorbiaceae	*Exococaria sp.* L.	South Africa	This study
Euphorbiaceae	*Antidesma pulvinatum* Hillebr.	Hawaii	Pemberton and Turner, (1989)
Euphorbiaceae	*Antidesma venosum* E.Mey. ex Tul.	South Africa	This study
Euphorbiaceae	*Erythrococca natalensis* Prain	South Africa	This study
Euphorbiaceae	*Glochidion ferdinandii* (Muell.Arg.) F.M.Bailey	Australia	Walter and O'Dowd, (1992a)
Euphorbiaceae	*Macaranga capensis* (Baill.) Sim	South Africa	This study
Euphorbiaceae	*Manihot esculenta* Crantz	USA	Magalhaes et a., (2002); Onzo et al., (2003)
Fagaceae	*Nothofagus menziesii* (Hook.f.) Oerst.	Australia, New Zealand	Sampson and Mclean, (2013)
Fagaceae	*Nothofagus fusca* (Hook.f.) Oerst.	Australia, New Zealand	O'Dowd and Willson, (1991); Sampson and Mclean, (2014)
Fagaceae	*Quercus agrifolia* Nèe	USA	Pemberton and Turner, (1989)
Fagaceae	*Quercus rubra* L.	USA	O'Dowd and Willson, (1997)
Fagaceae	*Quercus velutina* Lam.	USA	O'Dowd and Willson, (1998)
Flacourtiaceae	*Kiggelaria africana* L.	South Africa	This study

Hamamelidaceae	*Distylium lepidotum* Nakai	Ogasawara Islands	Pemberton and Turner, (1989)
Heteropyxidaceae	*Heteropyxis sp.* Harv.	South Africa	This study
Icacinaceae	*Pennantia corymbosa* J.R.Forst. & G.Forst.	New Zealand	Sampson and Mclean, (2014)
Icacinaceae	*Pennantia cunninghamii* Miers	Australia	Walter and O'Dowd, (1995)
Juglandaceae	*Juglans mandshurica* Maxim.	Korea	O'Dowd and Pemberton, (1998)
Lauraceae	*Cinnamomum camphora* (L.) J.Presl	China, Japan, Australia, South Africa	O'Dowd and Willson, (1989); Pemberton and Turner, (1989); Nishida et al., (2005); Nishida et al., (2006)
Lauraceae	*Cryptocarya laevigata* Blume	Australia	Walter and O'Dowd, (1992a); (1995)
Lauraceae	*Cryptocarya foveolata* C.T.White & W.D.Francis	Australia	Walter and O'Dowd, (1995)
Lauraceae	*Crytocarya triplinervis* R,Br.	Australia	O'Dowd and Willson, (1989); Walter and O'Dowd, (1995)
Lauraceae	*Endiandra cowleyana* F.M.Bailey	Australia	O'Dowd and Willson,(1989); (1991)
Lauraceae	*Endiandra discolor* Benth.	Australia	Walter and O'Dowd, (1992a); (1995)
Lauraceae	*Laurus nobilis* L.	Mediterranean	Pemberton and Turner, (1989)
Lauraceae	*Ocotea bullata* (Burch.) E. Meyerbin Drege	South Africa	This study
Lauraceae	*Persea americana* Mill.	USA	Agrawel, (1997)
Lecythidaceae	*Couroupita guianensis* Aubl.	Bangladesh	Rozario, (1995)
Maesaceae	*Maesa lanceolata* Forssk.	South Africa	This study
Magnoliaceae	*Magnolia hypoleuca* Siebold & Zucc.	Japan	Toyoshima and Amano, (2006)
Meliaceae	*Cedrela odorata* L.	West indies	Pemberton and Turner, (1989)
Meliaceae	*Dysoxylum fraserianum* (A.Juss.) Benth.	Australia	Walter and O'Dowd, (1992a)
Meliaceae	*Synoum glandulosum* (Sm.) A. Juss.	Australia	Walter and O'Dowd, (1992a)
Meliaceae	*Toona ciliata* M.Roem.	Australia, Bangladesh	O'Dowd and Willson, (1989); Walter and O'Dowd, (1992a); Rozario, (1995)

Moraceae	*Ficus comitis* King	South Africa	This study
Moraceae	*Ficus sp.* L.	South Africa	This study
Myrsinaceae	*Rapanea howittiana* F.Muell. ex Mez.	Australia	Walter and O'Dowd, (1992a)
Myrtaceae	*Syzygium smithii* (Poir.) Nied.	Australia	Walter and O'Dowd, (1992a)
Myrtaceae	*Syzygium cordatum* Hochst.ex C.Krauss.	South Africa	This study
Oleaceae	*Chionanthus floveolatus* (E.Mey.) Stearn	South Africa	This study
Oleaceae	*Chionanthus peglerae* (C.H.Wright) Stearn	South Africa	This study
Oleaceae	*Chionanthus sp.* L.	South Africa	This study
Oleaceae	*Fraxinus velutina* Torr.	Arizona	Pemberton and Turner, (1989)
Oleaceae	*Jasminum angulare* Vahl.	South Africa	This study
Oleaceae	*Jasminum fluminense* Vell.	South Africa	This study
Oleaceae	*Jasminum didymum* G.Forst.	Australia	O'Dowd and Willson, (1989)
Oleaceae	*Jasminum pubescens* (Retz.) Willd.	Dhaka	Rozario, (1995)
Oleaceae	*Notelaea longifolia* Vent.	Australia	Walter and O'Dowd, (1992a)
Oleaceae	*Olea capensis* L.	South Africa	This study
Oleaceae	*Olea foveolata* E.Mey.	South Africa	This study
Oleaceae	*Olea woodiana* Knobl.	South Africa	This study
Pittosporaceae	*Pittosporum revolutum* Aiton	Australia	Walter and O'Dowd, (1992a)
Rhamnaceae	*Rhamnus alaternus* L.	Spain	Martinez-Solis et al., (1993)
Rhamnaceae	*Rhamnus myrtifolia* Willk.	Spain	Martinez-Solis et al., (1993)
Rhamnaceae	*Rhamnus ludovici-salvatoris* Chodat	Spain	Martinez-Solis et al., (1993)
Rhamnaceae	*Rhamnus prinoides* L'Hér.	South Africa	This study
Rhamnaceae	*Rhamnus sp* L.	South Africa	This study
Rosaceae	*Prunus padus* L.	Korea	O'Dowd and Pemberton, (1998)
Rosaceae	*Prunus sargentii* Rehder	Korea	O'Dowd and Pemberton, (1999)
Rosaceae	*Prunus serotina* Ehrh.	USA	O'Dowd and Willson, (1997)

Rosaceae	*Prunus virginiana* L.	USA	O'Dowd and Willson, (1998)
Rosaceae	*Rubus moorei* F.Muell.	Australia	Walter and O'Dowd, (1992a)
Rosaceae	*Rubus australis* A.Kern.	New Zealand	Sampson and Mclean, (2014)
Rosaceae	*Rubus cissoides* Pau	New Zealand	Sampson and Mclean, (2014)
Rosaceae	*Rubus schmideliodes* A.Cunn.	New Zealand	Sampson and Mclean, (2014)
Rosaceae	*Stephanandra incisa* (Thunb.) Zabel	Korea	O'Dowd and Pemberton, (1998)
Rubiaceae	*Apodytes dimidiata* E.Mey. ex Arn.	South Africa	This study
Rubiaceae	*Bobea elatior* Gaudich.	Hawaii	Pemberton and Turner, (1989)
Rubiaceae	*Burchellia sp.* L.f.	South Africa	This study
Rubiaceae	*Burchellia bubalina* (L.f.) Sims	South Africa	This study
Rubiaceae	*Calodendrum capense* (L.f.) Thunb.	South Africa	This study
Rubiaceae	*Canthium ciliatum* (D.Dietr.) Kuntze	South Africa	This study
Rubiaceae	*Canthium inerme* (L.f.) Kuntze	South Africa	This study
Rubiaceae	*Canthium mundianum* Cham. & Schltdl.	South Africa	This study
Rubiaceae	*Canthium odoratum* (G.Forst.) Seem.	Pacific Islands	Pemberton and Turner, (1989)
Rubiaceae	*Canthium spinosum* (Klotzsch ex Eckl. & Zeyh.) Kuntze	South Africa	This study
Rubiaceae	*Canthium vanwykii* Tilney & Kok	South Africa	This study
Rubiaceae	*Coddia rudis* (E.Mey. ex Harv.) Verdc.	South Africa	This study
Rubiaceae	*Coddia rudis* (E.Mey. ex Harv.) Verdc.	South Africa	This study
Rubiaceae	*Coffea arabica* L.	Brazil, Africa, Australia, Arabia, Japan, New Guinea	Pemberton and Turner, (1989); O'Dowd and Willson, (1989); (1991); Nakamura et al., (1992); Romero and Benson, (2005); Matos et al., (2005); Ferreira et al., (2011)
Rubiaceae	*Coffea canephora* Pierre ex A.Froehner	Brazil	Matos et al., (2005)
Rubiaceae	*Coffea liberica* Hiern	Tropical west Africa	Pemberton and Turner, (1989)
Rubiaceae	*Gardenia taitensis* DC.	Society islands	Pemberton and Turner, (1989)

Rubiaceae	*Morinda jasminoides* A.Cunn.	Australia	Walter and O'Dowd, (1995)
Rubiaceae	*Gardenia thunbergia* Thunb.	South Africa	This study
Rubiaceae	*Psychotria capensis* (Eckl.) Vatke	South Africa	This study
Rubiaceae	*Psychotria horizontalis* Sw.	Panama	Richards and Coley, (2012)
Rubiaceae	*Psychotria loniceroides* Sieber ex DC.	Australia	Walter and O'Dowd, (1992a)
Rubiaceae	*Randia benthamiana* F.Muell.	Australia	Walter and O'Dowd, (1995)
Rubiaceae	*Rothmannia globosa* (Hochst.) Keay	South Africa	This study
Rubiaceae	*Rothmannia capensis* Thunb.	South Africa	This study
Rubiaceae	*Rothmannia sp.* Thunb.	South Africa	This study
Rubiaceae	*Rubiaceae sp.* L.	South Africa	This study
Rubiaceae	*Tecoma capensis* (Thunb.) Lindl.	South Africa	This study
Rubiaceae	*Tricalysia delagoensis* Schinz	South Africa	This study
Rubiaceae	*Tricalysia capensis* (Meisn. ex Hochst.) Sim	South Africa	This study
Rubiaceae	*Tricalysia lanceolata* (Sond.) Burtt Davy	South Africa	This study
Rubiaceae	*Trichocladus sp.* A.Rich. ex DC.	South Africa	This study
Rubiaceae	*Coprosma baueri* Endl.	China, Japan, New Zealand	O'Dowd and Willson, (1989); Pemberton and Turner, (1989)
Rubiaceae	*Coprosma acutifolia* Hook.f.	New Zealand	O'Dowd and Willson, (1989); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma antipoda* W.R.B.Oliv.	New Zealand	O'Dowd and Willson, (1989); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma arborea* Kirk	New Zealand	O'Dowd and Willson, (1989); O'Connell et al., (2010); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma areolata* Cheeseman	New Zealand	O'Dowd and Willson, (1989); O'Connell et al., (2010); Sampson and Mclean, (2014)

Rubiaceae	*Coprosma australis* (A.Rich.) Rob.	New Zealand	O'Dowd and Willson, (1989); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma banksii* Petrie	New Zealand	O'Dowd and Willson, (1989); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma chathamica* Cockayne	New Zealand	O'Dowd and Willson, (1989); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma cheesmanii* W.R.B.Oliv.	New Zealand	O'Dowd and Willson, (1989); O'Connell et al., (2010); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma ciliata* Hook.f.	New Zealand	O'Dowd and Willson, (1989); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma colensoi* Hook.f.	New Zealand	O'Dowd and Willson, (1989); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma crassifolia* Colenso	New Zealand	O'Dowd and Willson, (1989); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma depressa* Colenso ex Hook.f.	New Zealand	O'Dowd and Willson, (1989); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma dodonaeifolia* W.R.B.Oliv.	New Zealand	O'Dowd and Willson, (1989); O'Connell et al., (2010); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma elatirioides* de Lange & A.S.Markey	New Zealand	O'Dowd and Willson, (1989); O'Connell et al., (2010); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma foetidissima* J.R.Forst. & G.Forst.	New Zealand	O'Dowd and Willson, (1989); O'Connell et al., (2010); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma grandifolia* Hook.f.	New Zealand	O'Dowd and Willson, (1989); O'Connell et al., (2010); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma hirtella* Labill.	Canada	O'Dowd and Willson, (1990)
Rubiaceae	*Coprosma linariifolia* (Hook.f.) Hook. f.	New Zealand	O'Dowd and Willson, (1989); O'Connell et al., (2010); Sampson and Mclean, (2014)

Rubiaceae	*Coprosma longifolia* A.Gray	Hawaii	O'Dowd and Willson, (1989); Pemberton and Turner, (1989)
Rubiaceae	*Coprosma lucida* J.R.Forst. & G.Forst.	New Zealand, South Africa	O'Dowd and Willson, (1989); Monks et al., (2007); O'Connell et al., (2010)
Rubiaceae	*Coprosma macrocarpa* Cheeseman	New Zealand	O'Dowd and Willson, (1989); O'Connell et al., (2010); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma microcarpa* Hook.f.	New Zealand	O'Dowd and Willson, (1989); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma parviflora* Hook.f.	New Zealand	O'Dowd and Willson, (1989); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma petiolata* Hook.f.	New Zealand	O'Dowd and Willson, (1989); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma polymorpha* W.R.B.Oliv.	New Zealand	O'Dowd and Willson, (1989); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma propinqua* A.Cunn.	New Zealand	O'Dowd and Willson, (1989); O'Connell et al., (2010); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma repens* A.Rich.	Australia, New Zealand	O'Dowd and Willson, (1989); Rowles and O'Dowd, (2009); O'Connell et al., (2010)
Rubiaceae	*Coprosma rhamnoides* A.Cunn.	New Zealand	O'Dowd and Willson, (1989); O'Connell et al., (2010); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma rigida* Cheeseman	New Zealand	O'Dowd and Willson, (1989); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma robusta* Raoul	New Zealand	O'Dowd and Willson, (1989); O'Connell et al., (2010); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma rotundifolia* A.Cunn.	New Zealand	O'Dowd and Willson, (1989); O'Connell et al., (2010); (2015)
Rubiaceae	*Coprosma rubra* Petrie	New Zealand	O'Dowd and Willson, (1989); Sampson and Mclean,

			(2014)
Rubiaceae	*Coprosma rugosa* Cheeseman	New Zealand	O'Dowd and Willson, (1989); O'Connell et al., (2010); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma serrata* H.St.John	New Zealand	O'Dowd and Willson, (1989); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma serrulata* Hook.f. ex Buchanan	New Zealand	O'Dowd and Willson, (1989)
Rubiaceae	*Coprosma spathulata* A.Cunn.	New Zealand	O'Dowd and Willson, (1989); O'Connell et al., (2010); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma tayloriae* A.P.Druce ex G.T.Jane	New Zealand	O'Dowd and Willson, (1989); O'Connell et al., (2010); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma tenuicaulis* Hook.f.	New Zealand	O'Dowd and Willson, (1989); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma tenuifolia* Cheeseman	New Zealand	O'Dowd and Willson, (1989); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma virescens* Petrie	New Zealand	O'Dowd and Willson, (1989); Sampson and Mclean, (2014)
Rubiaceae	*Coprosma wallii* Petrie	New Zealand	O'Dowd and Willson, (1989); Sampson and Mclean, (2014)
Rubiaceae	*Ixora undulata* Roxb.	Bangaladesh	Rozario, (1995)
Rubiaceae	*Keetia gueinzii* (Sond.) Bridson	South Africa	This study
Rubiaceae	*Morinda citrifolia* L.	Australia	Pemberton and Turner, (1989)
Rubiaceae	*Morinda jasminoides* A.Cunn.	Australia	Walter and O'Dowd, (1992a)
Rubiaceae	*Oxyanthus speciosus* DC.	South Africa	This study
Rubiaceae	*Pavetta capensis* (Houtt.) Bremek.	South Africa	This study
Rubiaceae	*Pavetta cf. eylesii* S.Moore	South Africa	This study
Rubiaceae	*Pavetta gardeniifolia* Hochst. ex A.Rich.	South Africa	This study

Rubiaceae	*Pavetta lanceolata* Eckl.	South Africa	This study
Rubiaceae	*Pavetta revoluta* Hochst.	South Africa	This study
Rubiaceae	*Pavetta kotzei* Bremek.	South Africa	This study
Rubiaceae	*Psydrax livida* (Hiern) Bridson	South Africa	This study
Rubiaceae	*Psydrax obovata* (Klotzsch ex Eckl. & Zeyh.) Bridson	South Africa	This study
Rubiaceae	*Plectroniella armata* (K.Schum.) Robyns	South Africa	Tilney et al., (2012)
Rubiaceae	*Randia cochinchinensis* (Lour.) Merr.	Asia	Pemberton and Turner, (1989)
Rubiaceae	*Rudgea cornifolia* (Kunth) Standl.	USA	Pemberton and Turner, (1989)
Rubiaceae	*Rudgea eugenioides* Standl.	Brazil	Moraes et al., (2010)
Rubiaceae	*Hyperacanthus amoenus* (Sims) Bridson	South Africa	This study
Rubiaceae	*Tarena dallachiana* (F.Muell. ex Benth.) S.Moore	Australia	O'Dowd and Willson, (1989)
Rubiaceae	*Timonius timon* (Spreng.) Merr.	Australia	O'Dowd and Willson, (1989)
Rutaceae	*Zanthoxylum schinifolium* Siebold & Zucc.	Korea	O'Dowd and Pemberton, (1998)
Salicaceae	*Dovyalis longispina* (Harv.) Warb.	South Africa	This study
Salicaceae	*Dovyalis sp.* E.Mey. ex Arn.	South Africa	This study
Sapindaceae	*Acer campestre* L.	Italy	Duso et al., (2004)
Sapindaceae	*Acer mono* Maxim.	Korea	O'Dowd and Pemberton, (1998)
Sapindaceae	*Acer palmatum* Thunb.	Japan	Sudo and Osakabe, (2011)
Sapindaceae	*Acer pseudosieboldianum* (Pax) Kom.	Korea	O'Dowd and Pemberton, (1999)
Sapindaceae	*Acer rubrum* L.	USA	Prado et al., (2015)
Sapindaceae	*Acer saccharum* Marshall	USA	O'Dowd and Willson, (1997)
Sapindaceae	*Acer tegmentosum* Maxim.	Korea	O'Dowd and Pemberton, (1998)
Sapindaceae	*Cupania vernalis* Cambess.	Brazil	Romero and Benson, (2005)

Sapindaceae	*Guioa lasioneura* Radlk.	Australia	O'Dowd and Willson, (1989)
Sapindaceae	*Guioa acutifolia* Radlk.	Australia	O'Dowd and Willson, (1989)
Sapindaceae	*Sarcopteryx stipitata* (F.Muell.) Radlk.	Australia	Walter and O'Dowd, (1992a)
Sapindaceae	*Allophylus dregeanus* (Sond.) De Winter	South Africa	This study
Sapindaceae	*Allophylus natalensis* (Sond.) De Winter	South Africa	This study
Sapindaceae	*Allophylus decipiens* (E.Mey.) Radlk.	South Africa	This study
Sapindaceae	*Allophylus decipiens* (E.Mey.) Radlk.	South Africa	This study
Scrophulariaceae	*Halleria lucida* L.	South Africa	This study
Scrophulariaceae	*Hebe townsonii* Cockayne &Allan	New Zealand	Sampson and Mclean, (2014)
Smilacaceae	*Smilax australis* R.Br.	Australia	Walter and O'Dowd, (1992a)
Solanaceae	*Capsicum annuum* L.	Brazil, Netherlands	Faraji et al., (2002)Ferreira et al., (2011)
Sterculiaceae	*Commersonia fraseri* J.Gay	Australia	Walter and O'Dowd, (1992a)
Styracaceae	*Styrax japonica* Siebold & Zucc.	Korea	O'Dowd and Pemberton, (1998)
Symplocaceae	*Symplocos chinensis* (Lour.) Druce	Korea	O'Dowd and Pemberton, (1999)
Tiliaceae	*Grewia occidentalis* L.	South Africa	This study
Tiliaceae	*Tilia amurensis* Rupr.	Korea	O'Dowd and Pemberton, (1998)
Ulmaceae	*Ulmus davidiana* Planch.	Korea	O'Dowd and Pemberton, (1999)
Verbenaceae	*Vitex sp.* L.	South Africa	This study
Verbenaceae	*Vitex lucens* Kirk	New Zealand	Sampson and Mclean, (2014)
Verbenaceae	*Clerodendrum glabrum* E.May.	South Africa	This study
Violaceae	*Rinorea angustifolia* (Thouars) Baill.	South Africa	This study
Vitaceae	*Cissus antarctica* Vent.	Australia	Walter and O'Dowd, (1992a)
Vitaceae	*Cissus hypoglauca* A.Grey	Australia	Walter and O'Dowd, (1992a)
Vitaceae	*Cissus sterculiifolia* (F.Muell. ex Benth.) Planch.	Australia	Walter and O'Dowd, (1995)
Vitaceae	*Rhoicissus digitata* (L.f.) Gilg & M. Brandt	South Africa	This study

Vitaceae	*Rhoicissus revoilii* Planch.	South Africa	This study
Vitaceae	*Rhoicissus rhomboidea* (E.Mey ex Harv.) Planch.	South Africa	This study
Vitaceae	*Rhoicissus tomentosa* (Lam.) Wild &R.B. Drumm	South Africa	This study
Vitaceae	*Vitis acerifolia* Raf.	USA	Karban et al, (1995)
Vitaceae	*Vitis aestivalis* Michx.	USA	Karban et al, (1995)
Vitaceae	*Vitis arizonica* Engelm.	USA	Karban et al, (1995)
Vitaceae	*Vitis berlandieri* Planc.h	USA	Karban et al, (1995)
Vitaceae	*Vitis californica* Benth.	USA	Karban et al, (1995)
Vitaceae	*Vitis champinii* Planch.	USA	Karban et al, (1995)
Vitaceae	*Vitis cinerea* (Englem) Engelm. ex Millardet	USA	Karban et al, (1995)
Vitaceae	*Vitis doaniana* Munson	USA	Karban et al, (1995)
Vitaceae	*Vitis girdiana* Munson	USA	Karban et al, (1995)
Vitaceae	*Vitis labrusca* L.	USA	Karban et al, 1995
Vitaceae	*Vitis linsecumii* Buckley	USA	Karban et al, (1995)
Vitaceae	*Vitis monticola*(Buckley	USA	Karban et al, (1995)
Vitaceae	*Vitis mustangensis* Buckley	USA	Karban et al, (1995)
Vitaceae	*Vitis palmata* Vahl.	USA	Karban et al, (1995)
Vitaceae	*Vitis riparia* Michx.	USA,	O'Dowd and Willson, (1997); Norton et al., (2000); Norton et al., (2001); English-Loeb et al., (2002);(2005); Malidossian et al., (2005) Parolin et al., (2014a); Bresch et al., (2015)
Vitaceae	*Vitis rotundifolia* Michx.	USA	Karban et al, (1995)
Vitaceae	*Vitis rupestris* Scheels	USA	Karban et al, (1995)
Vitaceae	*Vitis smalliana* L.H.Bailey	USA	Karban et al, (1995)

Vitaceae	*Vitis treleasei* Munson ex L.H.Bailey	USA	Karban et al, (1995)
Vitaceae	*Vitis vinifera* L.	USA	Karban et al, (1995); Melidossian et al., (2005)
Vitaceae	*Vitis vulpina* L.	USA	O'Dowd and Pemberton, (1998)
Vitaceae	*Vitis amurensis* Rupr.	Korea	O'Dowd and Pemberton, (1998)
Vitaceae	*Coleospermum paniculatum* Lindl.	Australia	Walter and O'Dowd, (1995)
Winteraceae	*Tasmannia insipida* R.Br ex DC.	Australia	Walter and O'Dowd, (1992a)

4. The association between leaf domatia and mites: Is it a mutualism?

Mites are an ancient group of arthropods and the fossil record of mites collected from amber suggest that mites have been associated with plants since the Triassic period (Schmidt et al., 2012; Sidorchuk et al., 2015). Fossil leaves of Elaeocarpaceae and Lauraceae collected in southern Australia also reveal that oribatid mites have been associated with domatia since the Eocene period (O'Dowd et al., 1991). Most fossils of mites associated with plants suggest that earlier mites were fungivorous and scavengers and they have since evolved to include predators, parasites, and plant feeders (Walter and O'Dowd, 1995; Krantz and Walter, 2009). About 45 000 species of mites have been described (Maraun et al., 2007). In tropical and temperate forests floors and canopies the species richness of mites is estimated to exceed that of all other arthropods (Walter and O'Dowd, 1995; Walter and Proctor, 1998; Walter and Behan-Pelletier, 1999).

Mites are widely distributed on plants and may be found on the bark, stems and on leaves of woody plants. Sudo and Osakabe (2011) showed that mite assemblages in wild vegetation preferred the underside of leaves, because the abaxial leaf surfaces of trees were more hairy and abundant in pollen, an alternative food resource for phytoseiid mites, rather than adaxial surface. In plants with leaf domatia, mites are usually associated with these structures. Even though other arthropods may be found inside domatia, the majority of arthropods associated with leaf domatia are mites (O'Dowd and Willson, 1989). For example, in north Asia, 96-99% of arthropods encountered inside domatia were mites (O'Dowd and Pemberton, 1998). Leaf domatia play a significant role in influencing mite assemblages found on leaves (Loughner et al., 2008; 2010; Parolin et al., 2014a; 2014b). They alter mite distribution, abundance and reproduction. In plants that bear leaf domatia, the concentration of mites is always significantly higher in domatia than in vein axils of plants lacking leaf domatia (O'Dowd and Willson, 1991; Norton et al., 2000; Richards and Coley, 2012). In broad-leaved deciduous forests in Korea, mite abundance and reproduction was significantly higher on leaves with domatia than those without domatia (O'Dowd and Pemberton, 1998).

Regardless of the domatia type, the most commonly found guild of mites on plants with domatia are predatory species (Phytoseiidae and Stigmaeidae which feed on other mites) and insects or mycophagous mite species (Tarsonemidae) which feed on fungi and other micro-organisms found on leaf surfaces (Pemberton and Turner, 1989; O'Dowd and Willson, 1991; Walter and O'Dowd, 1992b; Rozario, 1995; O'Dowd and Pemberton 1998; Matos et al, 2006). These mite species are beneficial to the plant's fitness and evidence of their benefit has been shown in numerous studies (Pemberton and Turner, 1989; Walter and O'Dowd, 1995; Agrawel, 1997; Norton 2000; Monks, 2007; O'Connell et al., 2015).

Pemberton and Turner (1989) found that 84% of the plant species growing in California, Hawaii, and Costa Rica had predatory species that are beneficial to plants. Walter and O'Dowd (1992a) observed higher numbers of phytoseiid mites on leaves with domatia compared to leaves without. Monks et al. (2007) found high densities of fungivorous mites inside domatia of *Coprosma lucida* and that these mites significantly reduced fungal hyphae on the plant. The addition of artificial domatia in cotton plants resulted in an increased abundance of predatory mites and a subsequent decrease in herbivorous mites when compared to plants without artificial domatia (Agrawal et al., 2000). Pemberton and Turner (1989) suggested that beneficial mites are pre-adapted to use domatia, because unlike herbivorous mites which can make their own structures, they need these pre-made structures to protect their eggs from desiccation. Phytophagous (Eriophyidae and Tenuipalpidae) mites may also be found within leaf domatia and these feed on leaf surface and may cause damage to plants (Pemberton and Turner, 1989). Thus this association between plant with domatia and mites is believed to be a mutualism and there are benefits for both plants and mites.

4.1. What are the benefits for mites?

Many hypotheses have been proposed to explain the association that exist between host plants and mites (Walter, 1996; O'Dowd and Pemberton, 1998; Matos et al., 2006). Leaf domatia are thought to provide mites with shelter or refuge from other predatory insects and other mites, and a safe place for oviposition and moulting. This hypothesised benefit is widely accepted and many studies have shown evidence that supports it (O'Dowd and Willson, 1991; Walter and O'Dowd, 1992a, Norton at al., 2000; Bresch et al. 2015). In 20 North American species of grapevines, the density of Phytoseiid mite was positively correlated with the presence of leaf domatia and vein hairs suggesting that these mites use the structures for shelter (Karben et al., 1995). Norton et al. (2001) examined the inhabitants of leaf domatia of *Vitis riparia*. Common leaf mites were found inside the domatia and these were interpreted as evidence that the mites use domatia as hideaway places in the presence of predatory bugs and beetles, which were also found on the leaves of this species. Walter and O'Dowd (1992a) observed higher numbers of phytoseiid mites on leaves with domatia compared to leaves without. In almost every instance, the mites laid their eggs inside the domatia. Grostal and O'Dowd (1994) observed that the predatory mite *Metaseilus occidentalis* (Nesbitt) always laid its eggs inside domatia, suggesting that the mites use these structures as protective places in the presence of spotted spider mites. In an experiment to test mite preference for banker plants, the predatory mites *Neoseiulis califomicus* (McGregor) and *Phytoseiulus persimilis* (Dosse) chose plants with leaf domatia (Bresch et al., 2015). A banker plant is a plant that is purposely incorporated within a crop system with the intention to increase biocontrol of pests. The plant does this by regulating pests and fostering high densities of the natural enemy of crop pests (Parolin et al., 2012).

Walter and O'Dowd (1992a) conducted a blocking experiment on leaves to reduce the number of domatia available to mites and their results showed a reduction in the number of phytoseiid mites on blocked leaves. Also, the blocking experiment negatively affected the number of eggs present inside domatia. Grostal and O'Dowd (1994) observed similar results, where oviposition by *Metaseiulus occidentalis* was reduced on leaves with blocked domatia. In another comparative study looking at blocked domatia of certain rainforest species, fungivorous mites were observed to be six times more common on leaves with intact domatia than on adjacent leaves with blocked domatia, providing evidence that leaves with domatia are more attractive to beneficial mites than are those without domatia (Walter and O'Dowd, 1995).

Hair-tuft type domatia are proposed to trap and collect air-borne pollen grains and fungal spores and thus providing mites with readily available food sources (Romero and Benson, 2005). It is very unlikely that this benefit is realised by mites, because not all domatia types have trichomes and also a few pollen grains may be trapped in the trichomes of leaf domatia (Romero and Benson, 2005). However, Duso et al. (2004) presented partial evidence for this benefit in their study of phytoseiid mites and pollen relationships, which showed that natural hedgerows trapped pollen. Major peaks of pollen abundance were followed by increased phytoseiid mite abundance suggesting that a relationship exists between pollen availability and phytoseiid abundance (Duso et al., 2004). Kreiter at al. (2002) found high pollen densities inside the domatia of grapevines and suggested that the pollen was important in maintaining phytoseiid mites. Similarly, Addison et al. (2000) found that trichomes enhanced pollen densities on the leaves of *Malus pumila* Mill., an apple cultivar, and that pollen was an important supplementary food source in addition to the eriophyid *Aculus schlechtendali* (Nalepa) for phytoseiid mites. Roda et al. (2003) also showed that apple tree leaves having numerous trichomes or domatia frequently harbour greater numbers of phytoseiid mites than plant with leaves that lack these structures, because trichomes increase the capture of pollen or fungal spores that serve as alternative food.

Leaf domatia are believed to affect the microclimate of leaves by maintaining favourable humidity levels and thus creating suitable conditions for mites and to prevent desiccation of their eggs (O'Dowd and Willson, 1991; Norton et al., 2000; Grostal and O'Dowd, 1994). This benefit may be especially important in the inactive stages during moulting where mites may be vulnerable to physical extremes. There is evidence that mites and their eggs are sensitive to low relative humidity, but little direct evidence exists to support this hypothesis. Grostal and O'Dowd (1994) showed reduced oviposition by predatory mites at low relative humidity suggesting that domatia provide mites with a safe place during periods of extreme environmental conditions. Another study by Croft et al. (1993) showed that eggs and larvae of phytoseiid mites were sensitive to relative humidity. For all four species of phytoseiid mites subjected to humidity assays, egg and larvae mortality increased with decreasing humidity (Croft et al., 1993). Furthermore, Williams et al. (2004) found that egg hatching in some phytoseiid mites was susceptible to low relative humidity. Rowels and O'Dowd (2009) found contrasting results. They found that mites were present in domatia irrespective of the relative humidity levels that the leaf was subjected to. However, mite reproduction increased when leaves were subjected to 70% relative humidity as opposed to leaves exposed to lower relative humidity, suggesting that relative humidity alone cannot explain the association between mites and domatia bearing plants. Furthermore, Norton et al. (2001) showed that humidity assays involving phytoseiid mites did not show evidence for the microclimate benefit from domatia.

It has been hypothesised that mites may obtain food in the form of metabolites provided by plants through specialised structures inside domatia (O'Dowd and Willson, 1991; Tilney et al., 2012). Tilney et al. (2012) observed channel-like structures made up of thick cuticular folds in cell walls of epidermal cells lining the domatia of *Plectroniella armata*. These structures are thought to be associated with increased surface area to facilitate some translocation of compounds and metabolites. These findings also indicate that some form of communication may be possible between domatia of this plant and their inhabitants (Tilney et al., 2012). This benefit is highly unlikely as most species that bear domatia lack the structures and enzymes needed to facilitate such exchange of compounds (Anderson, 2005; Nishida et al. 2006). This is the only published study that found channel-like structures inside domatia as evidence for some sort of communication structures inside domatia and it is unlikely that such benefit is realised. Even so, this avenue of research has received little attention and more work still needs to be conducted to further ascertain this benefit.

4.2. What are the benefits for plants?

The benefit of being involved in the mutualism for plants is believed to be increased plant fitness because mites reduce herbivory and pathogen loads on leaves. Over 90% of mite taxa found to inhabit leaf domatia are mycophagous and predatory and these mites help reduce damage on leaf surfaces by feeding on fungi and phytophagous mites that may cause harm to the plant leaves. This decreases the damage caused by plant enemies on plants with leaf domatia (O’Dowd and Willson, 1991; Norton et al., 2000; Monks, 2007; O’Connell et al., 2015). Mites thus appear to act as plant “bodyguards” and protect plant leaves from damage, potentially reducing the resources a plant has to allocate to defence or replacing lost tissues and allowing them to reallocate their resources to vegetative growth and reproduction.

Evidence for this proposed benefit has been shown in semi-natural experimental systems in several studies. Cotton plants with artificial domatia added to them had increased populations of predatory mites, and their cotton yield was enhanced compared to plants without domatia (Agrawal et al., 2000). Norton et al. (2000) examined the abundance of the mycophagous mite, *Orthotydeus lambi* (Baker), in domatia of *Vitis riparia* Michx. and the impact that the mite had on a key fungal pathogen of the plant species, grape powdery mildew. Their experimental manipulations involved blocking a number of domatia with glue and leaving some open as a control. Higher densities of mites were observed in plants with intact domatia. Most importantly, plants with intact domatia had a significant reduction in leaf area covered in grape mildew than plants with blocked domatia. This study was the first to show direct benefit to plants through increased abundance of mycophagous mites, which resulted in the decrease in fungal attack. In addition, a study by Romero and Benson (2004) found less damage on leaves with intact domatia and an increase in leaf area damage caused by phytophagous mites in leaves of *Cupania vernalis* Cambess. (Sapindaceae) that had blocked domatia. The increased damage was due to fewer numbers of predatory mites on leaves with blocked domatia compared to intact domatia. Monks et al. (2007) and O'Connell et al. (2015) showed that the presence of fungivorous mites inside the domatia resulted in fewer fungal spores on leaves in *Coprosma lucida* and that these mites were as effective as fungicide at controlling fungal hyphae. Similarly, English-Loeb et al. (2007) found that fungivorous mites could effectively reduce powdery mildew loads in grapes.

Lundström (1887) also suggested that plants may benefit from the excretions or exudates left behind by mites and that plants may take up these nutrients, as it occurs in some plant–ant associations (Pemberton and Turner, 1989). However, no study has been conducted to show direct evidence for this benefit and it is very unlikely that this process could occur, as domatia lack the necessary enzymes and structures to facilitate the breakdown and uptake of compounds unlike plants with ant mutualism (Pemberton and Turner, 1989). Furthermore, domatia lack cuticular gaps and pores that could facilitate absorption of aqueous compounds similar to those in carnivorous plants such as *Roridula dentata* L. and *R. gorgonias* Planch. (Anderson, 2005). Tilney et al. (2012) suggested some form of communication in the form of translocation of compounds that may exist between domatia and mites, but provided no evidence for any particular enzymes that may assist this translocation.

5. Leaf domatia research in Africa

Most literature on leaf domatia and mite mutualism comes from developed countries. Research globally has focused on experimentally evaluating the association between leaf domatia-bearing plants and predatory mites, assessing the benefits that plants derive and exploring how this mutualism can be manipulated to enhance its role in the protection of crop plants (Norton et al., 2000; English-Loeb et al., 2002; Onzo et al., 2003; Cortesero et al., 2000). Some research has focused on the generation of lists of plant species with leaf domatia and the diversity of mites found within the domatia of these species (Castro and Moraes, 2007; Pemberton and Turner, 1989; O’Dowd and Willson, 1991; O’Dowd and Pemberton, 1998; Nishida et al., 2006).

However, there is little published data on leaf domatia and their associated mites from South Africa and the rest of Africa. There is only a single paper by Tilney et al. (2012) which indicates that one plant species has epidermal structures in its leaf domatia, which suggests the plant might be providing mites with some form of reward. Also, Professor Ueckermann and colleagues have collected and described mites from southern Africa and South Africa including new species from the Tydeidae (*Peralorryia*, *Lorryia* Oudemans) Anystidae, Camerobiidae, Phytoseiidae (*Kampimodromus* Nesbitt, *Amblyseius* Berlese, *Platyseiella* Muma) to name a few from various hosts including species with domatia (Ueckermann et al., 1979a; Ueckermann et al., 1979b; Ueckermann and Loots, 1985; 1987; Ueckermann, 1990; Du Toit et al., 1998; Ueckermann and Grout 2007). There are no other data on mite diversity and the leaf domatia-mite mutualism and thus Africa presents a range of research opportunities.

6. Project aims

The literature reviewed provides insight on the nature of the leaf domatia and mites association, how it operates and the benefits of the mutualism. However, it also gives rise to many further questions particularly about what drives the composition and diversity of mite communities found in plants with leaf domatia. This is a big knowledge gap in our holistic understanding of this association. This project thus aims to fill that gap and address some of the questions raised. This study is one of a few that studies mite communities and surveys the plant species involved in the leaf domatia–mite mutualism. Furthermore, it is also the first study to document and research the mutualism in South Africa. This will be done by addressing the following aims and objectives, which each formed a sub-project and their results are presented in 8 data chapters:

- First, the anatomical structure of leaf domatia from six selected plant species will be examined. The epidermal structure of the different domatia types will be also studied for the presence of modifications in their cuticle in an attempt to provide further evidence of exchange of metabolites between plants and mites. This work is presented in Chapter 2 and will be one of a few studies that document the anatomy of the different domatia types found across angiosperms.

- Chapter 3 aims to investigate whether mites found inside lead domatia are host specific or not. The key question for the study is: “Does each tree species host have a specific mite or mite assemblages, and do mites prefer a certain leaf domatia type?” This study will provide us a better understanding of how the plant-mite mutualism manifests itself and shed light on the co-evolution of this mutualism.
- The study then investigates the distribution of mite species across different vegetation types found within the Forest and the Albany Thicket biomes of South Africa. This work is presented in Chapter 4 and the key question is: “Do different vegetation sites and types differ in their mite diversity and species composition?” This study will highlight the diversity of mites associated with some of South Africa’s vegetation types and how mite lineages differ between different habitats with different vegetation structure.
- Chapter 5 surveys the distribution of mites within the tree canopy. The question addressed here is: “Do mites prefer a specific place in the tree canopy and does the microclimate in the tree canopy affect the distribution of mites?” This study will give us a better understanding of how mites respond to canopy microclimate and where mites are likely to be found within the tree canopy. This information will also have important implications for sampling approaches of future studies surveying mites.
- In Chapter 6, seasonal patterns in mite diversity and abundance are examined in three South African plants with leaf domatia. The key question for the study is: “Does mite abundance and diversity vary with season?” This work is important as it will provide insight into how and when mites utilise domatia, allow us to predict periods of mite pest explosion in plants, and also be used to improve sampling strategies in future mite-domatia studies..
- Lastly, the project examines mite abundance and diversity in coffee plantations and in a neighbouring forest patch in order to highlight the importance of this mutualism in commercial plants. The key question for this study was: “Do coffee plantations have a different suite of mites than adjacent forest and does the coffee plantation support higher diversity of mites compared to the adjacent forest site?” Presented in Chapter 7, this work

is an applied example documenting the plant-mite mutualism in an economically important plant that originates from a foreign country.

New South African species of mites found in different South African forests were also collected and are yet to be described and will be published at a later stage.

Chapter 2: A comparative study of the structure of Leaf Domatia in six South African woody plant species

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1. Introduction

Leaf domatia are common features in dicotyledonous plants and are frequent in several families including the Rubiaceae, Vitaceae, Annonaceae and Bignoniaceae to name a few (Romero and Benson, 2005). These structures are morphologically diverse both between families and even within plant species (O'Dowd and Willson, 1989; Nishida et al., 2006). They take the form of pits (which are invaginations of the leaf surfaces that extend to the mesophyll), a dense tuft of hairs, cavities which extend beneath expanded veins, and rolled vein margins. However, a combination of these types in one species is also common (O'Dowd and Willson, 1989; Romero and Benson, 2005).

Many ecological studies have attempted to clarify the function of leaf domatia in the interaction between plants and mites (Pemberton and Turner, 1989; O'Dowd and Willson, 1991; Walter and O'Dowd, 1992a; Walter, 1996; Norton et al., 2000; Romero and Benson, 2005). However, only a handful of studies have focused on the anatomy and ontogeny of leaf domatia (O'Dowd and Willson, 1989; Nakamura et al., 1992; Martinez-Solis et al., 1993; Nishida et al., 2006; Moraes et al., 2011). These studies describe these structures and suggest that their formation is characterised by cell differentiation and active cell division in the lower mesophyll.

A comparative study by Nishida et al. (2006) on the anatomy and development of the different types of domatia in *Cinnamomum camphora* (L.) J.Presl. is the only study to date that provides a better understanding of the changes that occur at a cellular level when plants are developing these structures. Unusually for leaf domatia bearing plants, *C. camphora* produces four domatia types (pouch, pubescent pit or hair tuft-type, glabrous pit-type, and dish-type or cavities) within a single leaf, which makes this species a good choice for anatomical studies. In their findings, Nishida et al. (2006) found that the upper mesophyll tissue, the lower mesophyll tissues and the tissues filling the rim opening, make up the anatomy of the domatia types they studied. Differences between domatia types were recognised as a consequence of differences in the cell types of the upper mesophyll tissue and in the size and number of cells of the rim tissue (Nishida et al., 2006).

Additional studies are thus needed in order for us to gain an increased understanding of how plants benefit from producing these structures and how they facilitate the relationship between plants and mites. This is particularly important as some studies suggest that plants may be providing mites with food in the form of metabolites through specialised structures inside domatia (O'Dowd and Willson, 1991; Tilney et al., 2012). Tilney et al. (2012) observed channel-like structures made up of thick cuticular folds in domatia of *Plectroniella armata* (K.Schum.) Robyns. They concluded that these structures are associated with increased surface area to facilitate some translocation of compounds and metabolites, and that these structures indicate that some form of communication may be possible between the domatia of this plant and their inhabitants (Tilney et al., 2012). This however, is the only study that presented indirect evidence for some sort of structures inside domatia that may facilitate the mutualism. More anatomical studies are needed to explore the structure of leaf domatia in other species.

The aim of this particular study was to examine the anatomy of six plant species with different domatia types. Similar to Tilney et al. (2012), the study also examined the epidermal structure of the different domatia types for presence of modifications in their cuticles in an attempt to provide further evidence of the exchange of metabolites between plants and mites.

2. Methods

Six plants species namely *Coffea arabica* L., *Gardenia thunbergia* Thunb., *Rothmannia capensis* Thunb., *Rothmannia globosa* (Hochst.) Keay from Rubiaceae, *Ocotea bullata* (Burch.) E. Meyer in Drege from Lauraceae and *Tecoma capensis* (Thunb.) Lindl. from Bignoniaceae, were selected for this study. These plants were chosen because various other studies that form part of this thesis were already been conducted on them. The specimens from these species were examined using Light Microscopy, Scanning Electron Microscopy and Transmission Electron Microscopy. For each species, a maximum of four leaves were collected. *Coffea arabica* specimens were collected at Beaver Creek in Port Edward; *G. thunbergia, R. capensis, R. globosa, O. bullata* and *T. capensis* were collected at the University of Pretoria gardens.

2.1. Preparation for Light Microscopy

A small section of the leaf containing the domatia and surrounding tissue was cut from each specimen and fixed in formaldehyde, Acetic acid, and alcohol (FAA) for a week. A series of alcohol solutions were used to dehydrate the leaves, starting with 30% butanol. Daily changes of 50%, 70%, 90% then 100% n-butanol were made and the alcohol was then replaced with paraplast wax. The leaves were embedded in the wax to provide support during sectioning. Serial sections of 8 µm were cut from the leaves using an ultra-microtome. In order to view the cell wall and cytoplasm, the sections were stained with safranin and fast green. Permanent mounts were produced using Entellan® (Product 7961, E. Merck, Darmstadt) rapid mounting medium. The sections were examined using an Olympus Light Microscope and photographs of the modified regions of domatial epidermis were taken with a camera at a fixed magnification.

2.2. Preparation for Scanning Electron Microscopy

Leaf sections were prepared for the Scanning Electron Microscope (SEM) to study the epidermis, and look for evidence of cuticular folds inside leaf domatia. Thin sections were cut through the domatia opening and these were fixed in 2.5% glutaraldehyde/formaldehyde solution for an hour and then washed with a phosphate buffer three times for 15 minutes in each wash. The sections were then put in 1% osmium tetroxide solution for another hour. Osmium tetroxide functions as a secondary fixative and it reacts with lipids. The leaf material was then washed with phosphate buffer three times and then dehydrated using a graded series of ethanol (30%, 50%, 70%, 90% and 3x100%) for 15 minutes in each concentration. The leaves were then left in 100% ethanol for 30 minutes and then after a mixture of 50:50 hexomethyldisilazane (HMDS) and 100% ethanol was added to the samples and the samples were allowed to rest for an hour. Pure HMDS was then added to the samples twice and they were left to rest for another hour. When HMDS was then added for the second time, the samples were left open to dry overnight. The samples were then mounted on aluminium stubs with double-sided carbon tape and coated with carbon. The stubs were then viewed under the SEM and high resolution images were taken.

2.3. Preparation for Transmission Electron Microscopy

Fresh leaf sections were prepared for Transmission Electron Microscopy (TEM) to study the epidermis and look for evidence of cuticular folds inside leaf domatia. These samples were fixed in glutaraldehyde/formaldehyde solution and dehydrated in an ethanol series in the same way as mentioned above. After that the leaf material was embedded in LR white resin solution in ethanol. Embedding was a stepwise process and the resin solution was increased in concentration (20%, 40%, 60%, 80%, and 100%) every hour. The plant material was transferred into gelatine capsules and fresh 100% resin was added and these were placed in the oven to polymerize for 36 hours. The resin block was then trimmed, sectioned and the contrast of the sections was enhanced with uranyl acetate and lead citrate. These were then viewed using a TEM and photographs were taken at fixed magnifications.

3. Results

3.1. Morphology of domatia based on Light Microscopy

The studied plant species possessed different types of domatia, and even though they may have appeared morphologically similar, their anatomical structure differed. Under a dissecting microscope the domatia of *C. arabica* appeared as a hairless pocket, while *O. bullata*, *R. capensis* and *G. thunbergia* had a pouch/pit-type domatia with trichomes. On the other hand, *R. globosa* and *T. capensis* had a hair-tuft domatia. The light microscopy study revealed the internal anatomical structure of these different domatia types (Figures 5-10). The results observed with regards to the three domatia types represented by these study species are discussed below.

Pocket-type domatia: The transverse section of the domatia of *C. arabica* revealed that the domatia form a pronounced cavity that extends deep beneath the veins. The domatia are hairless and big enough (about 270 µm) to accommodate several mites at once (Figure 5b). The histology indicated that the domatia comprised of a single upper epidermal layer, two palisade mesophyll layers, five or six spongy mesophyll layers, three layers of tightly packed rectangular mesophyll cells labelled domatia tissues (LD), and a single layer of the lower epidermis (Figure 5c). The lower epidermis of the domatia appeared to have a thickened cuticle in comparison to non-domatial areas. The rim of the domatia is filled with enlarged collenchymatous cells of different sizes labelled rim tissue (RT) (Figure 5d). For comparison, a transverse section of non-domatial lamina is given in Figure 5a. The non-domatial lamina consisted of a single layer of upper epidermal cells, one layer of palisade mesophyll cells and eight or nine layers of spongy mesophyll and one lower epidermal layer. The main difference between non-domatia and domatial lamina is that the intercellular airspace of spongy mesophyll was smaller than that of non-domatial lamina.

Pouch-type domatia: Like the pocket-type domatia, the pouch-type consisted of five histological parts; the upper epidermis, palisade mesophyll, spongy mesophyll, the domatial tissue, and lower epidermal layer. However, unlike in the pocket-type, the lower epidermis of the pouch-type had trichomes. All three plants with the pouch-type domatia had a single layer of upper epidermal cells, two layers of palisade mesophyll cells and a single layer of the lower epidermis. The rim tissue consisted of enlarged collenchyma cells (Figures 6-8). However, the main differences between the species were that *G. thunbergia* (Figure 6) had about three layers of tightly packed spongy mesophyll, while *O. bullata* (Figure 7) had about eight layers of spongy mesophyll and these were loosely packed and with noticeable intercellular air space in-between. *Rothmannia capensis* (Figure 8) had four layers of spongy mesophyll and these cells were also tightly packed. Trichomes in both *G. thunbergia* and *R. capensis* were abundant inside the domatia and the cuticle appeared thickened (Figures 6d and 7d). In *O. bullata* trichomes were restricted to the rim opening and were absent deep inside the pocket and the cuticle appeared thickened throughout the leaf and on both sides of the leaf (Figure 8). Also the cuticle was slightly stained with safranin. For comparison the non-domatial lamina for these plants are given in Figures 6a, 7a and 8a.

Hair tuft-type: No cell differentiation and modifications were seen on the hair tuft type domatia of *T. capensis* and *R. globosa*. The domatial lamina in both species consisted of a single layer of epidermal cells, about one layer of palisade mesophyll, three layers of spongy mesophyll and a layer of lower epidermis. In *T. capensis* the lower epidermis consisted of branching trichomes (see Figure 9). On the other hand, the domatia of *R. globosa* consisted of a deep invagination, similar to that of the pouch-type domatia with simple trichomes on the lower epidermis (Figure 10).

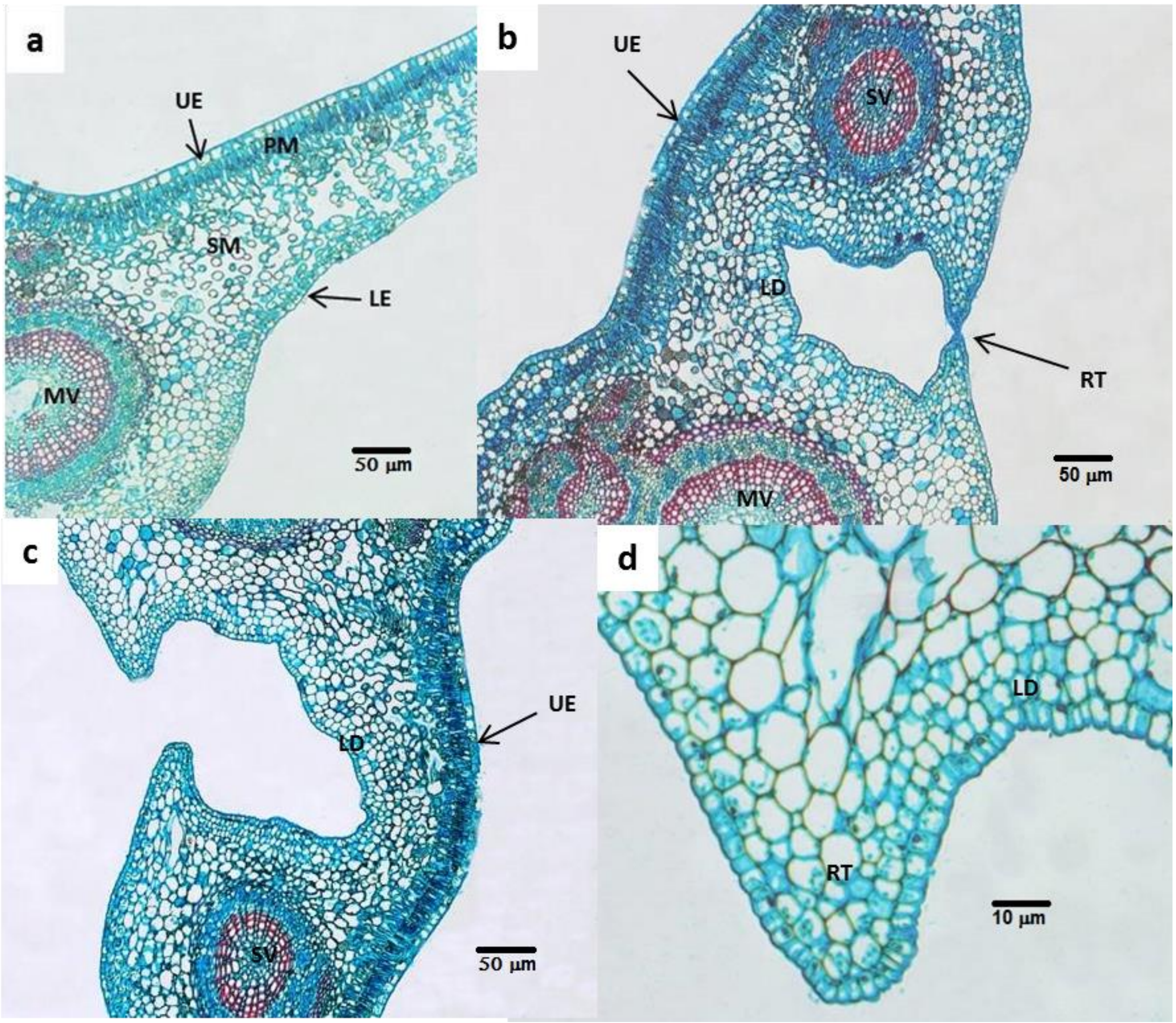

Figure 6: *Transverse section of the pocket-type domatia in Coffea arabica; (a) non-domatial area, (b) shows the pocket with the rims connected together by a few cells, (c) shows domatia opening, and (d) is a close-up picture of the rim tissue cells. LD: leaf domatial tissue, LE: lower epidermis, MV: mid-vain, UE: upper epidermis, PM: palisade mesophyll layer, RT: rim tissue, SM: spongy mesophyll layer and SV: secondary vein.*

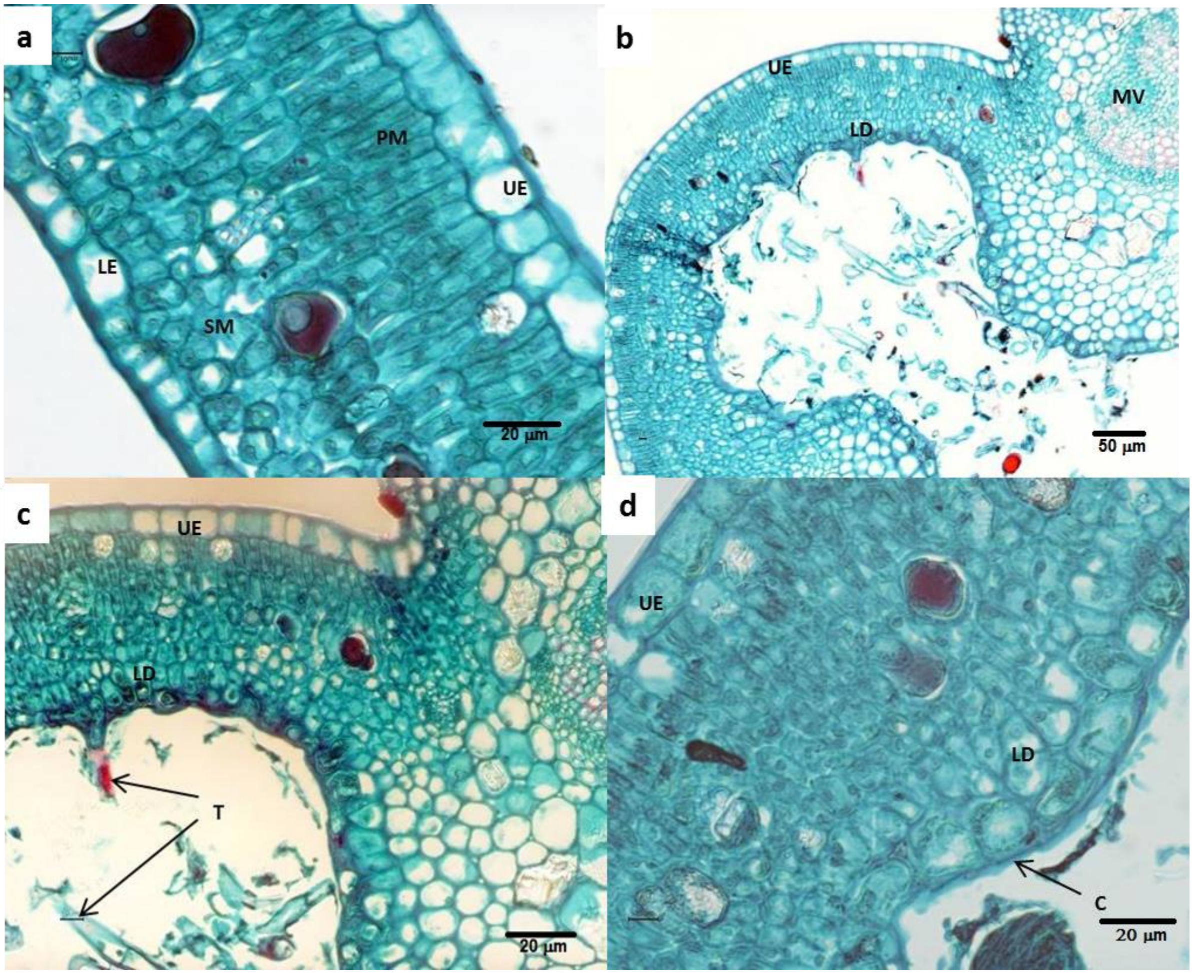

Figure 7: *Transverse section of the pouch-type domatia in Gardenia Thunbergia; (a) non-domatial area, (b) domatial opening, (c) high magnification of domatial tissue showing trichomes, and (d) shows thickened cuticle inside domatia. C: cuticle, LD: leaf domatial tissue, LE: lower epidermis, MV: mid-vain, UE: upper epidermis PM: palisade mesophyll layer, RT: rim tissue, SM: spongy mesophyll layer; and T: trichomes.*

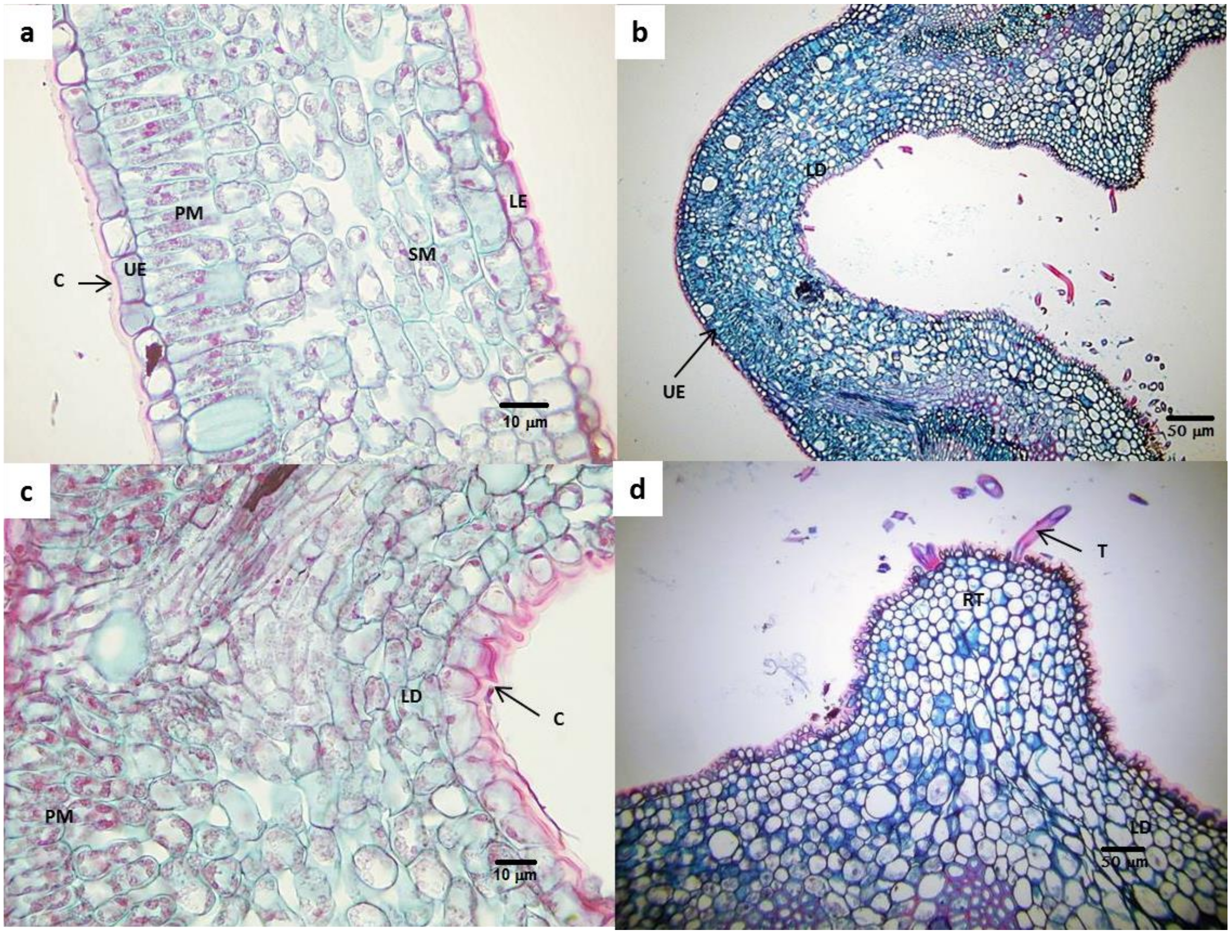

Figure 8: *Transverse section of the pouch-type domatia in Ocotea bullata; (a) non-domatial area, (b) domatial opening, (c) domatial tissue at high magnification, and (e) a close-up image of the rim tissue showing trichomes. C: cuticle, LD: leaf domatial tissue, LE: lower epidermis, UE: upper epidermis PM: palisade mesophyll layer, RT: rim tissue, SM: spongy mesophyll layer; and T: trichomes.*

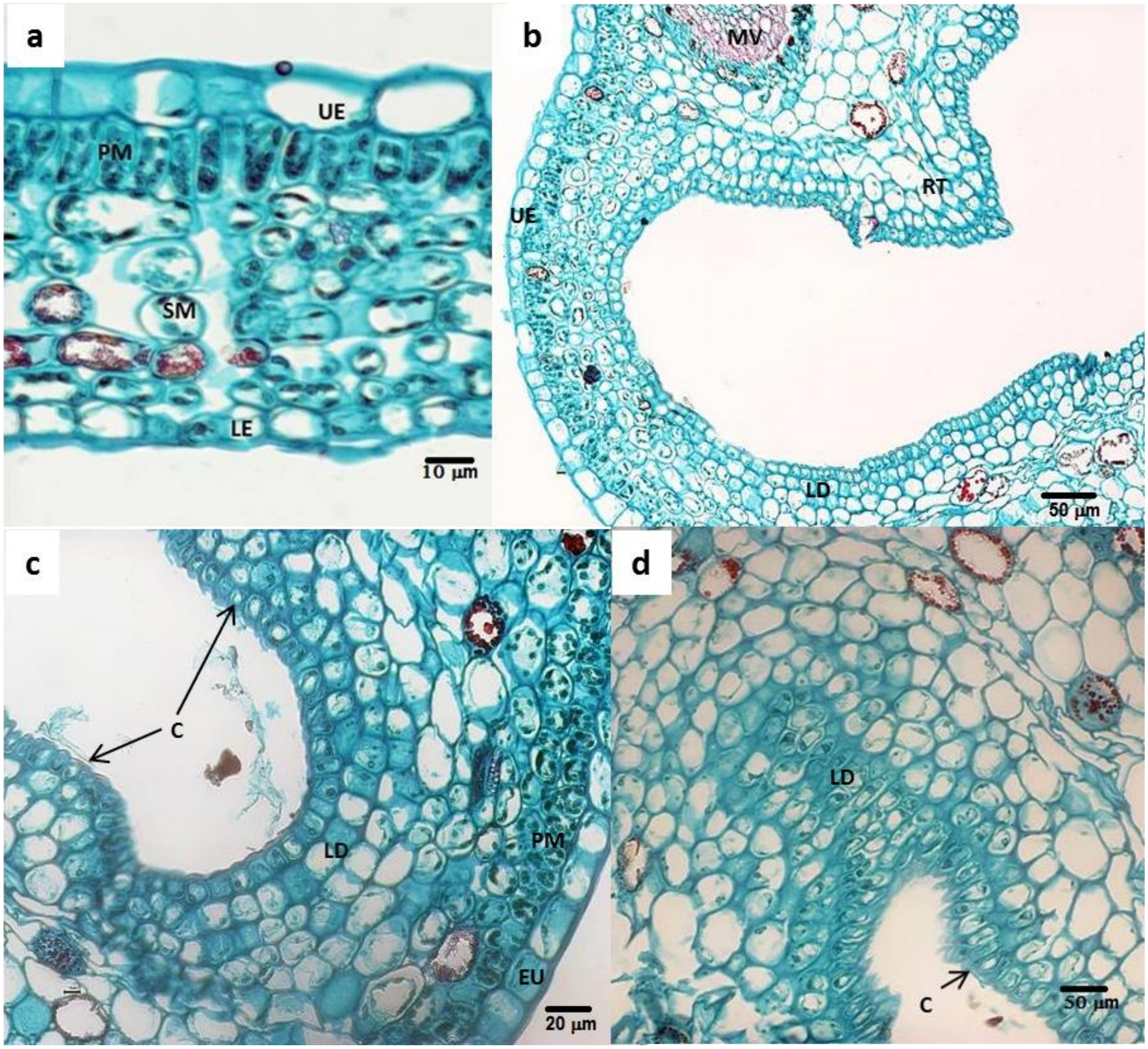

Figure 9: *Transverse section of pouch-type domatia in Rothmannia capensis ;(a) non-domatial area, (b) domatial opening, (c) domatial tissue at high magnification, and (d) shows rapidly dividing cells inside domatia and a thick cuticle. C: cuticle, LD: leaf domatial tissue, LE: lower epidermis, MV: mid-vain, UE: upper epidermis, PM: palisade mesophyll layer, RT: rim tissue and SM: spongy mesophyll layer.*

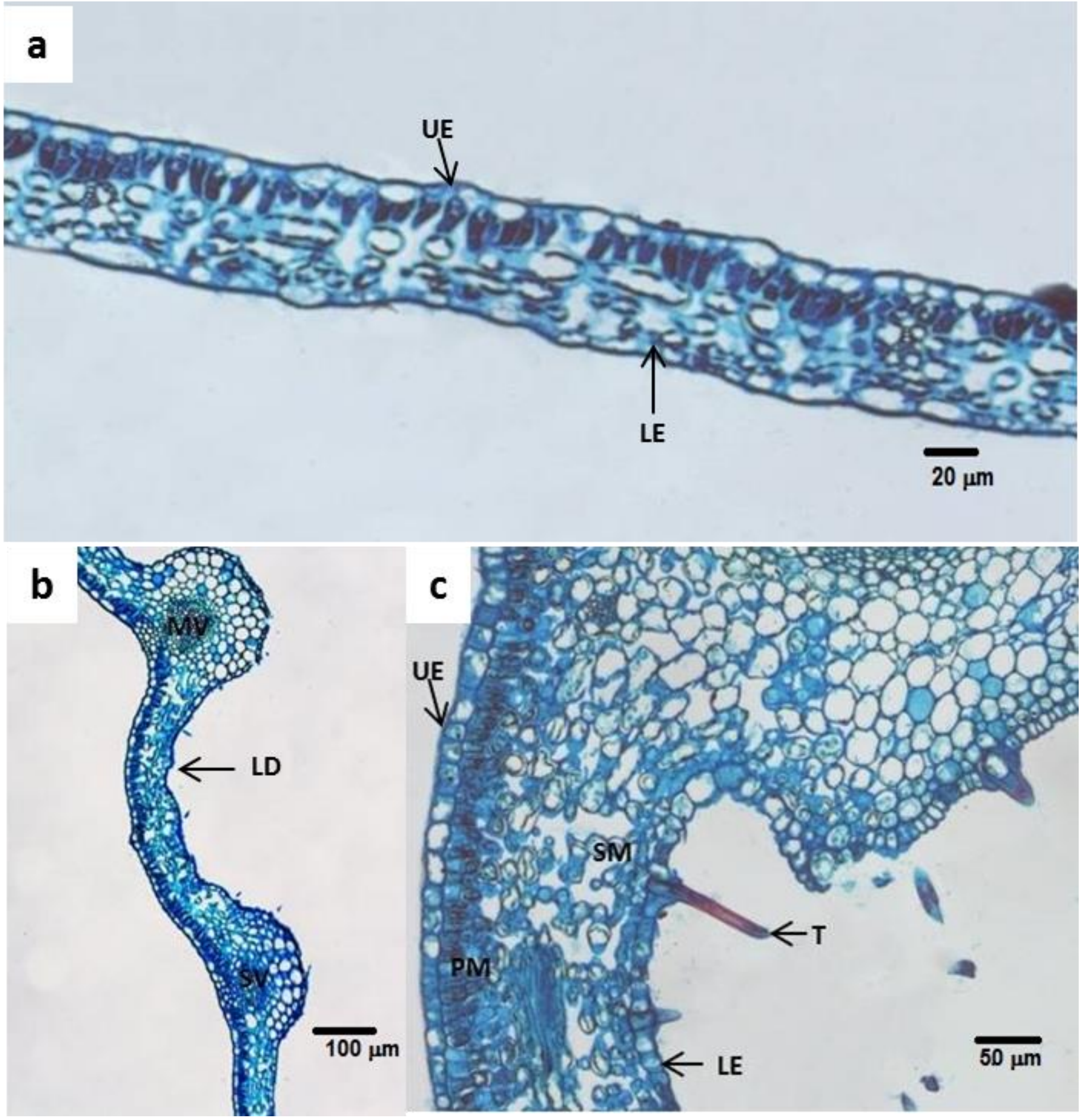

Figure 10: *Transverse section of the hair tuft-type domatia in Tecoma capensis; (a) non-domatial area, (b) domatia in low magnification, and (c) high magnification of domatial area showing trichomes. LD: leaf domatial tissue, LE: lower epidermis, MV: mid-vain, UE: upper epidermis PM: palisade mesophyll layer, SM: spongy mesophyll layer, SV: secondary vain, and T: trichomes.*

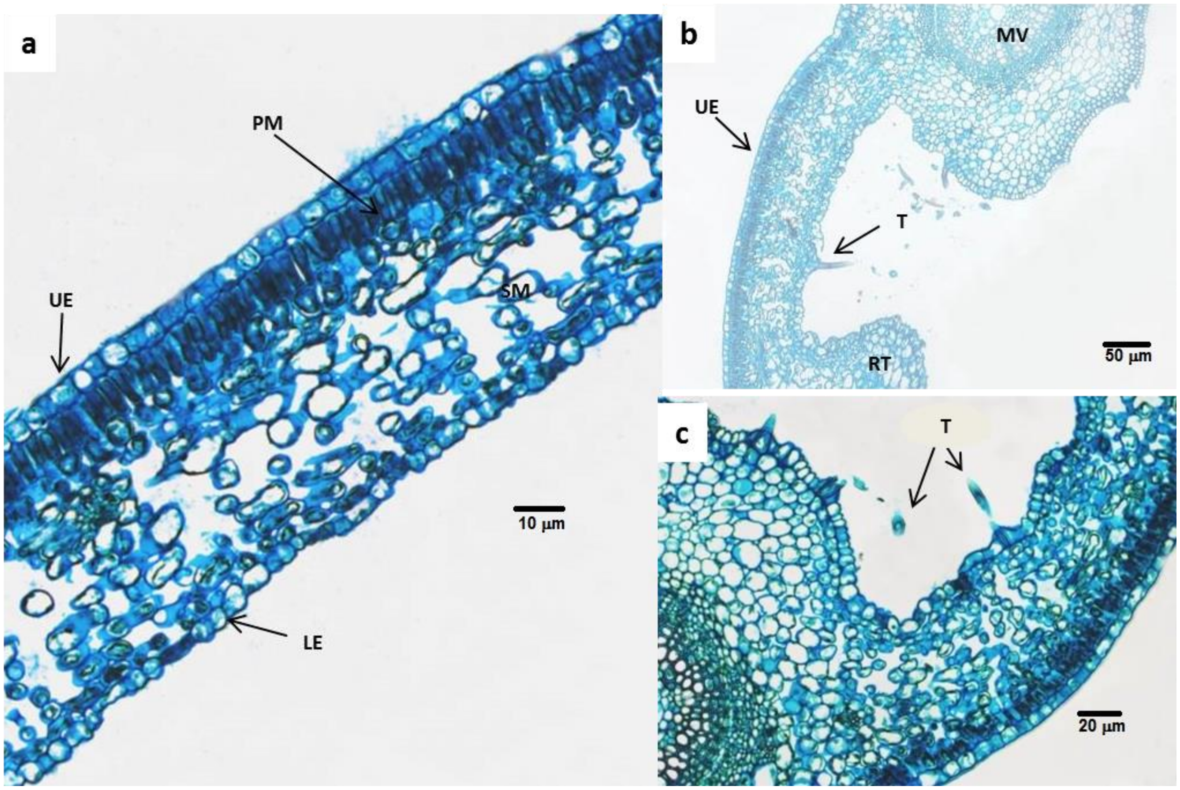

Figure 11: *Transverse section of the hair tuft-type domatia in Rothmannia globosa; (a) non-domatial area, (b) shows the domatial opening, and (c) is a close-up image of the domatial area showing trichomes. LD: leaf domatial tissue, LE: lower epidermis, MV: mid-vain, UE: upper epidermis, PM: palisade mesophyll layer, RT: rim tissue, SM: spongy mesophyll, and T: trichomes.*

3.2. Electron microscopy study of the anatomical modification of the epidermis and cuticle found inside leaf domatia

Unfortunately, there were some challenges with sample preparation for the TEM study and, after many attempts and time constraints, this approach was abandoned. Only a few pictures from four (*R. globosa*, *R capensis*, *C. arabica* and *G. thunbergia*) out of the six study plants were obtained. The results from the TEM study of the domatia revealed that the study plants did possess cuticular folds in the epidermis of leaf domatia. In *R. capensis* the cuticular folds were found consistently inside the domatia, but were not present in the non-domatial epidermis on the underside of the leaf (Figures 11a and b). The cuticular folds were not restricted to the domatial area, but were also seen in some areas on the adaxial side of the leaf (Figures 11e and f). However, these were not as pronounced as in the domatial area. The SEM study also suggested that the epidermis of this plant is highly folded inside the domatia when compared to non-domatial areas (see Figures 14c and e).

In *C. arabica* the cuticle of the domatia was seen to be folded in some areas, but not elsewhere (Figure 12a). These can also be seen under the SEM (Figure 13c). Similarly, the cuticular folds were present on domatia of *G. thunbergia* and *R. globosa*, but were not regularly seen (Figures 12c-f). While good pictures from TEM preparation for the other study plant were not obtained, the SEM pictures of the domatial epidermis of *T. capensis* suggested that this plant also possess cuticular folds around the domatial area which can be clearly seen in Figure 15d. The folds in the epidermis were not seen outside the domatia under SEM (Figure 15f). The SEM study suggested that these folds may also be present in the domatia of *G. thunbergia*; *R. globosa* and even *O. bullata* (see Figures 13, 14 and 15).

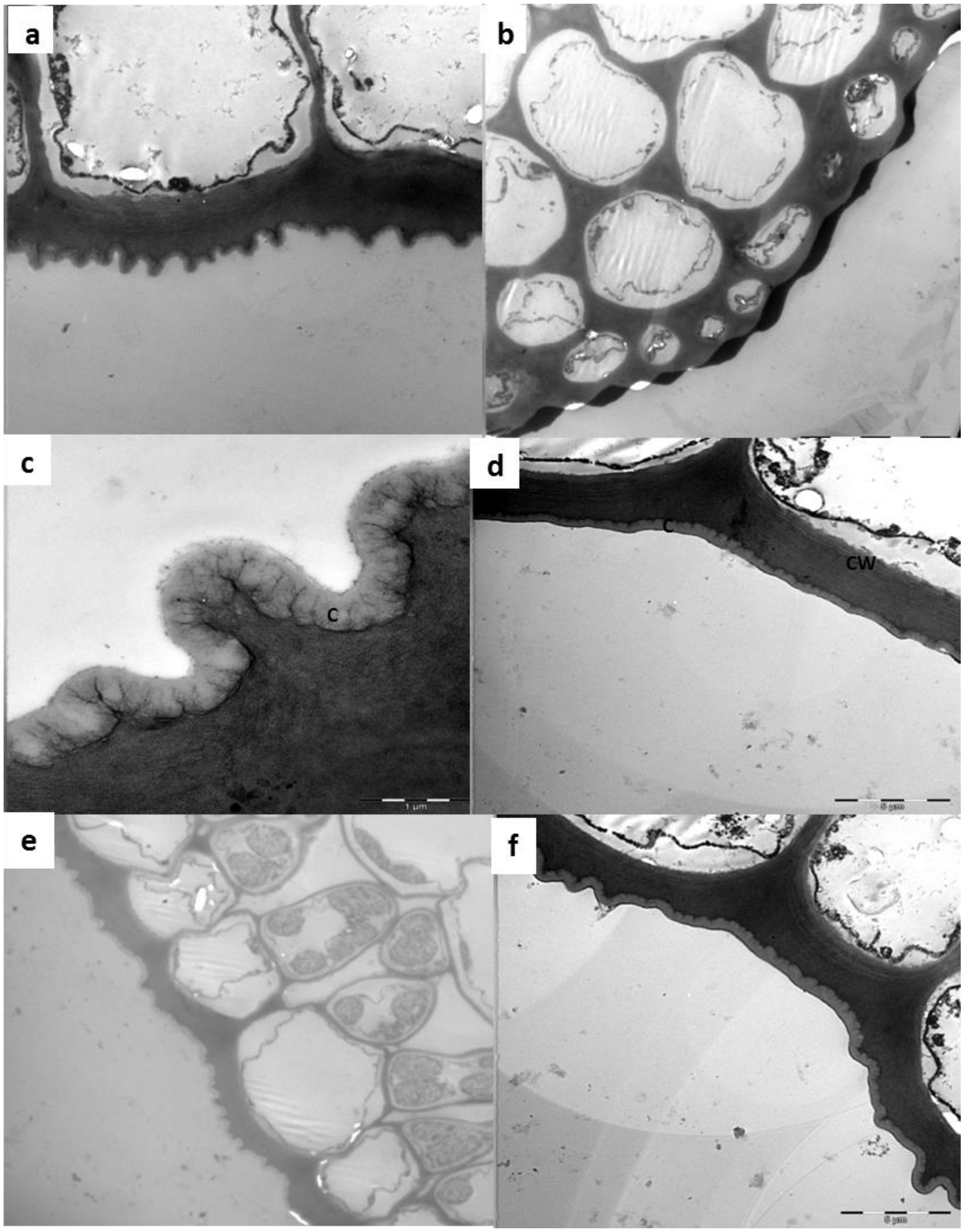

Figure 12: *TEM images of a section through the domatia of Rothmannia capensis, (a) shows cuticular fold present in the epidermis of the domatia, (b) shows the structure of non-domatial epidermis, (c) is a close-up image of the epidermal cuticular folds found inside the domatia, and (d) is a close-up image of non-domatial epidermis. The cuticular fold were not restricted to domatial epidermis. Images (e and f) show that the cuticular folds were also present on the upper surface of the leaf. C: cuticle and CW: cell wall.*

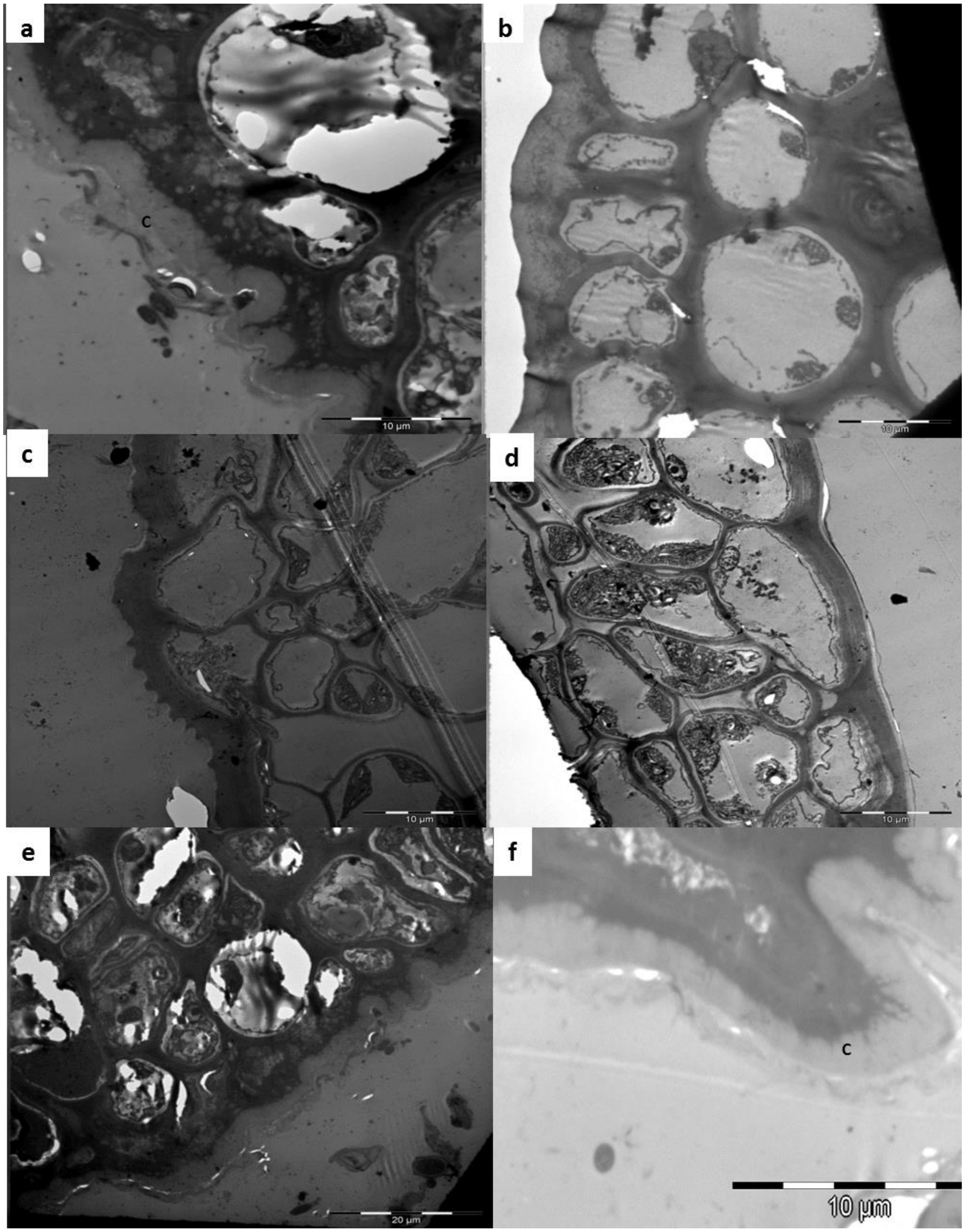

Figure 13: *TEM picture of the domatial epidermis of (a) Coffea arabica. Cuticular folds can be seen inside the domatia however, these are not consistently found all throughout the domatium, (b) non-domatial epidermis of Coffea arabica (cuticular folds are absent), (c) cuticular fold in domatial epidermal layer of Gardenia thunbergia, (d) non-domatial epidermis of Gardenia thunbergia, (e) domatial epidermal layer of Rothmannia globosa, and (f) is a magnified image of the cuticular fold of Rothmannia globosa. C: cuticle). 7*

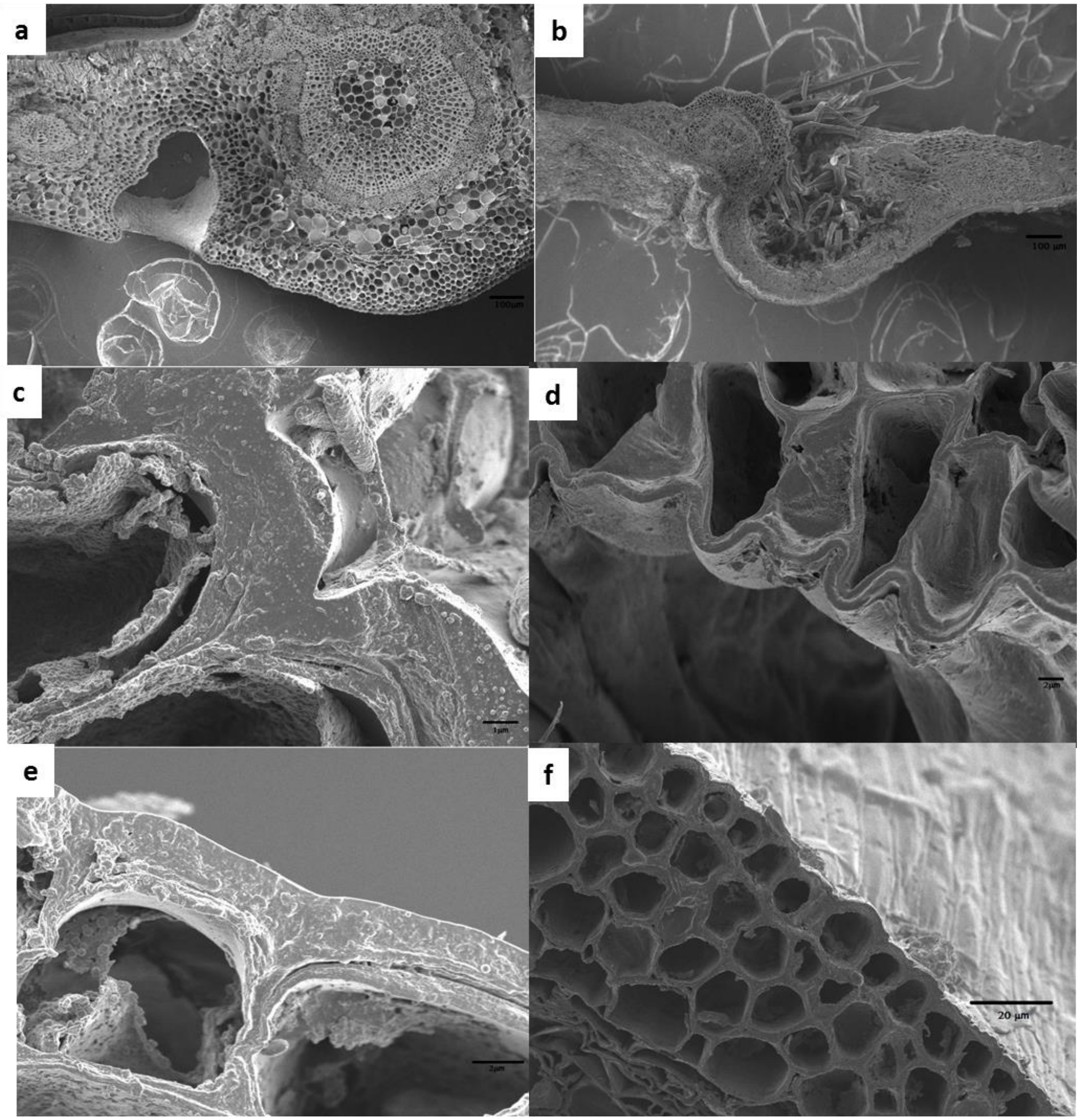

Figure 14: *SEM micrographs showing transverse sections through the domatia of (a) Coffea arabica and (b) Gardenia thunbergia, (c) magnified images of the domatial epidermis of Coffea arabica, and (d) is a magnified image of the domatial epidermis of Gardenia thunbergia and, (e and f) show the non-domatial epidermis of Coffea arabica and Gardenia thunbergia, respectively.*

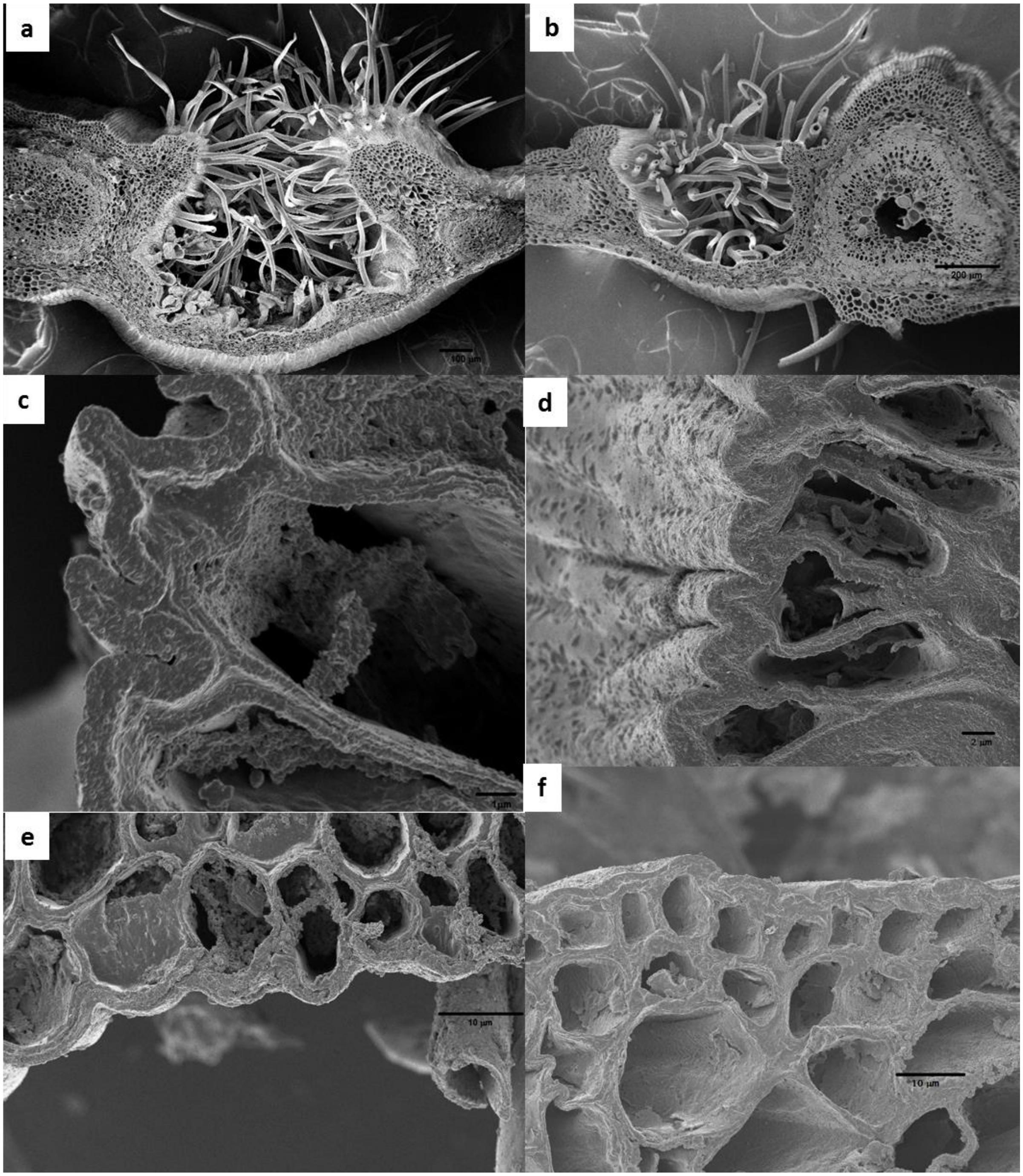

Figure 15: *SEM micrographs showing transverse sections through the domatia of (a) Rothmannia capensis and* (b) *Rothmannia globosa, (c) is a magnified image of the domatial epidermis of Rothmnnia capensis, and (d) is a magnified image of the domatial epidermis of Rothmannia globosa, (e and f) show the non-domatial epidermis of Rothmannia capensis and Rothmannia globosa, respectively.*

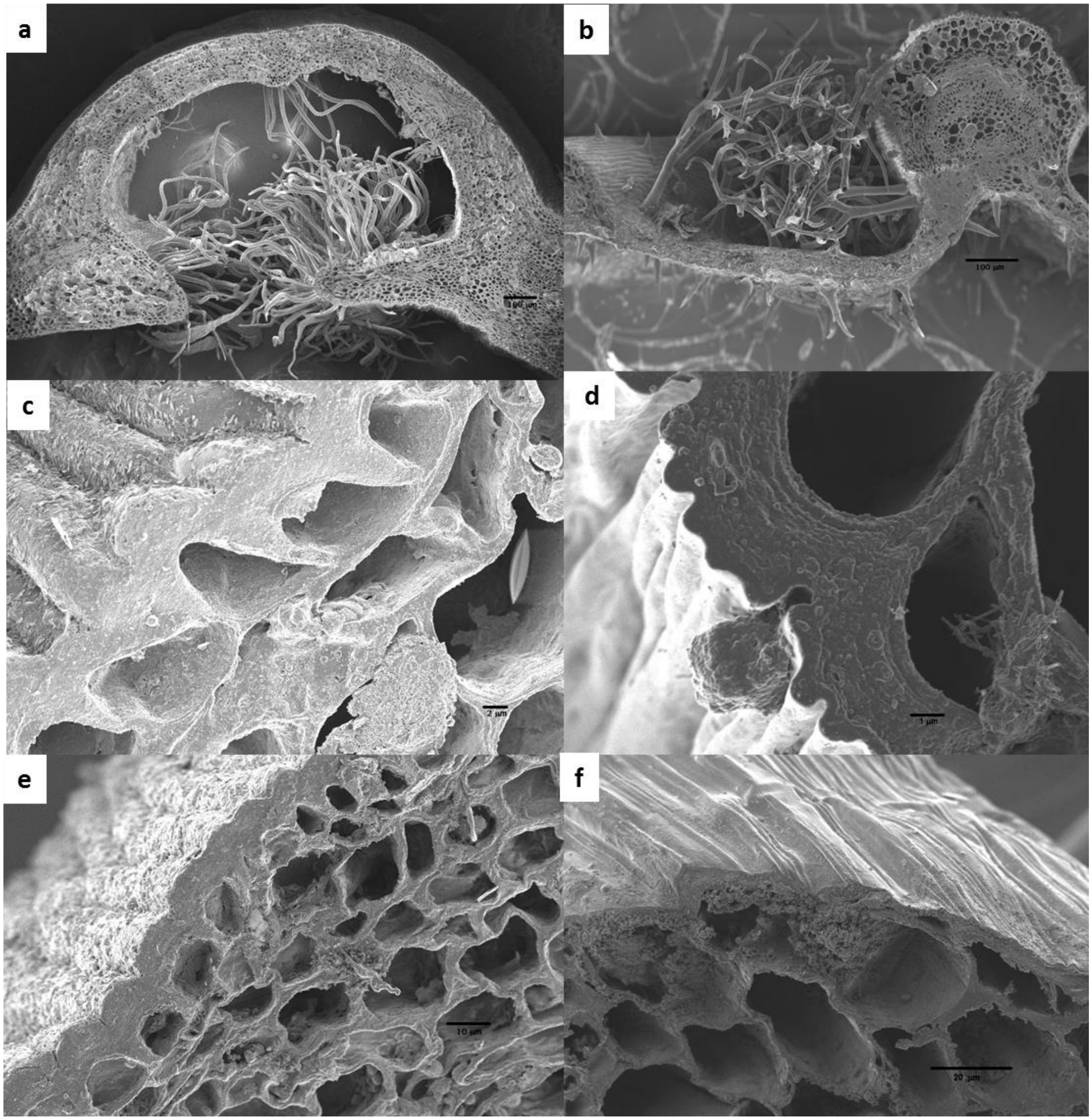

Figure 16: *SEM micrographs showing transverse sections through the domatia of (a) Ocotea bullata and (b) Tecoma capensis, (c) is a magnified image of the domatial epidermis of Ocotea bullata and (d)is a magnified image of the domatial epidermis of Tecoma capensis, (e and f) shows the non-domatial epidermis of Ocotea bullata and Tecoma capensis, respectively.*

4. Discussion

4.1. Differences between domatia types

This study provides insight into anatomical differences in the structure of the different domatia types studied. The major differences in the structure of domatia in relation to non-domatial lamina in all the plants samples were either one or more of the following: (1) the presence of an invagination on the lower surface, (2) presence of simple and branched trichomes, (3) tightly compact parenchyma cells which made up the domatial tissue, (4) collenchyma cells which formed the rim tissue and in some cases, (5) a thickened cuticular layer (see Figures 5 to 10). Nishida et al. (2006) suggested that the invagination forms as a result of differences in cell growth rates between the upper and lower regions of the leaf lamina, and that the lower epidermis and lower mesophyll divide more rapidly compared to the upper mesophyll and epidermis and this results in the formation of the cavity. Similarly when looking at the base of the domatia or the edge where the pocket starts (with the exception of *T. capensis*) we observed tightly packed cells of different sizes and we speculate that, also in these plants, domatia form as a result of rapidly dividing cells in the domatial region of the leaf.

Trichomes were present in five of the plants sampled and were a common feature between the pouch-type and the hair tuft-type domatia. The main difference between species was that in *G. thunbergia*, *R. capensis* and *R. globosa* (all Rubiaceae) the trichomes covered the entire domatia (Figure 6, 8 and 10), and this might be a family specific feature. On the other hand, in *O. bullata* (Lauraceae) (Figure 7), trichomes were restricted only to the rim of the domatia. In *T. capensis* (Bignoniaceae) trichomes were more complex and they appeared branched (see Figure 16a). An unpublished preliminary study examining the ontogeny of leaf domatia in four species (*Psychotria capensis*, *Gardenia thunbergia*, *Coprosma baueri*, and *Pavetta revoluta*) from the Rubiaceae revealed that trichomes inside domatia develop early and can be fully developed after only one month and that the number of trichomes increases with leaf age (Holmes and Barker, 2010). The pocket-type domatia of *Coffea arabica* was hairless. However, Nakamura et al. (1992) and To Ngoc Anh (1966) observed trichomes in the domatia of *Coffea arabica* and suggested that their formation was due to slowing of growth on the leaf surface during the early development stage.

Domatial tissue was a common feature in both the pocket-type and the pouch-type domatia comprising of about three layers of tightly packed rectangular cells, and these cells appeared relatively small in comparison to rim tissues cells. This layer was very distinct from the non-domatial lamina in all the plants sampled except in *T. capensis*. This finding echoes that of Sampson and Mclean (1965) who also studied seven New Zealand species and found that the domatia were characterised by modified tissue that may not be found anywhere else in the leaf lamina. Nishida et al. (2006) and To Ngoc Anh (1966) also observed this structure in their studies. These observations suggest that this feature might be universal in plants that possess these domatia types. The rim tissues of both the pocket-type and the pouch-type domatia consisted of enlarged collenchyma cells and the cuticles inside the domatia appeared thickened. Moraes et al. (2011) also observed a thick cuticle in the domatia of *Rudgea eugenioides* Standl. In comparison, the hair-tuft type domatia lacked the domatial tissue, and rim tissue; and the domatia were marked by the presence of highly abundant trichomes.

This study did not examine the development of domatia on young leaves. Nishida et al. (2006) and others suggest that domatia form when leaves are still in bud, and over time they develop in complexity. Furthermore, Nishida et al. (2006) showed that differences in the formation of the different domatia types found in *Cinnamomum camphora* were associated with initial timing of trichome growth and the degree to which the rim tissue develops and differentiates in the upper mesophyll cells. In their study, they found that the pouch-type domatia is the first to develop followed by the pit-type domatia. The dish-type only develops later on after the veins had appeared. In the pouch type, the development of the domatia starts with an invagination created by actively dividing cells in the domatium region. Subsequently, the cells in the lower part of the mesophyll begin to divide actively forming the rim tissues. The last stage in the development process of the pouch-type domatia is the growth of trichomes around the rim. In the pit-type, the domatia formation is initiated by elongation of cells of the upper mesophyll followed by cell differentiation into palisade cells. In the pubescent type, trichomes begin to grow at the same time as the invagination. In the dish-type domatia the cells of the upper mesophyll elongate before the formation of the domatia is initiated and the rim tissue is developed (Nishida et al., 2006).

4.2. Cuticular folds: are they evidence for plant-mite communication?

Tilney et al. (2012) studied the ultrastructure of domatial epidermis and the cuticle and observed pronounced and regular folding inside the domatia of *Plectroniella armata* (Rubiaceae). Within the folds were electron-dense non-cellulosic branching fibrils extending across the cuticle towards the cavity of the domatia. These channel-like structures and folds were thought to be an indication that some form of communication may be possible between domatia and mites which are usually their inhabitants (Tilney et al., 2012). The study observed similar structures in TEM study of the domatial cuticle. In *R. capensis* (also Rubiaceae), in particular, the cuticular fold was found consistently inside the domatia and were very pronounced on the underside of the leaf (Figures 11a and c). Unlike in *P. armata* these cuticular folds were not restricted to the domatial area, but were also seen in some areas on the adaxial side of the leaf (Figures 11e & f), but these were not as regular and as pronounced as in the domatial area. Because these folds are present also outside the domatia, it is less likely that these structures form part of a specialized domatial system involved in plant-mite communication. In *C. arabica, G. thunbergia* and *R. capensis* the cuticle of the domatia was seen to be folded in some areas, but not in other areas (Figure 12). Furthermore, SEM pictures of the domatial epidermis of *T. capensis* suggest that this plant also possesses cuticular folds around the domatial area and this can be clearly seen in Figure 15. The fact that these features were not regularly seen in domatia of these plants further suggests that these features are not an inherent part of the domatia.

The study was unable to examine study species for the presence of electron dense micro-fibrils inside the cuticular folds due to the difficulties experienced with TEM sample preparation and time constraints. Therefore, the study cannot conclusively say whether they possess the electron dense non-cellulosic branching fibrils as observed by Tilney et al. (2012).

5. Concluding remarks

This study provides useful insights into the anatomy of leaf domatia. The study species namely *C. arabica*, *G. thunbergia*, *R. capensis*, *R. globosa* (all Rubiaceae), *O. bullata* (Lauraceae) and *T. capensis* (Bignoniaceae) had different domatia types and there were some distinct differences in their anatomy. Even though the anatomy of the different plants differed, the key feature that distinguishes domatia is the presence of an extra layer of tissue in the lower epidermis and the presence of an invagination.

All the plants sampled had a similar association with mites. Thus learning about domatia anatomy will help better understand their evolution and association with mites. Results from the study are in agreement with those obtained previously (Tilney et al., 2012, Nishida et al., 2006) and show that domatia have a thick cuticle and cuticular folds. These are thought to be concrete evidence that plants secrete substances that the mites feed on. Unfortunately, due to the limitations of this study, it was impossible to perform an extensive examination of these structures. Since we did not perform any experimental studies that measure flow of radioactively-marked isolates inside these structures, we cannot conclusively say whether these modifications are involved in compound exchange. Even so, we believe that these structure cannot be linked with, and do not form part of a plant-mite communication. This suggestion is based on the fact that the folded cuticle was not restricted only to domatial lamina. More observation of the domatial cuticles are required to determine whether these structures form part of plant-mite communication.

Chapter 3: An assessment of mite-host specificity between leaf domatia types and host tree species

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1. Introduction

Cooperative interactions between organisms have been extensively studied by ecologists and evolutionary biologists (Bronstein et al., 2006). Amongst the most cited and well-known examples of these include mutualisms between plants and insects (Bernays and Graham, 1988). For example, many flowering plants depend on bees and other insects for pollination, seed dispersal, and reproduction (Bronstein et al., 2006). In this chapter another one of the many mutualisms that exists between plants and arthropods is considered, the mutualism between plants with leaf domatia and mites that inhibit these features.

Many studies have shown that they are involved in a mutualism with plants that bear leaf domatia and that these structures play a significant role in influencing mite assemblages found on plants (Pemberton and Turner, 1989; O'Dowd and Willson, 1991; Walter and O'Dowd, 1992b; Walter, 1996, Norton et al., 2000; Romero and Benson, 2005). Leaf domatia alter the distribution, abundance, and reproduction of mites and the concentration of mites on plants that bear domatia is always significantly higher in domatia than on vein axils of plants lacking leaf domatia (O'Dowd and Willson; 1991; O'Dowd and Pemberton, 1998; Norton et al., 2000). Leaf morphology is believed to have a strong influence on mite presence, as shelter may be more important than food availability (Beard and Walter, 2001).

A phenomenon of great importance that is often related to, and that facilitates interactions between species, is host specificity. Host specificity is fundamental to the study of plant-arthropod evolution and in ecology. Many studies have shown that domatia are important incubators of a wide range of mite species and that some mites may be associated differentially with certain plants (Rosenthal and Platts, 1990; Bakker and Klein, 1993; Beard and Walter, 2001; Li et al., 2016). For example, Bakker and Klein (1993) found that two *Typhlodromus* (Scheuten) species preferred cassava plants regardless of the availability of prey. Beard and Walter (2001) found strong host plant specificity trends in some species of mites, and specifically in the genus *Neoseiulus* (Hughes). Furthermore, Skoracka et al. (2010) based on collection and host plant records, showed that eriophyid mites prefer closely related hosts and

99% were recorded from one host plant family. Nevertheless, there is still little information on host specificity of mites found on plants with leaf domatia. Therefore this study addresses the following questions:

a) Does each tree species have a specific mite associated with it?
b) Do certain mites prefer a specific domatia type?

This study will provide a better understanding of the plant-mite mutualism and will shed light on whether the plant-mite co-evolution has resulted in a one-plant-one-mite relationship.

2. Methods

Mite abundance and diversity was evaluated from 10 plant species sampled in the Alexandria Forest and the Grahamstown Botanical Gardens, Eastern Cape, during the period between August and September, 2014. Two individuals per tree species were sampled and 20 leaves were collected and examined from each of the different plant species with various domatia types. The leaves of each tree sampled were placed in a zip-lock bag and stored in a cool bag, and then viewed under a dissecting microscope on the same day the sampling took place. Mites found inside domatia were counted before they were collected for identification. Mites were removed and collected from leaves using a pipette and a drop of alcohol, and mounted on a slide using PVA mounting medium or mounted on a stub and viewed under the Scanning Electron Microscope. The slides were used to identify mites to family or species level. The different mite species found were noted and mite abundance was determined from mite count data.

3. Results

3.1. Mite abundance in sampled plants

The study assessed the following plant species which possessed different domatia types: *Canthium ciliatum* (D.Dietr.) Kuntze, *Gardenia thunbergia* Thunb., *Ocotea bullata* (Burch.) E. Meyer in Drege, *Olea capensis* L., *Psychotria capensis* (Eckl.) Vatke, *Rhoicissus rhomboidea* (E.Mey ex Harv.) Planch., *Rhoicissus tomentosa* (Lam.) Wild & R.B. Drumm., *Rothmannia capensis* Thunb., *Rothmannia globosa* (Hochst.) Keay and *Tecoma capensis* (Thunb.) Lindl., (Table 2). Figure 16 shows the average number of mites found on leaves from these species. For most of the plant species sampled mite abundance was low, and *T. capensis*, *G. thunbergia* and *R. globosa* were the only plants with high abundance and an average of more than ten mites per leaf.

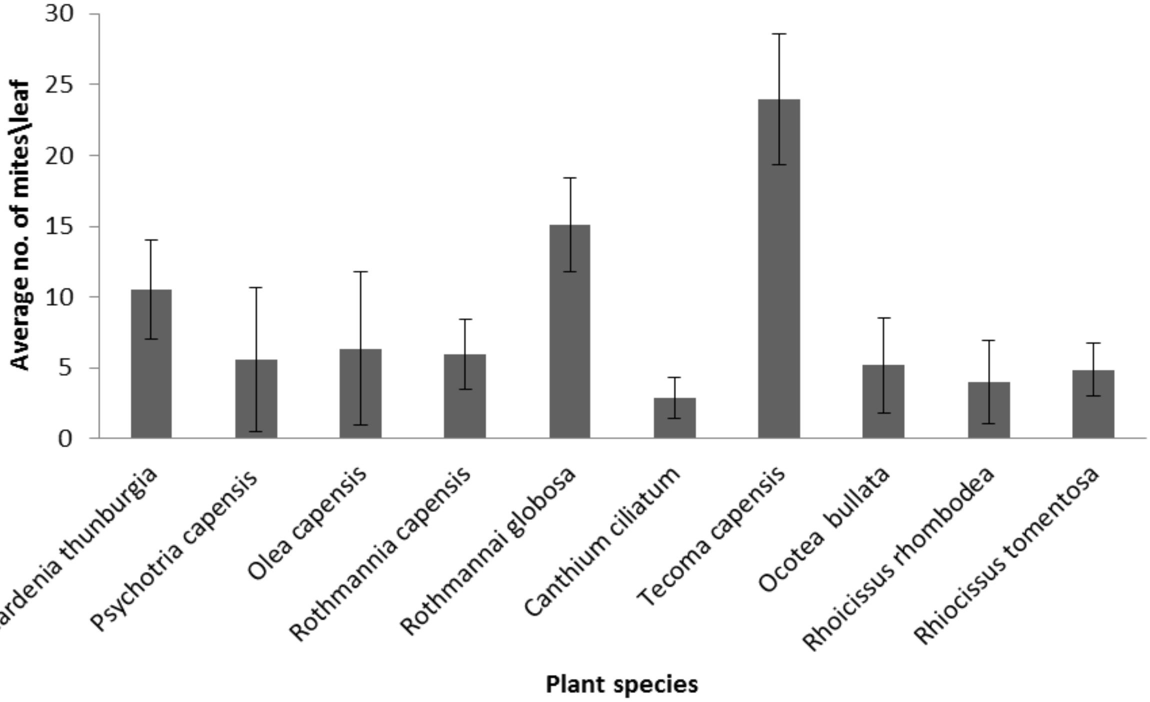

Figure 17: *Average mite abundance on selected plant species with leaf domatia. The values are based on 20 leaves sampled and 2 individuals were sampled per tree species. Error bars show standard deviation.*

3.2. Mite specificity: Does each tree species have a specific mite associated with it?

Table 2 contains a list of the mites collected from the tree species sampled. More than one species of mite were collected from the domatia in all sampled plant species, and most of the mite species were found occupying more than one plant species. These plants were associated with a diverse range of mite species and the majority of the mites found inside the domatia of the tree species were predatory in nature (Figure 17). *Euseius addoensis* (MucMurtry) from Phytoseiidae and *Agistemus tranatalensis* (Meyer &Ryke) from Stigmaeidae were the most common mites in most of the tree species and across the different domatia types sampled. Phytophagous and mycophagous mites were also occasionally observed from some of the plants and these consisted of individuals from –T*etranychus* (Dufour) species From Tetranychidae, an unknown species From the Eriophyidae and *Brevipalpus* (Donnadieu) species from Tenuipalpidae, families. Also individuals with a saprophytic feeding behaviour, *Siculobata sicula* (Berlese) from Oribitei was found from domatia of some of the trees sampled (Table 2).

3.3. Do certain mites prefer a specific domatia type?

Mites showed no preference for domatia type, and most of the mites were commonly found across the different types of domatia represented in this study (Table 2). For example, *Euseius addoensis* was found in the pit-type, hair tuft-type, and pouch-type domatia. Similarly, *Agistemus tranatalensis* was common inside these domatia types. *Brevipalpus sp*. (Tenuipalpidae), *Amblyseius anomalus* (van de Merve) from Phytoseiidae, *Tetranychus* specie from Tetranchidae, *Tydeus grabouwi* (Meyer & Ryke) from Tydeidae, and an unknown species (Eriophyiodae) were the only mites that were found in one domatia type. *Brevipalpus sp.* and *Tydeus grabouwi* were found in the raised dome, *Tetranychus sp.* and the unknown mite were found in the Hair tuft type domatia and *Amblyseius anomalus* occurred only in the pit-type domatia (Table 2).

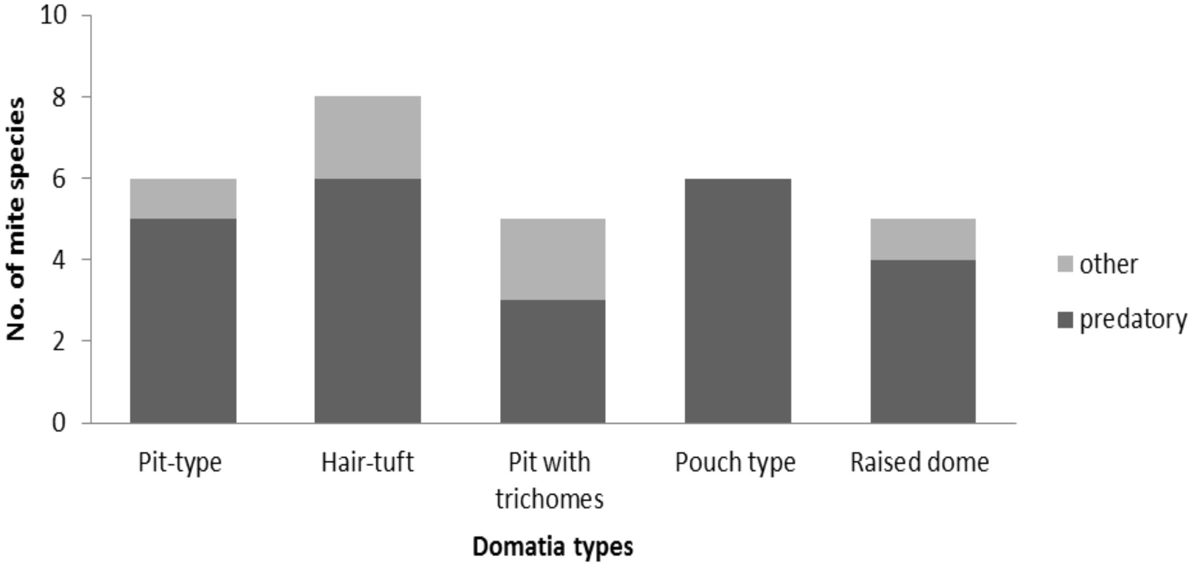

Figure 18: *Total number of mite species and the proportion of predatory mites found in the sampled domatia types.*

Table 2: *Mite species found in association with the different domatia types sampled from 10 different tree species and (the total number of individual mite species collected from 40 leaves from the two individuals sampled per tree species).*

Pit-type	**Hair-tuft**	**Pit with trichomes**	**Pouch- type**	**Raised dome**
O. capensis	***T. capensis***	***C. ciliatum***	***G. thunbergia***	***R. tomentosa***
Agistemus tranatalensis Meyer (18) *Oribatei* - saprophytic mite (14) *Amblyseius anomalus* van der Merwe (77)	*Euseius addoensis* van der Merwe (122) *Typhlodromus microbullatus* van der Merwe (100) *saproglyphus sp. (105)* *Agistemus tranatalensis* Meyer (18) *Eriophyiodae - Unknown* (118)	*Tetratriophtydeus myacanthus* Ueckermann (32) *Euseius addoensis* van der Merwe & Ryke (26)	*Anystis baccarum* Linnaeuas (52) *Cunaxidae -nymph* (16) *Agistemus tranatalensis* Meyer (10) *Bunaxella zebedielensis* Den Heyer (34) *Lourus citricolus* Ueckermann & Grout (48) *Euseius addoensis* van der Merwe (35)	*Cultroribella sp.* (32) *Tydeus grabouwi* Meyer & Ryke (18) *Brevipalpus sp.* (25) *Lourus citricolus* Ueckermann & Grout (22)

P. capensis	**R. globosa**	**O. bullata**	**R. capensis**	**R. rhomboidea**
Typhlodromus vescus van der Merwe (6) *Tydeus munsteri* Meyer & Ryke (41) *Euseius addoensis* van der Merwe &Ryke (21)	*Amblyseus neosuilus* (77) *Euseius addoensis* van der Merwe (67) *Tetranychus sp.* (66) *Tydeus monsteri* Meyer & Ryke (44) *Saproglyphus sp.* (28)	*Euseius addoensi* van der Merwe (40) *Siculobata sicula* Berlese (30) *Acaridae -nymph* (13)	*Agistemus tranatalensis* Meyer (12) *Bunaxella zebedielensis* Den Heyer (24) *Lourus citricolus* Ueckrmann & Grout (13) *Euseius addoensis* van der Merwe (122)	*Tydeus monsteri* Meyer & Ryke (25) *Siculobata sicula* Berlese (4) *Tydeus grabouwi* Meyer & Ryke (12) *Anystis baccarum* Linnaeus (11)

4. Discussion

This study tested whether there was evidence of plant host specificity in 10 South African plants, and whether mites preferred certain domatia types over others. Similar to other studies (O’Dowd and Willson, 1989; O’Dowd and Pemberton, 1998; Loughner et al., 2008; 2010; Parolin et al., 2014a), the results showed that South African plants that bear domatia are reservoirs for a high diversity of mites, and the plant species sampled were associated with more than one species of mites (Table 2). Mite abundance (Figure 16) and species richness also varied between these species.

These results complement other studies from Asia and North America (O’Dowd and Pemberton, 1998, Castro and Moraes, 2007 O’Dowd and Willson; 1991, Norton et al., 2000) which showed that most forest plants are associated with more than one mite species. Furthermore, these studies showed that the majority of mites found in association with domatia are predatory in nature and therefore beneficial to plants (Figure 17). Pemberton and Turner (1989) found that 84% of plant species with leaf domatia had beneficial mites. Walter and O’Dowd (1992a) observed higher numbers of phytoseiid mites on leaves with domatia and that mites used these structures as nest sites to lay their eggs (Walter and O’Dowd, 1992a).

Despite the fact that the majority of mites found inside domatia were potentially beneficial, other species which are plant feeding and saprophytic were also found in this study. These results suggest that domatia may also be utilised by potentially harmful mites. Similarly, Nishida et al. (2005) found a diverse range of mites with different feeding habits inside the domatia of *Cinnamomum camphora* (L.) J.Presl. Pemberton and Turner (1989) also found Tenuipalpidae and Eriophyidae mites in 19% (6 of 31) of the plants they sampled.

Some studies have shown evidence for what seems to be domatia type preference in some species of mites (Walter and O’Dowd, 1995; Karban et al., 1995; Kasai et al., 2002; Nishida et al., 2005). This is hypothesised to be due to the size of the domatia opening, which may influence which mite fauna inhabit the domatia, and that domatia of various shapes may offer different benefits to mites. Nishida et al. (2005) showed that different domatia types were associated with different mite fauna in *Cinnamomum camphora*. In their study Eriophyidae and Tarsonemidae mites were abundant in pouch domatia, Stigmaeidae mites were abundant in the pubescent pit domatia, and Phytoseiidae mites (*Amblyseius sojaensis* Ehara) were frequent in the glabrous pit domatia and the dish domatia. Similarly, Karban et al. (1995) observed high densities of predatory (Phytoseiidae) mites in vein pockets, particularly the hair tuft-type domatia. Kasai et al. (2002) suggest that Eriophyidae mites prefer domatia with small openings because they serve as refuge and a hideaway place from predatory mites which are bigger and may not fit inside domatia. Despite these observations from other studies, our results provide no evidence of preference by mites to certain domatia types.

5. Conclusion

Many organisms have co-evolved and have developed a one-on-one mutualism with plants. Our results show that this is not the case in the plant-mite mutualism, but rather this interaction is opportunistic and any species needing refuge can make use of these structures. The ten studied South African plant species were associated with more than one mite, and mites showed no preference for any of the domatia types and plant species represented in the study. Nonetheless, these results are preliminary and we emphasise the need for more extensive studies on this, because the knowledge of host ranges and specificity is essential to better understanding the interactions between leaf domatia bearing plants and mites and their co-evolution. Results from studies such as these would enhance our understanding of the diversity of the plant-mite interaction and offer better insight into how effectively we may use mites in bio-control strategies.

Chapter 4: Do different vegetation types of South Africa support different mite communities?

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1. Introduction

The remarkable variation in South Africa's topography, climate, altitude and latitude has resulted in a diverse vegetation that is exceptionally rich with over 11 700 plant taxa and over 1,1000 000 species on record in the national database (Powrie et al., 2012). As a result, South Africa boasts a wide range of vegetation biomes ranging from deserts to grasslands and forests. These biomes have been well-surveyed by botanists, and there is a general understanding of their biodiversity. For example, the Succulent Karoo Biome has the highest diversity of succulent plants in the world and is the most species-rich semi-desert on our planet (Mucina and Rutherford, 2006). The Forest Biome is the smallest biome (less than 3% of land surface is covered by forests) in South Africa and is one of the highly diverse and species rich biomes in the country (Rutherford et al. 2006). It contains a number of species ranging from big yellowwood trees to acacias, some of which are rare or endangered (Mucina and Rutherford, 2006). The Savanna Biome is the largest and is characterized by a grassy ground layer and a distinct upper layer of woody plants comprised of species of *Acacia*, *Combretum* and *Colophospermum*, and many more. Other vegetation biomes found in South Africa include the Grassland Biome, Nama Karoo, Fynbos Biome, Desert Biome, Albany Thicket Biome, and the Indian Ocean Coastal Belt (Figure 18). The distinction between these biomes is based on distinctive vegetation structure exposed to similar macroclimatic patterns, and taking into account also characteristic levels of disturbance such as grazing and fire (Rutherford et al. 2006). Most of these biomes include woody species which may be associated with mites, yet little is known about the diversity of mites found in the different vegetation types of South Africa.

Mites are an important component of the ecosystem and they contribute significantly to biodiversity (Lindo and Winchester, 2006). In tropical and temperate forests floors and in canopies the species richness of mites is estimated to exceed all other arthropods and usually canopy mites complement soil mite biota (Walter and O'Dowd, 1995; Walter and Proctor, 1998; Walter and Behan-Pelletier, 1999).

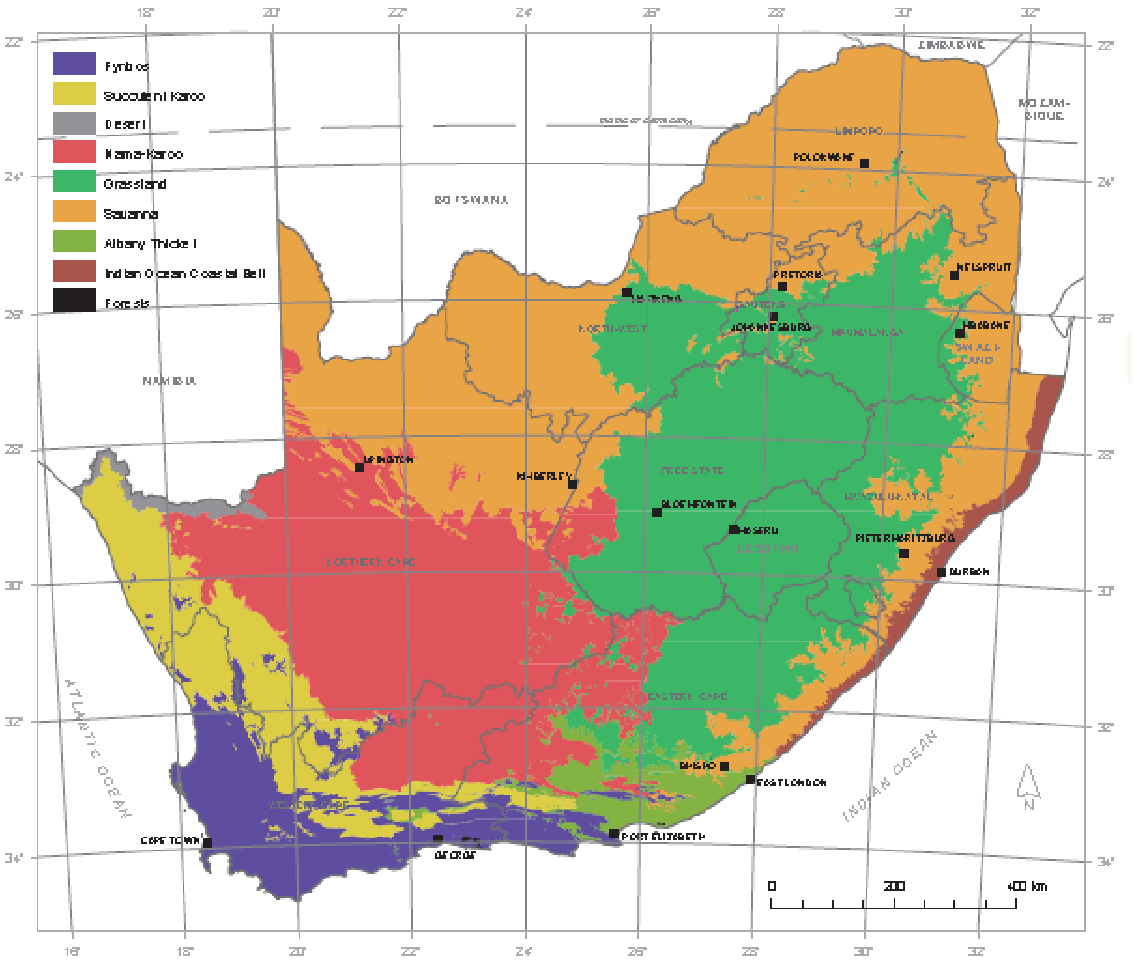

Figure 19: *Biomes of South Africa (Source: Mucina and Rutherford, 2006).*

This chapter assesses whether different vegetation types from various localities harbour different mites. Generally, the mites studied here, which are associated with domatia, inhabit woody species and thus are expected to be found in Savannas, Albany Thicket, Fynbos, and in Forest Biome. However, most plant species that possess leaf domatia have been reported from forests. The main woody component of the Fynbos and Savanna biomes lacks families with leaf domatia. Thus, in this study the Albany Thicket and Forest vegetation types were sampled to examine whether different sites and different vegetation types support different mites. It is thus hypothesised that:

1. Each site would harbour distinct mites because of their spatial separation and due to the fact that different sites are exposed to different environmental conditions.
2. The different vegetation types will have a distinct suite of mites, because of dissimilarities in vegetation composition and microclimate.

Vegetation types vary in terms of vegetation structure, soil properties, and climate variables and all these properties may have an influence in mite biota (Walter, 1999). To date no other study has looked at the distribution of mites communities across different vegetation types.

2. Methods

2.1. A description of the study area and the various vegetation types sampled

The plant and mite collections were conducted in different vegetation types within South Africa. Most of the sampling was conducted in the Eastern Cape and the Western Cape of South Africa. A few other sites were sampled in the forests of Limpopo, Mpumalanga, and KwaZulu-Natal. In total, 33 localities representing nine vegetation types (six forest and three thicket vegetation types) were visited over the course of the sampling period (Figure 19, Table 3).

2.1.1. Northern Coastal Forests

These are distributed in KwaZulu-Natal and the Eastern Cape Provinces along the seaboards of the Indian Ocean. They occur on coastal rolling plains at altitudes ranging from about 10-150 m. They also occur on stabilised coastal dunes (Figure 20). The coastal plains are species-rich and are dominated by *Drypetes natalensis* (Harv.) Hutch., *Englerophytum natalense* (Sond.) T.D.Penn., *Albizia adianthifolia* (Schum.) W.Wight and *Diospyros inhacaensis* F.White. On dunes, these forests have well-developed tree, shrub, and herbaceous layers. In these environments, *Mimusops caffra* (E.Mey ex A.D.C.) Kutze, *Vachellia kosiensis* (P.P.Sw. ex Coates Palgr.) Kyal. & Boatwr. and *Psydrax obovata* (Klotzsch ex Eckl.) are the most common constituents of the tree layer and *Brachylaena discolor* var. *discolor*(DC., *Chrysanthemoides monilifera* subsp. *Rotundata* (DC.) Norl., *Carissa bispinosa* subsp. *Bispinosa* (L.) Desf. ex

Brenan, and *Euclea natalensis* A.DC. amongst others are frequent in the understorey. Also, herbaceous vines and woody climbers such as *Senegalia kraussiana* (Meisn ex Benth.) Kyal. & Boatwr., *Artabotrys monteiroae* Oliv., and *Rhoicissus tomentosa* (Lam) Wild & R.B. Drumm. are important structural determinants of these forests (Mucina and Rutherford, 2006).

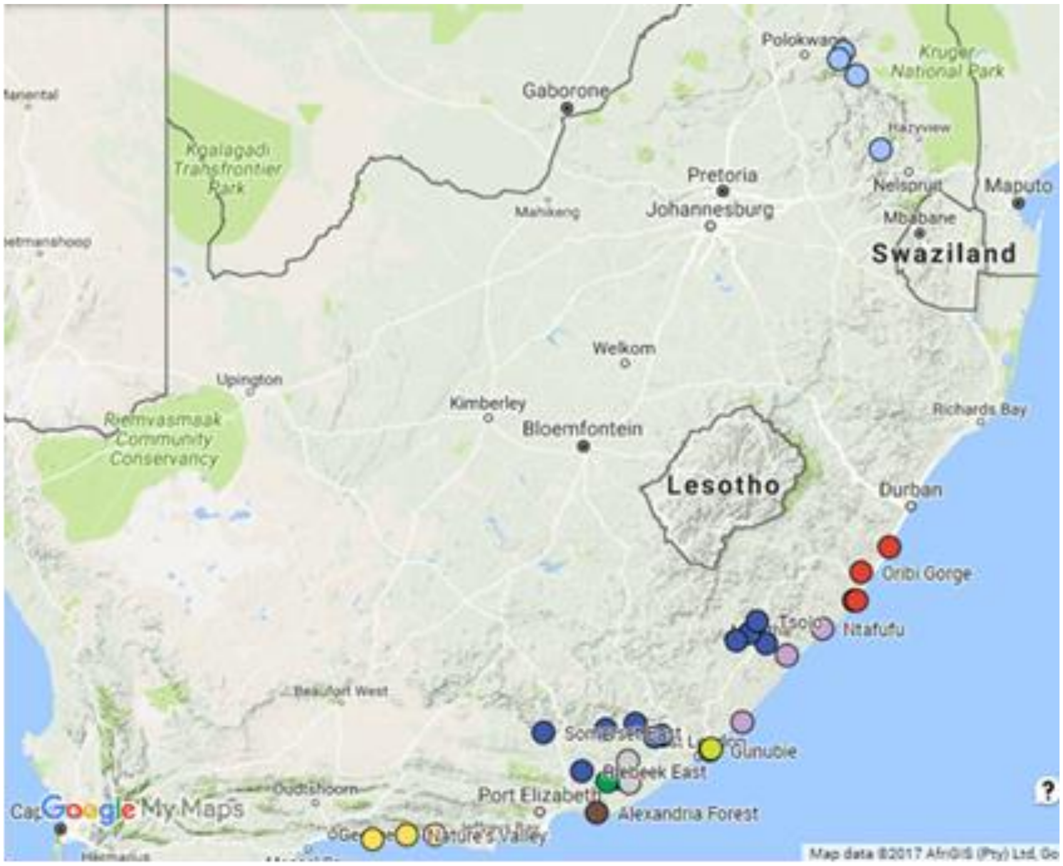

Figure 20: *A google map of South Africa showing the study sites visited. The light blues icons show Northern Mistbelt Forests, red icons are Scarp Forests, purple icons are Northern Coastal Forests, blue icons are Southern Mistbelt Forests, lime icons are Coastal thicket, the grey icons shows the Great Fish Thicket, green icons are Kowie Thicket, brown icons are Southern Coastal Forests and the yellow icon are Southern Afromontane Forest. (source: Google maps).*

2.1.2. Northern Mistbelt Forests

This group of forests is found in Free State, KwaZulu-Natal, Mpumalanga, North-West, Gauteng and Limpopo Provinces (Figure 19). They are restricted to mountain kloofs and low ridges with the majority of the patches occurring at altitudes between 1 450 m and 1 900 m, and outliers at low altitudes ranging from 1100m and around 2000 m. They are relatively species-poor forests when compared to other South African forests and their canopy is usually dominated by *Podocarpus latifolius* (Thunb.) R.Br. ex Mirb., *Olinia emarginata* Burtt Davy, *Halleria lucida* L. and *Scolopia mundii* Warb. On the drier mountain slopes the dominating species are *Pittosporum viridiflorum* Sims, *Celtis africana* Burm.f., *Mimusops zeyheri* Sond., *Nuxia congesta* R.Br. ex Fresen. and *Combretum erythrophyllum* (Burch.) Sond. (Mucina and Rutherford, 2006).

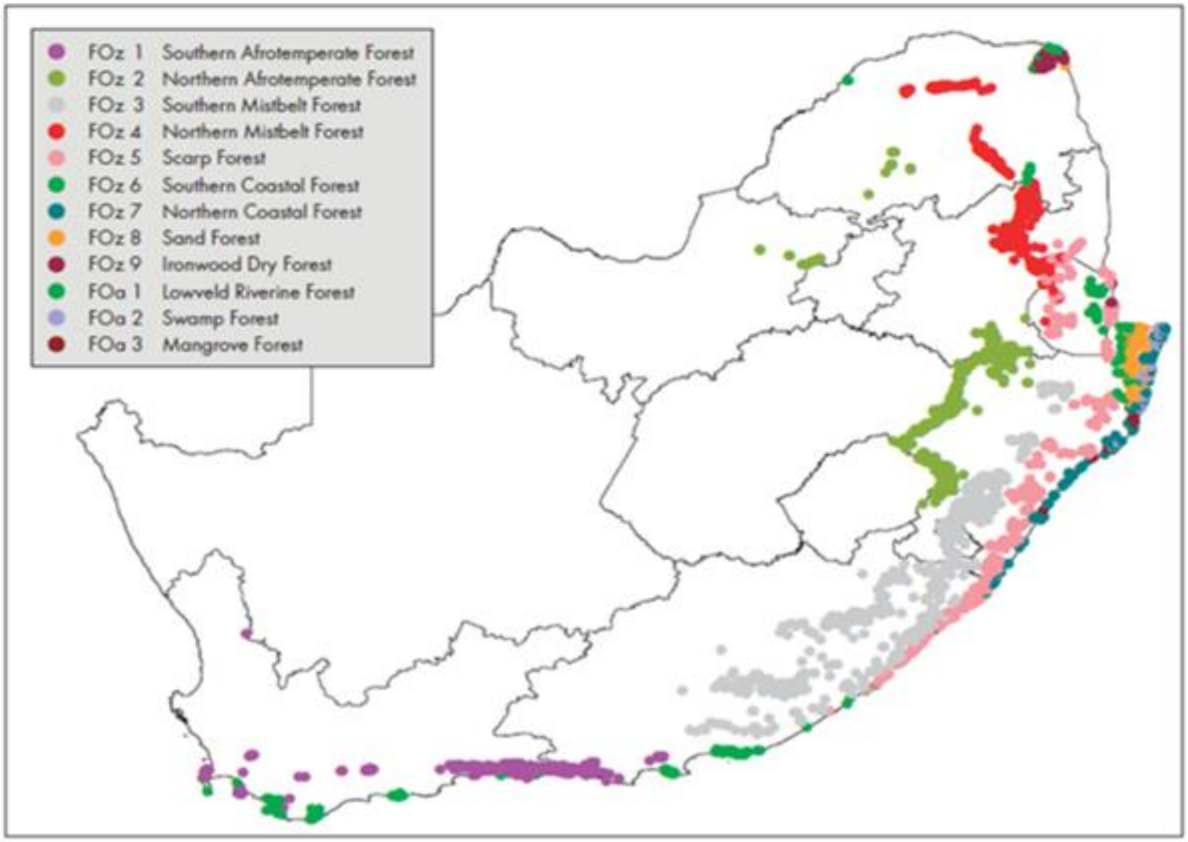

Figure 21: *Distribution of South Africa's forest vegetation types (Mucina and Rutherford, 2006).*

2.1.3. Scarp Forests

These are Subtropical Forests found in the Eastern Cape, KwaZulu-Natal and Mpumalanga Provinces. They are often associated with overhanging walls of rocks, scarps and coastal platforms (Figure 19). Most of the patches occur at low altitudes between 50 m and 600 m. They are species-rich and structurally diverse, with well-developed canopy and understorey tree layers, but a poorly developed herbaceous layer. The most common species are *Buxus macowanii* Oliv., *B. natalensis* (Oliv.) Hutch., *Drypetes gerrardii* Hutch., and *Rothmannia globosa* (Hochst.) Keay (Mucina and Rutherford, 2006).

2.1.4. Southern Afromontane Forests

These forests are found in the Eastern Cape and Western Cape and they also include the Tsitsikamma-Knysna forest complex (Figure 19). They occur on sheltered seaward slopes, plateau and coastal scarps at altitudes ranging from 10 m to 600 m. These forests are dominated by yellowwoods (*Afrocarpus falcatus* (Thunb.) C.N.Page and *Podocarpus latifolius* (Thunb.) R.Br. ex Mirb.) and other species such as *Ocotea bullata* (Burch.) E. Meyer in Drege and *Olea capensis* L. The shrub understorey and herb layers are well developed, especially in wet habitats. They have less species than mist-belt forests (Mucina and Rutherford, 2006).

2.1.5. Southern Coastal Forests

This group of forests is found in the Eastern Cape and Western Cape Provinces along the coastal plains between Alexandria and Van Stadens River gorge (west of Port Elizabeth). They occur at low altitude (between 20 m and 340 m for most patches) areas close to the sea and in deeply incised river valleys in the Albany region surrounded by subtropical succulent thickets. The dominating species are *Celtis africana* Burm.f., *Sideroxylon inerme* L., *Mimusops caffra* E.Mey ex A.DC. and *Dovyalis rotundifolia* (Thunb.) Harv. The forest patches found in the eastern regions of the distribution area are comprised of a well-developed low-tree and shrub as well as herbaceous layers and in the western regions they are floristically and structurally impoverished (Mucina and Rutherford, 2006).

2.1.6. Southern Mistbelt Forests

These are made up of forest patches of various sizes (most are found at altitudes between 1 000 m and 1 400 m), and are found in KwaZulu-Natal and in the Eastern Cape. They grow on shaded habitats on the south- and southeast-facing slopes with altitudes spanning 850 m–1600 m. These forests are located along the Great Escarpment (Amathole, Transkei Escarpment) and in KwaZulu-Natal they are sandwiched between the Drakensberg Montane Forests and Northern KwaZulu-Natal Mistbelt Forests. The dominating plant species in the west are *Afrocarpus falcatus* and a range of other deciduous and semi-deciduous species such as *Celtis africana*, *Calodendrum capense* (L.f.) Thunb., *Vepris lanceolata* G. Don and *Zanthoxylum davyi* Waterm. Further east towards KwaZulu-Natal Midlands the canopy layer changes and *Podocarpus henkelii* Stapf ex Dallim. & A.B.Jacks. becomes more common (Mucina and Rutherford, 2006).

2.1.7. Coastal Thicket

The coastal thicket is found in the Eastern Cape Province within 15 km of the Indian Ocean coastline, from Kei Mouth to the Sundays River. This vegetation is found on gentle slopes (altitude 10 m–400 m) and dissected hilltop slopes close to the coast. It is dominated by short grasslands punctuated by scattered bush clumps or solitary *Vachellia natalitia* (E.Mey.) Kyal. & Boatwr trees. Other tree species commonly found include *Brachylaena elliptica* (Thunb.) Less, *Canthium spinosum* (Klotzsch ex Eckl. & Zeyh.) Kuntze, *Erythrina caffra* Thunb. and *Euphorbia triangularis* Desf. ex A.Berger, (Mucina and Rutherford, 2006).

2.1.8. Great Fish Thicket

This vegetation is found in the Eastern Cape Province and mainly in the lower Great Fish River and Keiskamma River Valleys (altitude 0 m–1 000 m) and extends northwards up to Cookhouse and into the southernmost part of the Cradock District. The distribution also includes the lower reaches of the Koonap River and part of its upper reaches immediately north of Adelaide, as well as parts of the Kat River and Little Fish River Valleys. This vegetation has a well-developed layer of woody trees, shrubs and a succulent component and the most common species is

Portulacaria afra. This species is soon replaced by *Euphorbia bothae* Lotsy & Goddijn in more arid areas. In incised river valley slopes, *P. afr*a is replaced by woody elements and tall emergent *Euphorbia tetragona* Haw. and *E. triangularis*. There is distinct clumping of the vegetation, which is linked to zoogenic mounds, formed by termites, earthworms, and mole rats (Mucina and Rutherford, 2006).

2.1.9. Kowie Thicket

Kowie Thicket is found on dry steep and north-facing slopes (altitude 0 m–700 m) along the river valleys from near the Great Fish River Mouth to Kenton-on-Sea, extending inland up these valleys past Grahamstown to just past Riebeeck East and Alicedale to north of the Zuurberg. This tall thicket is dominated by succulent species of Euphorbia species and Aloes with a thick understorey composed of thorny shrubs, woody lianas (*Capparis* L., *Secamone* R.Br., *Rhoicissus* Planch, *Aloe* L.), and shrubby succulents (Crassulaceae, Asphodelaceae). The more moist south-facing slopes support thorny thickets dominated by low evergreen trees (*Cussonia* Thunb., *Euclea* L., *Hippobromus* Eckl. & Zeyh., *Pappea* Eckl. & Zeyh., *Ptaeroxylon* Eckl. & Zeyh, *Schotia* Jacq.) and shrubs (*Azima* Lam., *Carissa* L., *Gymnosporia* Chodat, *Putterlickia* Endl.) with fewer succulent shrubs and trees. This vegetation type has a poorly developed herbaceous layer (Mucina and Rutherford, 2006).

2.2. Field visits and sampling of mites

Field visits were undertaken periodically commencing in January 2013 until October 2015. At each site, forest walks were undertaken and trees were examined for the presence and absence of leaf domatia. From tree species that were found to possess leaf domatia, 20 leaves were collected from each individual tree and immediately placed in a zip lock bag and stored in a cool backpack. The leaves were examined for the presence of mites as soon after collection (usually the same day they were collected). The leaves were examined under a dissecting microscope and mites found inside the domatia and the surrounding leaf blade were collected, counted and either mounted on slides and sent to the Agricultural Research Council (ARC) Biosystematics division for identification or fixed on a stub and viewed under a Scanning Electron Microscope. Also herbarium voucher specimens of all the plants sampled were collected to verify tree species identity, and to make sure all host plants were correctly identified Vouchers are housed at the Schonland Herbarium (GRA) in Grahamstown.

2.3. Mite species data analyses

A list of all mite species collected from each of the plant species from the different sampling sites was collated and a presence-absence data matrix was produced where “1” represented the presence of a mite in a sampled tree and “0” its absence . These data were then used to perform resemblance analysis using D3 Chord distance (as resemblance measure) in the Plymouth Routines in Multivariate Ecological Research (PRIMER) software (Clarke and Gorley, 2006). A dendrogram plot was produced from a hierarchical cluster analysis using the “group average” linkage to identify relationships and similarities in mite biota between sites and the vegetation types sampled. The cluster analysis groups the samples (i.e. sampled sites) into clusters and produces a dendrogram with branch length proportional to the degree of dissimilarity.

A similarity percentage analysis (SIMPER) was also conducted to determine which mite species are characteristic of the different vegetation types and to identify which mites contribute most to the similarity between vegetation types and within sample sites. In this regard Bray-Curtis resemblance was used. This analysis allows us to identify which species contribute to the observed patterns of similarity and gives percentage similarity.

Table 3: *The sampled vegetation types, their broad climatic variables and the number of sites visited. For the list of all plant species sampled at each vegetation type see Appendix 3.*

Vegetation type	No of sites visited	Climatic and habitat variables
Coastal Thicket	3	The area is geologically complex and includes Beaufort Group mudstone and sandstone in the northeast, Nanaga Formation arenite and sand in the west and Bokkeveld, Witteberg and ecca sandstone and shale in between, and a thin strip of Quaternary sand along the coast. The mean annual precipitation (MAP) is 677 mm, mean annual temperature (MAT) is 17.8° C and mean frost days (MFD) are 2 days.
Great Fish Thicket	1	Found mostly on shallow clay soils derived from the Adelaide and estcourt formation mudstone and arenite. MAP is 449 mm, MAT is 17.1° C and MFD are 7 days.
Kowie Thicket	2	Found on clay soils derived from sandstone and shale and on Dwyka tillite. MAP is 517 mm, MAT is 17.5° C and MFD are 3 days.
Northern Coastal Forest	4	Found on well-developed sandy-loamy soils on sedimentary rocks of the Karoo Super group and Jurassic intrusive dolerites as well as on Holocene marine sediments. MAP is 1044 mm and MAT is 21° C.
Northern Mistbelt Forests	4	Found on highly weathered, clay soils mainly derived from shales, quartzite, dolomite, granite and diabase. MAP is 1084 mm and MAT is 14.8° C.
Scarp Forest	2	Found on shallow nutrient poor soils derived from Natal Sandstone outcrops as well as syenitic granite, rhyolite of the jozini formation and other Karoo sedimentary rocks. MAP is 1030 mm and MAT is 19° C
Southern Afromontane Forest	5	Soils varying from shallow Mispah, Glenrosa and Houwhoek forms to sandy humic fernwood form, derived from Table Mountain group sandstones and shales of the Cape Super group and partly also from Cape Granite. MAP is 836 mm and MAT is 16° C.

Southern Coastal Forests	1	Well-drained sandy soils originating from sedimentary rocks of Alexandria and Nanaga formations of the Algoa Group. MAP is 730 mm and MAT is 17° C.
Southern Mistbelt Forests	11	Soils are deep, loamy and with high nutrient status, developed on weathered dolerite intrusions or mudstones, shales and sandstones of the Karoo Super group. MAP is 988 mm and MAT is 15.7° C.
Total	**33**	

3. Results

A total of 62 mite species belonging to 15 families were collected. Of these mites, 15 appear to be previously undescribed species (Appendix 1 & 2). Finding so many probable new species highlights the lack of knowledge of our South African mite biota. Within the Thicket Biome, the Grahamstown site in the Eastern Cape had the highest species richness and within the Forest Biome species richness was highest in Alexandria (Eastern Cape) and Lekgalameetse in Limpopo. Kowie Thicket and the Southern Coastal Forests showed the highest mite species richness (Table 4).

In total 258 plant specimens representing 82 plant species were sampled from the sites visited. The highest number of plant species with leaf domatia was collected in Port Edward, Alexandria and Fort Fordyce (Table 5). Appendix 3 gives the list of the mite host plants collected from each site and vegetation type visited. The different vegetation types each had different species compositions, but some of the plants species (eg. *Canthium ciliatum, Grewia occidentalis, Rhoicissus tomentosa*) were common to all the sites sampled. The largest proportion of the plant species with leaf domatia collected belonged to Rubiaceae.

Table 4: *Average mite species richness in each of the vegetation types sampled.*

Forest types	**Mite species richness**	**Thicket vegetation types**	**Mite species richness**
Northern Coastal Forest	5.3	Coastal Thicket	9.7
Northern Mistbelt Forests	14.5	Great Fish Thicket	5.0
Scarp Forest	15.0	Kowie Thicket	27.0
Southern Afromontane Forest	6.6		
Southern Coastal Forests	25.0		
Southern Mistbelt Forests	10.3		

3.1. Relationships between sampled sites

Figure 21 shows relationships and similarities between visited sites based on the mite biota found on the plant species collected as well as between the different vegetation types. The cluster analysis revealed no clear distinction or grouping between the sampled sites (Figure 21). All sampled sites were found to have similar mite biota. Interestingly, some places that are geographically closer together grouped and formed recognizable clusters. These places included Langeni, Baziya and Mount Frere; Ntafufu and Mgazana; as well as Maidan Dam and Pirie Forest (Figure 21). This suggests that these places share similar mite biota compared to the other sites.

Table 6 gives the results of the similarity percentage analysis (within group similarity) and the top three mite species that contributed to the similarity at the different sites. This analysis allows us to account for which species explain similarities and dissimilarities between the groups. All the sampled sites had low percentage value and a few mites contributed to the similarity. This suggests that each of the trees sampled had different mites on their domatia and only a few mites were distributed across all the plant species. In Umgazana, Ngcobo, Baziya, Mount Frere, and Stellenbosch, similarity between samples (individual trees) was zero (Table 6).

Table 5: *The study sites visited with sampling date, number of plants species collected and the number of mite species at each site.*

Vegetation types	**Study site**	**Sampling date (dd/m/yr)**	**No. of plant species sampled**	**No. of mite species**
Coastal Thicket	Tolomnga	25/09/14	9	10
	Gunubie	26/09/14	5	10
	Kwelerha	27/09/14	6	9
Great Fish Thicket	Coombs farm	18/04/14	7	5
Kowie Thicket	Albany State Forest	12/05/13	11	21
	Grahamstown	4/09/14; 25/ 09/14 and 01/10/14	6	33
Northern Coastal Forest	Ntafufu	28/04/14	9	8
	Pietermaritzburg	20/01/15	1	2
	Umgazana	02/05/14	9	4
	Durban	26/03/15	7	7
Northern Mistbelt Forests	Buffelskloof	18/01/15	14	17
	Lekgalameenste	17/01/15	12	22
	Haenertsburg	16/01/15	9	13
	Venda	15/01/15	5	6
Scarp Forest	Oribi Gorge	26/03/15	7	10
	Port Edward	23/03/15	18	20
Southern Afromontane Forest	Nature's Valley	8/07/14	13	11
	Knysna	9/07/14	2	4
	Stellenbosch	11/11/14	4	6
	Bedfort	9/10/14	5	6
	Kambi	14/10/14	3	6
Southern Coastal Forests	Alexandria	28/08/14	16	25
Southern Mistbelt Forests	Tsitsa Falls	05/03/14	9	8
	Beggars Bush	02/08/2013	6	7
	Fort Fordyce	25/04/14 and 9/10/14	16	14
	Langeni	13/10/14 and 7/03/13	8	13
	Ngcobo	04/03/13	4	8
	Baziya	06/03/13 and 09/03/14	5	9
	Mount Frere	7/03/14	4	10
	Maiden Dam	17/ 04/13 and 11/10/14	9	13
	Pirie Forest	15/04/13 and 10/10/14	12	19
	Somerset East	8/10/14	3	5
	Hogsback	10/10/14	5	7
total			**258**	**368**

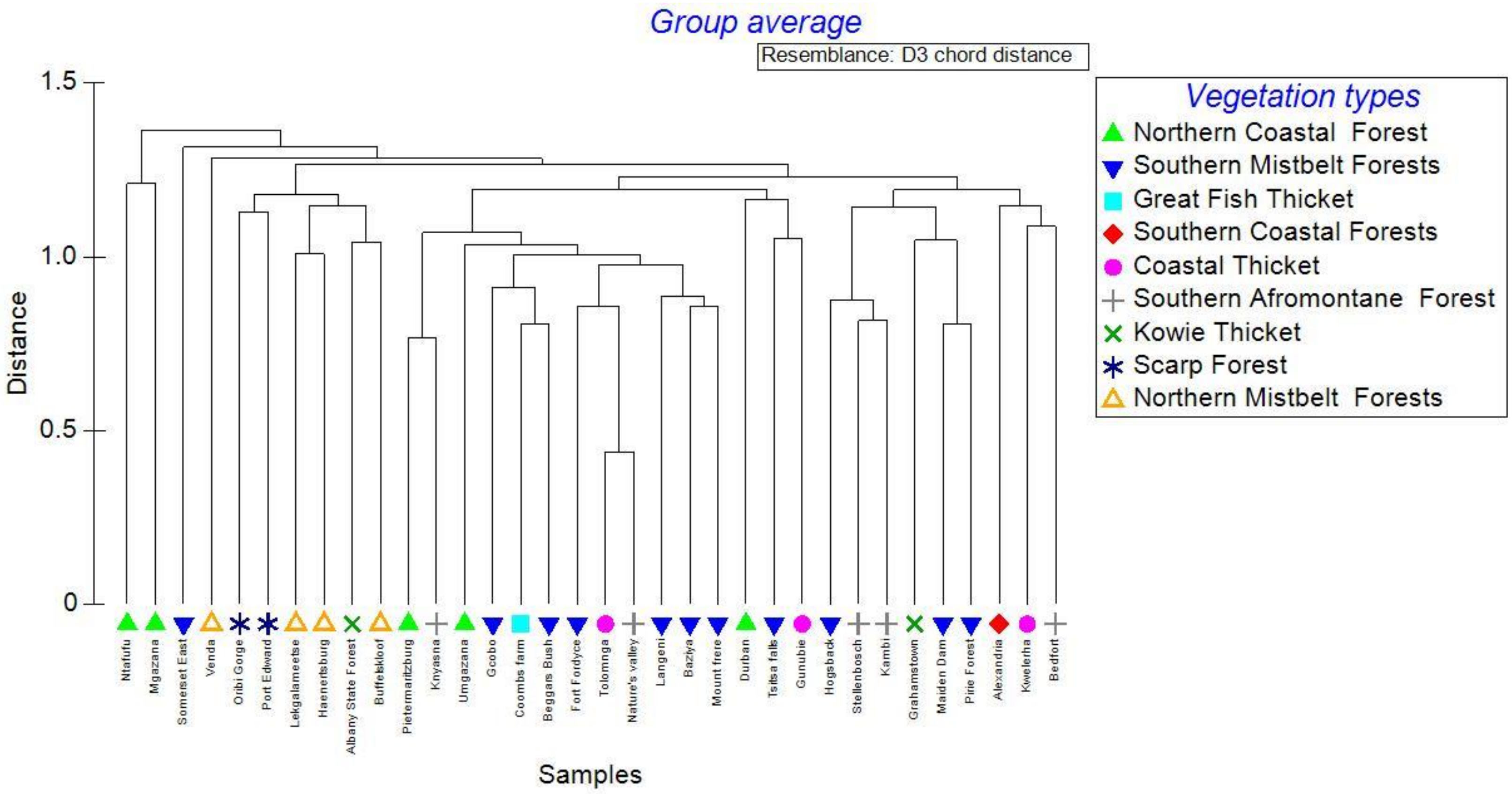

Figure 22: *A dendrogram showing relationships and similarities between sample sites and vegetation types sampled.*

Table 6: *Average within group similarity amongst the different vegetation types sampled and the mite species contributing to within group similarity. Results obtained from the SIMPER analysis.*

Sites	**Average within group similarity (%)**	**Contribution of species**
Ntafufu	46.76	Oribatidae
		Oribatula tibialis (Nicolet)
		Phytoseiidae
		Amblyseus neoankaratrae (van der Merwe & Ryke)
		Amblyseius anomalus (van der Merwe)
Tsitsa falls	21.11	Tenuipalpidae
		Cenopalpus sp. (probably new)
		Cunaxidae
		Bunaxella zebedielensis (Den Heyer)
		Tetranychidae
		Tetranychus sp.
Coombs farm	6.06	Tetranychidae
		Tetranychus sp.
Alexandria	10.31	Anystidae
		Anystis baccarum (Linnaeus)
		Iolinidae
		Pronematus ubiquitous (McGregor)
		Lamelareidae
		Cultroribella sp.
Tolomnga	17.37	Phytoseiidae
		Typhlodromus microbullatus (van der Merwe)
		Oribatidae
		Oribatei sp.
		Erythraeidae
		Unknown sp.
Nature's valley	6.67	Phytoseiidae
		Typhlodromus microbullatus (van der Merwe)
		Tetranychidae
		Tetranychus sp.
		Oribatei
		Unknown (probably new)
Knysna	66.67	Oribatei
		Unknown (probably new)
Beggars Bush	7.41	Cunaxidae
		Bunaxella zebedielensis (Den Heyer)
Fort Fordyce	7.98	Cunaxidae
		Bunaxella zebedielensis (Den Heyer)
		Lamelareidae
		Cultroribella sp.
		Stigmaeidae
		Mullederia centrata (Mayer)

Langeni	17.76	Tetranychidae
		Tetranychus sp.
		Cunaxida
		Bunaxella zebedielensis (Den Heyer)
		Phytoseiidae
		Typhlodromus microbullatus (van der Merwe)
Maiden Dam	17.13	Micreremidae
		Micreremus juvenile
		Phytoseiidae
		Typhlodromus microbullatus (van der Merwe)
		Typhlodromus sp.
Pirie Forest	16.63	Micreremidae
		Micreremus sp.
		Stigmaeidae
		Agistemus tranatalensis (Meyer)
		Phytoseiidae
		Typhlodromus microbullatus (van der Merwe)
Albany State Forest	20.24	Phytoseiidae
		Typhlodromus incisivus (van der Merwe)
		Tydeidae
		Tydeus sp.
		Tetranychidae
		Tetranychus sp.
Durban	39.52	Phytoseiidae
		Typhlodromus microbullatus (van der Merwe)
		Typhlodromus apoxys (van der Merwe)
		Typhlodromus sp.
Oribi Gorge	49.97	Phytoseiidae
		Typhlodromus crasus (van der Merwe)
		Amblyseius (probably new)
		Oribatei
		Unknown sp.
Port Edward	37.23	Phytoseiidae
		Amblyseius anomalus (van der Merwe)
		Phytoseius munteriensis (van der Merwe)
		Stigmaeidae
		Agastimus sp. (probably new)
Buffelskloof	26.09	Phytoseiidae
		Neosius natalensis
		Typhlodromus crasus (van der Merwe)
		Tetranychidae
		Oligonychus sp.
Lekgalameetse	16.35	Phytoseiidae
		Typhlodromus crasus (van der Merwe)
		Stigmaeidae
		Agistemus sp (probably new)

		Cunacidae	*Triophtydeidae sp.*
Haenertsburg	15.34	Oribatei	Unknown sp.
		Phytoseiidae	*Eusius rhusi* (van der Merwe)
		Stigmaeidae	*Agastimus new2*
Venda	26.33	Tydeidae	*Brachytydeus sp.* (probably new)
		phytoseiidae	*Typhlodromus praeacutus* (van der Merwe)
		Tetranychidae	*Tetranychus sp*. (probably new)
Somerset East	50.00	Tenuipalpidae	Probably new genus/species
		Tydeidae	*Brachytydeus sp*. (probably new)
Hogsback	19.67	Stigmaeidae	*Agistemus tranatalensis* (Meyer)
		Oribatei	(saprophytic mites, probably new)
Gunubie	18.89	Phytoseiidae	*Typhlodromus microbullatus* (van der Merwe)
		Acaridae	nymph
		Oribatei	(saprophytic mites, probably new)
Kwelerha	2.67	Oribatei	(saprophytic mites, probably new)
Bedfort	6.67	Tydeidae	*Brachytydeus sp.* (probably new)
Kambi	11.11	Stigmaeidae	*Agistemus sp.* (probably new)
Grahamstown	14.09	Tetranychidae	*Tetranychus sp.*
		Acaridae	nymph
		Phytoseiidae	*Typhlodromus microbullatus* (van der Merwe)
Pietermaritzburg	Less than 2 samples		
Umgazana	All the similarities are zero		
Ngcobo	All the similarities are zero		
Baziya	All the similarities are zero		
Mount frere	All the similarities are zero		
Stellenbosch	All the similarities are zero		

3.2. Relationships between vegetation types

Figure 21 also shows us similarities between the vegetation types sampled. Again there were no distinct clusters formed suggesting that these vegetation types has similar mites. However, there were some recognizable clusters comprising of sites from the Scarp Forests and Northern Mistbelt Forests (Figure 21). The close proximity in the grouping of these sites suggests that they have similar mites and are somewhat distinct from the other vegetation types.

Table 7 gives the results from the similarity percentage analysis and the mite species that contributed to the similarity of the different vegetation types. This analysis account for which species explain similarities and dissimilarities between the groups. The within group average similarity values for the different vegetation types sampled was low and this suggests that the sites which make up the forest groups differed greatly between each other. Moreover, only a few mite species accounted for the separation of the different vegetation types (Table 7).

Table 7: *The Average within group similarity amongst the different vegetation types sampled and mite species contributing to within group similarity.*

Vegetation	**Average within group similarity (%)**	**Contribution of species**
Coastal Thicket	7.65	Phytoseiidae *Typhlodromus microbullatus* (van der Merwe) Hemileiidae *Siculobata sicula* (Berlese) Oribatei *Oribatid sp*.
Great Fish Thicket	6.06	Tetranychidae *Tetranychus sp.*
Kowie Thicket	11.32	Tetranychidae *Tetranychus sp.* *Prosochyla herbuni* (Meyer) Phytoseiidae *Typhlodromus incisivus* (van der Merwe)
Northern Coastal Forest	14.76	Phytoseiidae *Typhlodromus microbullatus* (van der Merwe) *Amblyseus neoankaratrae* (Ueckermann & Loots) Oribatei *Oribatula tibialis* (Nicolet)
Northern Mistbelt Forests	8.79	Phytoseiidae *Typhlodromus crasus* (van der Merwe) *Neosius natalensis* Oribatei *Oribatid sp.*
Scarp Forest	23.50	Phytoseiidae *Amblyseius anomalus* (van der Merwe) *Phytoseius munteriensis* (van der Merwe) *Typhlodromus crasus* (van der Merwe)
Southern Afromontane Forest	6.27	Oribatei Unknown (probably new) Phytoseiidae *Typhlodromus microbullatus* (van der Merwe) Tetranychidae *Tetranychus sp.*
Southern Coastal Forests	10.31	Anystidae *Anystis baccarum* (Linnaeus) Tetranychidae *Tetranychus sp.* (probably new) Hemileiidae

		Siculobata sicula (Berlese)
Southern Mistbelt Forests	5.79	Cunaxidae *Bunaxella zebedielensis* (Den Heyer) Tetranychidae *Tetranychus sp.* Stigmaeidae *Agistemus tranatalensis* (Meyer)

4. Discussion

The association between mites and leaf domatia has been widely described from the Northern Hemisphere (North America and East Asia) (Pemberton and Turner, 1989; O'Dowd and Willson, 1991; Walter and O'Dowd, 1992a; O'Dowd and Pemberton, 1998; Matos et al., 2006). In Africa, including South Africa, only a few studies have been conducted on this phenomenon. Generally, forests support diverse assemblages of mites that comprise of multiple lineages of plant grazers, scavengers, parasites, and predators. These mites usually complement the mite fauna of the forest floor (Walter and Behan-Pelletier, 1999). However, little is known about differences in the diversity of these lineages between vegetation types. The aim of this study was to document this mutualism in South African woody vegetation, and to assess whether the different forests and thicket vegetation types found in South Africa harboured different mites.

Our results showed that South African tree species were associated with substantial diversity of mite species (see list of mites in Appendix 1 and 2). However, contrary to what was expected, the different vegetation types and sites did not differ in terms of their mite communities (Figure 21) and only slight differences in mite species composition between the sites and vegetation types were found. These were sites from the Scarp Forest and Northern Mistbelt Forests which clustered together suggesting that their mite composition was different from the other vegetation types. These results were further supported by the SIMPER analysis, which suggests that only a few species of mites contributed to within group similarity between the sites and vegetation types sampled (Table 6 and 7). Interestingly places that are in close proximity geographically and that were sampled around the same period grouped together suggesting that they have similar mites.

Nonetheless, these results imply that the vegetation types generally do not support distinct mite communities and that most of the plants species sampled from the different sites had similar mite species composition and diversity. The study proposes the following explanations for these results which may or may not be mutually exclusive:

1. Because mites are often associated with birds and insects and can hitchhike on birds and other insects and migrate greater distances (Walter and Proctor, 2013; a phenomenon known as phoresy) it can be expected that mite diversity across a region is relatively uniform. In fact many mite pests are transported in this way and one example is that of the European red mite which was found to spread via hitchhiking on other insects and birds, and in some cases mites can be dispersed over short distances by wind (Botha and Learmonth, 2005). This may be particularly the case with phytophagous mites which may not necessarily be phoretic (Liu et al. 2016). If that is the case, then mites would be able to cross barriers between different vegetation types. Most of the mites encountered in the study were widespread and common across the different sites, and this suggests that they may be able to disperse over a wide range and across vegetation types.

2. The timing of sampling may have affected the results. The sampling period for the study spanned two years and forest visits were undertaken starting in March 2013 until March 2015 (Table 5). Some studies suggest that mite species diversity and species composition change every season and every year (Toyoshima and Amano, 2006; Duso et al., 2004). Furthermore, our study of seasonal patterns in mite diversity on three South African plant species showed that mite communities change over the seasons (Chapter 6). The study also showed that environmental variables such as temperature and rainfall affect mite communities found at a particular time (Chapter 6). If that is the case, then one would have to sample the different sites during the same period and around the same season in order to get comparable results. This was not possible in this study as there were many places to visit across long distances. For future studies, researchers should conduct their sampling around the same time to try and eliminate seasonal changes.

3. Another factor that may have influenced the results is the composition of host plants at the different sites sampled is that the vegetation types sampled differed in terms of vegetation structure and composition. However, the majority of the leaf domatia bearing

plant species found at the sampled sites were widely distributed taxa. These included species from the genus *Rhoicissus*, *Canthium*, *Olea*, and *Grewia* and majority of the plants sampled were from the Rubiaceae family. If mites prefer a certain host plant it is expected that the study would find similar mites in many of the sites visited. However, the results in Chapter 3 showed that mites are not host specific but rather widely distributed cross different plant species.

5. Conclusion

This study highlights the gap that exists in our knowledge of the distribution and diversity of South Africa's mite biota. During the course of the study over 50 mite species were sampled and 15 other mites which are believed to be new and undescribed species were found. However, limitations in the sampling strategy mean that it is not possible to determine whether different sites and vegetation types support a distinct suite of mites. This is due to possible sampling flaws and weaknesses which we became aware of after the study commenced (variation in sampling season). Nevertheless, this study provides us with new insights as it is the first to compare mite biota from different vegetation types. This is important because in order for us to get a better understanding of the leaf domatia-mite mutualism and the diversity of mites found across regions, knowledge of how mites compliment the different vegetation types and biomes is required.

Chapter 5: Do mites prefer a specific place in the tree canopy?[1]

1. Introduction

Forests canopies alter environmental and climatic variables; such as solar radiation, air and soil temperature, rainfall, air humidity and wind; create a micro climate inside forests (Parker, 1995). The greatest changes to the micro-climate are brought about by adult stands with closed canopies and high leaf area indices (Aussenac, 2000). Forest trees may also modify their canopy microclimate along a vertical gradient, and air temperature generally declines with canopy depth due to within-crown shading. In coniferous forests, it has been observed that atmospheric temperature drops by about 0.25 to 1.8 °C with every metre of height you move down the tree canopy (Zweifel et al., 2002; Harley et al., 1996). Canopy structure therefore has a direct effect on the climate surrounding individual leaves and on the large-scale environment of forest regions. These changes in microclimate play an important role in determining the diversity of microorganisms, insects, birds, and vascular epiphytes found in forest canopies (Nadkarni, 1994).

Forest canopies also support diverse arthropods assemblages and these are usually distinct from those found on the forest floor (Arroyo et al., 2010). Inventories of arthropods have shown that forest canopies contain a high abundance and diversity of arthropods and that these insects may respond to environmental gradients within the forest from the top of the canopy to forest floors (Dial et al., 2006). These animals are thus an important component of forests, as they carry out a range of vital ecosystem services including decomposition and nutrient cycling in above ground deposits of litter and soils (Walter and Behan-Pelletier, 1999; Dial et al., 2006).

The majority of arthropods found in forests canopies are associated with leaves. The phylloplane of leaves may provide a wide range of insects with suitable micro habitat, and the surfaces of leaves are an important environment due to their diversity of anatomical, morphological and physiological properties (Pereira et al., 2002). These structures may support a rich arthropod fauna which is usually dominated by mites. Within forests canopies, mites exceed all other

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[1] Published as: S. Situngu and N. Barker. 2016. Position, position, position: Mites occupying leaf domatia are not uniformly distributed in the tree canopy. South African Journal of Botany, 108: 23–28. http://dx.doi.org/10.1016/j.sajb.2016.09.012 (See copy in Appendix 4)

arthropods in species abundance (Beaulieu et al., 2010; Walter and Behen-Pelletier, 1999), and mite assemblages comprise multiple lineages of predators, scavengers, grazers, animal associates, and plant parasites. Most of the mites encountered on leaves graze on phylloplane fungi are predatory to other mites. Harmful phytophagous mites may also be found (Krantz and Walter, 2009).

Some plant species possess leaves which bear structures known as leaf domatia (Figure 22). These often house large numbers of predatory mites, perhaps because this microhabitat provides higher relative humidity than surrounding air (Walter and Behan-Pelletier, 1999; Pemberton and Turner, 1989; O'Dowd and Wilson, 1991; Walter and O'Dowd, 1992b; O'Dowd and Pemberton, 1998; Matos et al., 2006). These structures influence the distribution and diversity of mites found on leaves.

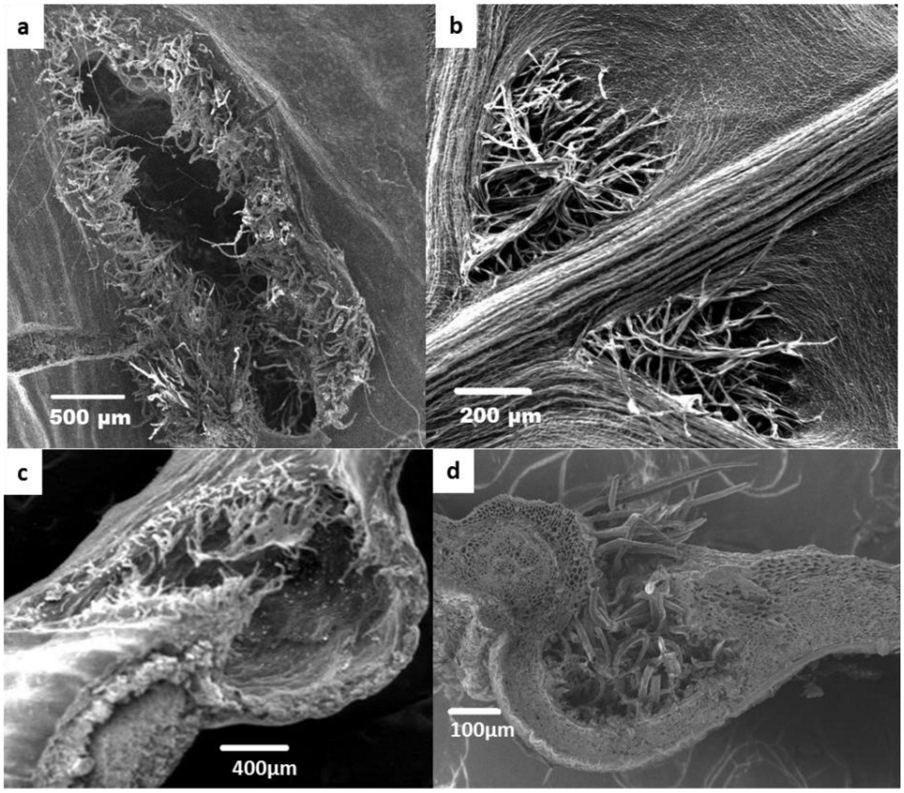

Figure 232: *Scanning Electron Micrographs of leaf domatia of the study species; (a) and (c) shows the domatium of Ocotea bullata and (b) and (d) is Gardenia thunbergia.*

Studies assessing the abundance of mites within forests have focused mainly on comparing the mite biota between the canopy biota and forest floor biota (Lindo and Winchester, 2006; Arroyo et al., 2010; Beaulieu et al., 2010). These studies have shown that there is a distinct variation in mites assemblages and specifically in mesostigmatid assemblages between canopy habitats and forest floor habitats. However, to the best of our knowledge, there is no information on the abundance of mites and their distribution within the tree canopy, or about how they respond to the environmental gradients from the ground to the top of the canopy. This study thus has the following aims:

1.1. To assess whether mite diversity and abundance is distributed uniformly throughout the canopy, irrespective of tree species. This was done using the species *Ocotea bullata* (Burch.) E. Meyer in Drege (Lauraceae) and *Gardenia thunbergia* Thunb. (Rubiaceae).
1.2. To assess whether mite diversity and abundance at different positions in the canopy of *Gardenia thunbergia* varies across seasons.
1.3. To measure changes in temperature and humidity at different positions in the canopy of *Gardenia thunbergia*, and to determine if these might influence mite diversity and abundance in this tree species.

2. Methods

Both *Ocotea bullata* and *Gardenia thunbergia* have pit-type domatia, surrounded by trichomes (Figure 22). *O. bullata* (commonly known as Stinkwood) is restricted almost entirely to South Africa, where it occurs from the forest on the Table Mountain (Cape Town) to the northern regions of the country. It is more common in moist and well drained forests, and is one of the dominant species within Southern Afro-montane forests (Mucina and Rutherford, 2006). It is also of economic importance, being used for building, making furniture, and its bark is an important traditional medicine (Geldenhuys, 2004). Field sampling of *Ocotea bullata* was conducted in the Tsistikama forest, Western Cape, South Africa, on the 9th of September 2013 where a stand of these trees were felled as part of another research program on this species. Because of the rarity of this species, it was sampled only once.

Gardenia thunbergia is an evergreen shrub or small tree that grows up to 6m in height, and is found in both Afromontane and Coastal forests of the Eastern Cape and KwaZulu-Natal regions of South Africa. The roots and leaves are used as traditional medicine and the hard wood is used to make tools (Boon, 2010). The trees sampled here are located in the Grahamstown Botanical Gardens, in the Eastern Cape, South Africa.

Five individuals of each species were selected for sampling. Before sampling, the north facing side of each tree was marked and then 10 leaves each were sampled from the North, South, East and West outside points, as well as the bottom inside and top of the canopy. *O. bullata* trees were between 15 to 19.10 meters in height, while *G. thunbergia* trees were over three meters. In the case of *O. bullata*, the leaves were sampled immediately after the tree was felled, and a ladder was used to access the leaves of *G. thunbergia*. Only mature but not senescent leaves were sampled. These leaves were placed in a labelled zip lock bag and kept cool. As soon after collection as possible, all the domatia present in each leaf were viewed under a dissecting microscope and the mites found on and inside leaf domatia were counted. The number of mites found in each leaf was recorded from each sampling position and the mean number of mites per canopy location was calculated. A non-parametric Kruskal-Wallis ANOVA was performed on STATISTICA version 2010 to compare mite abundance between all the canopy locations for each species.

The population of *Gardenia* was sampled on two different occasions. On the first occasion, five individual trees were sampled in the early austral spring on 4 September 2014 and on the second occasion in summer on 26 February 2015. Prior to sampling, seven iButton data loggers were placed in one of the trees at the positions of North, West, South, East, Inside, Top and Bottom of the canopy. The iButtons were glued in perforated plastic containers, and were then loosely covered in two layers of white cloth to minimize the effects of direct sunlight on the device. They were hung within the canopy. The iButtons were programmed to record temperature and relative humidity every 15 minutes. These were left on the trees for 14 days, and removed immediately after the leaves were sampled and assessed for mite diversity and abundance. Unfortunately, budgetary constraints restricted the use of this technology to a single tree.

Mites were also collected for identification purposes. To this end Scanning Electron Microscopy (SEM) was employed. Mites specimens selected to be viewed under the SEM were first dehydrated using 90% alcohol and then after air dried, they were mounted into a stub using graphite tape. The stub was then sputter coated with gold and observed under the TESCAN Vega TS 5136LM. Photographs were taken and image analysis was possible through the Scadium software. The photographs taken were then used for mite identification.

3. Results

3.1. Mite abundance in relation to canopy position in *O. bullata* and *G. thunbergia*

Figure 23 shows mite abundance from the different canopy locations of both *O. bullata* and *G. thunbergia*. For *O. bullata* no significant difference was found in mite abundance in terms of direction (East, West, North and South), p value <0.05 (Figure 23a). However, differences were found in the vertical distribution of mites in the tree canopy, with the canopy top having significantly fewer mites (p value <0.001). Furthermore, the mite abundance at the top of the canopy also differed from that of the East, West, North and South direction ($p \leq 0.02$).

Similar results were found for *G. thunbergia* (Figure 23b and c), which had significantly lower mite abundance at the top of the canopy (p value <0.001) on both sampling occasions. In addition, the bottom leaves of the tree canopy had significantly higher mite numbers (p value <0.0001). The west side of the canopy was found to also contain fewer mites on the first sampling occasion and was not significantly different from the top position. However, on the second sampling occasion, the West side was significantly different from the top position (p value = 0.04).

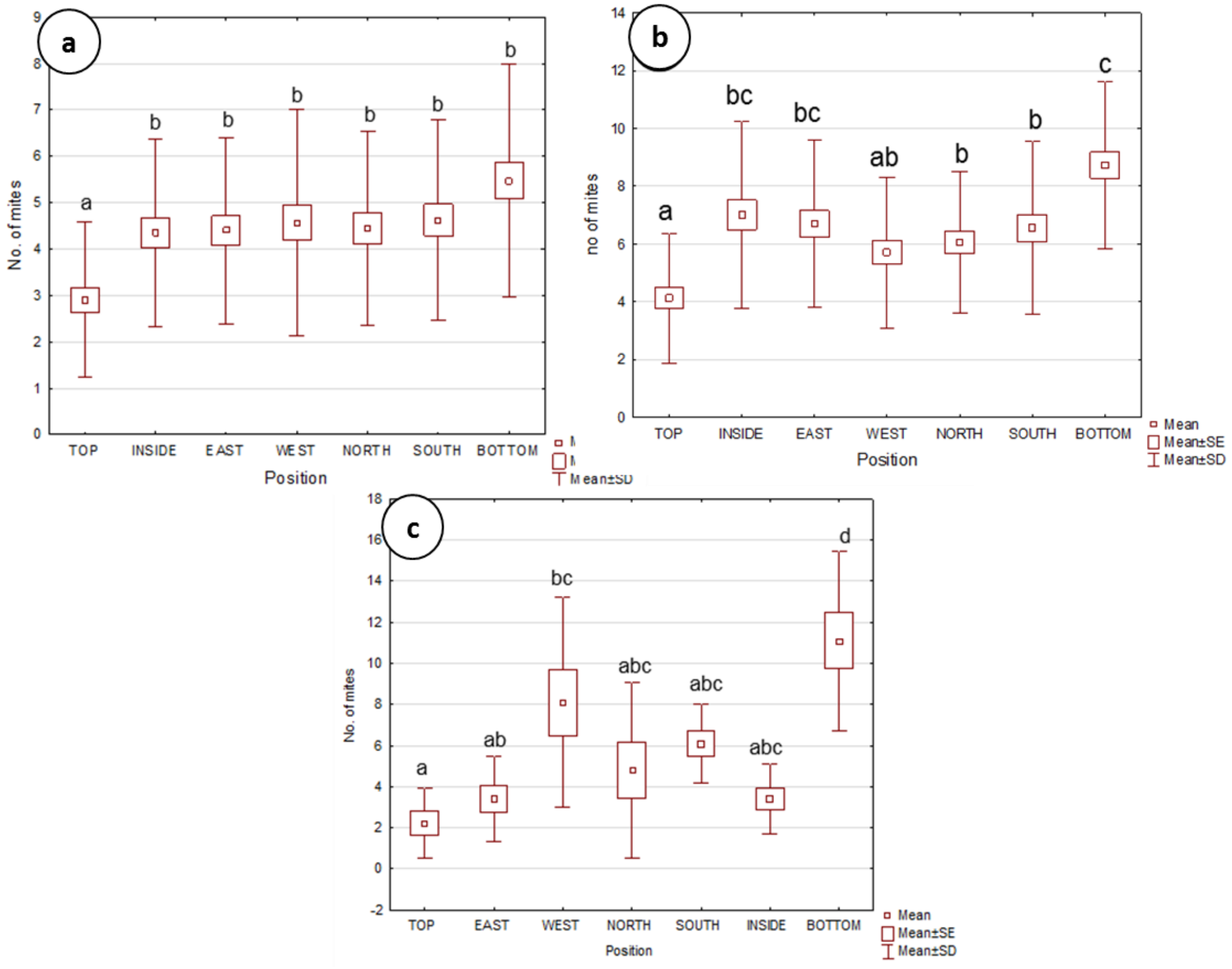

Figure 243: *Box and whisker plots showing the average number of mites found in leaves of (a) Ocotea bullata, and (b and c) Gardenia thunbergia at each tree canopy sampling location i.e. North, South, West and East aspect and from top, inside and bottom of canopy.*

3.2. Mite diversity in relation to canopy position

Mite diversity also varied within the canopy (Tables 8 and 9). Some taxa were found in all canopy sampling sites, while others were found at only some sites within the canopy. Only one taxon, *Bunaxella zebedielensis* was rare, and appeared to be highly restricted in its distribution, being found at the "inside" canopy position in September (Table 8) and the "South" position in February (Table 9).

Table 8: *The identity and total number of mites collected from 10 leaves at each canopy sampling point from five trees of Gardenia thunbergia (sampled on 4 September 2014).*

Family ***(species nam)***	**North**	**South**	**East**	**West**	**Top**	**Bottom**	**Inside**
Eriophyidea *Aculus comatus* (Nalepa)	124	64	70	108	85	133	54
Phytoseiidae *Euseius addoensis* (Van der Merwe & Ryke)	16	20	27	8	13	17	22
Stigmaeidae *Agistemus tranatalensis* (Meyer)	10	20	6	14	3	18	5
Phytoseiidae *Amblyseius anomalus* (van der Merwe)	33	48	42	32	26	79	32
Anystidae *Anystis baccarum* (Linnaeus)	25	23	21	26	35	33	25
Iolinidae *Lourus citricolus* (Ueckermann & Grout)	71	131	56	62	79	67	78
Cunaxidae *Bunaxella zebedielensis* (Den Heyer)	4	4		7	2	2	2
Oribatideae Oribatula sp.							3
Acaridae - nymph		1		1	2		3

Table 9: *The identity and total number of mites from 10 leaves at each canopy sampling point from the G. thunbergia tree in which iButtons were placed (Sampled on 26 February 2015).*

Family *(species name)*	**North**	**South**	**East**	**West**	**Top**	**Bottom**	**Inside**
Eriophyidea *Acalus comatus* (Nalepa)	14	15	8	43	6	74	
Phytoseiidae *Euseius addoensis* (van der Merwe and Ryke)	2	4	11	4	7		6
Stigmaeidae *Agistemus tranatalensis* (Meyer)	5	2	2	10	2		
Phytoseiidae *Amblyseius anomalus* (van der Merwe)	11	5	11	7	5	23	4
Anystidae *Anystis baccarum* (Linnaeus)	5	14	2	1		13	7
Iolinidae *Lourus citricolus* (Ueckermann & Grout)	11	18		2		1	14
Cunaxidae *Bunaxella zebedielensis* (Den Heyer)		3					
Oribatideae Oribatula sp.							3

3.3. Environmental variability within the canopy of *Gardenia thunbergia*

The first few days after the iButtons were placed on the tree, the weather was cold and wet. Thereafter the weather improved, and data from a period of the last 10 successive days was used in subsequent analyses. Unfortunately, the data logger on the West side of the tree was stolen or removed, so no data for this position is available. Table 10 summarises the recorded variables. As can be seen, the top, East and North facing aspect were the warmest, while the South, inside and bottom were coolest positions. There was a six degree centigrade difference between the warmest and coolest positions. The top, east and inside position had the lowest mite numbers, while the cooler sites (bottom and South) had more mites and greater diversity. When mite number and mite diversity was plotted against the environmental variables, these were found to be most strongly correlated with maximum temperature, variance in temperature and minimum relative humidity (Figure 24).

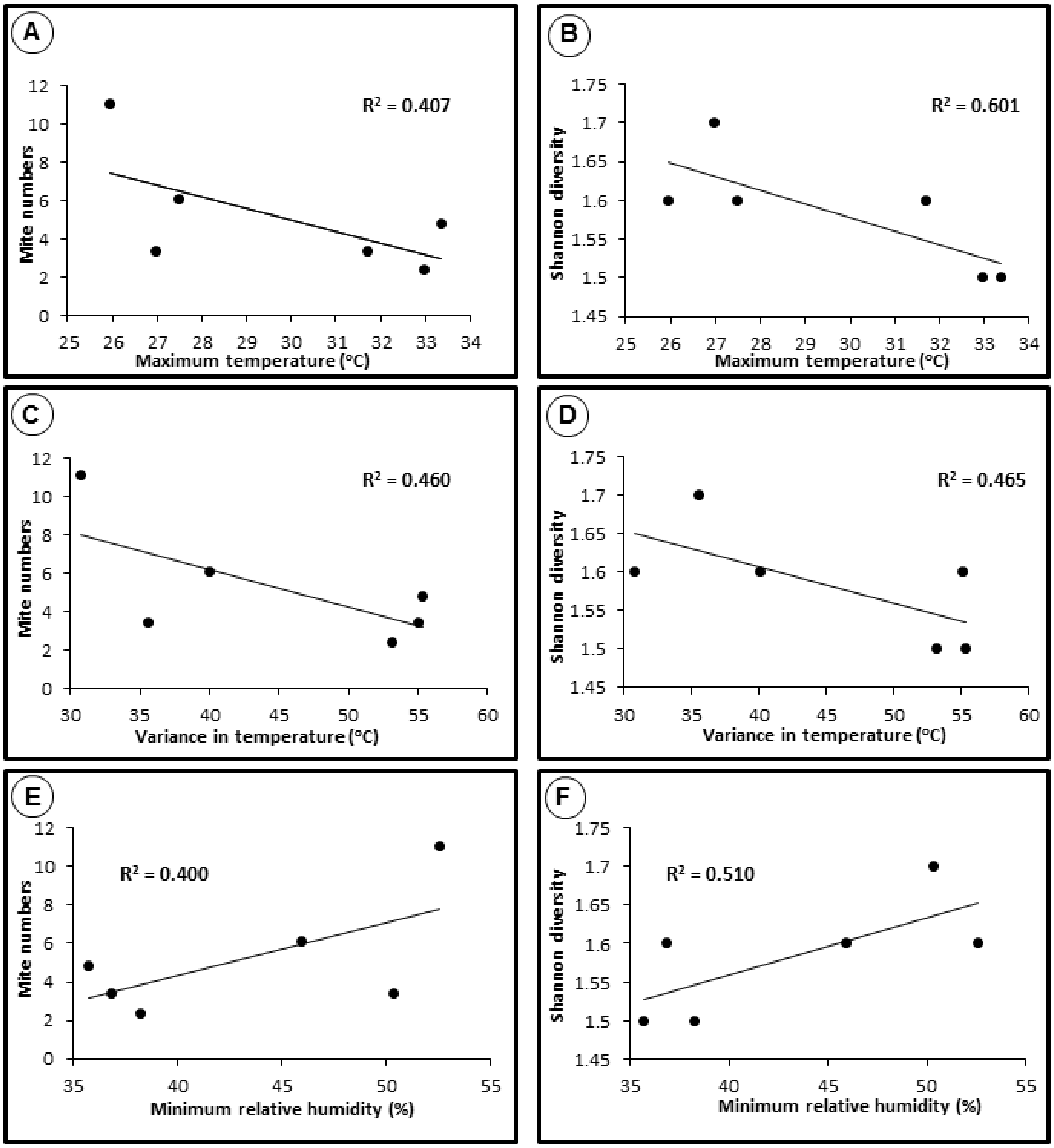

Figure 254: *Correlation plots showing relationships between mite abundance, Shannon diversity index, temperature and relative humidity for the sampled canopy position of Gardenia Thunbergia.*

Table 10: *I-Button data showing climatic variation at the different positions of the canopy of G. thunbergia.*

	North	**South**	**East**	**Top**	**Bottom**	**Inside**
Average Max Temp	33°C (±7.04)	27°C (±4.86)	32°C (±5.62)	33°C (±7.20)	26 ºC (±5.04)	27°C (±5.20)
Average min temp	13°C (±4.36	13°C (±4.46)	12°C (±2.84)	14°C (± 4.36)	14°C (±4.20)	14°C (±4.24)
Average Max Re Humidity	98 (± 2,42)	102 (±1.96)	100 (± 1.80)	95 (±1.22)	99 (±1.94)	98 (±2.02)
Average Min Re Humidity	36 (±18.42)	45 (±11.55)	37 (±9.66)	38 (±12.45)	52 (±13.38)	50 (± 12.79)
Var. Temp	55.30	40.03	55.05	53.12	30.75	35.57
Var. Re Humidity	397.37	440.31	530.85	5359.07	307.81	328.83

4. Discussion

Little is known about the mite biota found within forest habitats (Arroyo et al. 2010), especially the forests of South Africa. An improved understanding of where mites are found within the canopy of trees is important, as it may enable a better understanding of how the protective mutualism between plants and mites might work and how mites provide protection to host plants. Such knowledge may also be of value when planning future sampling strategies for mite studies, as diversity and even species composition may vary depending on the canopy position sampled. The results show that mite communities are more abundant and diverse at the lower and internal parts of the tree canopy where it is cooler and more humid (Figure 23 and 24). The study proposes two (not mutually exclusive) explanations for this result:

Firstly, this might be a factor of leaf age; mites may accumulate on older leaves that are found at lower levels and at the bottom of the canopy. Parolin et al., (2011) found a positive relationship between leaf maturity and the presence of the predatory mite *Amblyseius californicus* on *Viburnum tinus.* Mites were more frequent on old and mature leaves which possessed more domatia than young leaves. Moreover, mites may prefer older leaves because the domatia of older leaves are more developed than domatia on newly formed leaves. In an ontogenetic study on the domatia of *Gardenia thunbergia*, it was found that domatia form on leaves when they are very small (less than 1 cm in length), and that as the leaves age, the domatia increase in size and complexity, with an increase in trichome abundance (Barker and Holmes, 2010). It was also found that the number of domatia present on leaves of this species increases only slightly over the life of the leaf.

The role of trichomes associated with domatia is not fully understood, but it has been suggested that trichomes may provide signal to mites that leaf domatia are developed and ready to provide refuge to mites and thus be involved in the mutualistic relationship, due to evidence that mite abundance increased with leaf age (Nishida et al., 2005). Alternatively, trichomes may aid in retaining moisture and high levels of humidity within domatia. However, we endeavoured to sample only mature leaves and these factors may not be an adequate explanation for the observed differences.

Secondly, mites choose to reside at lower levels of tree canopy because the microclimate is more favourable (cooler, more humid and shaded) (Aussenac, 2000). Forest microclimate (patterns of temperature, moisture, wind and light) plays an important role in influencing insects and arthropod habitat selections (Chen et al., 1999; Fukui, 2001). In the shade there is less UV radiation and the rain penetrates less and more slowly compared to the top of canopy where sunlight and rain may impact the canopy and dry out or wash away mites. Furthermore, winds at the top of the canopy are stronger and could eliminate mites from leaves at the upper part of the tree.

Onzo et al., (2003) observed high densities of herbivorous mites lower down on older leaves of Cassava plants. They suggest that these mites migrated to older leaves in the presence of higher densities of predatory mites. However, contrary to the findings of this study, they observed high densities of predatory mites in the plant apex during the day and these predatory mites migrated down to forage at night. They suggest that these mites preferred to reside in the closed leaves of the apex during the day because they provide a safe refuge from harsh environmental conditions.

Desiccation is a major environmental hurdle for invertebrates especially during moulting. It has been reported that higher relative humidity allows insect larvae to survive and grow better than at low humidity (Fukui, 2001). It is hypothesized that the relative humidity inside shelter of a leaf domatium is significantly higher than that of the leaf surface. At the top of the canopy, the environmental gradients may be stronger, thus reducing humidity and increasing light levels such that mites avoid these parts of the tree.

5. Conclusion

To the best of our knowledge, this study is the first to document mite distribution within the canopy of domatia bearing tree species, and to correlate diversity and abundance to environmental variables. Our results show that mites may respond to intra-canopy microclimate, avoiding canopy positions of greater temperature, and lower humidity, and that this pattern is observed in multiple plant species, and across seasons. However, this result must be viewed as preliminary, as similar studies on additional tree species are required to confirm these findings. In addition, it would prove insightful to document the diurnal movements of mites and how they may correlate with canopy microclimate variables. Based on these results, we suggest that future studies on mites and leaf domatia should employ a consistent sampling approach, and avoid the sampling of exposed and environmentally variable portions of the canopy.

Chapter 6: Seasonal changes in foliar mite communities found in *Gardenia thunbergia* Thunb., *Rothmannia globosa* (Hochst.) Keay and *Tecoma capensis* (Thunb.) Lindl. over a period of two years

1. Introduction

This study investigates seasonal patterns in mite communities found on three plants with leaf domatia. Several studies have shown how structures such as leaf domatia affect the abundance and diversity of arthropods, including mites, found on leaves of forest trees (Walter, 1996; Pemberton and Turner, 1989; O'Dowd and Willson, 1991; Walter and O'Dowd, 1992b; O'Dowd and Pemberton 1998; Norton et al., 2000; Romero and Benson, 2005). For example, Karban et al. (1995) showed that leaf surface characteristics, particularly the density of vein hairs, influenced population densities of phytoseiid mites on grapes. Leaf domatia are known to be more attractive to these beneficial mites (Walter and O'Dowd, 1995). Rasmmy and Elbonhawy (1974) found that hair tuft-type domatia enhance the development and foraging success of phytoseiid mites. Also, leaf hairs interfere with the movement of parasitoid insects and the feeding activity of herbivorous insects and affect predatory mite communities.

However, there is insufficient understanding of how seasonality affects mite abundance and diversity. Most studies that examined mite assemblages found on leaf domatia are based on a single sampling period and little is known about seasonal fluctuations in mite communities within these structures. Only a few studies on this topic have been published (Duso at al., 2004; Toyoshima and Amano, 2006; Duso et al., 2010).

It is hypothesized that mites are affected by climatic conditions, such as temperature and relative humidity (Situngu and Barker, 2016), and changes in seasons play an important role in structuring mite assemblages (Toyoshima and Amano, 2006). In temperate zones, mites appear on deciduous trees annually in spring and the diversity and abundance of these communities changes every season. Seasonal fluctuations in mites are affected by the availability of food and fungal flora present on leaves as some mites are dependent on fungi as a food source (Toyoshima and Amano, 2006). Similarly, Duso et al. (2004) showed seasonal fluctuations in the abundance of phytoseiid mites, documenting high densities of mites in hedgerows from late April to late

June (winter) and low densities in September and October (spring). The high abundance was related to pollen availability.

In a later study, Duso et al. (2010) observed seasonal patterns in plant mite communities on a deciduous shrub *Viburnum erosum* Thunb. over a two year period. Their results showed that fungivorous mites (Winterschmidtidae, Tydeidae) were found in the domatia constantly throughout the sampling season from March to November. Phytoseiid mites were found within domatia of the leaves from May to November and the eriophyid mites were found in domatia only in spring and autumn. These results suggest that seasonal fluctuation in mite density and communities within domatia of this plant are present, but narrow. Toyoshima and Amano (2006) investigated seasonal dynamics of phytoseiid mites on *Magnolia* L. trees and found that the mite *Amblyseius orientalis* (Ehara) showed some seasonal fluctuations and was found in low numbers in spring and increased gradually in autumn (Toyoshima and Amano, 2006). However, there are no studies conducted in Africa and there is a gap in our understanding of seasonal patterns of mite diversity.

This study documented changes in mite communities found on *Gardenia thunbergia*, *Rhothmannia globosa* and *Tecoma capensis* over a period of two years. It is hypothesized that mite diversity and abundance will vary as the seasons change, and that these changes will be correlated with climatic variables.

2. Methods

2.1. Study site and climate variables

The study was conducted in Grahamstown, Eastern Cape, South Africa. The three plants sampled are located within the Rhodes University campus and the adjacent Grahamstown Botanical Gardens. Grahamstown has a semi-arid climate. The area is located on the eastern periphery of the winter and summer rainfall zone and as a result it receives rain throughout the year (Mucina and Rutherford, 2006). Normally, Grahamstown receives about 466mm of rain per year and most of this is received in winter. On average, August is the wettest month and the driest month is December (Figure 25) Grahamstown is located on the fringes of the arid Karoo and thus the area can be extremely hot in summer with average daily maximum temperatures of up to 26.8 °C. It gets quite cold in winter and average daily temperatures may drop to 5.6°C. On average, the warmest month is January and the coolest month is July. March is the most humid month and June is the least humid month (weather-and-climate, 2016).

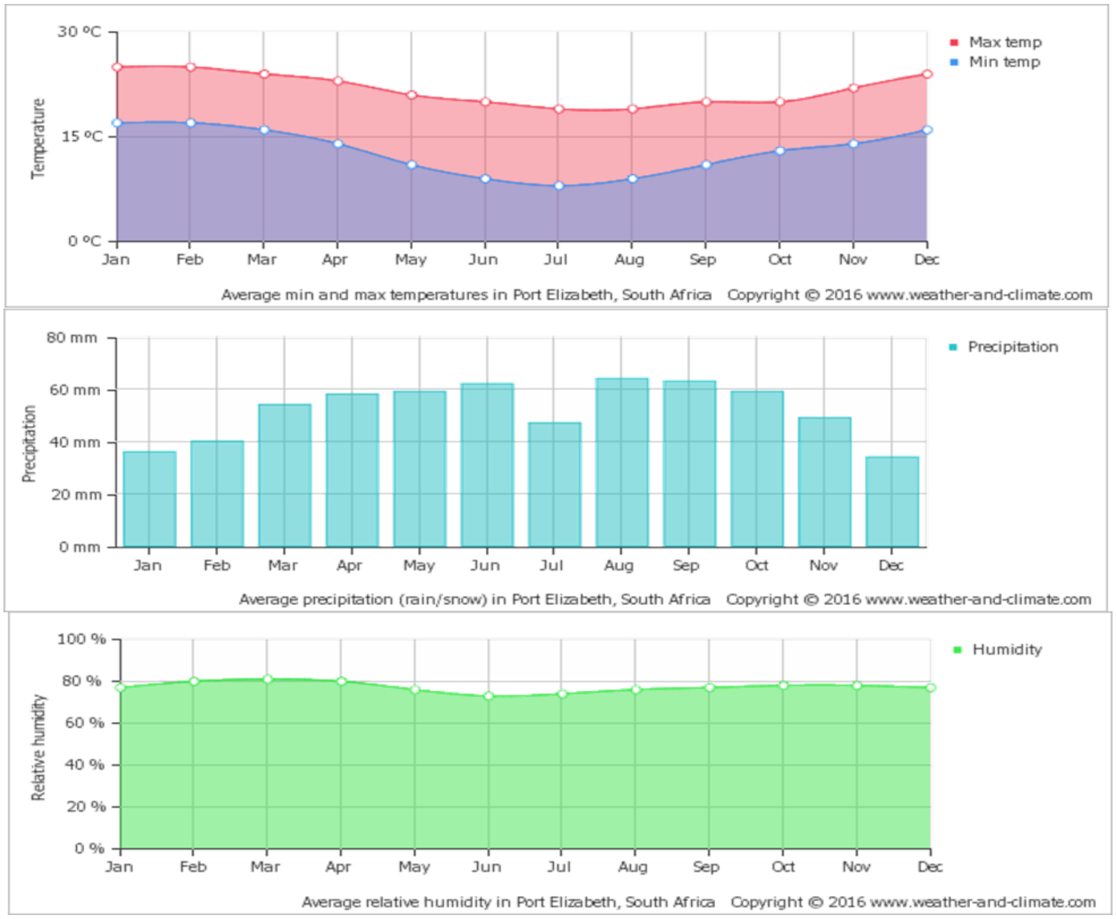

Figure 265: *Monthly minimum and maximum temperatures, monthly precipitation and humidity for Grahamstown. (Source: weather-and-climate.com, 2016)*

2.2. Plant species description

Three plant species; *Gardenia thunbergia*, *Rothmannia globosa* (both Rubiaceae) and *Tecoma capensis* from the Bignoniaceae family were selected for the study. A single tree of each of the plants species was selected because they could be easily accessed throughout the sampling period. Also, these species are evergreen plants and produce leaves throughout the year, making them ideal species for this type of study.

Gardenia thunbergia is an evergreen shrub or small tree that grows up to 7 m tall. This tree is found in both afro-temperate and coastal forests of the Eastern Cape and KwaZulu-Natal regions of South Africa. It has a smooth whitish stem and short rigid branchlets (Boon, 2010). The leaves are glossy dark green, hairless, and feel thinly leathery. These are usually carried in whorls of 3 or 4 crowded near the ends of the branchlets. They have a wavy margin, and tape abruptly to a rounded or blunt tip, while the base tapers gradually onto the stalk. The veins are conspicuous and the margins are wavy. Hairy pit-type domatia are present in vein axils. The roots and leaves are used as traditional medicine and the hard wood is used to make tools (Boon, 2010).

Rothmannia globosa is found in evergreen forest and along forest margins in the Eastern Cape, and as far north as the Limpopo Province and as far east as Swaziland. This tree can grow up to 15 m tall and has dark greyish-brown stems with rectangular markings. Leaves are glossy and dark green, often with yellowish to maroon veins on the underside. The base and apex of leaves are tapered. Hair tuft-type domatia are present in vain axils. The tree produces whitish flowers with a sweet scent (Boon, 2010).

Tecoma capensis is an evergreen Liana found in forest margins, in sub-tropical thicket and in coastal dunes of the Eastern Cape all the way to the tropics of Africa. The shrub is multi-stemmed with a pale brown bark and grows up to 5 m tall. It has shiny dark green compound leaves that have oval leaflets with blunt teeth (Dharani, 2002). The apex of the leaf is usually pointed, while the base is rounded and wedge shaped. The leaves have hair tuft-type domatia on vein axils. This plant produces tubular flowers in terminal clusters. The flowers vary in colour from red, deep orange to yellow (Dharani, 2002; Boon, 2010).

2.3. Field Sampling

Sampling was conducted on a single individual for each of the three plant species mentioned above. One specimen per species was sampled because it takes a long time to process the leaves and sampling more trees would be time consuming, potentially compromising the accuracy of mite counts. Sampling period started on the first week of March 2014 and ended on the 23rd of

August 2015. Twenty leaves were collected all around the plant every second week from each of the trees and viewed under the dissecting microscopes on the same day of collection. The total number of mites found inside domatia was counted and the different morpho-species of mites observed inside domatia were also noted. A representative sample of the mites found inside the domatia were collected and sent to Professor Ueckermann at the Agricultural Research Council (ARC) for identification. Some individuals were mounted on a stub and viewed under the Scanning Electron Microscope (SEM) or were alternatively mounted using Polyvinyl Alcohol (PVA) and viewed under a light microscope. In some cases SEM photos were taken and these also assisted in mite identification.

2.4. Data analysis

Mite counts were used to calculate the average number of mites found in each leaf as a representation of mite abundance. The number of individuals present for each mite species was also recorded and this data was used to calculate species richness and the Shannon-Weiner diversity index. Shannon-Weiner diversity index was calculated because it accounts for both abundance and evenness of the sampled species. This was done in order to show seasonal patterns in species abundance and diversity over the sampling period. The seasons were defined in the following terms: summer (December to February), autumn (March to May), winter (June to August) and spring (September to November).

Climate data for Grahamstown were obtained from the Grahamstown Weather Station located in the Grahamstown Army Base. From these data we extracted values for daily minimum and maximum temperatures, relative humidity recorded at 8h00 and at 14h00, rainfall the day before and accumulative rainfall over the preceding two week prior to sampling. These data were used to perform correlation matrices using STATISTICA 13 to determine whether there were relationships between these environmental variables and mite abundance and diversity.

A list of all mite species collected from each plant species during the sampling period was collated into a presence-absence matrix in excel and used to perform a multivariate analysis. A non-metric multidimensional scaling (NMDS) ordination plot was created to identify relationships and similarities in mite biota between the host tree species and between seasons.

For the NMDS, Chord distance resemblance was used, a minimum stress value of 0.01 and a Kruskal fit scheme of two were selected. A similarity percentage analysis (SIMPER) was also used to determine which mite species are characteristic of the different trees and identifying which mites contribute most to the similarity between sample host plants. The parameters for this analysis were Bray-Curtis resemblance and Cut off for low contributions was at 90%. All the analyses were carried out using PRIMER 6 software package (Clarke and Gorley 2006).

3. Results

3.1. Seasonal patterns in mite abundance

The results from this study show that mite abundance and mite species composition in *Tecoma capensis*, *Rothmannia globosa,* and *Gardenia thunbergia* differed between the plant species sampled and varied with seasons. In *T. capensis,* three peaks in mite abundance were observed; the first peak started in April 2014 during the autumn season and dropped in September in the beginning of spring (Figure 26a). The population peaked again in November 2014 during summer and a smaller peak was also observed in May 2015 in the middle of autumn. The standard deviation in mite abundance was great due to large variations between mite counts from the 20 replicate leaves that were sampled (Figure 26a). In *R. globosa*, the mite populations increased during the spring and summer months for both sampling years (Figure 27). This increase was observed starting in October 2014 until May of 2015 (Figure 27a). *Gardenia thunbergia* also had the highest mite abundance in spring and summer of 2014 - 2015 starting in October 2014 until March 2015 (Figure 28a).

When compared, *T. capensis* had the highest abundance from March until October of 2014 (Figure 26a). This was during autumn and all through to mid-spring season of 2014. *R. globosa* had stable populations of mite and their abundance did not exceed the other two sampled plants during the study period. *G. thunbergia* only had the highest mite abundance briefly during November and December of 2014 and again in August of 2015 (Figure 28a).

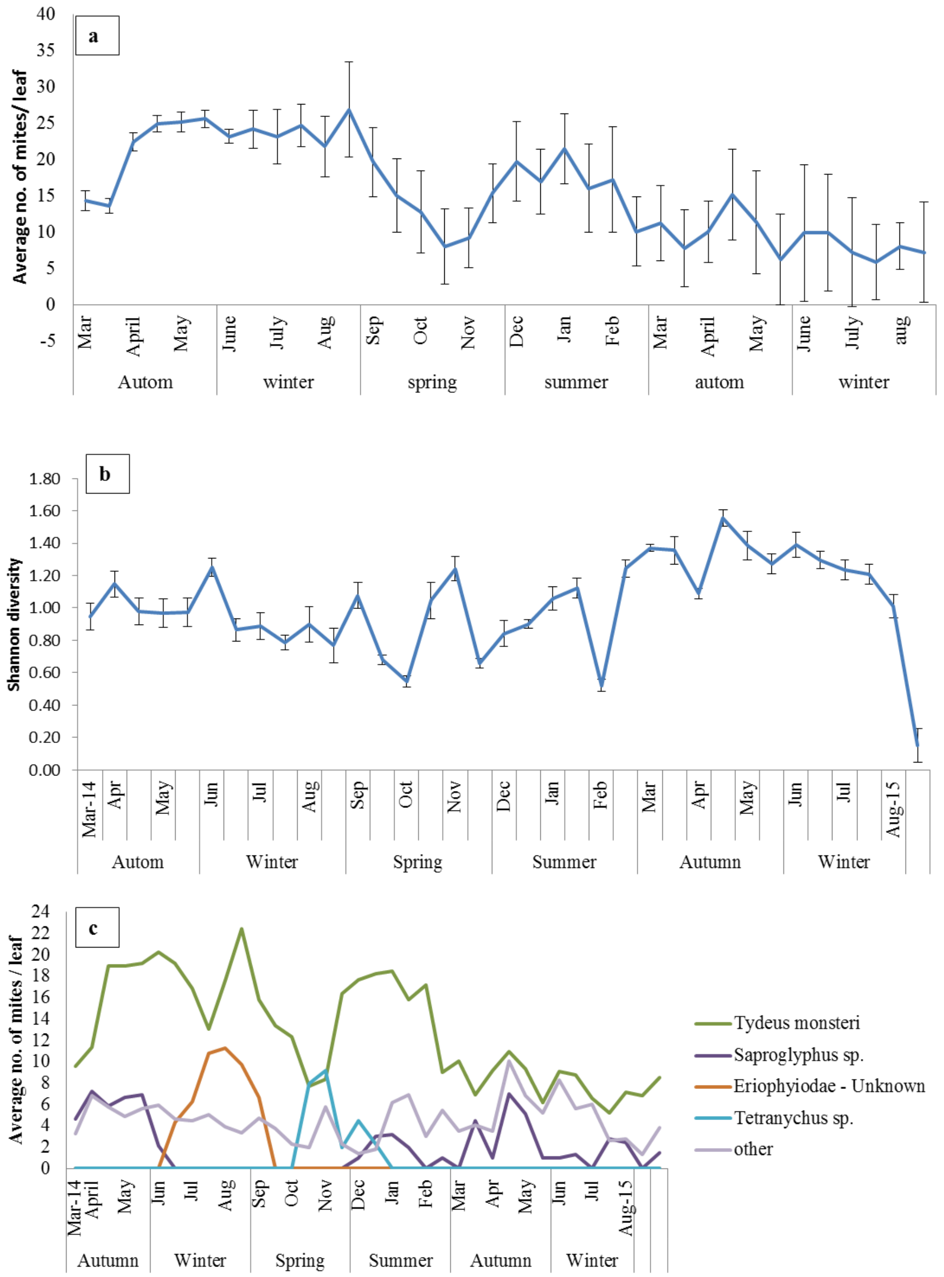

Figure 27: *Seasonal changes in mite abundance and diversity in Tecoma capensis, (a) Total number of mite per leaf, (b) Shannon-Weiner diversity index and (c) Abundance of the different species of mites found on T. capensis.*

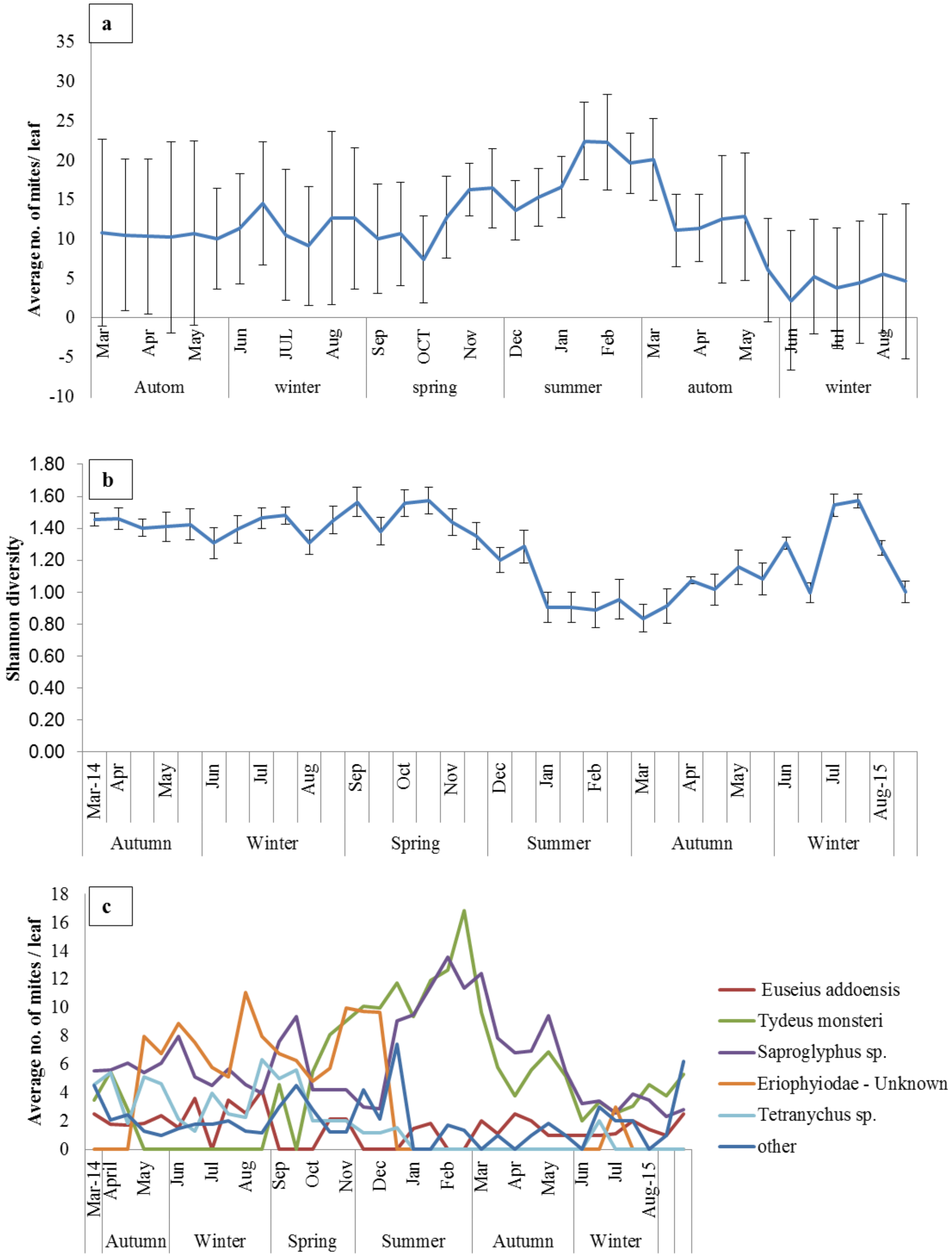

Figure 287: *Seasonal changes in mite abundance and diversity in Rothmannia globosa, (a) Total number of mite per leaf, (b) Shannon-Weiner diversity index, and (c) Abundance of the different species of mites found on the domatia.*

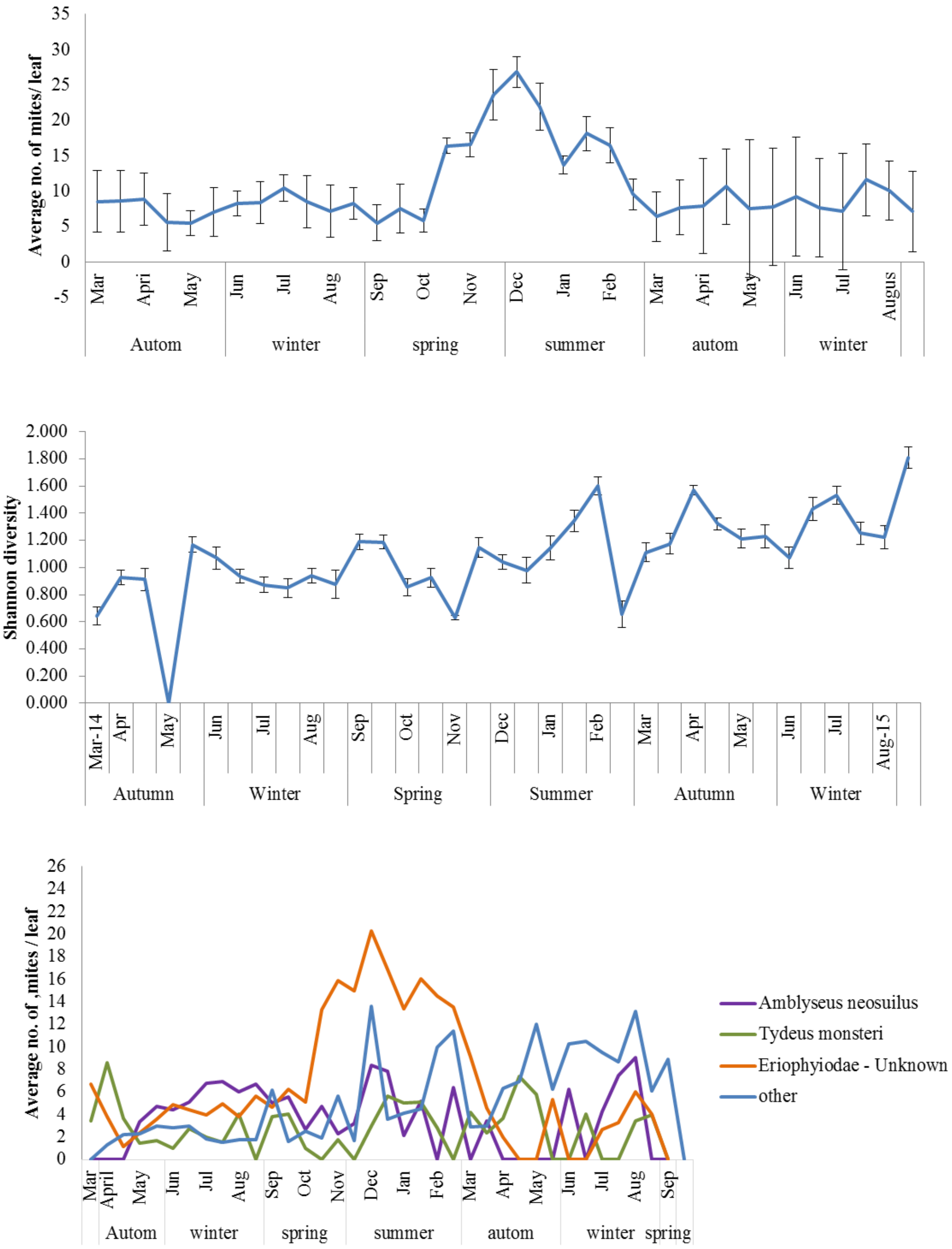

Figure 298: *Seasonal changes in mite abundance and diversity in Gardenia thunbergia, (a) Total number of mites per leaf, (b) Shannon-Weiner diversity index and (c) Abundance of the different species of mites found on the domatia.*

3.2. Seasonal patterns in mite diversity

The Shannon-Weiner diversity index in *T. capensis* varied throughout the sampling period. Mite diversity was low in October 2014 and February 2015. This peaked and was highest during the autumn months of 2015 (Figure 26c). Different mite species were found all through the sampling period. In *T. capensis* the *Tydeus monsteri* (Tydeidae) was the most common mite species all through the sampling period (Figure 26b). An unknown mites (Eriophyiodae) was only found in winter of 2014. The *Saproglyphus* mite (Wintischmitdae) was present only in the autumn months of 2014 and re-appeared again in December and their numbers peaked again in autumn of 2015. The *Tetranychus* mites (Tetranychidae) were found only from mid-October 2014 to mid-January of 2015 and dominated during autumn and winter of 2014 (Figure 26b). Other mite species were found to be present on the leaves of *T. capensis,* but in very low numbers. These are listed in Appendix 5.

Mite diversity in *R. globosa* remained the same in 2014 and did not differ much. Starting in January 2015, it declined a little and was lowest in autumn of 2015 (Figure 27b). The different mite species found inside the domatia of this plant during the sampling period are listed in Appendix 5 and their abundance varied within the seasons. During autumn and winter months of 2014, the unknown species (Eriophyidae) was the most abundant species found on the leaves of this tree (Figure 27c). This mite was encountered on leaves of *R. globosa* until January 2015 and then disappeared. In spring of 2015 populations of *Tydeus monsteri* started to peak and the highest numbers were observed in summer. The population declined again in the autumn months. A *Saproglyphus* mite (Winterschmidtiidae) was observed throughout the sampling period, but higher numbers were found in the summer and autumn months of 2015. Other species of mite were also found, but in low numbers (Appendix 5).

The Shannon diversity of *Gardenia thunbergia* varied throughout the sampling period and was highest in winter of 2015 (Figure 28b). The unknown species from the Eriophyidae family persisted all through the sampling period and was the most common species in spring and summer during the period of October 2014 to February of 2015. *Tydeus monsteri* was also present and was only common during a brief period in April 2014. *Amblyseus neosuilus* was the most common species in winter of 2014. Nine species of mites were found in association with this plant over the sampling period and a complete list is given in Appendix 5.

Mite diversity in *R. globosa* was the highest compared to the other sampled plants species (Figure 27b). Compared to the other plants, *T. capensis* had the lowest diversity throughout the sampling period except in autumn of 2015 (Figure 26b). In *G. thunbergia*, Shannon diversity was high in winter of 2015 (Figure 28b).

3.2.1. Comparing mite diversity between trees and between seasons

A NMDS plot was produced from a resemblance matrix based on the mite biota found on the plant species sampled over the different seasons. The results (Figure 29) showed that mite species composition differed over the sampling months between the trees. Samples from *T. capense* and *G. thunbergia* separated in the view of axes 1 and axes 2 while *G. thunbergia* and *R. globosa* are not. This suggests that *T. capense* and *G. thunbergia* have different mite biota. Table 11 gives average similarity and contribution of species to average similarity of the different trees sampled. These results suggest that the trees had similar mite species composition all through the sampling months.

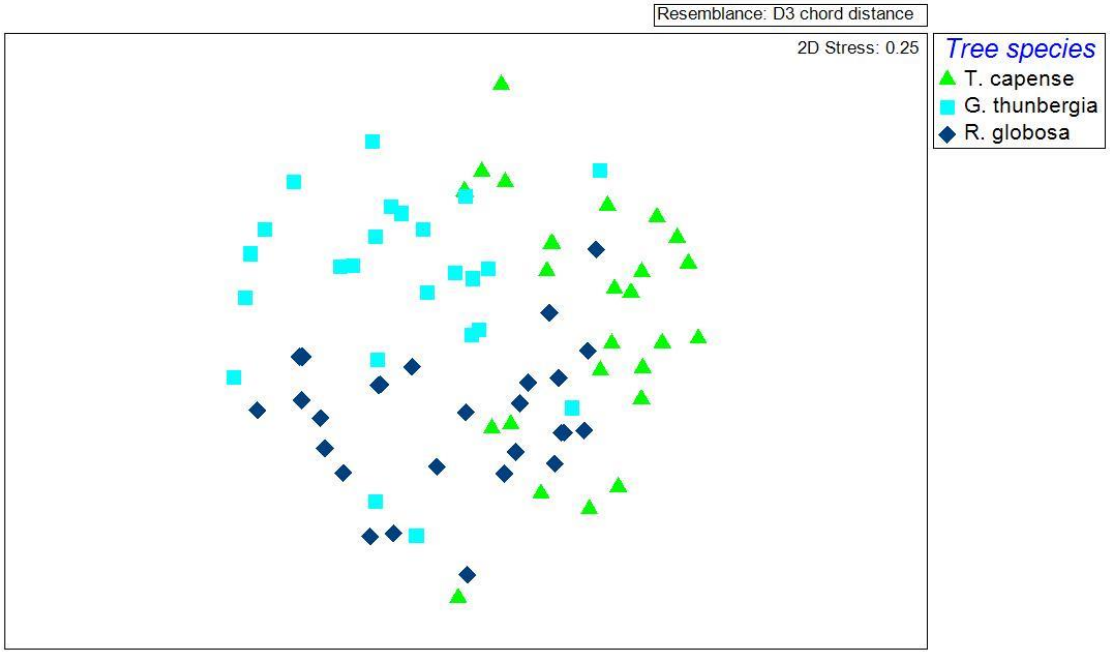

Figure 30: *Non- metric Multidimensional Scaling (NMDS) plot showing relationships in mite communities between the tree species sampled over a period of 17 months (March 2014 – August 2015). Each point represents a 2-week sample period.*

Tyres monsteri, *Euseius addoensis*, and *Saproglyphus* mite were the most common species found on all three plants and they contributed to within group similarity in both *T. capensis* and *R. globosa* (Table 11). *Tydeus monsteri* and *Euseius addoensis* are both predaceous mites and are beneficial to plants. These mites belong to the Tydeidae and Phytoseiidae families. *Saproglyphus* is saprophytic and not beneficial to the plants. These may thus be considered to be generalist

The unknown species (Eriophyidae) and *Amblyseus neosius* were common in *G. thunbergia* and they contributed to within group similarity. Both the *Tetranychus* mite and unkown mite from the Eriophyidae family are plant feeding and may cause serious damage to plants. On the other hand, the *Amblyseus* mites are predacious and feed on other small arthropods. Thus, majority of the mites in all trees were predatory.

Table 11: *The contribution (in percentages) of the different mite species sampled to the average within group similarity (similarity between months) amongst the trees sampled.*

Tree species	**Average within group similarity (%)**	**Contributing species (% for individual contribution)**
T. capensis	60.02	Tydeidae *Tydeus monsteri* Meyer & Ryke (34.4) Phytoseiidae *Euseius addoensis* Van der Merwe & Ryke (29.9) Winterschmidtidae *Saproglyphus* sp. (10.2)
R. globosa	64.77	Winterschmidtidae *Saproglyphus* sp. (34.3) Phytoseiidae *Euseius addoensis* Van der Merwe & Ryke (20.6) Tydeidae *Tydeus monsteri* Meyer & Ryke (19.5)
G. thunbergia	60.86	Eriophyiodae Unkown (27. 4) Tydeidae *Tydeus monsteri* Meyer & Ryke(21.2) Phytoseiidae *Amblyseus neosius* van der Merwe (15.7)

3.3. Relationships between mite abundance and diversity and environmental variables

The climate data from the Grahamstown Weather Station was correlated with mite abundance and diversity (see Appendix 6 and 7 for correlation plots). The results for *Tacoma capensis* suggest that minimum daily temperatures, relative humidity recorded at 8h00 and accumulative rainfall over two weeks prio to sampling had an influence in patterns of mite abundance and Shannon-Weiner diversity index of this tree (Table 12 and 13). Although these correlations are not strong, they were found to be statistically significant when a correlation matrix test was performed using STATISTICA. The general patterns suggest that higher mite abundance occurred at low minimum temperature and the higher the minimum temperature the lower the average number of mites (Table 12). In contrast, the Shannon diversity index increased with an increase in daily minimum temperature (Table 13). There was not a lot of variation in minimum temperature between sampling days and most recorded points were around the value 8°C to 15°C.

Both mite abundance and Shannon diversity increased with an increase in relative humidity (Tables 12 & 13). During most of the sampling days relative humidity ranged from 65% to 100% and there were a few days where it was significantly low. Accumulative rainfall was also negatively correlated with mite abundance in *T. capensis* and more frequent rainfall (measured as number of days it rained over the preceding 14 day period) resulted in less mites encountered on the leaves (Table 12).

In *R. globosa*, maximum daily temperature and minimum temperatures were negatively correlated with mite abundance and positively correlated with diversity. On the other hand, relative humidity at 8h00 was positively correlated with mite abundance and diversity. Mite abundance increased with an increase in both minimum and maximum temperature (Table 12) and the Shannon diversity decreased with increasing minimum and maximum temperature (Table 13). In addition, the Shannon diversity index was only significantly correlated with relative humidity and these results suggested that mite diversity increases with relative humidity (Table 13).

For *G. thunbergia*, mite abundance was correlated with maximum temperature, minimum temperature and relative humidity at 8h00 (Table 12). Shannon diversity was significantly correlated with only minimum temperature and relative humidity (Table 13). Both minimum and maximum temperatures were positively correlated with mite abundance. Similarly, Shannon diversity increased with an increase in both minimum temperature as well as relative humidity. When mite abundance was correlated with relative humidity, mite abundance decreased as relative humidity increased (negative correlation). P-values and R-values for all correlations are given in Table 12 and 13.

Table 12: *Correlation matrix results showing R^2, P-value and T for the plant species sampled. In this analysis, the different independent environmental variables were correlated with mite abundance (dependent variable) from the three plant species sampled. Values in bold are statistically significant at $p \leq 0.05$.*

		T. capensis	***R. capensis***	***G. thunbergia***
Min. Temp	R^2	**0.15**	**0.22**	**0.19**
	P	**0.006**	**0.0008**	**0.001**
	T	**-2.84**	**3.59**	**3.29**
Max. Temp	R^2	0.070	**0.28**	**0.23**
	P	0.071	**0.0001**	**0.0007**
	T	-1.84	**4.16**	**3.62**
RH @ 8h00	R^2	**0.13**	0.076	**0.09**
	P	**0.015**	0.06	**0.036**
	T	**2.54**	-1.92	**-2.16**
RH @ 14h00	R^2	0.009	0.0034	0.0008
	P	0.50	0.69	0.85
	T	0.67	-0.39	-0.18
Rain day before	R^2	0.04	0.001	0.0002
	P	0.17	0.82	0.92
	T	-1.38	-0.22	-0.094
Accumulative rainfall over 2 week period	R^2	**0.10**	0.035	0.026
	P	**0.027**	0.20	0.28
	T	**-2.28**	1.29	1.08

Table 13: *Correlation matrix results showing R^2, P- values and T for the plant species sampled. In this analysis, the different independent environmental variables were correlated with Shannon Diversity Index (dependent variable) from the three plant species sampled. Values in bold are statistically significant at $p \leq 0.05$.*

		T. capensis	***R. globosa***	***G. thunbergia***
Min. Temp	R^2	**0.11**	**0.24**	**0.11**
	P	**0.023**	**0.0005**	**0.025**
	T	**-2.36**	**-3.75**	**-2.32**
Max. Temp	R^2	0.0028	**0.13**	0.037
	P	0.722	**0.011**	0.19
	T	-0.35	**-2.67**	-1.31
RH@8h00	R^2	**0.12**	**0.19**	**0.17**
	P	**0.019**	**0.002**	**0.004**
	T	**2.43**	**3.25**	**3.03**
RH@14h00	R^2	0.047	0.0003	0.014
	P	0.13	0.89	0.42
	T	-0.18	-0.128	-0.79
Rain day before	R^2	0.0007	0.010	0.0003
	P	0.85	0.49	0.91
	T	-0.18	-0.68	-0.11
Accumulative rainfall over 2 week period	R^2	0.0001	0.010	0.0003
	P	0.94	0.49	0.90
	T	-0.069	-0.69	0.109

4. Discussion

4.1. Abundance and diversity patterns

Understanding seasonal variation in mite abundance and diversity is important in determining how and when mites utilize structures such as domatia. This knowledge is important in dealing with pest mites and trying to predict periods of pest explosions and how predatory mites which are usually associated with domatia can effectively control these (Kishimoto, 2002; Yaninek et al., 1987). This is particularly the case in commercially important plants. The results show that seasonal fluctuation in mite abundance and diversity occurs in the three South African plant species sampled (Figures 26, 27 & 28). These seasonal patterns in mite abundance differed between mite assemblages as well as between the plant species sampled. Even so, it appears that mite population increases during spring and summer months.

Our results are consistent with the results from studies by Toyoshima and Amano (2006) and Duso et al. (2010) who also showed that seasonal dynamics exist in some species of mites and that certain mites may colonize leaves at specific times throughout the seasons. It is unclear what influences seasonal patterns in mite abundance and why some mite species are found at certain times in the domatia of the different plants sampled. The study proposes that leaf domatia type and leaf age may somewhat explain these results. *T. capensis* and *R. globosa* have a hair-tuft leaf domatia, while *G. thunbergia* has a pit-type domatia with trichomes. Toyoshima and Amano (2006) suggested that the presence–absence of leaf hairs may influence mite abundance and diversity on leaves. This might be the case in these species as all the sampled plants had hairs in their domatia. Trichomes influence the presence of mites inside domatia, because they may aid in retaining moisture and high levels of humidity within domatia and thus creating favourable conditions for mites and their eggs (O'Dowd and Willson, 1991; Norton et al., 2000; Grostal and O'Dowd, 1994). Other factors that may influence mite diversity and abundance include density of mite prey, weather conditions and availability of alternative food sources such as pollen or fungi (Duso et al., 2010). Duso et al. (2004) and Toyoshima and Amano (2006) found that the seasonal fluctuations in mite abundance were related to the availability of pollen and fungi.

The mite communities found in the plants were similar throughout the sampling period, but different mites were found at different times during the sampling period. The host trees, and in particular *G. thunbergia* and *T. capense*, separated out and formed recognizable clusters based on their mite biota even though the groups were in close proximity to each other (Figure 30). This suggests that *T. capense* and *R. globosa* harboured a distinct mite biota e and that plant host specificity may be another factor to consider when looking at seasonal distribution of mites. However, the results from Chapter 3 showed that majority of the mites we sampled were not particularly host specific and thus host specificity alone cannot explain this observed patterns. Another explanation for this observation could be these plants had distinct mites because they produce different domatia types. It is more likely that this pattern was due to a combination of factors.

4.2. Abundance and diversity patterns in relation to environmental variables

The peaks in mite abundance were synchronized during some months in all three plants, particularly during some spring and summer months (Figures 26, 27 and 28). This observed pattern suggests that factors other than domatia type affect the presence of mites on domatia bearing leaves. We propose that these factors are environmental variables such as temperature, relative humidity, and rainfall. However, different patterns were observed between the plants sampled in relation to these environmental variables and the results were not consistent. For example, mite abundance was positively correlated with minimum temperature in both R. *globosa* and *G. thunbergia*, but this was not the case in *T. capensis* (Table 12). Also Shannon diversity increased with an increase in minimum temperature in both *T. capensis* and *G. thunbergia*, but decreased in *R. globosa* (Table 13). We suspect that these results were not consistence because macroclimate data was used for the correlations. Nonetheless, the results show that daily temperature, relative humidity, and rainfall affect the presence and the diversity of mites on leaf domatia on these plants (also see appendices 6 and 7).

These results support earlier results in Chapter 5 that showed that variance in temperature, and relative humidity influence the distribution of mites within the tree canopy. Moreover, in *T. capensis* mite abundance was negatively correlated with accumulative rainfall (Table 11). Rainfall is known to wash mites from leaves, leading to low mite abundance and diversity. As a result mites are known to favour the underside of leaves so as to avoid rain and ultra-violet radiation (Suzuki et al., 2009; Onzo et al., 2010). Yaninek (1987) found that generally the cassava green mite decreased during the wet seasons and that rainfall was the major cause of mite mortality during their sampling period.

Only a few studies have looked at how environmental variables affect mite abundance on leaves. Most studies that assess how these variables affect mites focus on litter or soil communities. Even so, results from these studies provide some insight on how climatic conditions may affect mite communities. Irmler (2006) found that temperature and precipitation influenced the assemblages of mites found on soils. Similarly, Stamou and Sgardelis (1989) found temperature to be the main variable that controls seasonal patterns in Oribatid mite populations found on forest litter. Huhta and Hanninen (2001) observed that mites react differently to environmental conditions and that some species were more sensitive to temperature, while others are affected by moisture. Uvarov (2003) found that different temperature regimes affected the population dynamics of mites and a trade-off existed between reproduction and adult mite survival.

5. Implications and recommendations

This study is the first to demonstrate seasonal patterns in mite diversity and abundance in African plant species. The patterns observed provide us with some insight into the complex interaction between plants and mites. The implications are summarised below.

- Firstly, the results are consistent with those of other studies which show that domatia are important features as they determine mite communities found on plants and promote high abundances of predatory mites. For this reason, a number of studies (Parolin et al., 2012; 2014b; 2015; Avery et al., 2014; Loughner et al., 2010) suggest the use of plants with domatia as banker plants in agricultural systems to promote healthy mite communities and in the management of pest mites.
- These results emphasize the need to understand how environmental factors such as temperature and rainfall affects mites communities, and can be important when predicting periods of pest mite explosions in economically important crops such as grape and coffee that bear leaf domatia.
- These results suggest that changes in weather patterns may affect the abundance of mites on plants and this needs to be considered when trying to find solutions for pest mites.
- Our results have implications for sampling strategies to be employed when surveying plants for their mite biota. From these results, repeated sampling periods are required to ensure that as many species of mite as possible are collected.
- Lastly, our results emphasize the need for a greater understanding of the natural interactions between mites and their host plants if we are to successfully utilize them in biocontrol systems.

In conclusion it is important to note that more work is needed if we are to get a better understating of how mite communities found inside domatia change over the seasons as this subject is poorly studied. During this study we encountered some challenges and we suggest some recommendations for future studies. Firstly, we suspect that maybe the two-week sampling approach was not suitable for the study and this was too-long a period in-between sampling times. For future studies we suggest that sampling every week would be more appropriate and would allow one to get a clearer picture of the seasonal patterns. Also we could only sample a single individual for the selected study species as mite counting and identification is time consuming. It would have been better to have replicates and we suggest future studies sample at least three individuals per plant species. This will allow one to determine if the observed patterns are plant specific, species specific or general. We also suggest that future studies utilise iButtons to monitor climatic variables to get more reliable environmental data. Another interesting idea would be to couple this study with laboratory experiments where mite communities are monitored under controlled conditions. This would allow the comparison between laboratory and field experiments.

Chapter 7: An assessment of mite host specificity and diversity on *Coffea arabica* L.

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1. Introduction

The commercial coffee plant, *Coffea arabica* L. (Rubiaceae), originates from Ethiopia. Today it is cultivated worldwide in more than 50 countries (Vega et al., 2003; 2007). Leaves of this crop species bear pit-type domatia (Figure 30) and coffee is one of many economically important species that have been shown to benefit from the mutualism with mites (O'Dowd, 1994; Agrawel., 1997; Norton et al., 2000; Onzo et al., 2003; Vega et al. 2009; Oliveira et al., 2014). Other economically important plants known to be associated with mites include grapes, avocado, sweet pepper and cassava (Agrawel, 1997; English-Loeb et al., 1999; Onzo et al., 2003; Ferraira et al., 2008). This mutualism is important in coffee, because it may provide some relief from one of the most important diseases of coffee plants, namely the coffee leaf rust fungus (*Hemilleia vastatrix* Berk. & Broome) and also from phytophagous mites (Oliveira et al., 2014).

Coffee is commonly associated with and attacked by the coffee red mite, *Oligonichus ilicis* (McGragor), which is a common pest in many countries that produce coffee (O'Dowd, 1994; Fahl et al., 2007; Romero et al., 2011; Oliveira et al., 2014). Even so, coffee leaves have been shown to harbour more beneficial mites than pest mites (Pemberton and Turner, 1989; Matos et al., 2006; Mineiro et al., 2008). In north Queensland, the majority of the mites found inside the domatia in coffee were primarily predatory from the families Stigmaeidae, Phytoseiidae, and Bdellidae (O'Dowd, 1994). In Brazil, coffee is associated with the predatory mite *Amblyseius herbicolus* (Chant), and this mite controls the pest mites *Brevipalpus phoenicis* (Geijskes) and *Oligonychus ilicis*. Also, Mineiro et al. (2008) found the predatory mites *Euseius citrifolius* (Denmark & Muma) and *Euseius concordis* (Chant) to be the most frequent species in a coffee plantation in the State of São Paulo, Brazil.

The aim of this chapter was to highlight the possible economic importance of the plant-mite mutualism using coffee as a study plant. This was done by addressing the following questions:

a) Do coffee plantations have a different suite of mites when compared to an adjacent forest patch?

b) Does the plantation support higher diversity of mites compared to the adjacent forest site sampled?

c) Do coffee plants have the same trophic guilds of mites as the plants in the indigenous forest?

The study will give us insight in the context of mite migration and selection of an exotic host by indigenous mites. Based on the literature and from the results in the chapters presented above, it is hypothesised that the coffee plantations will harbour beneficial mites and primarily predatory species that are similar to those found in the adjacent forest.

Figure 31: *A photograph of the coffee plant and the leaf. Domatia (circled in red) are visible on the upper surface of the leaf.*

2. Methods

Beaver Creek Coffee Plantation in Port Edward, KwaZulu-Natal Province, South Africa was selected as the study site, because of its close proximity to the indigenous Umtamvuna Forest. Four transects were laid out across the plantation; two transects in the middle of the plantation and two at the edge of the plantation. Both the edge of the plantation closest to the forest as well as in the middle of the plantation were sampled to test whether there would be more or different mites at the edge of the plantation compared to the middle. 20 leaves were sampled from each of the 10 individual trees across the transect line. In addition, forest walks in the neighbouring

Umtanvuna Forest were undertaken and plant species found to possess leaf domatia were sampled. The leaves were viewed under a dissecting microscope and mites found were collected, counted and then identified. Mite species abundance (average number of mites per leaf), composition and the Shannon diversity index for both sites were determined.

A list of all mite species collected from the two sites was compiled and these data were used to perform a resemblance analysis (normalised D3 Chord distance) in the PRIMER software. From this analysis a dendrogram plot was produced using the "group average" linkage to identify relationships and similarities in mite biota between the sites and host tree species sampled. This analysis was conducted on the mite species data (presence - absence data) where each sampling unit was one plant. In each plant 10 leaves were sampled.

3. Results

3.1. Does the coffee plantation have a different suite of mites?

Twenty seven different plant species were sampled from Umtanvuna forest. The results from the assessment of host specificity in *Coffea arabica* showed that this plant is associated with more than one species of mite (Table 14). The samples from the coffee plantation formed two groups in the multivariate analysis. Samples from the edge of the coffee plantation clustered together and were mostly similar to *Olea capensis* and *Macaranga capensis*. The two samples from the middle of the coffee plantation were incorporated in another major cluster along with other specimens from the natural forest (Figure 31). Table 14 gives a list of the entire mite species collected from coffee plants and from Umtanvuna Forest.

3.2. Does the plantation support a higher diversity of mites?

In total, seven different species of mites were collected from leaves of coffee and 20 mite species were found on the forest samples (Figure 32). The adjacent forest had a higher Shannon diversity index than the coffee plantation (Figure 33) and when comparing the two sample sites within the coffee plantation, leaves collected in the middle of the plantation had more mites and a higher diversity index compared to samples collected at the edge (Figure 32 and 33). Table 4 shows that some mite species found in the coffee plantations were also found in the adjacent forest. Two mite species (*Muniederia centrata* and *Ueckernmannseus* sp1*)* were uniquely found in the coffee plantation and on the other hand, 15 mite species were found only in the adjacent forest.

Table 14: *A list of the mite species collected from the coffee plantations and the neighbouring Umthanvuna Forest.*

***Coffea arabica* plantation**	**Umtanvuna Forest**
Stigmaeidae	Tetranychidae
Agestimus sp. (probably new)	Oligonychus sp.(probably new)
Muniederia centrata (Meyer)	*Tetranychus* sp.
Cunaxidae	*Tetranychus* nymph
Bunaxella guini (Heyer &Castro)	Cunaxidae
Phytoseiidae	*Bunaxella guini* (Heyer & Castro)
Euseus addoensis (van der Merwe & Ryke)	*Rubroscirus* sp.
Ueckernmannseus sp1. (Ueckermann)	Phytoseiidae
Typhlodromus crassus (van der Merwe)	*Amblyseius anomalus* (van der Merwe)
Phytoseius munteriensis (van der Merwe)	*Euseius addoensis* (van der Merwe &Ryke)
	Ueckermannseus sp2. (Uekermann)
	Phytoseius munteriensis (van der Merwe)
	Typhlodromus canthoseius (van der Merwe)
	Typhlodromus crassus (van der Merwe)
	Eriophyidae
	Eriophydae sp.
	Eupodidae
	Eupodes sp.
	Oribatidae
	Oribatei sp.
	Cheyletidae
	Prosochyla hepburnii (Lawrence)
	Stigmaeidae
	Agistemus tranatalensis (Meyer)
	Tenuipalpidae
	*Brevipalpus sp. (*probably new)
	Tydeidae
	Tydeus munsteri (Meyer & Ryke)
	Triophtydeidae
	Tetratriophtydeus myacanthus (Ueckermann)
	Winterschmidtidae
	Saproglyphus sp.

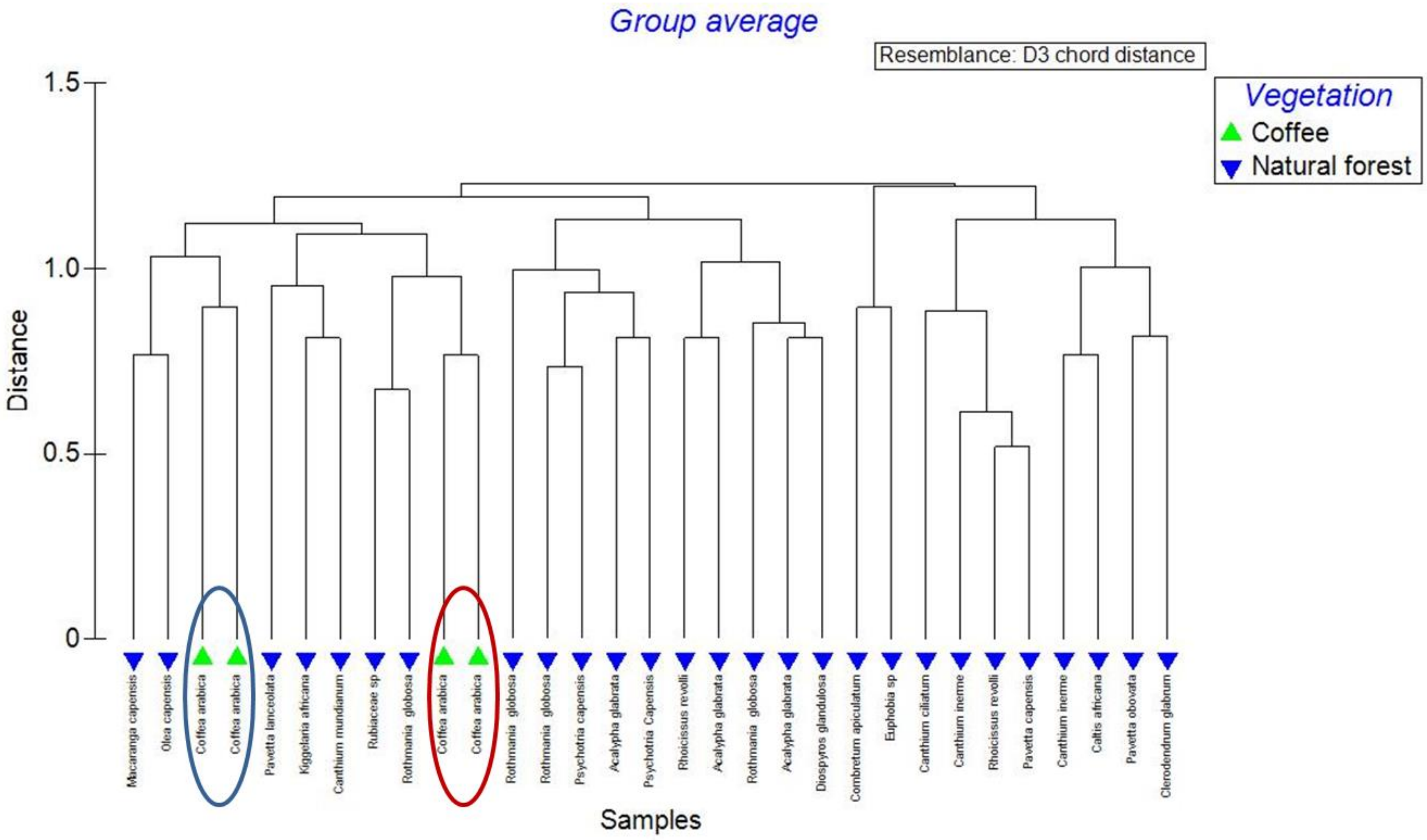

Figure 32: *A dendrogram showing similarities between coffee sample sites and the different plant species sampled from the adjacent indigenous forest. The multivariate analysis was based on mite species encountered on the leaves of the different individual plants sampled. Coffee Samples from the edge are circled in blue and samples from the middle of the plantation are the ones in the red circle.*

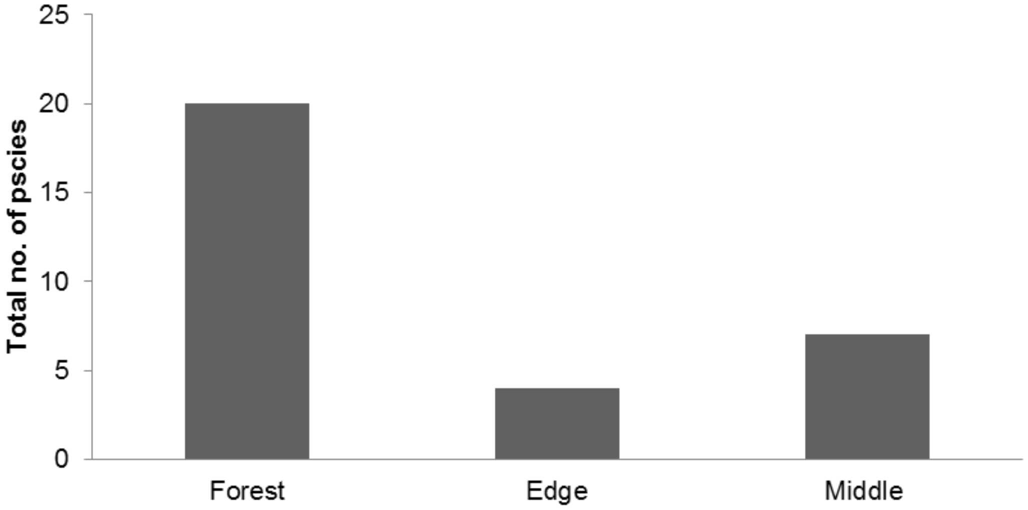

Figure 33: *Total number of mite species collected from the Umtanvuna Forest and two sites in the Coffea arabica plantation.*

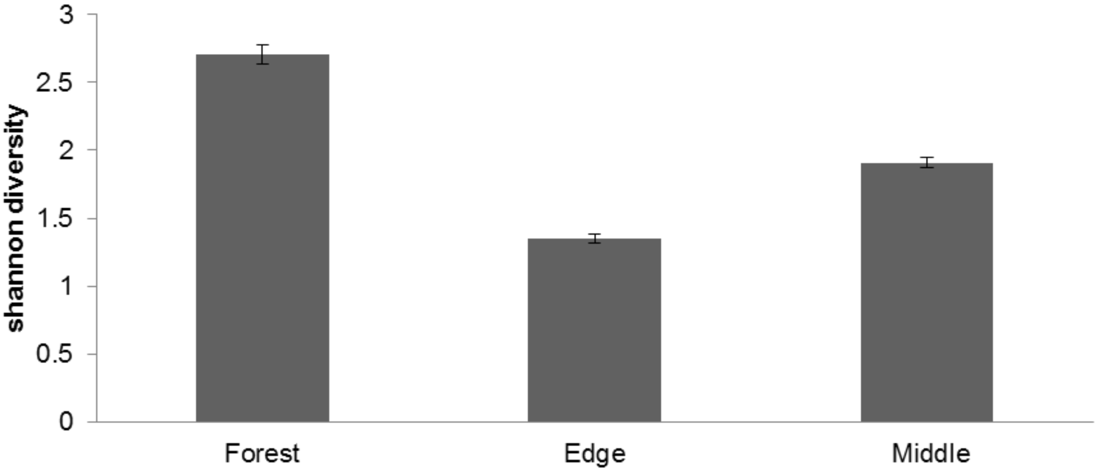

Figure 34: *Shannon diversity index of the two sites in the Coffea arabica plantation and neighbouring Untanvuna forest.*

4. Discussion

4.1. Does the plantation support a higher diversity of mites?

Coffee arabica had more than one mite species occupying its domatia (Table 14). However, the coffee plantation had low mite species diversity (Figure 33) when compared to the neighbouring indigenous forest. The mites collected from the coffee plants belonged to the families Phytoseiidae, Stigmaeidae and Cunacidae and these are commonly found in leaf domatia all over the world. These are all predatory mites and mites from these families have previously been shown to be associated with coffee plantations (O'Dowd, 1994; Mineiro et al., 2008; Vega et al., 2009). Matos et al. (2006) found that coffee domatia had a positive effect on the abundance of predatory mites and plants with higher densities of domatia harbour more predatory mite and fewer prey mites. These results further support the hypothesis that domatia attract beneficial mites that act as the plants bodyguards and highlight the importance of this mutualism in economically important species. A similar study investigating the relationship between domatia availability and foliar mite assemblage in native forest, plantation forest, and pasture by O'Connell et al. (2010a) found that native forests supported higher numbers of mite species than either plantation forest or pastoral grasses.

This beneficial mutualism has been shown in other commercial plants including grapes, cassava and even in cotton (Agrawal et al., 2000; English-Loeb et al., 2002; Onzo et al., 2003). Avocado, (*Persea americana* Mill.) plant leaves with domatia were found to be associated with more predatory mites and fewer herbivorous mites than those without domatia. Onzo et al. (2003) showed that predatory mites help protect cassava plants by reducing herbivorous mites on young leaves that are the most photosynthetically active and force herbivorous mite to move down the plant to less profitable older leaves. Norton et al. (2000) found high densities of the beneficial mites *Orthotydeus lambi* (Baker) on grape plants with intact domatia than on plants with blocked domatia. English-Loeb et al. (1999) and Melidossian et al. (2005) have shown that tydeid mite suppresses powdery mildew on the fruit and foliage of *Vitis vinifera* (L.) and that the mite could be an important bio-control agent for the grape powdery mildew which is a hazardous pathogen of cultivated and wild grapes. Furthermore, grape vines with bigger domatia are resilient to pathogenic fungi, because they support larger communities of beneficial mites (English-Loeb et al., 2002).

In our study the mites found in association with coffee were all predatory. However, other studies examining the occupants of coffee domatia also observed phytophagous mite such as *Oligonichus ilicis* and *Brevipalpus phoenicis* which cause damage to the leaves (Oliveira et al., 2014; Matos et al., 2005; Chagas et al., 2003). The study did not find any of these mites in the study site. Some potentially harmful mites were found in Umtanvuna Forest, but these did not seem to have migrated to the plantation. Coffee plants appeared to be healthy and free from damage or pathogens (see Figure 30). From this we speculate that predatory mites found in the plantation were successful at keeping the plants healthy.

4.2. Does the coffee plantation have a different suite of mites?

When compared to the adjacent forest patch coffee plants formed two distinct clusters; one with samples collected at the edge of the plantation and the other cluster with samples from the middle of the plantation (Figure 31). Two mite species (*Muniederia centrata* and *Ueckernmannseus* sp1) were uniquely found in the coffee plantation and five species (*Agestimus sp.* (probably new) *Bunaxella guini, Euseus addoensis, Typhlodromus crassus, Phytoseius munteriensis*) were found in both the adjacent forest and in the coffee plantations (Table 4). These results suggest that to some extent certain mites had preference for coffee plants over the indigenous species found in the adjacent forest patch. Some of these mites (Stigmaeidae and Phytoseiidae) found on coffee plants are indigenous to South Africa suggesting that this exotic plant is not associated with its own mite species from another country. Rather, mites are able to migrate and establish in exotic host species and that this plant can still benefit from the mutualism.

Interestingly, plants at the edge of the coffee plantation did not cluster together with those from the middle of the plantation and they had fewer mites and a low Shannon diversity compared to plants in the middle of the plantation. This suggests that mites found on plants at the edge may be subjected to negative edge effects. An edge effect results when both biotic and abiotic conditions change along the boundary between two habitats and consequently affecting the distribution, biodiversity and ecosystem functionality of species in both habitats (Murcia 1995).

A negative edge effect results when species found the edge of the habitat patch are exposed to an increased risk of parasitism, disease, increased predation, adverse microclimate conditions, and increased competition from invasive species (Harper et al., 2005). We suspect that this was also the case in this study and that this was due to the fact that the mites at the edge of the plantation would have been exposed to harsher environmental conditions such as strong winds, increased exposure to rainfall and sunlight as well as lower relative humidity than in the middle of the plantation. Mites are delicate and are susceptible to these extreme conditions and thus avoid such habitats. Many species have been shown to suffer from edge effects (Murcia 1995; Ries and Sisk, 2004; Harper et al., 2005; Tian et al., 2011). For example, a study on ground-dwelling arthropods showed that grassland species of spiders, centipedes and ground beetles were affected by the edge and this influence was evident up to 15m from the habitat edge (Lacasella et al., 2015). To the best of our knowledge this study is the first to show reduced mite diversity due to edge effect.

5. Conclusion

The aim of the study was to demonstrate the importance of the mutualism between leaf domatia and mites in commercial plants such as coffee. The results showed that the coffee plantations at Beaver Creek were associated with only predatory mites, some of which were indigenous to South Africa. This suggests that the plantations are able to be successfully colonised by indigenous beneficial mites. These results highlight the importance of this mutualism in commercial plants. Moreover, it provides some evidence that indigenous mites are able to colonise and establish the beneficial mutualism on exotic species as shown in other studies. This is important as it implies that even in a foreign country; mites may be effective at controlling pests as an alternative to chemical control.

Chapter 8: Synthesis and Opportunities for Future Research

Since Lundström's (1887) hypothesis, in which he suggested that plants with leaf domatia are involved in a protective mutualism with mites and that leaf domatia provided mites with shelter, while mites, in turn, decreased the amount of herbivore and pathogen loads on leaf surfaces, more studies on this subject have been conducted. Overwhelming evidence suggests that leaf domatia are primarily used by mites as a place of refuge. The findings of this study provide us with great insights into the ecology of mites associated with leaf domatia in South Africa and can be summarised as follows:

- Leaf domatia bearing plants are very common in the forest and the thicket biomes in South Africa and were found all over the country. They were also observed in plant species from the Combretaceae, which is common in the savanna biome (see Table 1 in Chapter 1). Plants from nine families were associated with 62 different mite species belonging to 15 different families. Also, over the duration of the project, 15 potentially new mite species were collected, which will be described in a peer-reviewed journal article. These results contribute immensely to our knowledge of the diversity of South African plants that produce leaf domatia. These results also give us a better understanding of the biological diversity of mites that inhabit these plants. The data collected here is particularly important as the region has been understudied and highlights the lack of knowledge of our South African mite biota.

- The anatomical study provided useful insights into the structure of leaf domatia. Examination of six species with various types of leaf domatia suggested that the key features which distinguish domatia are the presence of an extra layer of tissue in the lower epidermis, a thick cuticle, cuticular folds, presence of trichomes, and an invagination (Chapter 2). These results are in line with those obtained by previous studies (Tilney et al., 2012; Nishida et al., 2006). Additional ontogenetic studies would augment these observations and help explain how domatia are formed by plants.

- An examination of ten South African plant species showed that the association between leaf domatia and mites is opportunistic and that mites have no preference for any particular domatia type. Furthermore, mites are generally not host specific (Chapter 3) even through some species of mites may sometime be associated with a particular plant. The results were consistent with results from other studies which showed that the majority of mites found inside domatia are predatory mites. The presence of predatory mites within leaf domatia can improve the overall fitness of plants and this has been shown in numerous studies.

- It is not clear whether different vegetation types support a distinct suite of mites. The study assessing the distribution of mites across vegetation types showed that the different vegetation types and sites visited did not differ markedly in terms of their mite biota. Nevertheless, these results provide new insights into mite distribution and abundance in South Africa's vegetation, which can help us better understand the plant-mite association from an African perspective (Chapter 4).

- Furthermore, this study reveals that mites may respond to intra-canopy microclimate and preferred to reside in the lower areas and inside the tree canopy, avoiding exposed areas in an attempt to minimise exposure to hash environmental conditions (Chapter 5).

- The seasonality study (Chapter 6), as shown in other studies conducted in other countries, suggests that mite communities found in association with domatia change as the year progresses and over the seasons. These fluctuations in abundance and diversity of mites vary between plant species. The results of this study suggest that more mites are found during periods when temperatures are warm and relative humidity is high. On the other hand, rainfall, low temperatures and low relative humidity were found to have a negative impact on mite abundance and diversity.

- Finally, the results from Chapter 7 showed that mites are able to colonise and establish the beneficial mutualism on exotic species. This is important as it ascertains that economically important plants that are cultivated in foreign countries can still benefit from this mutualism.

1. Implications for future studies

This study is the first of its kind to study and document a range of aspects of the leaf domatia-mite mutualism in South Africa. However, during this study we encountered some challenges and the results presented here are preliminary, more work still needs to be done.

1. More effort needs to be directed towards formulating studies that look at periodic changes associated with mites found in leaf domatia. Seasonality studies are important because fungal flora present on leaves changes seasonally and may affect the types of mites present on leaves at a particular time. These types of studies are also important particularly when dealing with pest control of spider mites and controlling pathogenic diseases in both our natural vegetation and commercial species. Our results suggest that changes in weather patterns affect abundance of beneficial mites on plants and that needs to be considered when trying to find solutions for pest mites.

2. Our results are consistent with results from other studies that showed that the majority of mites found inside domatia are predatory mites. These results also indicate that African plants with domatia may play an important role in controlling pest mites if they coexist with other plants of economic importance. This is because leaf domatia facilitate and promote predator-prey relationships in mite communities found on plants. The presence of predatory mites within leaf domatia can improve the overall fitness of plant and that has stimulated interest in bio-control research. Leaf domatia are heritable traits and thus can be artificially selected and genetically engineered to enhance abundance of predatory mites and improve the efficiency of bio-control of plant pests. This is an important subject and we encourage future studies on this.

3. The results presented here are some of the first to document mite distribution within the canopy of domatia bearing tree species, and to correlate diversity and abundance to environmental variables. Thus based on these results, we suggest that future studies on mites and leaf domatia should employ a consistent sampling approach, and avoid the sampling areas of the canopy which are simply easily accessible or exposed to harsh environmental conditions.

In conclusion, this work which emanates from South Africa is very important as it presents a series of studies that make a meaningful contribution to our understating of the plant-mite relationships and fills the geographical gap in the global knowledge of mite diversity. The study has provided valuable data and insights on the distribution and diversity of South Africa's mite biota and mite ecology and forms the basis from which new studies can emerge to further document and test this mutualism in Africa. We encourage future studies to undertake intense collection initiatives to get a better understating of the diversity of mites South Africa.

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Appendices

Appendix 1: A List of all the mites species collected during the course of the study from the different sites visited.

Mite Species	**Feeding guild**
Acaridae	
Nymph	Stored product mites
Tyrophagus plutrescentiae (Schrank)	Fungivorous
Anystidae	
Anystis baccarum (Linnaeus)	Predacious
Ascidae	
Asca sp.	Stored product mites
Bdellidae	
Unknown sp.	Predacious
Cheyletidae	
Cheletomimus wellsi (Baker)	Predacious
Prosocheyla hepburni (Lawrence)	Predacious
Cunaxidae	
Nymph	Stored product mites
Bunaxella quini (Heyer &Castro)	Predacious
Bunaxella zebedielensis (Den Heyer)	Predacious
Nymph *(Rubroscirus)*	Predacious
Unknown sp.	Predacious

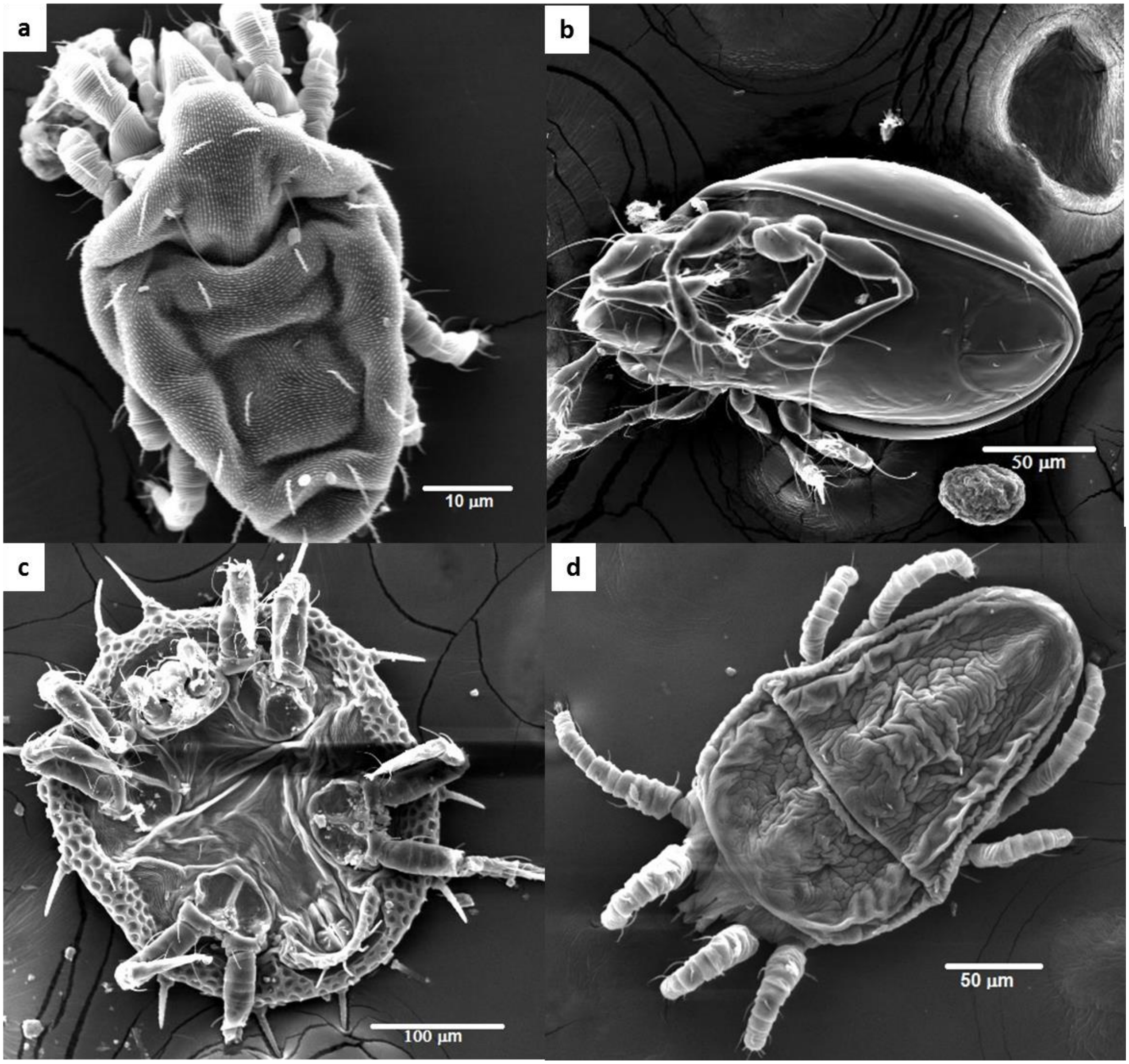
Eriophyidae	
Aculus comartus (Nalepa)	Plant feeding
Unknown sp.	Plant feeding
Erythraeidae	
Unknown sp.	Predacious
Eupalopsellidae	
Eupalopsellus brevipilus (Meyer & Ryke)	Predacious
Eupodidae	
Eupodia sp.	Algiphagous
Hemileiidae	
Siculobata juvenile	Probably Mycophagous/ Saprophytic
Siculobata sicula (Berlese)	Probably Mycophagous/ Saprophytic
Iolinidae	
Lourus citricolus (Ueckermann & Grout)	Predacious
Pronematus ubiquitus (McGregor)	Predacious
Lamellareidae	
Cultroribella sp.	Unknown
Micreremidae	
Juveniles *(Micreremus)*	Mycophagous
Micreremus sp.	Mycophagous
Oribatidae	Mycophagous/ Saprophytic
Oribatei sp. (probably new)	Mycophagous/ Saprophytic
Oribatula tibialis (Nicolet)	

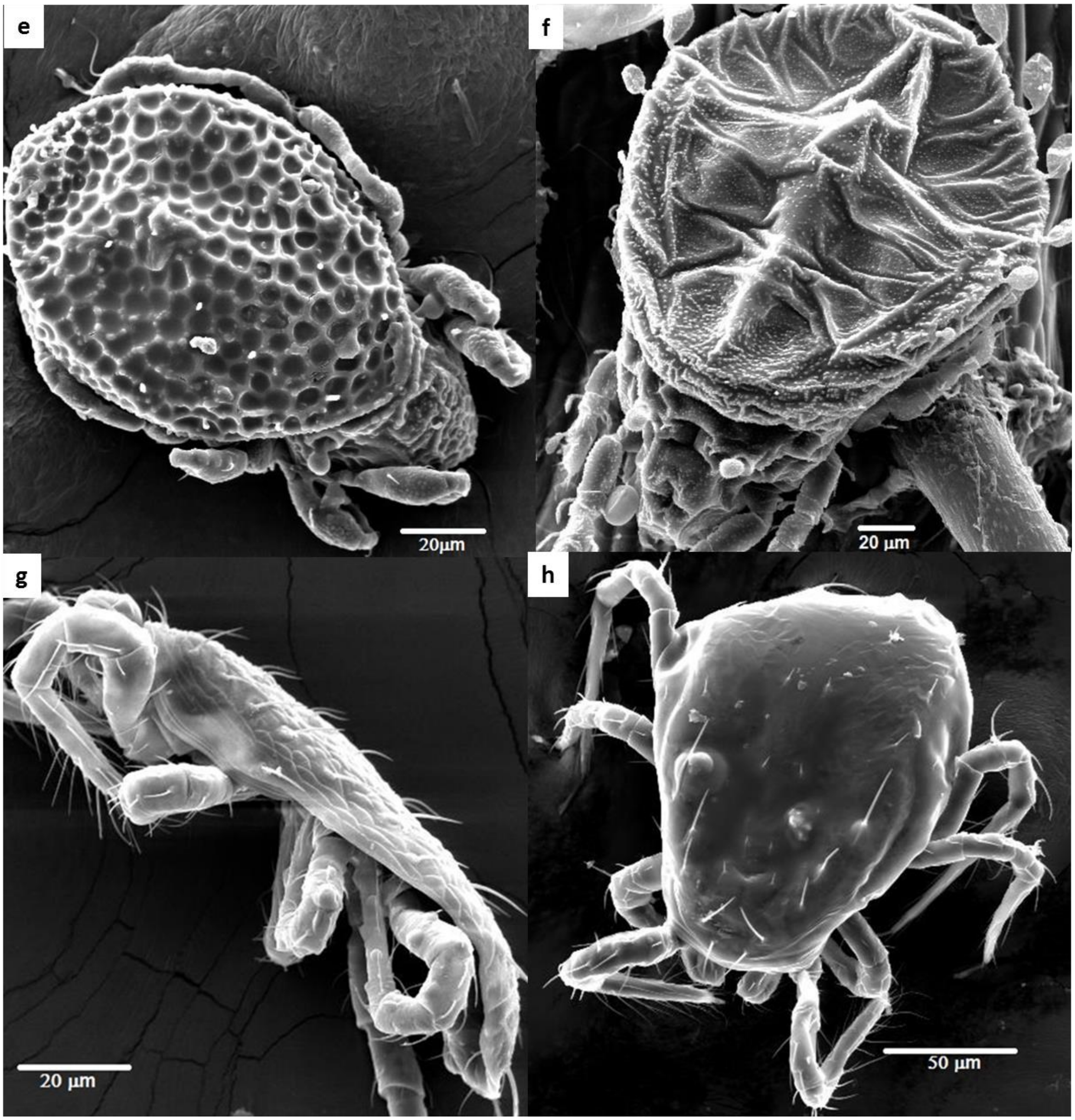
Phytoseiidae	
Amblyseius anomalus (van der Merwe)	Predacious
Amblyseius herbicolus (van der Merwe)	Predacious
*Amblyseius neokaratrae (*Ueckermann &Loots)	Predacious
Amblyseius sp.	Predacious
Euseius addoensis (van der Merwe)	Predacious
Euseius arbicorlus(van der Merwe)	Predacious
E*useius rhusi* (van der Merwe)	Predacious
Euseius sp. (probably new)	Predacious
Neo*seius natalensis*(van der Merwe)	Predacious
Phytoseius munteriensis (van der Merwe)	Predacious
Typhlodromus apoxys (van der Merwe)	predacious
Typhlodromus canthoseius (van der Merwe)	predacious
Typhlodromus crasus (van der Merwe)	Predacious
Typhlodromus incivivus (van der Merwe)	Predacious
Typhlodromus microbullatus (van der Merwe)	Predacious
Nymph (*Typhlodromus*)	Stored product mites
Typhlodromus praeacutus (van der Merwe)	Predacious
Typhlodromus sp1.	Predacious
Typhlodromus sp2.	Predacious
Typhlodromus sp3. (probably new)	Predacious
Typhlodromus vescus (van der Merwe)	Predacious
Ueckermanneus sp1. (Ueckermann & Grout)	Predacious
Ueckermanneus sp2. (Ueckermann &Grout)	Predacious

Stigmaeidae	
Agistemus sp1. *(*probably new)	Predacious
Agistemus sp2. (probably new)	Predacious
Agistemus tranatalensis (Meyer)	Predacious
Eryngiopus bibens (Meyer)	Predacious
Eryngiopus sp. (probably new)	Predacious
Mullederia centrata (Meyer)	Predacious
Mullederia sp. (probably new)	Predacious
Tarsonemidae	
Fungitarsonemus sp1. (probably new)	Fungivorous/algivorous
Fungitarsonemus sp2. (probably new)	Fungivorous/algivorous
Tarsonemidae	
Tarsonemus sp.	Plant feeding
Prostigmata sp.	Plant feeding
Tenuipalpidae	
Brevipalpus Phoenicis (Donnadieu)	Plant feeding
Brevipalpus sp1. (probably new)	Plant feeding
Cenopalpus sp2. (probably new)	Plant feeding
Unknown (Probably new)	Plant feeding
Tetranychidae	
Nymph	Stored product mites
Oligonychus sp1.	Plant feeding
Oligonychus sp2.(probably new)	Plant feeding
Tetranychus sp1. (probably new)	Plant feeding
Tetranychus sp2. (probably new)	Plant feeding
Unknown (probably new)	Plant feeding

Triophtydeidae	
Tetratriophtydeus immanis (Ueckermann)	Predacious
Tetratriophtydeus myacanthus (Ueckermann)	Predacious
Tydeidae	
Brachytydeus sp1. (probably new)	Predacious
Brachytydeus sp2. (probably new)	Predacious
Brachytydeus sp3.	Predacious
Orfareptydeus stephani (Ueckermann & Grout)	Predacious
Tydeus Africanus (Baker)	Predacious
Tydeus grabouwi (Meyer & Ryke)	Predacious
Tydeus munsteri (Meyer & Ryke)	Predacious
Tydeus sp1.	Predacious
Tydeus sp2. (probably new)	Predacious
Tydeus sp3. (probably new)	Predacious
Wintorschmidtidae	
Agastimus sp.	Fungivorous
Unknown sp.	Fungivorous

Appendix 2: Scanning Electron Microscope images of some species of mites observed inside leaf domatia of different plant species. (A) Tydiedae (*Tydeus* sp.), (B) Oribatei, (C) Stigmaeidae (*Mullederia* sp.), (D) Tenuipalpidae (*Tenuipulpus* sp.), (E) Unknown (possibly *Oribatei*) , (F) Unknown (possibly *Caleremaeus* sp.), (G) Phytoseiidae**,** (H) Phytoseiidae (*Typhlodromus* sp.), (I) Unknown and (J) Wintorschmidtidae (*Saproglyphus* sp.).

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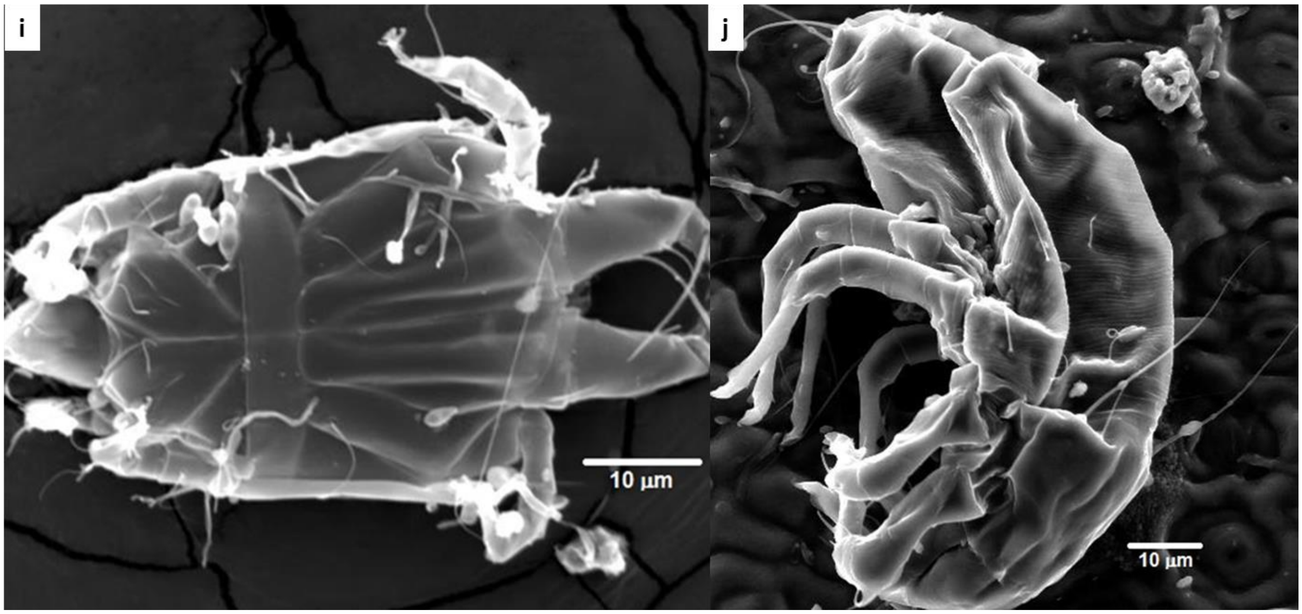
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Appendix 3: A list of plant species collected from the different sites and vegetation types sampled.

Plant species	Veg type (Coastal Thicket)			Veg type (Great Fish Thicket)	Veg type (Kowie Thicket)		Veg type (Northern Coastal Forest)			
	Site1 (Tolomnga)	Site2 (Gunubie)	Site3 (Kwelerha)	Site4 (Coombs Farm)	Site5 (Albany State Forest)	Site6 (Grahamstow n)	Site7 (Ntafufu)	Site8 (Pietermaritz burg)	Site9 (Umgazana)	Site10 (Durban)
Acalypha glabrata										
Allophyllus dregeanus										
Allophyllus natalensis										
Allophylus decipiens										
Antidesma venosum										
Apodytes dimidiata										
Berchellia sp.										
Burchellia bubalina										
Calodendun capense										
Caltis africana										
Canthium ciliatum										1
Canthium inerme	1		1	1	1	1				1
Canthium mundianum					1				1	1
Canthium spinosum										
Canthium vanwykii										
Chinanthus floveolatus										
Chionanthus peglerae										
Chionanthus sp.										
Cinnamomum camphora										
Clerodendrum glabrum										
Cloristalys rhomnoides										
Coddia rudis					1					1
Combretum apiculatum										
Combretum kraussii										
Combretum sp.				1						
Coprosma lucida						1				
Diospyros glandulosa	1									

Dlueggea verrucosa										
dovyalis longispina					1					
Dovyalis sp.										
Ehretia rigida	1		1	1	1				1	
Erythrococca natalensis					1					
Euphobia sp	1									
Excocaria										
Ficus comnata										
Ficus sp										
Gardenia thunbergia		1				1	1			
Grewia occidentalis	1	1	1		1				1	1
Halleria lucida										
Hatarophyxis sp										
Hyperacanthus amoenus										
Jasminum angulare				1	1					
Jasminum fluminerse										
Keetia gueinzii										
Kiggelaria africana							1			
Macaranga capensis										
Mackeya bella										
Maesa lanceolata							1			
Ocotea bullata								1		
Olea capensis										
Olea faveolata	1		1							
Olea woodiana										
Oxyanthus latifolius							1			
Oxyanthus speciosus										
Pavetta capensis										
Pavetta cf. eylesii										
Pavetta gardeniifolia							1			
Pavetta lanceolata										1
Pavetta revoluta		1								
Pavetta kotzei										
Plectriniella armata										
Psychotria capensis				1	1				1	1
Psydrax livida										
Psydrax Obuvata	1						1		1	
Rhoicissus digitata	1		1	1					1	
Rhoicissus revolli									1	
Rhoicissus	1	1	1			1	1		1	

rhomboidea										
Rhoicissus tamentosa							1		1	
Rhumnus prinoides										
Rhumnus sp					1					
Rinorea angustifolia										
Rothmania globosa						1				
Rothmannia capensis							1			
Rothmannia sp										
Rubiaceae sp										
Syzygium peglerae										
Tecoma capensis					1	1				
Tricalysia capensis										
Tricalysia delagoensis										
Tricalysia lanceolota		1								
Trichocladus sp										
Vitex sp										

	Veg type (Northern Mistbelt Forest)				**Veg type (Scarp Forest)**		**Veg type (Southern Afromontane Forest)**				
Species (Leeaf domatia type)	Site11 (Buffelskloof)	Site12 (Lekggalametse)	Site13 (Heanertsburg)	Site14 (Venda)	Site15 (Oribi Gorge)	Site16 (Port Edward)	Site17 (Nature's Valley)	Site18 (Knysna)	Site19 (Stellenbosh)	Site20 (Bedfort)	Site21 (Kambi)
Acalypha glabrata		1				1					
Allophyllus dregeanus											
Allophyllus natalensis							1				
Allophylus decipiens											
Antidesma venosum											
Apodytes dimidiata											
Berchellia sp.											
Burchellia bubalina								1			
Calodendun capense											
Caltis africana						1					
Canthium ciliatum		1		1	1		1			1	
Canthium inerme	1					1				1	
Canthium mundianum		1		1		1					
Canthium spinosum										1	
Canthium vanwykii						1					
Chinanthus floveolatus						1					
Chionanthus peglerae			1								
Chionanthus sp.							1				
Cinnamomum camphora											
Clerodendrum glabrum						1					
Cloristalys rhomnoides	1										
Coddia rudis											

Combretum apiculatum						1					
Combretum kraussii	1										
Combretum sp.											
Coprosma lucida									1		
Diospyros glandulosa						1					
Dlueggea verrucosa											
dovyalis longispina											
Dovyalis sp.											
Ehretia rigida				1					1		
Erythrococca natalensis											
Euphobia sp					1	1					
Excocaria											
Ficus comnata											
Ficus sp	1										
Gardenia thunbergia					1						1
Grewia occidentalis					1					1	1
Halleria lucida											
Hatarophyxis sp	1	1									
Hyperacanthus amoenus											
Jasminum angulare											
Jasminum fluminerse											
Keetia gueinzii		1	1								
Kiggelaria africana	1				1		1		1		
Macaranga capensis						1					
Mackeya bella		1	1								
Maesa lanceolata	1	1									
Ocotea bullata							1	1			
Olea capensis	1					1	1			1	1
Olea faveolata											
Olea woodiana							1				

Oxyanthus latifolius											
Oxyanthus speciosus		1									
Pavetta capensis											
Pavetta cf. eylesii				1							
Pavetta gardeniifolia											
Pavetta lanceolata						1					
Pavetta revoluta											
Pavetta kotzei											
Plectriniella armata											
Psychotria capensis	1				1	1					
Psydrax livida			1								
Psydrax Obuvata						1					
Rhoicissus digitata							1				
Rhoicissus revolli	1					1	1				
Rhoicissus rhomboidea			1		1		1				
Rhoicissus tamentosa	1	1	1								
Rhumnus prinoides	1						1				
Rhumnus sp		1									
Rinorea angustifolia			1								
Rothmania globosa						1					
Rothmannia capensis		1									
Rothmannia sp							1				
Rubiaceae sp						1					
Syzygium peglerae											
Tecoma capensis	1			1			1		1		
Tricalysia capensis		1	1								
Tricalysia delagoensis											
Tricalysia lanceolota											
Trichocladus sp			1								

Vitex sp	1										

Species (Leeaf domatia type)	**Veg type (Southern Coastal Forest)**	**Veg type (Southern Mistbelt Forest)**										
	Site22 (Alexandia Forest)	Site23 (Tsitsa Falls)	Site24 (Beggars Bush)	Site25 (Fort fordyce)	Site26 (Langeni Forest)	Site27 (Gcobo)	Site28 (Baziya)	Site29 (Mouth Frere)	Site30 (Maidan Dam)	Site31 (Pirie Forest)	Site32 (Somerset East)	Site33 (Hogsback)
Acalypha glabrata												
Allophyllus dregeanus		1										
Allophyllus natalensis		1										
Allophylus decipiens	1											
Antidesma venosum												
Apodytes dimidiata				1								
Berchellia sp.												1
Burchellia bubalina			1							1		
Calodendun capense												
Caltis africana	1									1		
Canthium ciliatum	1	1		1				1		1		1
Canthium inerme	1			1					1	1		
Canthium mundianum	1			1						1		
Canthium spinosum			1			1						
Canthium vanwykii												
Chinanthus floveolatus				1								
Chionanthus peglerae												
Chionanthus sp.												
Cinnamomum camphora												
Clerodendrum glabrum												
Cloristalys rhomnoides												
Coddia rudis				1								
Combretum												

apiculatum												
Combretum kraussii												
Combretum sp.		1										
Coprosma lucida												
Diospyros glandulosa												
Dlueggea verrucosa												
dovyalis longispina												
Dovyalis sp.												
Ehretia rigida	1								1			
Erythrococca natalensis												
Euphobia sp												
Excocaria												
Ficus comnata												
Ficus sp												
Gardenia thunbergia	1									1		
Grewia occidentalis	1	1								1	1	1
Halleria lucida						1						
Hatarophyxis sp												
Hyperacanthus amoenus												
Jasminum angulare	1		1									
Jasminum fluminerse	1											
Keetia gueinzii												
Kiggelaria africana		1		1			1	1				1
Macaranga capensis												
Mackeya bella												
Maesa lanceolata												
Ocotea bullata				1	1		1			1		
Olea capensis		1		1					1	1		
Olea faveolata												
Olea woodiana												
Oxyanthus latifolius												
Oxyanthus speciosus					1							

Pavetta capensis					1	1						
Pavetta cf. eylesii												
Pavetta gardeniifolia												
Pavetta lanceolata	1			1			1					
Pavetta revoluta									1			
Pavetta kotzei				1								
Plectriniella armata												
Psychotria capensis			1	1	1		1		1	1		
Psydrax livida												
Psydrax Obuvata												
Rhoicissus digitata	1			1					1		1	
Rhoicissus revolli				1					1			
Rhoicissus rhomboidea		1	1	1	1			1	1	1	1	
Rhoicissus tamentosa	1		1						1			
Rhumnus prinoides		1		1	1			1		1		
Rhumnus sp	1											
Rinorea angustifolia												
Rothmania globosa	1											
Rothmannia capensis							1					
Rothmannia sp												
Rubiaceae sp												
Syzygium peglerae												
Tecoma capensis	1											
Tricalysia capensis					1	1						
Tricalysia delagoensis					1							
Tricalysia lanceolota												1
Trichocladus sp												
Vitex sp												

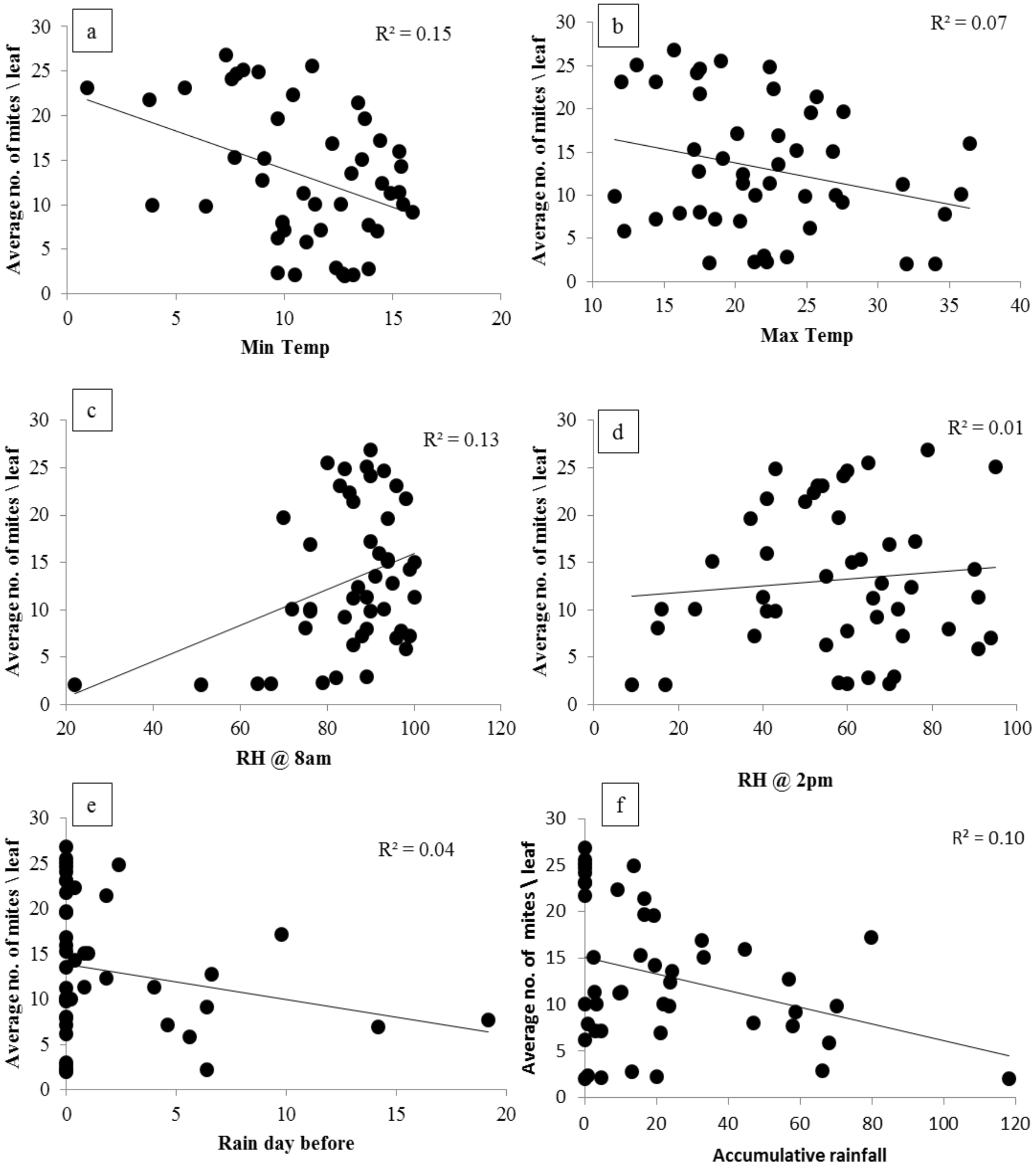
Appendix 4: Published paper: Situngu, S., Barker, N.P. 2016. Position, position, position: Mites occupying leaf domatia are not uniformly distributed in the tree canopy. *South African Journal of Botany*, 108: 23-28. (See attachment in the next page).

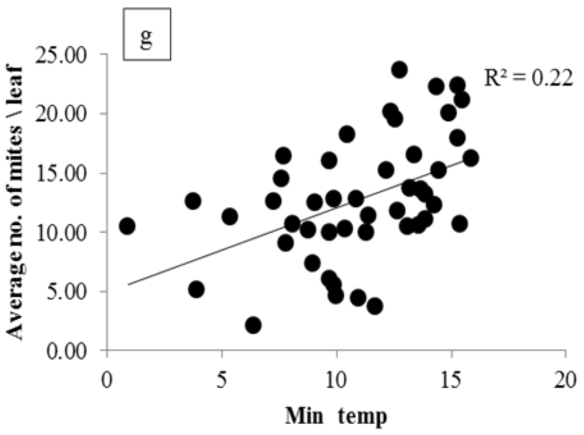
Appendix 5: A List of mite species found in domatia of the sampled tree species.

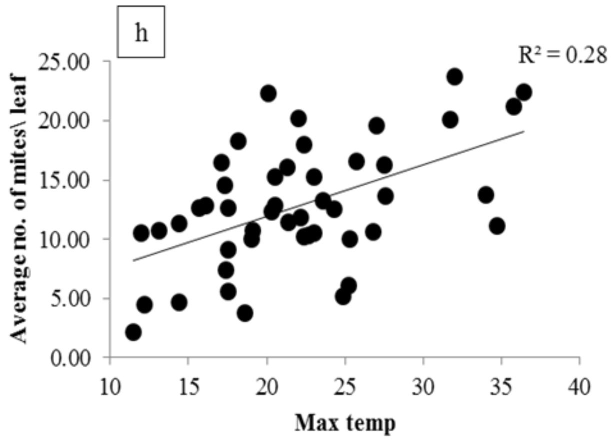
Plant species	Mites collected	Feeding guild
Tecoma capensis	Phytoseiidae	
	Amblyseus neosuilus	Predacious
	Euseius addoensis	Predacious
	Typhlodromus microbullatus	Predacious
	Tetranychidae	
	Panonychus ulmi	Phytophagous
	Tetranychus sp.	Phytophagous
	Tetranychus sp. (probably new)	Phytophagous
	Tydeidae	
	Tydeus monsteri	Predacious
	Wintorschmidtidae	
	Saproglyphus sp.	Fungivorous
	Stigmaeidae	
	Agistemus tranatalensis	Predacious
	Eriophyiodae	
	Unknown	Plant feeding
	Cunaxidae	
	Bunaxella zebedielensis	Predacious
	Tenuipalpidae	
	Brevipalpus sp. (probably new)	Plant feeding
	Triophtydeidae	
	Tetratriophtydeus myacanthus	Predacious
Rothmannia globosa	Phytoseiidae	
	Amblyseus neosuilus	Predacious
	Euseius addoensis	Predacious
	Typhlodromus microbullatus	Predacious
	Tetranychidae	
	Tetranychus sp.	Phytophagous
	Tydeidae	
	Tydeus monsteri	Predacious
	Wintorschmidtidae	
	Saproglyphus sp.	Fungivorous
	Stigmaeidae	
	Agistemus tranatalensis	Predacious
	Eriophyiodae	
	Unknown	Phytophagous
	Oribatei	

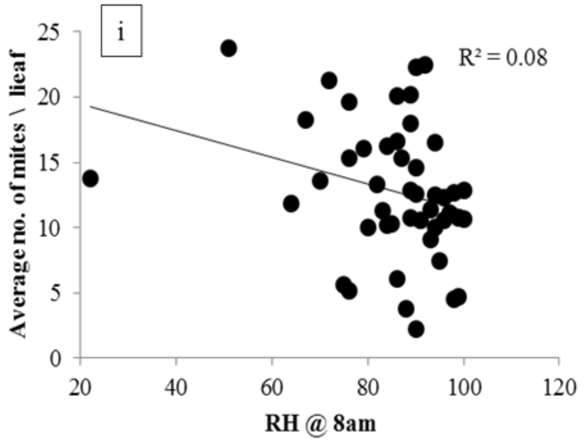
	Oribatis sp.	Mycophagous/ Saprophytic
	Triophtydeidae	
	Tetratriophtydeus myacanthus	Predacious
Gardenia thunbergia	Phytoseiidae	
	Amblyseus neosuilus	Predacious
	Euseius addoensis	Predacious
	Typhlodromus microbullatus	Predacious
	Tetranychidae	
	Tetranychus sp. (probably new)	Phytophagous
	Tydeidae	
	Tydeus monsteri	Predacious
	Wintorschmidtidae	
	Saproglyphus sp.	Fungivorous
	Stigmaeidae	
	Agistemus tranatalensis	Predacious
	Eriophyiodae	
	Unknown	Phytophagous
	Triophtydeidae	
	Tetratriophtydeus myacanthus	Predacious
	Hermileiidae	
	Siculobata sicula	Mycophagous/ Saprophytic

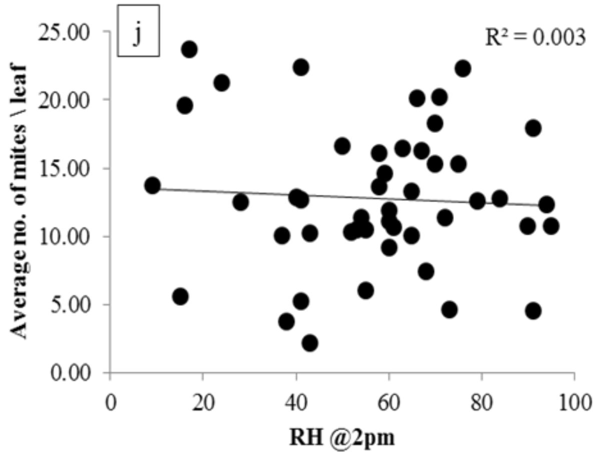
Appendix 6: Correlation plots showing relationships between environmental variables and mite abundance in (A - F) *Tecoma capensis*, (G - L) *Rothmania globosa* and (M - R) *Gardenia thunbergia.*

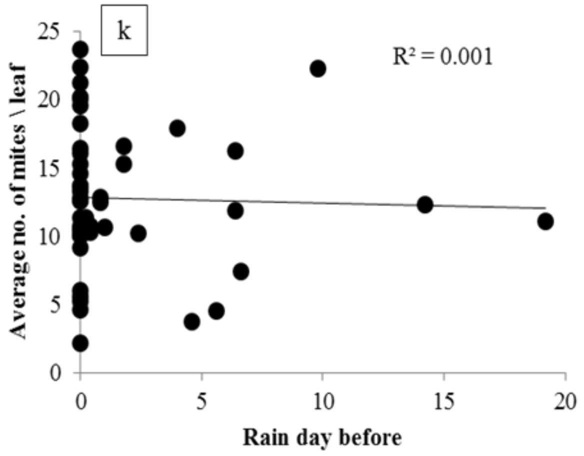


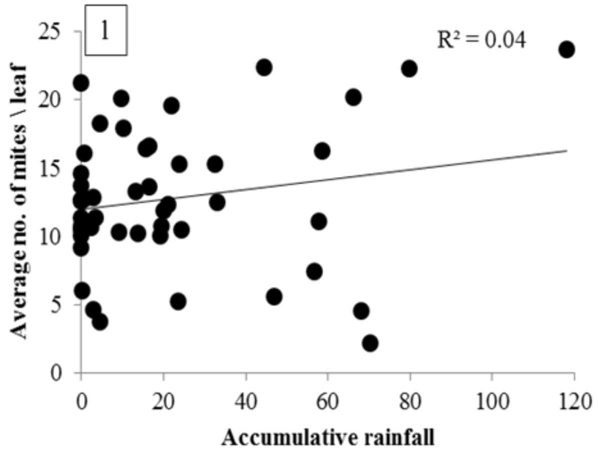

g
R² = 0.22
Average no. of mites \ leaf
0.00
5.00
10.00
15.00
20.00
25.00
0
5
10
15
20
Min temp

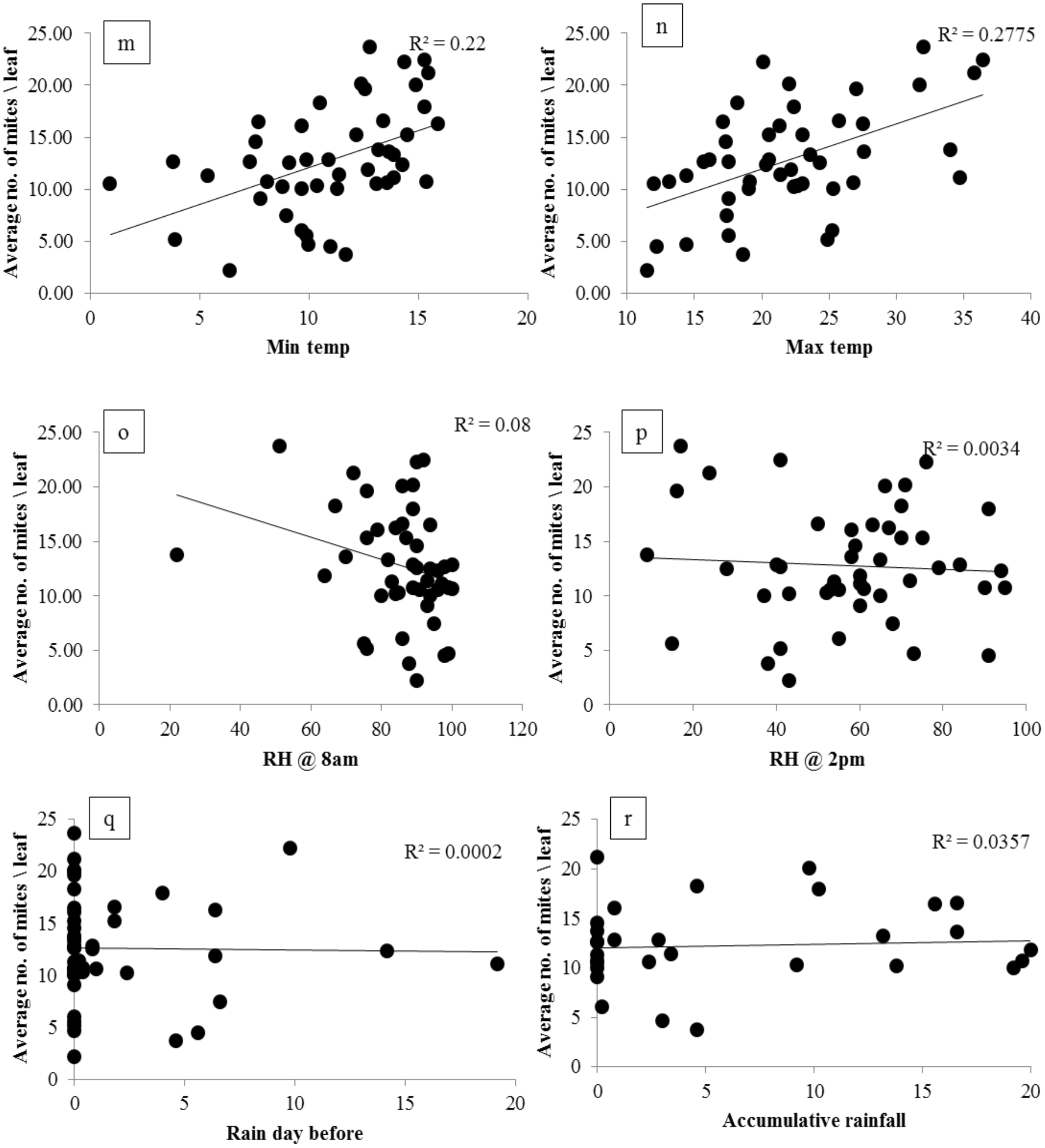

h
R² = 0.28
Average no. of mites\ leaf
0.00
5.00
10.00
15.00
20.00
25.00
10
15
20
25
30
35
40
Max temp


i
R² = 0.08
Average no. of mites \ lieaf
0
5
10
15
20
25
20
40
60
80
100
120
RH @ 8am

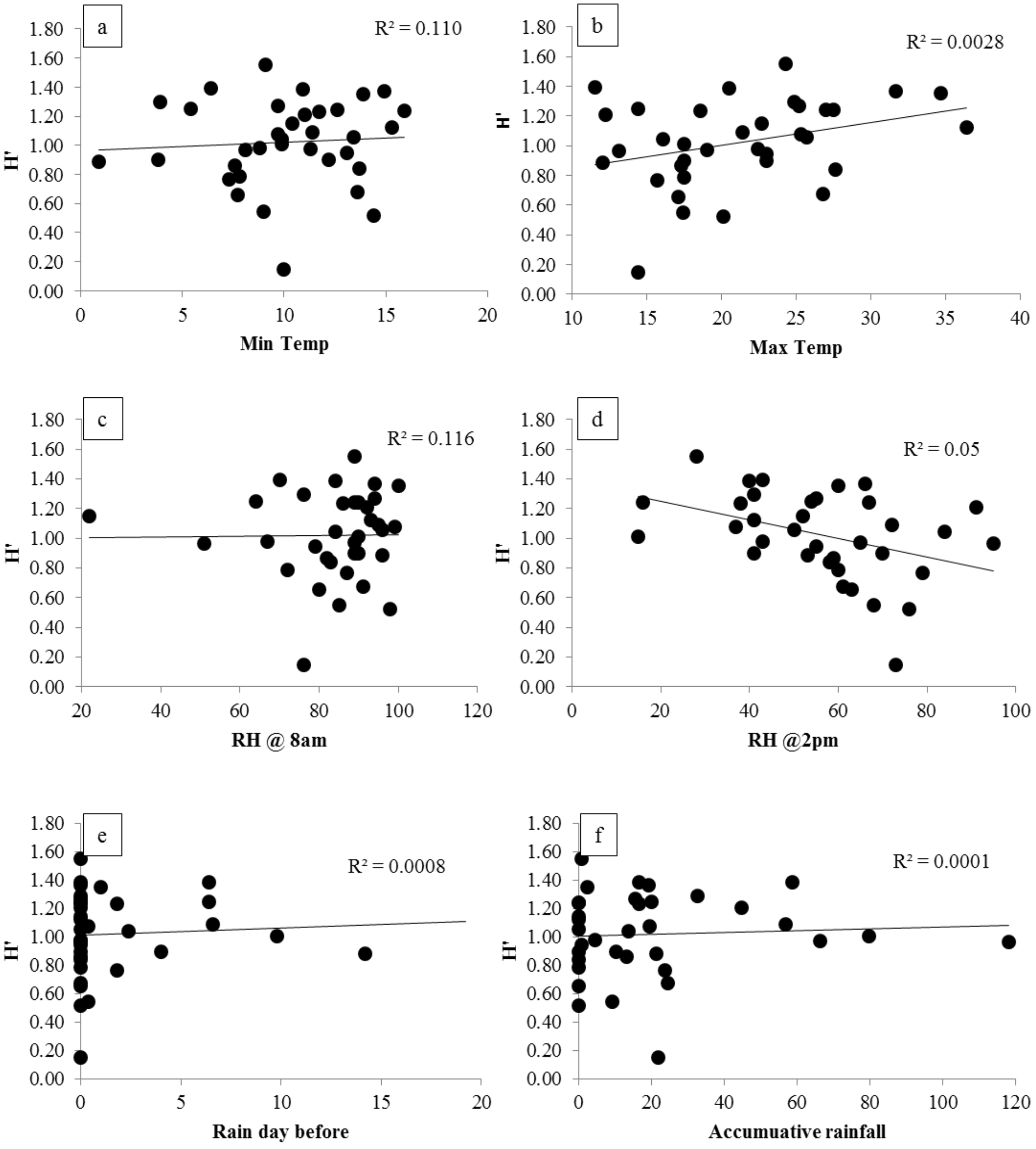

j
R² = 0.003
Average no. of mites \ leaf
0.00
5.00
10.00
15.00
20.00
25.00
0
20
40
60
80
100
RH @2pm

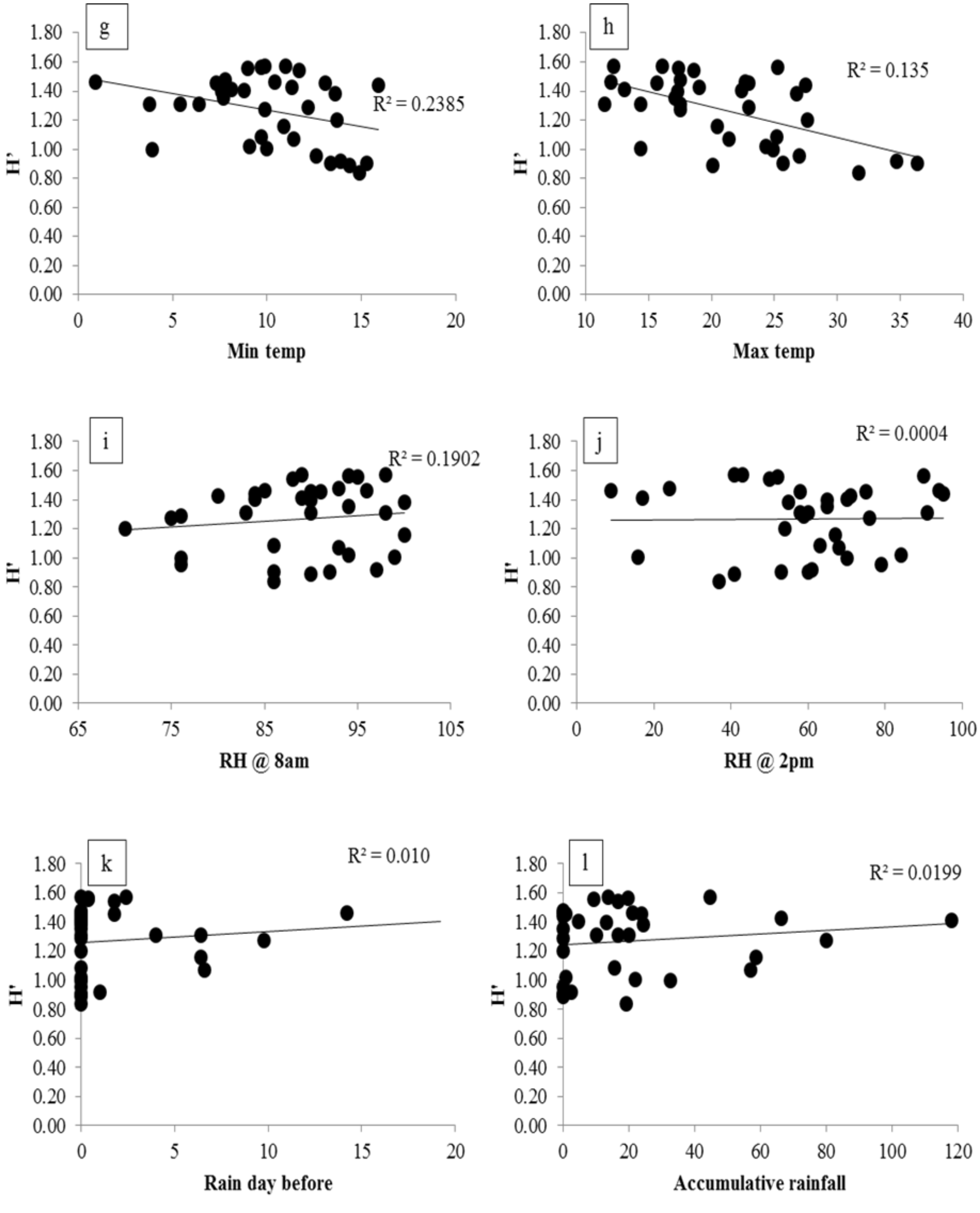

k
R² = 0.001
Average no. of mites \ leaf
0
5
10
15
20
25
0
5
10
15
20
Rain day before


l
R² = 0.04
Average no. of mites \ leaf
0
5
10
15
20
25
0
20
40
60
80
100
120
Accumulative rainfall


m
R² = 0.22
Average no. of mites \ leaf
25.00
20.00
15.00
10.00
5.00
0.00
0
5
10
15
20
Min temp
n
R² = 0.2775
Average no. of mites \ leaf
25.00
20.00
15.00
10.00
5.00
0.00
10
15
20
25
30
35
40
Max temp
o
R² = 0.08
Average no. of mites \ leaf
25.00
20.00
15.00
10.00
5.00
0.00
0
20
40
60
80
100
120
RH @ 8am
p
R² = 0.0034
Average no. of mites \ leaf
25
20
15
10
5
0
0
20
40
60
80
100
RH @ 2pm
q
R² = 0.0002
Average no. of mites \ leaf
25
20
15
10
5
0
0
5
10
15
20
Rain day before
r
R² = 0.0357
Average no. of mites \ leaf
25
20
15
10
5
0
0
5
10
15
20
Accumulative rainfall

Appendix 7: Correlation plots showing relationships between environmental variables and mite Diversity in (A - F) *Tecoma capensis*, (G - L) *Rothmannia globosa* and (M - R) *Gardenia thunbergia.*




g
H'
Min temp
R² = 0.2385
h
H'
Max temp
R² = 0.135
i
H'
RH @ 8am
R² = 0.1902
j
H'
RH @ 2pm
R² = 0.0004
k
H'
Rain day before
R² = 0.010
l
H'
Accumulative rainfall
R² = 0.0199
0.00
0.20
0.40
0.60
0.80
1.00
1.20
1.40
1.60
1.80