Fly Pollination of Generalist Daisies in the Greater Cape Floristic Region

By

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Thesis presented in partial fulfilment of the requirements for the degree of Master of Science
in the Faculty of Science at Stellenbosch University

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March 2023

Declaration

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Abstract

The majority of flowering plant species rely on insect pollen vectors for reproduction. A key factor in determining the dependence of plants on certain pollinators is understanding the specialisation of plant-pollinator interactions. Generalist pollinated plants are visited by a wide diversity of pollinators, and so do not necessarily exclude visitation from insects which are not offering effective pollination services. This ultimately results in effectiveness hierarchies where some effective pollinators are most important for ecological persistence and individual fitness of plants, while many other visitors may contribute little to reproduction. Daisies (Asteraceae) are a classic example of generalist pollinated plants due to their radially symmetrical and open access inflorescence morphology which allows visitation from a variety of insect visitors. However, in contrast to this expectation of generalism, recent work suggests that the dominant annual daisy species of the Namaqualand mass flowering displays may be strongly reliant on few fly pollinators (particularly Bombyliidae: Mariobezziinae, and Tabanidae: *Rhigioglossa*) for reproduction. To test whether daisies in the Western Cape are also specialised, I investigate the extent to which dominant annual daisies in the Western Cape spring flowering displays are dependent on flies as pollinators.

In Chapter 2, I examine the prevalence of flies in daisy pollination across the Greater Cape Floristic Region. Based on recent studies conducted in Namaqualand, I expect daisy visitor communities to be diverse, but dominated by flies. In this chapter, I ask what the diversity and composition of flower visiting insect communities associated with spring flowering annual GCFR daisies is. By conducting surveys of pollinator communities across the Western Cape, I find that the dominant, showy annual daisy species of the spring flowering displays are visited by an average of eight visitor species, making them relatively generalized compared to the daisies of Namaqualand. Secondly, I ask how these communities are structured across daisy genera/species and across space. I find strong variation in the pollinator communities across space, which is characteristic of generalized pollination systems, and substantial overlap in visitor communities across daisy genera. Lastly, I ask if flies are consistently the most abundant visitors to daisies across the GCFR. As expected, flies were found to be one of the dominant daisy visitor groups. However, beetles (particularly Nitidulidae and Melyridae) were far more abundant visitors than flies. Surprisingly bees, that are dominant daisy pollinators globally, were virtually absent as visitors to the annual mass flowering daisies of the GCFR.

In Chapter 3, I build on my findings in chapter 2, and determine the relative importance of flies and beetles for the reproduction of *Dimorphotheca pluvialis*, a dominant daisy species across the Greater Cape Floristic Region, by quantifying their pollination effectiveness. While previous studies have used pollinator visitation rates and/or frequencies as a measure of a pollinator's importance, in this chapter I experimentally examine both the quantity (visitation rates) and quality (single visit pollen deposition and seed set) components to fully understand the role that these visitors play in female plant fitness. Overall, I find that Nitidulidae beetles and Mariobezziinae flies are offering similar levels of pollination effectiveness. This is certainly a surprising result since nitidulids have previously been disregarded as effective pollinators due to their low mobility, small body size and low overall hairiness. However, this study finds that nitidulids are not only active visitors, but they also carry substantial pollen loads which are ultimately deposited on receptive stigmas.

Taken together, this study provides the first baseline data on the pollination systems of the foundational daisy species in the Western Cape spring flowering displays. This thesis confirms the importance of Mariobezziinae bee flies as daisy pollinators, and further highlights the importance of nitidulid beetles, which have previously been assumed to be ineffective pollinators, in the persistence of the spring-mass flowering, annual daisies of the GCFR. Further investigation into the relative importance of flies and beetles as selective agents on daisy floral traits in the GCFR is required.

Opsomming

Die meerderheid blomplant spesies is afhanklik van insekte as stuifmeel vektore vir voortplanting. Die spesialisering van plant-bestuiwer interaksies is die hoof faktor om die afhanklikheid van plante op verskeie bestuiwers te verstaan. Algemene plante word deur ‘n wye verskeidenheid bestuiwer besoek, maar hierdie bestuiwers verskaf nie noodwendig effektiewe bestuiwingsdienste nie. Dit veroorsaak verskeie vlakke van doeltreffendheid, waar sekere effektiewe bestuiwers die belangrikste is vir die voortbestaan van ‘n spesifieke plant spesie in ‘n gemeenskap en ander insekte min tot geen bydra lewer tot voortplanting nie. Magrietjies (Asteraceae) bly ‘n klassieke voorbeeld van algemene bestuifde plante, as gevolg van die morfologie met betrekking tot die radiale simmertrie en oop, bekombare bloeiwyse, wat vir ‘n wye verskeienheid insekte toegang bied. In teenstryding met die verwagting van insek bestuiwing van blomme met ‘n algemene morfologie, het onlangse navorsing getoon dat jaarlikse magrietjie spesies van Namakwaland se massa blomtyd ‘n sterk afhanklik is van slegs a paar vlieg bestuiwers toon (spesifiek Bombyliidae: Mariobezziinae, en Tabanidae: *Rhigioglossa*) vir voortplanting. In hierdie proefskrif, ondersoek ek die mate waartin jaarlikse bloeiwyse magrietjies in die Wes-Kaapse lente se massa blom vertonings, afhanklik is van vlieë as bestuiwers.

In Hoofstuk 2, ondersoek ek die algemene voorkoms van vlieë met betrekking to magrietjie bestuiwing in die Groter Kaapse Floristiese Streek (GKFS). Gebaseer op die onlangse studies in Namakwaland, verwag ek dat magrietjie besoeker gemeenskappe uiteenlopend sal wees, met die oorgrote meerderheid wat uit vlieë spesies bestaan. In die hoofstuk, vra ek ook wat die uiteenlopendheid en samestelling van blom besoekers van die insek gemeenskappe is, wat geassosieer word met die massa lente blomtyd van eenjarige GKFS magrietjies. Opnames van die Wes-Kaapse bestuiwer-gemeenskappe van die dominante magrietjie genusse, bewys dat die dominante, pronkerige jaarlikse magrietjie spesies tydens die Kaapse massa blomtyd deur ‘n gemiddeld van agt insekte besoek word. Dit dui aan dat die bestuiwers van hierdie magrietjies nie so gespesialiseerd is in vergelyking met die Namakwaland magrietjies.

Tweedens, vra ek hoe die magrietjie gemeenskappe geskruktueer is met betrekking to genusse en spesies in die landskap. Die bestuiwer gemeenskappe het sterk uiteenlopendheid patrone gewys oor die landskap, wat kenmerkend is van algemene bestuiwer sisteme, met aansienlike oorvleueling van besoeker gemeenskappe oor magrietjie genusse.

Laastens, vra ek of vlieë konsekwent die mees volopste besoekers is van magrietjies oor die GKFS. Soos ek verwag het, was vlieë een van die dominante magrietjie besoeker groepe. Alhoewel, kewer (spesifiek Nitidulidae en Melyridae) besoekers, by verre meer volop was as vlieë. Verbasend genoeg was bye besoekers, wat wêreldwyd dominante bestuiwers van magrietjies is, basies afwesig as besoekers van die jaarlikse massa blomme prag van eenmalige magrietjies van die Wes-Kaap.

In Hoofstuk 3, bou ek voort op die bevindinge van Hoofstuk 2. Hier bepaal ek wat die relatiewe belangrikheid is van vlieë en kewers vir die voorplanting sukses van *Dimorphotheca pluvialis*, ‘n dominante magrietjie spesie van die Groter Kaapse Floristiese Streek, deur hul bestuiwing doeltreffendheid te meet. Alhoewel vorige studies bestuiwer besoeke en/of frekwensies gebruik het om bestuiwer belangrikheid te meet, het ek in hierdie hoofstuk, eksperimenteel ondersoek ingestel vir beide die hoeveelheid (hoeveel besoeke) en kwaliteit (eenmalige besoek stuifmeel neerslag en hoeveelheid sade) komponente om ‘n meer holisties samevatting te bekom van die rol van hierdie besoekers op vroulike plant fiksheid. Oor die algemeen, het ek gevind dat Nitidulidae kewers en Mariobezziinae vlieë om en by dieselfde vlakke van bestuiwing doeltreffendheid bied. Dit is ‘n verrassende bevinding, omdat nitidulids voorheen as effektiewe bestuiwers verontagsaam is, a.g.v. lae mobiliteit, klein liggaamsgrootte en omdat hulle oor die algemeen min hare het. Hierdie studie vind egter dat nitidulids nie net aktiewe besoekers is nie, maar ook heelwat stuifmeel dra wat uiteindelik op die ontvanklike stempel neergesit word.

In samevatting, verskaf hierdie studie pionieer data van bestuiwingsisteme van ‘n baie algemene magrietjie spesies in die Wes-Kaapse lente blomering vertonings. Die proefskrif bevestig die belangrikheid van Mariobezziinae by-vlieë as magrietjie bestuiwers, en onderstreep die belangrikheid van nitidulid kewers, wat voorheen as ondoeltreffende bestuiwers gekenmerk was in die onderhouding van die jaarlikse lente blommeprag van jaarlikse magrietjies van die Wes-Kaap. Verdere studies word benodig om die relatiewe belangrikheid van vlieë en kewers as spesifieke agente vir magrietjie blom-eienskappe in die Wes-Kaap te betaal.

Acknowledgements

First, I would like to thank my supervisor, Prof Allan Ellis, for his unwavering guidance and support along the road to completing this thesis. His vast knowledge and passion for the subject contributed greatly to shaping this project.

I would also like to thank my field assistants, Luke Siljeur and Jess Muller, for their tireless efforts during sampling. You both made my time in the field a positive and fun experience. Thank you also to my lab group for your encouragement and critical comments.

Thank you to my team at Grootbos Foundation for your support. Your understanding and reassurance in the final months of completing this thesis did not go unnoticed, and I am truly grateful to be working with such an incredible team.

Last, but certainly not least, I would like to thank my parents, Barry and Bev Vlotman. They have offered unwavering love and support despite all the challenges we have faced in the past years. I am also grateful to my wonderful grandmother, Winifred Addinall, whom I am greatly indebted to for constantly encouraging me to be better.

This project was funded by the Royal Museum of Central Africa. A massive thank you to RMCA for funding my studies, as well as additional training in Afrotropical fly taxonomy and systematics. Permits were provided by CapeNature (CN35-87-17692, CN44-87-17693). Thank you to Bridget Thompson for providing accommodation in Langebaan.

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Chapter 1:

General Introduction

Introduction

Insect pollinators play an essential role in the reproduction of many flowering plants (Ollerton et al., 2011). Despite bees being the main focus of the majority of pollination research, recent studies highlight the importance of flies as pollinators (Raguso, 2020; Banda et al., 2021). In fact, Inouye et al. (2015) suggests that flies display many of the same flower visiting behaviours as other insect pollinators and are overall effective pollinators in both natural and agricultural ecosystems.

Daisies (Asteraceae) are the most diverse plant family in the Cape Floristic Region (Manning and Goldblatt, 2012). The West Coast of South Africa is well-known for the annual spring mass-flowering displays, which are dominated by annual Asteraceae species. This event attracts tourists, both locally and globally, contributing greatly to the tourism industry. Daisy inflorescences offer open access to floral rewards, such as pollen and nectar, to a wide diversity of pollinator types, and are often considered to be generalist pollinated. Although generalisation dominates pollination systems (Waser et al., 1996), the vast majority of previous pollination studies have focused on specialist pollinated plants (Ollerton et al., 2006), while generalist pollination, which is characteristic of daisy-pollinator interactions, is less understood.

Throughout this thesis, I explore the diversity of insect visitors that are associated with the dominant daisy genera of the spring mass flowering displays in the Cape Floristic Region, South Africa. Recent studies conducted on the pollination systems of GCFR daisies find that flies dominate the visitation to populations of annual daisies (Kemp et al., 2019; Ellis et al., 2021). I, therefore, specifically focus on the pollination importance of *Corsomyza* and *Megapalpus* beeflies (Bombyliidae: Mariobezziinae) and pollen-feeding beetles (Coleoptera: Nitidulidae) compared to other insect visitors.

In this introductory section, I discuss what is known about generalised plant-pollinator interactions, and more specifically the pollination system of daisies in the context of the Greater Cape Floristic Region. I also explore the term “pollination effectiveness” and I provide a definition of the term in the context of this study. Pollination syndromes are discussed, and I define the pollination syndrome of daisies. Finally, I describe the study system, the Greater Cape Floristic, and what is known about the pollination systems of daisies. I also discuss the diversity of insect pollinators in the GCFR.

Generalisation versus specialisation in plant-pollinator interactions

Ecological generalisation or specialisation in pollination refers to the number of effective pollinator species with which a plant interacts, while functional (or functional-group) generalisation or specialisation refers to the functional diversity of pollinators that visit a plant (Ollerton et al., 2006; Armbruster, 2017). By these definitions, one would consider a plant that is visited and effectively pollinated by just one pollinator species or functional group to be highly specialised. Furthermore, some ecologically generalised plants (i.e. those that interact with many effective pollinators) may be functionally specialised to a narrow pollinator taxonomic (or functional) group (Ollerton et al., 2006). For this reason, Armbruster (2017) suggests that generalisation and specialisation occur along a continuum rather than as a rigid dichotomy.

From the perspective of the plant, a pollinator should preferably specialise during individual foraging bouts in order to securely transfer conspecific pollen between individuals (Waser, 1978; Morales and Traveset, 2008). However, when scaling up to plant individual lifetimes and entire species, the incentive to simultaneously generalise increases in order to guarantee that pollen transfer remains stable over a long period (Waser et al., 1996). Similarly, a pollinator's incentive to generalise increases when scaling up from individual foraging bouts to pollinator lifespans and to species over long time periods in order to ensure the maintenance of floral resources over time (Waser et al., 1996). Brosi (2016) therefore argues that specialisation and generalisation can occur simultaneously across the individual to community scales. For example, in a population of pollinators, individual pollinators may specialise over short term foraging bouts while simultaneously generalising over their lifetime (Brosi, 2016).

Pollination systems may also vary in specificity along spatial and temporal scales. Gomez et al. (2009), for example, found that the generalist plant *Elysium mediohispanicum* (Brassicaceae) showed significant spatial variation in its pollinator assemblage structure across different populations, with different populations showing functional specificity for different pollinator functional groups. Similarly, populations of the generalist *Hormathophylla spinosa* (Cruciferae) in Sierra Nevada, Spain are visited by separate pollinator guilds across altitudinal gradients (Gomez and Zamora, 1999). Lower elevation populations of *H. spinosa* were more frequently visited by nectarivorous pollinator guilds, while higher elevation populations interacted more frequently with pollinivores (Gomez and Zamora, 1999). Furthermore, a study of *Lavandula latifolia* (Labiatae), in the Mediterranean woodlands of southeastern Spain, revealed marked temporal variation, both seasonally and annually, in its pollinator assemblage structure (Herrera, 1988). Lepidopteran pollinators were the dominant pollinators for a year, while Hymenopterans dominated pollinator assemblages for the remainder of the six-year study (Herrera, 1988). Additionally, Lepidopterans dominated pollinator assemblages mid-summer with Hymenopterans taking over as autumn began (Herrera, 1988).

Due to the reliance of flowers on pollinators, and vice versa, it comes as no surprise that coevolutionary forces play a role in the diversification of both. Geographic selection mosaics are an important driving force in coevolutionary dynamics and are thus an essential part of the Geographic Mosaic Theory of Coevolution (Thompson, 2005). In generalist plant systems, spatial variation in mutualistic plant-pollinator interactions is the result of changes in pollinator assemblage structure, diversity and abundance across space (Price et al., 2005; Gómez et al., 2009) . As a result, different populations of the same species may experience selection pressures for differing phenotypic traits. For example, populations of *E. mediohispanicum* interact with different pollinator assemblages across populations, and these differences resulted in the selection of different phenotypic traits (e.g. corolla size, flower number and stalk height) (Gómez et al., 2009). On the opposite end, antagonistic interactions (i.e., plant-herbivore interactions) can also impose selection pressures. A study investigating the mutualistic and antagonistic interactions among populations of the previously mentioned *H. spinosa* in Spain found that in populations with low herbivore pressure, there was a pollinator-mediated selective pressure for more flower heads per plant (Gómez and Zamora, 2000). Conversely, in populations with high herbivore pressure, there was strong selection for an increase in thorn density (Gómez and Zamora, 2000).

Previous studies have attempted to quantify the degree of specialisation in widely regarded generalist pollination systems. Some of these studies essentially estimate visitor richness (Pettersson, 1991; Struck, 1992) or visitation frequency (Gómez et al., 2007, 2009), and do not account for a few species dominating visitation networks. For example, Pettersson (1991) found that the plant *Silene vulgaris* is pollinated by more than 26 moth species, however, there is no consideration of each species' relative abundance or effectiveness and it is not clear if visitation is strongly dominated by a few moth species. Few studies have constructed species accumulation curves to examine whether sampling provided an accurate estimation of the pollinator assemblage (Gómez et al., 2007, 2009). Since generalist pollination systems also vary along spatial scales (Price et al., 2005; Moeller, 2005, 2006; Gómez et al., 2009, Ellis et al 2021), it also becomes important to examine changes in pollinator diversity and abundance between geographically separated plant populations (Price et al., 2005; Moeller, 2006; Gómez et al., 2009).

Pollination effectiveness

Plant-pollinator interactions are generally considered to be mutualistic (Bronstein, 1994). Ideally, a pollinator provides a beneficial service to a plant (i.e. pollen transfer), and in return receives a reward (usually food) (Woodcock et al., 2014). However, mutualisms are complex, and in some cases, are not equally beneficial to both parties. This is certainly the case in plant-pollinator interactions where plant species accept pollination services without providing rewards (deceptive pollination) (Jersáková et al., 2006), and where flower visitors frequently exploit floral rewards without providing an effective pollination service (e.g. nectar robbers and pollen thieves) (Hargreaves et al., 2009). The “leaky bucket” metaphor of Thomson (2003) aptly compares pollinators to buckets that vary in size and the number of holes through which they leak. A pollen thief is, therefore, an example of a very large bucket with very many holes, where much of the pollen that is collected is lost before it can be deposited on a receptive stigma (Thomson, 2003). In these cases, pollen thieves cause pollen quantity limitation (Hargreaves et al., 2009), which reduces the reproductive success of plants (Hargreaves et al., 2010). By making their rewards exclusively available to the most effective pollinator, specialist plants significantly reduce their chances of pollen theft. In the case of generalist plants however, pollen loss increases since they are not excluding less effective pollinators. This essentially results in effectiveness hierarchies where some effective pollinators become more important than those which are less effective.

Pollination effectiveness comprises of two components, namely; the quantity component (the pollinator-plant interaction frequency), and the quality component (the pollinator’s single visit performance). Some pollination biologists have used relative visitation frequency/rate or abundance to measure a pollinator’s importance in the system (Stebbins, 1970; Pauw et al., 2020) . Although this does not consider the full picture of a pollinator’s effectiveness, it does provide a powerful insight into a pollinator’s relative importance, since Olsen (1997) found that pollinator importance is strongly driven by the quantity component. Other studies have used the number of pollen grains deposited on stigmas per single visit or pollen loads on pollinator bodies as estimators of the quality component (Olsen, 1997; Armbruster, 1998; Minnaar et al., 2019; Valverde et al., 2019; Pauw et al., 2020). Very few studies, however, take both components into account when estimating a pollinator’s effectiveness, and some argue that seed set is the desirable outcome and is a more valuable measure of a pollinator’s effectiveness (Dieringer, 1992; Keys et al., 1995; Olsen, 1997; Valverde et al., 2019).

While pollinator effectiveness examines a pollinator's relative importance for the female plant fitness component (i.e., seed set), this does not necessarily take into account the pollinator-mediated male fitness component (effective pollen transfer), which is crucial in driving the evolution of floral traits (Minnaar et al., 2019; Ellis et al., 2021). Minnaar et al. (2019) clearly outlines the male fitness pathway, from pollen production to ovule fertilization, necessary for reproductive success in animal-pollinated plants. Vector-mediated pollen transfer between plants is a significant step towards pollination success, and is also characterised by multiple avenues of pollen loss which weakens male fitness (Minnaar et al., 2019). Before pollen transfer takes place, pollen will be left behind after a visit, or lost through consumption by a pollinator (Minnaar et al., 2019). Finally, a pollen vector must successfully place the pollen within the stigma contact area for pollen germination and ovule fertilization to take place (Minnaar et al., 2019).

Pollination syndromes

Angiosperms have developed diverse ways to attract pollinators. If a species can develop a distinctive set of floral signals that advertise a reward, pollinators are more likely to revisit and pollinate that species. When a pollinator tends to exclusively visit a certain flower species, or morph within a species; thereby bypassing other flower species that could be more rewarding (Waser, 1986), it is known as floral constancy. Pollinator preference can result in two evolutionary responses from flowers: floral advergence (floral mimicry) or floral convergence (pollination syndromes) (Schiestl and Johnson, 2013). Mimicry is an adaptation where one plant (a mimic) develops a similarity to another (a model) in order to increase the mimic's fitness by eliciting certain behaviour by the pollinator (operator) (Schiestl and Johnson, 2013). A floral mimic can either be deceptive (no reward, equivalent to Batesian mimicry) or rewarding (equivalent to Müllerian mimicry) (Jersakova et al., 2009; Schiestl and Johnson, 2013) and may elicit feeding (Nilsson, 1983), sexual (Vereecken and Schiestl, 2008; Peakall *et al.*, 2010) or oviposition behaviour (Urru et al., 2011) from the operator in order to be visited and pollinated.

The second response to floral constancy and pollinator floral preferences is pollination syndromes, which are described by Waser et al. (1996) as suites of floral traits that are selected for by certain pollinator functional groups. Studies in the Northern Hemisphere reveal that this kind of floral evolution occurs at the order level (e.g. flies vs ants vs beetles) and above (e.g. insects vs birds) (Waser et al., 1996). In a Northern Hemisphere context, one can see that compared to taxonomic groups, pollinator functional groups offer a more informative indication of pollination syndromes, and thus if a plant is specialised or generalised (Fenster et al., 2004).

In southern Africa, however, pollination syndromes can occur at the level of individual pollinator species (Johnson and Steiner, 2003). For example, many Iridaceae species in southern Africa are exclusively pollinated by a single long-proboscid fly species (Goldblatt and Manning, 1999).

Pollination syndromes imply convergent evolution (Ollerton et al., 2006) and therefore, a level of specialisation. Generalist pollinated plants, therefore, do not fall within this category since they inherently attract, reward and are pollinated by a wide array of different pollinators. However, pollinator hierarchies exist within these systems, where a diversity of pollinators may visit and be rewarded by the plant, but only a subset of pollinators are actually providing effective pollination services. In these cases, generalist plants may exhibit some degree of functional specialisation and so those effective pollinators will act as the main selective agents of floral traits across plant communities (Kemp et al., 2019; Ellis et al., 2021).

The pollination syndrome of daisies

Daisies are the most diverse and successful plant family in the world (Willis, 2017) and have extremely derived and unique floral structures (Ellis and Johnson, 2009; De Jager and Ellis, 2014; Kemp et al., 2019), presumably adapted for effective pollination, and yet have received little attention from pollination biologists. Specialist pollination interactions are well studied due to their tractable and elegant design (Manning and Goldblatt, 1996; Waser et al., 1996; Goldblatt and Manning, 2000), but generalists make up the majority of pollination interactions (Waser et al., 1996) and are more complex and variable, perhaps making them less attractive to pollination ecologists. Daisies have what some biologists consider to be a “generalist” morphology; a simple, radially symmetrical capitulum, offering easy access to pollen and nectar (Ollerton et al., 2006), implying that a wide range of visitors may utilize such rewards. Pollination ecologists, however, use “generalization” to define the number of visitors (Waser et al., 1996), not taking floral morphology into account. While both these definitions are not necessarily mutually exclusive, Ollerton et al. (2007) argues that some plants with apparently “generalist” morphologies may be more specialised than expected.

Daisies are generalists, and are thus visited by diverse insect communities which exhibit changes in pollinator abundance, diversity and assemblage structure across space (geographic selection mosaics) (Price et al., 2005; Thompson, 2005; Gómez et al., 2009; Kemp et al., 2019; Ellis et al., 2021). As such, variation in the dominant pollinator functional groups across populations of the same plant species, which are attracted to different visual or morphological cues, will select for varying floral traits (most effective pollinator principle) (Stebbins, 1970), which can ultimately result in speciation (Johnson, 2006). This has been previously found for daisies, where pollinators play a key role in the selection of daisy ray morphology (Andersson and Widen, 1993; Andersson, 2008; De Jager and Ellis, 2014; Kemp et al., 2019).

Globally, daisies are visited by a wide taxonomic diversity of insects, including Lepidoptera, Hymenoptera and Diptera (Torres and Galetto, 2002; Figueroa-Castro and Cano-Santana, 2004; Figueroa-Castro et al., 2016; Guerrina et al., 2016; Usharani and Raju, 2018). The majority of daisies that have received attention in the Northern Hemisphere are pollinated by bees and butterflies (Schmitt, 1983; Boldt and Robbins, 1987; Olsen, 1997; Freitas and Sazima, 2006). Hymenopterans and Lepidopterans probably dominate these visitation networks due to their relatively high diversity compared to southern Africa (Cottrell, 1985; Ollerton et al., 2007). However, evidence suggests that daisy-fly pollinator interactions in South Africa may be more specialised than alpine and arctic fly pollination systems (Larson et al., 2001).

Study system: Greater Cape Floristic Region

The floral diversity of the GCFR

The Greater Cape Floristic Region (GCFR) is comprised of two biodiversity hotspots, the Cape Floristic Region (CFR), which is located at the southern tip of Africa, and the Succulent Karoo (SK) Biome, located along the south-western border of Southern Africa (Myers et al., 2000; Born et al., 2007). The CFR is made up of a land area of 90 720 km^2 and hosts 9 383 plant species, with around 68% species being endemic (Manning and Goldblatt, 2012). Asteraceae is the largest plant family in the CFR with 1 077 species, 669 (62%) of which are endemic to the area (Manning and Goldblatt, 2012). The SK Biome hosts 3 715 native plant species (40.4% being endemic) (Snijman, 2013). Asteraceae (495 species) is the second largest plant family in the SK, behind Aizoaceae (658 species), and constitutes 6% of the Biome's total plant species (Desmet, 2007; Rundel and Cowling, 2013; Snijman, 2013). The dominant annual species of *Dimorphotheca*, *Arctotheca*, *Arctotis* and *Ursinia*, all show remarkable intraspecific variation in capitulum morphology (De Jager and Ellis, 2014; Kemp et al., 2019) as a result of pollinator mosaics across geographical regions (De Jager and Ellis, 2014; Kemp et al., 2019; Ellis et al., 2021).

The West Coast of Southern Africa is well-known for the annual spring mass-flowering displays, which are dominated by Asteraceae. This event attracts tourists, both locally and globally, contributing greatly to the tourism industry. Passive nature-based tourism (scenic appreciation) is valued at more than five billion rand a year, making up almost 60% of the CFR’s total biodiversity value (Turpie et al., 2003). These spring mass flowering displays therefore make up an important part of South Africa’s ecotourism industry. Understanding the pollination interactions that determine seed set in these annual daisies is, thus, of both ecological and economic value.

Pollination specialisation in the GCFR

Studies conducted in Namaqualand find that the daisy genera that dominate the annual daisy displays are visited by a wide diversity of insect pollinators, as expected for generalists. Struck (1992) sampled six perennial daisy species which were each visited by nine different pollinator species on average, belonging to Hymenopteran, Dipteran and Coleopteran orders. Over half of those pollinator species carried more than 80% conspecific pollen of their respective host plants suggesting they may be effective pollinators (Struck, 1992). However, recent work found that the annual daisy communities of Namaqualand are unusual in that they are dominated by a single fly pollinator (Kemp, et al., 2019; Ellis et al., 2021). A study of the floral visitors to six *Gorteria diffusa* morphs across Namaqualand also found that *Megapalpus capensis*, *Apis mellifera* and a tabanid *Rhigioglossa* species were frequent and active visitors and all carried conspecific pollen, and therefore contributing somewhat to pollination (Ellis and Johnson, 2009). However, *Megapalpus capensis* (Bombyliidae) was the most abundant visitor to this daisy species. *G. diffusa* is easily recognised by the raised black spots in the capitula resembling beetles, giving the species its name: beetle daisy. Studies show that these spots resemble female members of *M. capensis* and attract males by eliciting mate-seeking behaviour (Johnson and Midgley, 1997; Ellis and Johnson, 2010). Similar results were found for the polymorphic *Ursinia calenduliflora*, where *M. capensis* males were dominant visitors to the spotted morphs of this daisy species (De Jager and Ellis, 2014). Furthermore, flower colour patterns of Namaqualand daisy communities are strongly influenced by a geographic mosaic of fly pollinators with different colour preferences (Kemp et al., 2019; Ellis et al., 2021).

It should be noted, however, that variations in floral traits among populations of the same species are not always dependent on pollinator shifts. The distinct floral forms of *G. diffusa* are located in separate areas across the Greater Cape Floristic Region. After investigating the reason for this geographic variation, Ellis & Johnson (2009) found that it was not a result of variation in pollinators assemblages since the pollinator assemblages in 11 of the 14 floral forms were dominated by the same fly species, *Megapalpus capensis*. In this case, the extensive floral variation is not a result of evolutionary shifts between pollinators (Ellis and Johnson, 2009), but may be a result of more subtle differences in pollinator populations (e.g. differences in abundance or behaviour).

Pollination effectiveness in GCFR daisies

Studies conducted on the pollination ecology of Namaqualand daisies found that only a subset of all insect visitors (predominantly flies) are important for plant reproductive success. However, these conclusions are usually based on visitation rates (Kemp et al., 2019) or relative visitation frequencies (Struck, 1992; Gibson, 1999; Ellis and Johnson, 2009; Ellis et al., 2021) as metrics of pollinator importance in these systems. The quantity component alone is not an adequate indicator of a pollinator's single visit performance and its importance for seed production (Dieringer, 1992; Keys et al., 1995; Olsen, 1997; Valverde et al., 2019). Goldberg (1996) and Gibson (1999) took this one step further by incorporating the proportion of conspecific pollen loads carried by a pollinator, however, there have been no attempts to estimate each pollinator's single visit performance. Therefore, there is a substantial knowledge gap in the pollination effectiveness of the dominant insect visitors to GCFR daisy communities.

Daisy pollinators in the GCFR

Flies

The CFR may be the centre for fly speciation in southern Africa (Bowden, 1978). There are 40 tabanid species present in the Western Cape, with around 16% being endemic to the area (Usher, 1972). Extremely long-proboscid tabanids and nemestrinids are well-known pollinators in the GCFR, and thus their role in the pollination of many plant species, particularly Geraniaceae, Iridaceae and Orchidaceae, has been well-studied (Usher, 1972; Manning and Goldblatt, 1996; Goldblatt and Manning, 1999, 2000, 2006). In Namaqualand, for example, 28 endemic flower species are exclusively pollinated by nemestrinid flies (Manning and Goldblatt, 1996). Members of the tabanid genus *Rhigioglossa*, however, have short proboscises in comparison, which may explain their tendency to visit the shallow flowers of daisies (Chainey, 1987). Further than this, their role in pollination within the South African context is not well investigated.

Bombyliid flies of the subfamily Mariobezziinae are important pollinators of many self-incompatible daisy species of Namaqualand and thus play a role in their persistence in the ecosystem (Ellis and Johnson, 2009; De Jager and Ellis, 2014; de Waal et al., 2015; Kemp et al., 2019). Beeflies show high levels of endemism in the SK (Hesse, 1938; Struck, 1994a) and have formed strong plant-pollinator interactions with many flower species (Johnson and Midgley, 1997; Goldblatt and Manning, 2013). The interaction between *Gorteria diffusa* and its dominant beefly pollinator is one of the most investigated daisy-fly pollinator systems in South Africa (Johnson and Midgley, 1997; Midgley and Johnson, 1998; Ellis and Johnson, 2009, 2012; de Jager and Ellis, 2012). Another study in Darling, South Africa revealed that Bombyliidae were the most frequent visitors to the daisy species, *Ursinia anthemoides* (Goldberg, 1996). Kemp et al. (2019) found that the insect visitor composition of daisy communities in Namaqualand are dominated by the beefly *Megapalpus capensis* and the horsefly *Rhigioglossa*. Furthermore, Ellis et al. (2021) found that *Corsomyza* flies dominate visitation to *Dimorphotheca* daisies across Namaqualand, while *Megapalpus capensis* and monkey beetles are frequent visitors to *Ursinia* daisies. There is in fact a strong geographic mosaic of pollinators with different flower colour preferences which have potentially shaped the variation in daisy inflorescence colour across the Namaqualand landscape (Kemp et al., 2019; Ellis et al., 2021).

Other insect visitors

Besides flies, generalist flowers in the GCFR are frequently visited by monkey beetles (Scarabaeidae: Hopliini) which are largely endemic to southern Africa (Scholtz and Holm, 1985). A large majority of monkey beetles species are concentrated in the SK (Rundel and Cowling, 2013) and are potentially responsible for the pollination of a diverse range of endemic plants (Struck, 1994a ; Colville et al., 2002), specifically annual Asteraceae species (Mayer et al., 2006). An assessment of the geographical variation in the insect pollinators of Asteraceae across the GCFR found that monkey beetles were important pollinators across the region (Gibson, 1999). Furthermore, solitary bees (Hymenoptera: Apoidea) are the dominant flower-visiting insects in the SK (Struck, 1994a).

Objectives of research chapters

The overarching aim of this thesis is to fill a knowledge gap about keystone dipteran pollinators and their role in the maintenance of the ecologically and economically important daisy diversity of the GCFR in South Africa.

In Chapter 1, I investigate how widespread fly pollination is in spring mass flowering daisies in the GCFR. First, I assess the diversity and composition of flower visiting insect communities associated with spring flowering annual GCFR daisies. Second, I determine whether flies are consistently the most abundant visitors on daisies. This was done by conducting diversity surveys and visitation observations of the main annual flowering daisy genera found across the Western Cape. Since daisies are widely presumed to be generalists (Ollerton et al., 2006), I expect them to be associated with a wide taxonomic and functional diversity of visitors. Under this generalization hypothesis, I also expect limited differentiation between visitor communities associated with different daisy species within regions, but would expect substantial compositional turnover across regions if insect communities exhibit high beta diversity, as has been demonstrated for other GCFR insect groups (Kemp et al., 2017).

In Chapter 2, I experimentally assess the importance of flies, relative to other flower visiting insects, in daisy pollination. First, I combine the visitation rate data from the broad pollinator surveys, with assessment of pollen loads on visitor taxa, to rank pollinators. Second, I experimentally compare the effectiveness of pollinator groups using single visit experiments on a population of *Dimorphotheca pluvialis*, a dominant daisy species across the GCFR. Due to the dominance of flies as pollinators in the spring flowering daisy communities of the GCFR (Goldberg, 1996; Gibson, 1999; Ellis and Johnson, 2009; Kemp et al., 2019), I expect flies to be more effective pollinators compared to other insect taxa.

Figure 1.1: The remarkable intraspecific diversity in capitulum morphology of *Dimorphotheca*, *Arctotis* and *Arctotheca*. A = *Dimorphotheca pluvialis* (mulberry form), B = *Dimorphotheca pluvialis* (candles form), C = *Dimorphotheca pluvialis* (cape town form), D = *Arctotis hirsuta* (no ring form), E = *Arctotis hirsuta* (ring form), F = *Dimorphotheca sinuata* (tricolor form), G = *Dimorphotheca sinuata* (kardoesie form), H = *Dimorphotheca sinuata* (naartjie form), I = *Arctotheca calendula* (langebaan form), J = *Arctotheca calendula*.

Chapter 2:

Determining the diversity and importance of insect pollinators of spring mass-flowering daisies across the Greater Cape Floristic Region

Abstract

Specialised plant-pollinator interactions dominate southern Africa and are well studied. Daisies, however, are often thought to be both ecological and phenotypic generalists due to their open access inflorescence morphology that facilitates visitation and resource acquisition by a wide diversity of insect species. However, previous studies on the pollination systems of Namaqualand mass-flowering annual daisies have found that they are relatively specialised, being visited mainly by flies (Bombyliidae: Mariobezziinae, and Tabanidae: *Rhigioglossa*). The purpose of this study was to determine the diversity and importance of different pollinators for seed production of the dominant daisy genera of spring-mass flowering displays of the Western Cape. I found that the dominant showy daisy species of the Western Cape are associated with a mean of eight different pollinator species, making them relatively generalised compared to the daisies of Namaqualand, which are visited by an average of three species. Furthermore, there is strong evidence for high spatial turnover in the insect visitor communities to daisies across various spatial scales. Overall, flies always contributed to daisy visitation, however less frequently than beetles. Therefore, further assessment of the pollination effectiveness of these dominant pollinators is required to determine whether beetles and flies are offering similar levels of effective pollination services.

Introduction

Plants are immobile and thus rely on abiotic and biotic pollen and seed vectors for reproduction. Insects are important pollen vectors and play a key role in the maintenance of angiosperm diversity since the majority of flowering plant species are dependent on insect pollination for the production of seeds (Burd, 1994; Ollerton et al., 2011; Rodger et al., 2021). Consequently, a decline in pollinator availability would result in the reduction of plant reproductive output (Rodger et al., 2021). A critical factor in determining the dependence of plants on certain pollinator species is the level of specialisation of plant-pollinator interactions. Plant-pollinator interactions may be the outcome of a strong co-evolutionary process where plants evolve traits to attract the most effective pollinator and these pollinators evolve traits that allow them to effectively exploit floral resources (Stebbins, 1970). In this case, plants and their pollinators will evolve towards specialisation. Alternatively generalisation may be the rule rather than the exception as pollination interactions usually result from diffuse coevolution (Waser et al., 1996; Gómez et al., 2007; Ollerton et al., 2007). Johnson and Steiner (2003) argue that the "generalisation" view of pollination systems results from a geographical research bias towards cooler temperate regions in the Northern Hemisphere. Studies conducted on pollination systems in southern Africa, for example, show that plants are far more specialised, with many plants relying on a single pollinator (Manning and Goldblatt, 1996; Goldblatt and Manning, 1999; Johnson and Steiner, 2003; Geerts and Pauw, 2009; Pauw and Stanway, 2015).

Specialisation most often results when a plant's specialised phenotype restricts visitation to phenotypically specialised pollinators. The long tubular flower of *Gladiolus angustus* (Asparagales: Iridaceae), for example, makes its nectar exclusively available to the extremely long proboscid fly *Moegistorhynchus longirostris* (Diptera: Nemestrinidae) (Goldblatt and Manning, 1999). In contrast, plants with a generalist pollination phenotype are able to support visitation from a wide diversity of pollinator species and functional types (Fenster et al., 2004; Ollerton et al., 2007; Armbruster, 2017), but there are some circumstances in which phenotypically generalised plants can be relatively specialised ecologically (i.e. have a limited diversity of visitors). First, in many generalist systems, pollinator communities can vary geographically as a result of changes in pollinator composition, abundance and diversity (Price et al., 2005; Moeller, 2005, 2006; Gómez et al., 2009, Ellis et al 2021). This results in populations of the same plant species being visited by (and potentially specialising on) different visitor species/functional groups across space. Second, competition plays a key role in the evolution and ecology of generalised plant-pollinator mutualisms (Sargent and Ackerly, 2008;

Jones et al., 2012). When different pollinator species compete for the same limited resource, competitive exclusion by dominant species can displace other pollinator species (Gause, 1934; Hardin, 1960; Johnson and Bronstein, 2019). This may result in a typically generalist pollinated plant species displaying specialised pollination.

Daisies (Asteraceae) are considered a classic example of ecological and phenotypic pollination generalists, due to their open access phenotype which does not necessarily exclude visitation from any insects (Ollerton et al., 2006). However, some foragers may be filtered out as potential visitors. Some long-tongued pollinators, especially Hymenoptera and Diptera, may select against visiting daisies since foraging efficiency is strongly dependent on size matching between proboscis length and daisy tube length (Klumpers et al., 2019). Globally, daisies are visited by a wide taxonomic diversity of insects (Torres and Galetto, 2002; Figueroa-Castro and Cano-Santana, 2004; Figueroa-Castro et al., 2016; Guerrina et al., 2016; Usharani and Raju, 2018). In xeric environments like Mexico, some daisy species are visited by up to 41 insect taxa (average of 34 insect species across 4 daisy species) (Figueroa-Castro and Cano-Santana, 2004). A study in Italy found that *Berardia sunacaulis* (Asteraceae) was visited by 13 different insect taxa (Guerrina et al., 2016), and in India, one daisy species (*Synedrella nudiflora*) had up to 17 different species of insect visitors (Usharani and Raju, 2018). Torres and Galetto (2002) found an average of 13 insect visitor species across 20 daisy species in Argentina. In all of the previously mentioned studies, insect visitors belonged to between 3 and 5 insect orders, mainly Lepidoptera, Hymenoptera and Diptera, which strongly suggests that daisies are true taxonomic and functional generalists globally. Furthermore, it has been found that during visitation bouts, these visitors make contact with stigmas (Hawkeswood, 1987), depositing pollen which ultimately results in fruit set (Schmitt, 1983; Olsen, 1996; Guerrina et al., 2016; Courtice et al., 2020). While the dominant showy daisy species of Namaqualand are visited by a wide taxonomic diversity of insects (average of 15 insect species across 4 daisy species, Ellis et al (2021)), these visitation networks are dominated by a select few visitor species (de Waal et al., 2015; Kemp et al., 2019), mainly Mariobezziinae flies and monkey beetles (Johnson and Midgley, 1997; Ellis and Johnson, 2009; Ellis et al., 2021), and on very rare occasions are also visited by bees (Ellis and Johnson, 2009; Ellis et al., 2021). Most notably, however, butterflies are completely absent from these visitation networks.

In the Western Cape, daisies are of both economic and ecological importance. The West Coast of South Africa is well-known for the spring mass-flowering displays which are dominated by daisies. These mass-flowering displays attract large numbers of tourists annually, contributing greatly to South Africa’s passive nature-based tourism. Scenic appreciation, of which these mass-flowering events play an important role, makes up the majority of the Cape Floristic Region's biodiversity value (Turpie et al., 2003). Asteraceae are the most diverse angiosperm family in the Cape Floristic Region (CFR) (986 species), of which 62% are endemic to the area (Goldblatt, 1997) and are, therefore, contributing greatly to the diversity of Cape flora. Furthermore, daisies are an important food source (Larson et al., 2001), and mating and congregation site (Johnson and Midgley, 1997; Johnson and Dafni, 1998), for endemic insect species. Self-incompatible annual daisy species seem to dominate these mass-flowering displays and are therefore dependent on pollinators to persist in the community (de Waal et al., 2015). The few studies conducted on the daisy communities of Namaqualand suggest that these dominant daisy species are relatively specialised on fly pollinators (particularly Bombyliidae: Mariobezziinae, and Tabanidae: *Rhigioglossa*) (Struck, 1992, 1994a, 1994b; Johnson and Midgley, 1997; Ellis and Johnson, 2009; Kemp et al., 2019). However, these studies are focused on relatively few species and little information exists on the daisy pollination systems in the southern parts of the GCFR.

In this study, I investigate the prevalence of flies in daisy pollination across the Greater Cape Floristic Region. Firstly, I ask what the diversity and composition of flower visiting insect communities associated with spring flowering annual GCFR daisies is, and how these communities are structured across daisy genera/species and across space. If daisies are indeed generalized, I expect them to be associated with a wide taxonomic and functional diversity of visitors. I also expect limited differentiation between visitor communities associated with different daisy species within regions, but substantial compositional turnover across regions if insect communities exhibit high beta diversity, as has been demonstrated for other GCFR insect groups (Kemp et al., 2017; Kemp and Ellis, 2017). Secondly, I ask if flies are consistently the most abundant visitors to daisies across the GCFR. Based on the limited number of existing studies of GCFR daisy pollination (Struck, 1994; Kemp et al., 2019; Ellis et al., 2021), I expect flies to be the most abundant visitors to daisies.

Methodology

Study system

This study took place during spring 2020 and 2021 in the Western Cape Province of South Africa. Sampling was conducted in three regions with mass flowering spring annual daisy displays, namely; 1) the Langebaan-Velddrif region ("West Coast"), 2) the Breede river valley in the Worcester vicinity ("Breede Valley") and 3) the Olifants river valley between

Clanwilliam and Citrusdal ("Olifants Valley") (Figure 2.1). Sites that contained one or more members of the dominant daisy genera constituting the spring flowering displays (*Arctotheca*, *Arctotis*, *Gazania*, *Ursinia* and *Dimorphotheca*) were randomly selected. Spatially structured variants (i.e., floral morphotypes) within each daisy species were treated as unique taxa and therefore, the terms "species" and "morphotype" may be used interchangeably within this study. In 2020, I randomly selected ten sites in each of the previously mentioned regions with the primary goal of assessing regional variation in the daisy visiting insect community. In 2021, I resampled at a select few sites across the West Coast in order to assess changes in the insect community across years. I also sampled an additional 15 sites in the West Coast (25 in total) which were chosen in order to allow evaluation of divergence in insect communities across daisy species/morphotypes in this region. In total, I sampled at 45 sites across the Western Cape.

A total of 15 daisy species belonging to 6 genera, of which 5 species comprised multiple phenotypically distinct floral morphotypes were sampled across all three regions. Members of *Dimorphotheca cuneata*, *Gazania krebsiana*, *Arctotis breviscapa* and *Arctotheca prostrata* are perennials while all other species are annuals. Floral forms of species such as *Dimorphotheca sinuata* (7 forms), *Dimorphotheca pluvialis* (2 forms), *Arctotheca calendula* (2 forms), *Arctotis flaccida* (2 forms) and *Arctotis hirsuta* (2 forms) were treated as unique taxa.

Sampling

To quantify visitor communities on dominant annual daisy species/morphotypes at each site, a walked survey approach was used (Ellis and Johnson, 2009, Ellis et al 2021). On sunny days when inflorescences were open and pollinators were active (between 10.00 and 16.00), two observers walked transects through a daisy population, inspecting every inflorescence encountered for insect visitors. These surveys were carried out systematically, sampling 20 inflorescences at a time in order to survey a 1000 inflorescences per species of each targeted daisy species at each site. Individuals of each insect visitor morphospecies were counted and two representatives of each were captured. Pinned insects were sorted to morphospecies and identified to family level. Bombyliidae and Tabanidae were identified to genus level using family keys (Chainey, 2017; Evenhuis and Lamas, 2017a). Hymenoptera were grouped into bee and wasp taxa. All incidental non-flower feeding insects such as Hemiptera and Orthoptera, as well as any immature insects were excluded from analysis. Voucher specimens of all insect visitors are housed at department of Botany and Zoology at Stellenbosch University.

Data analysis

1) What is the diversity and composition of flower visitor communities associated with GCFR daisies, and how are these communities structured across daisy genera and across regions?

First, I explored visitor richness and diversity at the population level to determine how specialised the individual daisy populations are. I calculated the observed insect species richness, visitor abundance (number of visitors per 1000 flowers) and visitor alpha diversity for each daisy population. Alpha diversity was calculated based on Hill numbers of Shannon diversity index because it is not biased towards rare or common species (Jost, 2007). I then tested my sampling effort by constructing rarefaction curves (the number of species as a function of the number of samples) and calculated Chao expected richness for each population sampled. Samples of 20 consecutive inflorescences inspected for visitors were used as the sampling unit for constructing rarefaction curves. To illustrate where the sampled daisy populations fall along the specialisation continuum, frequency histograms of the visitor richness, Chao estimated richness and alpha diversity results were constructed. I then used ANOVAs to test for differences in population-level visitor richness, abundance and alpha diversity across 1) daisy species/morphotype where three or more populations were sampled, 2) daisy genera which were sampled at three or more populations, and 3) across regions. I also estimated the richness of visitors to morphotypes/species of daisies where multiple populations were sampled using rarefaction curves (one sample = 20 sampled inflorescences) and the Chao diversity estimator. Species-level curves were compared to population-level rarefaction curves to assess the extent to which community richness estimates at the species-level are a function of increased sampling or population-level turnover of the associated visitor communities.

Thereafter, I compared the visitor community changes across daisy genera and regions. First, insect community beta diversity was calculated across each daisy population using the "hillR" package in RStudio (Li, 2018). These results were then used to determine the Horn similarity index which quantifies the overlap of effective visitor species between sampling units (Tuomisto, 2010). This index is defined as:

$$^{1}D_{\beta} = \frac{(ln2 - H_{\beta Shan})}{ln2}$$

where $H_{\beta Shan}$ is Shannon beta diversity based on Hill numbers. Values of 1 indicate complete similarity in visitor communities across daisy populations.

To test the expectation that generalist daisies should make use of different visitor communities across space, I used Mantel tests to examine changes in visitor community beta diversity within daisy genera with increasing geographic distance. I then calculated Horn similarity of the insect communities that visit daisy populations within and between both daisy genus and region. To determine whether the turnover in visitor communities across regions is substantially greater than between insect communities associated with different daisy genera, a Kruskal-Wallis test was used to compare the beta diversity of visitor communities associated with daisy populations within and between daisy genera and regions. I also compared the composition of flower visitors across regions and daisy genera by conducting a permutational multivariate analysis (PERMANOVA) of the Bray-Curtis similarity measure in R statistical software (R Core Team, 2020) using the package "vegan" (Oksanen et al., 2020). The test was permutated 999 times and region and daisy genus were used as predictors. A post-hoc pairwise analysis was then conducted using the packages "devtools" (Wickham et al., 2021) and "pairwiseAdonis" (Martinez Arbizu, 2020) to test which regions and daisy genera were significantly different. To visualise the patterns of similarity in the visitor communities across regions and daisy genus, a non-metric multi-dimensional scale (NMDS) plot was constructed using the Bray-Curtis similarity index.

Lastly, I explored whether plant species exhibit differing levels of specialisation and whether species/morphotypes are associated with different visitor communities. Since most of the samples were collected in the West Coast region, I only used data from the West Coast daisy sites in this analysis in order to evaluate species-level differences in the visitor communities without any region effects. First, the daisy visitor compositional structure was compared across daisy species/morphotypes that were sampled across 3 or more populations using a PERMANOVA of the Bray-Curtis similarity measure. The test was permuted 999 times and daisy species/morphotype was used as the predictor. Thereafter, a post-hoc analysis was run to test which daisy species/morphotypes were visited by significantly different insect assemblages. An NMDS plot was constructed to visualise these patterns. Then, I calculated the Horn similarity across insect visitor communities associated with 1) repeat samples taken from the same daisy population, 2) different populations of the same daisy species/morphotype, 3) different daisy species/morphotypes at the same site, and 4) different daisy species/morphotypes across different sites. I compared the Horn similarities across these group using a Kruskal-Wallis test to examine whether the insect visitor communities associated with each daisy species/morphotype differs across space.

2) Are flies consistently the most abundant visitors to GCFR daisies?

First, I determined the relative abundance of all the visitor groups (visitor group abundance/total abundance) to each daisy genus sampled and constructed a stacked bar graph to visualise the relative importance of different insect groups. I then ran an ANOVA on relative fly abundance (fly abundance/total abundance) to test for significant differences across region, daisy genus and species. Thereafter, a post hoc test of the daisy genus variable was conducted to determine which daisy genera differ in their interaction with flies. Second, I determined the visitation density of flies (number of individuals per flower) and ran an ANOVA to test whether the visitation of flies differs significantly across daisy genera, daisy species and across regions.

Results

1) What is the diversity and composition of flower visiting communities associated with GCFR daisies, and how are these communities structured across daisy genera and across regions?

A total of 119 unique pollinator morphospecies (9545 individuals) were recorded during surveys of 78 625 capitula across all three regions (Table 2.1). A total of 95 morphospecies (7307 individuals) were caught in the West Coast, 33 morphospecies (1108 individuals) in the Breede Valley and 29 morphospecies (1130 individuals) in Olifants Valley. Monkey beetles were by far the most speciose group of visitors (31 morphospecies), followed by melyrid beetles (27 morphospecies), while bibionid flies only comprised of one morphospecies. Interestingly, both Empidid flies and wasps were present in the West Coast, but completely absent from both the Breede Valley and Olifants Valley regions. Overall, nitidulid beetles were the most abundant visitors (3213 individuals), followed by bombyliid flies and melyrid beetles (2001 and 1657 individuals respectively). Moreover, bees were the least abundant group of visitors in each region.

Local ecological specialisation

The mean visitor abundance per flower ranged from 0.03 for *Dimorphotheca pinnata* to 0.27 for *Dimorphotheca sinuata kardoesie* (mean = 0.12 (±0.02), Table 2.2). The mean species richness of visitors on daisy populations ranged from 3 for *Dimorphotheca cuneata* to 13.25 for *Dimorphotheca pluvialis candles* (overall mean = 7.67 (±0.57)). Chao richness estimators were substantially higher ($3 < x < 30.8$, mean = 11.04 (±1.33)), indicating that visitor communities comprise many rarely encountered taxa. Alpha diversity estimates of effective species numbers were lower ($1.67 < x < 5.85$, mean = 3.95 (±0.25)), again suggesting that the distribution of individuals across insect visitor species is uneven.

A frequency histogram of the Chao richness for each daisy population is skewed to the right, showing that the majority of daisy populations are associated with insect communities with a Chao estimate of between 5 and 10 (Figure 2.2). While there was no significant difference in the estimated Chao richness across daisy genera (ANOVA, $df = 4$, $F = 2.26$, $p = 0.07$), there was a significant difference across daisy species (ANOVA, $df = 28$, $F = 2.33$, $p < 0.05$). Post-hoc analysis found that *Dimorphotheca pluvialis candles* is significantly different from *D. pluvialis whiteS* ($p < 0.05$). The visitor abundance did not differ significantly across daisy genera (ANOVA, $df = 4$, $F = 2.38$, $p = 0.06$), or across daisy species (ANOVA, $df = 8$, $F = 1.5$, $p = 0.19$).

A frequency histogram of the alpha diversity results for each daisy population is clearly skewed to the right, showing that while the sampled daisy populations are associated with insect communities with an alpha diversity between 1 and 6, with the majority of the daisy populations are associated with a lower alpha diversity (between 3 and 5) of insect visitors (Figure 2.2). There was no significant difference in the alpha diversity of visitors to different daisy genera (ANOVA, $df = 4$, $F = 1.77$, $p = 0.15$) and daisy species (ANOVA, $df = 8$, $F = 0.81$, $p = 0.6$). Furthermore, most daisy populations had less than eight visitor morphospecies (Figure 2.2). There was no significant difference in the visitor morphospecies richness across daisy genera (ANOVA, $df = 4$, $F = 2.52$, $p = 0.05$) or daisy species (ANOVA, $df = 8$, $F = 1.37$, $p = 0.24$).

ANOVA analysis of the population-level differences across regions found that there was a significant difference in the visitor morphospecies richness (ANOVA, $df = 2$, $F = 11.86$, $p < 0.001$), estimated Chao visitor richness (ANOVA, $df = 2$, $F = 13.89$, $p < 0.001$) and alpha diversity (ANOVA, $df = 2$, $F = 7.22$, $p < 0.01$) across regions. All metrics were significantly higher for West Coast daisy populations than for those in the Breede or Olifants valleys ($p < 0.05$).

In the majority of populations where 1000 inflorescences were sampled, the rarefaction curves reached saturation suggesting that the sampling effort at the population level was sufficient to capture all common visitor species (Appendix A). Furthermore, Chao estimated richness was similar to or slightly greater than the observed richness for most populations (Appendix A), suggesting that a few rare species were not captured during sampling.

Range wide specialisation

While population richness is relatively low (mean = 8.61 visitors, Table 2.2), the richness of the community of visitors across the range of a daisy species is much higher (mean = 27.78 (±3.35) visitors, Table 2.3). The species rarefaction curves are not just an extension of the population-level curves (Appendix B), suggesting that the higher richness range-wide is not a sampling effect but likely results from spatial turnover in the insect community.

Visitor community composition changes across daisy genera and regions

Mantel tests revealed that there was a significant positive correlation between geographic distance and population visitor beta diversity for *Dimorphotheca*, *Arctotheca* and *Ursinia* (Table 2.4), suggesting that geographically separated populations associate with more dissimilar pollinator communities than populations that are close together. Overall, turnover in insect visitor community composition between the sampled daisy populations was high (Figure 2.3). Insect community similarity was highest within regions, whether comparing daisy populations from the same or different genera (Figure 2.3). Insect communities associated with daisy populations in different regions were significantly more dissimilar and this was particularly the case when comparing across daisy genera (Figure 2.3).

Insect visitor composition was significantly influenced by region (PERMANOVA, $F = 3.663$, $p < 0.01$, Figure 2.4), with the West Coast being significantly different from both Olifants Valley ($F = 4.57$, $p < 0.01$) and Breede Valley ($F = 3.448$, $p < 0.01$). PERMANOVA results also suggest that visitor composition varies across daisy genera ($F = 1.366$, $p < 0.01$), however these differences are less obvious (Figure 2.4). Pairwise post-hoc analysis shows that *Dimorphotheca* is significantly different from *Arctotis* ($F = 2.263$, $p < 0.05$) and *Arctotheca* ($F = 2.234$, $p < 0.05$).

Visitor community composition changes across daisy species

PERMANOVA showed that daisy species ($F = 1.7$, $p < 0.01$) has a significant effect on visitor composition in the West Coast region (Figure 2.5). Post hoc analysis shows that the insect visitor community associated with *Dimorphotheca pluvialis candles* is significantly different from the insect community associated with *Arctotis hirsuta ring* ($F = 3.15$, $p < 0.05$). A nitidulid beetle species (NIT1) is shown to be most influential in distinguishing the visitor composition of *Dimorphotheca pluvialis candles* apart from *Arctotis hirsuta ring* (Figure 2.5).

Horn similarity varied significantly across site by species comparison categories (Kruskal-Wallis, chi-squared = 26.152, df = 3, p < 0.001). The insect visitor communities from repeat sampling (across years) of the same daisy species at the same site were significantly more similar than comparisons of the same (p < 0.001) or different daisy species across sites (p < 0.001), although they were not significantly more similar than insect communities associated with different daisy species co-flowering at the same site (Figure 2.6). Insect community similarity was significantly higher when comparing populations of the same daisy species across sites than when comparing different daisy species (p < 0.001; Figure 2.6).

2) Are flies consistently the most abundant visitors to GCFR daisies?

Relative importance of different pollinator groups

All daisy genera received visits from most insect groups; however, each visitor group differed in their relative visitation. The relative visitor abundance of insect groups to *Arctotis* is more evenly abundant. *Dimorphotheca* daises were dominated by flies and received more Bombyliid fly visits compared to other groups, while *Gazania* populations are visited by more Tabanid flies (Figure 2.7). Beetles are also dominant visitors to all daisy genera, of which nitidulid and melyrid beetles are most abundant. *Ursinia* populations are visited more by monkey beetles (Scarabaeidae: Hopliini) compared to other daisy genera. Furthermore, bees were found to visit *Arctotheca* and *Arctotis* populations, with very low abundances.

Importance of flies

Both fly visitation rate (mean = 0.05 flies per flower) and relative fly abundance (mean = 0.34) is highest in the West Coast (Figure 2.8). Flies made up a similar percentage of total visitors in Olifants Valley (mean = 0.26) and Breede Valley (mean = 0.259), however fly visitation rate in Olifants Valley was lower than Breede Valley (mean = 0.018 and 0.026, respectively). There was, however, no significant difference in relative fly abundance (Kruskal-Wallis, chi-squared = 1.855, p = 0.396) or fly visitation rate (Kruskal-Wallis, chi-squared = 4.033, p = 0.133) between regions.

Dimorphotheca daisies received the highest fly visitor relative abundance (fly abundance/total visitor abundance) compared to other daisy genera, while *Arctotheca* received the lowest

(Figure 2.9). There was a significant genus effect on fly visitor abundance (Kruskal-Wallis, chi-squared = 10.02, df = 4, $p < 0.05$). Post-hoc pairwise comparisons found the fly visitor abundance to *Dimorphotheca* to be significantly different from both *Arctotheca* ($p < 0.05$) and *Arctotis* ($p < 0.01$). Furthermore, the fly visitation rate (number of individuals per flower) was highest for *Arctotis*. Although, *Ursinia* populations are associated with the second highest relative fly abundance, it receives the lowest fly visitation rate compared to other daisy genera (Figure 2.9). There was a significant daisy genus effect for fly visitation rate (Kruskal-Wallis, chi-squared = 12.33, df = 4, $p < 0.05$). Fly visitation rate to *Dimorphotheca* was significantly different from *Arctotheca* ($p < 0.05$) and *Ursinia* ($p < 0.01$).

On average flies made up 75% of the visits to *Dimorphotheca pluvialis whiteS*, and 50% of the visits to *Dimorphotheca pluvialis cape town* populations (Figure 2.10). While flies made up around 45% of the visits to *Ursinia sp1*, their visitation rates are lowest (mean < 0.01 flies per flower). Flies had the highest visitation rate to *Arctotheca calendula langebaan* (mean = 0.09 flies per flower), followed closely by *Dimorphotheca pluvialis candles* (mean = 0.08 flies per flower). Daisy species did not have a significant effect on either relative fly abundance (ANOVA, F = 1.622, df = 6, $p = 0.18$) or fly visitation rate (Kruskal-Wallis, chi-squared = 7.871, df = 6, $p = 0.248$).

Discussion

1) What is the diversity and composition of flower visiting communities associated with GCFR daisies, and how are these communities across daisy genera and across regions?

Previous studies on the daisy-pollinator interactions in the Greater Cape Floristic Region (GCFR) have been limited to Namaqualand and have found that daisy pollination is unusually specialised (Johnson and Midgley, 1997; Kemp et al., 2019; Ellis et al., 2021). In contrast, several lines of evidence from this study suggest that daisies in the Western Cape exhibit more generalised pollination typical of daisies globally. Daisy populations of the Western Cape are associated with a wide taxonomic diversity of insect visitors (a mean of eight insect species from up to three insect orders). Namaqualand daisies are visited almost exclusively by Mariobezziinae flies (*Corsomyza* and *Megapalpus*) with less frequent visits by beetles and bees (Struck, 1994a; Johnson and Midgley, 1997; Ellis and Johnson, 2009). In other areas of the world, daisies are commonly pollinated by multiple insect species, from a variety of insect orders, mainly Hymenoptera, Diptera, Coleoptera and Lepidoptera (between 13 and 41 insect species per daisy species) (Torres and Galetto, 2002; Basteri and Benvenuti, 2010; Guerrina et al., 2016; Usharani and Raju, 2018; Courtice et al., 2020). While CFR daisies exhibit somewhat generalised pollination typical of daisies globally, the striking result from this study is the absence of Lepidoptera and the very limited contribution of Hymenoptera. In this respect, the community of visitors in the GCFR differs strikingly from the global understanding of daisy pollination, and the absence of these groups, which globally are most frequent daisy pollinators, perhaps render the GCFR daisy pollination less generalised taxonomically.

Spatial variation in pollinator communities is characteristic of generalist pollinated plants (Moeller, 2005, 2006; Price et al., 2005; Gómez et al., 2014). This study provides evidence for strong spatial variation in the insect visitor communities at various spatial scales. Different populations of the same daisy species/morphotype are associated with different insect communities (i.e., there is high spatial turnover within species) and thus range-wide species richness estimates (i.e., gamma diversity) are also high. Turnover in the visitor communities visiting the same genera across regions is also greater than the turnover between daisy genera within regions. This is likely a reflection of changes in visitor assemblage structure and abundances across space (Price et al., 2005; Ellis et al., 2021), since insect community turnover increases with an increase in distance. Herbivorous insect communities have previously been found to exhibit high levels of turnover across short spatial scales in the CFR (Kemp et al., 2017; Kemp and Ellis, 2017). This high insect beta diversity was found to be strongly influenced by plant turnover across local scales, but not at regional scales (Kemp and Ellis, 2017). Since the same daisy genera are represented in each region and region-level turnover in insect visitors is greater than genus-level turnover, this is unlikely to be a reflection of plant turnover across regions and is perhaps a response to the same biogeographical factors within each region (McGlynn, 2010; Kemp and Ellis, 2017).

The Horn similarity between daisy species within a site was not significantly different from repeat samples within the same population, providing some evidence that daisies are using different subsets of the same visitor community due to competition among pollinators and/or plants for the same resource (Johnson and Bronstein, 2019), an expectation under generalism. However, there was a significant difference in the visitor community composition across daisy species in the West Coast region, and Horn similarities in the insect visitor communities across daisy species were low overall, showing that there is high differentiation in the visitor communities associated with different daisy species. While all daisy genera are visited by most insect pollinator groups, beetles seem to dominate visitation to orange and yellow daisies, and flies were more abundant on white daisies. Furthermore, bees were found to only visit *Arctotheca* and *Arctotis* daisies. Some daisy visitors, such as monkey beetles (Colville et al., 2002), Mariobezziinae flies (Ellis et al., 2021) and honey bees (Hill et al., 1997), show innate colour preferences, suggesting that while the daisy morphology does not exclude visitation from certain pollinators, certain daisy species may still be selected against by some insect pollinators.

2) Are flies consistently the most abundant visitors to GCFR daisies?

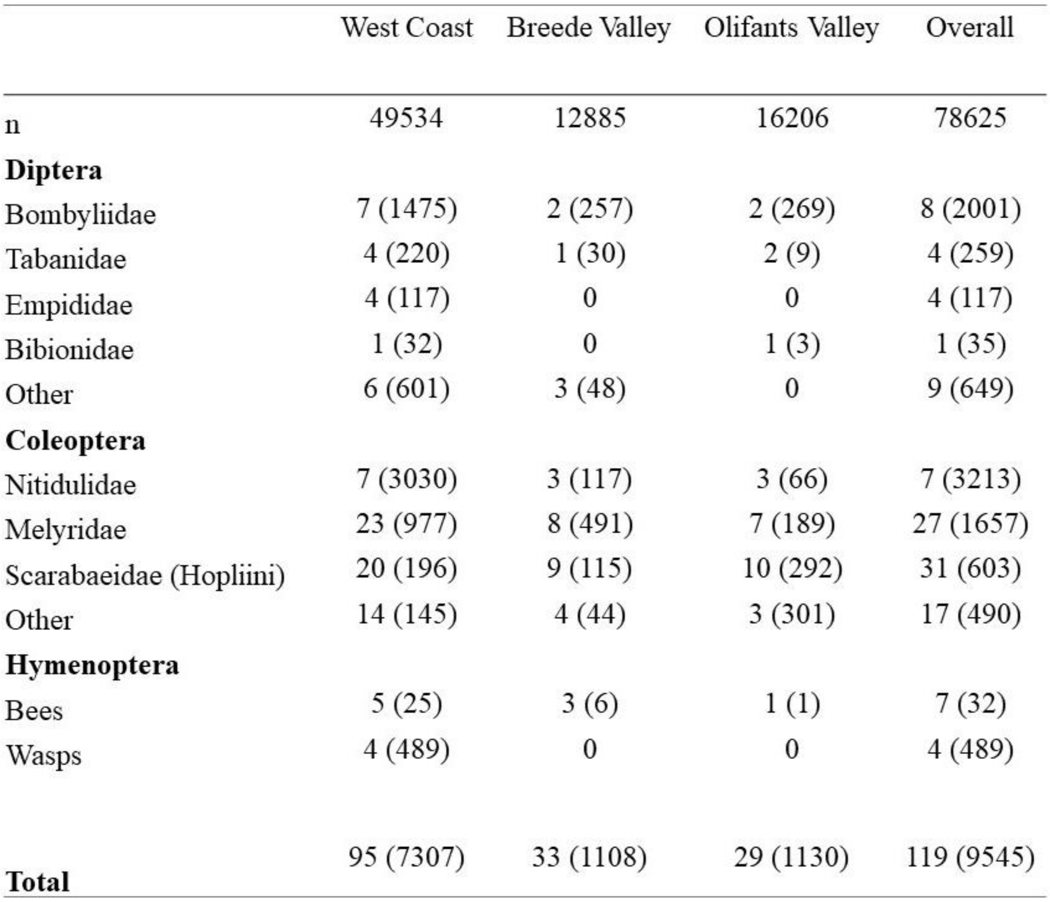
Daisy populations in the West Coast received the highest fly visits and relative fly abundance. This may be due to the fact that *Dimorphotheca* daisies, which dominated West Coast sites, received the greatest fly abundance. This is not surprising since a previous study showed that flies dominate visitation to *Dimorphotheca* in Namaqualand (Ellis et al., 2021). Ultimately, though, flies contributed less frequently to daisy visitation than beetles across the genera and regions sampled here. This contrasts to the dominance of Mariobezziinae beeflies as well as *Rhigioglossa* horseflies in Namaqualand (Kemp et al., 2019). Interestingly though, while butterflies and bees have been found to be important visitors to daisies in other Mediterranean and arid areas globally (Schmitt, 1983; Boldt and Robbins, 1987; Freitas and Sazima, 2006; Figueroa-Castro et al., 2016;), bees represented the lowest proportion of insect visitation and Lepidopteran visitors were completely absent as visitors to the focal daisy species in the study. This is unsurprising in the GCFR context, since previous studies investigating the visitor diversity of Namaqualand daisies show that butterflies do not visit the dominant showy daisy species and bees represent a small proportion of visitors (Johnson and Midgley, 1997; Ellis and Johnson, 2009; Ellis et al., 2021). While it is important to note that in these studies, only the dominant showy daisy species were investigated, which ultimately is a small subset of the total Cape daisy species, in the global context, this is certainly an unusual case, since the daisy phenotype does not necessarily exclude visitation from certain pollinators (Ollerton et al., 2006). This pattern may be a result of some unique circumstances which exist within the Cape Floristic Regions (CFR). Firstly, the CFR contains an extremely high plant diversity (Goldblatt, 1997; Cowling et al., 1998) packed densely into a relatively small area with most of these plant species flowering in spring. This overload of options for insect pollinators may have resulted in butterflies and bees forming more rewarding plant-pollinator interactions with other flowering plants. For example, it has been found that generalist bees avoid foraging on daisies due to the unfavourable and protective properties of daisy pollen which makes its digestion difficult for bees (Muller and Kuhlmann, 2008). Furthermore, some specialist bees may be filtered out as potential visitors due to low foraging efficiency as a result of poor size matching between proboscis length and floret tube length (Klumpers et al., 2019). Secondly, insect herbivory is generally low in fynbos due to the low nutrient content and palatability of the sclerophyllous leaves (Morrow, 1983; Cottrell, 1985). In fact, Cottrell (1985) suggests that the low foraging quality of fynbos underpins the relatively low butterfly abundance in the Cape due to low larval food plant nutrition (Anderson et al., 2014). Lastly, *Corsomyza* and *Megapalpus* flies are endemic to southern Africa (Evenhuis and Lamas, 2017b), which may have resulted in a case of competitive exclusion, with bombyliid flies establishing themselves as the main flying insect pollinator of daisies.

Conclusion

This study provides the first description of the insect pollinator diversity associated with spring mass-flowering daisy communities across the Western Cape. While previous evidence suggests that the GCFR daisy-pollinator interactions are unusually specialised, this study shows that daisy pollination in the Western Cape is relatively generalised, although the composition of visitors is unusual. Visitor communities are dominated by flies and beetles, with a striking absence of bees and butterflies. Further assessment of the pollination effectiveness of these dominant pollinators is required to fully understand their importance in the maintenance of daisy diversity in the Western Cape.

Tables

Table 2.1: Morphospecies richness (and abundance) of insect visitors to daisies in the West Coast, Breede Valley and Olifants Valley regions, as well as overall. Sample sizes (n) refers to the number of inflorescences inspected in each region.



	West Coast	Breede Valley	Olifants Valley	Overall
n	49534	12885	16206	78625
Diptera				
Bombyliidae	7 (1475)	2 (257)	2 (269)	8 (2001)
Tabanidae	4 (220)	1 (30)	2 (9)	4 (259)
Empididae	4 (117)	0	0	4 (117)
Bibionidae	1 (32)	0	1 (3)	1 (35)
Other	6 (601)	3 (48)	0	9 (649)
Coleoptera				
Nitidulidae	7 (3030)	3 (117)	3 (66)	7 (3213)
Melyridae	23 (977)	8 (491)	7 (189)	27 (1657)
Scarabaeidae (Hopliini)	20 (196)	9 (115)	10 (292)	31 (603)
Other	14 (145)	4 (44)	3 (301)	17 (490)
Hymenoptera				
Bees	5 (25)	3 (6)	1 (1)	7 (32)
Wasps	4 (489)	0	0	4 (489)
Total	95 (7307)	33 (1108)	29 (1130)	119 (9545)

Table 2.2: The mean population visitor richness, estimated chao, visitor abundance and alpha diversity (based on hill numbers) (±SE) for each plant species/floral morphotype sampled across regions. Number of populations sampled (n) for each daisy species are also shown.

	N	Species richness	Chao	Abundance per flower	Alpha diversity
Dimorphotheca					
Dimorphotheca cuneata	1	3	3	0.08	1.67
Dimorphotheca pinnata	2	3.5 (±0.5)	3.75 (±0.75)	0.03 (±0.01)	2.3 (±0.62)
Dimorphotheca pluvialis cape town	4	8.25 (±2.63)	8.84 (±2.53)	0.18 (±0.06)	3.25 (±0.86)
Dimorphotheca pluvialis whiteS	5	6.8 (±1.62)	8.04 (±2.76)	0.07 (±0.01)	3.87 (±0.9)
Dimorphotheca pluvialis candles	4	13.25 (±1.97)	30.8 (±8.45)	0.21 (±0.04)	3.6 (±0.21)
Dimorphotheca sinuata kardoesie	1	8	8.163	0.27	2.52
Dimorphotheca pluvialis mulberry	7	9.71 (±1.64)	14.28 (±2.64)	0.11 (±0.03)	4.8 (±0.62)
Dimorphotheca sinuata naartjie	1	6	6.99	0.06	3.37
Dimorphotheca sinuata peachBotter	2	7 (±1)	7.24 (±0.76)	0.05 (±0.02)	4.58 (±0.33)
Dimorphotheca sinuata peachGifberg	1	4	4	0.04	2.78
Dimorphotheca sinuata tricolor	2	8.5 (±1.5)	11.45 (±4.45)	0.08 (±0.02)	5.09 (±1.2)
Arctotheca					
Arctotheca calendula	9	8.22 (±1.37)	10.75 (±2.16)	0.1 (±0.02)	3.97 (±0.77)
Arctotheca calendula langebaan	3	8.33 (±1.45)	15.03 (±6.88)	0.15 (±0.11)	5.62 (±1.27)
Arctotheca prostrata	1	6	6.98	0.12	3.01
Arctotis					
Arctotis breviscapa	2	11.5 (±5.5)	13.11 (±6.86)	0.1 (±0.06)	5.85 (±2.76)
Arctotis flaccida no ring	1	8	17.71	0.09	5.73
Arctotis flaccida ring	1	11	18.79	0.43	4.41
Arctotis hirsuta no-ring	2	12 (±2)	18.89 (±2.79)	0.21 (±0.07)	5.56 (±0.27)
Arctotis hirsuta ring	7	10.29 (±1.41)	15.39 (±2.14)	0.17 (±0.07)	5.03 (±1.07)
Ursinia					
Ursinia cakilefolia	2	5.5 (±1.5)	5.99 (±1.99)	0.05 (±0.003)	3.7 (±1.91)
Ursinia sp1	3	6.67 (±2.91)	8.63 (±3.83)	0.04 (±0.03)	3.69 (±0.86)
Ursinia sp2	1	5	6.98	0.05	3.71
Gazania					
Gazania krebsiana	3	6 (±1.73)	9.27 (±4.05)	0.07 (±0.04)	2.84 (±0.89)

Table 2.3: The mean population-level species richness and estimated Chao results, compared to the species-level (or range-wide) species richness and estimated Chao for each of daisy species with multiple sampled populations. Sample size (n) refers to the number of populations sampled for each daisy species.

	n	Population-level species richness	Species-level species richness	Population-level Chao	Species-level Chao
Dimorphotheca					
Dimorphotheca pluvialis cape town	4	8.25 (±2.63)	19	8.84 (±2.53)	19.9 (±1.46)
Dimorphotheca pluvialis whiteS	5	6.8 (±1.62)	17	8.04 (±2.76)	26.95 (±10.22)
Dimorphotheca sinuata candles	4	13.25 (±1.97)	40	30.8 (±8.45)	106.45 (±49.21)
Dimorphotheca sinuata mulberry	7	9.71 (±1.64)	36	14.28 (±2.64)	71.92 (±33.33)
Arctotheca					
Arctotheca calendula	9	8.22 (±1.37)	25	10.75 (±2.16)	30.98 (±6.46)
Arctotheca calendula langebaan	3	8.33 (±1.45)	20	15.03 (±6.88)	47.78 (±21.2)
Arctotis					
Arctotis hirsuta ring	7	10.29 (±1.41)	36	15.39 (±2.14)	80.89 (±30.2)
Ursinia					
Ursinia sp1	3	6.67 (±2.91)	18	8.63 (±3.83)	42.39 (±30.91)
Gazania					
Gazania krebsiana	3	6 (±1.73)	12	9.27 (±4.05)	14.24 (±3.38)

Table 2.4: Mantel test results showing increasing dissimilarity of insect visitor communities with increasing geographic distance between populations. Significant p-values in **bold** and n refers to the number of sampled populations.

Daisy genus	n	Mantel statistic r	P-value
Dimorphotheca	20	0.259	**0.001**
Arctotheca	12	0.363	**0.011**
Arctotis	7	0.267	0.066
Ursinia	3	0.469	**0.022**
Gazania	3	-0.387	0.667

Figures

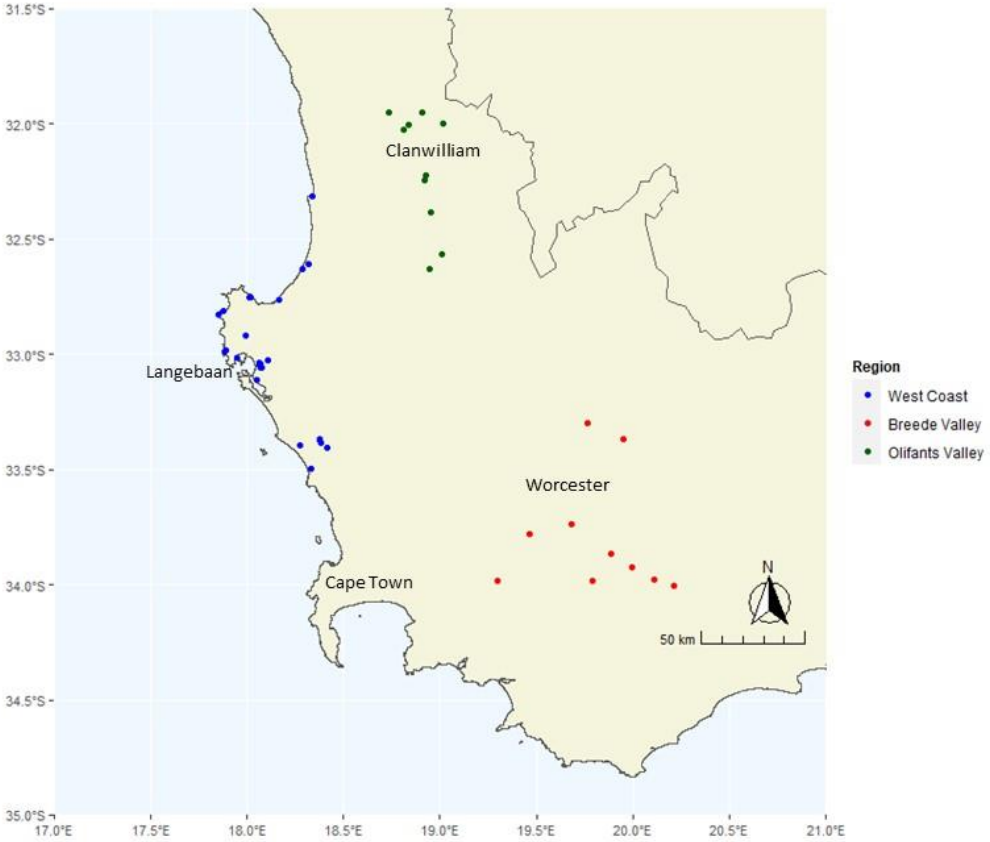


Figure 2.1: A map showing the location of study sites across the Greater Cape Floristic Region. The West Coast region contain 25 sites, and the Breede Valley and Olifants Valley regions both contained 10 sites each.

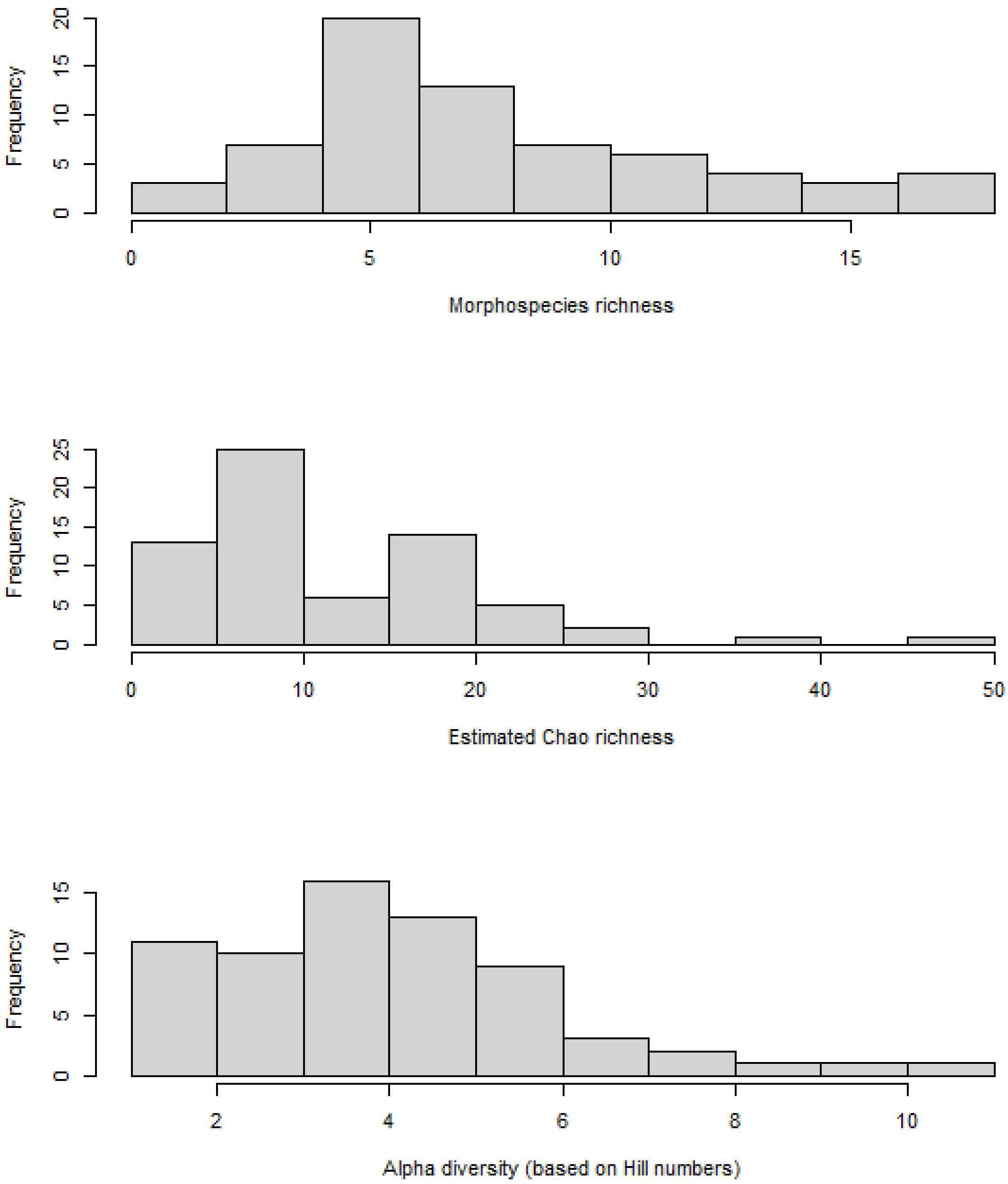


Figure 2.2: Frequency histogram of the visitor morphospecies richness, estimated Chao richness and alpha diversity (effective species) for each daisy population sampled.

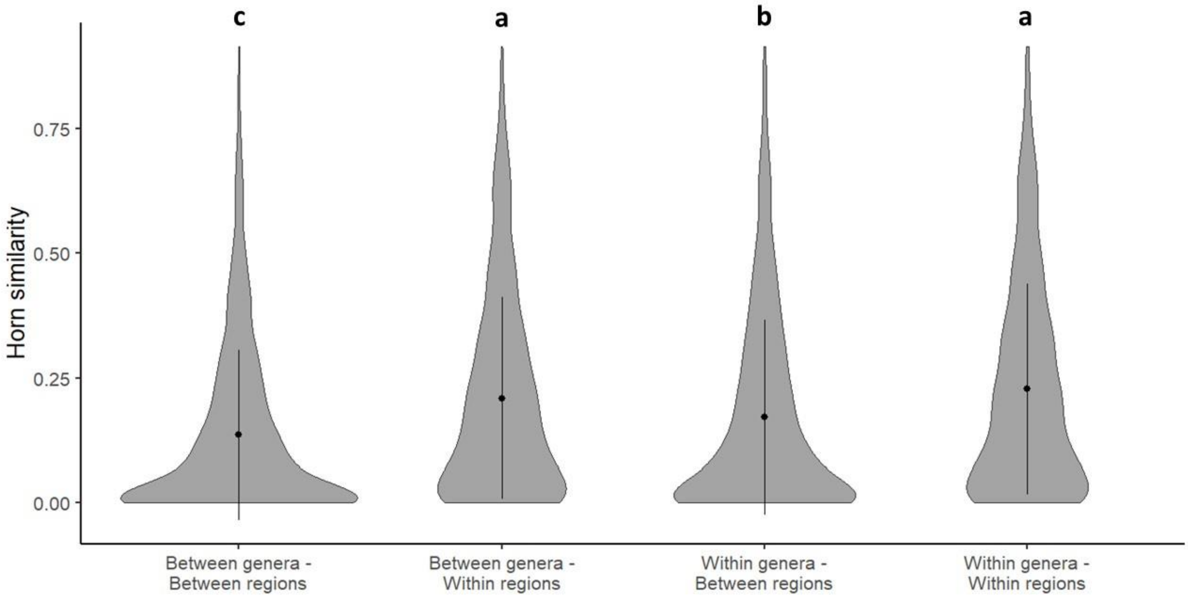


Figure 2.3: Horn similarity of insect visitor communities between and within regions and daisy genera. Index values of 1 indicate complete overlap in visitor communities between daisy populations. Black dots represent means and black lines represent standard deviation. Letters represent significant differences in the Horn similarity from Kruskal-Wallis analysis. The shape of each plot shows the frequency distribution of the population beta diversity data.

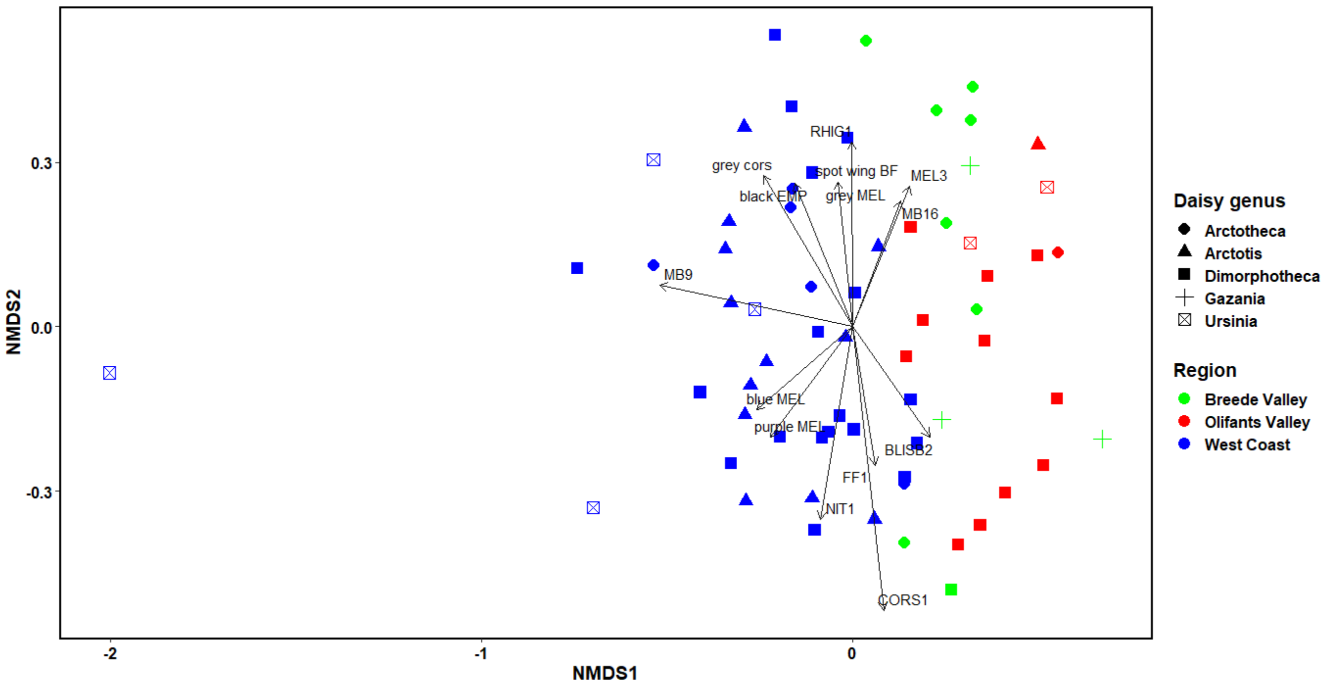


Figure 2.4: A non-metric multidimensional scale (NMDS) plot showing the insect visitor composition to daisy genera across the three regions. Those insect morphospecies that distinguish visitor communities from one another are also shown. RHIG = *Rhigioglossa* sp, MEL = Melyridae sp, MB = Hopliini sp, BLISB = Meloidae sp, CORS = *Corsomyza* sp, NIT = Nitidulidae sp, FF = fruit fly.

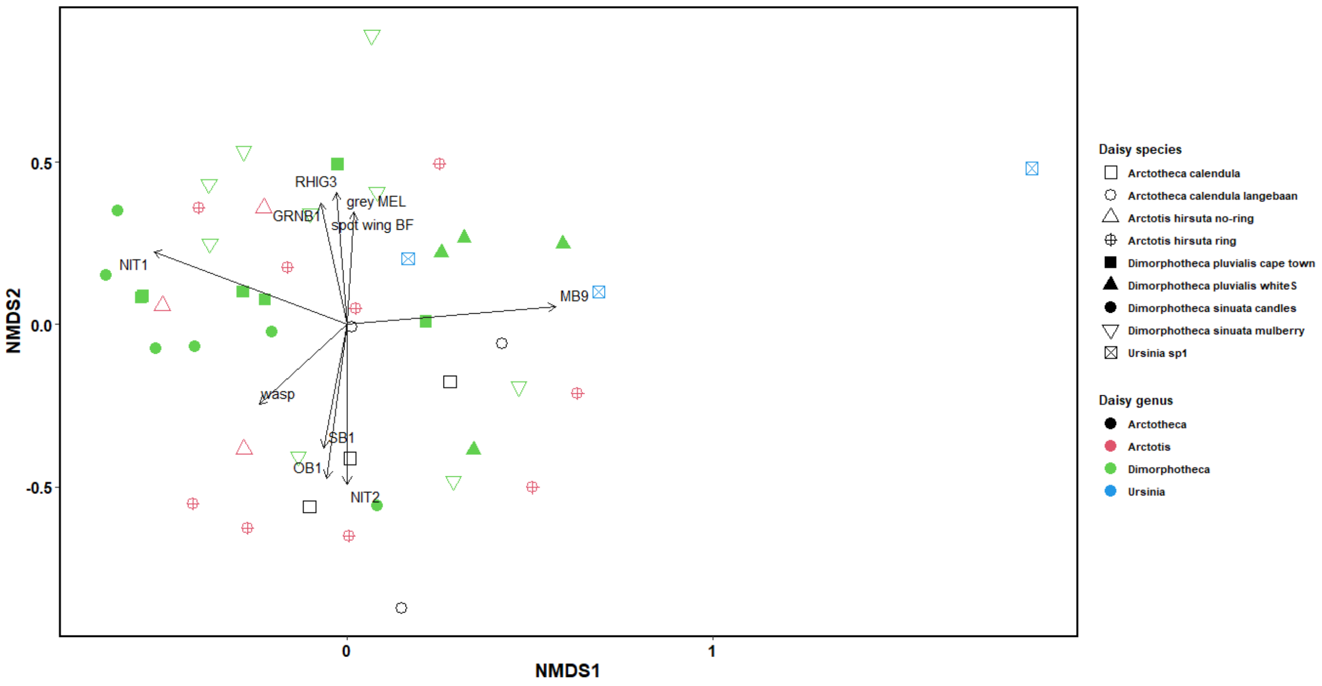


Figure 2.5: A non-metric multidimensional scale (NMDS) plot showing the insect visitor composition to daisy species where multiple populations were sampled in the West Coast. Those insect morphospecies that distinguish visitor communities from one another are also shown. CORS = *Corsomyza* sp, RHIG = *Rhigioglossa* sp, EMP = Empididae sp, GRNB = Melyridae sp, STRIPB = Melyridae sp, grey MEL = Melyridae sp, NIT = Nitidulidae sp, MB = Hopliini sp, OB = Nitidulidae sp, SB = Nitidulidae sp, spot wing BF = Bombyliidae sp.

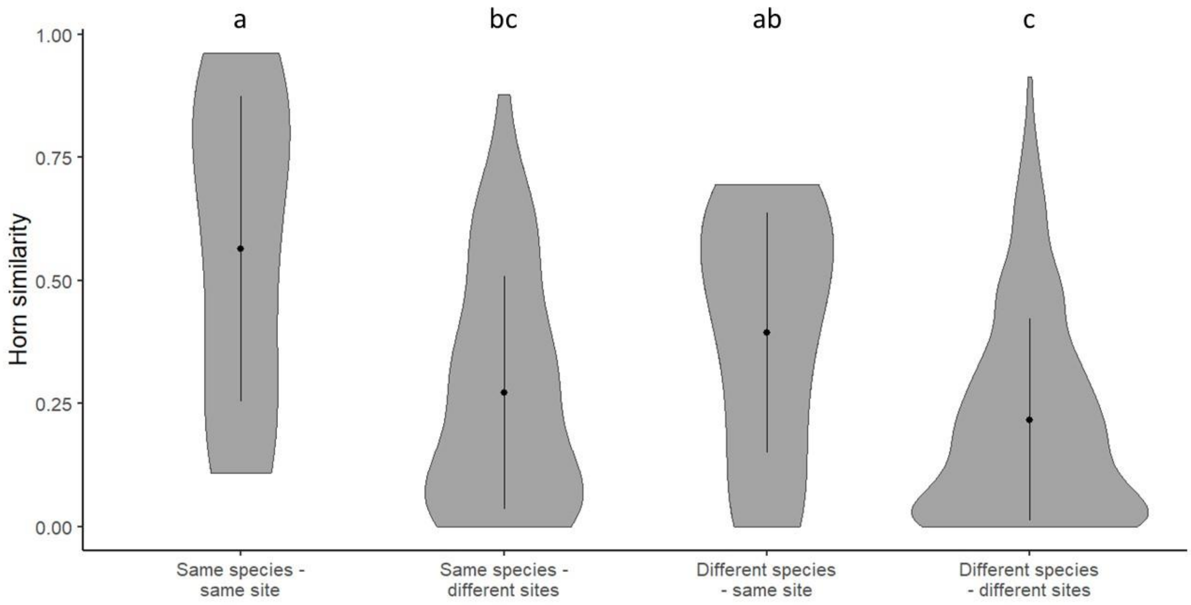


Figure 2.6: Insect visitor community similarity across daisy species in the West Coast region. An index of 0 represents a complete turnover in visitor communities between populations. Black dots represent means and thin black lines represent the standard deviation. The shape of the grey plots shows the frequency distribution of the data. Letters indicate significant differences across groups.

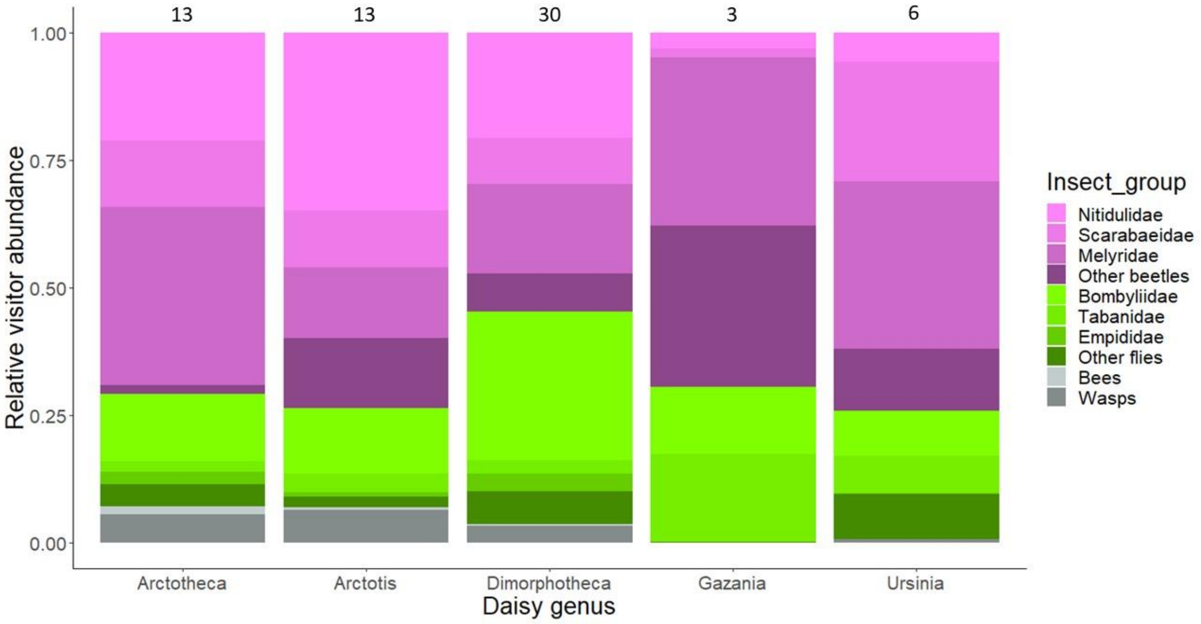


Figure 2.7: Stacked bar plot showing the relative visitor abundance (insect visitor abundance/total visitor abundance) for each daisy genus. Numbers above bars refer to the number of populations sampled for each genus (n = 65 populations).

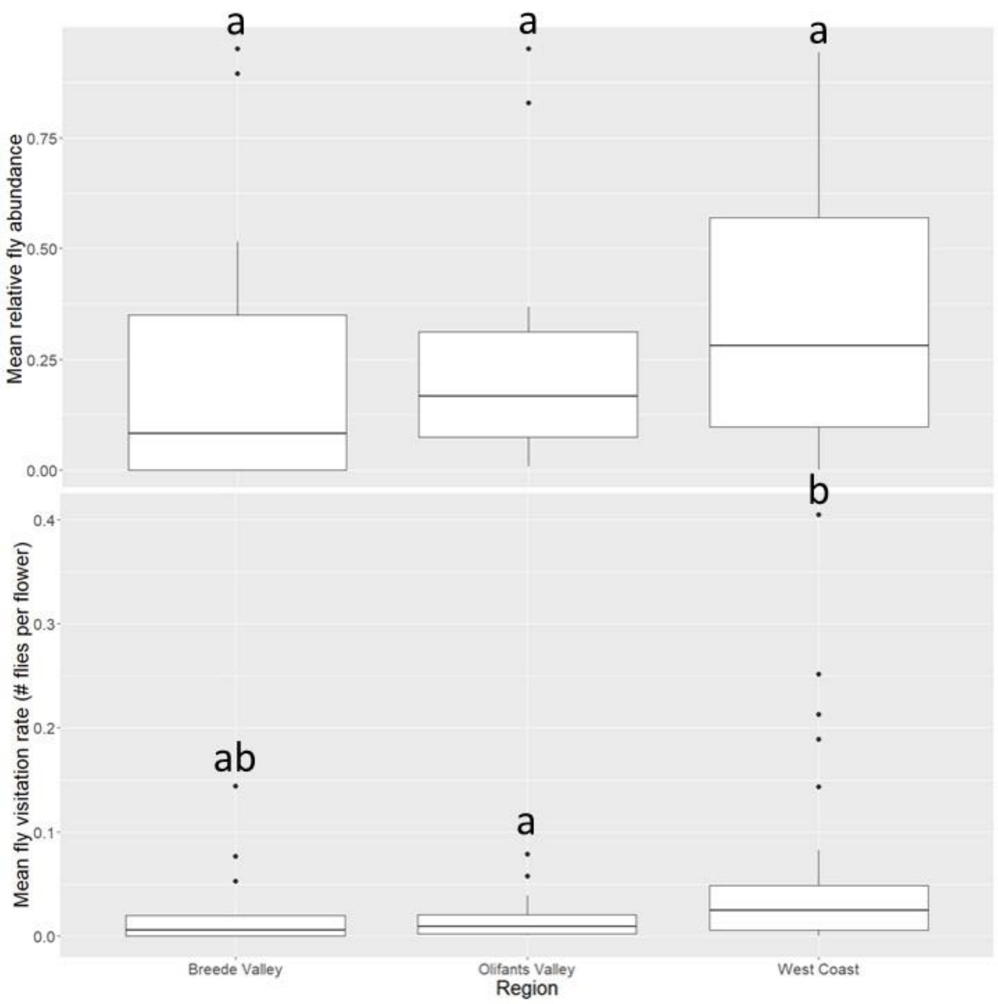


Figure 2.8: Mean (±SE) relative fly abundance and fly visitation rate (number of flies per flower). Letters above boxes indicate significant differences between regions based on pairwise post-hoc tests, with significance level of $p < 0.05$.

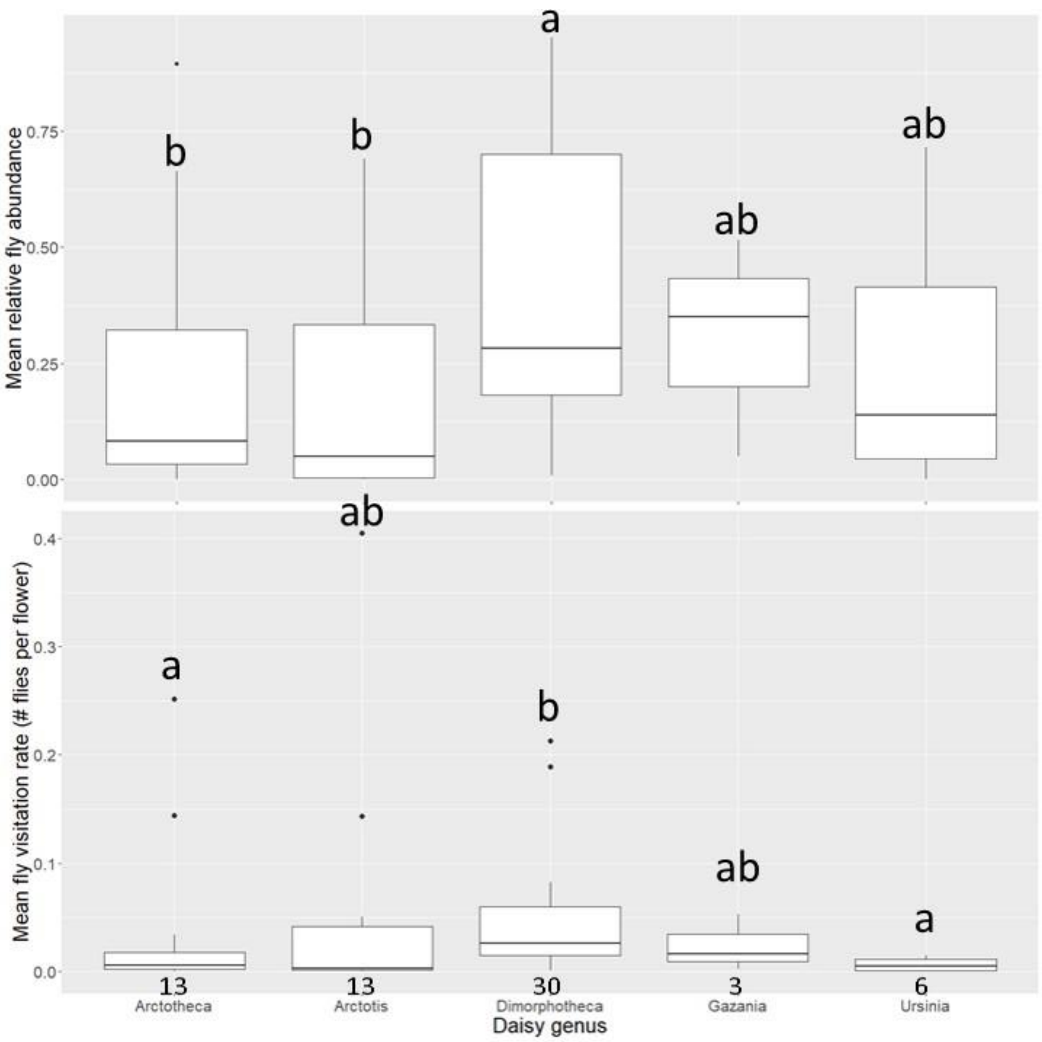


Figure 2.9: Mean (±SE) relative fly abundance and fly visitation rate (number of flies per flower) for each daisy genus. Numbers below boxes refer to the number of populations sampled per daisy genus (n). Letters above boxes indicate significant differences between daisy genera based on pairwise post-hoc tests, with significance level of $p < 0.05$.

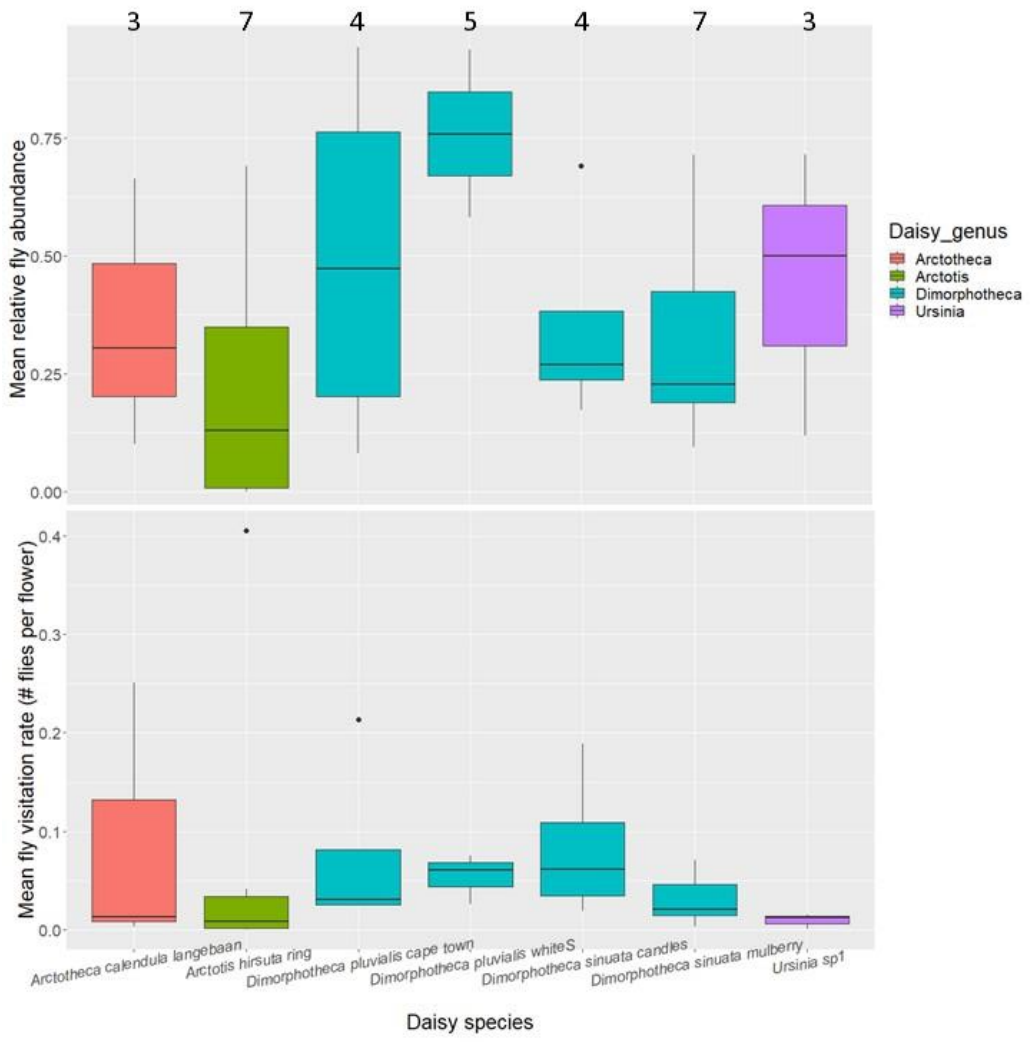


Figure 2.10: Mean (±SE) relative fly abundance and fly visitation rate (number of flies per flower) for each daisy species in the West Coast. Numbers above boxes refer to the number of populations sampled for each daisy species (n). There were no significant differences across daisy species for both fly abundance and visitation rate.

Chapter 3:

Pollination effectiveness of important pollinators of spring mass-flowering daisies in the Greater Cape Floristic Region

Abstract

A pollinator's effectiveness is comprised of both quantity (frequency of interaction) and quality (single visit performance) components. Because daisies have generalised floral phenotypes that do not exclude visitation from less effective pollinators, visitor communities should comprise hierarchies of pollinators that differ in the quantity and/or quality components of effectiveness. This is the case for the annual daisies of the Western Cape that are visited by a diversity of insect visitors that potentially differ in their effectiveness. Previous studies have found that Mariobezziinae beeflies are the dominant visitors to daisies of the Greater Cape Floristic Region, suggesting that they are possibly the most effective pollinators. However, these studies are based solely on the quantity component of effectiveness. The aim of this study was to experimentally compare the pollination effectiveness of the dominant visitors on *Dimorphotheca pluvialis*, by firstly ranking visitors based on their individual pollinator importance indices, and secondly, by experimentally comparing their single visit pollen deposition and resulting seed set. Nitidulid beetles and *Corsomyza* flies were the most frequent visitors. Since nitidulid beetles are significantly smaller in size with far fewer pollen-trapping hairs, compared to *Corsomyza* flies, they have previously been discounted as effective pollinators. In contrast, I found that while *Corsomyza* flies carried significantly more pollen than nitidulids, these groups did not differ in the quality component of pollination effectiveness. Thus, both Mariobezzid beeflies and nititulid beetles are likely important for seed set and persistence of *D. pluvialis* spring flowering displays, but further investigation into the pollinator-facilitated male component of plant fitness is required to fully understand the relative importance of these pollinator groups as selective agents shaping divergence of floral traits in this system.

Introduction

The pollination effectiveness of flower visitors measures their relative contribution to a plant's pollination success (Ne'eman et al., 2010). Some insect-pollinated plants have evolved highly specialised phenotypes and are visited by a single specialist pollinator which provides the most effective pollination service. These specialised plant-pollinator interactions are well-studied in southern Africa (Manning and Goldblatt, 1996; Johnson and Midgley, 1997; Goldblatt and Manning, 1999, 2000; Johnson and Steiner, 2003; Goldblatt and Manning, 2013), while generalised plant-pollinator interactions have not received the same attention. Generalist pollinated plants do not possess specialised phenotypes that may exclude visitation from less effective pollinators, which ultimately results in effectiveness hierarchies. Pollinator effectiveness hierarchies are a result of differences in each pollinator's morphology, physiology, foraging behaviour and plant-pollinator phenotype matching (Primack and Silander, 1975; Herrera, 1987; Young, 1988; Klumpers et al., 2019) and determining the variation in pollination effectiveness helps to separate those visitors providing genuine pollination services from ineffective visitors and pollen thieves (Inouye, 1980; Thomson, 2003; Hargreaves et al., 2009).

Previous studies have defined *pollinator effectiveness* as a product of two components; 1) the quantity component which depends on the frequency of interaction, and 2) the quality component which measures pollinator performance per single visit (King et al., 2013; Ne'eman et al., 2010). Studies have used a visitor's visitation rate/frequency or relative abundance to estimate the quantity component (Stebbins, 1970; Pauw et al., 2020), while the number of pollen grains deposited on stigmas during a single visit or pollen loads on pollinator bodies have been frequently used as estimators of the quality component (Olsen, 1997; Armbruster, 1998; King et al., 2013; Minnaar et al., 2019; Valverde et al., 2019; Pauw et al., 2020). However, pollen loads, or single visit deposition, do not account for quality of pollen delivered to stigmas. For example, pollen quality may be limiting if pollinators are depositing inadequate amounts of outcross pollen (Minnaar et al., 2019), or are depositing high numbers of incompatible pollen (i.e., pollen from another plant species) (Waser, 1978). Furthermore, some pollen vectors may not make contact with each stigma or stigma branch and might deposit pollen outside of the receptive stigma contact area (Minnaar et al., 2019). Some pollinators may also lose pollen passively (e.g. when flying from one flower to the next), or actively through grooming behaviours and pollen consumption (Koch et al., 2017; Minnaar et al., 2019). Therefore, some argue that resultant seed set is a more important measure of a pollinator's performance since it is the desired consequence of a pollinator visit (Dieringer, 1992; Keys et al., 1995; Olsen, 1997; Valverde et al., 2019). Therefore, in this study, an *effective pollinator* from the quality component perspective, is a pollinator, that during one visitation bout, deposits a large number conspecific pollen grains to a receptive stigma which results in high seed set.

Plants with generalist pollination phenotypes, of which the daisy (Asteraceae) capitulum is an archetypal example, offer easily-accessible rewards to visitors (Fenster et al., 2004; Ollerton et al., 2006; Armbruster, 2017;) and as a result many visiting species are likely to remove and deposit pollen during visitation, and could thus be effective pollinators. In these cases, those pollinators that visit most frequently are perhaps likely to offer the most effective pollination services (Stebbins, 1970). However, because the diverse visitors to generalist flowers are likely to differ in their foraging behaviour and morphology, they are likely to also differ in the quality component of effectiveness (Young, 1988; Minnaar et al., 2019). Flower feeding beetles, such as monkey beetles (Scarabaeidae: Hopliini), for example, often embed themselves in daisy florets, feeding on pollen, ovules and ray florets, resulting in damage to the florets and thus a reduced seed set (Picker and Midgley, 1996). Since pollen is also a primary floral reward for many daisy visitors (Crowson, 1988; Picker and Midgley, 1996; Johnson and Midgley, 1997; Evenhuis and Lamas, 2017b), it goes without saying that pollen will be lost through consumption before it reaches a receptive stigma. Additionally, functional morphological components, such as body size and hairiness, likely also influence removal and deposition of pollen. Insects with a larger body size will deposit a significantly greater number of pollen grains compared to smaller insects (Földesi et al., 2021), and insects that are hairier are also able to trap and transport more pollen grains (Holloway, 1976; Stavert et al., 2016). Thus, the diverse visitor communities on generalist plants, like daisies, are likely to straddle hierarchies of effectiveness based on both quantity and quality components of pollinator effectiveness.

Globally, the few studies that have characterised effectiveness hierarchies of daisy pollinators suggest that bees are the most effective pollinators of daisies (Olsen, 1997; Maruyama et al., 2018). For example, while *Aspilia joylana* (Asteraceae) in Brazil is visited by a wide taxonomic and functional diversity of pollinators, as expected for the generalist phenotype of daisies, it is predominantly visited by bees (Maruyama et al., 2018). Olsen (1997) found that visitation to a population of *Heterotheca subaxillaris* (Asteracaeae) in Texas was dominated by five bee species. Based on their relative abundance and seed set after a single visit, a *Dialistus* bee species was ranked as being the most important pollinator (Olsen, 1997). However, few studies have attempted to fully measure pollination effectiveness based on both the quality and quantity components.

Like daisies globally, the spring mass-flowering, showy annual daisy species of the Greater Cape Floristic Region are visited by a wide diversity of insect species due to their generalist phenotype (Kemp et al., 2019; Chapter 2). However, unlike daisies globally, visitation is not dominated by bees, and instead *Mariobezziinae* beeflies, *Rhigioglossa* horseflies, nitidulid beetles and monkey beetles (Scarabaeidae: Hopliini) are frequent visitors (Struck, 1992; Picker and Midgley, 1996; Johnson and Midgley, 1997; Mayer et al., 2006; Ellis and Johnson, 2009; Kemp et al., 2019; Ellis et al., 2021; Chapter 2). These insects are therefore considered important pollinators, although this is largely based on the quantity component of effectiveness i.e. visitation rates (Kemp et al., 2019) or relative visitation frequencies (Struck, 1992; Gibson, 1999; Ellis and Johnson, 2009; Ellis et al., 2021). Only two studies have attempted to determine the effectiveness hierarchies of these dominant visitors, in both cases using pollen loads as a crude approximation of the quality component of effectiveness (Goldberg, 1996; Gibson, 1999).

In this chapter, I experimentally compare the pollination effectiveness of bee flies (Bombyliidae: Mariobezziinae) and sap beetles (Nitidulidae), the dominant visitors to daisies in the Western Cape (Chapter 2), to other daisy visitors. First, I examine quality (pollen loads) and quantity (visitation rates and frequency) components of effectiveness to rank each pollinator's *pollinator importance*. Second, I experimentally test each pollinator's effectiveness by determining their individual single visit pollen deposition and contribution to seed set. Mariobezziinae flies and nitidulid beetles differ functionally and thus likely differ in the quality component of effectiveness. Mariobezziinae flies are active, larger insects and are densely covered in hairs, while Nitidulid beetles are significantly smaller in size with less hairier bodies and are assumed to be relatively immobile (Proctor et al., 1996). I therefore expect Mariobezziinae flies to be the most important and effective pollinators due to their larger body size and overall hairiness.

Methodology

Study system

The focal daisy species of this study is *Dimorphotheca pluvialis*, an annual species that is abundant across the West Coast and flowers during spring, from late July to late September. Plants are self-incompatible (Hof and Lange, 1998), so the transfer of outcross pollen by pollinators is necessary for seed production. Furthermore, inflorescences have male-sterile ray florets that mature first, which allows for the quantification of outcross pollen deposition on stigmas, unlike in the bisexual disc florets where pollen is presented on the stigma.

Experiments were conducted during spring 2021 in Langebaan, South Africa (33°02’36.2’’S, 18°04’16.8’’E; Figure 3.1). Seed set experiments were conducted on a population of *Dimorphotheca pluvialis* (Cape Town form), while pollen deposition experiments were conducted on *Dimorphotheca pluvialis* (mulberry form) inflorescences because their ray stigmas were easily visible. In order to test the expectation that flies are the most effective pollinators I quantified effectiveness of the major groups of insect visitors identified during the diversity surveys of Chapter 2, which included flies (Bombyliidae: Mariobezziinae) and nitidulid beetles.

Pollinator importance

To determine pollinator importance hierarchies, visitation rates, visitation frequency and pollen loads were determined for insect visitors on *Arctotis* and *Dimorphotheca* annual daisy species at seven sites located in the West Coast. *Arctotis* and *Dimorphotheca* were the dominant flowering species at these sites. In 2020 and 2021, I observed 20 discrete patches of the target daisy species at each site for 10 minute periods (total of 143 observation periods for both species = 23.8 hours of observations) between 10:00 and 16:00 to record the visitation of all insect visitors (Ellis and Johnson, 2009). The number of inflorescences was also counted in each observation patch. These visitation observations were conducted on multiple daisy species that occur at a site (a total of five daisy species/morphotypes sampled across seven sites). For these analyses, spatially structured variants (i.e., floral morphotypes) within each daisy species were treated as unique taxa. As visitors entered the observation area, I recorded their identity and the number of capitula they visited within that time period. Insects were then caught for identification once they left the observation area. Visitation rates were calculated as the number of visits per inflorescence per hour. I then used diversity survey data collected in 2020 and 2021 at the same sites to calculate visitation frequency (number of individuals per inflorescence) for each of the main visitors for each daisy population (see Chapter 2 for survey method details). Observations and surveys at sites 10, 11 and 25 were carried out on the same day. For sites 2 and 12, observations were carried out a month after surveys, and for sites 3 and 5, observations occurred a year after surveys were conducted.

The most common visitors (five representatives of each species) identified during the diversity surveys (based on visitation frequencies) were collected at seven sites across the West Coast and were collected directly from the inflorescence using Eppendorf vials. These vials were then filled with 95% ethanol to preserve both the insects and pollen they carried. The insects were thoroughly washed to ensure all pollen was removed from the body. Thereafter, the Eppendorf tubes were placed in a drying oven overnight at 60°C to evaporate the ethanol. A small piece of fuchsin gelatin was dropped into the Eppendorf and melted in a warm water bath. Once the gelatin had solidified, it was used to wipe up all the pollen grains left in the Eppendorf. The gelatin was then melted onto a microscope slide and a cover slip was placed on top. I then systematically counted the total number of daisy pollen grains using a light microscope at 40x magnification. Daisy pollen was identified according to their characteristic spiky shape. Those insects collected at sites with just one daisy species were examined first in order to identify that daisy species' pollen. These were used as a reference library to separate host daisy pollen loads from pollen of other species at sites with more than one daisy species flowering. Pollen grains of co-occurring daisy species at a site could be confidently separated based on size and shape. Pollen grains consistent with *Dimorphotheca* pollen loads consisted of extremely sharp and spiky protrusions on the exine (Appendix D), while pollen grains from *Arctotis* were larger in diameter and had less sharp protrusions (Appendix D).

The following statistical analyses were conducted in R statistical software (RStudio Team, 2021). First, I ran separate Generalised Linear Mixed Models (GLMM) on *Arctotis* and *Dimorphotheca* with population as a random factor to determine whether there was a significant difference in the mean visitation rates across (i.e., mean number of visits per inflorescence per hour) and visitation frequencies (i.e., number of individuals per inflorescence surveyed at each site) across insect visitors within each daisy genus. Thereafter, I conducted a Tukey post-hoc test to examine which insect visitors differed significantly from one another using the multcomp package in R (Hothorn et al., 2008), in order to determine whether some insects were visiting each genus at significantly greater rates and frequencies. I then ran a Generalised Linear Mixed Model (GLMM) with conspecific pollen loads as the response variable and site as a random factor to test for differences in the conspecific pollen loads carried across insect visitors. Pollinator importance (PI) for each of the main insect visitors across sites was then calculated using the formula:

$$\mathrm{PI} = \mathrm{V_r} \times \mathrm{H_L}$$

where V_r is the mean visitation rate of the target pollinator species (average visits per flower per hour), and H_L is the mean conspecific pollen load (the average number of conspecific pollen grains carried by a pollinator).

Pollen deposition effectiveness

The number of pollen grains deposited after a single visit by a pollinator can provide insight into that pollinator's importance in the system. Since each ray floret only contains one ovule, only one pollen grain is needed to fertilise each floret. However, those stigmas which receive greater numbers of pollen grains will have a greater chance of fertilisation and perhaps increased quality of the resulting seed (Björkman, 1995). Therefore, quantifying the number of pollen grains a pollinator may deposit on each stigma provides valuable information on the importance of that visitor. To determine pollen deposition effectiveness, I collected newly opened, virgin *Dimorphotheca pluvialis* (mulberry form) inflorescences (disc florets closed) and placed them into Eppendorf vials filled with water. These were then presented to visitors in a population of *Dimorphotheca pluvialis* (cape town form), the same site where the following "seed set" experiments were conducted, until each received one visit from a target pollinator. The mulberry form was used for deposition experiments because their ray stigmas clearly stuck out beyond the ray floret, making pollen deposition easy to quantify. Thereafter, I inspected the ray florets under a microscope to determine 1) the proportion of stigmas that received pollen and 2) the number of pollen grains on each "pollinated" stigma.

First, I calculated the proportion of "pollinated" ray stigmas after a single visit from an insect (the number of stigmas with pollen/total number of stigmas). I then ran a GLMM on the number of pollinated stigmas with the visitor species as a predictor and the number of rays as a random factor. A Tukey post-hoc test was used to examine which insects visited the most ray stigmas during a single visit. Of those stigmas that received pollen, I then calculated the mean number of pollen grains deposited after a single pollinator visitation bout. A GLMM was used to test for differences in the number of pollen grains deposited by different visitor species, with stigmas as the level of replication and inflorescence as a random factor. A Tukey post-hoc test was used to further determine which visitors deposited the most pollen grains after a single visit.

Seed set

Ultimately seed set is the desired consequence of a pollinator's visit and so measuring seed set after a single visit is an important measure of the quality component. To prevent visitation from pollinators, I placed pollinator exclusion cages over discrete patches of young unopened *Dimorphotheca pluvialis* (Cape Town form) inflorescences (total of eight cages with an average of 11 inflorescences each). These cages were made using wooden dowels as a frame, covered in a fine mesh which still allowed sunlight, wind and rain through. Cages were knocked into the ground to secure them and sand was placed on the edges of the frame to prevent insects from crawling under the mesh. Each daisy head was labelled with a unique identity code. Once the flower heads fully opened, I presented each patch to visitors for two hours or until each flower had received a visit, during which time I recorded the identity of each visitor and the number of inflorescences it visited in the patch. Thereafter, I replaced the cage over the patch and left the inflorescences to mature. Only the ray seed set was considered for analysis, since the ray florets all opened on the same day, unlike disc florets which open and mature sequentially. The number of swollen ray floret ovaries per infructescence were counted as a measure of ray seed set (see De Waal et al., 2014).

One cage (9 inflorescences) was not exposed to pollinators, and served as a control for possible pollen transfer by small insects inside the cages. Additionally, any inflorescences that did not receive a recorded visit in the exposed cages were included in analysis as a “no visit” treatment (24 inflorescences, a total of 33 “no visit” inflorescences). I also included uncaged patches (n=30 inflorescences) which received unlimited pollinator visitation.

During these experiments, four main visitor species in three functional groups were dominant, namely; a *Corsomyza* species, *Megapalpus capensis* (Bombyliidae: Mariobezziinae), a Nitidulidae species (Coleoptera) and a parasitoid wasp species (Hymenoptera: Ichneumonidae: Tersilochinae). Therefore, inflorescences from the caged experiments were grouped according to different pollinator visit treatments, namely: 1) single *Corsomyza* visits, 2) single *Megapalpus* visits, 3) single nitidulid beetle visits, 4) single Tersilochinae visits 5) unlimited visitation, and 6) no visits. To test for differences in the number of seeds set across treatments, I used the lme4 package in RStudio to conduct a GLMM with number of nuts (ray seeds) as the dependent variable, treatment as a predictor and number of ray florets as a random factor. A Tukey post-hoc analysis was then run using the “multcomp” package to determine where these significant differences lie. Second, to determine whether an increasing number of visits increases seed set regardless of visitor identity, I ran a linear regression on the percentage of ray seeds set with number of visits as the predictor variable.

Results

Pollinator importance

Nitidulid beetles had highest visitation rates on *Dimorphotheca* (Figure 3.2, Table 3.2). These beetles visited at significantly higher rates than other beetle, fly and Hymenopteran taxa. In *Arctotis*, *Rhigioglossa* flies had higher visitation rates compared to *Corsomyza*, Empididae and *Megapalpus capensis* flies. They also visited at significantly higher rates compared to other wasp and bee taxa. However, their visitation rates were not significantly greater than a Tersilochinae wasp species, or other beetle and fly taxa (Figure 3.2).

Generalised Linear Mixed Model analyses showed that there was a significant difference in the visitation frequencies across insect visitors to *Dimorphotheca* (Chi-squared = 39.02, df = 11, $p < 0.001$). Nitidulid beetles had the highest visitation frequencies compared to all other taxa (Figure 3.2). In *Arctotis* populations, beetles had higher visitation frequencies than other visitors, however not significantly so. Overall, there was no significant differences in the visitation frequencies of insect visitors to *Arctotis* (Chi-squared = 15.01, df = 11, $p = 0.18$).

There was a significant difference in the conspecific pollen loads carried by insects (GLMM, Chisq = 30485, df = 4, $p < 0.001$). *Corsomyza* flies had the highest pollen loads compared to other main insect visitors, while empidid flies had the overall lowest conspecific pollen load. (Figure 3.3). Melyridae beetles and Tersilochinae wasps did not significantly differ in their pollen loads (Figure 3.3). Due to their high visitation rates, nitidulid beetles overall had the highest PIs (Table 3.1, 3.2). Overall, empidid flies had the lowest PIs due to both their low pollen loads and visitations rates.

Pollen deposition effectiveness

There was a significant difference in the proportion of stigmas pollinated during single visits across insect species (GLMM, Chisq = 13.598, df = 2, $p < 0.01$, Figure 3.4). Mariobezziinae flies and nitidulid beetles pollinated a similar proportion of ray stigmas. Post-hoc pairwise analyses found that Tersilochinae wasps deposited pollen on significantly fewer stigmas compared to Mariobezziinae flies ($p < 0.05$) and Nitidulidae beetles ($p < 0.01$) (Figure 3.4). Furthermore, there was a significant difference in the number of pollen grains deposited per "pollinated" stigma (GLMM, Chisq = 11.169, df = 2, $p < 0.01$). Mariobezziinae flies and nitidulid beetles deposited a similar number of pollen grains on average (Figure 3.4).

Tersilochinae wasps deposited the lowest number of pollen grains compared to other insect species ($p < 0.001$) (Figure 3.5).

Seed set

There was a significant difference in the number of seeds set after a single visit from each insect visitor (GLMM, Chisq = 147.18, df = 5, $p < 0.001$). Post-hoc analysis found that seed set is elevated above the baseline (i.e., no visits) only after a single visit by *Corsomyza* flies (Figure 3.6). Furthermore, the number of seeds set after a single visit from *Corsomyza* flies and nitidulid beetles did not differ significantly from those inflorescences which received unlimited visits. Seed set after a single visit from *Corsomyza* flies is significantly greater than the percentage seeds set after a single visit from Tersilochinae wasps, however a single visit from other taxa did not result in significantly less seed set compared to *Corsomyza* flies. There was a significant relationship between the percentage of ray seeds set and the number of visits (LM, F = 10.47, $R^2 = 0.11$, $p < 0.01$), with a 5.66 % increase in seed set for every visit (Figure 3.7).

Discussion

Overall, Mariobezziinae beeflies are delivering marginally better quality effectiveness, but nitidulids are significantly more important for the quantity component, and are thus likely equally important for the persistence of Cape mass-flowering daisy species (Table 3.2), rejecting my hypothesis that mariobezzid flies are more effective pollinators than nitidulids. The previous assumptions that nitidulids are less important (or not important) pollinators because of their low mobility (Proctor et al., 1996), small size, and low hairiness overall (Mayer et al., 2006) are unfounded. In fact, the results of this study suggest that they have higher visitation rates and similar qualitative effectiveness than flies for seed set and the ultimate persistence of Western Cape daisies.

Furthermore, the results of this study suggest that, as expected for generalist pollinated plants (Motten, 1983; Herrera, 1987; Keys et al., 1995; Fumero-Cabán and Meléndez-Ackerman, 2007; Valverde et al., 2019), spring flowering annual daises are characterised by pollinator effectiveness hierarchies. The community of visitors on *Dimorphotheca* and *Arctotis* daisy displays exhibit significant variation in the quantity component of effectiveness, and while all visitors do carry pollen and could thus function as pollinators, pollen loads varied significantly across pollinator taxa. While the two dominant pollinators on *Dimorphotheca pluvialis* have similar quality effectiveness levels, the Tersilochinae wasp had the lowest quality effectiveness, despite having high visitation rates. Furthermore, despite carrying substantial pollen loads, single visit experiments suggest that this wasp is contributing significantly less to seed set than flies and nitidulid beetles. These wasps visited a significantly lower proportion of stigmas compared to other insects, and also deposited significantly fewer pollen grains compared to mariobezzid flies and nitidulid beetles which ultimately resulted in significantly fewer seeds after a single visit. These wasps have a relatively small body size compared to other visitors and are not covered in "pollen-trapping" hairs. Furthermore, these wasps were only observed laying eggs within the inflorescence, and not feeding on pollen or nectar and so they are likely visiting daisies to deposit eggs on the beetle larvae which inhabit inflorescences (Jervis et al., 2001). They did not visit each floret individually and were therefore unable to effectively deposit many pollen grains within the stigma contact areas. These results also suggest that pollen loads provide an incomplete picture of the quality component of pollinator effectiveness.

Single visit deposition and seed set were perhaps surprisingly high, with an average of 50% of florets receiving pollen and setting fruit after single visits from the dominant *Corsomyza* and Nitidulidae pollinators. This suggests that behaviour on daisy inflorescence is such that both groups contact stigmas of multiple florets, and that outcross pollen is deposited on sequential stigmas. Apart from their high visitation rates, field observations indicated that nitidulid beetles were also highly active in the daisy capitulum, purposefully walking to each ray floret along the line of purple colouration at the base of the rays, possibly a result of mate seeking behaviour by the beetles. Little information exists on nitidulids and their flower visiting behaviour, however, previous work has shown that pollinating beetles, such as monkey beetles, are attracted to light-coloured flowers with dark centres (Picker and Midgley, 1996; Goldblatt et al., 1998; Van Kleunen et al., 2007). *Dimorphotheca pluvialis* daisies have white rays, with dark purple colouration at the base, giving a bulls-eye appearance. These darker bases also reflect light similarly to the way nitidulid beetle elytra do. These two components combined suggest that apart from food rewards, nitidulids possibly visit daisies as aggregation sites in an attempt to find a mate, and in the process will deposit pollen grains on sequential stigmas around the daisy capitulum. It was also noted that *Corsomyza* flies followed a pattern of "head-bobbing" behaviour (i.e., probing each daisy floret for nutritive rewards), moving sequentially around the capitulum, which results in pollen grains being deposited on receptive stigmas along the way.

Despite high single visit effectiveness, the results show that receiving repeated visits from pollinators, regardless of their identity, offers seed set benefits. This suggests that the quantity components (i.e., visitation rate and visitation frequency) are potentially the more important determinants of the importance of daisy pollinators, as has been suggested previously for other generalist plants (Olsen, 1997; Maruyama et al., 2018). As I only quantified seed set of ray florets, which are open simultaneously, this effect is likely to be even stronger when considering total seed set, given that disk florets open sequentially and thus require repeated visitation over multiple days.

Interestingly, I recorded relatively high seed set (34%) in inflorescences that had not received a visit from the observed insects. While this could simply reflect experimental effects such as missed observations of visits, or insects trapped in the cages after exposure, or insects emerging in the cages, it could also point to an alternative contributor to seed set in these systems. Partial selfing is an obvious possibility, but unlikely given that previous work has demonstrated complete sporophytic self-incompatibility in *Dimorphotheca* annuals (Hof and Lange, 1998; De Waal et al., 2014). Other possibilities include wind mediated contact between inflorescences of neighbouring plants, a real likelihood given the density of flowering displays. Alternatively, the importance of small insect pollinators, like thrips and collembola, has been underestimated even though they are present in daisy inflorescences and likely to be able to move through the mesh exclusion cages.

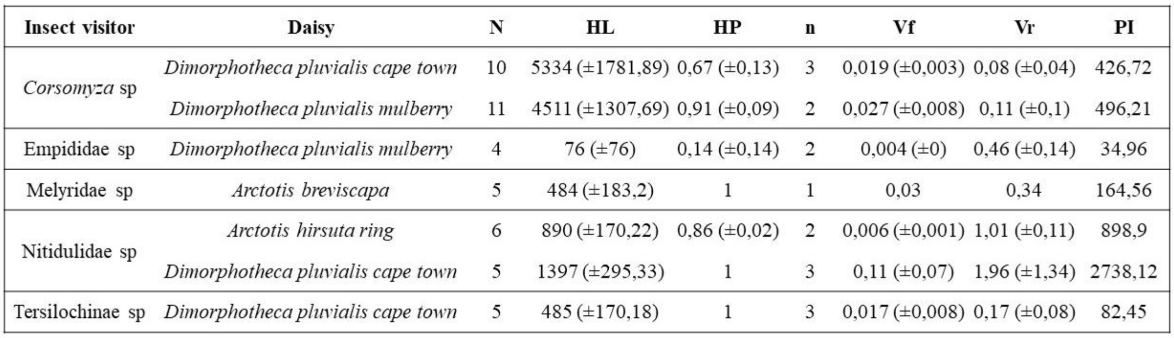
My results suggest that few pollinators likely contribute most of the seed set and are thus most important for the persistence of Cape daisies. However, since all visitors carry pollen and do deposit some, it is unlikely that the absence of dominant pollinator groups will result in seed set failure. The female component of pollination (i.e., seed production) is critical for the persistence of plants in a system. If the results of this study are generalizable beyond the observed daisy population, then Nitidulidae beetles are likely most important for seed set. However, the pollinator-facilitated male component of pollination (i.e., pollen transfer) is crucial in the evolution of plant reproductive traits (Minnaar et al., 2019). Previous studies on pollinator-driven floral traits exhibited by Namaqua showy daisy communities found that Mariobezziinae beeflies and *Rhigioglossa* horseflies are driving flower colour patterns across communities (Kemp et al., 2019; Ellis et al., 2021). Before pollen is deposited on a receptive stigma, pollen grains can be lost through various means, for example through grooming behaviours and pollen consumption (Minnaar et al., 2019). Nitidulid beetles (Crowson, 1988) and Mariobezziinae flies (Johnson and Midgley, 1997; Evenhuis and Lamas, 2017a) are both pollinivores, and so much of the pollen presented may be consumed before reaching a receptive stigma. However, due to the lack of long, dense hairs on the body of nitidulid beetles, much of the pollen may also be lost passively when moving between flowers. On the other hand, the larger and hairier bodies of *Corsomyza* flies allow them to carry higher pollen loads (Holloway, 1976; Stavert et al., 2016; Földesi et al., 2021), much of which is ultimately deposited on receptive stigmas. Thus, it is possible that Mariobezziinae flies are still important for the male fitness component and are therefore the main selective agents on floral traits.

Conclusion

Previous studies on the pollination ecology of the showy daisies of the Greater Cape Floristic Region have suggested that Mariobezziinae flies are offering the most effective pollination services (Johnson and Midgley, 1997; Ellis and Johnson, 2009; Kemp et al., 2019; Ellis et al., 2021). However, these studies have not experimentally compared the pollination effectiveness of these flies to other daisy visitors. In this study, I have found that both *Corsomyza* flies and nitidulid beetles offer similar levels of pollination effectiveness for daisies of the West Coast. This result is surprising since nitidulid beetles have previously been disregarded as important insect visitors (Mayer et al., 2006). Further investigation into the male component of plant fitness is required to fully determine the relative importance of these pollinators as selective agents on daisy floral traits.

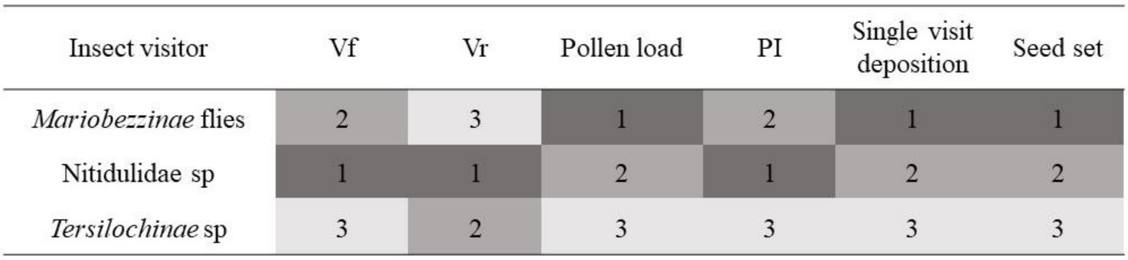
Tables

Table 3.1: Pollinator importance (PI) for the dominant visitors (i.e., those visitors with the highest visitation frequencies) to daisy species/morphotypes across six sampled sites. H_L is the average conspecific pollen load on each insect body, H_P refers to the average pollen load purity (the average proportion of conspecific pollen grains in the total pollen load carried by an insect), V_f is the average visitation frequency (number of individuals per inflorescence) and V_r is the average visitation rate across populations (visits per flower per hour) for that insect visitor across daisy populations. PI is the product of H_L and V_r. Sample size (N) refers to the number of individuals inspected for pollen loads, while n refers to the number of populations surveyed (V_f)/observed (V_r) for each daisy species.



Insect visitor	Daisy	N	HL	HP	n	Vf	Vr	PI
Corsomyza sp	*Dimorphotheca pluvialis cape town*	10	5334 (±1781,89)	0,67 (±0,13)	3	0,019 (±0,003)	0,08 (±0,04)	426,72
	Dimorphotheca pluvialis mulberry	11	4511 (±1307,69)	0,91 (±0,09)	2	0,027 (±0,008)	0,11 (±0,1)	496,21
Empididae sp	*Dimorphotheca pluvialis mulberry*	4	76 (±76)	0,14 (±0,14)	2	0,004 (±0)	0,46 (±0,14)	34,96
Melyridae sp	*Arctotis breviscapa*	5	484 (±183,2)	1	1	0,03	0,34	164,56
Nitidulidae sp	*Arctotis hirsuta ring*	6	890 (±170,22)	0,86 (±0,02)	2	0,006 (±0,001)	1,01 (±0,11)	898,9
	Dimorphotheca pluvialis cape town	5	1397 (±295,33)	1	3	0,11 (±0,07)	1,96 (±1,34)	2738,12
Tersilochinae sp	*Dimorphotheca pluvialis cape town*	5	485 (±170,18)	1	3	0,017 (±0,008)	0,17 (±0,08)	82,45

Table 3.2: The main insect visitors to *Dimorphotheca pluvialis* are ranked based on the main pollination effectiveness metrics examined, with 1 being the highest and 3 the lowest. V_f refers to the visitation frequency (number of individuals per inflorescence), V_r is the visitation rate (visits per inflorescence per hour) and PI refers to the pollination importance (product of the average conspecific pollen load and visitation rate).



Insect visitor	Vf	Vr	Pollen load	PI	Single visit deposition	Seed set
Mariobezzinae flies	2	3	1	2	1	1
Nitidulidae sp	1	1	2	1	2	2
Tersilochinae sp	3	2	3	3	3	3

Figures

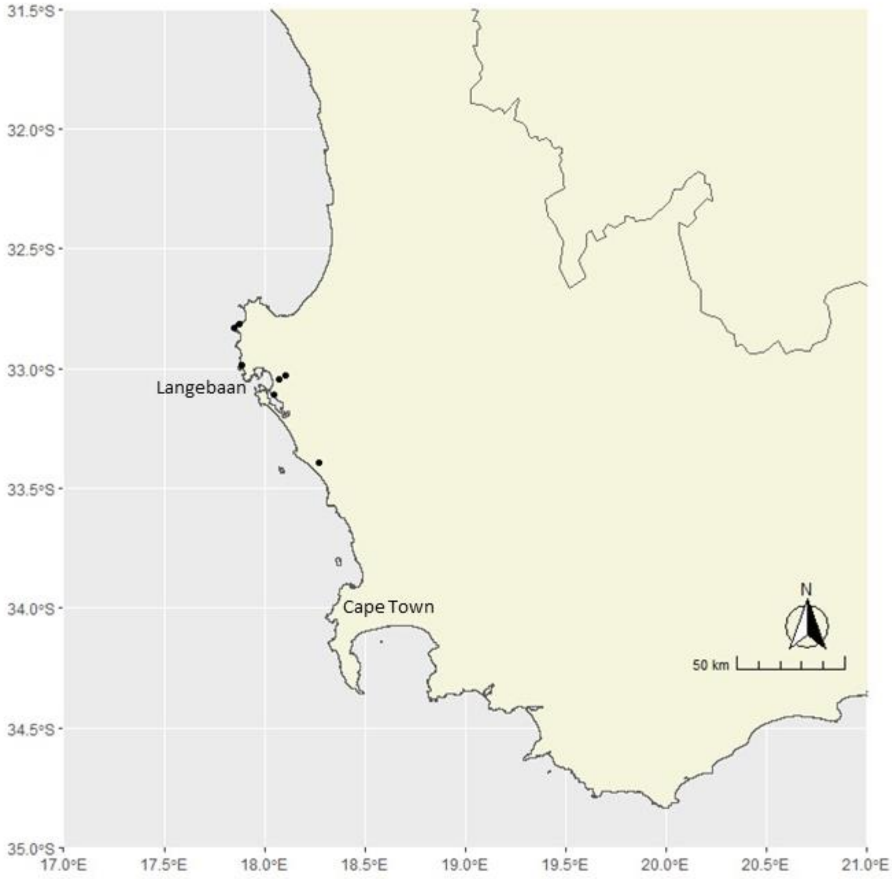


Figure 3.1: A map showing the location of seven sites in the West Coast region where visitation rate sampling took place.

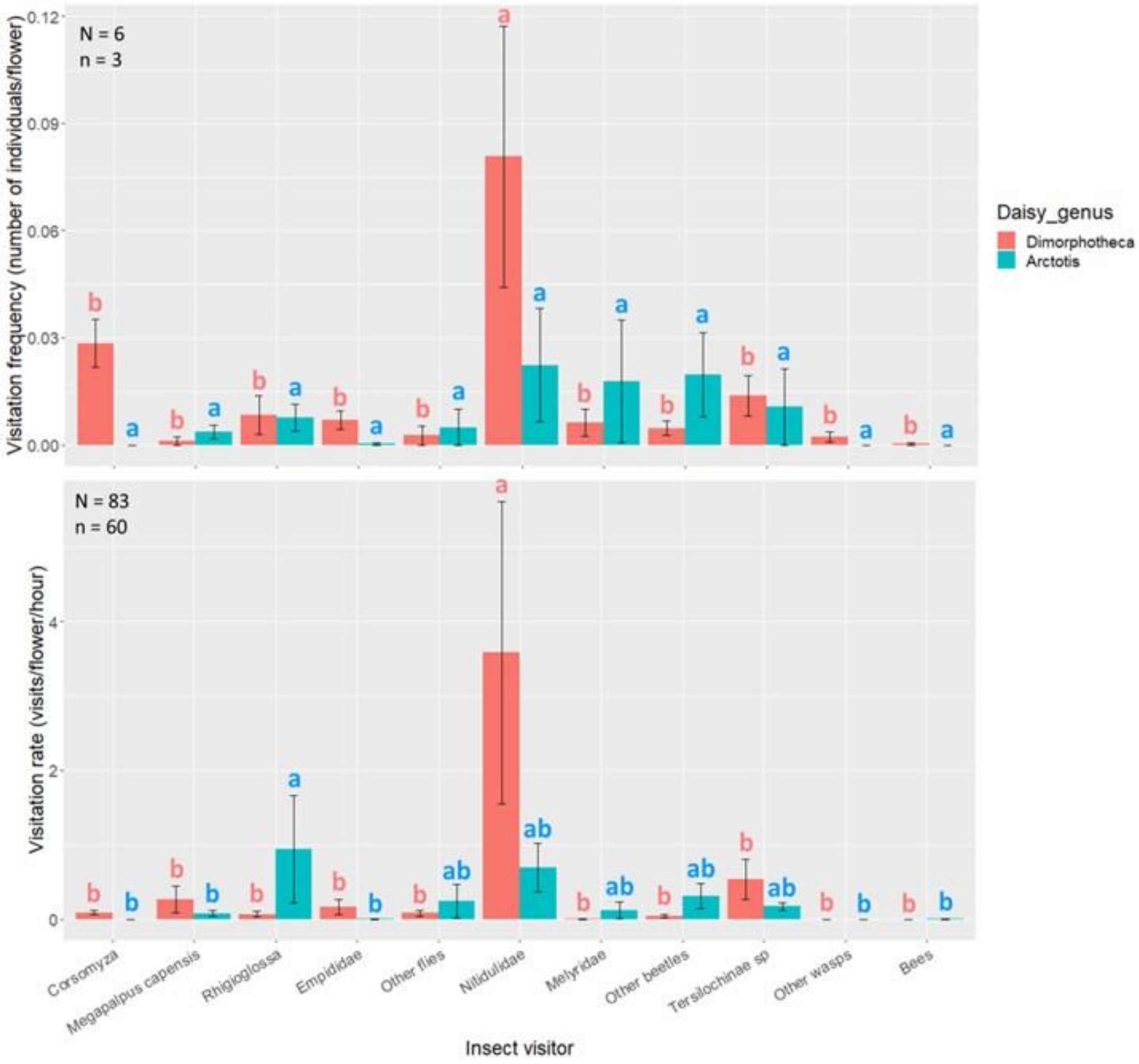


Figure 3.2: The average (±SE) visitation frequency (number of individuals/flower) and average (±SE) visitation rate (visits per inflorescence per hour) for each of the main insect visitors across daisy genera. Pink letters above bars indicate significant differences in the V_f and V_r across insect visitors to *Dimorphotheca*, blue letters indicate significant differences across insect visitors for *Arctotis* ($p < 0.05$). Upper case N and lower case n refer to sample sizes for *Dimorphotheca* and *Arctotis* respectively. For visitation frequency, sample sizes are the number of populations surveyed for each genus. For visitation rates, sample sizes refer to the number of observation periods carried out for each daisy genus.

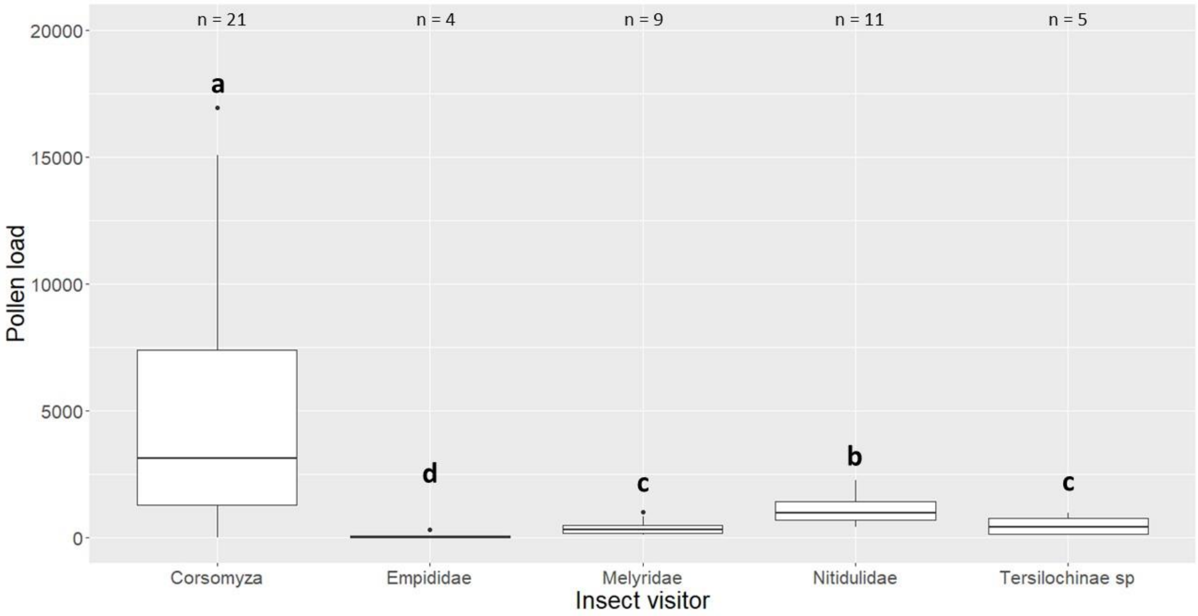


Figure 3.3: Conspecific pollen loads carried by the main insect visitors identified during diversity surveys. Sample size (n) refers to the number of individuals inspected for pollen for each insect visitor. Boxes show the interquartile range, and whiskers end in the maximum and minimum. The centre black line shows the median, and black dots represent outliers. Different letters above boxplots show significant differences in the pollen loads carried by different insects, based on a significance level of $p < 0.05$.

Figure 3.4: The proportion of total ray floret stigmas of *D. pluvialis* (mulberry form) that received pollen grains (ie “pollinated” stigmas) after a single visit from an insect pollinator. Boxes show the interquartile range, and whiskers end in the maximum and minimum. The solid black centre line represents the median. Different letters above bars indicate significant differences between insect species based on Tukey’s post-hoc tests, with a significance level of $p < 0.05$. Sample size (n) refers to the number of inflorescences that received a single visit by each insect visitor (or no visit for control).

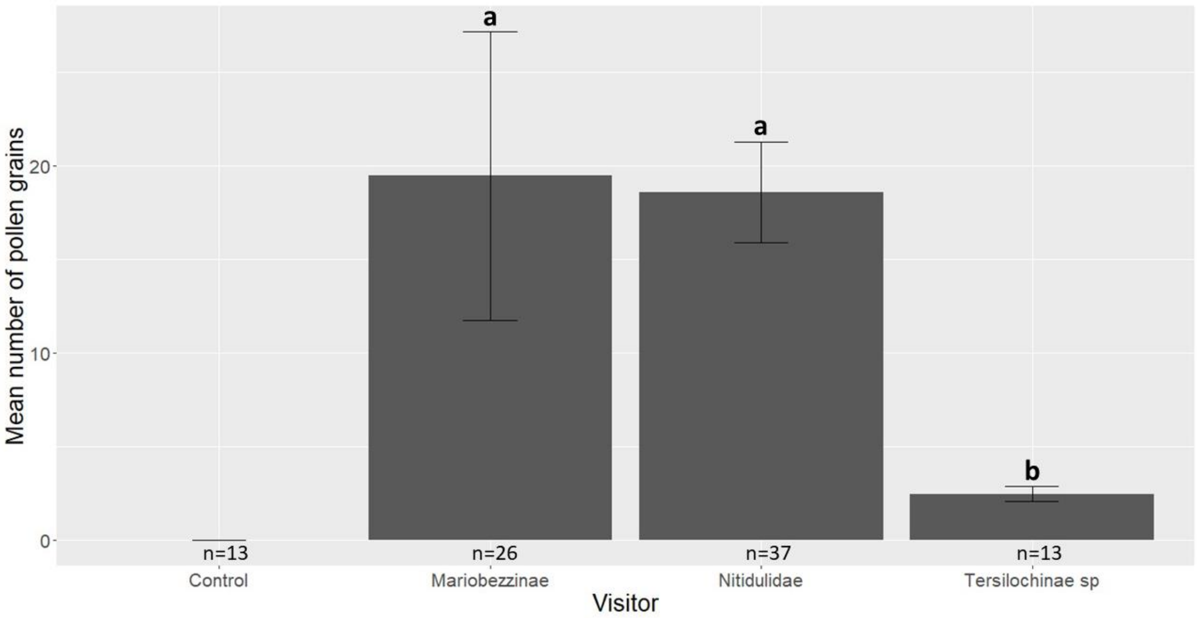


Figure 3.5: The mean (±SE) number of pollen grains deposited per "pollinated" stigma across insect visitors. Letters above bars represent significant post-hoc results, based on a significance level of $p < 0.05$. Sample size (n) refers to the number of stigmas that received pollen grains after a single visit by each insect (or no visits for control).

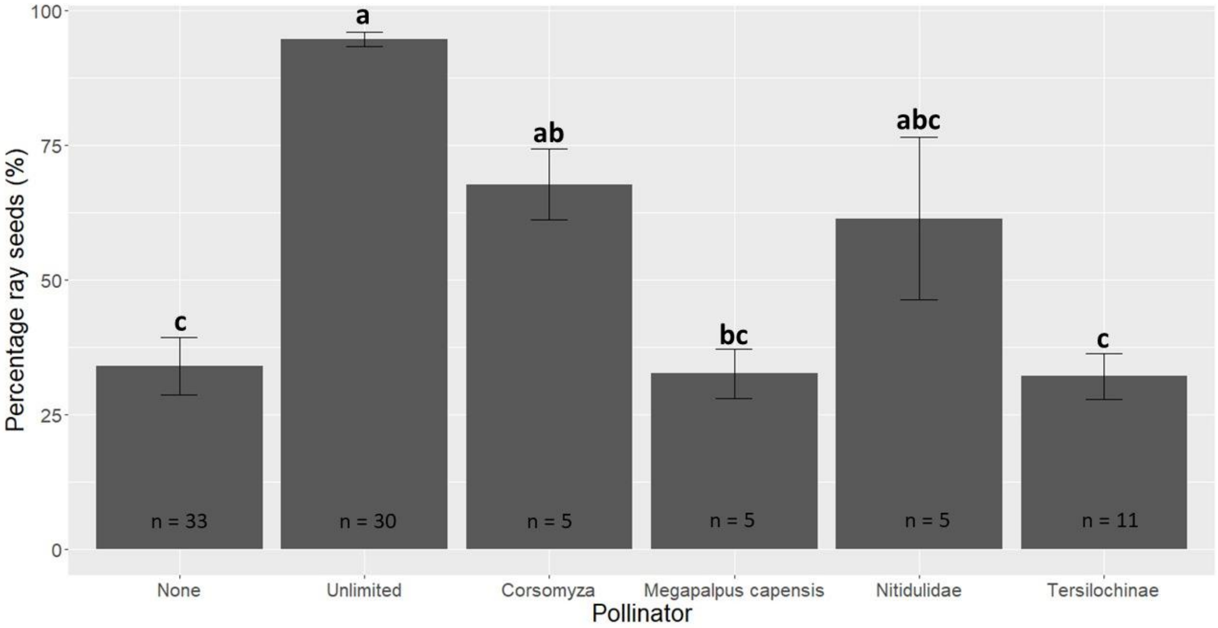


Figure 3.6: The mean (±SE) percentage of ray florets that set seed after a single visit from each insect visitor. Sample sizes (n) for each treatment are shown at the bottom of each bar. Letters above each bar indicates significant differences across insect visitors based on Tukey's post-hoc pairwise tests, with a significance level of $p < 0.05$.

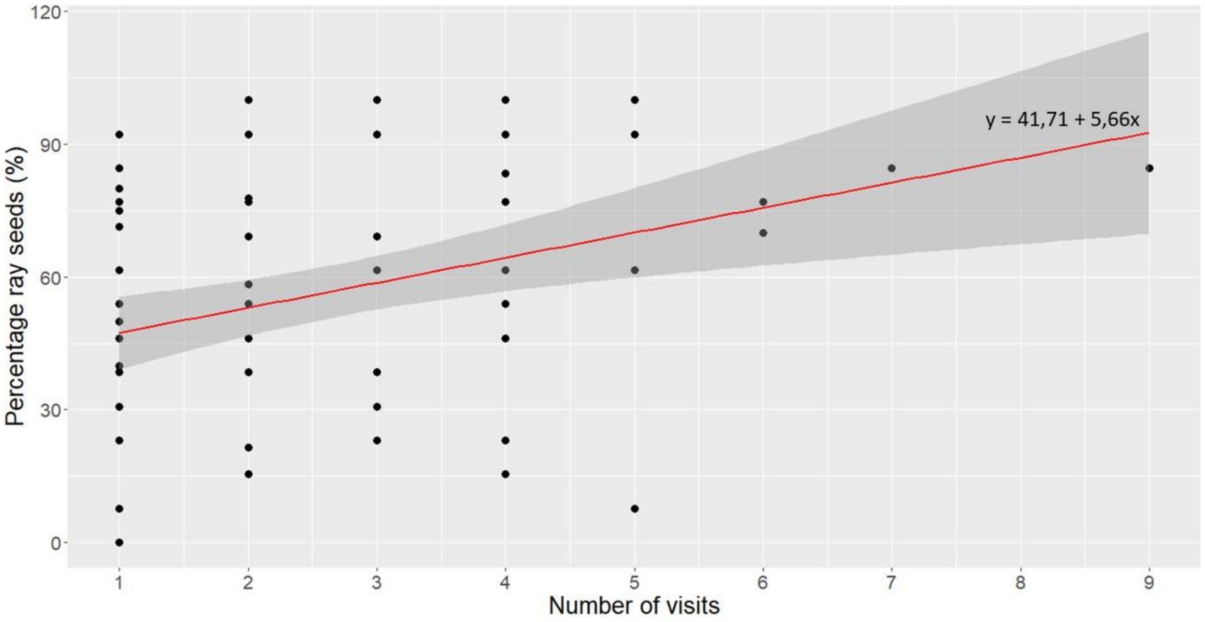


Figure 3.7: The percentage of ray florets producing seeds with increasing number of visits by floral visitors. The regression line (linear model) in red with standard error (grey shade).

Chapter 4:

General Conclusions

A key factor in understanding the dependence of plants on certain pollinators is determining the level of specialisation of the plant-pollinator interaction. Generalist pollinated plants do not necessarily exclude less effective visitors and so determining the importance of dominant pollinators is complex. At one level, Western Cape annual daisies are relatively specialised on two insect orders, namely Diptera and Coleoptera. At species level, however, Western Cape daisies correspond more with global daisy studies, being visited by an average of 8 visitor species. This is in contrast to the more specialised pollination of Namaqua daisies which are almost exclusively dominated by Mariobezziinae flies (*Corsomyza* and *Megapalpus*) (Struck, 1994a; Johnson and Midgley, 1997; Ellis and Johnson, 2009; Kemp et al., 2019; Ellis et al., 2021). This is possibly due to a taxon sampling effect, since daisy studies in Namaqualand are biased towards *Gorteria diffusa*, that is highly specialised on *Megapalpus capensis* flies attracted to the raised, black spots on the orange ray florets (Johnson and Midgley, 1997; Ellis and Johnson, 2009). This pattern of higher visitor diversity in the southern GCFR relative to the Northern parts, may be further exacerbated by the lower non-xerophilous insect diversity in the more arid region of Namaqualand (Tankard and Rogers, 1978). Although functional specialisation was not specifically tested in this study, Western Cape daisies seem to be effectively pollinated by two different functional groups, beetles and flies. While both groups feed on both nectar and pollen, these two groups differ in body size, hairiness and mouthpart length (Gómez et al., 2009). This is similar to global daisy pollinator networks that are dominated by bees and butterflies, which also differ in these key morphological traits.

With the exception of Tersilochinae wasps, Hymenoptera were rare visitors and Lepidoptera were completely absent, making these daisies unique compared to global daisies which are dominated by bee and butterfly pollinators (Schmitt, 1983; Boldt and Robbins, 1987; Freitas and Sazima, 2006; Figueroa-Castro et al., 2016). This is unsurprising in the southern African context since Namaqualand daisies are also rarely visited by bees and butterflies (Johnson and Midgley, 1997; Ellis and Johnson, 2009; Ellis et al., 2021). Butterfly diversity is low in fynbos due to the low nutrient quality of sclerophyllous leaves, making them unpalatable to caterpillars (Morrow, 1983; Cottrell, 1985 in Anderson et al., 2014). However, this cannot explain the absence of bees since the bee diversity of the winter rainfall regions of southern Africa is high (Kuhlmann, 2009). While bees may be visiting other daisy species not included in this analysis, some research suggests that bees may avoid daisies due to the unfavourable properties of daisy pollen which makes it difficult to digest (Muller and Kuhlmann, 2008).

In Chapter 3, I showed that Nitidulidae beetles and Mariobezziinae flies offered similar levels of pollination effectiveness to a population of *Dimorphotheca pluvialis*. In this chapter, I examined the quality components for *Dimorphotheca pluvialis* only, however, other beetles such as Melyridae were found to be frequent visitors to *Arctotis* daisies. Based on the results of this study, which found that pollinator importance is strongly driven by the quantity component, it may be that a different suite of insects would be important for the maintenance of orange daisy species in the western cape. According to the visitation rates and frequencies recorded in this study, *Arctotis* daisies may be more reliant on beetles such as Nitidulidae, Melyridae and monkey beetles for pollination in the West Coast. Further investigation into the pollination effectiveness of *Arctotis,* and other orange daisy genera, is required to fully understand the importance of beetles in the pollination of GCFR daisy communities.

As expected for generalist pollinated plants, I found limited differentiation in pollinator communities across co-occurring daisy species. However, I also found that most daisy visitors carried a high proportion of conspecific pollen, suggesting that pollinators are showing some level of floral constancy. Another explanation is that individual pollinators are in fact exhibiting floral constancy, suggesting that individual pollinators will continue to visit flowers within a single species throughout their lifetime in order to minimize search time and energy expenditure (Levin and Anderson, 1970). However, this is probably unlikely since Mariobezziinae flies have previously been shown to have a lack of floral constancy in Namaqualand systems (Ellis and Johnson, 2012). The more likely explanation is the patchy nature of co-flowering daisy species. Because daisy species tend to be quite aggregated, most visiting insects may spend most of their time on a single daisy species, even if they don’t exhibit floral constancy. So even though the visitor assemblage structure across co-flowering daisy species is similar in terms of species present, individuals within each insect species may tend to visit one daisy species throughout their individual lifetime, resulting in high pollen load purity. Furthermore, previous studies have found that high pollinator functional diversity results in higher seed set due to spatio-temporal niche complementarity (Hoehn et al., 2008; Blitzer et al., 2016), suggesting that the high diversity of pollinators visiting daisies across the GCFR actually facilitates conspecific pollen transfer and, therefore, high seed set. This thesis provides the first insights into the pollination ecology of the spring mass-flowering, annual daisy species of the Western Cape. I found that Mariobezziinae flies and nitidulid beetles are equally critical for the persistence of mass-flowering, annual daisy species of the Western Cape. While it was hypothesised that Mariobezziinae flies would be important in this system, based on studies conducted in Namaqualand, my study is the first to highlight the importance of Nitidulidae beetles in the reproduction of *Dimorphotheca pluvialis*, a dominant daisy species in the Greater Cape Floristic Region. Since this study focusses specifically on the female fitness component reproduction, research into the relative contribution of these two effective visitors to male plant fitness is a critical next step into understanding what is driving the evolution of floral traits across the Western Cape.

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Appendix A:

Chapter 2: Rarefaction curves and Chao estimators for all daisy populations sampled

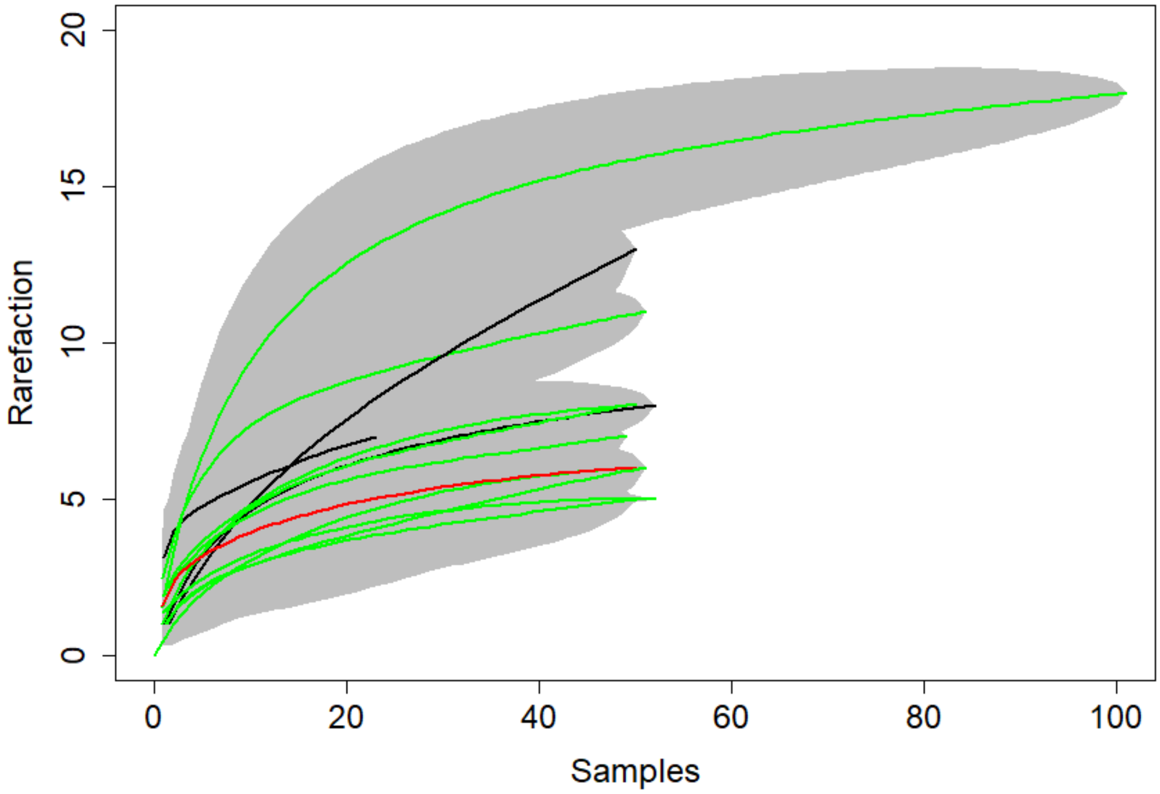

Figure S2.1: Rarefaction curves for each population sampled of *Arctotheca*. Black lines = *Arctotheca calendula langebaan*, green = *A. calendula*, red = *A. prostrata*.

Table S2.1: Observed visitor richness and estimated Chao (±SE) for each population of Arctotheca sampled.

	Population	**Species richness**	**Chao**	**±SE**
Arctotheca calendula langebaan	WC7	6	6,478	1,274
	WC15	8	9,962	3,675
	WC23	11	28,64	23,157
Arctotheca calendula	WC18	11	15,412	7,059
	WC22	18	25,921	11,550
	W2	7	7,980	2,195
	W3	5	5,980	2,159
	W5	8	8,163	0,528
	W6	8	10,939	4,377
	W7	5	5	0
	W8	6	6,980	1,839
	C8	6	10,412	7,059
Arctotheca prostrata	W10	6	6,980	2,180

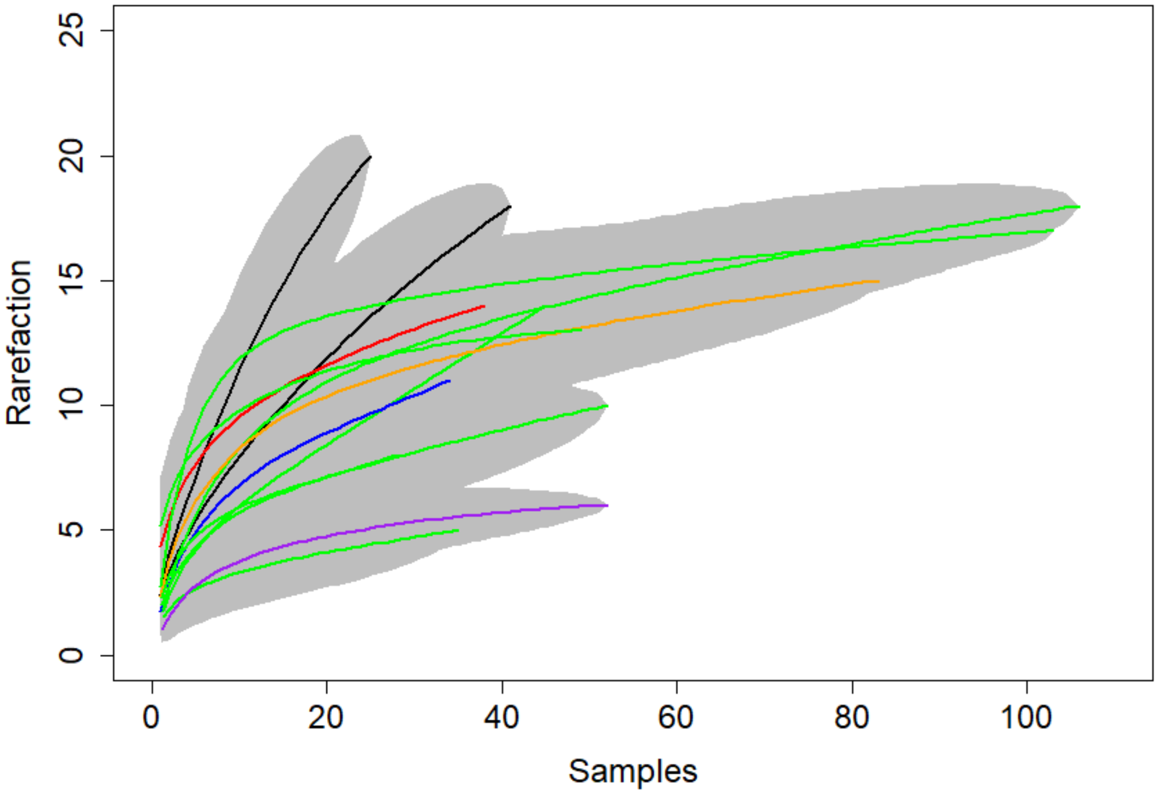

Figure S2.2: Rarefaction curves for each population of *Arctotis*. Black = *Arctotis hirsuta* no-ring, green = *A. hirsuta* ring, blue = *A. flaccida* no-ring, red = *A. flaccida* ring, orange = *A. sp 1*, purple = *A. breviscapa*

Table S2.2: Observed visitor richness and estimated Chao (±SE) for each population of Arctotis sampled.

	Population	Species richness	Chao	±SE
Arctotis hirsuta no-ring	WC1	10	16,098	7,378
	WC2	14	21,68	7,205
Arctotis hirsuta ring	WC3	8	22,667	13,221
	WC10	15	18.962	5,245
	WC11	14	18,446	7,11
	WC13	5	5,971	2,141
	WC14	8	10,893	4,313
	WC16	9	13,413	7,061
	WC17	13	17,408	7,053
Arctotis flaccida no-ring	WC8	8	17,706	9,837
Arctotis flaccida ring	WC9	11	18,789	11,364
Arctotis breviscapa	WC12	17	19,971	4,513
	C7	6	6,245	0,718

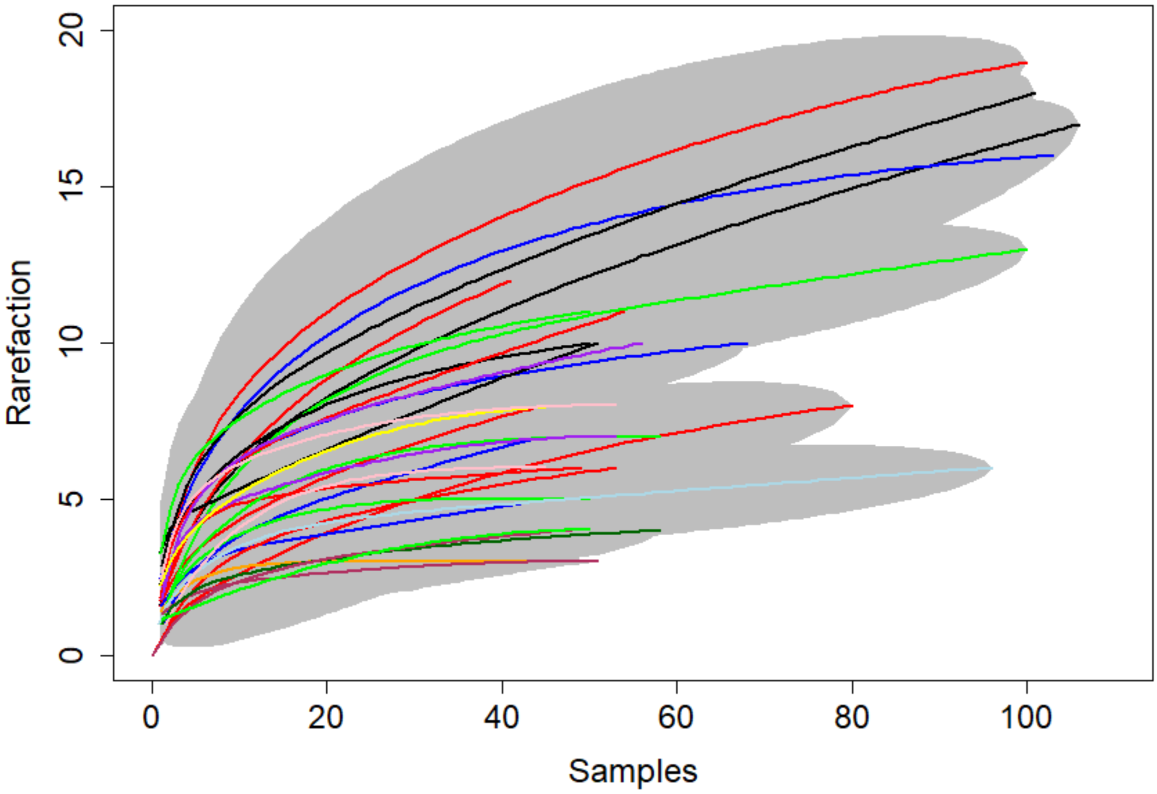

Figure S2.3: Rarefaction curves for each population of *Dimorphotheca*. Black lines = *Dimorphotheca pluvialis* candles, blue = *D. pluvialis* cape town, red = *D. pluvialis* mulberry, green = *D. pluvialis* whiteS, orange = *D. cuneata*, purple = *D. sinuata* tricolor, yellow = *D. sinuata* kardoesie, light blue = *D. sinuata* naartjie, pink = *D. sinuata* peachBotter, maroon = *D. pinnata*, dark green = *D. sinuata* peachGifberg.

Table S2.3: Observed visitor morphospecies richness and estimated Chao (±SE) for each population of Dimorphotheca sampled.

	Population	Species richness	Chao	±SE
Dimorphotheca pluvialis candles	WC1	15	39,269	30,819
	WC20	10	11,960	3,672
	WC21	10	22,255	16,810
	WC22	18	49,683	39,210
Dimorphotheca pluvialis cape town	WC2	7	7	0
	WC3	5	5,978	2,154
	WC7	5	5,978	2,154
	WC12	16	16,396	0,861
Dimorphotheca pluvialis mulberry	WC4	6	6,988	1,851
	WC5	10	19,756	9,930
	WC6	6	7,956	3,664
	WC13	6	7,962	3,676
	WC19	18	21,094	3,625
	WC24	11	23,269	16,829
	WC25	11	12,960	3,672
Dimorphotheca pluvialis white south	WC14	13	18,940	7,117
	WC23	5	5	0
	WC25	6	6	0,447
	C1	7	7	0
	C10	4	4,245	0,718
Dimorphotheca cuneata	W4	3	3	0
Dimorphotheca sinuata tricolor	C1	7	7	0
	C2	10	15,893	7,020
Dimorphotheca sinuata kardoesie	C3	8	8,163	0,528
Dimorphotheca sinuata naartjie	C4	6	6,990	2,199
Dimorphotheca sinuata peachBotter	C5	8	8	0
	C7	6	6,489	1,298
Dimorphotheca pinnata	C6	3	3	0
	C9	4	4,490	1,299
Dimorphotheca sinuata peachGifberg	C8	4	4	0,426

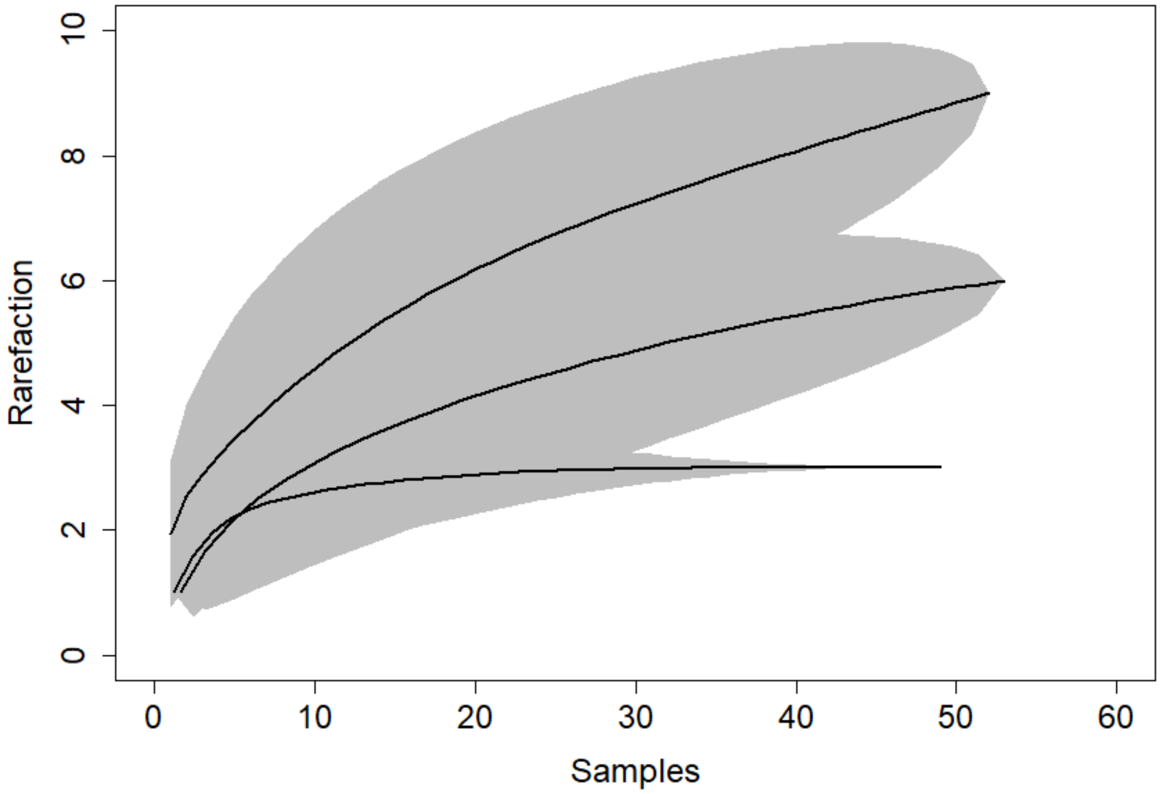

Figure S2.4: Rarefaction curves for each population of *Gazania krebsiana*.

Table S2.4: Observed visitor richness and estimated Chao (±SE) for each population of *Gazania krebsiana* sampled.

	Population	Species richness	Chao	±SE
Gazania krebsiana	W1	9	16,846	11,444
	W6	6	7,962	3,676
	W9	3	3	0

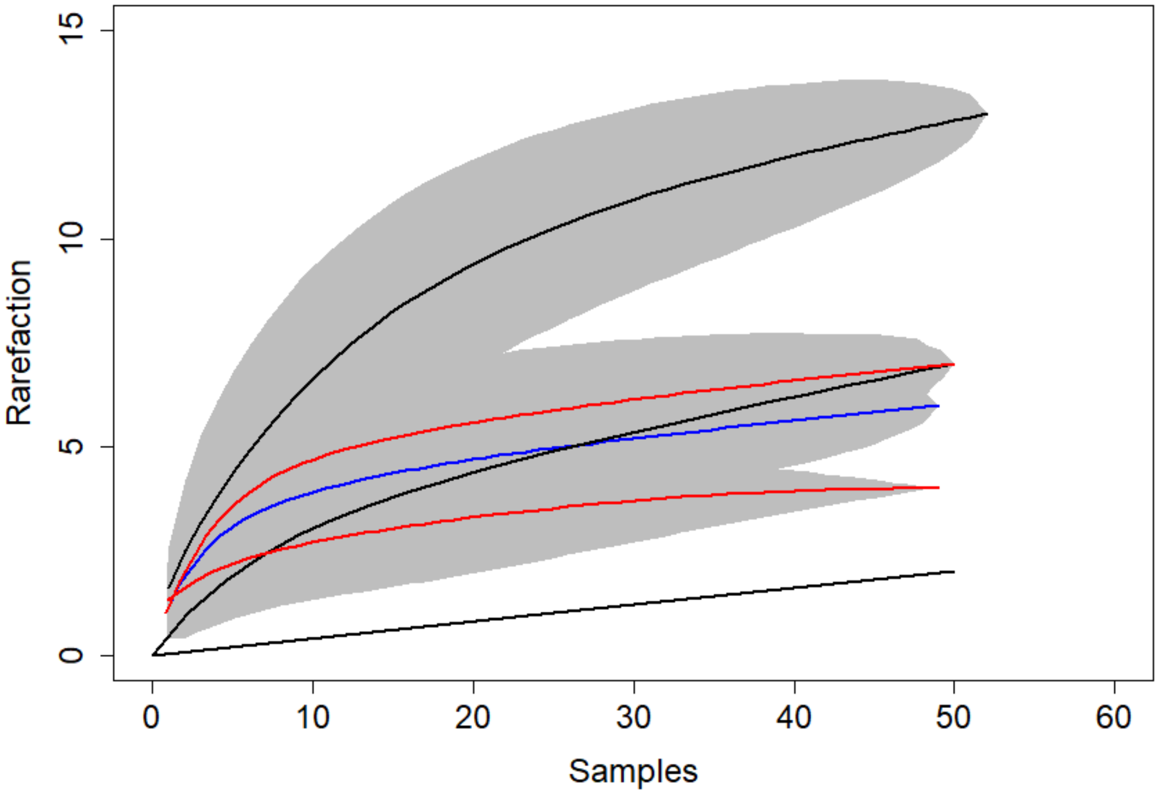

Figure S2.6: Rarefaction curves for all sampled population of *Ursinia*. Black lines = *Ursinia sp1*, blue = *U. sp2*, red = *U. cakelifolia*.

Table S2.6: Observed visitor richness and estimated Chao (±SE) for each population of *Ursinia* sampled.

	Population	Species richness	Chao	±SE
Ursinia sp1	WC12	12	15,923	5,197
	WC23	2	2,980	2,003
	WC25	6	8,940	4,333
Ursinia sp2	WC12	6	6,980	2,179
Ursinia cakelifolia	C3	7	7,980	2,196
	C4	4	4	0

Appendix B:

Chapter 2: Overall species- and population-level rarefaction curves for each daisy species with multiple populations sampled

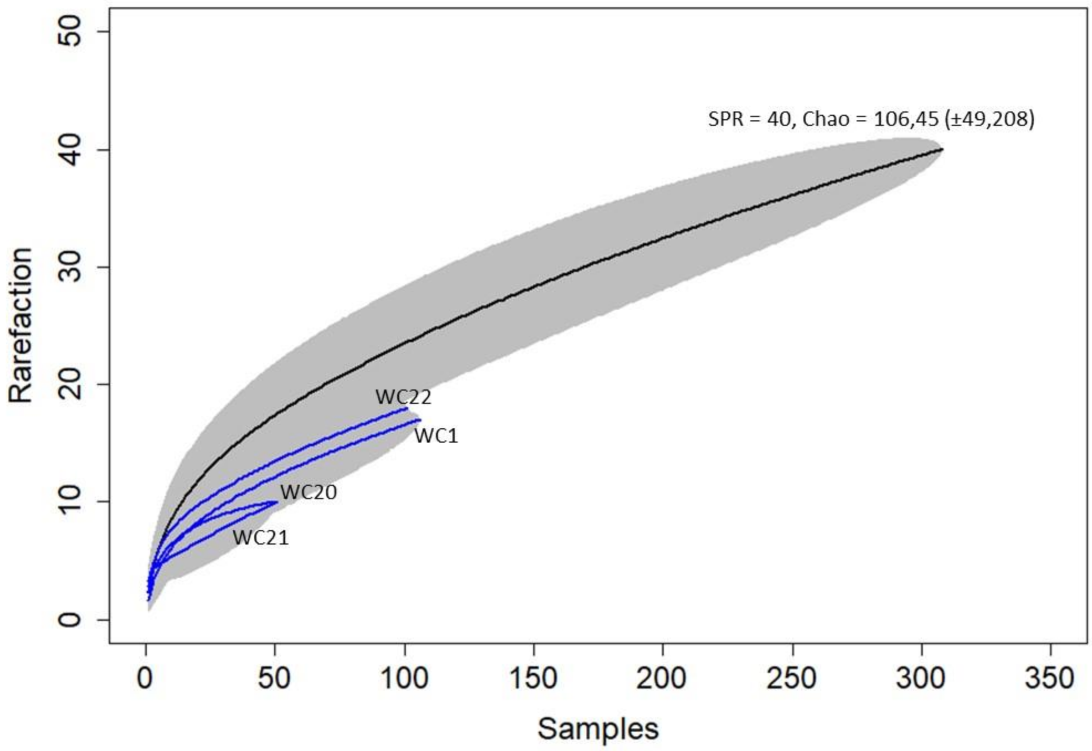

Figure S2.7: Rarefaction curves for all populations of *Dimorphotheca pluvialis* candles sampled (blue lines), as well as the overall species level curve (black lines). SPR = insect species richness. SPR and Chao results for the species overall. The following are the SPR (and Chao±SE) results for each population sampled. WC1 = 17 (49.700±39.228), WC20 = 10 (11.96±3.672), WC21 = 10 (22.255±16.81), WC22 = 18 (49.683±39.210).

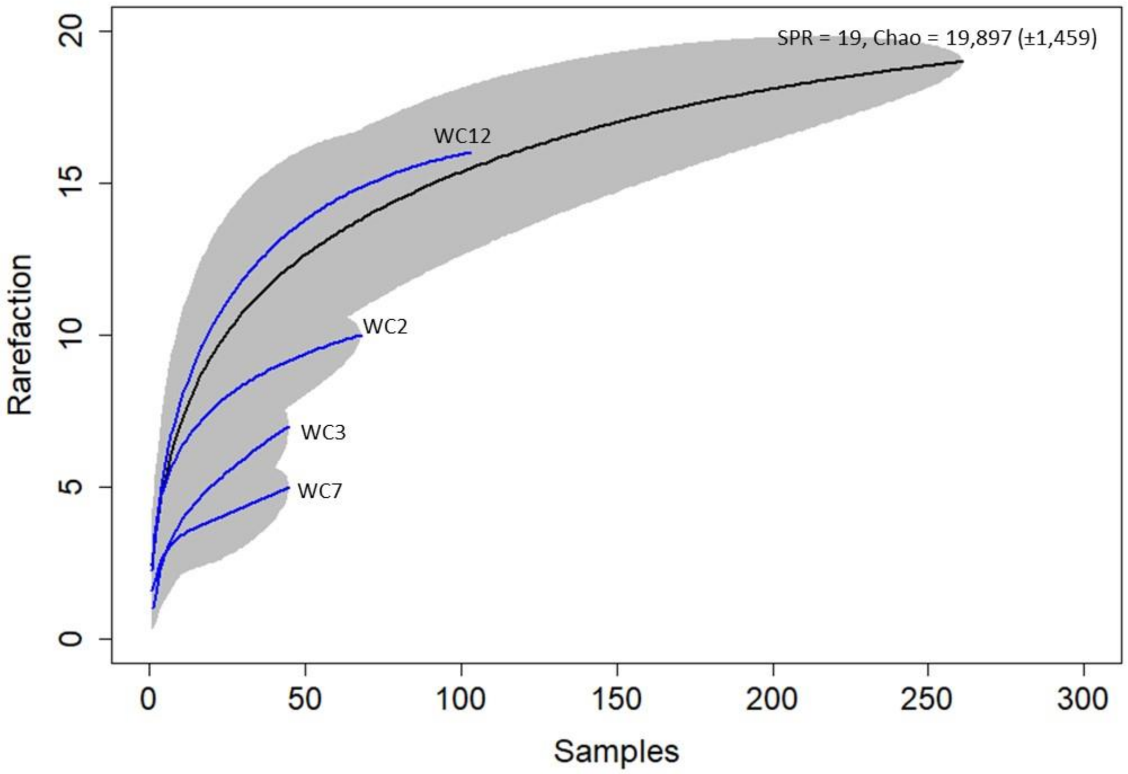

Figure S2.8: Rarefaction curves for all populations of *Dimorphotheca pluvialis* cape town sampled (blue lines), as well as the overall species level curve (black lines). SPR = insect species richness. SPR and Chao results for the species overall. The following are the SPR (and Chao±SE) results for each population sampled. WC2 = 10 (11.971±3.691), WC3 = 7 (12.867±6.925), WC7 = 5 (5.978±2.154), WC12 = 16 (16.396±0.861).

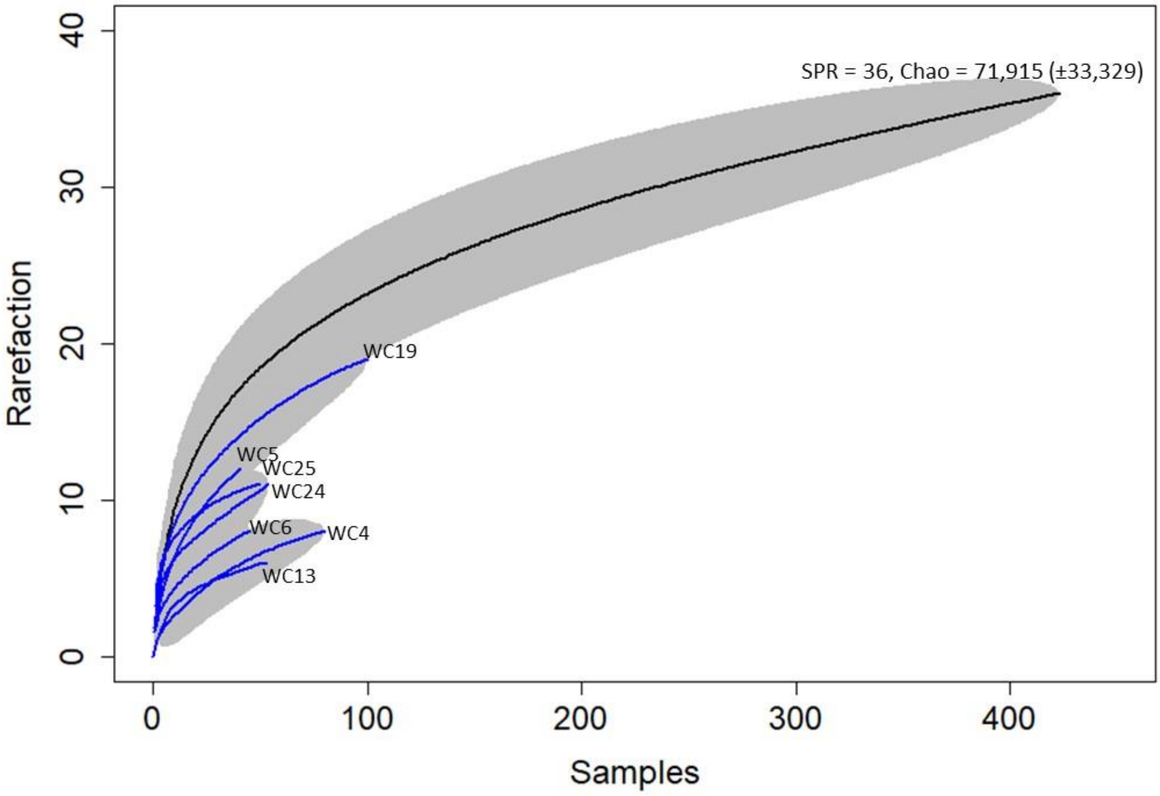

Figure S2.9: Rarefaction curves for all populations of *Dimorphotheca pluvialis* mulberry sampled (blue lines), as well as the overall species level curve (black lines). SPR = insect species richness. SPR and Chao results for the species overall. The following are the SPR (and Chao±SE) results for each population sampled. WC4 = 8 (9.481±2.267), WC5 = 12 (26.634±13.259), WC6 = 8 (10.2±3.328), WC13 = 6 (7.962±3.676), WC19 = 19 (21.475±2.933), WC24 = 11 (23.269±16.829), WC25 = 11 (12.96±3.672).

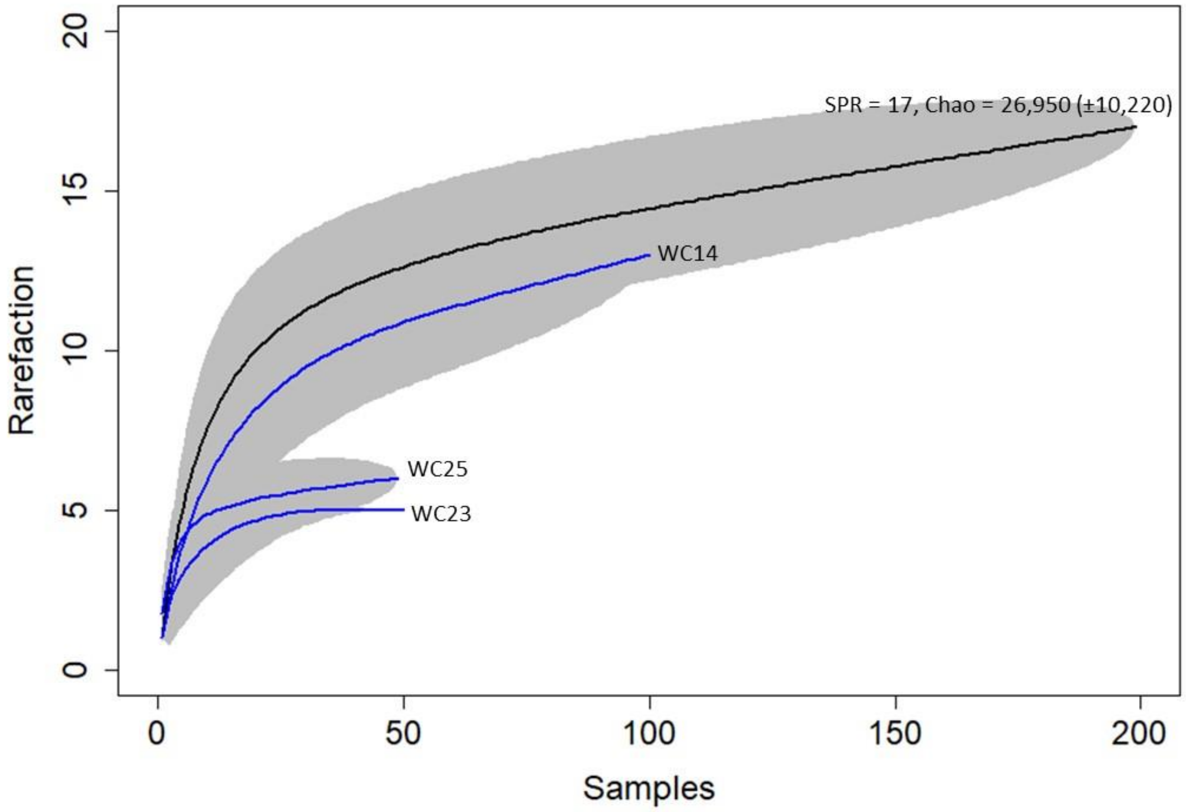

Figure S2.10: Rarefaction curves for all populations of *Dimorphotheca pluvialis* whiteS sampled (blue lines), as well as the overall species level curve (black lines). SPR = insect species richness. SPR and Chao results for the species overall. The following are the SPR (and Chao±SE) results for each population sampled. WC14 = 13 (18.94±7.117), WC23 = 5 (5±0), WC25 = 6 (6±0.447).

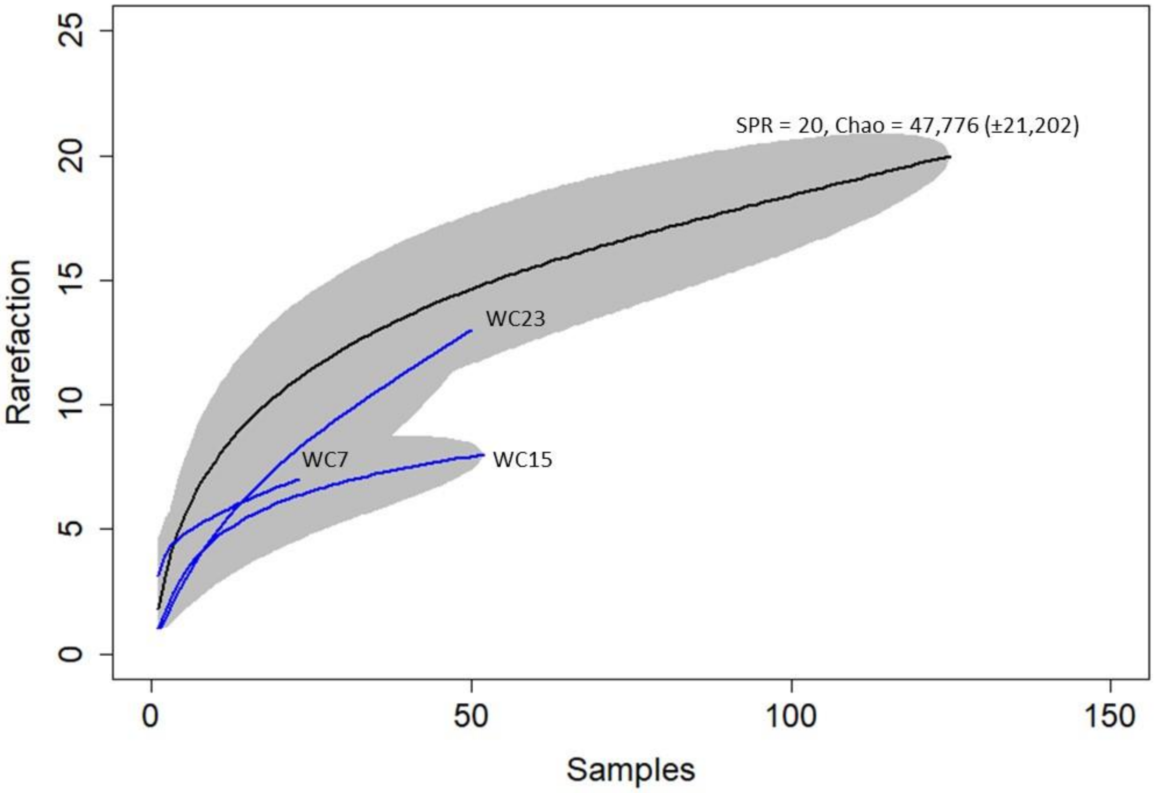

Figure S2.11: Rarefaction curves for all populations of *Arctotheca calendula* langebaan sampled (blue lines), as well as the overall species level curve (black lines). SPR = insect species richness. SPR and Chao results for the species overall. The following are the SPR (and Chao±SE) results for each population sampled. WC7 = 7 (8.913±3.591), WC15 = 8 (9.962±3.675), WC23 = 13 (44.36±38.814).

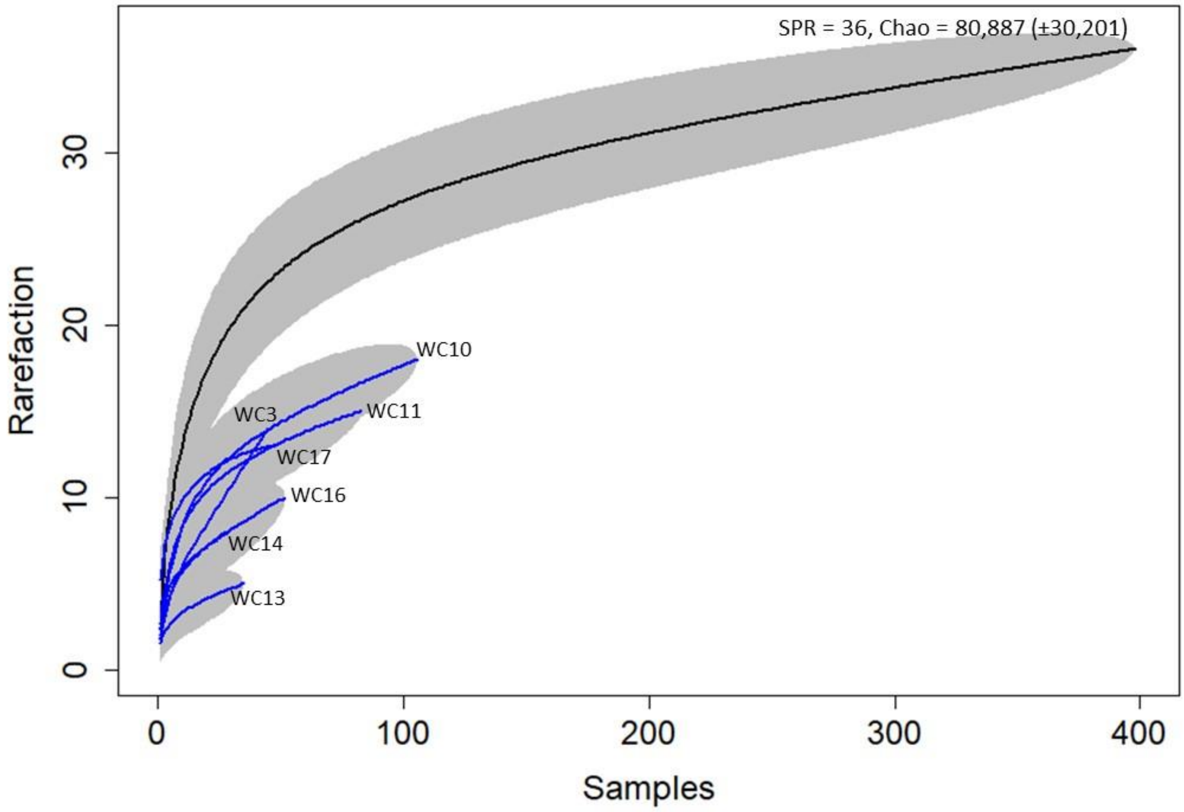

Figure S2.12: Rarefaction curves for all populations of *Arctotis hirsuta* ring sampled (blue lines), as well as the overall species level curve (black lines). SPR = insect species richness. SPR and Chao results for the species overall. The following are the SPR (and Chao±SE) results for each population sampled. WC3 = 14 (58±29.422), WC10 = 18 (26.915±10.082), WC11 = 15 (22.904±11.526), WC13 = 5 (5.971±2.141), WC14 = 8 (10.893±4.313), WC16 = 10 (17.846±11.444), WC17 = 13 (17.408±7.053).

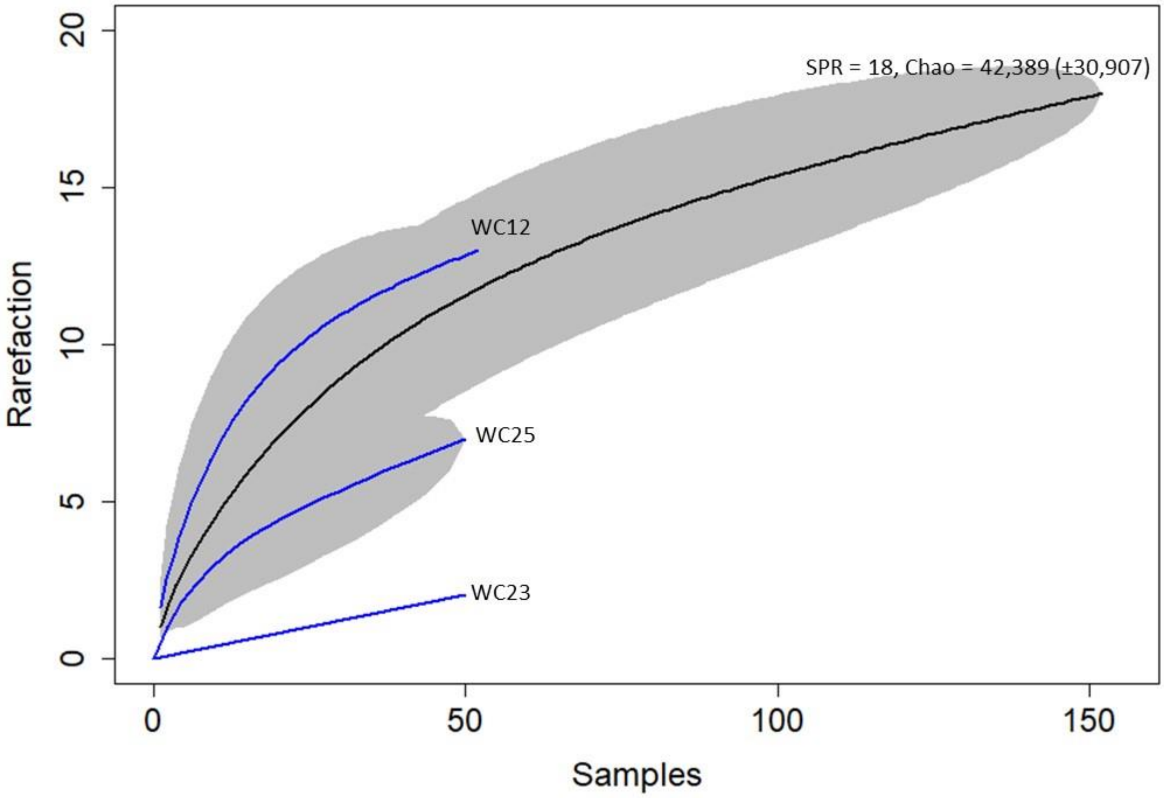

Figure S2.13: Rarefaction curves for all populations of *Ursinia sp1* sampled (blue lines), as well as the overall species level curve (black lines). SPR = insect species richness. SPR and Chao results for the species overall. The following are the SPR (and Chao±SE) results for each population sampled. WC12 = 13 (16.923±5.197), WC12 = 2 (2.98±2.003), WC25 = 7 (12.88±6.94)

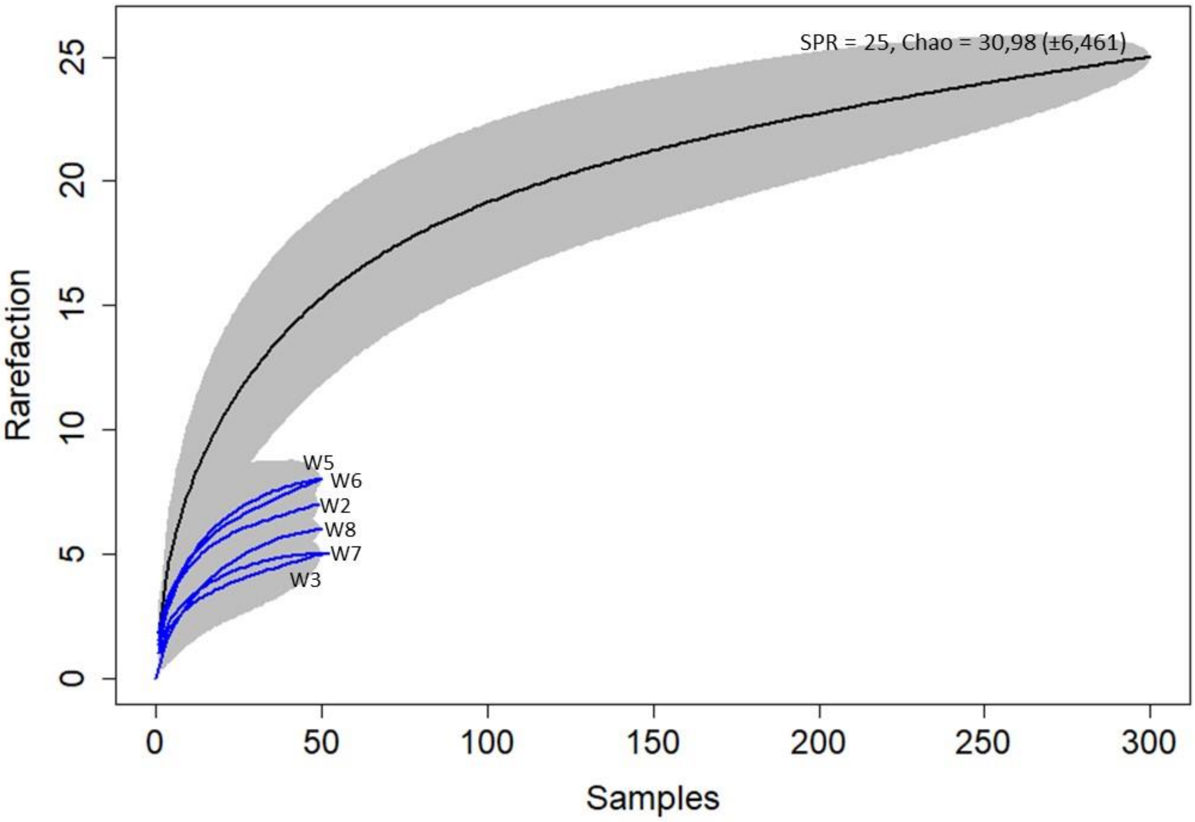

Figure S2.14: Rarefaction curves for all populations of *Arctotheca calendula* sampled (blue lines), as well as the overall species level curve (black lines). SPR = insect species richness. SPR and Chao results for the species overall. The following are the SPR (and Chao±SE) results for each population sampled. W2 = 7 (7.98±2.195), W3 = 5 (5.98±2.159), W5 = 8 (8.163±0.528), W6 = 8 (10.939±4.377), W7 = 5 (5±0), W8 = 6 (6.98±1.839).

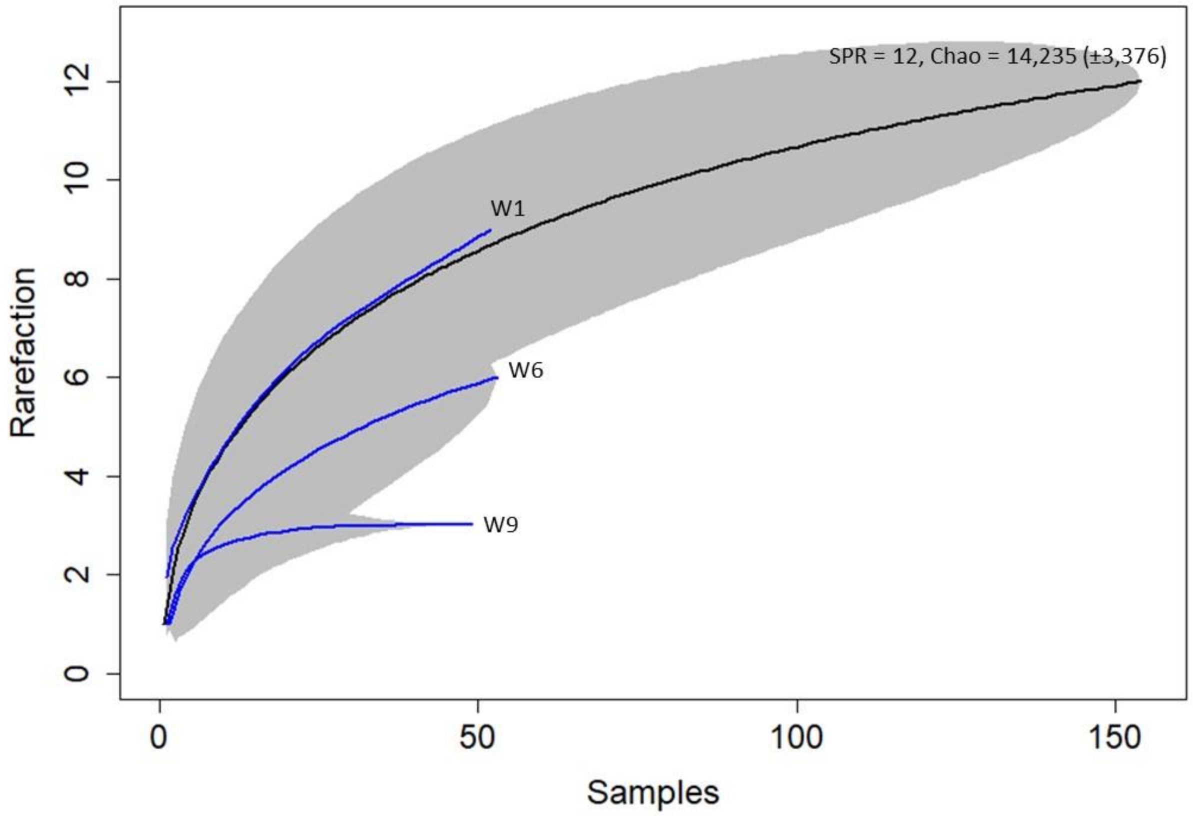

Figure S2.15: Rarefaction curves for all populations of *Gazania krebsiana* sampled (blue lines), as well as the overall species level curve (black lines). SPR = insect species richness. SPR and Chao results for the species overall. The following are the SPR (and Chao±SE) results for each population sampled. W1 = 9 (16.846±11.444), W6 = 6 (7.962±3.676), W9 = 3 (3±0).

Appendix C:

Chapter 2: Rarefaction curves for the genus-level interactions

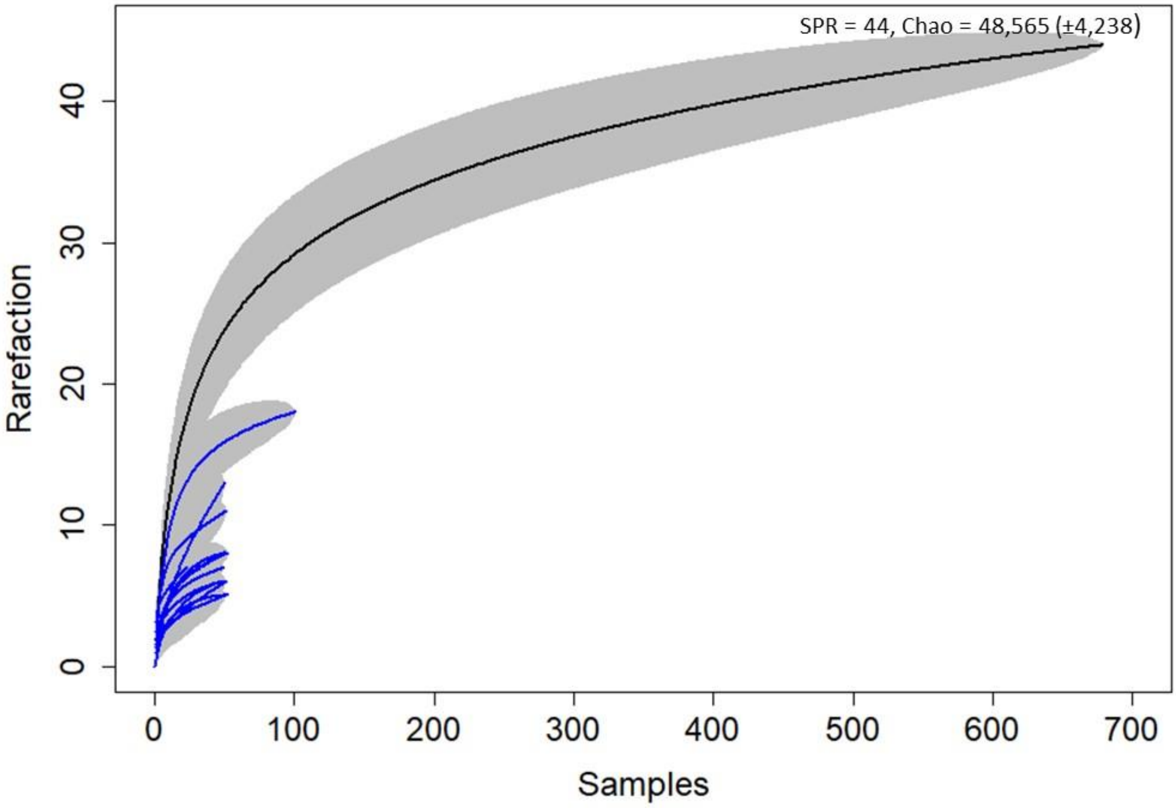

Figure S2.16: Rarefaction curves for the overall genus-level interaction, as well as all the populations sampled for *Arctotheca*.

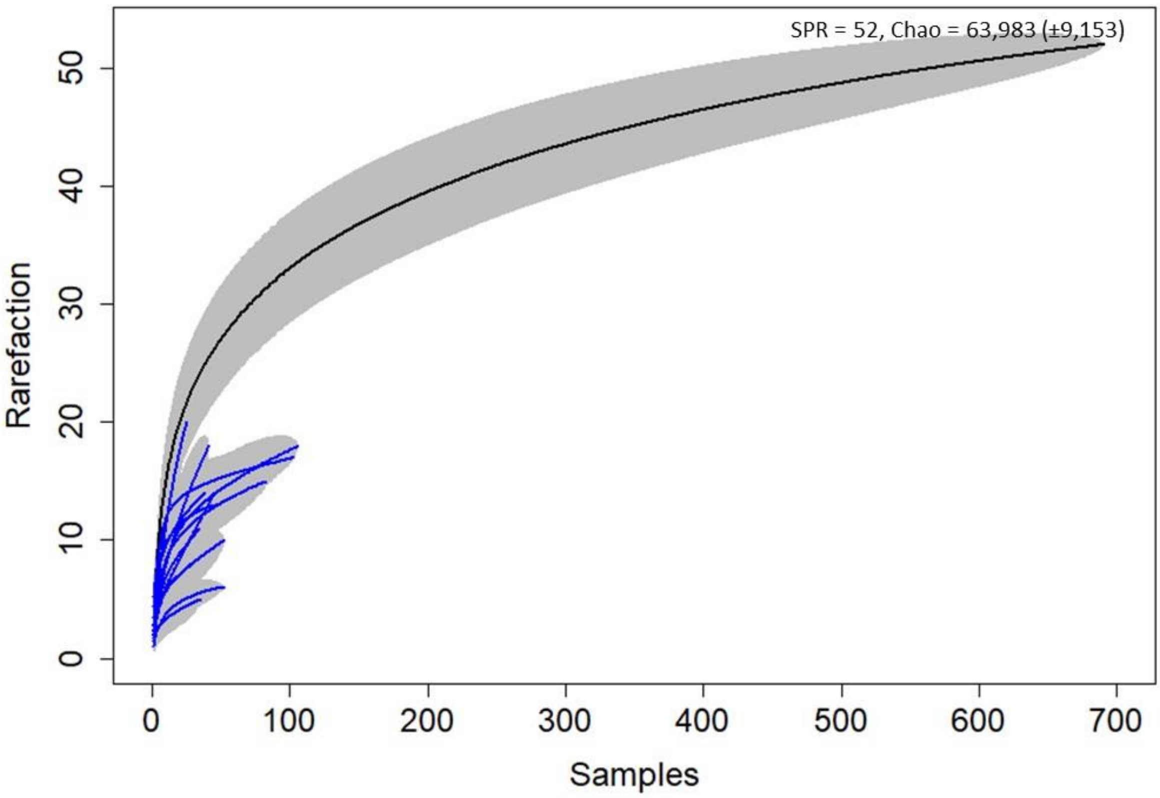

Figure S2.17: Rarefaction curves for the overall genus-level interaction, as well as all the populations sampled for *Arctotis*.

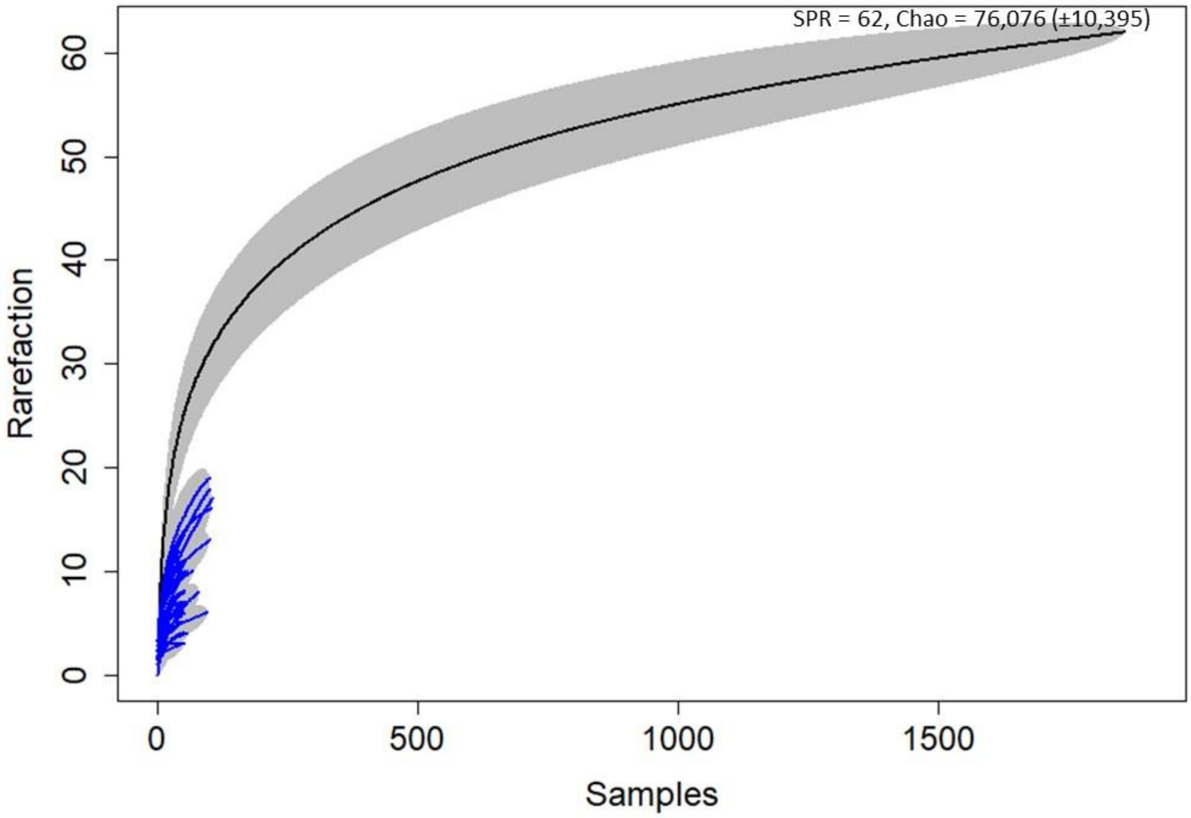

Figure S2.18: Rarefaction curves for the overall genus-level interaction, as well as all the populations sampled for *Dimorphotheca*.

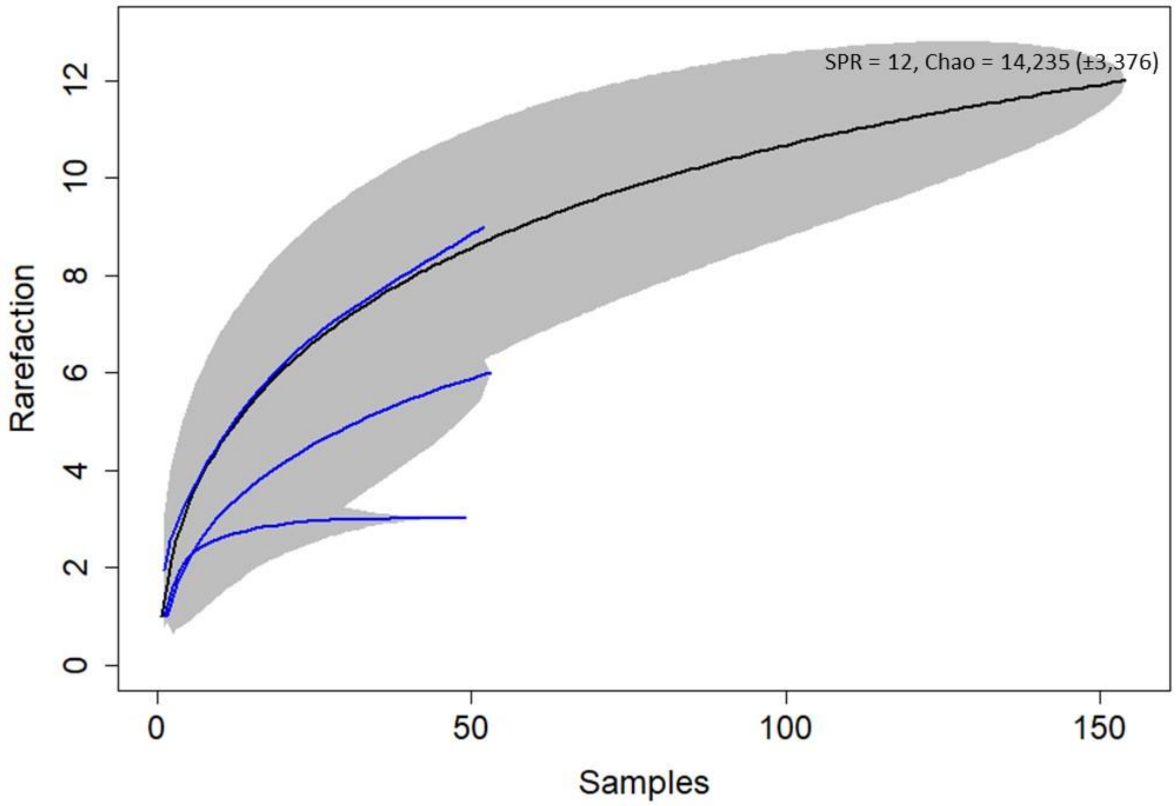

Figure S2.19: Rarefaction curves for the overall genus-level interaction, as well as all the populations sampled for *Gazania*.

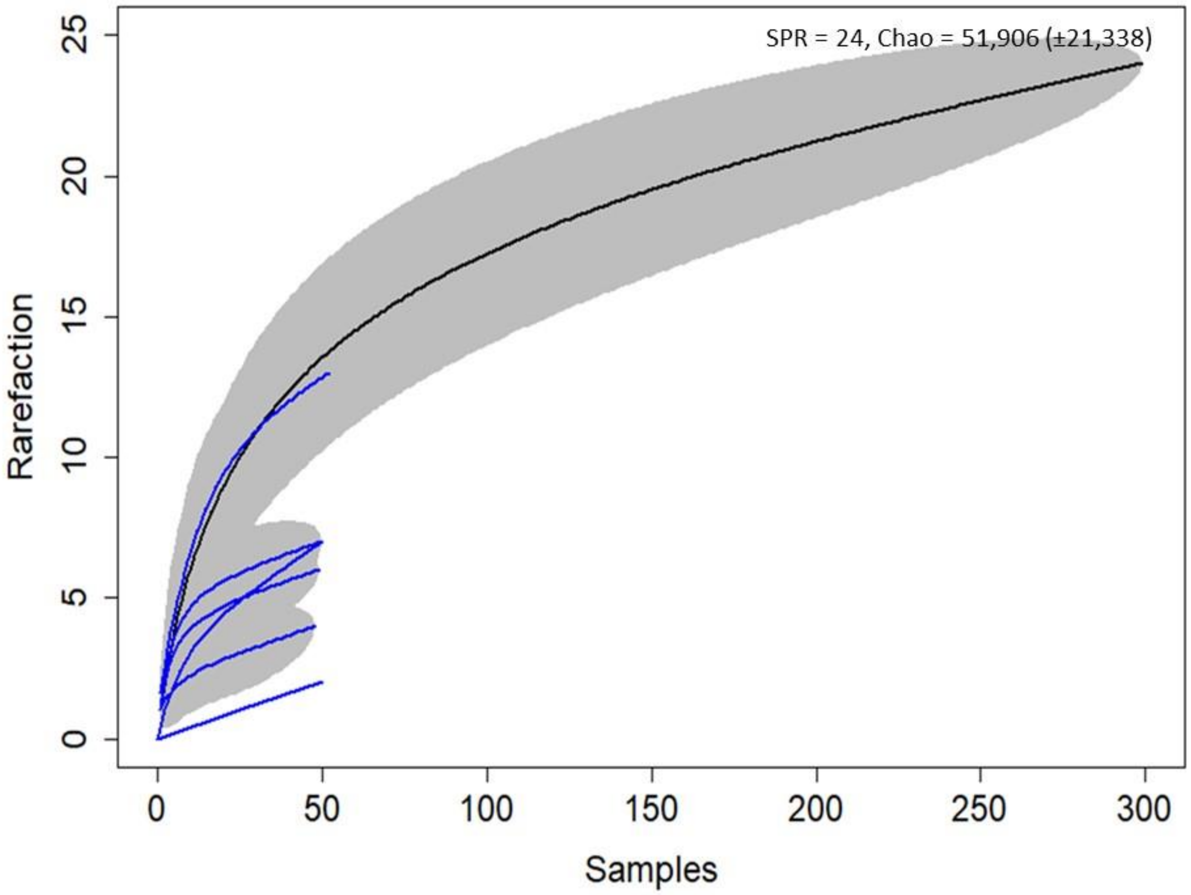

Figure S2.20: Rarefaction curves for the overall genus-level interaction, as well as all the populations sampled for *Ursinia*.

Appendix D:

Chapter 3: Images showing the differences in pollen morphology between *Dimorphotheca* and *Arctotis*

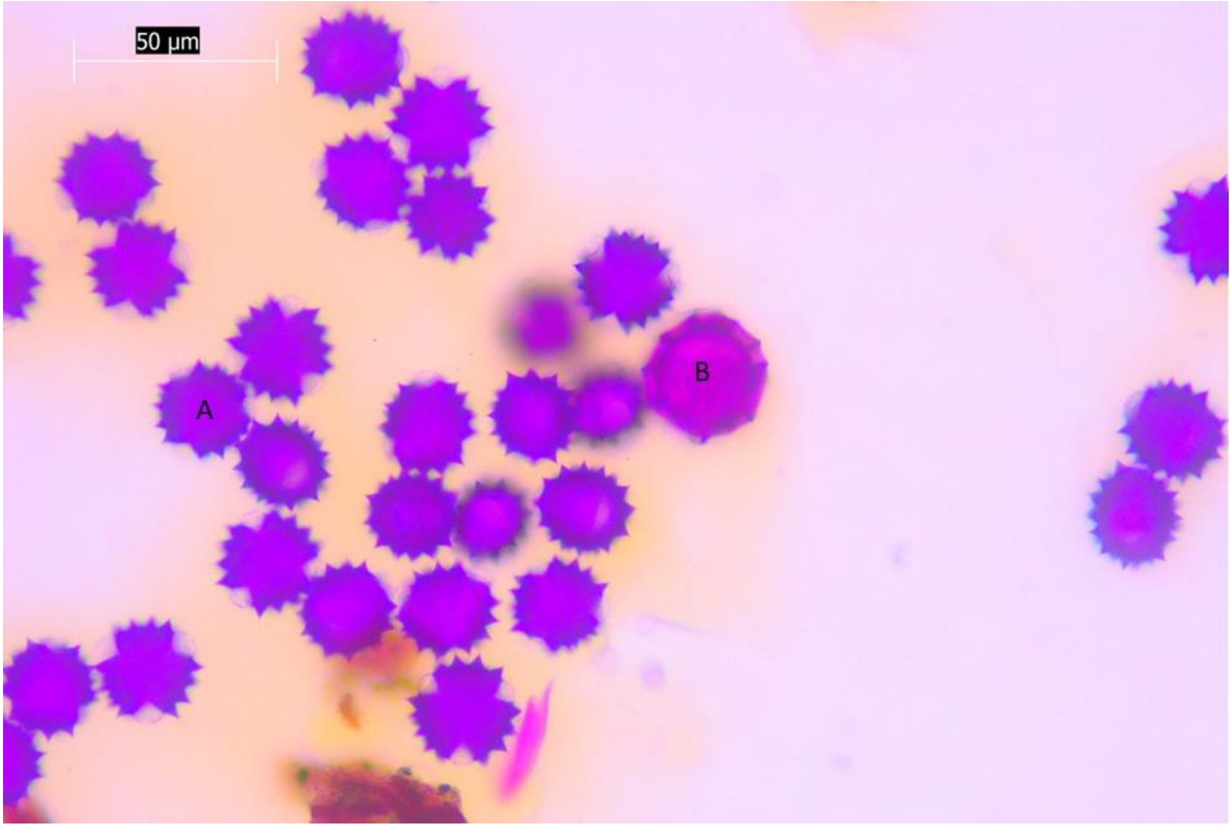

Figure S3.1: The pollen grains removed from the body of a *Corsomyza* fly collected off a *Dimorphotheca pluvialis* (mulberry form) daisy at a site with contained both *Dimorphotheca pluvialis* (mulberry form) and *Arctotis hirsuta* (ring form) populations (WC13). Those pollen grains consistent with *Dimorphotheca* pollen (A) show to have sharp, spiky protrusions. Pollen grain B is much larger than A, with an obviously less spiky exine (identity unknown).

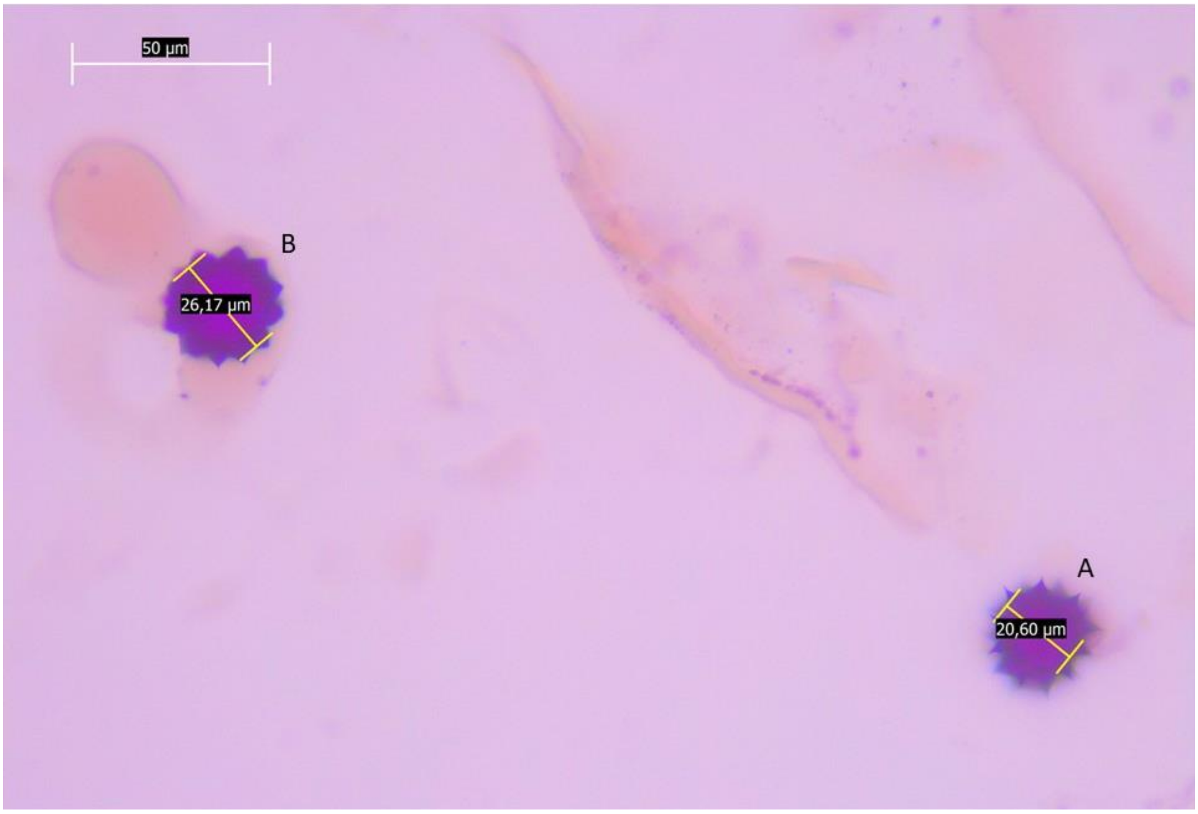

Figure S3.2: The pollen grains removed from the body of a Melyrid beetle collected off an *Arctotis breviscapa* daisy at a site containing both *Dimorphotheca pluvialis* (cape town form) and *Arctotis breviscapa* populations (WC12). Pollen grain A is consistent with *Dimorphotheca* pollen, while pollen grains B is consistent with *Arctotis* pollen. It is larger in diameter and has less pointy spikes on the exine.