






A bimodal pollination system enhances reproductive potential of translocated populations of an endangered grassland succulent



Authors:

Peter G. Jansen¹ 
 Stefan J. Siebert¹ 
 Frances Siebert¹ 
 Johnnie van den Berg¹ 
 Anine Jordaan² 

Affiliations:

¹Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa

²Laboratory of Electron Microscopy, Chemical Resource Beneficiation, North-West University, Potchefstroom, South Africa

Corresponding author:

Stefan Siebert,
 stefan.siebert@nwu.ac.za

Dates:

Received: 27 Nov. 2018
 Accepted: 02 Apr. 2019
 Published: 14 Aug. 2019

How to cite this article:

Jansen, P.G., Siebert, S.J., Siebert, F., Van den Berg, J. & Jordaan, A., 2019, 'A bimodal pollination system enhances reproductive potential of translocated populations of an endangered grassland succulent', *Koedoe* 61(1), a1562. <https://doi.org/10.4102/koedoe.v61i1.1562>

Copyright:

© 2019. The Authors.
 Licensee: AOSIS. This work is licensed under the Creative Commons Attribution License.

Read online:



Scan this QR code with your smart phone or mobile device to read online.

A major concern during the translocation of higher plant species is related to habitat suitability and the availability of pollination services. Should these not meet the requirements of the plant, then successful reproduction and establishment cannot occur. We studied an endangered succulent, *Frithia humilis*, which had previously been translocated to typical and atypical habitats, to assess the occurrence of potential pollinators at these sites. Insects visiting *F. humilis* flowers and showing signs of pollen were captured, preserved and studied using a scanning electron microscope. Pollen of *F. humilis* was searched for. Abundance and diversity patterns of these pollen carriers across edaphic habitats of translocated populations were compared with those in a natural occurring population. Pollination success of guilds was compared amongst translocated *F. humilis* populations by considering the number of seedlings in a new season. Across *F. humilis* populations, Hymenopteran species had the largest pollen loads, making this a Melittophilous pollination system, typical for the Aizoaceae. Additionally, Anemophilous syndrome was described for translocation sites which provide reserve pollinators. Fruit formation was more favourable in suitable edaphic habitat on Ecce sandstones. Presence of seedlings at both translocation sites was indicative of successful pollination events of the self-incompatible species, but recruitment was proportionally higher in suitable habitat. Habitat suitability, in the case of this threatened species, is more of a limiting factor than pollination services after a translocation event.

Conservation implications: Translocations are fast becoming an attractive alternative for developers. This study cautions that the presence of pollinators and successful reproduction in translocated populations are only effective if the populations were translocated to an ideal habitat.

Keywords: Aizoaceae; coal mining; conservation ecology; *Frithia humilis*; mesembryanthemaceae.

Introduction

Frithia humilis Burgoyne is an endangered, cryptic, dwarf succulent in the Aizoaceae (Burgoyne & Krynauw 2005; Burgoyne, Smith & Du Plessis 2000). It is endemic to the Rand Highveld Grassland of Gauteng and Mpumalanga in South Africa, specifically the area between Bronkhorstspuit, Ogies and Middelburg. Here it is restricted to flat sandstone plates of the Dwyka and Ecce formations in microhabitats comprising aggregates of weathered rock and organic materials (Burgoyne & Hoffman 2011). The succulent leaves of the species generally grow from beneath the soil surface (a typical window plant), seldom protruding more than 20 mm above ground level. The leaves are contractile, allowing the plant to retract into the soil where it is protected from desiccation and frost in the winter months (Burgoyne et al. 2000). Consequently, leaves of this species are only visible during active growth, specifically when flowering during the summer months (Figure 1a).

In 2008, a population of *F. humilis* was discovered at a coal mine in the north of eMalahleni after a mining license had already been granted (Harris et al. 2014). *In situ* conservation was impossible because of the destructive nature of open-cast mining practices. Considering the species' endangered status (Burgoyne & Krynauw 2005), translocation was regarded as a last resort to save the population (Godefroid et al. 2011; Gordon 1994). Translocation is the process whereby a population of living organisms is deliberately moved from one area to another suitable habitat within its existing distribution range (IUCN 2013). Consequently, conservation agencies translocated the population to pre-selected receptor sites (Burgoyne & Hoffmann 2011).

A rapid pre-translocation habitat assessment was conducted to assess the suitability of receptor sites in terms of substrate, ecological integrity, genetic impacts and long-term protection

(Burgoyne & Hoffmann 2011). A major critique of such rapid assessments is that in-depth assessments of pollinators are generally lacking (Forup et al. 2008), putting the translocated population at risk, especially in the case of a self-incompatible species such as *F. humilis* (Harris, Van den Berg & Siebert 2016). However, it could be argued that by the very nature of a translocation the number of plant individuals should be high enough to attract pollinators from elsewhere in the distribution range and provide for their dietary requirements (Menz et al. 2011).

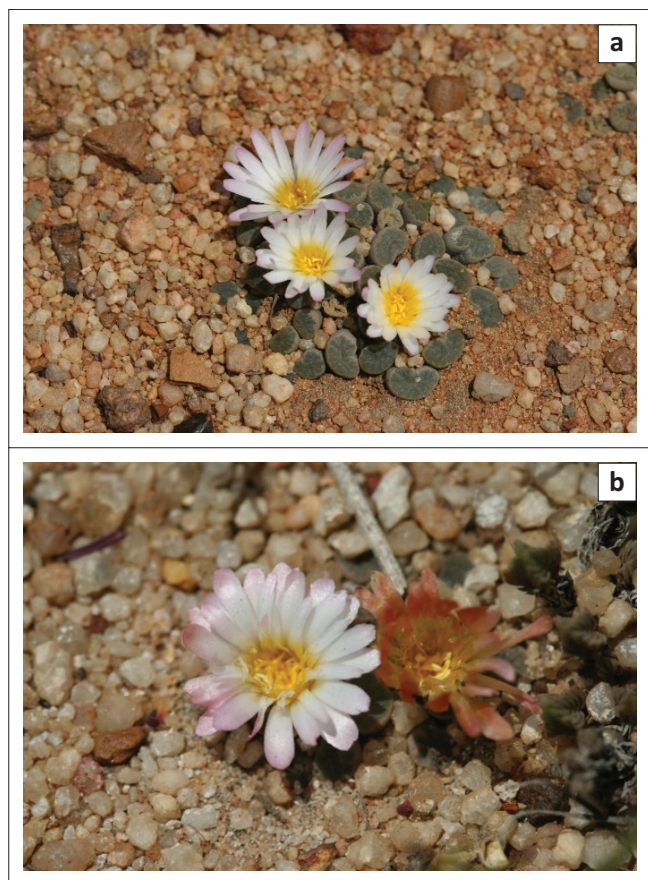
The translocation of *F. humilis* provided an ideal opportunity to test whether the pollination guild of the translocated populations is similar to that of a naturally occurring population. Knowledge of this provides a measure to gauge whether a population is only reproducing because of chance pollination, or it persists because of effective pollination (Montalvo et al. 1997). A poorly functioning pollination system can have several genetic consequences (Armstrong & Seddon 2008; Montalvo et al. 1997; Moritz 1999) and may sooner or later affect population viability, eventually leading to population extinction (Kearns & Inouye 1997).

Johnson (2010) has called for pollination research in South Africa to improve our understanding of the role of pollination niches for the maintenance of plant species diversity in a rapidly changing environment. This study heeds the call to supplement existing knowledge concerning

the pollinators of *F. humilis* (Harris et al. 2016). It highlights the importance of pollination studies in translocation initiatives and provides insight into the status of a biotic factor that could limit reproductive success after a founder population was established in a harsh environment.

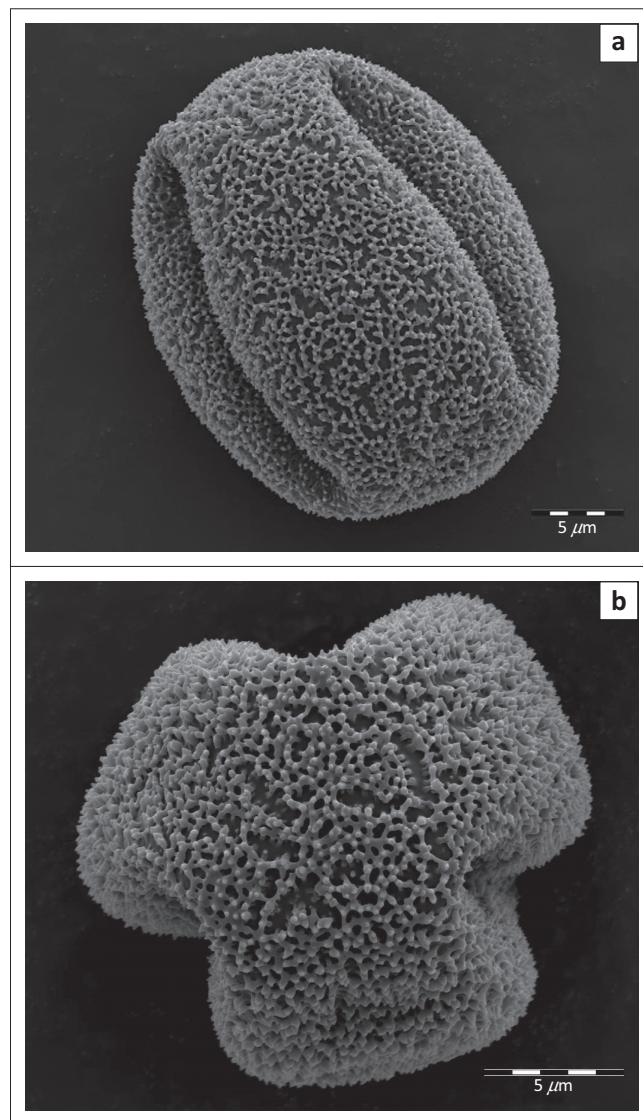
Flowers of *Frithia humilis*

The flowers of *F. humilis* are 15 mm–20 mm in diameter and white with a yellow centre (Figure 1a, b). The petals, especially towards the tip, are occasionally tinged pink. Flowers are either carried on very short stalks or are entirely stalkless. The pollen is yellow and tricolpate (Figure 2). The sepals resemble the leaves and form a short tube (Burgoyne et al. 2000). *Frithia* flowers are self-incompatible (Burgoyne et al. 2000), a common characteristic of South African Aizoaceae (Mayer & Pufal 2007). After pollination the flowers turn yellow or pink before expiring (Figure 1b), which may serve as an indication to pollinators that the flower is pollinated and without reward (Burgoyne et al. 2000).



Source: Photo courtesy of K. Smit.

FIGURE 1: *Frithia humilis* plants in flower (a) and displaying a fresh (left) and an expired flower (right) on the same plant (b).



Source: Photo courtesy of A. Jordaan.

FIGURE 2: Scanning electron micrographs of *Frithia humilis* pollen grains in its distal polar-equatorial view (a) and polar view (b).

Based on familial pollination syndromes (Hartmann 1991), *F. humilis* flowers could be Melittophilous because they are wide open, petals are shiny, whitish and open diurnally (from mid-morning to mid-afternoon) for several days. Pollen is less abundant than in most Melittophilous species but easily accessible. Further support for this syndrome is the self-incompatible sexual phase, with flowers reported to have a distinct male phase (protandrous) followed by a female phase approximately 4 days later (Hammer 1995; Hartmann 1991). Stigmata are initially shorter than stamens, elongating and emerging only at the beginning of the female phase when the male phase ends.

Research method and design

Study area and site selection

Two sites were chosen as receptor sites based on pre-selected criteria (Burgoyne & Hoffmann 2011) for plants translocated from a coal mine (Figure 3). Half of the affected population was translocated to typical Ecga Group sandstone which corresponded with the geology of the donor site. The other half of the population was translocated to less ideal outcrops of the sedimentary Wilge River Formation of the Waterberg Group to test whether other related rock types could serve as an alternative should mining activities warrant future translocations (Harris et al. 2014).

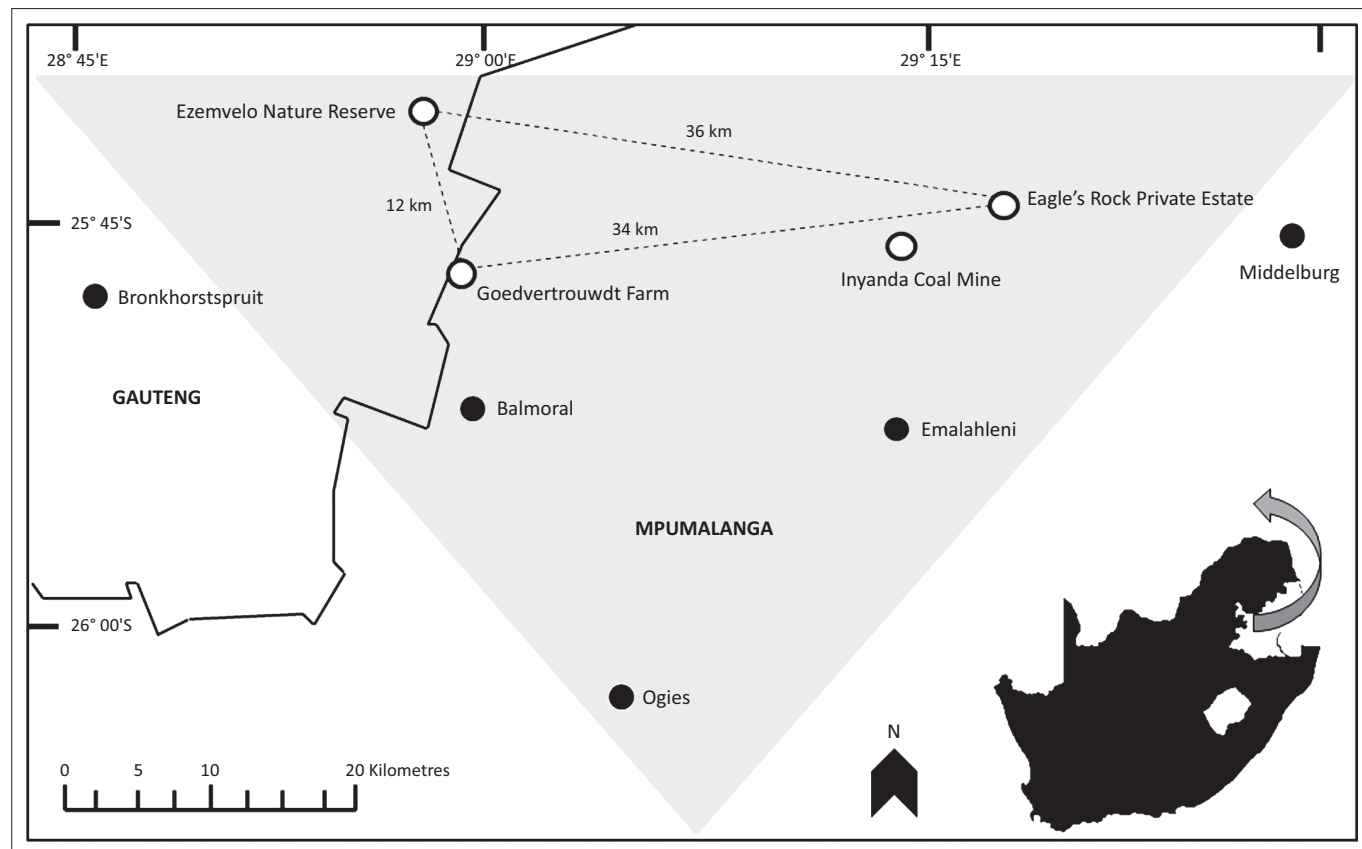
Insect observations

Qualitative observations for pollinators were made at the receptor sites of Goedvertrouwdt and Eagle's Rock, and a large, natural population at Ezemvelo Nature Reserve. As the flowers open only on sunny days, observations were made on non-overcast days of March 2016 and insects that visited the flowers were collected by hand. One observation session (of 3.5 h) was performed per day to coincide with the flowering time of the species for each population, on 3 suitable days (Table 1). Three 1-m² patches with most flowers were visually estimated and demarcated at each site for observation. One person per patch observed for pollinators for the entire period of anthesis from 11:30 to 15:00. Thus 10.5 h of observation was conducted per population. Insects alighting or foraging on flowers were captured using an insect net and those species observed for the first time were euthanised in a killing jar with ethyl acetate. These specimens and a set of duplicates were then stored in individually marked containers for later

TABLE 1: Overview of time spent and flower visitors recorded during the survey.

Locality	Mean number of plants per 1 m ²	Total hours of observation	Number of flower visitors	Number of species
Eagle's Rock	62	10.5	6	4
Goedvertrouwdt	54	10.5	14	6
Ezemvelo Nature Reserve	429	10.5	26	4
Total	-	31.5	46	9

Note: Daily observations were made between 11:30 and 15:00. Three observations were made per site.



Source: Photo courtesy of S. van Eeden.

FIGURE 3: Localities of the receptor sites (Ecga sandstone at Goedvertrouwdt Farm and sedimentary rock at Eagle's Rock Private Estate) and control population at Ezemvelo Nature Reserve on Ecga and Dwyka sandstone in South Africa. The donor population at Inyanda Coal Mine is indicated and the shaded grey triangle represents the approximate endemic distribution of *Frithia humilis*. Distances between study populations are indicated.

identification and pollen assessments. Repeat visitors of the same species were only counted and released.

Verification of pollen and insect identification

All euthanised specimens were photographed with a Nikon AZ1000 stereomicroscope to assist in identification and scanned for pollen grains. Particular attention was paid to the head and legs, where pollen was most likely to be found. Those specimens carrying pollen were sputter-coated with a thin gold-palladium layer and inspected under an FEI Quanta FEG 250 scanning electron microscope (SEM) and micrographs of relevant pollen loads were taken. Duplicate insect specimens were pinned and labelled as prescribed by Uys and Urban (2006) and submitted to the Biosystematics Division of the Plant Protection Research Institute of the Agricultural Research Council (ARC) for identification (Appendix 1). Identification to species level was hampered by the taxonomic impediment whereby large numbers of species still require classification or have gone undiscovered (Eardley, Gikungu & Schwarz 2009). Verification of *F. humilis* pollen was based on pollen micrographs in Burgoyne et al. (2000).

Plant counts

In each of the three subplots per population, all the adult plants and flowers were counted according to the technique described by Harris et al. (2014). The fruits were counted per subplot 30 days later. To estimate seed set per capsule in each subplot, 20 capsules were removed from plants bordering on the subplot (after 6 months) and the seeds were counted to determine the mean number of seeds per capsule. The sites were revisited after the first follow-up rains in October 2017 to count the seedlings per subplot.

Ethical considerations

Ethical clearance was obtained from the North-West University. This article followed all ethical standards for research without direct contact with human or animal subjects.

Results

Pollinator diversity

A total of 46 insects were captured whilst alighting on *F. humilis* flowers. These comprised nine different species of

which six were hymenopterans and three were dipterans (Table 2). No lepidopterans were captured during this survey although species were observed making darting visits to *Frithia* flowers. Harris et al. (2016) did record two butterfly species alighting on *Frithia* flowers but these were void of pollen.

At Goedvertrouwdt, 14 individuals belonging to six species were recorded, with *Exoprosopa eluta* (Loew) (Hymenoptera: Bombyliidae) being the most frequent visitor (five records) of *Frithia* flowers (Table 2). Species of *Lipotriches* (Gerstaecker) (Hymenoptera: Bombyliidae) and *Quartinia* (André) (Hymenoptera: Vespidae) were recorded the most at Eagle's Rock Private Estate (Table 2). At Ezemvelo Nature Reserve, 26 individuals (four species) were captured, with *Amegilla fallax* (Smith) (Hymenoptera: Apidae) and *E. eluta* accounting for 65% of the visitations (Table 2).

Pollen carriers

Harris et al. (2016) already provided evidence that the bees *Amegilla fallax* and *Megachile niveofasciata* (Friese) (Hymenoptera: Megachilidae), together with the bee fly *E. eluta*, carry *F. humilis* pollen. Scanning electron microscope evidence reported here was, therefore, specifically aimed at the five newly captured flower visitors and also on recaptured *Lipotriches* sp. previously identified as a potential pollen carrier by Harris et al. (2016).

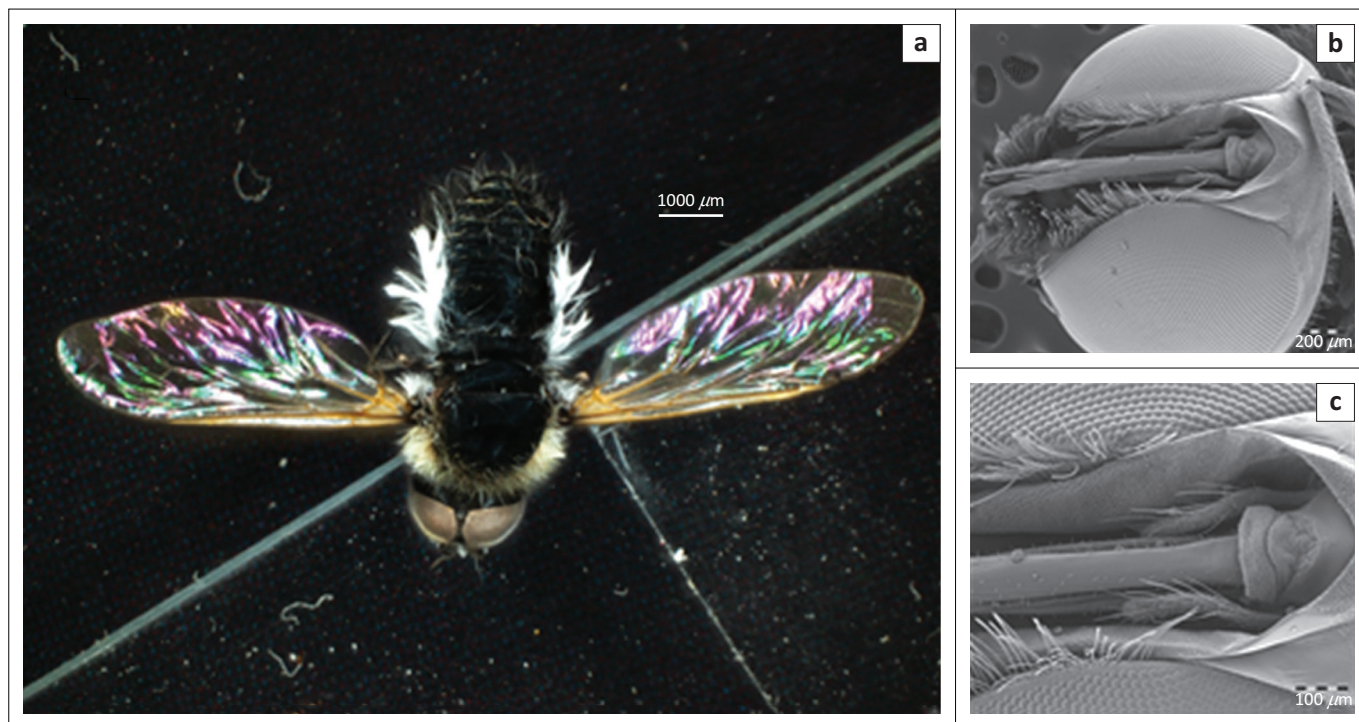
Frithia pollen was found in varying amounts on the selected insect specimens and these species are therefore considered pollen carriers (Table 2). Some pollen grains were visible on *Notolomatia* sp. (Figure 4), on and immediately adjacent to the mouth parts. Although only three pollen grains were found on the abdomen of *Paragus* sp. (Figure 5), a significant clump of *Frithia* pollen was found directly on the anus of the specimen. Pollen found on the *Quartinia* sp. (Figure 6) was sparsely distributed over its entire body. Copious amounts of pollen were found beneath the mouth parts of the *Ammophila* sp. (Figure 7). Halictidae specimens (*Seladonea* sp. and *Lipotriches* sp.) (Figures 8 and 9) were carrying copious amounts of *Frithia* pollen, particularly in their pollen baskets.

TABLE 2: *Frithia humilis* pollen carriers with collection locality and pollen load.

Order/Family	Species	Common name	Pollen placement	Pollen load	Study sites	Source
Diptera						
Bombyliidae	<i>Exoprosopa eluta</i> (Loew)	Bee fly	Eyes and head cavity	Low	E(1), G(5), ENR(8)	Harris et al. (2016)
	<i>Notolomatia</i> (Greathead) sp.	Bee fly	Eyes and proboscis	Low	G(1)	Reported here
Syrphidae	<i>Paragus</i> (Latreille) sp.	Hover fly	Body and anus	Low	E(1)	Reported here
Hymenoptera						
Apidae	<i>Amegilla fallax</i> (Smith)	Banded bee	Head, legs and eyes	Medium	G(2), ENR(9)	Harris et al. (2016)
Halictidae	<i>Lipotriches</i> (Gerstaecker) sp.	Sweat bee	Pollen baskets and legs	High	E(2), ENR(4)	Reported here
	<i>Seladonea</i> (Robertson) sp.	Sweat bee	Pollen baskets and legs	High	G(2)	Reported here
Megachilidae	<i>Megachile niveofasciata</i> (Friese)	Dauber bee	Abdomen	High	G(3), ENR(5)	Harris et al. (2016)
Sphecidae	<i>Ammophila</i> (W.Kirby) sp.	Thread-waist wasp	Mouth parts	Medium	G(1)	Reported here
Vespidae	<i>Quartinia</i> (André) sp.	Pollen wasp	Head, body and legs	Low	E(2)	Reported here

E, Eagle's Rock Private Estate; G, Goedvertrouwdt Farm; ENR, Ezemvelo Nature Reserve.

Note: Numbers in brackets per study site indicate the number of pollen carriers successfully captured and identified per site in March 2016. Low, <10; medium, 10–100; high, >100 pollen grains.



Source: Photo courtesy of P. Jansen.

FIGURE 4: (a) Stereomicrograph of *Notolomatia* sp. (Bombyliidae). Scanning electron micrographs of several *Frithia humilis* pollen grains on the eyes (b) and proboscis (c).

Pollination system

The two translocation sites and the control populations were situated in an area of approximately 200 km². Within this area, three bee species (*A. fallax*, *Lipotriches* sp. and *M. niveofasciata*) were recorded from at least two of the sites (Figure 10) and a bee fly species, *E. eluta*, was recorded from all three sites (Table 2). The 39 flower visits by these four species accounted for 85% of all recorded visitations. This indicates that the pollination system of *F. humilis* is dominated by generalist bee pollinators that carry medium to high loads of pollen. Although the bee fly was a more regular flower visitor (14 visits), its pollen load was low (Table 2). Reserve pollinators were observed at Goedvertrouwdt and Eagle Rock Private Estate (Figure 10). These pollinators were mostly flies and wasps and generally had medium to low pollen loads. A sweat bee (*Seladonea* sp.) was only recorded from Goedvertrouwdt and had a high pollen load. These five reserve pollinators accounted for 15% of flower visitations.

Pollination success

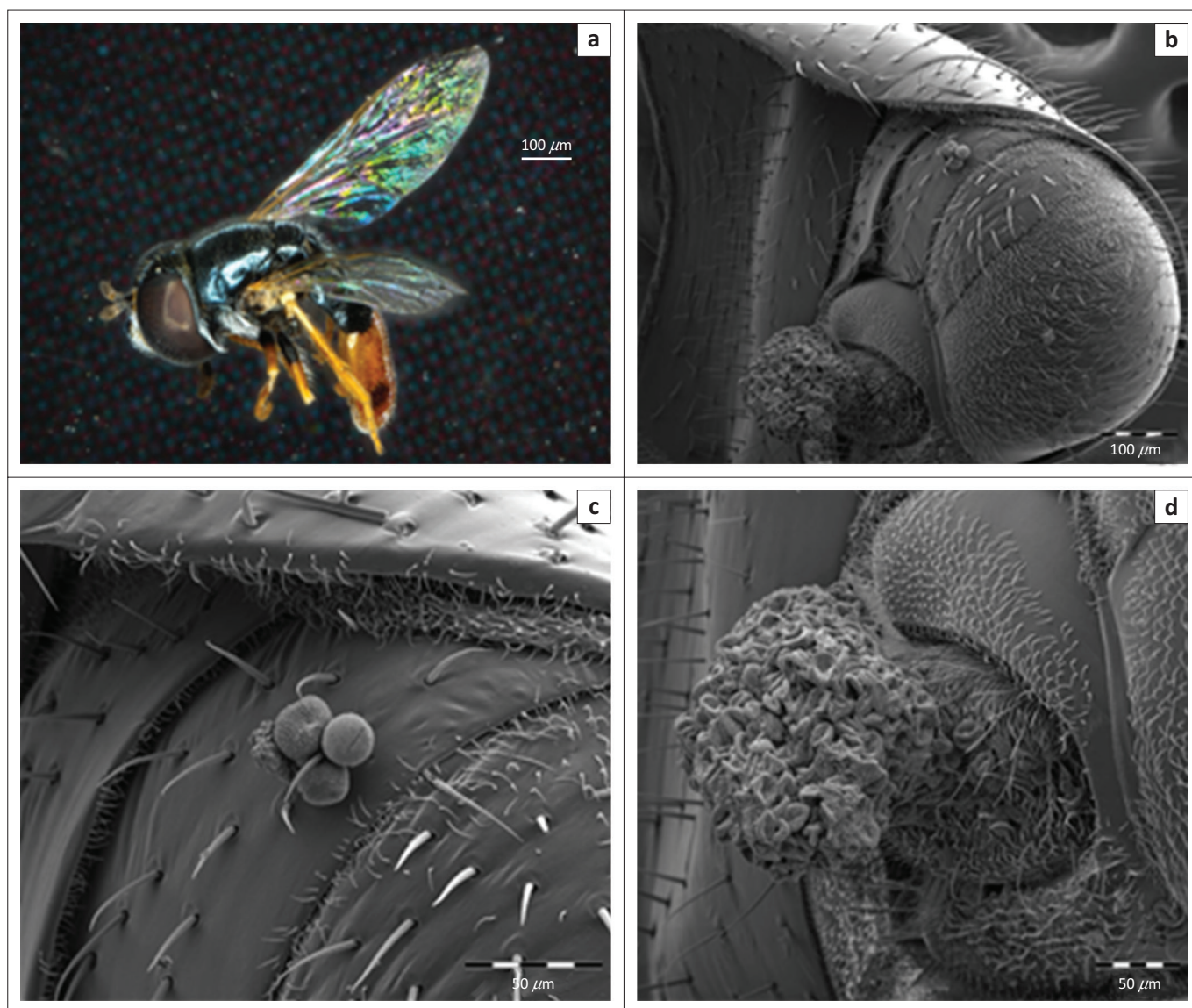
When mature plants, flowers, fruits and seedlings were compared across sites per 1 m², Ezemvelo Nature Reserve had significantly higher numbers, as would be expected for a natural population (Table 3). However, no significant differences were recorded between the typical and atypical habitats. Plants from Ezemvelo Nature Reserve had the highest number of flowers as a percentage of total number of mature plants, with fruit set as a percentage of flowers the highest for the sites at Goedvertrouwdt and Ezemvelo Nature Reserve (Table 3). As this study was conducted 7 years after

translocation, it may be assumed that the seed bank would make a minor contribution to recruitment and that recent seed production is a consequence of recent flowering seasons. Based on this premise, seedling emergence at Goedvertrouwdt receptor site as a percentage of seed numbers was considerably higher than that of the control population and is indicative of viable seed (Table 3). Considering the 7-year time span since translocation, the presence of six pollinators (Figure 10) and seedlings, it can be deduced that effective pollination is taking place.

Discussion

Flower visitation and pollen carriers

Exoprosopa and *Notolomatia* of the Bombyliidae are primarily Afrotropical in occurrence (Greathead & Evenhuis 2001). Larvae are either predators or parasitoids, whilst adult flies in most genera are adapted for nectar feeding. Female flies also feed on pollen for reproductive purposes and many of them have special adaptations for the collection of pollen (Greathead et al. 2006). Bombyliidae are most active during sunny middays that coincide with the anthesis of *F. humilis*. Little is known about their role as pollinators; it is generally believed that most of them are generalists (Szucsich & Krenn 2002) although some specialised relationships are known (Johnson & Steiner 1997). The small number of pollen grains on the *Notolomatia* specimen could be a consequence of the species or individuals (the inherent interest in pollen), the gender of the specimen (male flies may not be as interested in pollen, if at all) and the fact that the specimen was captured as soon as it landed on the flower, thereby shortening its potential visitation time.



Source: Photo courtesy of P. Jansen.

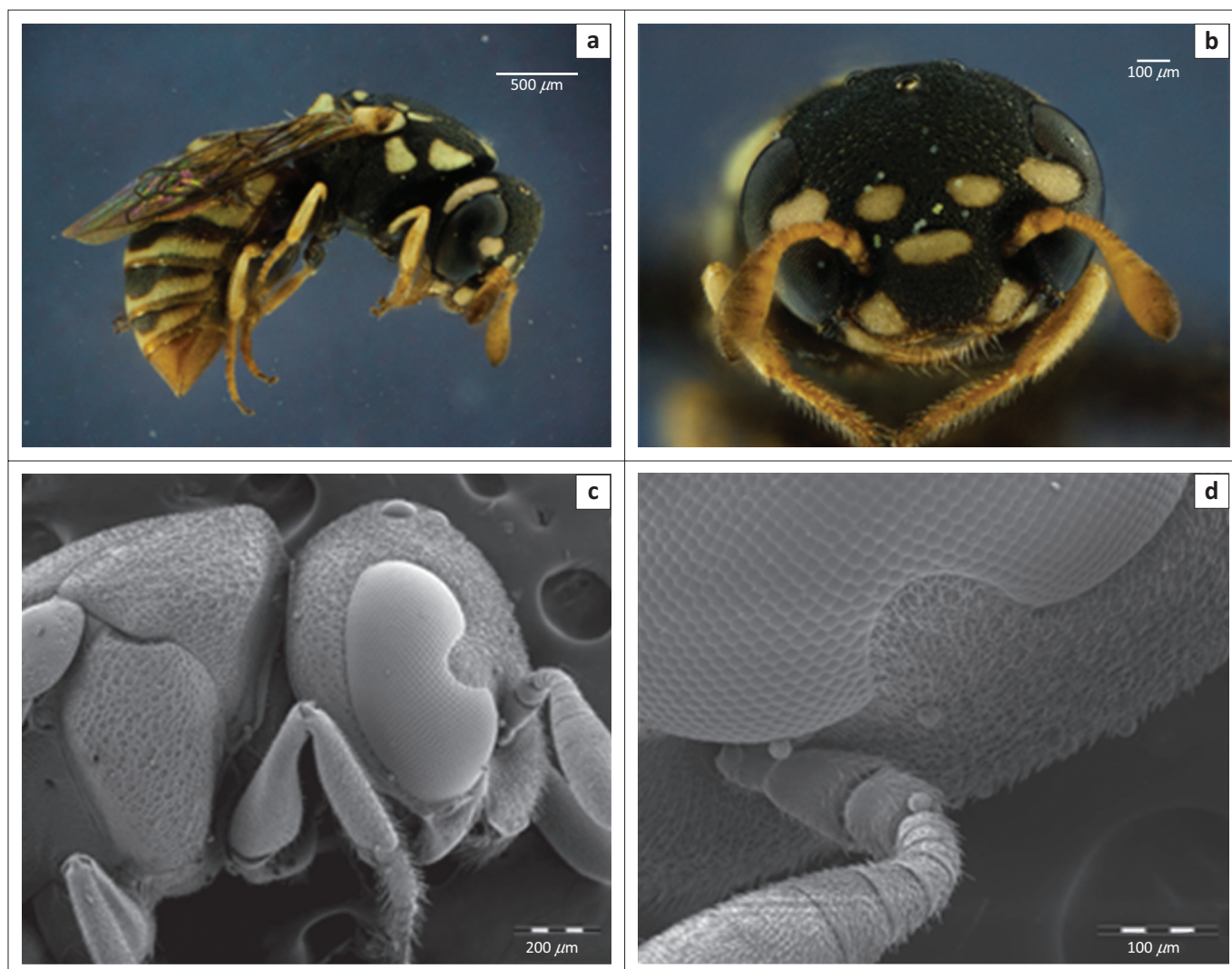
FIGURE 5: (a) Stereomicrograph of *Paragus* sp. (Syrphidae). Scanning electron micrographs of *Frithia humilis* pollen grains above the anus (b), on the body (c) and on the anus (d).

Larvae of the hover fly genus *Paragus* (Diptera: Syrphidae) are recognised as aphid predators (Hayat & Claussen 1997), whilst adult flies are nectar feeders. Female flies consume pollen because it is a rich protein source necessary for reproduction (Haslett 1989). Even if pollen is consumed and digested by all syrphids, it still retains its shape after passing through the gut, making identification possible (Holloway 1976). This explains the large cluster of pollen grains from *F. humilis* and other plant species on the anus of the specimen. The specimen only foraged for a short period before it was captured. Although the pollen load was low on the insect itself, the pollen cluster on the anus suggested that it had recently fed on *F. humilis* pollen.

The Halictidae, represented by the species *Lipotriches* and *Seladonea* in this study, is the second largest family of bees with over 3500 members. Many of them are pollen specialists with distinctive adaptations for collecting nectar and/or pollen from a small number of closely related plant species

(Danforth et al. 2008). In southern Africa, these non-apids bees visit numerous plant families but the most predominant is the Aizoaceae, along with Asteraceae, Fabaceae and Zygophyllaceae (Gess & Gess 2004a). Gess and Gess (2004a) reported that as many as 13 different plant families may be visited by a single species but visitation records pointed to preferences for certain families. *Seladonea* sp., for example, seemed to prefer Asteraceae compared with other families. However, when considering the species richness and diversity of the Halictidae, along with their polylectic manner of feeding, it can be concluded with a reasonable degree of certainty that they are important pollinators for many seed-bearing plants (Dikmen 2007). All of the collected Halictidae specimens displayed a typical pollen collection behaviour, which explains the copious amounts of pollen observed on their bodies and in their pollen baskets.

Ammophila is a genus of thread-waisted wasps in the Sphecidae well known to be parasitic and predatory in nature.



Source: Photo courtesy of P. Jansen.

FIGURE 6: (a, b) Stereomicrographs of *Quartinia* sp. (Vespidae). Scanning electron micrographs of several *Frithia humilis* pollen grains on the body (c) and the head and antenna (d).

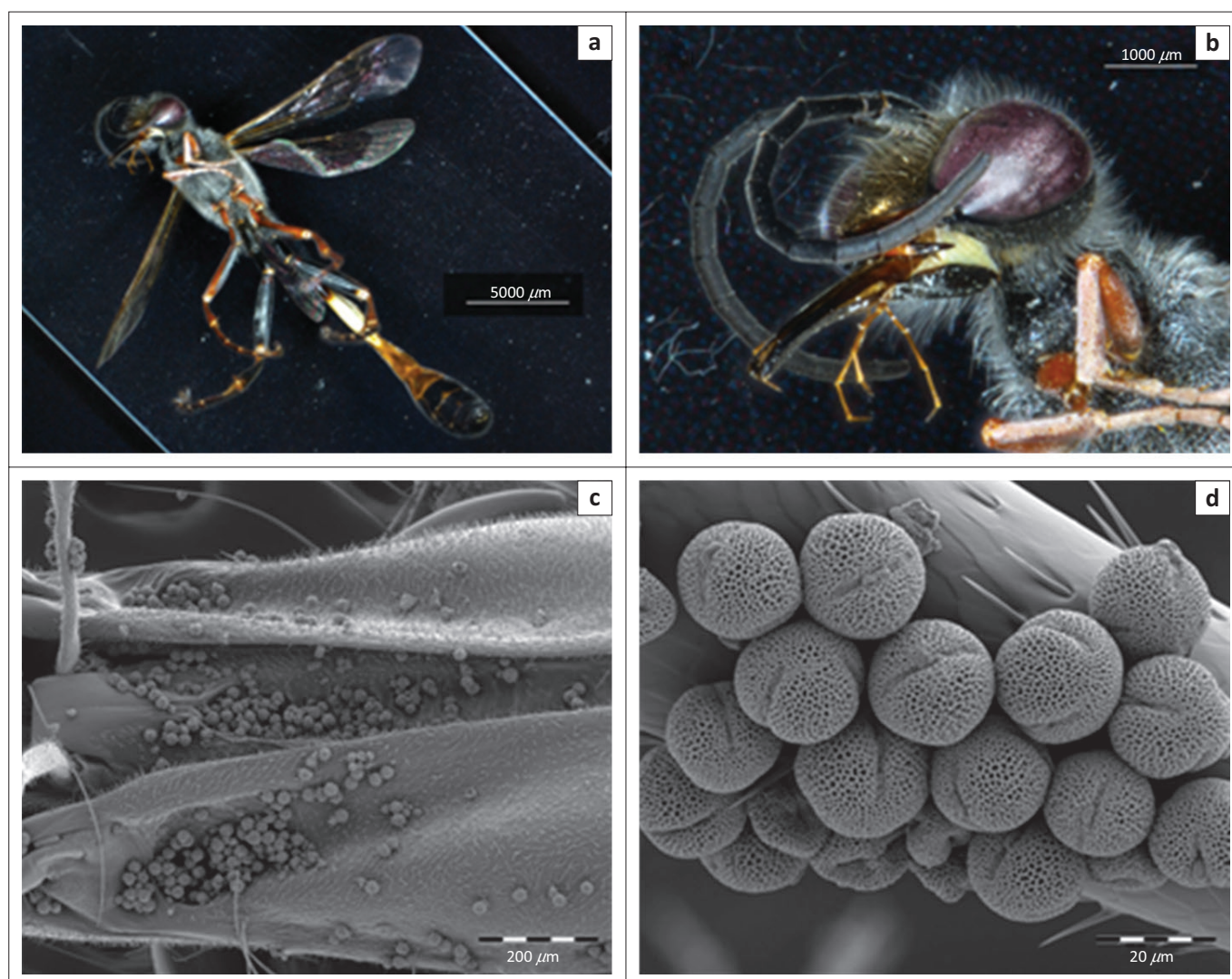
Preferences for specific flowers are known amongst the genera of Sphecidae but such relationships are not as strong as in bees or masarids (Bohart & Menke 1976). Sphecids, in general, feed on a variety of food including nectar, honeydew and bodily fluids of their prey. Genera with short tongues seek nectar from flowers with short corollas such as the Apiaceae, Asteraceae, Euphorbiaceae and Polygonaceae (Gess & Gess 1991). *Ammophila* has elongated mouthparts that enable it to feed on a greater variety of flowers. Flowers known to be visited by *Ammophila* include Acanthaceae, Apiaceae and Lamiaceae (Gess & Gess 1991; Herrera 1989; Weaving 1989). The captured *Ammophila* specimen was observed to make precise efforts to probe the nectaries of the *F. humilis* flower by circumnavigating the flower in such a way to ensure contact between the anthers and the underside of its mouth parts where pollen grains were found.

All species of pollen wasps (Vespidae), including those belonging to the genus *Quartinia*, are nectar feeders and pollen collectors (Gess & Gess 2010). Of the 40 species recorded to visit flowers, 75% preferred the Aizoaceae. *Quartinia* is also generally regarded as an effective pollinator

for many Aizoaceae species and numerous specialised relationships have been noted (Gess & Gess 2004b, 2010). During the survey, one of the *Quartinia* specimens continued to forage in a *F. humilis* flower even after the hand net was placed over it and the flower. Even though little pollen was found on the specimen, the fact that all species of *Quartinia* are pollen feeders and collectors indicate that this genus may be an efficient reserve pollinator of *F. humilis* (Gess & Gess 2010).

Pollination system

Although few specimens (46 individuals) of pollen carriers were captured during this study, our observations provided enough evidence to support the hypothesis presented by Hartmann (1991) that *F. humilis* belongs to the Melittophilous syndrome, thus being pollinated mostly by bee and bee-like pollinators. This was especially the case for the natural *F. humilis* population at Ezemvelo Nature Reserve, where bee pollinators were predominant. At translocated sites, additional observations were made of flies and the *Ammophila*



Source: Photo courtesy of P. Jansen.

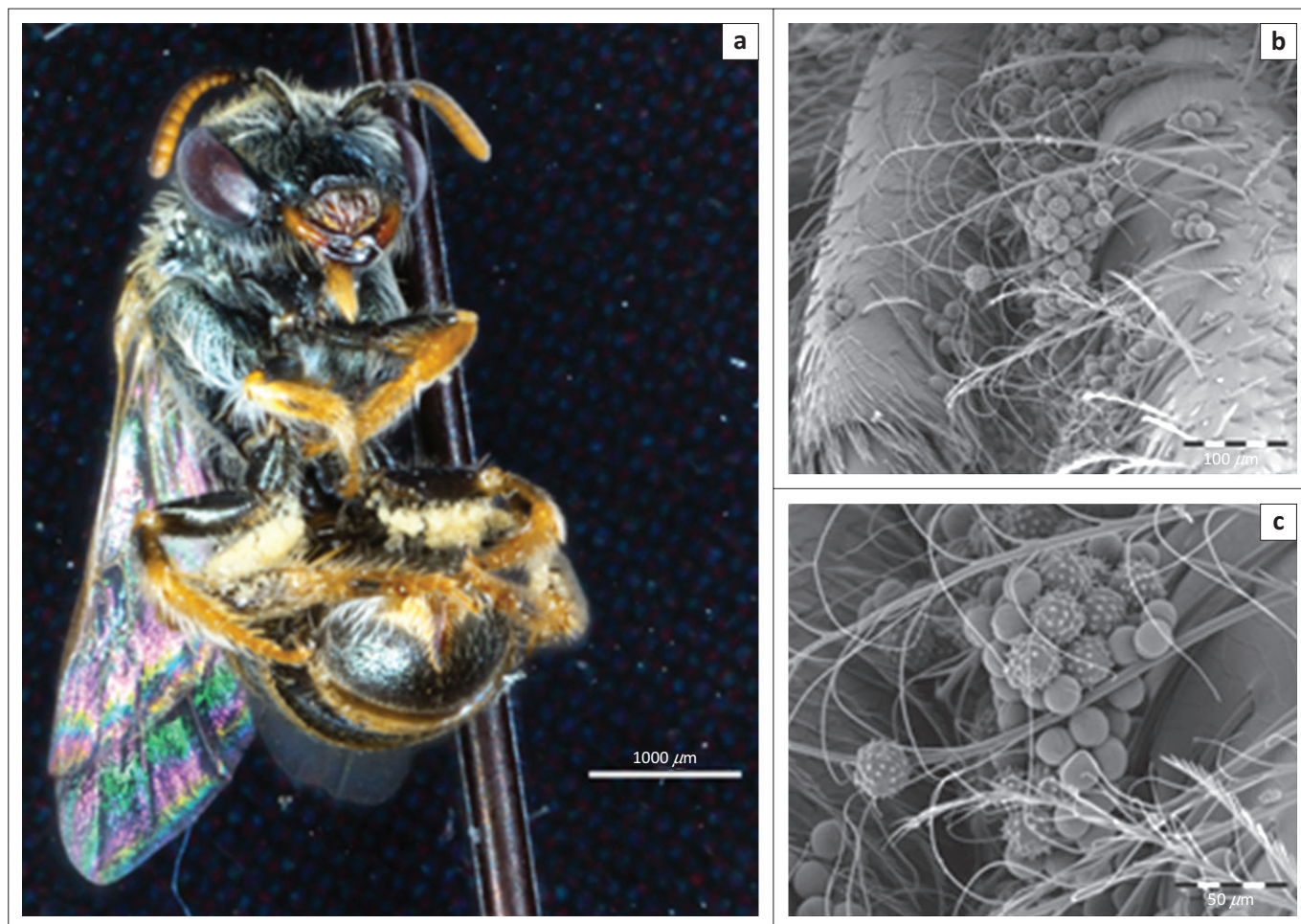
FIGURE 7: (a, b) Stereomicrographs of *Ammophila* sp. (Sphecidae). Scanning electron micrographs of *Frithia humilis* pollen grains on the underside of the mouth parts (c) and on a maxillary palp (d).

wasps which support the presence of Anemophilous syndrome and imply a bimodal pollination system. These ‘reserve’ pollinators (Johnson, Harris & Procheş 2009) associated with the Anemophilous syndrome provide plausible alternatives for the translocated populations.

However, it must be cautioned that even though specimens might carry *F. humilis* pollen, it does not conclusively prove that any of these species are pollinators. In addition, the amount of pollen on a pollinator’s body is regarded as an unreliable determinant of efficiency (Adler & Irwin 2006). Identifying a primary pollinator depends on the pollination efficiency of the species, which is defined as the amount of con-specific pollen transferred to a stigma in any one visit (Inouye et al. 1994). However, determining pollination efficiency is problematic because pollination is a highly variable ecological interaction and can be influenced by factors such as pollinator abundance across years, flower visit duration and frequency, contact with flower parts as well as pollen removal and deposition (Ivey, Martinez & Wyatt 2003).

With this in mind, the bees *Amegilla fallax*, *Megachile niveofasciata* and *Lipotriches* sp. that form part of the Melittophilous syndrome are cautiously proposed as primary pollinators of *F. humilis* because of their higher visitation frequencies and larger pollen loads. The sweat bee, *Seladonea* sp., could also be a primary pollinator because of its higher observed pollen loads, despite only being observed twice. Whilst pollen load may not be a direct indication of pollination efficiency, the larger pollen loads of bees compared with other *F. humilis* visitors were particularly evident on SEM micrographs. The number of observations recorded as visitation frequency is also in favour of bees compared with the various other species. One generalist pollinator, *E. eluta*, was observed at all sites but is likely an inefficient pollinator because of the limited amount of pollen found only on the eyes (Harris et al. 2016).

Further evidence for bee pollination is supported by the pollination system of a closely related species, *Bergeranthus multiceps* (Aizoaceae), from spring and autumn rainfall areas of the Eastern Cape Province, South Africa. This plant is also self-incompatible and flowers open between 15:30 and 18:30.



Source: Photo courtesy of P. Jansen.

FIGURE 8: (a) Stereomicrograph of *Seladonea* sp. (Halictidae). (b, c) Scanning electron microscope micrographs of *Frithia humilis* pollen grains in the pollen baskets.

Bees were determined to be the most important pollinators to this species, placing it in the Melittophilous syndrome (Peter et al. 2004). Other potential pollinators included butterflies, bees and hover flies belonging to the families Bombyliidae, Tachinidae and Syrphidae (Peter et al. 2004). This study and Harris et al. (2016) made similar observations for *F. humilis*.

Pollination syndromes may maximise successful pollination by a specific species whilst still reserving the option for chance pollination by a less suitable species. The non-Melittophilous *Frithia* pollen carriers are suggested to be reserve pollinators because of their frequent visits and exclusive occurrence in translocated populations. They are primarily wasp and fly species that form part of an Anemophilous syndrome. Whilst bees may be primary pollinators, the small size and isolation of *F. humilis* populations supports the idea that *F. humilis* is a generalist rather than a specialist, and it can rely on reserve pollinators as its flower structure allows pollination by a different syndrome. A bimodal pollination system has also been identified previously for a member of the Ruschioideae in the Aizoaceae (Zietsman 2013).

Pollination efficacy

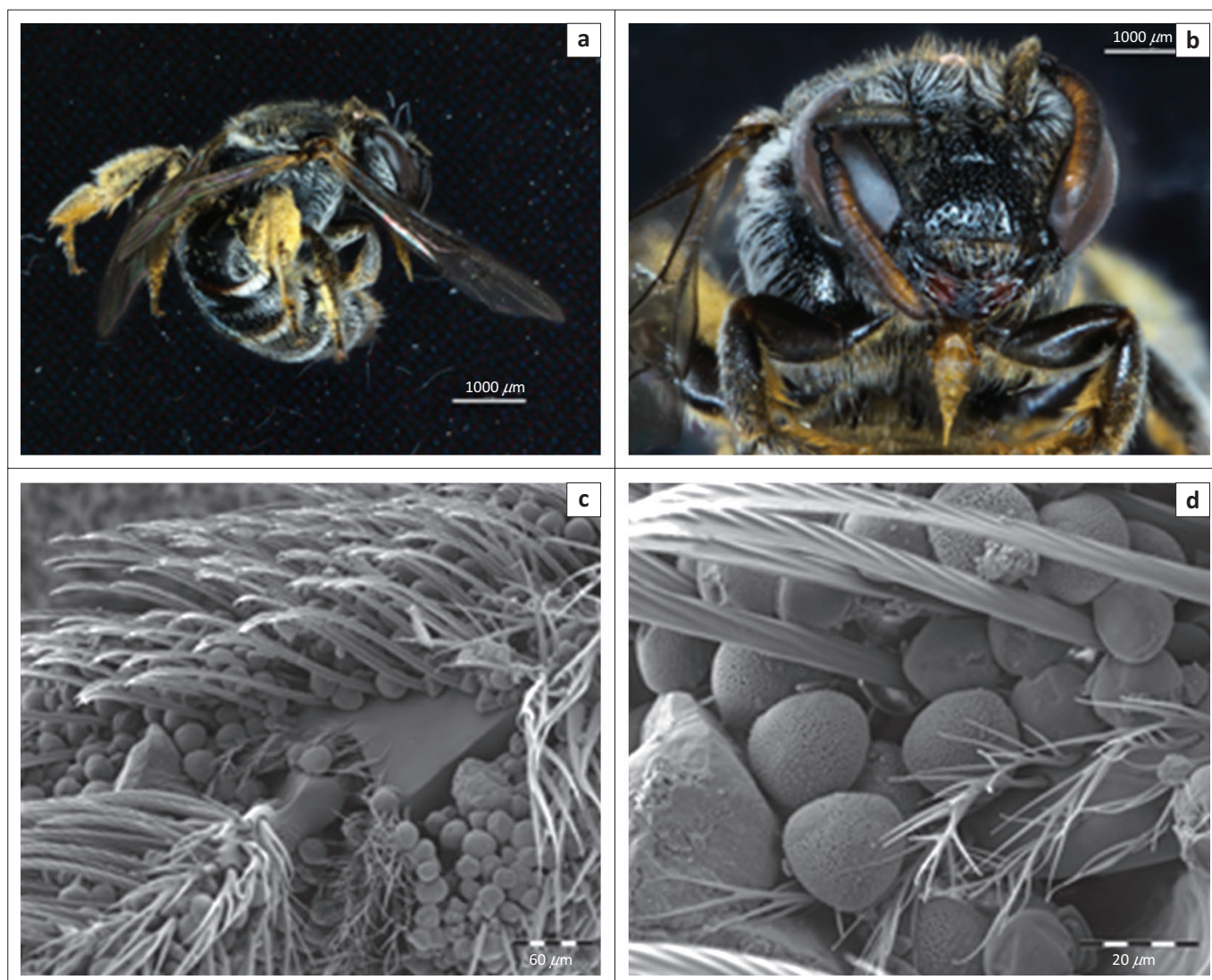
Pollination was equally successful at the natural and translocated Goedvertrouwdt populations although flower

density differed (Harris et al. 2014). This is in accordance with the flower density hypothesis (Pufal et al. 2008). Flower density is known to have a strong influence on pollinator visits (Kunin 1997) but the size of the population had little effect on pollination efficacy. In other words, more flowers do not imply higher proportional frequencies of pollination.

Seedling recruitment per seed quantity was the highest for Goedvertrouwdt, but this could be ascribed to lower plant densities and more suitable niches (Eriksson & Ehrlén 1992). Eagle's Rock has a different geology and as expected did not have ideal conditions for seedling germination and establishment (Harris et al. 2014). The presence of seedlings, therefore, is no indication of pollination efficacy but only that successful pollination takes place. This indicates that at least some of the pollen carriers recorded in this study are also pollinators.

Implications for translocation science

This study showed that plant populations translocated to atypical habitats and geology had lower levels of fruit formation despite having higher levels of flowering compared to translocated populations of typical habitat. Site selection is important and should be performed carefully to avoid plant stressors and nutrient deficiencies which may



Source: Photo courtesy of P. Jansen.

FIGURE 9: (a, b) Stereomicrograph of *Lipotriches* sp. (Halictidae). (c, d) Scanning electron micrographs of *Frithia humilis* pollen grains in the pollen baskets.

affect plant and flower health, and the availability of pollen (Mayer 2004). Reduced flowering can affect the number of pollinators that may visit a population and might limit outcrossing in small populations resulting in pollen transfer between relatives or not being pollinated at all (Kearns & Inouye 1997).

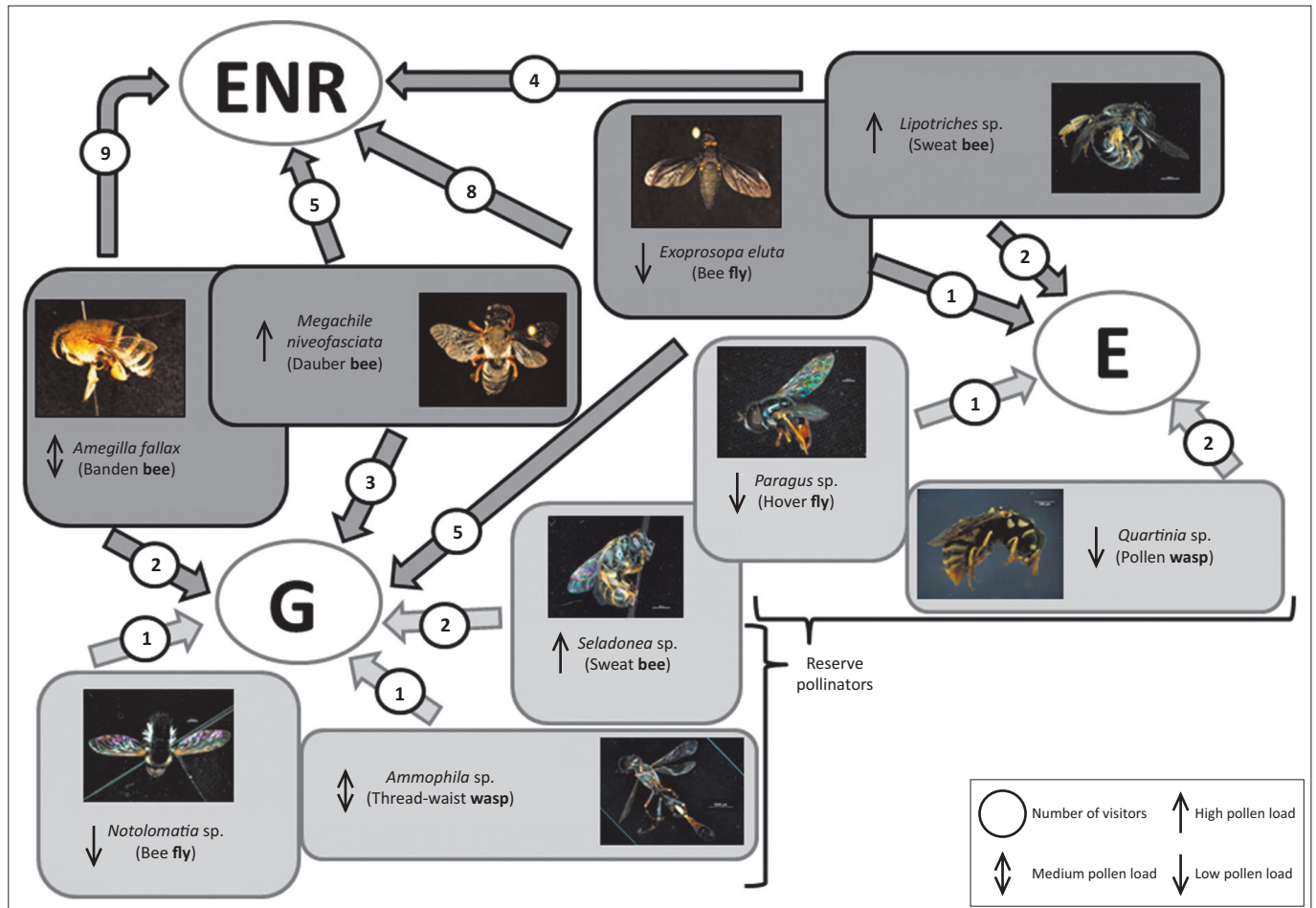
The sparsely populated translocated sites had higher levels of fruit set and seedling occurrence per individual than the natural population. This shows that it is important to consider transplant density for xenogamous species, as too high densities influence the available pollinators and could result in many unpollinated flowers, which could restrict gene flow in the newly established population. Pufal et al. (2008) suggested that decreased density of flowers increases pollination success and fruit set per plant as pollinators are known to be scarce, with a few taxa accounting for most of the flowers at a site (Herrera 1989).

Translocation requires relocating a species to areas within its existing distribution range (IUCN 2013). This study has

shown that generalist pollinators of *F. humilis* occur throughout its natural distribution range and are common and mobile enough to locate newly established populations. Translocation is therefore a low risk conservation option in terms of generalist plant species. Pollination is further enhanced by the bimodal pollination system that is characteristic for some of members of the Aizoaceae (Zietsman 2013), which makes them resilient to shifts within their distribution range.

Conclusion

Nine arthropod species have now been confirmed as *F. humilis* pollen carriers. Hymenopteran species had the largest pollen loads in pollen baskets and on front legs. This positioning of pollen ensures contact with the stigmata of the open flowers of *F. humilis* and could result in effective pollination. The pollination system is Melittophilous as would be expected for the family, with an Anemophilous syndrome providing reserve pollinators at translocation sites. The presence of seedlings at both translocation sites was indicative of successful pollination by insects of this self-incompatible species.



G, FIGURE 10: Pollination system of translocated *Frithia humilis* populations (G; E) in relation to a control population (ENR). Goedvertrouwdt Farm; E, Eagle's Rock Private Estate; ENR, Ezemvelo Nature Reserve.

TABLE 3: Mean number of mature plants, flowers, fruit, seed and seedlings per 1 m² for receptor sites and the control, as well as the flowering, fruiting and seeding percentage in brackets.

Locality	Mature plants	Flowers	Capsules	Seed	Seedlings
G	52 ± 18 ^a	11 ± 8 ^a (21%)	3 ± 1 ^a (27%)	438	62 ± 39 ^a (14%)
E	78 ± 33 ^a	25 ± 9 ^a (32%)	2 ± 1 ^a (8%)	292	18 ± 14 ^a (6.2%)
ENR	398 ± 163 ^{***}	184 ± 82 ^{***} (46%)	42 ± 27 ^{b*} (26%)	6132	185 ± 112 ^{b*} (3.1%)

Note: Mean seed set per capsule: 146 ± 12.. One-way analysis of variance; Unequal N Tukey test: ***p* < 0.005; **p* < 0.05.

Superscripts that differ denote significant variation

E, Eagle's Rock Private Estate; G, Goedvertrouwdt Farm; ENR, Ezemvelo Nature Reserve.

Pollination of flowers was proportionally similar between the varying population densities of natural and translocated populations on Ecce sandstone. This indicated that flower density did not influence fruit formation in this species. When the two translocated populations were compared, it was evident that fruit formation was more effective on Ecce sandstone. This indicated that reproductive success of this edaphic specialist decreases on non-typical rock habitats.

Micro-habitat conditions, planting density and locality within the species' natural distribution range require an in-depth understanding before sites are selected to enhance pollination probability. However, even if predictions based on this are not accurate, it can be assumed that generalist and 'reserve' pollinators are widespread and abundant enough

to locate translocated populations of *F. humilis* within the natural distribution range of the species. It can be concluded that pollinators are not limiting factors when this species is translocated.

Acknowledgements

The authors thank Dr Duncan MacFayden (Manager: Research and Conservation at E. Oppenheimer & Son) for providing access to the *Frithia* population at Ezemvelo Nature Reserve. They are grateful to the landowners for providing access to translocated populations at Eagle's Rock Private Estate and Goedvertrouwdt Farm and giving permission to collect insect specimens on their properties. Mr Scott van Eeden prepared the locality map and Mr Koos Smit photographed the species in habitat.

Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

P.G.J. conducted fieldwork and species identification and wrote part of the manuscript. S.J.S. designed the study,

conducted initial surveys and wrote part of the manuscript. F.S. contributed to the writing of the manuscript and data analyses. J.v.d.B. performed sorting of arthropod material and coordinated the identification of pollinators. A.J. conducted scanning electron microscopy.

Funding information

Financial assistance for this study was provided by the National Research Foundation (Grant UID: 103370).

Data availability statement

Data sharing is applicable to this article as new data were created or analysed in this study.

Disclaimer

Opinions expressed and conclusions arrived at in the article are those of the authors and are not necessarily to be attributed to the National Research Foundation (NRF).

References

- Adler, L.S. & Irwin, R.E., 2006, 'Comparison of pollen transfer dynamics by multiple floral visitors: Experiments with pollen and fluorescent dye', *Annals of Botany* 97(1), 141–150. <https://doi.org/10.1093/aob/mcj012>
- Armstrong, D.P. & Seddon, P.J., 2008, 'Directions in reintroduction biology', *Trends in Ecology and Evolution* 23(1), 20–25. <https://doi.org/10.1016/j.tree.2007.10.003>
- Bohart, R.M. & Menke, A.S., 1976, *Sphecid wasps of the world: A generic revision*, University of California Press, Berkeley.
- Burgoyne, P.M., Smith, G.F. & Du Plessis, F., 2000, 'Notes on the genus *Frithia* (Mesembryanthemaceae) and the description of a new species, *F. humilis*, in South Africa', *Bothalia* 30(1), 1–7. <https://doi.org/10.4102/abc.v30i1.531>
- Burgoyne, P.M. & Krynauw, S., 2005, *Frithia humilis* Burgoyne, National Assessment: Red List of South African Plants version 2017.1, South African National Biodiversity Institute, viewed 19 March 2018, from <http://redlist.sanbi.org/species.php?species=126-3>.
- Burgoyne, P.M. & Hoffmann, A., 2011, '*Frithia humilis* – notes on the translocation of a Red Listed succulent', *Aloe* 48(2), 38–42.
- Danforth, B.N., Eardley, C., Packer, L., Walker, K., Pauly, A. & Randrianambintsoa, F.J., 2008, 'Phylogeny of Halictidae with an emphasis on the African Halictinae', *Apidologie* 39(1), 86–101. <https://doi.org/10.1051/apido:2008002>
- Dikmen, F., 2007, 'The role and the importance of the family Halictidae (Apiformes: Apoidea) in pollination of natural and agricultural vegetation', *Melipona* 7, 16–19.
- Eardley, C.D., Gikungu, M. & Schwarz, M.P., 2009, 'Bee conservation in Sub-Saharan Africa and Madagascar: Diversity, status and threats', *Apidologie* 40(3), 355–366. <https://doi.org/10.1051/apido/2009016>
- Eriksson, O. & Ehrlén, J., 1992, 'Seed and microsite limitation of recruitment in plant populations', *Oecologia* 91(3), 360–364. <https://doi.org/10.1007/BF00317624>
- Forup, M.L., Henson, K.S., Craze, P.G. & Memmott, J., 2008, 'The restoration of ecological interactions: Plant–pollinator networks on ancient and restored heathlands', *Journal of Applied Ecology* 45(3), 742–752. <https://doi.org/10.1111/j.1365-2664.2007.01390.x>
- Gess, F.W. & Gess, S.K., 1991, 'A preliminary survey of the aculeate wasps and the bees of the lower reaches of the Nossob River Valley, Kalahari Gemsbok National Park, South Africa', *Koedoe* 34(2), 77–88. <https://doi.org/10.4102/koedoe.v34i2.424>
- Gess, S.K. & Gess, F.W., 2004a, 'A comparative overview of flower visiting by non-Apis bees in the semi-arid to arid areas of southern Africa', *Journal of the Kansas Entomological Society* 77, 602–618. <https://doi.org/10.2317/E7.1>
- Gess, S.K. & Gess, F.W., 2004b, 'Distributions of flower associations of pollen wasps (Vespidae: Masarinae) in southern Africa', *Journal of Arid Environments* 57(1), 17–44. [https://doi.org/10.1016/S0140-1963\(03\)00093-4](https://doi.org/10.1016/S0140-1963(03)00093-4)
- Gess, S.K. & Gess, F.W., 2010, *Pollen wasps and flowers in southern Africa*, SANBI Biodiversity Series 18, South African National Biodiversity Institute, Pretoria.
- Godefroid, S., Piazza, C., Rossi, G., Buord, S., Stevens, A.D., Aguraiju, R. et al., 2011, 'How successful are plant species reintroductions?', *Biological Conservation* 144(2), 672–682. <https://doi.org/10.1016/j.biocon.2010.10.003>
- Gordon, D., 1994, 'Translocation of species into conservation areas: A key for natural resource managers', *Natural Areas Journal* 14(1), 31–37.
- Greathead, D.J. & Evenhuis, N.L., 2001, 'Annotated keys to the genera of African Bombyliidae (Diptera: Bombyliidae: Mythicomyiidae)', *African Invertebrates* 42(1), 105–224.
- Greathead, D.J., Lovell, S., Barraclough, D., Slotow, R., Hamer, M. & Herbert, D., 2006, 'An ecological and conservation assessment of the fauna of Bombyliidae (Diptera) occurring in the Mkhuzi, Phinda and False Bay reserves, KwaZulu-Natal, South Africa', *African Invertebrates* 47(1), 185–206.
- Hammer, S., 1995, 'Mastering the art of growing mesembs', *Cactus and Succulent Journal* 67, 195–247.
- Harris, E., Siebert, S.J., Smit, J.H.L. & Van den Berg, J., 2014, 'Translocation of an endangered succulent plant species from sandstone outcrops earmarked for coal mining', *Journal of the Southern African Institute of Mining and Metallurgy* 114(11), 904–912.
- Harris, E., Van den Berg, J. & Siebert, S.J., 2016, 'Notes on the pollinators of *Frithia humilis* Burgoyne, an endangered Highveld succulent', *African Entomology* 24(1), 250–256. <https://doi.org/10.4001/003.024.0250>
- Hartmann, H.E.K., 1991, 'Mesembryanthema', *Contributions from the Bolus Herbarium* 13, 75–157.
- Haslett, J.R., 1989, 'Adult feeding by holometabolous insects: Pollen and nectar as complementary nutrient sources for *Rhingia campestris* (Diptera: Syrphidae)', *Oecologia* 81(3), 361–363. <https://doi.org/10.1007/BF00377084>
- Hayat, R. & Claussen, C., 1997, 'A new species and new records of the genus *Paragus* Latreille, 1804 from Turkey (Diptera: Syrphidae)', *Zoology in the Middle East* 14(1), 99–108. <https://doi.org/10.1080/09397140.1997.10637710>
- Herrera, C.M., 1989, 'Pollinator abundance, morphology, and lower visitation rate: Analysis of the "quantity" component in a plant–pollinator system', *Oecologia* 80(2), 241–248. <https://doi.org/10.1007/BF00380158>
- Holloway, B.A., 1976, 'Pollen-feeding in hover-flies (Diptera: Syrphidae)', *New Zealand Journal of Zoology* 3(4), 339–350. <https://doi.org/10.1080/03014223.1976.9517924>
- Inouye, D.W., Gill, D.E., Dudash, M.R. & Fenster, C.B., 1994, 'A model and lexicon for pollen fate', *American Journal of Botany* 81(12), 1517–1530. <https://doi.org/10.1002/j.1537-2197.1994.tb11462.x>
- International Union for Conservation of Nature (IUCN), 2013, *Guidelines for reintroductions and other conservation translocations*, IUCN, Gland.
- Ivey, C.T., Martinez, P. & Wyatt, R., 2003, 'Variation in pollinator effectiveness in swamp milkweed, *Asclepias incarnata* (Apocynaceae)', *American Journal of Botany* 90(2), 214–225. <https://doi.org/10.3732/ajb.90.2.214>
- Johnson, S.D. & Steiner, K.E., 1997, 'Long-tongued fly pollination and evolution of floral spur length in the *Disa draconis* complex (Orchidaceae)', *Evolution* 51(1), 45–53. <https://doi.org/10.1111/j.1558-5646.1997.tb02387.x>
- Johnson, S.D., 2010, 'The pollination niche and its role in the diversification and maintenance of the southern African flora', *Philosophical Transactions of the Royal Society B* 365(1539), 499–516. <https://doi.org/10.1098/rstb.2009.0243>
- Johnson, S.D., Harris, L.F. & Proches, S., 2009, 'Pollination and breeding systems of selected wildflowers in a southern African grassland community', *South African Journal of Botany* 75(4), 630–645. <https://doi.org/10.1016/j.sajb.2009.07.011>
- Kearns, C.A. & Inouye, D.W., 1997, 'Pollinators, flowering plants, and conservation biology', *Bioscience* 47(5), 297–307. <https://doi.org/10.2307/1313191>
- Kunin, W.E., 1997, 'Population size and density effects in pollination: Pollinator foraging and plant reproductive success in experimental arrays of *Brassica kaber*', *Journal of Ecology* 85(2), 225–234. <https://doi.org/10.2307/2960653>
- Mayer, C., 2004, 'Pollination services under different grazing intensities', *International Journal of Tropical Insect Science* 24(1), 96–103. <https://doi.org/10.1079/IJT20047>
- Mayer, C. & Pufal, G., 2007, 'Investigation of breeding systems for four Aizoaceae species in Namaqualand, South Africa', *South African Journal of Botany* 73(4), 657–660. <https://doi.org/10.1016/j.sajb.2007.06.001>
- Menz, M.H.M., Phillips, R.D., Winfree, R., Kremen, C., Aizen, M.A., Johnson, S.D. et al., 2011, 'Reconnecting plants and pollinators: Challenges in the restoration of pollination mutualisms', *Trends in Plant Science* 16(1), 4–12. <https://doi.org/10.1016/j.tplants.2010.09.006>
- Montalvo, A.M., Williams, S.L., Rice, K.J., Buchmann, S.L., Cory, C., Handel, S.N. et al., 1997, 'Restoration biology: A population biology perspective', *Restoration Ecology* 5(4), 277–290. <https://doi.org/10.1046/j.1526-100X.1997.00542.x>
- Moritz, C., 1999, 'Conservation units and translocations: Strategies for conserving evolutionary processes', *Heredity* 130(3), 217–228. <https://doi.org/10.1111/j.1601-5223.1999.00217.x>
- Peter, C.I., Dold, A.P., Barker, N.P. & Ripley, B.S., 2004, 'Pollination biology of *Bergeranthus multiceps* (Aizoaceae) with preliminary observations of repeated flower opening and closure', *South African Journal of Science* 100(11–12), 624–629.
- Pufal, G., Mayer, C., Porembski, S. & Jürgens, N., 2008, 'Factors affecting fruit set in Aizoaceae species of the Succulent Karoo', *Basic and Applied Ecology* 9(4), 401–409. <https://doi.org/10.1016/j.baee.2007.06.001>
- Szucsich, N.U. & Krenn, H.W., 2002, 'Flies and concealed nectar sources: Morphological innovations in the proboscis of Bombyliidae (Diptera)', *Acta Zoologica* 83(3), 183–192. <https://doi.org/10.1046/j.1463-6395.2002.00111.x>
- Weaving, A.J.S., 1989, 'Nesting strategies in some southern African species of *Ammophila* (Hymenoptera: Sphecidae)', *Journal of Natural History* 23(1), 1–16. <https://doi.org/10.1080/00222938900770011>
- Uys, V.M. & Urban, R.P., 2006, *How to collect and preserve insects and arachnids*, 2nd edn., Plant Protection Research Institute, Queensland.
- Zietsman, P.C., 2013, 'Reproductive biology of *Stomatium bolusiae* (Aizoaceae: Ruschioideae)', *Bothalia* 43(1), 23–27. <https://doi.org/10.4102/abc.v43i1.83>

Appendix starts on the next page →

Appendix 1

TABLE 1-A1: Specialist identifications of *Frithia humilis* pollen carriers not considered in Harris et al. (2016).

Family	Genus	Contributor	Comments	Date
Bombyliidae	<i>Notolomatia</i> sp.	Evenhuis, N.L. Bishop Museum, Honolulu, Hawaii, United States	Genus endemic to southern Africa with many species. Related to European <i>Lomatia</i> . In need of revision and therefore no accurate species names.	8/6/2016
Halictidae	<i>Lipotriches</i> sp.	Eardly, C. Plant Protection Research Institute of the Agricultural Research Council	Has not been revised and it is not possible to provide accurate species name.	31/5/2016
Halictidae	<i>Seladonea</i> sp.	Eardly, C. Plant Protection Research Institute of the Agricultural Research Council	Has not been revised and it is not possible to provide accurate species name.	31/5/2016
Syrphidae	<i>Paragus</i> sp.	Jordaens, K. Royal Museum for Central Africa, Tervuren, Belgium	Currently very difficult to name up to species level.	8/6/2016
Sphecidae	<i>Ammophila</i> sp.	Eardly, C.	No expert available to identify up to species level.	31/5/2016
Vespidae	<i>Quartinia</i> sp.	Gess, S.K. Albany Museum, Grahamstown, South Africa	Species not seen previously. Might be undescribed species as there are still many.	31/5/2016