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Investigation of Pollination Pathways in the Savanna Biome of the Kruger National Park, South Africa - a Potential Tool for the Interpretation of Holocene Fossil Pollen Archives

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1. Introduction

Southern Africa has a rich fossil heritage in sediments of different ages (McCarthy & Rubidge 2005). Important steps in human evolution and cultural development were identified, these evolutionary changes were often influenced by palaeoecological changes especially during the Quaternary (Scott and Neumann 2018, Mitchell 2002 and Parkington et al. 2013). Palaeoecologists aim to reconstruct environmental fluctuations that influence evolutionary changes of the ecosystem over time (Rull 2010 and Rull 1990), using several methods like isotope analysis, sedimentological analysis, faunal investigation and archaeobotanical investigations which include pollen, charcoal and phytolith analyses (Scott et al. 2015). Palynology is the analysis of organic-walled microfossils including pollen and spores (Erdtman 1963); it is a well-tested method to investigate vegetation changes and to deduct climate fluctuations as well as human impact (compare Rull 2010).

Plants use diverse pollen transportation pathways, e.g., wind, water, insects, birds, mammals, and reptiles (Kevan et al. 2013) and for this reason, the use of palynology for palaeoecological reconstruction often falls short of the understanding of pollen-vegetation pathways (Duffin and Bunting 2008). The form of pollen transportation, the quantity of pollen production and the distance over which the pollen may be dispersed varies considerably between species (Duffin and Bunting 2008). Understanding these pathways and their contributing factors such as pollen production is important for a reliable interpretation of Quaternary pollen archives (Duffin and Bunting 2008).

Pollen assemblage richness, diversity and evenness have been used by many palynologists as a proxy for past plant richness, by utilising rarefaction analysis-based on pollen stratigraphical data to determine and reconstruct past vegetations (Simberloff 1978, Heck et al. 1975, and Birks et al. 2016). Pollen diversity and abundance in honey samples have also been used by melissopalynologists as a proxy to determine the geographical and botanical origins of the honey (Dustmann and Von der Ohe 1993, Johannsmeier 2016). Melissopalynology is the study of pollen in nectar which is collected to make honey (Dustmann and Von der Ohe 1993, Johannsmeier 2016). Honey therefore is hypothesised to be a pollen assemblage archive that can be used to contribute to the understanding of the pollen-vegetation relationship. This idea will be explored later in the melissopalynology chapter (1.2.).

Only few studies have explored how recent pollen richness relates to the contemporary plant richness and as a result, those that have, agree that they can be used as a proxy for past environmental reconstruction (Birks et al. 2016) despite the disproportional representation of pollen rain to the surrounding vegetation (Julier et al. 2018 and Davis 1963). This disproportional representation of the environment in pollen assemblages is said to be due to the overrepresentation of wind-pollinated pollen taxa in sediment samples relative to their abundance in the vegetation (Julier et al. 2018, Bush and Rivera 2001), and the underrepresented of insect-pollinated pollen taxa in sediment pollen assemblages (Julier et al. 2018, Vincens et al. 2000). To facilitate an improved interpretation of fossil pollen archives, this study explores modern pollen-vegetation relationships in a savanna ecosystem in the Kruger National Park (KNP) (compared to these studies: Julier et al. 2018, Duffin and Bunting 2008, Duffin 2008).

A study in Ghana has compared modern pollen data to the surrounding savanna vegetation to improve the interpretation of fossil pollen records (Julier et al. 2018). The relationship between the pollen and vegetation abundance was estimated by dividing the average pollen abundance percentage in each trap by the basal area percentage of the same taxon in the vegetation area these were referred to as 'R-rel value' following Gosling et al. (2017). The values have been used to express the degree to which a taxon is under or over-representation (in Julier et al. 2018). The study revealed that the pollen assemblages in the savanna area revealed an over-representation of Combretaceae and an underrepresentation of *Pterocarpus, Terminalia* and *Uapaca*. Additionally, the savanna sediment trap also revealed highly similar pollen assemblages for the Poaceae and Combretaceae, both families contributed more pollen grains than other pollen taxa from the surrounding vegetation (Julier et al. 2018). These studies can be improved by a detailed account of more taxa within each landscape and more sites to be compared to pollen records (Duffin and Bunting 2008).

A previous study from the KNP has contributed to this improved approach to better interpret fossil pollen data by investigating the pollen dispersal and deposition for southern African savanna taxa (Duffin & Bunting 2008). The authors discovered that the pollen fall speeds, which depends on the pollen grain density, affects the pollen productivity, and therefore influences its deposition (Duffin & Bunting 2008), hence relative pollen productivity estimates (PPEs) were acquired for seven taxon groups (which include *Colophospermum mopane*, Poaceae, Combretaceae, Cyperaceae, Anacardiaceae, *Spirostachys africana* and *Senegalia/Vachellia* types), that are common in the KNP. The lowest PPEs were for the

insect pollinated *Senegalia/Vachellia* types and *Colophospermum mopane* and the highest were Anacardicaceae/*Spirostachys africana* and Combretaceae. Poaceae/Cypreceae had an average PPE compared to the highest PPE of Combretaceae (Duffin & Bunting 2008). These PPEs have quantitatively revealed the relative differences between the total number of taxa recorded in modern pollen surface sediment samples and the floristic richness of the pollen area (Duffin & Bunting 2008, Odgaard 1999), and this discrepancy is amplified in an environment like the KNP that has a higher floristic richness (Odgaard 1999). More contributing studies are necessary for the investigation of pollen-vegetation relationships and their interrelation to different pollination pathways.

The current study attempts to understand the pollen content of both honey and modern surface sediment with regards to the surrounding vegetation. We assume that honey is the most accessible source of insect-vegetation relationship and hypothesize that wind-pollinated taxa might be overrepresented in the surface sediment samples. The study was conducted at the Associated Private Nature Reserves (APNR) within the KNP. The sediment and honey samples were provided by Elephants Alive (Michelle D. Henley and Robin M. Cook), a South African based Non-Governmental Organization (NGO), our collaborators for this project, have the "mission to ensure the survival of elephants and their habitats and to promote harmonious co-existence between elephants and people" (http://elephantsalive.org/).

1.1 Palaeoecological Records

Palynology and other archaeobotanical methods like charcoal and phytolith analysis provide the most reliable representation of plant vegetation over a wide region for the reconstruction of (Norström et al. 2018, Baboolal 2014 and Scott et al. 2012). In South Africa although a variety of Quaternary pollen archives exist from lakes, wetlands, springs mounds and even deposits like caves sediments and dung heaps from all biomes of the subcontinent (Scott et al. 2012), information is limited due to the scarcity of suitable pollen traps (Scott 2016).

Quaternary pollen archives from the savanna biome of South African include the Tswaing Crater to the North of Pretoria (Scott 2016 and Scott 1982), Wonderkrater spring mount in Limpopo (Scott 1982), Scot's Farm on the edge of the Limpopo valley (Scott 1982) and Tate Vondo in the Zoutpansberg range (Scott 1982), (Figure 1, Scott et al. 2012). The most relevant records to this study are the Tswaing, Wonderkrater and the Limpopo Valley pollen records. Tswaing and Wonderkrater records are important because they are the longest savanna pollen records in South Africa (Partridge 1997 and Scott 1999). The Limpopo Valley pollen record is from the KNP, Limpopo National Park (PNL) and Mozambique (Ekblom et al. 2012), this record is significant because it is the closest savanna record to our study area (Figure 1).

The Tswaing pollen record is the longest and most continuous terrestrial Quaternary record in southern Africa. This important pollen archive is based on a core that was extracted from a lake in the centre of the Tswaing meteorite impact crater where the sediments stretched back > 200 000 years (Figure 2, Partridge 1997 and 1999). The pollen record shows a strong savanna woodland component with pollen of trees like *Spirostachys* and Combretaceae (Scott 1999). Additionally, the record shows a strong fluctuation of vegetation and climate over time, indicating warm and dry conditions (Metwally et al. 2014, Jacobs et al. 2008).

Wonderkrater is a spring mound consisting of peats deposits from the Driefontein farm in Limpopo with an age of c. 35 000 B.P. (middle to late Pleistocene) (Figure 1., McCarthy and Woodborne 2010, Scott 1982). The Wonderkrater pollen record is the second-longest pollen profile in the savanna biome after the Tswaing pollen archive (Scott 2016 and 1982). Savanna elements from this archive include Combretaceae, *Acacia* (now referred to as *Senegalia/ Vachellia* types), *Burkea*, Capparaceae, *Tarchonanthus* and *Spirostachys* (Truc et al. 2013). Pollen taxa like Poaceae, Combretaceae, *Tarchonanthus* and Capparidaceae, all reflect a warm climate with oscillating dryness (Puech et al.2017 and Scott 2016). The rise in

temperatures is reflected by an increase in taxa like Combretaceae, *Tarchonanthus* and Capparidaceae, together with a decrease in fynbos taxa and Chenopodiaceae (Baboolal 2014, Truc et al. 2013).

The lower Limpopo Valley southern African record is from 800 AD to the present. The lower Limpopo Valley's palaeoecological data (fossil pollen, spores, diatoms, and lithology) is from several hydrological systems in KNP and PNL (Ekblom 2008, Figure 1). Pollen types associated with savanna woodland include *Dodonaea, Sclerocarya* and *Brachyleana* (Ekblom 2008), corresponding to a decrease in moisture conditions (Ekblom et al. 2011, 2012).

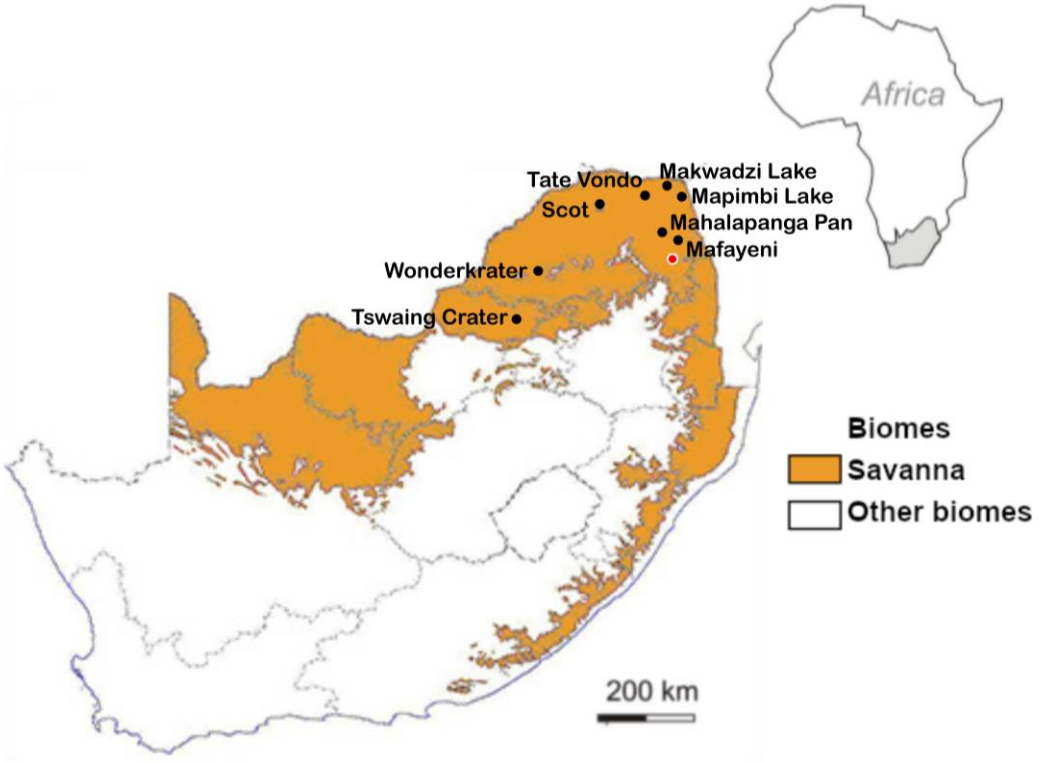

Figure 1. Locality map showing the Tswaing Crater, Wonderkrater, Scot, Tate Vondo and lower Limpopo Valley (Makwadzi Lake, Mapimbi Lake, Mahalapanga Pan and Mafayeni (after Ekblom et al. 2012)) proxy records in the savanna biome of South Africa in black dots (after Scott 2016) and the red dot is the study location.

The savanna biome harbours the longest pollen records in southern Africa (Scott 1999). Palaeoecological interpretation of fossil pollen assemblages from savanna biome deposits like the Tswaing crater and Wonderkrater can be improved by a better understanding of the pollen-vegetation relationships. For instance, present-day vegetation in KNP was surveyed around the pollen sampling site to compare present-day vegetation communities with pollen assemblages shown in modern surface sediment pollen samples to gain an improved basis for

the interpretation of past and long-term records (Zhao and Wefer 2016, Duffin and Bunting 2008, Bonny 1978). Understanding pollen transport pathways and representation in the savanna biomes is important because surface sediment pollen analysis from the KNP has shown that many trees like *Colophospermum mopane* and *Senegalia*/*Vachellia* spp. are poor pollen producers despite their relatively high presence in the vegetation (Duffin and Bunting 2008). Studies of *Senegalia*/*Vachellia* pollen morphology have shown that few large pollen polyades are produced per anther and as a result few are dispersed compared to pollen from wind pollinated plants; *Colophospermum mopane* also produces relatively few pollen grains (Duffin and Bunting 2008).

Senegalia/*Vachellia* species are predominantly insect-pollinated, and this is reflected in the fact that they do not release monads but polyades instead (Duffin and Bunting 2008). They also rely on wind for pollination (Melusi and Mojeremane, 2012). *Colophospermum mopane* is wind-pollinated (Duffin and Bunting 2008) but has also been observed to be partially pollinated by *Apis mellifera* (Jordaan et al. 2002). The greenish flowers of *Colophospermum mopane* are attractive to bees and its seasonal flowering is unpredictable which results to it absence for several years, and rainfall is the main contributing factor that controls the extent to which the plant flowers and secretes nectar (Johannsmeier 2016). The quantity of pollen production seems to determine the main type of pollen transportation pathway of *Colophospermum mopane* and *Senegalia*/*Vachellia* spp., and this has been reflected in their representation in surface sediment samples.

In terms of pollen taphonomy, corrosion affects the preservation of pollen, the thinner the exine the higher the chances for corrosion (Wilmshurst and McGlone 2005). Pollen corrosion includes various external factors (e.g., microbial attack, oxidation, mechanical forces, and high temperature), as well as internal factors inherent to the pollen grain (e.g., chemical and physical composition of the pollen wall) that lead to the breakdown of the pollen (Havinga 1967). For example, *Colophospermum mopane* (Breteler et al.1997) pollen grains have thin exines and are easily corroded (Wilmshurst and McGlone 2005). The higher the incidence of corrosion, the lower is the resistance of the pollen type over time (Wilmshurst and McGlone 2005).

Therefore, the type of pollination pathway a species depends on and the exine thickness (fragility to corrosion) of the pollen taxa determines the quantity that is found in surface samples and may be the reason for pollen rarity/underrepresentation in surface samples. Figure 2 below shows for southern Africa the unknown proportion of wind-transported pollen that ends up in surface sediment samples and the unknown proportion of insect-transported pollen that end up in surface sediment sample and honey samples and the unknown proportion of the overlap for both samples.

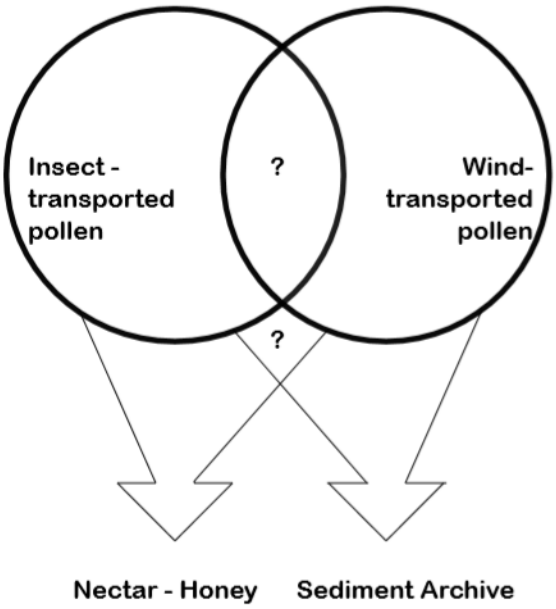

Figure 2. Diagram showing the unknown representation proportion of wind and insect transportation pollen in surface samples and honey samples in southern Africa.

1.2 Melissopalynology

Insect pollinators influence the stability, diversity, and function of natural ecosystems and agricultural plant communities (Saunders 2018). Bees are the primary pollinators in most ecological regions of the world (Greenleaf et al. 2007, Bawa 1990, Axelrod 1960). Apart from the fact that honeybees play a critical role as pollinators they also produce honey, wax (to produce honeycombs) and propolis (used by bees to seal gaps in the hive), as well as collect pollen and water (Pirk et al. 2014, Delaplane et al. 2000). Honeybees belong to the genus *Apis* which features nine species but only one is native to Africa, the common honeybee, *Apis mellifera* (Johannsmeier 2001).

Honeybees are spread across all southern African habitats with three sub-species, namely *Apis mellifera scutellata* in the largest part of the sub-continent whereas *Apis mellifera capensis* is usually limited to the southwestern Cape of South Africa (Ruttner 1986, Johannsmeier 2016). *Apis mellifera litorea* is distributed at the northern coastal KwaZulu Natal (KZN) (Eardly and Tribe 2001 in Johannsmeier 2001). The wide distribution range of *A. mellifera* has necessitated the species to adapt to numerous climates, that vary from cold temperatures, tropical conditions, high rainfall areas to semi-deserts. Each region has its distinctive floral season, that is complemented by *Apis mellifera* natural enemies (like wasps, ants, birds, wax moths, hive beetles, etc) and the characteristics of its nesting sites (nests in cavities in the ground, in hallow trees, rock crevices or under rocks, and occasionally builds exposed comb) (Eardly and Tribe 2001 in Johannsmeier 2001). Additionally, the *A. mellifera*'s limiting factor on the colony size is generally a lack of suitable nesting sites rather than a lack of food (Eardly and Tribe 2001 in Johannsmeier 2001).

Honeybees are generalist (Hausmann et al. 2016, Garibaldi 2013), although different colonies have specific plant preferences. Food preferences are influenced by the available plants in the surrounding area and the plant diversity that the bees are environed by (Gruter et al. 2011). The preferred foraging plant species depends on the rewards the plants have to offer like nutritional value (corresponding to the crude protein contained in pollen, see Johannsmeier 2016) and signals like the attractiveness of the flowers (Baude et al. 2011).

Bees forage on nectar and pollen for their nutritional sources (Ghosh et al. 2020). Nectar is the sugary fluid secreted by plants (including extra floral nectar) as a reward for pollinators especially prevalent within the flower. The foraged nectar undergoes modification inside the honey stomach of bees with the assistance of enzymes such as invertase, diastase and glucose

oxidase to result in honey (Ghosh et al. 2020, Winston 1991). Pollen is the primary source of protein, lipids, and micronutrients which is necessary for the health of bees (Ghosh et al. 2020, Ghosh and Jung 2017). Nutrient quantity and quality are the main key parameters to understand the foraging decision-making of insect foragers, including other factors like the availability of the flowers. Flowers colour, shape, morphology, display area and odour are important factors in determining insect food foraging (Ghosh et al. 2020, Willmer 2011, Brunet et al. 2015). This behaviour of bees is formally known as resource specialization, and nonparasitic bees (bees that forage for their food) are prone to resource specialization since their larval development depends on them foraging for adequate nutrition (Wcislo and Cane 1996).

Since pollen and nectar are an essential nutrient for honey bees, bee plant values are a quantitative tool used to inform beekeepers which plants in southern Africa are favoured by bees (Johannsmeier 2016). Substances such as pollen, nectar, extrafloral nectar and associated values, including the bee value of plants, are critical to beekeepers (refer to Table 1 for definitions and relevant values). These values are important to beekeepers, to evaluate which plants are good sources of nectar and pollen for the bee colonies, as well as other informative indicators like information about the extrafloral nectar and “bee factors” (definitions in Table 1, Johannsmeier 2016). The N and P values, EN and bee factor estimates are all various tools to assess the nutrient quantity and quality that determines the plants from which bees forage.

Table 1: Bee plant tools used to assess the nutrient quantity and quality that determines the plants from which bees forage (adapted after Johannsmeier & Mostert 2001, Johannsmeier 2016).

	Definition	Value range	Reference
Pollen values (P)	These values are based on the volume of bee-collected pollen, which is determined from the relative abundance, size, and evaluation of trapped pollen loads.	P0-P4, A "P0 (P zero)" value implies that the pollen is not collected by honeybees and P4 is frequently collected by honeybees.	Johannsmeier & Mostert 2001, Johannsmeier 2016
Nectar values (N)	Nectar values (N) of plants refer to the quantity of nectar available in a flower to honeybees.	N0-N4, A "N0" values implies no nectar, were as N1 = poor source, N2 = minor to medium source, N3 = medium to good source, N4 = very good source	Johannsmeier & Mostert 2001 and Johannsmeier 2016
Extrafloral nectar (EN)	Extrafloral nectar is secreted by nectaries that are located outside the flower, usually on the leaves.	Present or absent	Johannsmeier 2016
Bee factor	Bee factor is an estimated value based on the reliance on honeybee pollination a crop plant must obtain its maximum fruit or seed set.	Calculated as a percentage	Johannsmeier 2016

Furthermore, the distance between the plant species has not been regarded as a factor in the choice of plant species the bees forage from but the reward is the biggest determinant (Grüter and Ratnieks 2011). Consequently, as the number of times the foragers find no resources (low nectar sources) increases, so does the incentive to forage from the alternative plant source. This is a result of unfavourable weather conditions (like winter and periods categorised by the disappearance of important crops), depletion, diurnal cycles, etc. hence honey bees tend to continually return to the food source in case the rewards increase again, and the spatial information of the location (encoded in the foragers wiggle dance) is therefore not forgotten by the bee colony (Grüter and Ratnieks 2011).

Principle foods of any given animal population are regarded as the foods foraged on in the greatest quantity, although these foods may not be those that are the most preferred by the foragers (Petrides 1975). Preferred foods of an animal are foods which are proportionally more frequently detected in the diet than those that are abundant in the environment (Petrides 1975). Several indexes are used to calculate preferred foods, these indices produce special values which are used to rate food as preferred food or principal food. Special values are calculated by dividing the percentage of a certain food in the diet by the percentage available

in the environment (Petrides 1975). Foods that are proportionally few compared to the total food consumed are noted to be neglected and/or avoided for a number of reasons. Food that is eaten to the extent exactly proportional to its availability in nature is neither preferred nor neglected but is rather neural to the foraging species feeding preferences (Petrides 1975).

Preferred foods of animals are usually confused with principal foods in most studies and hence a few studies have considered the environmental abundance of the plants when determining which plants are preferred by bees (Petrides 1975). In this study, the botanical survey will give an overview of plants that are available in the environment thus allowing us to know which plants are selected for by the bees and which are neglected. This will shed light on the bee's feeding behaviour by knowing their preferred foods. The question is, are honeybee's generalists as speculated (by Hausmann et al. 2016, Garibaldi 2013) or do they have specific preferences in terms of foraged nectar and/or pollen for their dietary needs?

Understanding the foraging behaviour of bees is important to a melissopalynologist since pollen counts are the fundamental data used to make inferences about the bees and the factors that influence how they forage (Jamil Noor et al 2016, Jones and Bryant 2014). This is essential information for beekeepers and the economic component of honey for example to test for honey fraud (Ndlovu et al 2021), by comparing sold honey and its declared floristic/geographical origin to the known pollen concentration and pollen spectra found in the undiluted wild honey that has been verified to be produced in the corresponding region.

Variations of different pollen taxa abundance in honey samples have been noticed and it has been confirmed that variation in pollen density over time may be attributed to changes in density and richness of plant species that are foraging by bees due to seasonality (Aswini 2013). Therefore, during a peak of the flowering season, *Apis mellifera* has shown high foraging activities on few floral sources. While, during seasons like winter when the floral sources are reduced, bees must search for nutrient from more plant species, and this is reflected in the increase abundance of pollen types (Laura and Cynthia 2018). Even though the foraging range for honeybees is estimated to range from a minimum of 45m to a maximum of 5983m (Hagler et al. 2011), the average range is 6km minimum to 16 km maximum range according to other references (Bekman and Ratnieks 2000, Schneider 1990) but the range is still highly dependent on the plant species preferred by the bees (Onyango 2019). Furthermore, the nearness of a crop is an attractive factor particularly during unfavourable weather (Synge 1947).

Melissopalynology is analysis of pollen in nectar which is collected intentionally and accidentally to make honey. Pollen is intentionally collected by bees as a nutritional source (Ghosh et al. 2020), and pollen can be unintentionally collected if the bees become dusted with pollen that is not brushed off before they return to the hive or move on to another plant source (Westerkamp 1996). Nectar can also trap pollen that the bees can unintentionally collect because it is a sticky substance that pollen can easily adhere to (Johannsmeier 2016). Melissopalynology has been used as a standard method to test honey for its geographical and floristic origin (Ponnuchamy et al. 2014). This honey testing method has also been utilised to test its correlations with *in situ* climatic parameters like temperature and rainfall fluctuations since external factors affect pollination and pollinators (Ponnuchamy et al. 2014).

Honey is therefore a source of information for the identification of pollen taxa collected by bees (generally insect-pollinated taxa) (Ponnuchamy et al. 2014). However, the limitation of this study is that other insect pollinators like carpenter bees, bumblebees or butterflies will be not investigated due to time constraints. Using honey as a source of information to further understand the insect-transport pollination pathways using melissopalynology is crucial since honeybees are major pollinators within many ecosystems in Africa (Jones and Jones 2001 and Johannsmeier 2001).

The determination of the botanical and geographical origin of honey samples is accomplished by observing and counting the different pollen types identified and therefore revealing which plants the bees are preferring for nectar foraging (Dustmann and Von der Ohe 1993, Johannsmeier 2016). This idea will be further explored in this study by comparing the ratio of pollen taxa found in honey and surface sediment samples to the surrounding botanical survey findings. By quantitatively depicting how accurately the honey determines its geographical origin, the data will be displayed in a pie chart. Based on the literature it is expected that pollen from both wind and insect transportation pathways found in the honey produce a pollen spectrum that is unique to its specific region where it was collected by the bees (Igbe and Obasanmi 2014). Currently, in South Africa melissopalynological studies are scarce (see Ndlovu et al 2021). Melissopalynological studies are more common from other parts of Africa (e.g Ghana and Niger) (compare to Rasoloarijsao et al. 2017, Esterhuizen 2017, Onyango 2019). This has led to a discrepancy in the information about insect-transported pollen since they are potentially underrepresented in sediment archives (Duffin and Bunting 2008).

Surface sediment analysis and melissopalynological methods will clarify how much insect-transported pollen ends up in the sediment record and how much wind transported pollen ends up in honey (Figure 2), allowing the identification of the proportion of anemophilous (wind-pollinated) plants that bees collected pollen from the surrounding vegetation (eg. Olives trees) (Ghosh et al. 2020, Cuevas and Polito 2004). Wind transported pollen rich in protein might also be collected by honeybees but generally airborne pollen can potentially enter a hive by wind currents (Bryant and Jones 2001), whilst nectar may trap pollen of various plants which are blown into the beehives (Johannsmeier 2016 and unpublished data by Pencharz 2021 (Unbublished Honours report, study on honey samples from central Johannesburg)). These pollen grains are usually few when compared to the pollen carried into the hives by worker bees and some of these gains can be deposited into ripened honey (which is honey that has been through the ripening process of being converted from nectar to honey (Lichtenberg-Kraag 2014)) when it is being removed by the beekeeper (Johannsmeier 2016, Bryant and Jones 2001).

These discrepancies between the different pollen transportation pathways bring about a unique spectrum of pollen from both anemophilous and entomophilous taxa (wind and insect taxa) in honey that is unique for a specific region (Bryant and Jones 2010). Unpublished melissopalynological studies in the Pullen Nature Reserve in the savanna biome indicate that a certain amount of wind-transported pollen like Poaceae and Combretaceae end up in substantial quantities in honey (unpublished data Neumann and Balkwill 2019).

The study will explore the melissopalynological investigation on 38 honey samples (from three different seasons) from 12 beehives from the Associated Private Nature Reserves (APNR) (coordinate: Table 2.) in the savanna biome in Kruger National Park (KNP). This investigation will be accompanied by a palynological investigation of 12 sediment surface samples taken from immediately below the same beehives. Multivariant statistics e.g., NMDS and Rarefaction curves, were applied to the data to uncover the quantitative variabilities within the data. The limitation of this study is that it does not explore other insect-pollinators that exist in the savanna biome either than the bees as a point of reference to the insect-pollinating pathway. Another limitation is the varying quantities of the honey samples; not all quantities where sufficient (at least 10g.

1.2.1 Statistical Application

Many complexities exist when interpreting vegetational structures based on uncorrelated, relative pollen counts. The use of statistical techniques is important in the understanding of the intricate relationship between each plant taxon and how its pollen is either over- or under-represented in relative pollen counts (Bryant and Jones 2010). Uncertainties about how floristic vegetation composition is reflected by pollen data used for vegetational reconstruction has concerned palynologists and melissopalynologists (Todd and Vansell 1942). In many palynological studies, the challenge is the understanding of the relationship between the pollen plant sources and the recovered pollen data. Todd and Vansell (1942), found that plants do not contribute pollen equally to honey and nectar, they demonstrated that the relationship between plants and pollen found in honey is indirectly proportional. This paved the way for the later development of coefficient values in melissopalynology which is statistically equivalent to R-values (Todd and Vansell 1942, Bryant and Jones 2010). Unfortunately, R-values do not quantitively account for the underrepresentation of certain taxa in honey samples relative to the vegetational structure of a particular environment (Bryant and Jones 2010).

Since then, most melissopalynologists have neglected the use of other statistical analysing techniques besides percentages to better understand the under-representation of certain taxa found in honey (D'Albore 1998 and van der Ham et al. 1999). The reason for this traditional approach to melissopalynology is because the aim was to classify honey as either unifloral or multifloral rather than understanding pollen transportation pathways (Bryant and Jones 2010). Lately, Principal Component Analysis (PCA) has been used to determine the different botanical origins of the honey and to graphically display the discrepancies in their physiochemical components (Corvucci et al. 2015 and Shukla and Kumar 2020). All the above-mentioned methods are suitable for the awareness of the percentage composition of the data but more informative techniques like ecological biodiversity multivariant statistics which can expose the pollen community distribution represented by the pollen data and in doing so improving the interpretation of pollen data for past vegetations.

Non-Metric Multidimensional Scaling (NMDS) is considered as an appropriate ordination technique for count data because it is non-parametric (not based on an assumption of underlying distribution of the data) (Julier et al. 2018, Oksanen et al. 2015) and has been previously used to analyse fossil and modern pollen assemblages (Julier et al. 2018, Jardine and Harrington 2008, Schuler et al. 2014). For this study, NMDS is used to present the seasonal distribution of pollen collected all year since it allows the analysis of the distribution of the different groups (seasons).

The Shannon index was used to calculate diversities between seasons and honey hive samples since this metric is advantageous because it gives equal weight to scarce and abundant taxa (Julier et al. 2018, Morris et al. 2014). Additionally, an R package iNext (iNterpolation/EXTreapolation) which provides simple functions to compute and plot the seamless rarefaction curves for the most widely used members of the Hill number family (species richness (q=0), Shannon diversity (q=1) and Simpson diversity (q=2)). The Simpson diversity index is an abundance index that accounts for the proportion of species in a sample (Simpson 1949), whereas Shannon diversity is also an abundance index that is based on randomness present at a population site (Shannon 1948). They are both effective in determining the diversity of a species population. For this abundance data, the goal is firstly to make fair comparisons of diversity across multiple assembles (in this case different hives and sediment samples). Secondly the species richness is applied to compare the richness of all samples without excluding any data, specifically larger sample data compared to the rest of the data (Hsieh et al. 2016). These multivariant statistical techniques are therefore used in this study to develop a clear understanding of the pollen assemblage composition to better understand the pollen vegetation relationship in the savanna biome.

1.3 Aim

Aim of the current study is to analyse the pollen taxa found in honey samples and compare them to pollen spectra found in the sediment samples. The primary reason is to better understand pollen transportation pathways and the pollen-vegetation relationship. The secondary reason is to improve the interpretation of fossil pollen archives in the savanna biome. This study also intends to investigate the influence of seasonality and bee foraging behaviour from the pollen contained in the honey.

1.4 Objectives

i. To identify which specific pollen taxon are preferred by bees and how the frequency changes with seasonality.
ii. To identify the pollen spectra in the sediment samples and compare to the surrounding vegetation.
iii. To determine the ratio of insect-pollinated (entomophilous) taxa to wind-pollinated taxa in surface sediments and how it compares to honey sample

1.5 Hypotheses

Null Hypothesis: There is no significant proportional difference between the pollen taxa found in honey and sediment samples from the same location. If honey samples are used as a proxy to represent the surrounding vegetation, then seasonality will not have a direct influence on the pollen spectra in the savanna biome of South Africa.

iv. Honey and sediment samples can be used to understand the pollen-vegetation relationship within the study area.
v. The pollen-vegetation relationship from honey samples can expose the effect of the pollination pathways on the pollen-vegetation relationship by showing a higher representation of insect transported pollen than wind transported taxa.
vi. Surface sediment samples can expose the sampling biases of the surrounding vegetation. The sampling bias is influenced by different pollination pathways, for example the under-representation of entomophilous (insect-pollinated) taxa and the overrepresentation of wind-transported pollen in sediment deposits.
vii. Surface sediment samples are a good representation of the surrounding vegetation.

viii. Melissopalynology is indicative of the pollen collected by the bees and their preferred plants in the Lowveld region of southern Africa.

ix. Therefore, palynological studies on surface sediments and honey are both useful pollen and vegetation representation proxies that are useful to the reconstruction of past vegetation.

2. Materials

2.1 Study Site Location and Description

The honey was harvested and supplied by the NGO Elephants Alive from four seasons (10th December 2019, 15th January 2020, 19th March 2020, 1st July 2020, and 26th October 2020), at least 10 g were supplied from the same 12 hives for all seasons separately (total of 38 honey samples and 36 sediment samples, coordinates: Table 2, Figure 3). December and January samples are additional samples to the proposed three harvest seasons (autumn, winter and spring) and they are specifically harvested from only a single hive (H1: Monokane Hive) (Table 2). Approximately 383.50m west from the hives is a lucerne (*Medicago sativa*) field which is bordered by an old airstrip, most areas of the airstrip are bare, and others are covered in pioneer species and forbs. Further away from the hives, approx. 920m west is the Olifants River (Table 2, Figure 3).

Table 2. Coordinates of Beehives from the Beehive Site at the KNP Associated Private Nature Reserves

Beehive Number	Latititude	Longitude
1	-24,21712	30,82666
2	-24,21725	30,82668
3	-24,21774	30,82654
4	-24,21791	30,82678
5	-24,21811	30,82673
6	-24,21815	30,82641
7	-24,21814	30,82635
8	-24,21783	30,82588
9	-24,21773	30,82587
10	-24,21753	30,82603
11	-24,21745	30,82606
12	-24,21744	30,82621
13	-24,21701	30,82639

Figure 3. Esri map image of the hives and the nearby disturbed areas such as the lucerne field and the Olifants River (Geographic Information System (GIS) Esri tool (CapeFarmMapper (CFM) 2.6.2 -Elsenburg).

2.1.1 Climate, Vegetation, Geology and Soils of the Region

South Africa is highly sensitive to climate change since the semi-arid sub-continent is influenced by both transitional between temperate and tropical/sub-tropical atmospheric systems, over various temporal, and special scales. The continent is affected the cool Benguela Current along the western shore of the sub-continent and the warm Agulhas Current at the eastern and southern coast (Figure 4, Zhao 2017). The cool Benguela current triggers the induction of a winter rainfall zone at the southwestern tip of the continent (Zhao 2017). A transitional year-round rainfall zone stretching from the southern coast towards the interior of the continent and a summer rainfall zone in eastern South Africa continues along the coast of the Indian Ocean and further inwards (Figure 4, Zhao 2017).

South Africa has a rich plant biodiversity which is unparalleled by most other countries. This is reflected in the vegetation featuring 34 bioregions falling under 9 biomes due to a function of extreme climatic variations of rainfall > 150 mm in the west, to high rainfall > 700mm/year in the much more humid eastern part of the sub-content (Mucina and Rutherford 2006, Johannsmeier 2016, see figure 4.1). The savanna biome, where the current study is located, is often referred to as “bushveld” in South Africa (Schmidt et al. 2002). The savanna biome stretches from north-eastern South Africa upwards to eastern Africa and is characterised by strong seasonality with a summer rainfall (Mucina and Rutherford 2006). Savanna is dominated by woody vegetation with an open canopy with many tropical trees like Combretaceae, *Senegalia* and *Vachellia* species and a grassy understorey including species like *Themeda triandra* (Scott and Neumann 2018, Mucina and Rutherford 2006).

The Atlantic Ocean and Indian Ocean at the coasts of South Africa influence the macroclimatic regimes of the savanna biome region (Rutherford et al. 2006, Mucina and Rutherford 2006). The macroclimate of the biome is characterized by seasonal rainfall (which is described as the interchange of dry winter periods and wet summer) and a (sub)tropical thermal regime with absent to low frost incidences (Rutherford et al. 2006). Unsurprisingly, the thermal regimes and temperature differences reveal several major trends (Rutherford et al. 2006, Schulze and McGee 1978), for example an increased overall temperature towards the equator is one of the major trends that results in the highest yearly temperatures in the Mopane Savanna bioregion (Rutherford et al. 2006). Furthermore, the savanna biome, which is characterizing APNR, receives a mean annual rainfall of 400mm to 600mm and mean

monthly maximum and minimum temperatures of 39.5°C and -0.1°C respectively (Mucina and Rutherford 2006, Figure 4.1).

The study is carried out in the KNP which covers two bioregions within the savanna biome, the Mopane Veld, and the Lower Bushveld bioregions (Figure 2, Mucina & Rutherford 2006, Johannsmeier 2016). The Mopane Veld bioregions (SVmp) is dominated by Mopane trees (*Colophospermum mopane*) that are mainly situated in the northeast of KNP at the border to Mozambique (Rutherford et al. 2006). The Lowveld Bushveld bioregion (VI) is situated in the east along the Eswatini and Mozambique borders (Rutherford et al. 2006). The study will be conducted in the vegetation type Granite Lowveld (SVI 3) of the Low Bushveld bioregion (VI) within a frost-free region (Rutherford et al. 2006, Figure 4.2).

The vegetation of the site is classified as the Mopane Bushveld Savanna (*Colophospermum mopane* Bush Savanna) vegetation of the KNP (Schmidt et al. 2002, Figure 4). The underlying soils of the bushveld are loamy sand and sandy to clay (Schmidt et al. 2002). The vegetation and landscape around the beehives is characterised by woodlands on deep sandy uplands and the vegetation is characterised by tall shrubs and a few trees like *Combretum zeyheri, Terminalia sericea* and *Capiculatum* along with ground layers consisting of *Poponarthria squarrosa, Eragrostis rigidior* and *Tricholaena monachne.* The vegetation landscape is further surrounded by Dense thicket to open savanna can be found in the low-lying land with *Dichrostachys cinera, Senegalia nigrescens, Grewia bicolor* in the woody layers of the region (Mucina and Rutherford 2006). The dense herbaceous layer of the landscape contains predominantly *Aristisa congesta, Panicum maximum* and *Digitaria eriantha* on fine-textured soils (Mucina and Rutherford 2006).

The KNP is a fenced National Park in the midst of wide and varying landscapes and vegetation that has been invaded by several alien plant species (Foxcroft and Freitag-Ronaldson 2007). Currently, the most prevalent invasive species of the KNP are *Lantana camara, Opuntia stricta, Chromolaena ordorata, Azolla filiculoides, Eichhornia crassipers* and *Pistia stratiotes* (Foxcroft and Freitag-Ronaldson 2007). The most widespread invasive alien plant species in KNP were either introduced accidently along roads and rivers, or deliberately as ornamentals (Foxcroft et al. 2008). Two hundred and fifty eight invasive species have been documented in thirty-six tourist camps and staff villages of the KNP and they include plant species like Amaranthaceae (*Alternanthera denticulata, Alternanthera ficoidea, Alternanthera pungens*), Euphorbiaceae (*Euphobia heterophylla, Euphobia*

leucocephala, *Euphobia pulcherrima* and *Pedilanthus tithymaloides*), Asteraceae (*Helianthus annuus*), Malvaceae (*Hibiscus rosa-sinensis*, *Hibiscus schizopetalus*), Acanthaceae (*Justicia brandegeana*, *Odontonema strictum*), Haloragaceae (*Myriophyllum aquaticum*), Poaceae (*Pennisetum purpureum* and *Pennisetum setaceum*), Pandanaceae (*Pandanus baptistii* and *Pandanus utilis*), and Nyctaginaceae (*Mirabilis jalapa*) (Foxcroft et al. 2008). These ornamental plants are most likely to invade the urban vegetation, wildland ecosystems and the natural vegetation of one of the world's largest areas managed primarily for biodiversity conservation which is the KNP (Foxcroft et al. 2008). Furthermore, some of these neophytes (eg. *Ambrosia artemisiifolia*) can also be used as time markers in Holocene pollen archives (Neumann et al 2008, 2011, Scott et al 1982).

Bees forage from both native and exotic plants that are available in the ecosystem (Harmon-Threatt and Kremen 2015), hence some invasive species have been identified as bee plants. The bee plant crops from the semi-arid western region include *Medicago sativa* (alfalfa) which is a neophyte planted extensively in South Africa for grazing, hay, and seed production (Johannsmier 2016). *Helianthus annuus* (sun flower) is mainly grown on the highveld of the northern provinces of southern Africa, as well as *Citrus* spp. (lemons, oranges) which produces citrus honey that is obtained from orchards in the Northern Provinces and Mpumalanga. Bee plant weeds of the region include *Tribulus terrestris.*, *Echium lycopsis*, *Hypochoeris radicata*, etc (Johannsmeier 2001). Furthermore, the Mopane Bushveld study vegetation site is associated with some of the following known bee plants: bushwillows *Combretum* species(bushwillows), *Sclerocarya birrea* (marula), *Grewia* spp. (raisin bushes), *Senegalia mellifera* (black thorn), (*Boscia albitrunca*) shepherd's tree, *Dichrostachys cinera* (sickle bush), *Adansonia digitata* (baobab), *Vachellia tortilis* (umbrella thorn), *Colophospermum mopane* (mopane tree), (Johannsmeier 2016), and *Faurea saligna* (willow beechwood), (Johannsmeier 2001). According to Johannsmeier 2016, there were no known beekeepers in this veld type, but this study utilizes honey samples from beehives owned by Elephants Alive.

Lastly the geology of the region (SVI 3) from north to south is characterised by the Swazian Goudplaats Gneiss, Makhutswi Gneiss and Nelspruit Suite (granite gneiss and migmatite), and the further south it is characterised by the younger Mpuluzi Granite (Randian) which is from the major basement geology of the area (Mucina and Rutherford 2006, Figure 4.2). The Swazian Goudplaats Gneiss and Makhutswi Gneiss are the oldest known rocks in the KNP (Schutte 1986). In the uplands of the landscape the Archaean granite and gneiss rocks overlap

into sandy soils while the clayey soils with high sodium content are found in the lowlands (Rutherford et al. 2006).

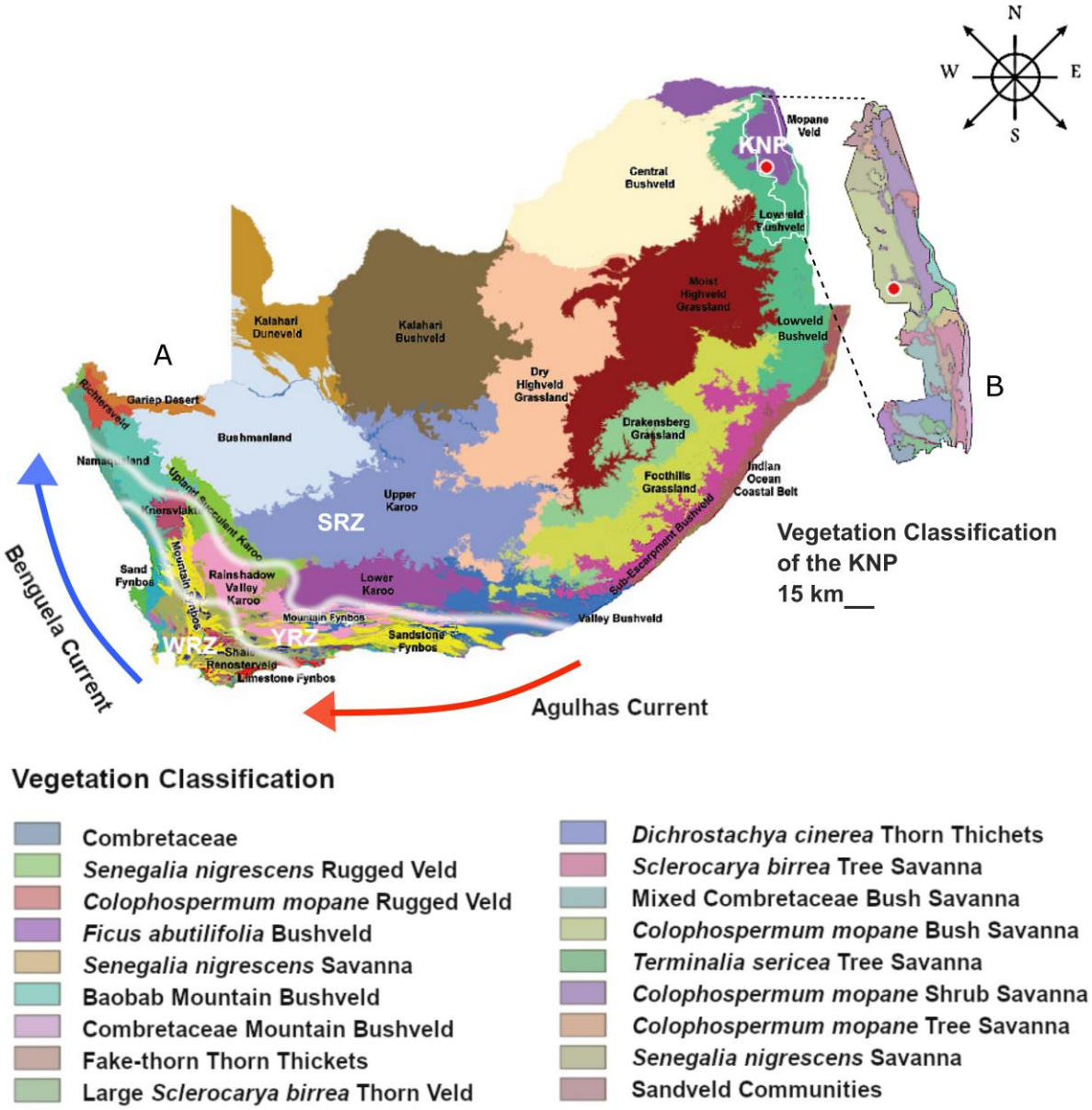

Figure 4. A (map on the left)**:** Bioregions of South Africa, Lesotho, and Swaziland (after Mucina and Rutherford 2006, Johannsmeier 2016) with oceanic currents (blue arrow: Benguela Current, red arrow: Agulhas Current). The rainfall zones are labelled as Winter rainfall zone (WRZ), year-round rainfall zone (YRZ), summer rainfall zone (SRZ) (after Zhao 2017). Study site/Associated Private Nature Reserve is indicated as a red dot within the B (map on the right): Greater Kruger National Park (KNP) with an extended vegetation classification map (after www.sanparks.org).

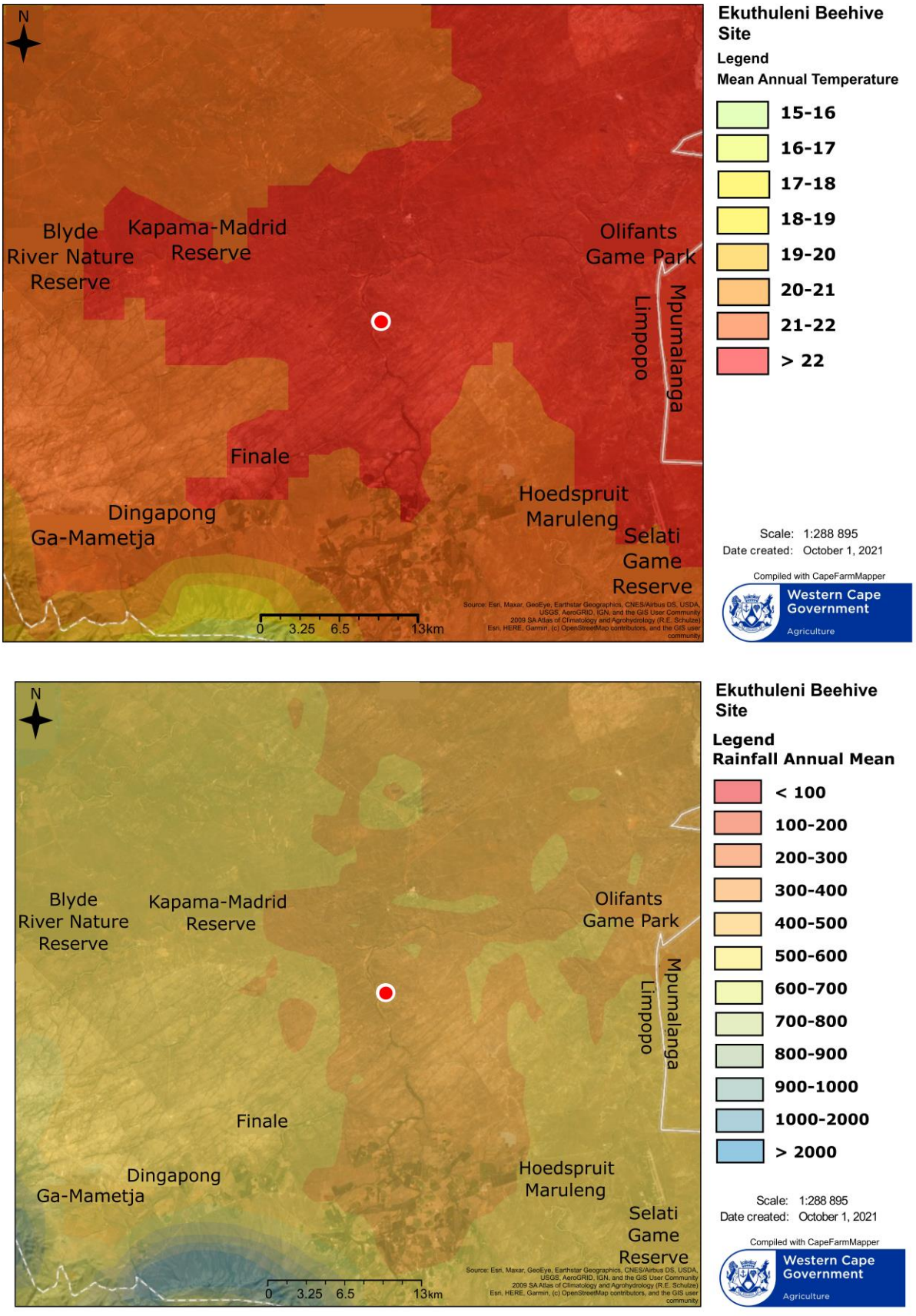

Figure 4.1 The mean annual temperature as °C (top) and mean annual rainfall in mm (bottom) maps of the study location (adapted from Schulze 2009 using the Geographic Information System (GIS) Esri tool (CapeFarmMapper (CFM) 2.6.2 -Elsenburg)). Red dot: Study site.

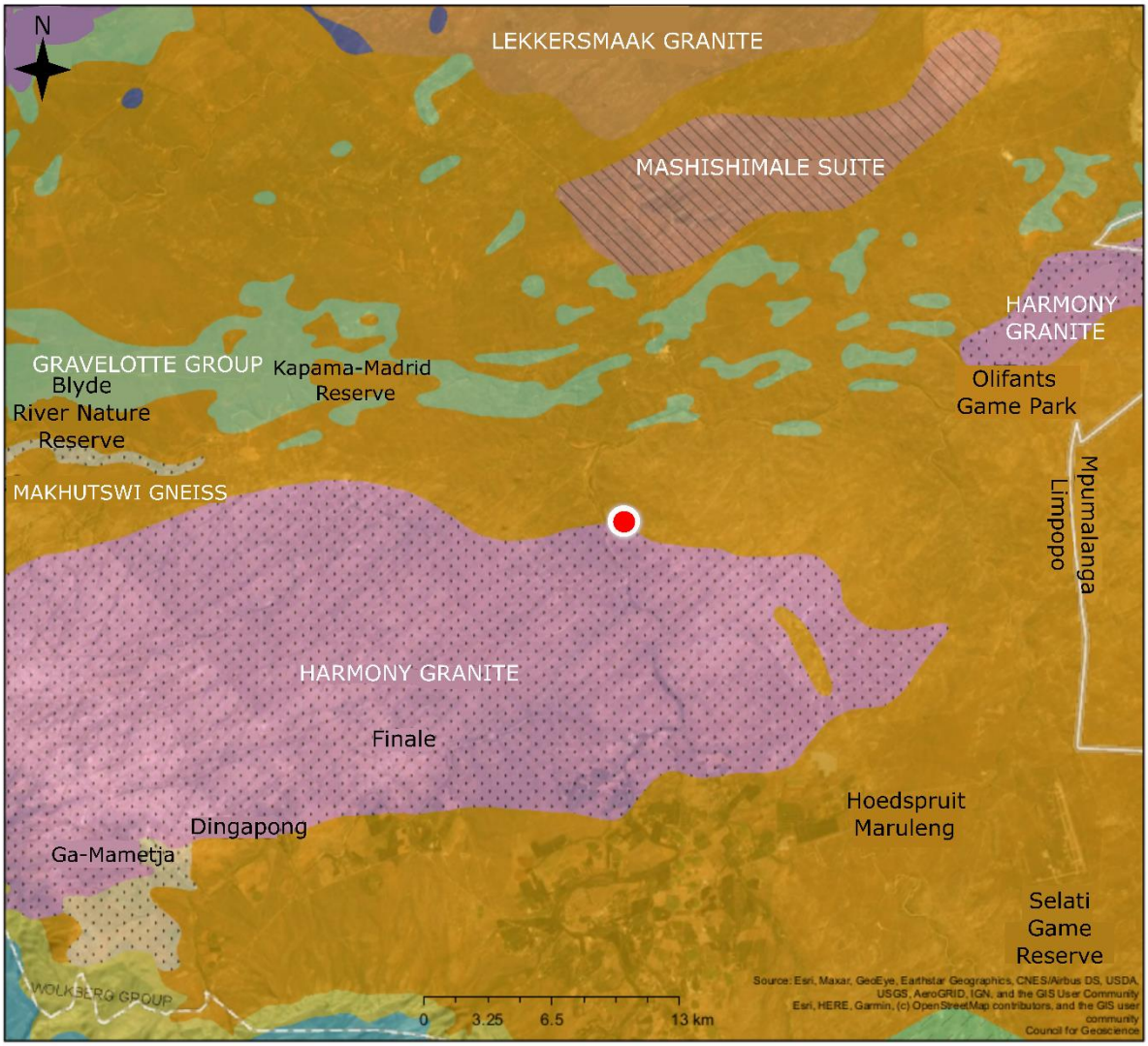

Figure 4.2 Geology map of the study location. The geological map is adapted from Council for Geoscience and the map was conducted using the GIS Esri tool (CFM 2.6.2 -Elsenburg). Red dot: Study site.

3. Methods

3.1 Botanical Survey in the Vicinity of the Hives

A preliminary botanical survey served to examine the surrounding vegetation to evaluate the degree of similarity between the contemporary vegetation composition and the collected pollen by bees with the focus on specific species that were frequently found in pollen samples (more than 1%). A vegetation study of the APNR location within the KNP has been conducted (the data is not specific or in great proximity to the location of the beehives, attached as an appendix, S. Figure 5) and this botanical survey aims to specifically identify the plant species around hive sites of the APNR (Figure 4).

The list of selected plants with a high relative abundance of species-specific pollen in the honey samples were identified along a 250m transect from four cardinal directions, North to South and from the West to East across the centre of all the beehives. Each of these species that occurred along the transect were given a coordinate and the distance between the bee plant and the closest hive-site (Figure 5). This survey method was adapted from Tabares et al. (2021). The survey was conducted from the centre of all hives together (since the hives are within a 150m range from each other) to avoid redundancy of the plants identified.

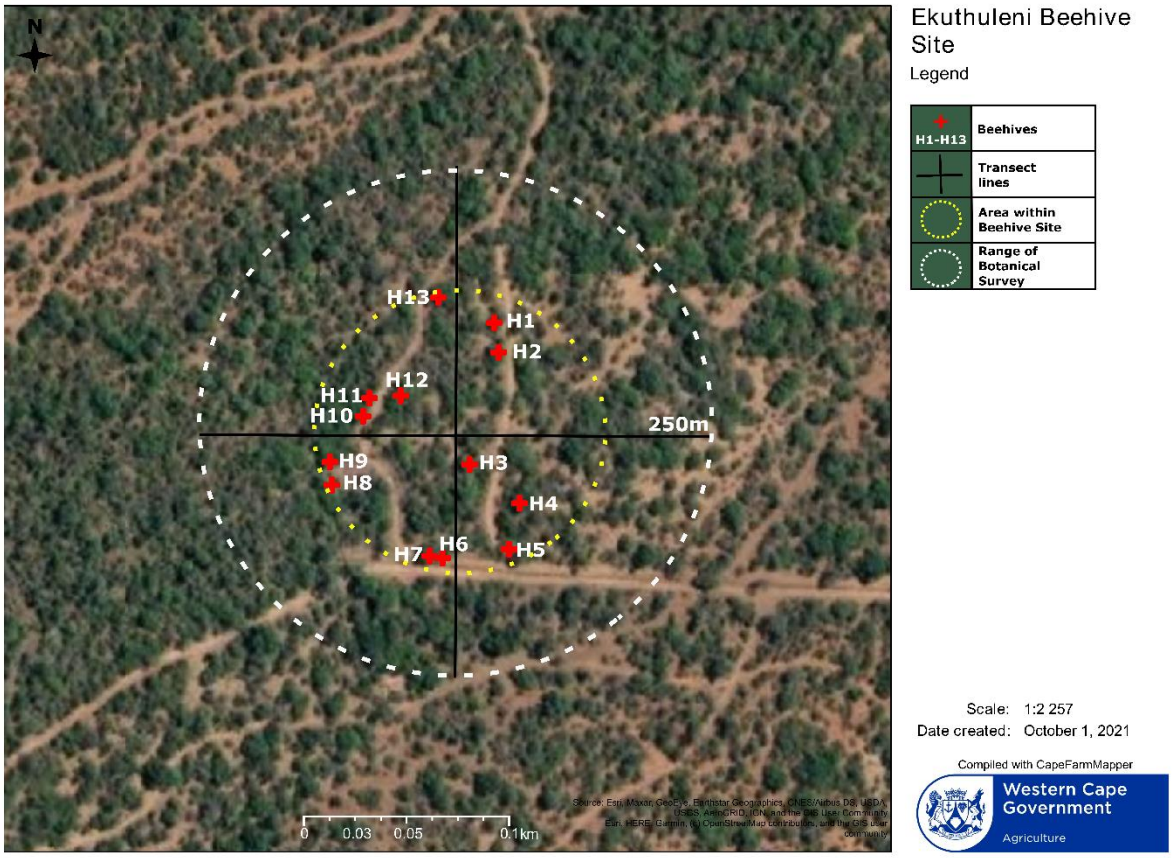

Figure 5. Preliminary Botanical Survey of a 250m transect within and beyond the beehive locations (Table 2 contains the hive coordinates). This diagram was drafted used the GIS Esri tool (CapeFarmMapper (CFM) 2.6.2 -Elsenburg.

3.2 Melissopalynological Processing

The processing of the 38 honey samples (12 honey sites from 3 different seasons plus an additional 2 from the summer harvest) was done by acetolysis. Acetolysis is used to destroy plant matter as well as lipids, carotenoid pigments (pollen kitt) and cell contents of pollen, this allows for a better identification and concentration of the pollen (Louveaux et al. 1978). The acetolysis mixture is prepared by adding 1 ml of sulphuric acid (H_2SO_4) to 9 ml of acetic anhydride ($(CH_3CO)_2O$) or a 1 (H_2SO_4): 9 ($(CH_3CO)_2O$) ratio (Louveaux et al. 1978).

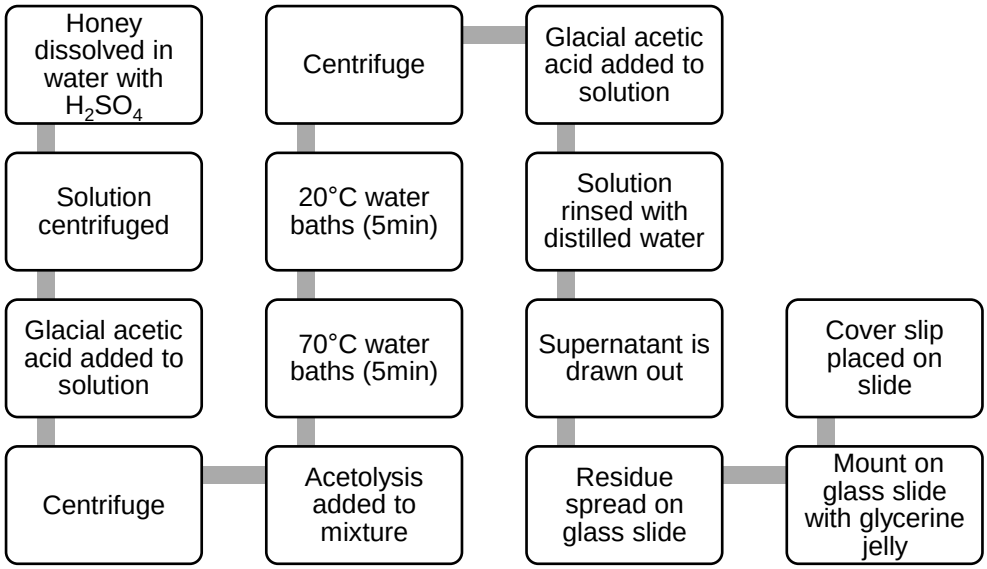

Figure 6.2 Simplified pollen processing steps for honey samples.

Slide preparation was carried out by dissolving 10g of honey in 15 ml of hot distilled water in a beaker, distilled water is preferred over normal water since normal water is a potential source of contamination and it might contain pollen (Kettering et al. 2002). Then 35ml of weak sulfuric acid (3ml of concentrated acid to 1000ml distilled water) was added to the solution which is transferred to a centrifuge tube to be centrifuged for 5 minutes at 3000 r/min (Lieux 1980). One *Lycopodium* spore tablet was dissolved in sulfuric acid for the calculation of the pollen concentration (Queiroz and Mateus 1998). Thereafter the supernatant liquid was drawn out with a pipette, leaving approximately 1 ml in the tube then the remaining weak sulfuric acid was continuously rinsed and centrifuged repeatedly until the solution finished.

This process was followed by the acetolysis procedure that firstly includes the preparation of the acetolysis mixture mentioned above. Then 15ml of glacial acetic acid was added to the solution and centrifuged at 3000r/min for 5minute. Thereafter 15ml of the acetolysis mixture was added to the solution in the tube. The tube was then placed in a 70 °C hot water bath for 5 minutes followed by another 5minute in a 20°C water bath. Thereafter the tube was centrifuged for 5 minute and then the supernatant liquid was drawn out with a pipette. The centrifuge tube was filled with 10ml of glacial acetic acid then centrifuged at 3000r/min for 5minute. The solution was finally rinsed three times with distilled water in the centrifuge before the supernatant was drawn out.

Once the supernatant is drawn out and discarded the residue was spread on a glass slide over an area of 20 x 20 mm then placed on a hot plate. Then drops of glycerine gelatine were placed in the centre of the slide with the residue, and then the glass cover is placed on the glass slide while it is on the hot plate. This prevents air bubbles from forming especially since they might diminish the clarity of the slide under the microscope. The residues of approximately 2ml are archived in Eppendorf tubes at ESI (Evolutionary Studies Institute, University of Witwatersrand).

3.3 Processing of Sediment Surface Samples

Modern surface sediment samples (total of 36) were collected directly under each beehive hung on a tree from an area of approximately 1 m^2 using a trowel which was regularly cleaned under running water to avoid contamination. Thereafter the 12 sediment samples were collected from three different seasons resulting in the analysis of 36 sediment sample. These were prepared for pollen analysis using the standard palynology processing procedure.

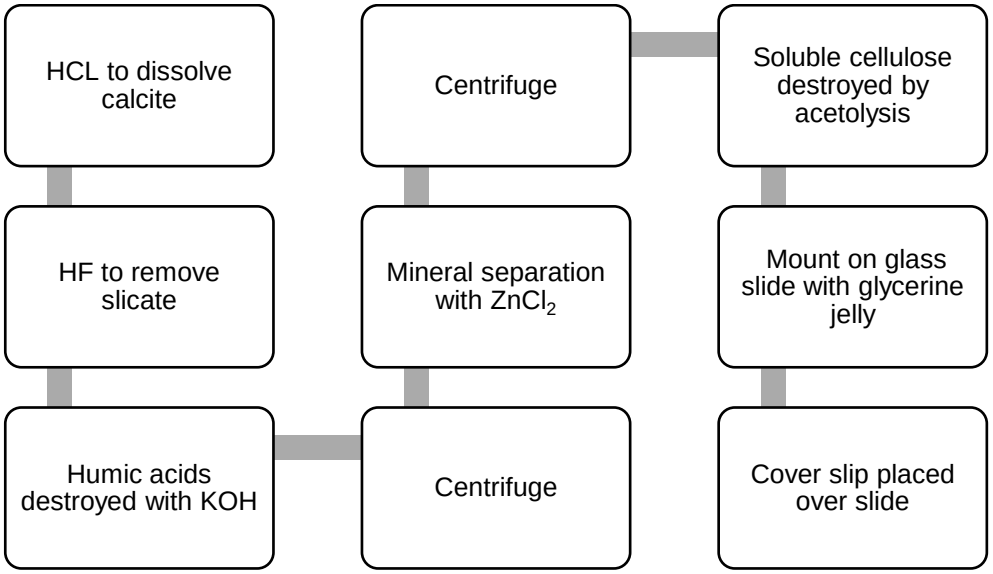

Figure 6.1 Simplified pollen processing steps for sediment samples (after Wood 1996).

Firstly, 10g of all sediment samples were transferred into a beaker and sieved with a 200μm mesh (to remove large particles like twigs, charcoal, roots etc.). Then 20-30ml of Hydrochloric acid (HCl) was added to the beaker to dissolve calcite from the sediment samples (Lund et al. 1975). One *Lycopodium* spore tablet was dissolved in HCl for the calculation of the pollen concentration in the sediment (Queiroz and Mateus 1998). Then the solution was transferred into a tube and rinsed three times with distilled water. Thereafter 10-20ml of 40% hydrofluoric acid (HF) was added to the solution and stirred constantly with a polythene rod to remove silicates and the HF solution was left to react overnight. Distilled water (10ml) was used to rinse the supernatant by centrifuging the solution at 3000r/min for 5minute three times repeatedly. Then 10-20ml of 10% potassium hydroxide (KOH) solution was added to the tube to remove humic acids, then it was centrifuged at 3000r/min for 5minute repeatedly with distilled water until the solution was clear.

Thereafter the heavy liquid separation (for mineral separation of the supernatant from the precipitate (waste)) method was done using zinc chloride ($ZnCl_2$). The supernatant was rinsed and centrifuged three times then cellulose was removed by acetolysis. 30-40 ml of acetolysis mixture (9:1 ratio of acetic anhydride and concentrated sulphuric acid) was added to the sediment in the tube. The tube was placed in a 70°C water-bath for 5 minutes then placed in a 20 °C water-bath for another 5 minutes then rinsed three times with distilled water.

Thereafter 10ml of glacial acetic acid (CH_3COOH) was added to the tube, the tube was then topped up with distilled water, then centrifuged at 3000rounds/min for 5minutes and then decanted into a dry beaker. The tube was finally filled with distilled water again and centrifuged for an additional 5 minute (repeated three times). The supernatant was sieved with a 10 microns sieve to remove debris and improve the pollen concentration on the slide. The remaining supernatant was drawn off using a pipette and the sediment was mounted on a glass slide over an area of 20 x20 mm with glycerine jelly (Figure 7.1 after Wood 1996).

The pollen concentration value was calculated for the October sediment samples and summer months honey samples (December and January) using *Lycopodium* spore markers which were added while the samples were processed. The use of the spores is to produce accurate quantitative estimations of the pollen assemblage within the samples (Queiroz and Mateus 1998). Pollen concentration values are estimated by the pollen analytical counting of a processed sample of known weight, to which the tablets of the known number of spores were added (Queiroz and Mateus 1998). These values give a general idea of the amount of pollen present. The pollen concentration value is calculated using the following format (adapted from Queiroz and Mateus 1998):

Equation 1

$$P_{con} = \frac{\textit{Lycopodium}\text{ spores added}}{\textit{Lycopodium}\text{ spores counted}} \times \frac{\text{pollen counted}}{\text{the volume (or weight) of the sample}}$$

3.3.1 Microscopic Analysis and Microphotography

The slides were investigated under the light microscope (Zeiss Axioscope 5) with a magnification of 1000x to view details of the pollen morphology. Detailed pictures of the pollen grains were done using an Olympus 2L04386 camera including image analysis software. The identification of taxa was done using the pollen reference collection at ESI as well as online guides (e.g Master Reference Collection from Global Pollen Project at https://globalpollenproject.org/Taxon) and references (e.g, Gosling et al. 2013, Bonnefille and Riolett 1980, Scott 1982). The 12 modern surface sediment samples (the rest of the 24 samples did not yield pollen sums > 15) and 38 honey samples were counted to a pollen sum of approx. 300 per slide then the data was graphed on several pie charts in R to display the relative abundance of different pollen taxa between seasons and samples. Pie charts have been used by several melissopalynologists including Brodscheider et al. 2019, Adeonipekun 2012 and many more publications to represent pollen composition over different seasons. Additionally, the counts from the slides were also used to determine the pollen concentration values of the samples.

Pollen concentration values of honey are used to allocate honey in different categories. This method is used by melissopalynologists to categorise the honey samples investigated (category definitions and values in Table 1.1).

Table 1.1 Honey pollen category definitions and pollen concertation values (adapted from Maurizio 1975)

Honey Category	**Number of pollen per 10 grams**	**Honey category interpretation**
Category 1	< 20000grains/10g	Usually honey in this category represents samples that have been pressure-filtered and honey from plant sources that produce minimal pollen. This category also include honey that was partly produced by sugar-feeding bees or that has been adulterated by adding sugar like high fructose syrup.
Category 2	20 000-100 000 grains per 10g	This category includes most of the honey produced in the world from floral sources.
Category 3	100 000-500 000 grains per 10g	Represents plant sources that produce a high quantity of pollen or the category indicates that some of the pure pollen contained in comb storage cells may have come into contact with the extracted honey.
Category 4	500 000-1000 000 grains per 10g	Indicates honey that is produced from few different plant sources that are extremely rich in pollen.
Category 5	> 1 000 000 grains per 10g	

3.3.2 Multivariate statistics

Multivariate statistics were used to identify the variance of the pollen taxa contained within the honey and the sediment samples followed by the descriptive taxa analysis of how both these samples compared to the botanical survey findings. The multivariant technique to compare the samples was performed in R using the vegan package, which is suitable for vegetation diversity analysis using functions like PERMANOVA (Dixon 2003). A permutational analysis of variance (PERMANOVA) multivariant technique was utilised to compare pollen taxa composition found in honey samples throughout different seasons using the adonis function (Oksanen et al. 2007).

This technique is a non-parametric test used to compare groups of objects and to test the dispersion of the sample groups; the calculated p-value indicates the significance of the assumed heterogeneity (Oksanen et al. 2007). The results were graphically displayed using a non-metric multidimensional scaling (NMDS), which is found in the MASS library in the Vegan package (Dixon 2003). An NMDS attempts to represent as closely as possible the pairwise dissimilarity between the honey sample groups. This test shows the significance of the differences between the honey samples throughout different seasons and as a result indicate if there is any correlation between seasons and the plants the bees prefer (Johannsmeier 2016).

ANOVA and adonis tests were used to test for the significance between the honey and sediment samples. The adonis function in R was used to test for variance using a distance matrix (Oksanen et al. 2007). Then the assumption for homogeneity of the multivariate data was tested using the ANOVA function in R (Faraway 2002). Anova and adonis tests showed the significance of the differences between the samples and therefore shed light on the preliminary studies that are focused on understanding pollen spectra in different samples. In doing so the indirect relationship between the surrounding vegetation and sediment pollen accumulation can be accounted for by comparing them to honey samples, to understand the influence of the pollen transportation pathways on pollen deposition.

4. Results

4.1 Melissopalynological Results

A total pollen sum of 13053 was counted across 38 samples from four different honey harvest months (10th December 2019, 15th January 2020, 19th March 2020, 1st July 2020, and 26th October 2020) including 12 surface sediment samples (October collection). The autumn and winter sediment samples did not yield satisfactory pollen counts <15 pollen grains per season. The honey samples in total yielded 32 pollen taxa from 20 families (Table 3 and 4). The pollen counts from all honey seasons has a mean of 333, range of 622, minimum 26 and a maximum of 648 pollen from about 38 different sample counts (number of hives). Each pollen type was identified to the lowest possible taxonomical level which was family, genus or where possible species level (Table 3). Morphological similarities that exist within a family do not always make it easy to identify the pollen type on a genus or species level. Genus of some species is mostly written as "genus name sp." or "genus name spp.", "genus name sp. means only one type was observed for the pollen but it could not be labelled to species level and "genus name spp.", means that more than one type was observed for the genus but varying types could not be identified to species level. In this investigation, they include the Euphorbiaceae and Myrtaceae which were only identified to the family level. These types can be observed in the photo plates attached below (Figure 6.1- 6.4).

Table 3 represents all pollen taxa percentage values for each harvest from the honey samples. Table 4 describes the pollen morphologies (including the pollen morphology author) of all taxa and presents the general ecological description of each taxon, the species flowering periods, and the reported pollinators of the species along with their bee plant values, which are values developed to quantify the preference of a plant species based on it pollen and nectar collected by bees, the values are after Johannsmeier 2016 (Table 4).

Pollen concentration value of 50 080 pollen grains per 10g of honey places the samples in Category 2. Category 2 contain between 20 000- 100 000 grains per 10g, this includes the majority of honey produced in the world from floral sources (Maurizio 1975).

Equation 1:

$$\text{Pcon} = \frac{\textit{Lycopodium}\text{ spores added}}{\textit{Lycopodium}\text{ spores counted}} \times \frac{\text{pollen counted}}{\text{the volume (or weight) of the sample}}$$

Pcon = 12000/75 x 313/10g = 5008, Pcon per 10g of honey = 50 080

Table 3. Percentage list of all the pollen taxa found in the December (D), January (Ja), March (M), July (Ju), and October (O) honey samples, FT-Flowering Time (adapted after various references presented in Table 3 and extended in S. Table 2), FDH- Flowering during harvest and BV-Bee plant value (after Johannsmeier 2016)

Honey Taxon		**D %**	**Ja %**	**M %**	**Ju %**	**O %**	**FT**	**FDH**	**BPV**
Acanthaceae	*Justicia flava*	0	0	0	1.07	0	1-12	Yes	N1-2, P0-2?
	Justicia decurrens	0	0	0	1.73	0	11-4	No	N1-2, P0-2?
Anacardiaceae	*Harpephyllum caffrum*	0	0.64	5.71	1.76	7.09	11-2	Yes	N0-1, P0-1
	Sclerocarya birrea	1.32	13.73	5.36	11.58	39.95	11-3, 9-11, 8-9	Yes	N0-3, P0-2
	Lannea sp.	0	0	0	7.10	0.34	11-3, 7-2	Yes	N0-3?, P0-3
Amaranthaceae	*Alternanthera* sp.	0	0	0	1.81	0	10-6	Yes	P0-1, N0-2
Asteraceae	*Gerbera* sp.	0	0	3.87	0.05	0	9-1, 1-12	Yes	N2, P2
	Helianthus sp.	0	0	0.39	0.18	0	9-4	Yes	N2-3, P0-3
	Ambrosia	0	0	0.01	0.05	0	9-12	No	N0, P3?
	Tubuliflorae	0	0	0.52	0.43	0	-	-	-
Burseraceae	*Commiphora* spp.	0	0	0	0	0.41	9-3	Yes	-
Caesalpinioideae	*Peltophorum africanum*	0	0	0	0.21	0	9-3	No	N0-2, P1-3
Celastreaceae	Celastreaceae	0	1.59	0	0.03	0.41	2-5	Yes	N0-1, P0-1
Combretaceae	*Combretum* spp.	97.4	7.99	63.34	67.36	46.66	8-2	Yes	N0-3, P0-3

Cyperaceae	Cyperaceae	0	0	0.19	0	0	10-5	No	N0, P0-2
Euphorbiaceae	*Euphorbia* spp.	0	0	1.81	1.25	2.43	10-3, 1-12	Yes	N0-2, P0-1
Fabaceae	*Senegalia* type	1	0.64	0.97	0.94	0.44	8-11	Yes	N0-4, P0-4
	Vachellia type	0.33	2.56	0.45	0.13	0.07	11-1	Yes	N0-4, P0-4
	(Mimosoideae), *Dichrostachys* sp.	0	0	0	0.18	0	10-2	No	N0-1?, P0-3
	Medicago sativa	0	71.88	0	0	0	11-3	Yes	N0-3, P0-2
Haloragaceae	*Myriophyllum* sp.	0	0	0.02	0.05	0	9-2	No	-
Lamiaceae	*Plectranthus* sp.	0	0	0.07	0	0.38	1-6	Yes	N0-3?, P0-2?
Malvaceae	*Dombeya* sp.	0	0	0.01	0.21	0	1-12, 3-8	Yes	N1-2, P0-2
	Hibiscus sp.	0	0	0.19	0.86	0	1-12	Yes	N2?, EN, P1?
	Grewia spp.	0	0	0.56	0.05	0.5	10-5	Yes	N0-3, P0-3
	Triumfetta sp.	0	0	0	0	0.01	12-5	No	N1-2?, EN, P1-3
Myrtaceae	Myrtaceae	0	0	0.52	0	0.38	1-3,6-12	Yes	N0-3?, P0-3?
Nyctaginaceae	*Phaeoptilum spinosum*	0	0	0	0.39	0	8-9	No	N?, P?

Platanaceae	*Platanus sp.*	0	0	0.25	0	0	9	No	N0, P0-1
Poaceae	Poaceae	0	0	15.12	0.96	0.10	12-6	No	N0, P0-2
Podocarpaceae	*Podocarpus*/ *Afrocarpus*	0	0	0.05	0	0	12-2	No	-
Proteaceae	*Protea/Faurea* sp.	0	0	0.72	0.07	0	6-10, 4-9,8-1	Yes	N0-3, P0-2
Salicaceae	*Salix* sp.	0	0.96	0	0	0.58	8-9, 8-10	No	N?, P1
Zygophyllaceae	*Tribulus sp.*	0.3	0	0	1.19	0	10-5	Yes	N2, P2

Table 4. A list of important exotic and abundant pollen taxa found in the December, January, March, July, October honey samples and October surface sediment samples. The pollen morphology, flowering period, ecology, bee-plant values, and the pollinators of the plants have been documented for each taxon. The extended table with all other rare pollen taxa identified is attached as a supplementary table (S. Table 2).

Anacardiaceae
Species and author-name: *Harpephyllum caffrum*, Halbritter (Halbritter 2016) [Figure 6.1 Q-T] **Morphological description:** Monad, isopolar, radially symmetric from the polar view and tricolpate (Halbritter 2016). The sculpture is striate (Halbritter 2016). Polar length is c. 20-28 µm (c. 20-25 µm own observation) and equatorial diameter is 20-30 µm (c. 20-26 µm own observation) (Halbritter 2016). **Growth form:** Evergreen tree up to 15m height (Schmidt et al. 2002) **Flowering period:** November to February (Schmidt et al. 2002) and November to April (Johannsmeier 2016) **Ecology:** *Harpephyllum caffrum* grows in frost-free areas, usually in riverine forest along the escarpment of the EC (EC), northwards through KZN, Eswatini, southern Mozambique, Limpopo and into Zimbabwe (Schmidt et al. 2002). Dioecious attractive ornamental trees used for streets and gardens (Johannsmeier 2016). **Pollinator:** The plant Is insect-pollinated and is visited by bees (Johannsmeier 2016, Rowell 2009). One record of bee visits has been reported from the southwestern Cape (Johannsmeier 2016). The species is part of a list of trees that are where observed to be visited by bees in Kirstenbosch National Botanical Garden in Western Cape (WC) and by a variety of references including South-WC and Pretoria (Johannsmeier 2005 and Henderson 2001). **Bee plant value:** N0 -1, P0 -1 (Johannsmeier 2016)
Species and author-name: *Sclerocarya birrea*, (A. Rich) Hochst (Gouwakinnou et al. 2011) [Figure 6.1 A-D] **Morphological description:** Monade, isopolar, radially symmetric from the polar view and tricolporate. The sculpture is striate with a thick exine, especially at the polar ends. Polar length of 30-35 µm and equatorial diameter of 20-23 µm (own observation), (Gosling et al. 2013). **Growth form:** Medium to large deciduous tree up to 18m tall (Schmidt et al. 2002). **Flowering period:** November to March (Shackleton 2002), September to November (Schmidt et al. 2002) respectively August to November (Johannsmeier 2016). **Ecology:** *Sclerocarya birrea* occurs in savannas of the South African Lowveld regions (Shackleton 2002). The tree has the widest distribution of any tree in the KNP with the greatest concentration on the basalt plains west of the Lebombo range in the south of the KNP (Schmidt et al. 2002). One of

the great trees of Africa, usually preserved by indigenous people for its valuable fruits and shade. The fruit, which is high in vitamin C, is utilised by animals and humans (Schmidt et al. 2002). A jelly preserve and an intoxicating drink are made from the juice. The seeds are used raw, cooked, or ground (Schmidt et al. 2002). Many insects use the tree to breed and feed on. The tree can be propagated from seed or using truncheons but is frost-sensitive (Schmidt et al. 2002).

Pollinator: Entomophilous species that produces sticky pollen and secretes nectar; the honeybee is a major pollinator (Helm et al. 2011, Hall et al., 2002).

Bee plant value: N0-3, P0-2 (Johannsmeier 2016)

Species and author-name: *Lannea* sp., Erdtman 1952 [Figure 6.1 I-J]

Morphological description: Isopolar grain, subprolate tricolporate, 33-35 µm polar axis and c. 23-25 µm equatorial diameter (own observation), striate sculpture (Pradeep 2014), (fine and long parallel striae compared to *Sclerocarya birrea* which has thicker striae in a wider distance from each other (own obervations)), thick exine c. 2 µm (Pradeep 2014).

Growth form: A deciduous tree that grows up to 15m tall (Schmidt et al. 2002)

Flowering period: November to March (Schmidt et al. 2002), July to February (Johannsmeier 2016)

Ecology: Savannas and grasslands of northern KZN through Swaziland, Mozambique, Zimbabwe, westwards through Mpumalanga, northern Gauteng, the Limpopo province, and Botswana (Schmidt et al. 2002).

Pollinator: *Apis mellifera adansonii* visits the flowers and collect the nectar and pollen from the plants (Fohouo et al. 2010). East African *Lannea* species yield nectar heavily during the early morning and in the late afternoon and producing a mild or light amber coloured honey (Johannsmeier 2016).

Bee plant value: N0-3 ?, P0-3 (Johannsmeier 2016)

Asteraceae

Species and author-name: *Gerbera* sp.*,* Southworth 1966 [Figure 6.4 D-E]

Morphological description: Tricolporate pollen grain with a granulate exine, they tend to be prolate to subprolate in shape. The pollen grain has a diameter of c. 45 -50 µm (own observation) and it has granules on the surface (Xu et al. 2018).

Growth form: Herbs (Johnson et al. 2014)

Flowering period: September to January (Johnson et al. 2014), January to December (Johannsmeier 2016)

Ecology: *Gerbera* sp. is an herb of the southern Africa grasslands (Johnson et al. 2014). The plants are distributed on sloping terrain, growing in the full shade of the forest of southern Africa (Johnson et al. 2014).

Pollinator: Pollinated by pollen foraging beetles (Teeri et al. 2006), specifically, the *Eriesthis* beetles and honeybees (Johnson et al. 2004).

Bee plant value: N2, P2 (Johannsmeier 2016)

Species and author-name: *Helianthus annua,* J. Torrey, and A. Gray

Morphological description: Tricolporate, suboblate to spheroidal and circular in polar view. With long colpi that is broad at the equator and narrow towards the ends. Long echinae, pollen diameter c. 30 µm (Palazzesi et al. 2007).

Growth form: Herb, cultivated (sunflower) (Johannsmeier 2016).

Flowering period: November to April (Johannsmeier 2016)

Ecology: Cultivated neophyte from North America, open environment, grassy fields of southern African (Du Toit 2008) that is mostly grown in the country as an ornamental plant that produces yellow flower heads (Johannsmeier 2016). The major production areas in South Africa are North West Province, Free State, Limpopo and Mpumalanga. Furthermore, the sunflower is planted in the eastern areas of southern Africa from early November to the end of December and mid-January it is planted in the western areas of the continent (Department of Agriculture, Forestry and Fisheries 2010).

Pollinator: Entomophilous, honeybee (*Apis mellifera* L), solitary bees and butterflies (Du Toit 2008, DeGrandi-Hoffman and Watkins 2000). Small multi-flowered bush that is rarely without bees, but it produces no nectar when it is at a moisture deficit stress (Johannsmeier 2016). After March the low temperatures of the highveld inhibit *Helianthus annua* nectar secretion but the honeybee colonies maintain their breeding activities (Johannsmeier 2016). Furthermore, the bee factor is 60% for commercial crops of this species and 95% for seed production (Johannsmeier 2016). Interestingly, inbred breeding lines produce relatively little nectar, which compromises pollination especially in the presence of competitive bee foraging (Johannsmeier 2016).

Bee plant value: N2-3, P0-3 (Johannsmeier 2016)

Species and author-name: *Ambrosia artemisiifolia,* Cookson 1947 ex Potonie 1960 [Figure 6.5 D-F]

Morphological description: Tricolporate, spheroidal to subprolate pollen grain 12-15 µm in diameter (Palazzesi et al. 2007), 18-20 µm in diameter (own observation). Long colpi and extending into the polar region. The sculpture is echinate with short spines (Palazzesi et al. 2007, Zavada and de Villiers 2000). The colpi are larger than the pores (Payne and Skvarla 1970, Payne 1963).

Growth form: Herb about 60-150 cm in height (Prasad et al. 2013)

Flowering period: September to December (Schmidt et al. 2002)

Ecology: The Asteraceae family is distributed throughout the Eastern Cape along the eastern coast of South Africa to southern Mozambique and reaches Mpumalanga (Schmidt et al. 2002). *Artemisia artemisiifolia* is a North American neophyte (Kovalev 1989), and it is naturalised in Limpopo to the coast of KZN, through to the disturbed soils of the Highveld (Johannsmeier 2016).

Pollinator: *Apis mellifera* L. (Villanueva-Gutiérrez et al. 2015), Kottner (1991) recorded it as a bee plant in the southwestern Cape: pellets orange-yellow, crude protein 11.6% (Johannsmeier 2016). *Ambrosia* is both wind and insect-pollinated (Friedman. and Barrett 2008.).

Bee plant value: N0, P3? and Kotter (1991) recorded it as bee plant in the southwestern Cape, with orange-yellow, crude protein 11.6% (Johannsmeier 2016).

Caesalpinioideae

Species and author-name: *Peltophorum africanum*, Hutchinson, and Dalziel 1958

[Figure 6.1 M-N, Figure 6.3 K]

Morphological description: Pollen tricolporate, polar diameter is c. 50-52µm (own observation: 40µm) and equatorial diameter is c. 35-37µm long (own observation), subprolate shape with a granulate annulus that is 3-4µm thick, 2-3µm thick, coarsely reticulate sculpture. Tectum is densely granulated and columellate (Orijemie 2018).

Growth form: Small to medium-sized tree (Johannsmeier 2016), 4-9m in height (Schmidt et al. 2002)

Flowering period: September to March (Johannsmeier 2016, Schmidt et al. 2002)

Ecology: In open bushveld at medium to low altitudes, often on sandy soils of KZN, Eswatini, Zimbabwe, Botswana, Namibia (Johannsmeier 2016, Schmidt et al. 2002).

Pollinator: Entomophilous, *Peltophorum africanum* is pollinated by bees (Hymenoptera: Apidae, Halictidae and Megachilidae) and scarab beetles (Coleoptera: Scarabaeidae) (Mawdsley and Sithole 2010).

Bee plant value: N0-2, P1-3 (Johannsmeier 2016)

Celastraceae

Species and author-name: Celastraceae

Morphological description: Radially symmetric, oblate spheroidal tricolporate pollen with long colpi. The pollen is medium size with a diameter ranging from 26µm to 30µm (Gavrilova et al. 2018), equatorial diameter 35-40 µm and polar diameter of 25-30 µm (own observation). The exine is columellate with a reticulate sculpture (Gavrilova et al. 2018).

Growth form: The family is dominated by medium-sized tree usually 5m to 25m (Schmidt et al. 2002)

Flowering period: February to May, for the two types that have been reported in the Limpopo and Mpumalanga province, namely *Cassine peragua* and *Catha edulis* (Schmidt et al. 2002).

Ecology: Widely distributed in the open veld, on rocky outcrops and along rivers in Highveld grasslands and forest margins. The genus spread throughout KNP, the whole of southern Africa including Zimbabwe (Roocroft and Roocroft 2010, Schmidt et al. 2002).

Pollinator: Entomophilous, pollinators are mainly Diptera and some Hymenoptera insects including bees (Konarska 2015). Celastraceae is probably not a good bee plant (N0-1, P0-1) but honeybees have been observed to collect nectar and pollen occasionally (Johannsmeier 2016).

Bee plant value: N0-1, P0-1 (Johannsmeier 2016)

Combretaceae

Species and author: *Combretum* spp. [Figure 6.1 E-H]

Morphological description: Subprolate pollen grain with a length of 20 – 23 µm and 18 – 20 µm width (own observation), trilobate-hexalobate in shape. Pollen grains are 6-heterocolporates. The exine is tectate with a psilate or faintly striate (own observation) sculpture (Schuler and Hemp 2016). There are many similar *Combretum* spp. pollen types in the honey samples which are morphologically very similar and are identified and described here together.

Growth form: Small tree up to 10 m high and it is rarely a shrub (Schemske 1980).

Flowering period: August to February (Johannsmeier 2016), different species flower in different intervals thought the year (Schmidt et al. 2002).

Ecology: Savanna, woodlands and open Mountain Bushveld in Limpopo and Mpumalanga, South Africa (Seibert et al. 2002). Spread across the savanna in KNP (Harley et al. 2003).

Pollinator: Amphiphilous and entomophilous, know pollinators include Insects, hummingbirds, and bats (Bernardello 1994, Ekeke and Agbagwa 2015). Bees forage during the morning (Johannsmeier 2016)

Bee plant value: N0-3, P0-3 (Johannsmeier 2016)

Euphorbiaceae

Species and author-name: *Euphorbia* sp., Kohler 1965 [Figure 6.3 I-J]

Morphological description: Tricolporate, spheroidal shape and reticulate ornamentation, large pollen grain with a thick exine which is 1.5 µm. Polar length is c. 45-50 µm and equatorial diameter of c. 38-40 µm (same as own observation) (Gosling et al. 2013, Saad and El-Ghazaly 1988).

Growth form: Shrub up to 4 m (Schmidt et al. 2002)

Flowering period: October to March, (Schmidt et al. 2002), January to December (Johannsmeier 2016)

Ecology: in the rocky outcrops and sometimes in the bushveld and grassland of the Limpopo province in South Africa (Semenya and Maroyi 2020, Schmidt et al. 2002).

Pollinator: Entomophilous. Insects specifically flies and *Apis mellifera* L. (Villanueva-Gutiérrez et al. 2015).

Bee plant value: N0-2, P0-1 (Johannsmeier 2016)

Fabaceae

Species and author-name: *Medicago sativa* (Alfalfa, Lucerne) [Figure 6.4 J-K]

Morphological description: Tricolporate, isopolar, radially symmetrical pollen that is elliptic in the equatorial view and triangular in polar view (Khan et al. 2020). Polar axis length of c. 21 µm (own observation is 22-24 µm) and equatorial axis length of c. 31 µm (own observation is 35-37 µm) (Khan et al. 2020).

Growth form: Short, bushy deep tap-rooted perennial (Bagavathiannan and Van 2009)

Flowering period: November to March (Hutton-Squire 2014, Johannsmier 2016)

Ecology: *Medicago sativa* has been planted extensively throughout South Africa for hay, grazing, and the production of lucerne seeds (Johannsmeier 2016). *Medicago sativa* has been listed as naturalized and casual alien plant in the Savanna Biome of southern Africa mainly in the EC, WC, Gauteng, FS and Northwest, native to Africa, Asia and Europe (Henderson 2007). A lucerne (*Medicago sativa*) field is located 213.14m west from the hives (Figure 4). This species has adapted to a wide variety of situations, from clayey to sandy soils and cold to hot areas (Crocker and Collett 2003).

Pollinator: *Medicago sativa* rely heavily on being pollinated by honeybees (Johannsmier 2016) and Alfalfa pollen is sticky and easily adheres to most of its insect pollinators (Bagavathiannan and Van 2009, Barnes et al., 1972). Which includes honeybees (*Apis mellifera*), leaf cutter bees (*Megachile rotundata*), alkali bees (*Nomia melander*) and bumble bees (*Bombus* spp.) are all effective pollinators for alfalfa (Bagavathiannan and Van 2009, Rincker et al., 1988). In the WC alfalfa has been recorded to be pollinated by solitary bees and honeybees (Watmough 1999). Additionally, *Medicago sativa* and *Helianthus annuus* are important agricultural crops of the South Africa honeybee industry because of their valuable nectar and pollen sources (Hutton-Squire 2014). For example, in the managed honeybee colonies of the Northern Cape, *Medicago sativa* is the highest ranked crop species foraged by bees (Hutton-Squire 2014).

Bee plant value: N0-3, P0-2 (Johannsmeier 2016)

Species and author-name: *Senegalia* type [Figure 6.2 A-B]

Morphological description: Polyads of 16 grains, the single grains of the polyade are pyramidal with the pointed proximal face. Porate apertures are present but not always visible. Psilate exine (Schuler and Hemp 2016). The polyade diameter is c. 55-60 µm (own observations).

Growth form: Tree (Mills et al. 2021)

Flowering period: August to November (Johannsmeier 2016)

Ecology: Widespread thought the arid bushveld biome of South Africa from the Northern Cape across the country to the Limpopo province and Mozambique (Schmidt et al. 2002) and the open grassland, *Senegalia* savanna within the central KNP predominantly *Senegalia nigrescens* (Mills et al. 2021).

Additionally, the African acacias have recently in 2003, been re-classified under the genera *Senegalia* which is characterised by recurved thorns and the *Vachellia* which is characterised by straight thorns (Johannsmeier 2016, Dyer 2014, and Maslin2003)

Pollinator: Anemophilous and entomophilous (Melusi and Mojeremane, 2012) but also collected by *Apis mellifera* (Villanueva-Gutiérrez et al. 2015, Georginah and Maanda 2015).

Bee plant value: N0-4, P0-4 (Johannsmeier 2016) and the information on the nutritional value of *Senegalia*/*Vachellia* tree pollen is minimal but practical experience on the trees has shown that brood production is stimulated and sustained (Johannsmeier 2016).

Species and author-name: *Vachellia* type [Figure 6.2 C-D]

Morphological description: Polyads of 16 pollen grains, the single grains of the polyade are pyramidal with pointed proximal face. Porate apertures are present but not always visible (Schuler and Hemp 2016). Scabrate exine with furrows (own observation on modern pollen from the ESI collection). Pollen diameter is c. 55-60 µm (own obervations).

Growth form: Tree 5-15m (Schmidt et al. 2002)

Flowering period: November to January (Johannsmeier 2016), October to February (Schmidt et al. 2002)

Ecology: Widespread thought the bushveld biome of South Africa from the Northern Cape across FS, North-West Province, Gauteng, Mpumalanga, KZN to the Limpopo province and Mozambique (Schmidt et al. 2002).

Pollinator: Anemophilous and entomophilous (Melusi and Mojeremane, 2012) but also collected by *Apis mellifera* (Villanueva-Gutiérrez et al. 2015, Georginah and Maanda 2015).

Bee plant value: N0-4, P0-4 (Johannsmeier 2016)

Species and author-name: *Dichrostachys sp.,* Wight, and Arnott 1908, Erdtman 1952 [Figure 6.1 O-P]

Morphological description: Isopolar, radially symmetrical grains. The polar view (amb) of the grain is circular to triangular, the equatorial view is circular or suboblate in shape ranging from c. 28 to 32 µm. Triporate pollen grain with a thin exine that is c. 2 – 3µm thick with an undulating or verrucate sculpture (Gosling et al. 2013, Jumah 1991 and Sorsa 1969).

Growth form: Shrubs or small trees that grow up to 7 meters in height (Cheek 2009).

Flowering period: October to February (Cheek 2009), October to April (Johannsmeier 2016).

Ecology: Distributed along the mountain bushveld and thornveld of the savanna biome of the KNP (Theron et al. 2020 and Rutherford et al. 2006). *Dichrostachys* sp. (*Dichrostachys cinerea*), although an indigenous taxon, is one of the major woody invasive species in southern African savannas (Wakeling and Bond 2007).

Pollinator: Amphiphilous and entomophilous, *Apis mellifera* worker bees and bats pollinate *Dichrostachys* sp. (Fleming et al. 2009 and Fohouo et al. 2008).

Bee plant value: N0-1 ?, P0-3 (Johannsmeier 2016)

Malvaceae

Species and author-name: *Dombeya* sp., Kearney 1951 [Figure 6.2 G-H]

Morphological description: Spherical triporate pollen grain, c. 28 µm- 30 µm in diameter (own observation). Exine has short spines about 0.5-1 µm (Christensen 1986, Scott 1982).

Growth form: Shrub or small trees up to 10m (Schmidt et al. 2002)

Flowering period: Year-round (Machado et al. 2008, Schmidt et al. 2002), March to August (Johannsmeier 2016)

Ecology: *Dombeya autumnalis* is distributed in the Blyde River and Olifants River gorges and Sekhukhuneland while other species are distributed on rocky outcrops, on forest margins, along rivers and in moist woodlands across the KZN, Swaziland, Gauteng, North-West and Limpopo (Schmidt et al. 2002). Additionally, *Dombeya* is spread across four different biomes of the Cape Floristic Region of South African, Baviaanskloof Conservation Area (Fynbos, Grassland, Thicket and Nama Karoo), (Proches and Cowling 2006).

Pollinator: Amphiphilous and entomophilous, bats at night and hummingbirds during the day (Buzato et al. 1994), also pollinated by honeybees (Machado et al. 2008).

Bee plant value: N1-2, P0-2 (Johannsmeier 2016)

Species and author-name: *Hibiscus* sp., Shaheen *et al* 2009 [Figure 6.3 A-B, G-H]

Morphological description: Spherical grain, 61.2- 62 µm in diameter, polypantoporate. Exine is 3-4 µm thick with 7-10 µm long spines that are broad at the base and gradually tapering towards the tips (Shaheen *et al* 2009, Christensen 1986, Scott 1982).

Growth form: Herb, shrub or small trees to 3m (Schmidt et al. 2002)

Flowering period: Year-round but mainly during summer months (Johannsmeier 2016, Machado et al. 2008, Schmidt et al. 2002)

Ecology: *Hisbiscus* sp. is found in open bushveld or on rocky terrains of the bushveld, in valleys and on rocky outcrops throughout the KZN, Swaziland, Gauteng, North-West and Limpopo province (Schmidt et al. 2002). *Hibiscus* sp. also grown in some Lowveld gardens (Johannsmeier 2016).

Additionally, Malvaceae is spread across the Cape Floristic Region of South Africa and the four different biomes of Baviaanskloof Conservation Area (Fynbos, Grassland, Thicket and Nama Karoo) (Proches and Cowling 2006).

Pollinator: Entomophilous, pollinated by honeybees (Machado et al. 2008).

Bee plant value: N2?, EN, P1? (Johannsmeier 2016), one report of honeybees utilising extrafloral nectar (the location of the nectarines was not indicated), (Johannsmeier 2016).

Species and author-name: *Grewia* spp., Erdtman 1952 (El-husseini 2006).

[Figure 6.2 K-L, Figure 6.4 N]

Morphological description: Pollen grains are isopolar, tricolporate, spheroidal to prolate and elliptical in equatorial view. The sculpture is reticulate, muri sharply ridged (tetragonal to pentagonal) with thin regular ridges (El-Husseini 2006). Polar diameter of c. 40µm (similar to own measurements) and equatorial diameter of c. 55µm (El-husseini 2006), 50 µm (own observation).

Growth form: Shrub and small trees up to 5m (Schmidt et al. 2002).

Flowering period: October to May (Johannsmeier 2016), January to June for *Grewia bicolor* (Schmidt et al. 2002) and December to August for the rest of the genus (Schmidt et al. 2002).

Ecology: Common in low bushveld or on the rocky or sandy soil of Limpopo Province, Mozambique, and Zimbabwe. *Grewia bicolor* is dominant in KNP (Schmidt et al. 2002).

Pollinator: Entomophilous, pollinated by honeybees (*Apis mellifera*) and two carpenter bee species (Xylocopa; Apidae), (Zietsman 1991).

Bee plant value: N0-3, P0-3 (Johannsmeier 2016), *Grewia* is the main pollen collected by honeybees in some bushveld localities during December (Johannsmeier 2016).

Myrtaceae

Species and author-name: Myrtaceae sp. (resembles the genus *Eugenia* sp.), Moar 1993

[Figure 6.3 E-F]

Morphological description: Peroblate, syncolporate-tricolporate with an equatorial diameter 15 -20 µm. Apertures are rounded and the exine surface is smooth (Gosling et al. 2013,
Moar and McGlone 2011). Apertures are rounded and the exine surface is smooth (Gosling et al. 2013, Moar and McGlone 2011).

Growth form: Shrubs and trees up to 4m-20m (Schmidt et al. 2002)

Flowering period: Flowering period varies between summer (November to March) and winter (June to August) depending on the flowering species of the Myrtaceae family (Schmidt et al. 2002),
Myrtaceae sp. flowers from July to December (Johannsmeier 2016) and *Eugenia* sp. flowers from January to March (Johannsmeier 2016).

Ecology: *Eugenia* sp. is distributed in east Africa and the east of South Africa towards Mozambique and it is distributed in a wide range of vegetation types like lowland moist forests, bushlands, riverine forest, coastal thickets and bushlands (Verdcourt 1999)

Pollinator: Entomophilous, *Apis mellifera*, social bees and native insects are the main pollinators of *Eucalyptus* (Johannsmeier 2016, Hingston et al. 2004, Moncur et al. 1995).

Bee plant value: N0-3?, P0-3? (Johannsmeier 2016)

Species and author-name: *Morella* sp. (pollen morphologically similar to *Casuarina* (Coetzee 1984)) [Figure 6.5 A-B]

Morphological description: Triangular grain with the average dimensions of 32µm (about 28µm from own observation) (Willard et al. 2004), triporate with rounded pores (Willard et al 2004), psilate surface structure with an exine thickness of 2.4µm (1µm from own Observations) (Willard et al 2004).

Growth form: Large shrub (Johannsmeier 2016), up to 2m (Hyde et al 2021)

Flowering period: August to September (Hyde et al 2021)

Ecology: *Morella* is currently the largest genus that has 50 species described and it is widely distributed in Africa, Europe, North America and Asia (Herbert 2005). All *Morella* species are woody shrubs or tree pioneers in nitrogen-poor soils such as sandy soils or gravelly sites (Gtari 2011). *Morella serrata* is distributed and used in southern Africa for asthma, coughing and shortness of breath (Silva 2015, Schmidt et al 2002). Widespread in tropical Africa to the WC of South Africa (Hyde et al 2021).

Pollinator: *Morella* sp. is wind pollinated, but the male plants produce quite a lot of pollen which attracts bees for pollen and nectar (Notten 2005, Johannsmeier 2005 and Henderson 2001).

Bee plant value: N0-3?, P0-2? (Johannsmeier 2016)

Pinaceae

Species and author-name: *Pinus* sp. [Figure 6.5 C]

Morphological description: Bisaccate monad pollen grain with a think exine, the exine patterns are regulate on the corpus (diameter of 20 - 35µm, own observation) but smooth on the sacci (diameter of 10-25µm from the corpus, own observation) (adapted from Nakagawa et al 2000).

Growth form: Tree that is 25 to 60m tall (Britannica 2020)

Flowering period: June to November (Johannsmeier 2016)

Ecology: The species was introduced into South Africa in the 1920's from Central America, and it is currently predominant moist summer rainfall areas in Mpulalanga and KZN, as well as on a smaller scale in EC province (Nyoka 2003). The species thrives on a wide variety of soils including those derived from dolomite, dolerite, quartzite, granite and sandstone (Nyoka 2003).

Pollinator: *Pinus* species are wind-pollinated (Williams 2009)

Bee plant value: N0, P0-2 and HD0-2, pollen grains are released in masses from the male cones and honey dew is scarcely collected if there are more attractive winter-flowering eucalyptus nearby. Pollen is only collected if a nectar source is available simultaneously this might be due to the low (7-13%) crude protein content of the species (Johannsmeier 2016).

Poaceae

Species and author-name: Poaceae (might be Wild grass based on the size range of the pollen grain) [Figure 6.4 M]

Morphological description: Spherical/oval pollen grains with a diameter that ranges between 25 µm and 40 µm (own observations) and 1-2 µm annulus diameter (Bastl et al. 2020, Joly et al. 2007).

Growth form: Grass that has an erect stem up to 6m tall (Bastl et al. 2020)

Flowering period: December to June (Mashau and Muthanyi 2015, Russell et al. 1990)

Ecology: Poaceae are also distributed in the savanna grassland of the Limpopo province in South Africa (Semenya and Maroyi 2020).

Pollinator: Anemophilous and entomophilous, predominantly wind but also insect pollinated (Kosina and Florek 2011).

Bee plant value: N0, P0-2 (Johannsmeier 2016)

Zygophyllaceae

Species and author-name: *Tribulus sp.,* Erdman 1952

Morphological description: Periporate, oblate – spheroidal c.t 35-38 µm in diameter, coarsely reticulate sculpture with brochi of varying sizes and 2-3 µm sized lumen (Erdman 1952, Agababian 1964, Semerdjieva et al. 2011).

Growth form: Herbaceous plant (Johannsmeier 2016)

Flowering period: October to May (Johannsmeier 2016)

Ecology: Wide distribution in the EC Province, FS, Gauteng, KZN, Limpopo Northern Cape, and WC (Raimondo et al. 2009), as well as the savanna of KNP (van Aardt et al. 2020). *Tribulus sp.* is a weed known to occur in disturbed areas (van Aardt et al. 2020, Van Wyk and Malan 1998).

Pollinator: Entomophilous (*Xylocopa darwinii* and *Apis mellifera*) and self-pollinating (Subbareddi et al. 1981, Scott 1982, Coetzee 1967, Semerdjieva et al. 2011).

Bee plant value: N2, P2 (Johannsmeier 2016), the species nutritious pollen and stimulative nectar promote brood production and they are usually small honey crops (Johannsmeier 2016).

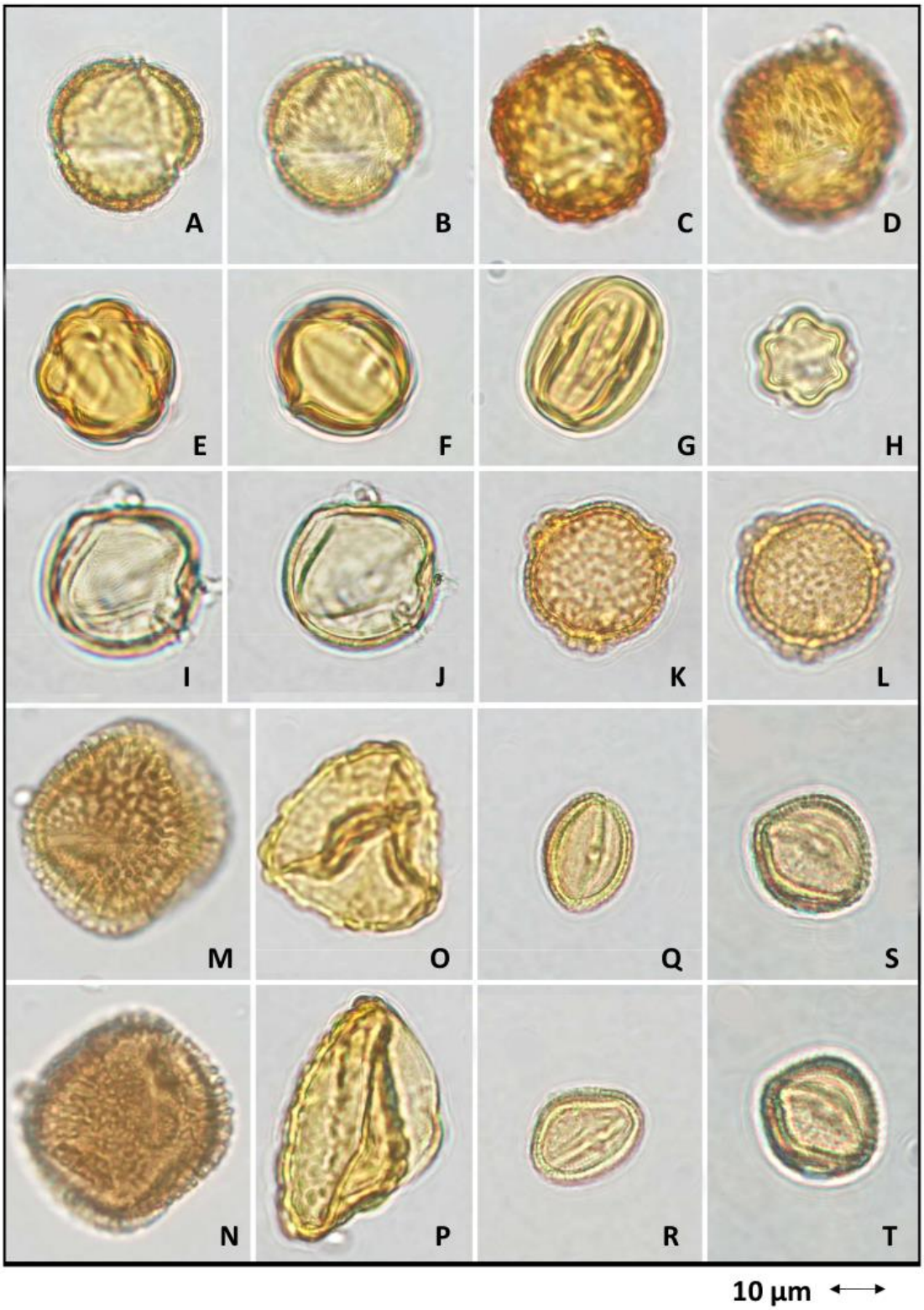

Figure 6.1 Photo plate 1.A-D: *Sclerocarya birrea*, E-H: Combretaceae, I-J: *Lannea* sp., K-L: *Commiphora* spp., M-N: *Peltophorum africanum*, O-P: *Dichrostachys* sp. and Q-T: *Harpephyllum caffrum*, Scale bar = 10µm, 1000x magnification.

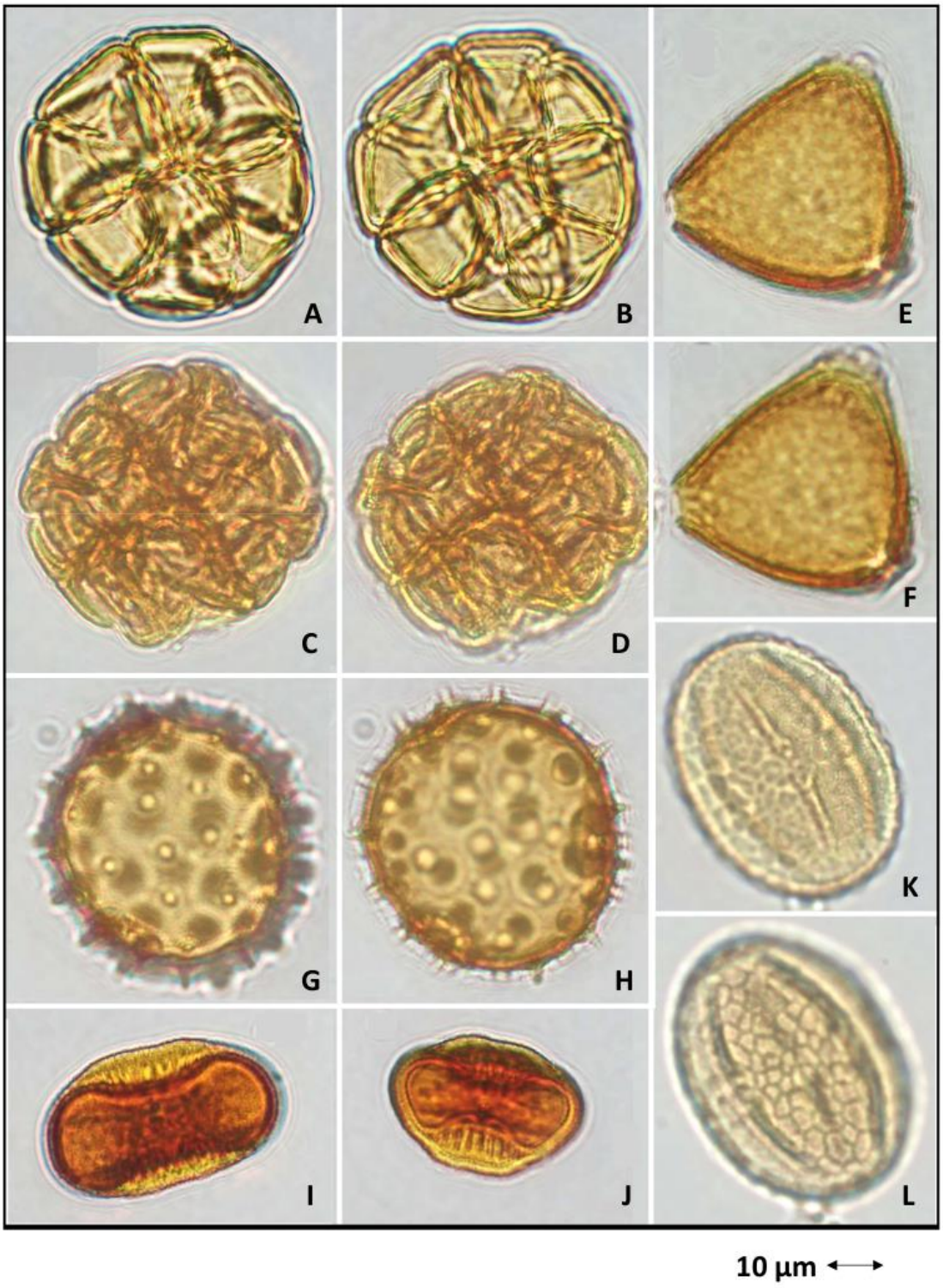

Figure 6.2 Photo plate 2. A-B: *Senegalia* type, C-D: *Vachellia* type, E-F: *Protea* sp., G-H: *Dombeya*, I-J: *Justicia flava*, K-L: *Grewia*, Scale bar = 10µm, 1000x magnification.

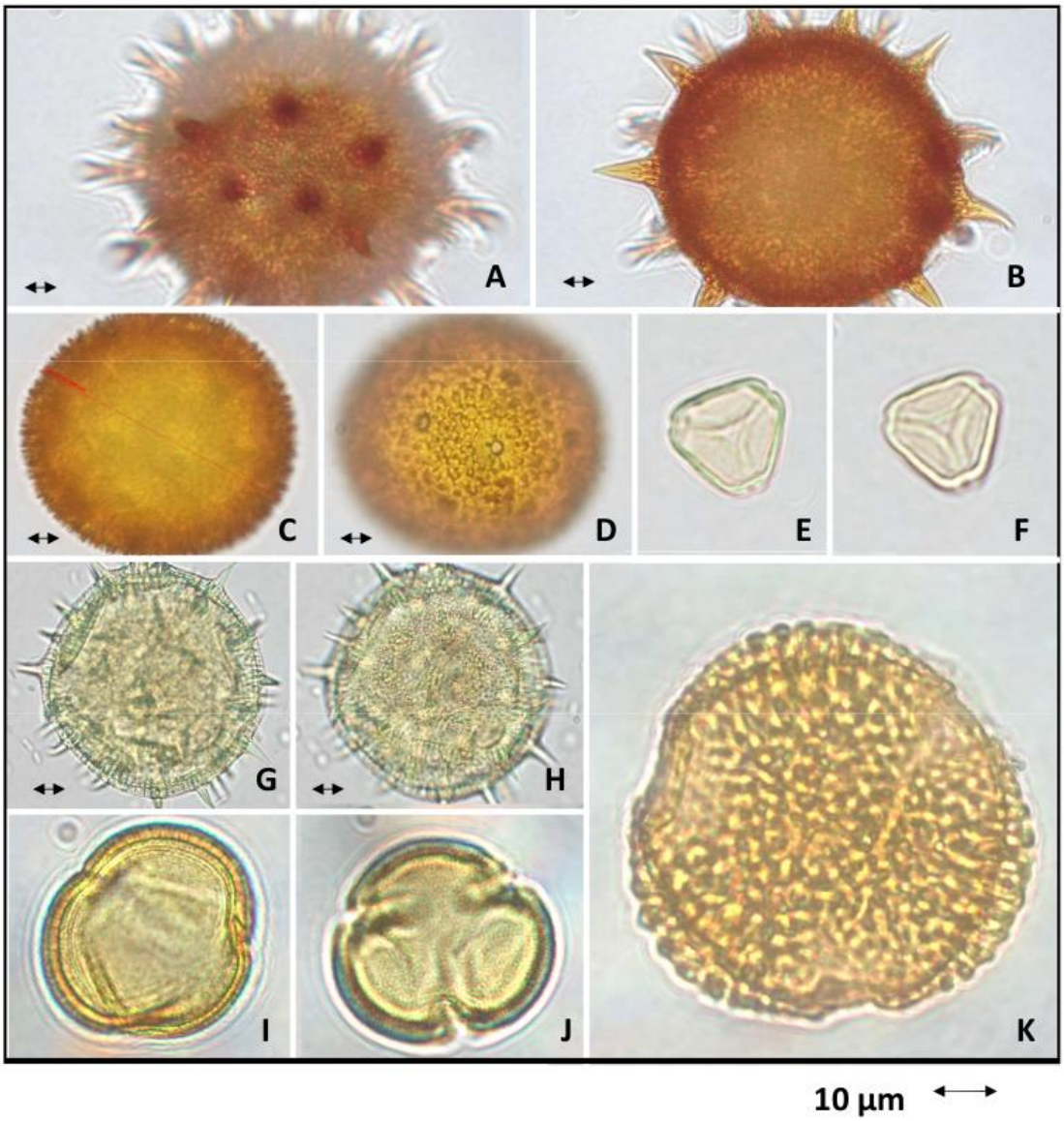

Figure 6.3 Photo plate 3. A-B: *Hibiscus* sp., C-D: *Phaeoptilum spinosum*, E-F: Myrtaceae (*Eugenia* sp.), G-H: Malvaceae (*Hibiscus* sp.), I-J: *Euphorbia* sp., K: *Peltophorum africanum*, Scale bar = 10µm, 1000x magnification

Figure 6.4 Photo plate 4. A-B: Tubuliflorae, C: *Alternanthera* sp., D-E: *Gerbera* sp., F: *Justica decurrens*, G-H: *Plectranthus*, I: *Salix*, J-K: *Medicago sativa* , L: Amaranthaceae, M: Poaceae, and N: *Grewia* sp.. Scale bar = 10µm, 1000x magnification

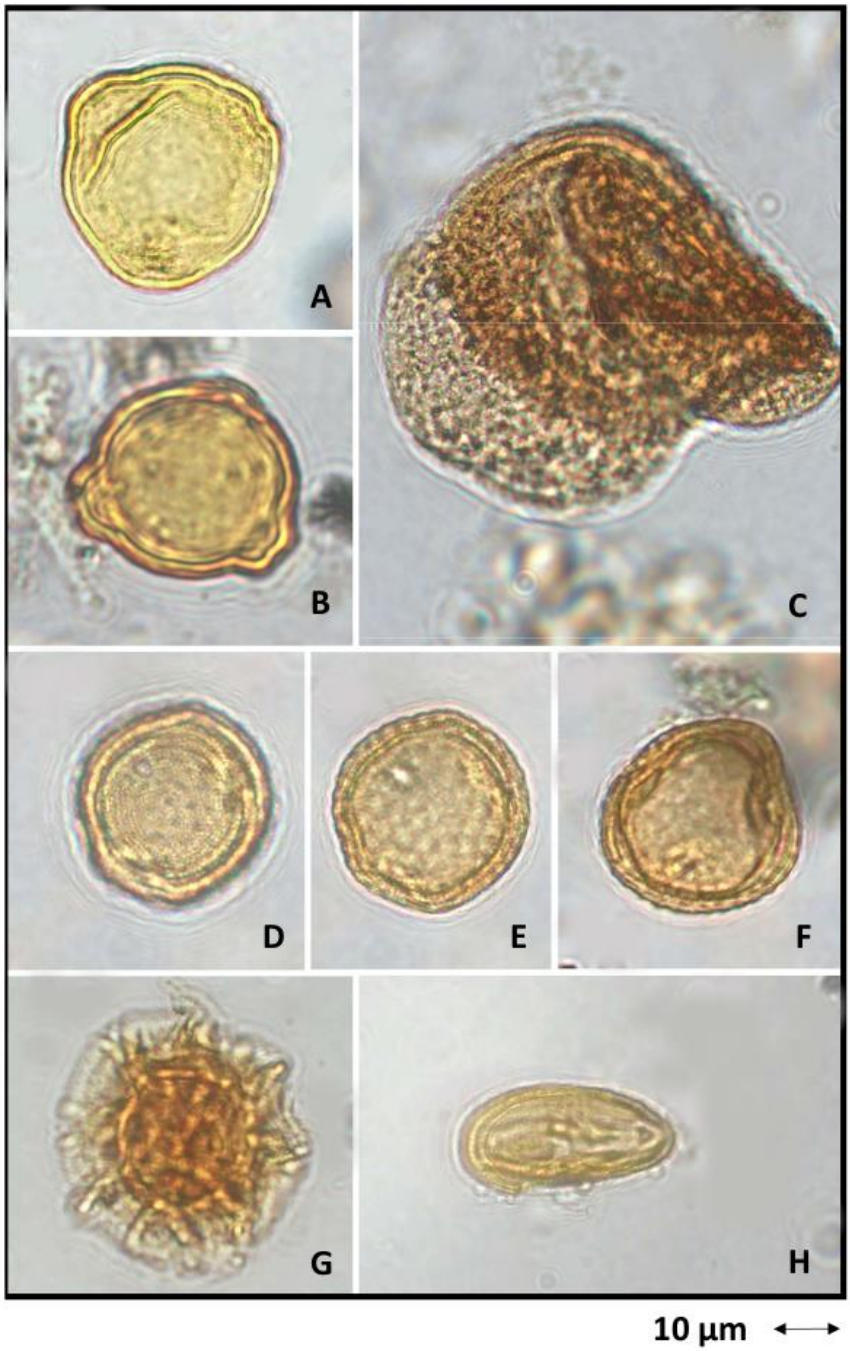

Figure 6.5 Photo plate 5. Sediment pollen photo plate, A-B: *Morella* type, C: *Pinus* sp., D-F: *Ambrosia artemisiifolia*, G: *Vernonieae* type and H: *Heteromorpha* sp., Scale bar = 10µm, 1000x magnification.

4.1.1 Honey Pollen Percentage Composition

The December, January, March, July and October honey samples have several similar pollen taxa like Combretaceae, which is highly abundant in all samples (56%), (Figure 7.1). Along with the dominance of three species from the Anacadiaeceae family (*Sclerocarya birrea* (14%), *Harpephyllum caffrum* (3%) and *Lannea* sp. (1,5%)), (Table 3 and 4, Figure 6.1). All harvest months have other several taxa in common that are not necessarily dominant in the samples like *Senegalia* type (0.8%), *Vachellia* type (0.7%), *Euphorbia* sp. (1.1%) and Poaceae (3%) (Table 3 and 4). The summer season (December and January harvests) has the least number of pollen types all year with eight different pollen taxa (Table 3, Figure 7.2 A), whereas the winter season (July) has the highest number of pollen types compared to the other seasons with 25 different pollen taxa (Table 3 Figure 6.2 C). Generally, the pollen spectra and predominant pollen taxa vary over different seasons (Table 3, Figure 7.2).

Based on the percentages of each pollen type in the sample, pollen types were classified into four categories. Honey pollen category A: Predominant pollen types comprised >45% of a sample. Category B: Secondary pollen types comprised 16–45% of a sample. Category C: important minor pollen types comprised 3–15% of a sample. Category D: Minor pollen types comprised <3% of a sample (Baum et al. 2011, Louveaux et al. 1970). Combretaceae (56%) is the only member of Category A, according to the percentage composition of the pollen all year. Category B represents *Sclerocarya birrea* (14%) as a secondary pollen type. Category C comprises Poaceae (3%), *Harpephyllum caffrum* (3%) and *Lannea* sp. (1.5%) and Category D includes the rest of the taxa (Table 3, Figure 7.1).

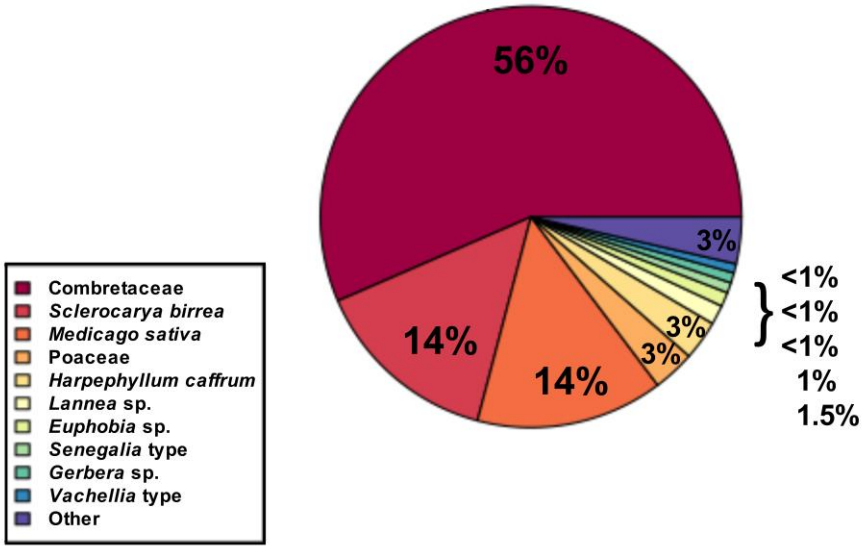

Figure 7.1 Pie chart plot displaying the pollen taxa percentage composition present in all honey samples throughout a year (2019-2020).

December harvest 2019

December 2019, honey harvest from the H1 hive (Table 2) has taxa such as Combretaceae (97%), *Sclerocarya birrea* (1.3%) and *Senegalia* type (1%) (Table 3). The December harvest has the lowest number of taxa, making it the least diverse harvest with only four taxa, including the ones mentioned and *Vachellia* type (0.3%).

January harvest 2020

The January 2020 samples from the H1 hive (Table 1 in the methods section) had 72% of *Medicago sativa*, *Sclerocarya birrea* (13%), Combretaceae (8%) as the most dominant type and *Medicago sativa* (71.88%) characterise this sample. The January harvest is the second-lowest sample regarding diversity with only eight pollen taxa, including Celastraceae (1.6%), *Vachellia* type (2.6%), *Salix* (1%), *Senegalia* type (0.6%) and *Harpephyllum caffrum* (0.6%), (Table 3 Figure 7.2 A).

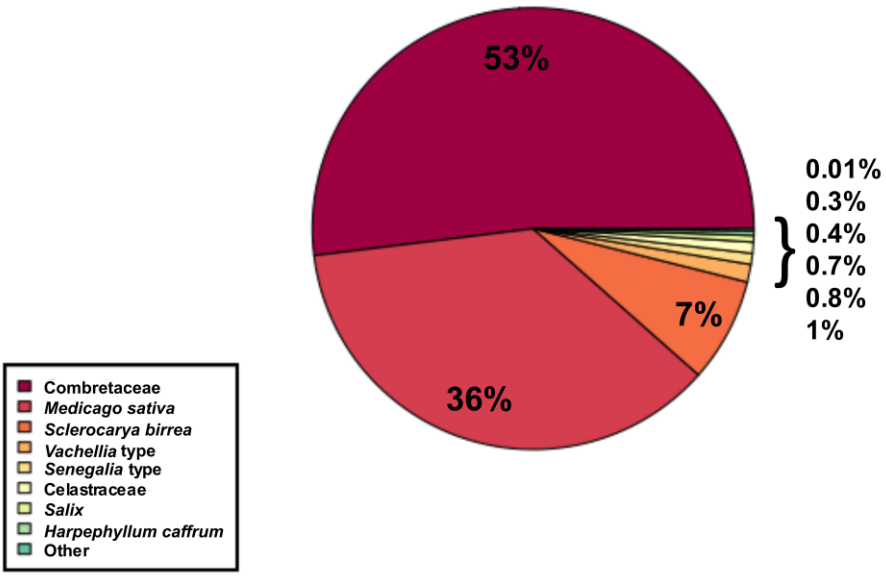

Figure 7.2 A. Pie chart plot displaying the pollen taxa percentage composition present in the Summer (January and December) honey samples, Other: *Tribulus* sp.

March harvest 2020

The March honey samples show a good representation of savanna taxa, with an abundance of Combretaceae (64%), Poaceae (15%), *Harpephyllum caffrum* (5.7%), *Sclerocarya birrea* (5.4%) and *Gerbera* sp. (3.9%). *Podocarpus* and *Myriophyllum* sp. were the only taxa distinct to this sample but occurred in small quantities (Table 3). Poaceae and *Gerbera* sp. dominate the March harvest in comparison to the other harvests which have < 0.6% Poaceae (July and October harvest) and <0.02 *Gerbera* sp. (only in the July harvest), (Table 3, Figure 7.2 B).

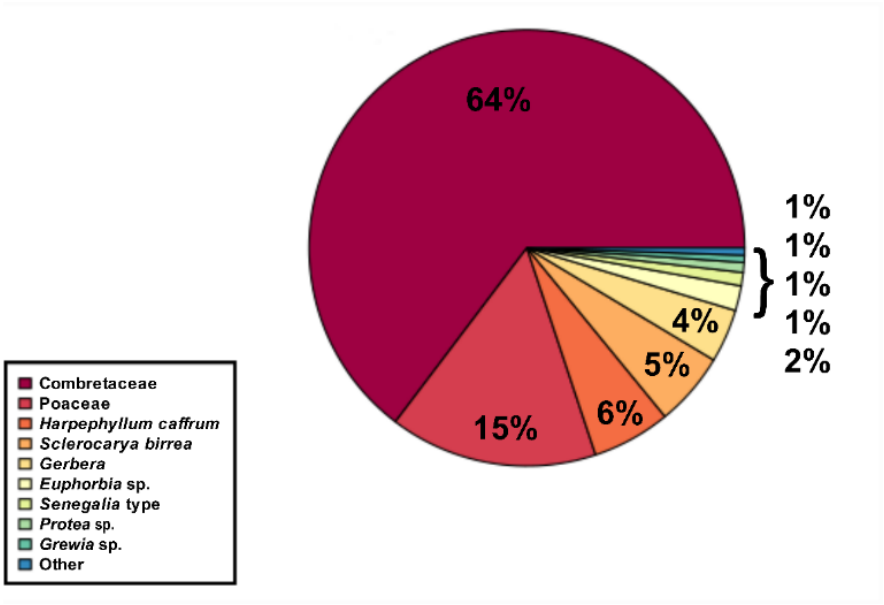

Figure 7.2 B. Pie chart plot displaying the pollen taxa percentage composition present in the Autumn (March) honey samples, Other: *Vachellia* type, *Protea/Faurea* sp., *Helianthus* sp., *Podocarpus/Afrocarpus*, *Hibiscus* sp., *Plectranthus*, *Myriophyllum* and Cyperaceae.

July harvest 2020

The July honey samples are dominated by pollen taxa like Combretaceae (67.4%), *Sclerocarya birrea* (11.6%), *Lannea* sp. (7.1%), *Alternanthera* sp. (1.8%) and *Harpephyllum caffrum* (1.8%) (Table 3 Figure 7.2 C). All mentioned pollen taxa are insect-pollinated (Table 3). The July harvest is distinctly dominated by the *Lannea* sp. (Anacardiaceae) which is present in the other samples but in fewer quantities. The July harvest has the higest number of pollen types in comparison to all the other harvests (Table 3) since it is the only harvest that has taxa like *Alternanthera* sp., *Tribulus* sp., Tubuliflorae, and *Phaeoptilum spinosum* (Table 3).

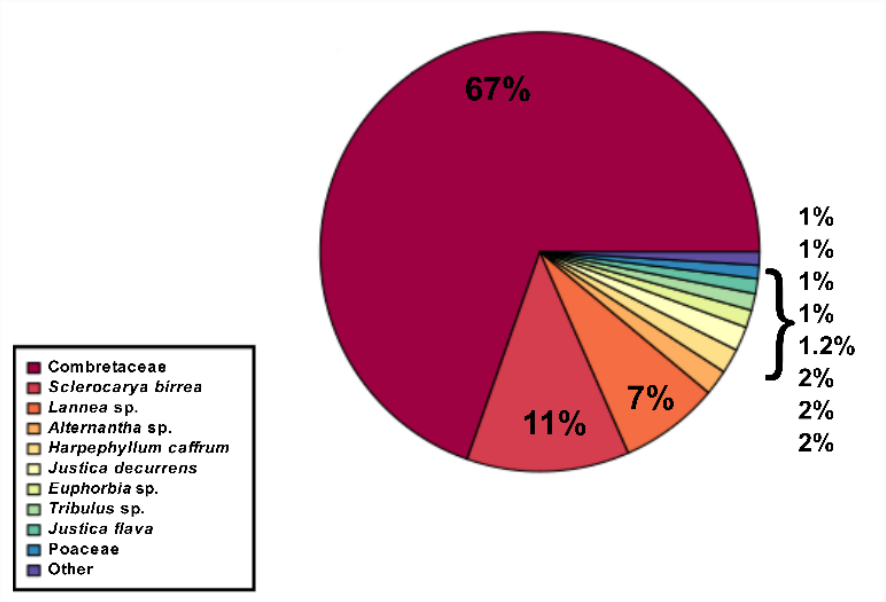

Figure 7.2 C. Pie chart plot displaying the pollen taxa percentage composition present in the Winter (July) honey samples, Other: *Vachellia* type, Poaceae, *Protea* sp., *Helianthus sp.*, *Ambrosia*, *Tubuliflorae, Myriophyllum*, *Gerbera* sp., *Dichrostachys* sp., Celastraceae, *Peltophorum africanum*, *Grewia* sp. and *Peltophorum africanum*.

October harvest 2020

The October harvest is dominated by Combretaceae (46.7%), *Sclerocarya birrea* (39%) and *Harpephyllum caffrum* (7%) (Table 1). *Triumfetta* sp. (0.04%) and *Commiphora* (0.4 %) are only found in the October harvest (Table 3). This harvest is the second least diverse harvest of all investigated seasons (first being the summer harvest), with only 12 different taxa (Table 3 Figure 7.2 D).

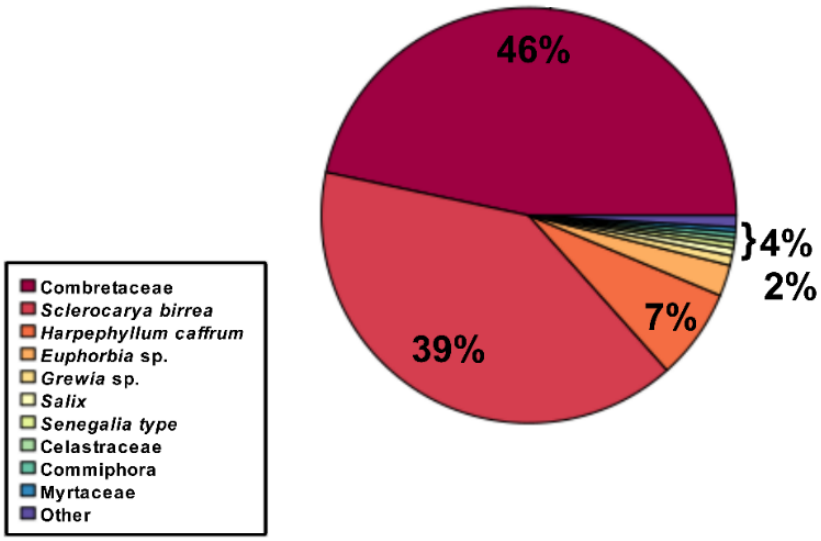

Figure 7.2 D. Pie chart plot displaying the pollen taxa percentage composition present the Spring (October), Other: *Vachellia* type, Poaceae, *Triumfetta* sp., *Lannea* sp. and *Plectranthus.*

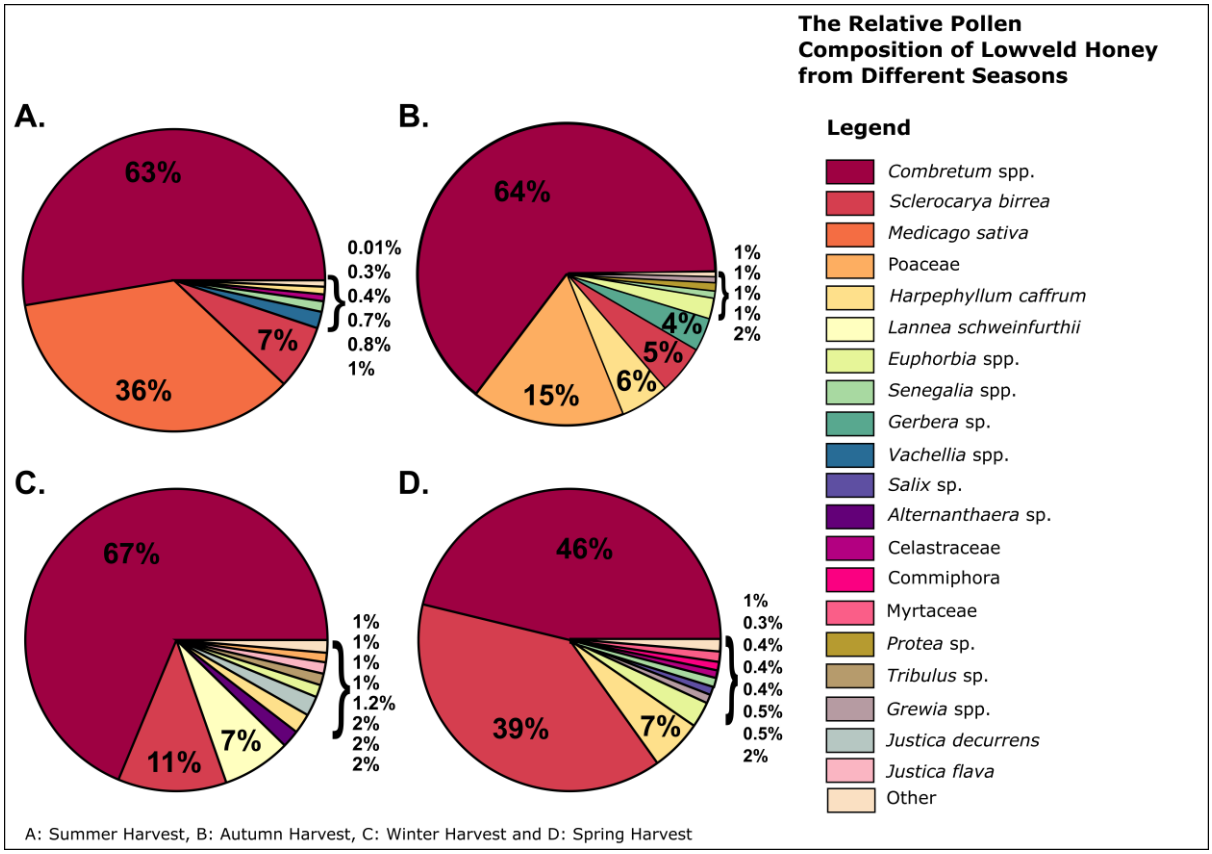

Figure 7.3. A summary display of all pie charts displaying the pollen taxa percentage composition present in four different seasons including the A: Summer (January and December) honey samples, Other represents: Tribulus sp. B: Autumn (March) honey samples, Other represents: *Vachellia* spp., *Helianthus annua*, *Podocarpus*/*Afrocarpus*, *Hibiscus* sp., *Plectranthus*, *Myriophyllum* sp. and Cyperaceae. C: Winter (July) honey samples, Other represents: *Vachellia* spp., Poaceae, *Protea*/*Faurea* sp., *Helianthus annua*, *Ambrosia artemisiifolia*, Tubuliflorae, *Myriophyllum* sp. *Gerbera* sp., *Dichrostachys* sp., Celastraceae, *Peltophorum africanum*, *Grewia* spp. and *Peltophorum africanum*. D: Spring (October), Other: *Vachellia* spp., Poaceae, *Triumfetta* sp., *Lannea* sp. and *Plectranthus* sp.

4.1.2 Honey Temporal Comparison (Non-Metric Multidimensional Scaling)

Seasonality correspondence to pollen diversity and composition is shown by the results of the Non-metric multidimensional scaling (NMDS) ordination method (Figure 8). The NMDS showed that a three-dimensional solution was sufficient to achieve low-stress values to enable the interpretation of seasonality (seasons as sample groups) on the pollen content in the honey (stress = 0.0875). The dispersion of the sample groups (seasonality) was significantly correlated with the NMDS analysis of pollen composition found in different harvests throughout different seasons. The stress value of 0.09 indicates a satisfying significance of the assumed heterogeneity (variation) of the different seasonal harvests (Figure 8).

The NMDS displays the variables (pollen species) and the different groups of the hives. These results are placed on the plot according to the Bray-Curtis distance matrix. The closer the species plot on the dimensional space to sample/season the more abundant the species is within that sample. Combretaceae, *Euphobia* sp., *Pletophorum africanum, Senegalia* type, *Vachellia* type, *Harpephyllum caffrum, Grewia* sp., *Plectranthus* and *Commiphora* sp. clustered within the March, July, and October samples (coloured polygons). Poaceae, Cyperaceae, *Gerbera* sp., Tubuliflorae and Myrtaceae are clustered at far edges of the autumn (March) samples (Figure 8).

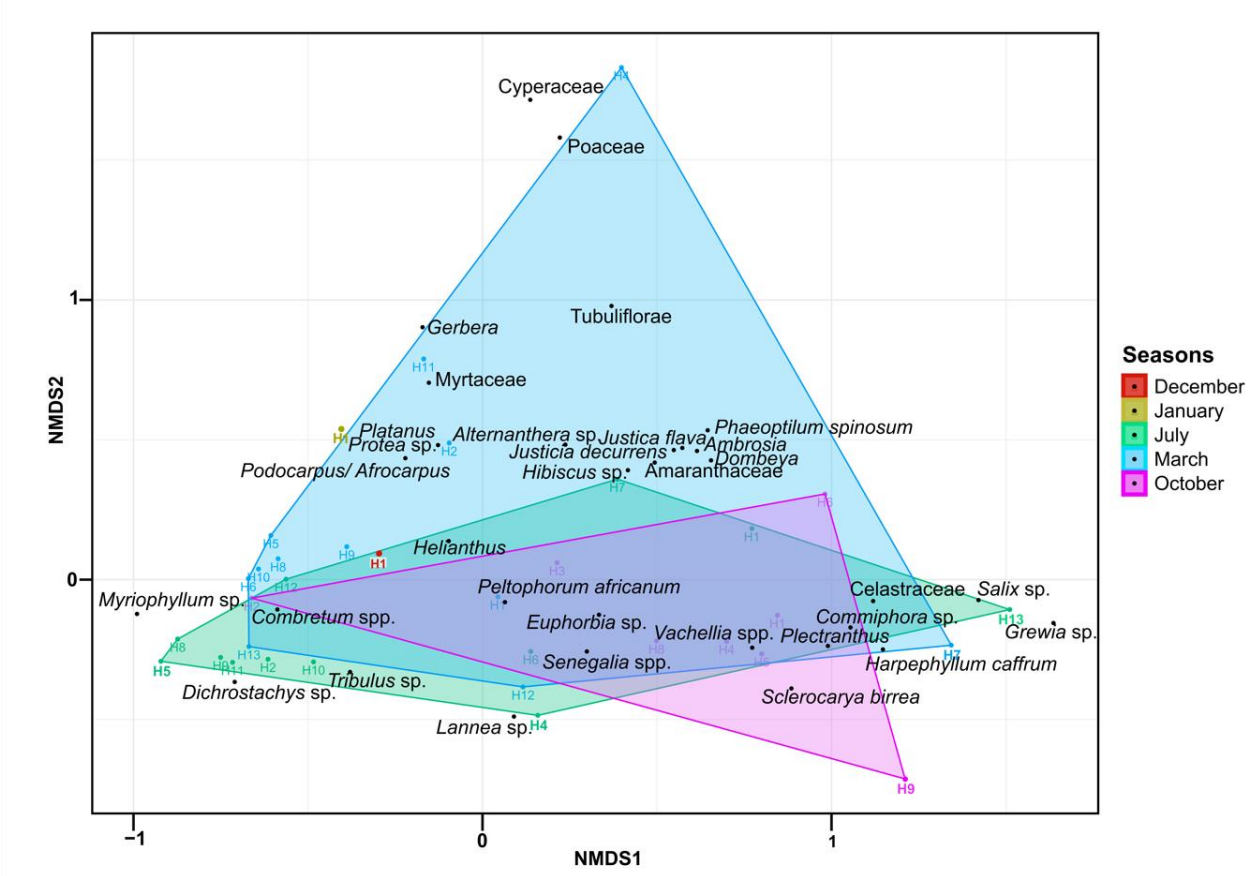

Figure 8. Non-metric multidimensional scaling (NMDS) plot displaying the Bray-Curtis distance between five honey seasonal samples collected all year from different hives of the APNR location within the KNP. The coloured dots represent the different hives corresponding to their seasons and the black dots represent different pollen taxa. The coloured polygon shapes group all the hives according to seasonality. Seasonality is displayed as December and January (Summer), July (Winter), March (Autumn) and October (Spring).

4.2 Surface Sediment Sample Results

The surface sediment samples where collected for the autumn, winter and spring season (total of 36 samples). The spring sureface sediment samples (12 samples) are the only seasonal collection that yielded statifactory pollen counts >50 per sample site. In contrast, the other seasons yielded < 15 pollen counts for the entire season. These counts where dominated by *Pinus* sp. and the results where not published since the pollen counts are not statistically robust.

Moreover, the pollen found in the spring (October) sediment samples is composed of thirty different pollen taxa from seventeen families, with three types unidentified and labelled as Px (e.g. Px20)(Table 5 and Figure 9). The most abundant pollen types are Poaceae (22.62%), Combretaceae (20.44) and *Seclerocarya birrea* (17.78). *Morella* sp. (1.05) and *Heteromorpha* sp. (1.71) are the only pollen species distinct to the sediment samples (Table 5 and Figure 9).

The pollen concentration values were clacuated for the sediment samples as Equation 1: Pcon = 12000 x 97 /38x10g = 3068, Pcon per 10g of sediment = 30 632.

Table 5. Percentage list of all the pollen taxa found in the October sediment samples.

Pollen Taxon		**October sediment samples (%)**
Acanthaceae	*Justica flava*	0.48
	Justicia decurrens	0.86
Anacardiaceae	*Harpephyllum caffrum*	0.57
	Sclerocarya birrea	17.78
Amaranthaceae	*Alternanthera* sp.	1.14
	Amaranthaceae	5.04
Apiaceae	*Heteromorpha* sp.	1.71
Asteraceae	*Gerbera* sp.	0.29
	Ambrosia	1.81
	Tubuliflorae	0.10
	Vernonieae type	0.01
Celastreaceae	Celastreaceae	0.57

Combretaceae	*Combretum* spp.	20.44
Cyperaceae	Cyperaceae sp.	2.09
Euphorbiaceae	*Euphorbia* sp.	1.05
Fabaceae	*Senegalia* type	1.62
	(Mimosoideae), *Dichrostachys* sp.	1.61
	Medicago sativa	0.67
Lamiaceae	*Plectranthus*	0.95
Malvaceae	*Dombeya* sp.	0.38
	Hibiscus sp.	0.09
	Grewia spp.	1.53
Myrtaceae	*Morella* sp.	1.05
Nyctaginaceae	*Phaeoptilum spinosum*	0.38
Pinaceae	*Pinus* sp.	11.12
Poaceae	*Phragmites* -type	22.62
Proteaceae	*Protea* sp.	0.09

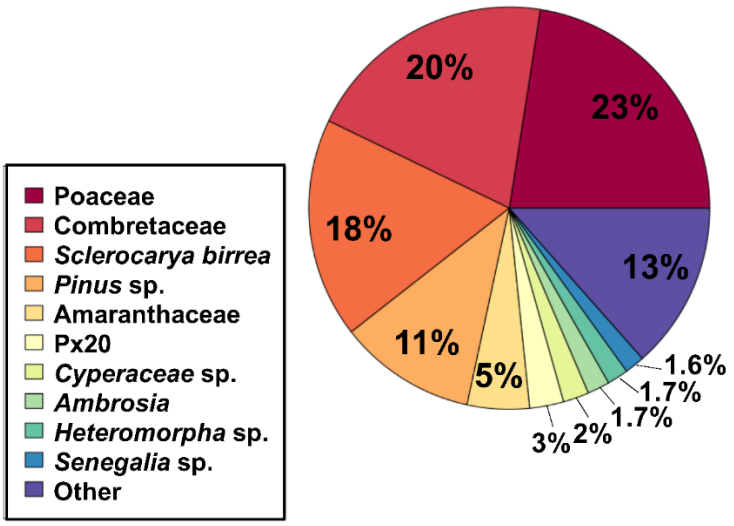

Figure 9. Pie chart plot displaying the pollen taxa percentage composition present in the October sediment samples, Other represents Protea sp., *Grewia* sp., *Harpephyllum caffrum*, *Euphorbia* sp., *Gerbera* sp., *Justica flava*, *Justicia decurrens*, *Dicrostachys* sp., *Plectranthus*, Celastraceae, *Phaeoptilum spinosum*, Podocarpus/Afrocarpus, Hibiscus sp., *Alternanthera* sp., Plectranthus, and *Morella* sp. Px20 represents an unidentified pollen type.

4.3 Pollen Species Richness and Diversity

The variation between the hives and the honey seasons was observed by evaluating the diversity of the pollen taxa for each sample. This was done using the Shannon Wiener diversity index technique (Figure 10). The pollen diversity of different hives was represented by a histogram throughout different seasons of the year (Figure 10). The Shannon Wiener diversity indices show that even though the hives are within a 120m range from each other, the pollen diversity found in their honey is still highly variable throughout different hives as shown by the figure 10 histogram with the Shannon Wiener diversity indices compared (Figure 10).

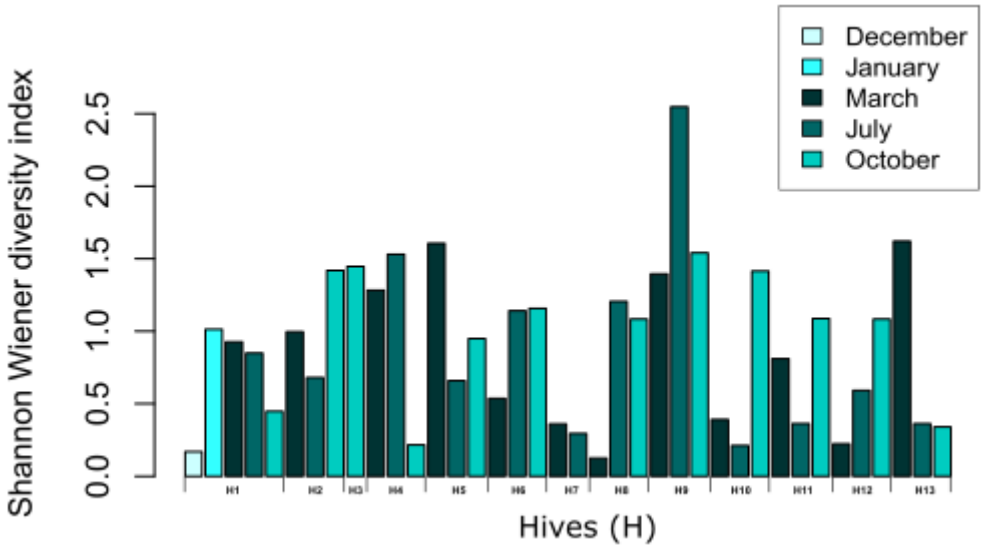

Figure 10. Shannon Wiener diversity indices are displayed on a histogram for all hives from different harvest seasons (December 2020 to October 2021).

The varying differences between months were observed from the taxa percentage table, the pie charts above and NMDS plot (Table 3, Figure 7.3 and 8). They reveal that the summer months (December and January harvest) have the lowest number of pollen types compared to other seasons throughout the year (Table 3, Figure 11 and 12). Specifically, the December harvest has the lowest number of pollen taxa of all samples, versus the July (Winter) harvest which has the highest number of pollen types. The July harvest is followed by the March (Autumn) and October (Spring) harvests in terms of having the second highest number of pollen types (Table 3 Figure 6.2 C). To compare the most diverse (number of pollen types) honey sample (July) and the least diverse sample (December and January), rarefaction curve (95% Confidence Intervals (CI)) graphs were generated to display the species diversity (according to Shannon's diversity indices) and species richness of all the seasons (Figure 11

and 12). These metrics were calculated using the iNEXT package in R. The Chao1 estimator was used to extrapolate species richness while the Chao-Jost estimator was used to extrapolate the samples' diversity (Hsieh et al., 2016).

According to the rarefaction curves, the summer season has the lowest species richness (January were 8.00 and December was 4.00) and Shannon's diversity (January was 1.06 and December was 1.84) compared to the autumn, winter, and spring seasons respectively (Figure 11, 12, 13 and 14). The winter season has the highest species richness (5.00 and 23.00) and diversity (varied between 2.09 and 4.17) (Figure 13). The other seasons, e.g., autumn (varies between 2.00 and 14.00) and spring (varies between 2.00 and 10.00), have a higher species richness than most summer samples and lower species richness than most winter samples (Figure 12 and 14). Furthermore, the species diversity of both autumn (varies between 1.08 and 3.60) and spring (varies between 1.16 and 3.99) is higher than most summer samples and lower than most winter samples (Figure 12 and 14).

Additionally, the sediment samples species richness varies between 13.00 and 17.00, while the species diversity varies between 7.48 and 7.73 (Figure 16). The richness and diversity rarefaction curve for the sediment samples show that the range between the samples with the highest and lowest variances is much smaller compared to the honey samples (Figure 16).

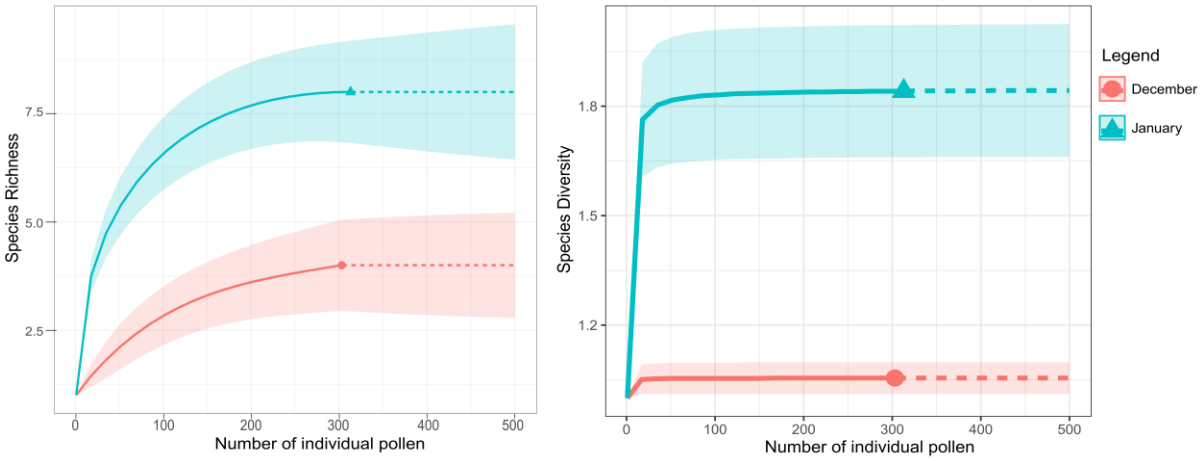

Figure 11: Rarefaction curves (95% CI) displaying differences in richness and diversity between two honey samples collected in summer (Summer samples include December and January). The left figure (q = 0) is comparing the season in terms of the Species Richness index and the right figure (q = 1) is comparing the season in terms of the Shannon diversity index.

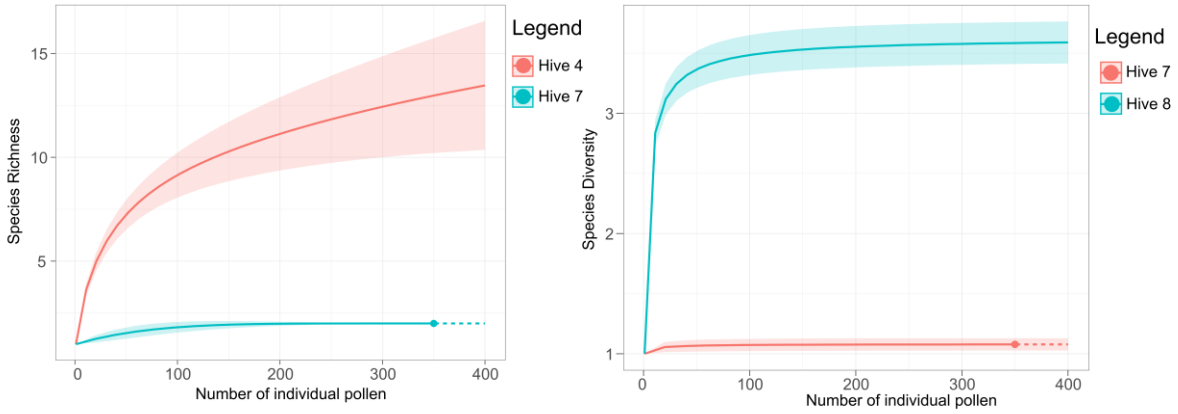

Figure 12: Rarefaction curves (95% CI) displaying differences in richness and diversity between two honey samples (hive 4 (highest species richness), hive 7 (lowest species richness and diversity) and hive 8 (highest diversity) collected in autumn (March samples). The left figure (q = 0) shows a comparison of the season in terms of the Species Richness index and the right figure (q = 1) is comparing the season in terms of the Shannon diversity index. Extensive graphs with rest of the hives are depicted in Supplementary Figure 1 (S. Figure 1).

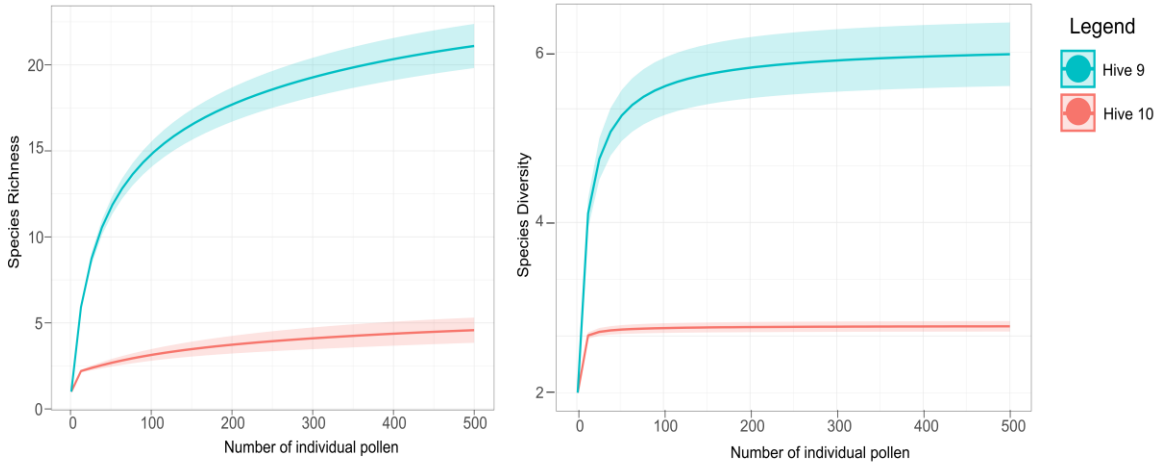

Figure 13: Rarefaction curves (95% CI) displaying differences in richness and diversity between two honey samples, hive 9 (with the lowest species richness and diversity) and hive 10 (highest species richness and diversity) collected in winter (July samples). The left figure (q = 0) is comparing the season in terms of the Species Richness index and the right figure (q = 1) is comparing the season in terms of the Shannon diversity index. Extensive graphs with rest of the hives are in Supplementary Figure 2 (S. Figure 2).

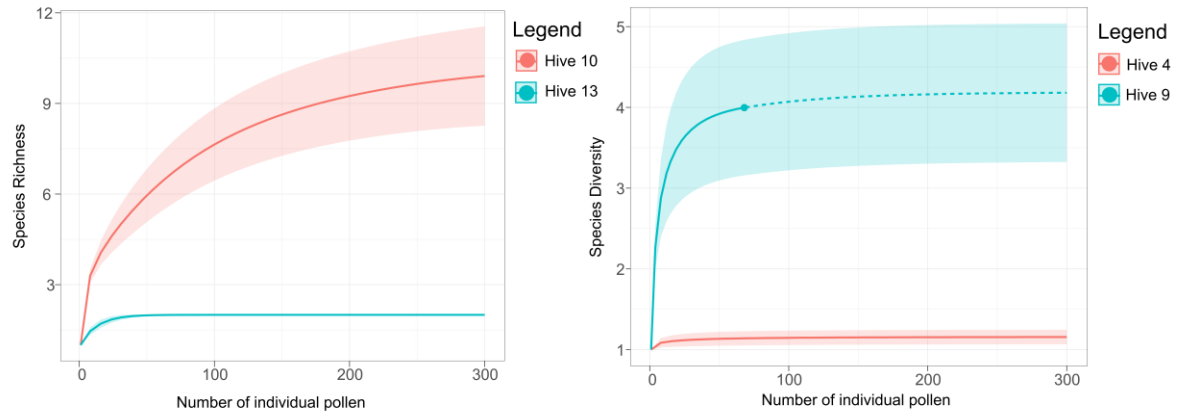

Figure 14: Rarefaction curves (95% CI) displaying differences in richness and diversity between two honey samples (hive 10 and hive 13 with the highest and lowest species richness respectively, hive 9 and hive 4 with the highest and lowest species diversity respectively) collected in spring (October samples). The left figure (q = 0) is comparing the season in terms of the Species Richness index and the right figure (q = 1) is comparing the season in terms of the Shannon diversity index. Extensive graphs with rest of the hives are in Supplementary Figure 3 (S. Figure 3).

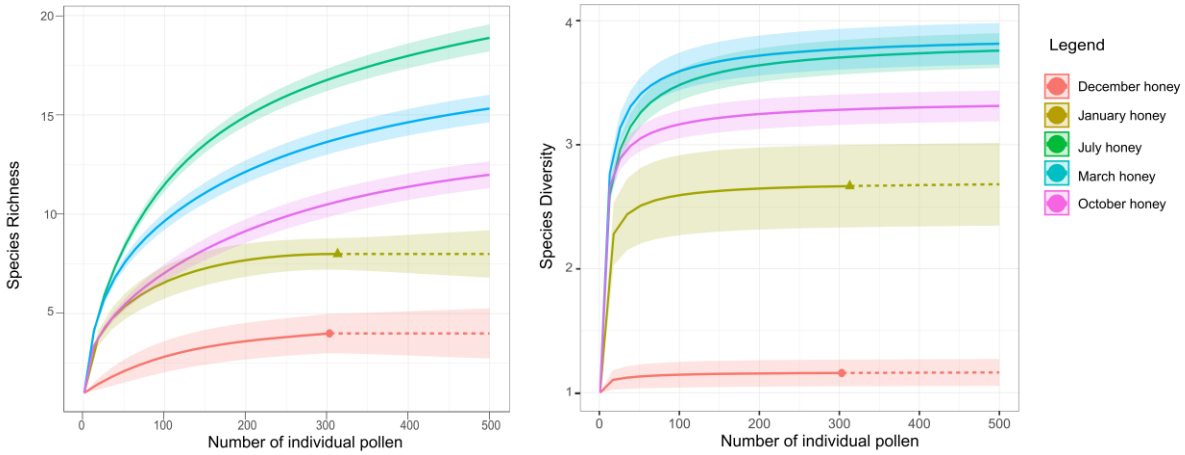

Figure 15: Rarefaction curves (95% CI) summary display of differences the species richness and diversity between all honey seasons (hives averaged for each season) collected throughout the year. Left figure (q = 0) is comparing the sites in terms of the Species Richness index, right figure (q = 1) is comparing the sites in terms of the Shannon diversity index.

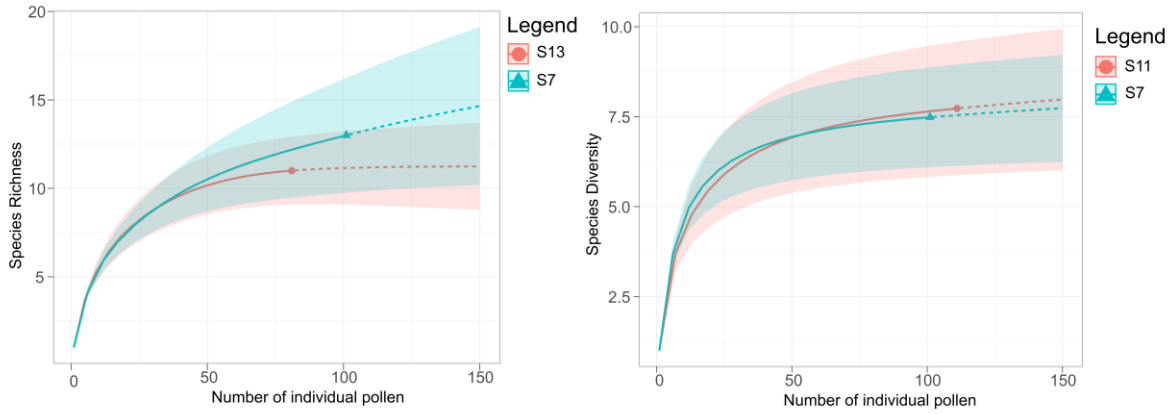

Figure 16: Rarefaction curves (95% CI) displaying differences in richness and diversity between two sediment samples, S7 (has the highest species richness and lowest diversity), hive 11 (has the highest species diversity) and hive 13 (has the lowest species richness) collected in spring (October samples). The left figure (q = 0) is comparing the sites in terms of the Species Richness index and the right figure (q = 1) is comparing the sites in terms of the Shannon diversity index. Extensive graphs with rest of the hives are in Supplementary Figure 4 (S. Figure 4).

The species richness and diversity of the sediment samples was compared to the averaged data from all the seasons since the different seasons were already plotted on an NMDS plot to display the variability between the seasons (Figure 8). The rarefaction curves depict the differences in species richness and diversity of the October sediments compared to the other seasons (Figure 17). The Shannon diversity indices for the October sediment samples are 7.00, while for the honey samples it ranged between 1.05 (December) and 2.61 (October) (Figure 17) which means that the diversity is higher in sediment sample compared to individual honey seasonal samples. The species richness index for the October sediment samples is 29.00, while the honey samples indices are between 4.00 (December) to 26.00 (July), (Figure 17). Both richness and diversity of the sediment samples are relatively high compared to all honey samples (Figure 17).

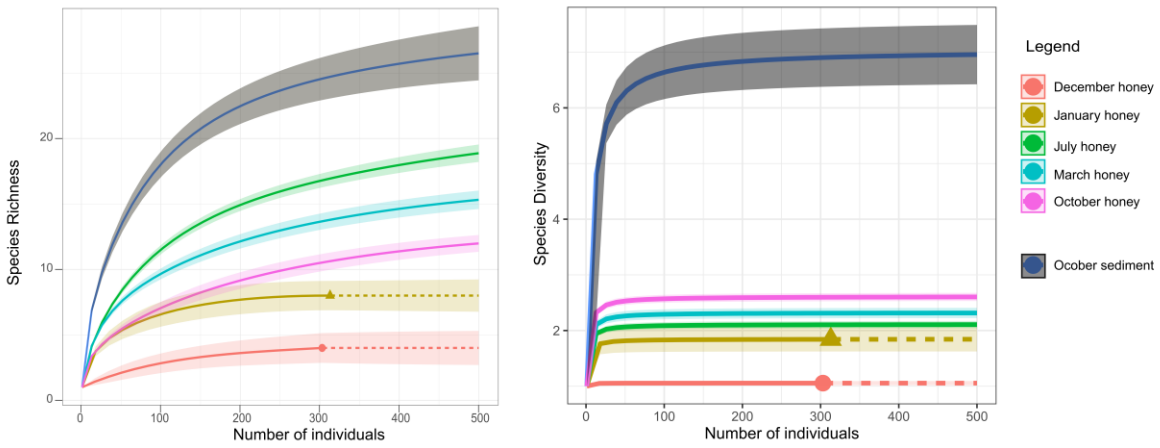

Figure 17: Rarefaction curves (95% CI) displaying differences in richness and diversity between all honey seasons (hives averaged for each season) collected thought out the year and one sediment sample collected in autumn. The right figure (q = 0) is comparing the sites in terms of the Species Richness index and the right figure (q = 1) is comparing the sites in terms of the Shannon diversity index.

The variance between the October sediment samples and October honey samples was further tested using the adonis and ANOVA tests of variance. The adonis function in R was used to test for variance using distance metrics with 999 permutations and it calculated that there is a significant difference (p-value =0.007) between the sample group (Table 5.1). Then assumption for homogeneity of the multivariate data was conducted with the ANOVA function in R. The p-value was calculated to be 0.04295, which means that homogeneity of the Sediment and honey samples cannot be assumed (Table 5.2).

Analysis of Variance Table

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Clusterdata$`Ocober sediment`	1	1601070	1601070	38.381	0.50249	0.007 **
Residuals	38	1585171	41715		0.49751	
Total	39	3186241			1.00000	
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						

Table 5.1. Adonis function variance results.

Response: Distances

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	10	1.08512	0.108512	2.4725	0.04295 *
Residuals	19	0.83386	0.043887		
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					

Table 5.2. Anova function variance results.

4.4 Surface Sediments Compared to Honey Samples (Presence/Absence of Pollen Types)

The presence/absence of pollen types from the October sediments are compared to the honey samples all year, this was conducted using the "incidence_freq" datatype in the iNEXT package from R (Table 5, Figure 18). The Species Richness, Shannon's diversity and Simpson's diversity was calculated based on the presence and absence of species types in either sample using the "incidence_freq" (instead of the abundance datatype like the rarefaction curve data above) to explore the compared species richness and diversity in the sediment samples and all the honey samples (instead of individual seasons). The species richness and diversity were the highest in honey samples (34) collected all year round compared to sediment samples (29) successfully calculated for a single soil collection (Table 5, Figure 18). These results were used to calculate the pollen percentage representation of a particular samples based on the total number of pollen types (40) from both samples.

Therefore, the percentage of pollen types present in either sample (sediment samples or all year honey samples) was calculated by adding the number of different pollen types divided by the total number of pollen types in both samples' times 100, to get a representative percentage of pollen types that are represented by a specific sample. Sediment samples account for the presence of 72% (29 pollen types/40 total pollen*100) pollen types from the total number of pollen types found in all samples honey samples and sediment sample. Whereas honey samples account for the presence 85% (34 pollen types/ 40 total pollen*100) pollen types from the total number of pollen types from all honey samples and sediment samples (Figure 18). About eleven pollen types that have been found in the honey samples are missing from the sediment samples (Figure 18 and 19), compared to four pollen types that are present in the sediment samples that are missing from the honey samples (Figure 18 and 19).

A total of 40 pollen types were found from both honey and sediment samples. Nine important and rare elements (*Peltophorum africanum, Lannea* sp., *Commiphora* spp., *Tribulus* sp., *Plectranthus* sp., *Helianthus annuus, Triumeffetta* sp., *Salix mucronata* and *Podocarpus/Afrocarpus*) were only found in the honey samples and four pollen types (*Morella* sp., *Heteromorpha* sp., *Pinus* sp. and *Vernonieae* type) were exclusively detected in the sediment samples (Figure 19). Twenty-four pollen types are present in both the honey samples all year and sediment samples (Figure 19 and 21). Honey samples collected multiple

times all year round have a higher species richness and diversity compared to sediment samples collected multiple times (Table 5). Additionally, the high pollen type variability in the honey samples is comparably higher in honey collected all year than honey collected for a specific season (Table 5 and Figure 17).

Table 5: Asymptotic Estimate table displays the Species Richness, Shanoon's diversity and Simpson's diversity for honey samples (all year) and October sediment samples. The "$AsyEst: asymptotic diversity estimates along with related statistics" values are generated from the incidence_freq data found in the iNext package in R.

Site	Diversity	Observed	Estimator	s.e.	LCL	UCL
October sediment samples	Species richness	29	29	0	-	-
	Shannon diversity	29	0	0	29	0
	Simpson diversity	29	29	0	29	29
Total honey samples (all year)	Species richness	34	34	0	-	-
	Shannon diversity	34	0	0	34	0
	Simpson diversity	34	34	0	34	34

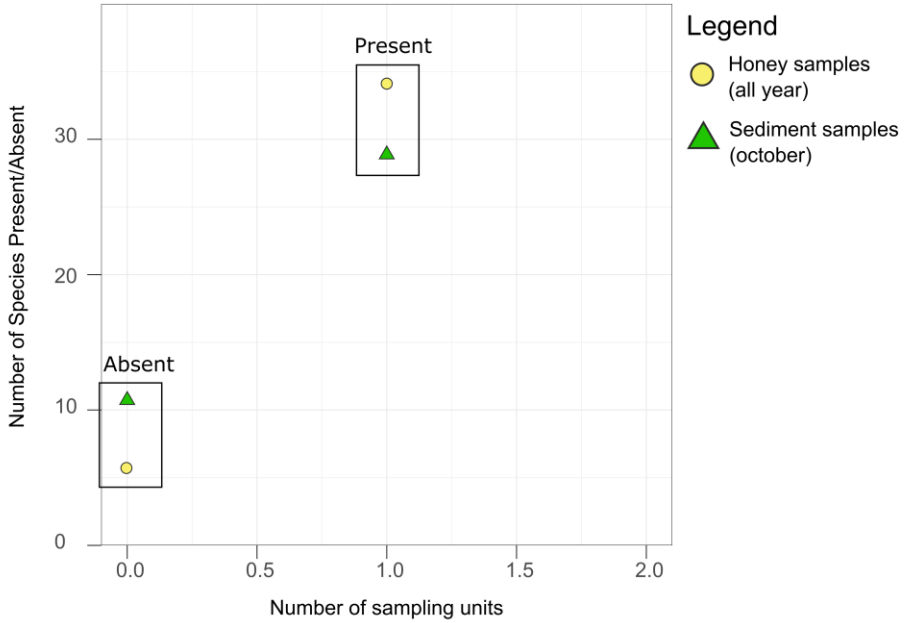

Figure 18A: The plot represents the number of species present/ absent for each sample. The plot was generated from the incidence_freq data found in the iNext package in R.

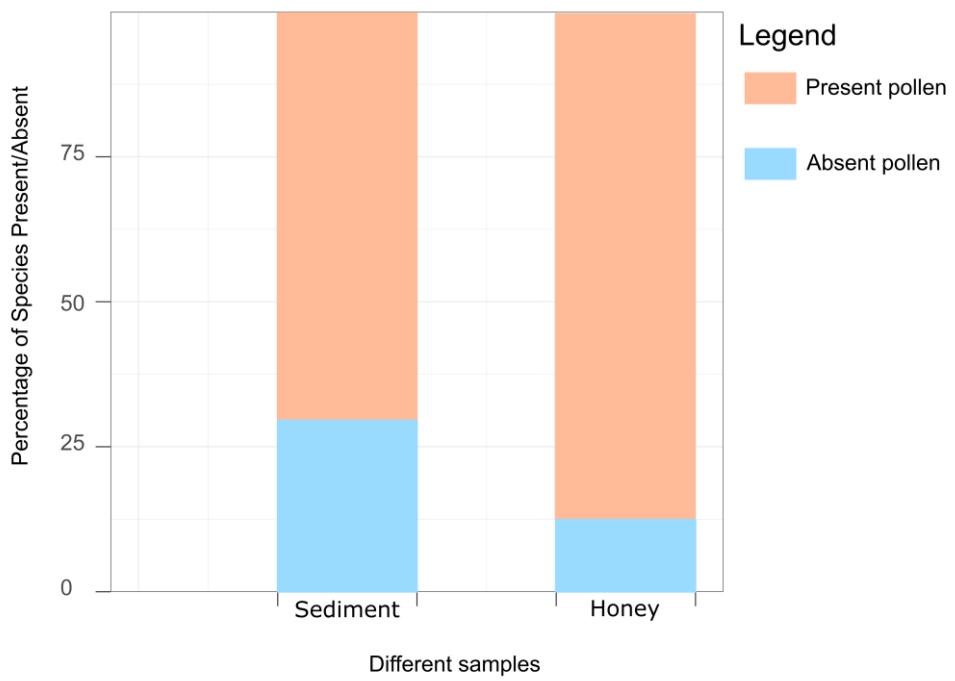

Figure 18B: Percentage cluster bar as a simplified version of figure 18A. It displays the percentage of present/absent pollen types in the honey samples and surface sediment samples.

4.5 Preliminary Botanical Survey

A preliminary botanical survey was conducted by Michelle D. Henley and Robin M. Cook on 12th of October 2021. Species that were identified along the transacts include *Senegalia nigrescens*, *Sclerocarya birrea*, *Euphorbia* sp., *Lannea schweinfurthii* and Combretaceae (Combretum spp.) (Figure 19). The location around the hives was surrounded by numerous Poaceae and *Grewia* spp. which were also growing within a meter of every beehive. *Senegalia nigrescens* (54%), *Scelrocarya birrea* (19%) and *Euphorbia* sp. (13%) were the most dominant species found and the least dominant were *Lannea schweinfurthii* (2%) and Combretaceae (2%), (Figure 19). Approximately 383.50m west from hive 9 is a lucerne field (*Medicago sativa*) and approximately 773.70m west is a *Vachellia xanthophloea* tree and 920m west from hive 9 is the Olifants River.

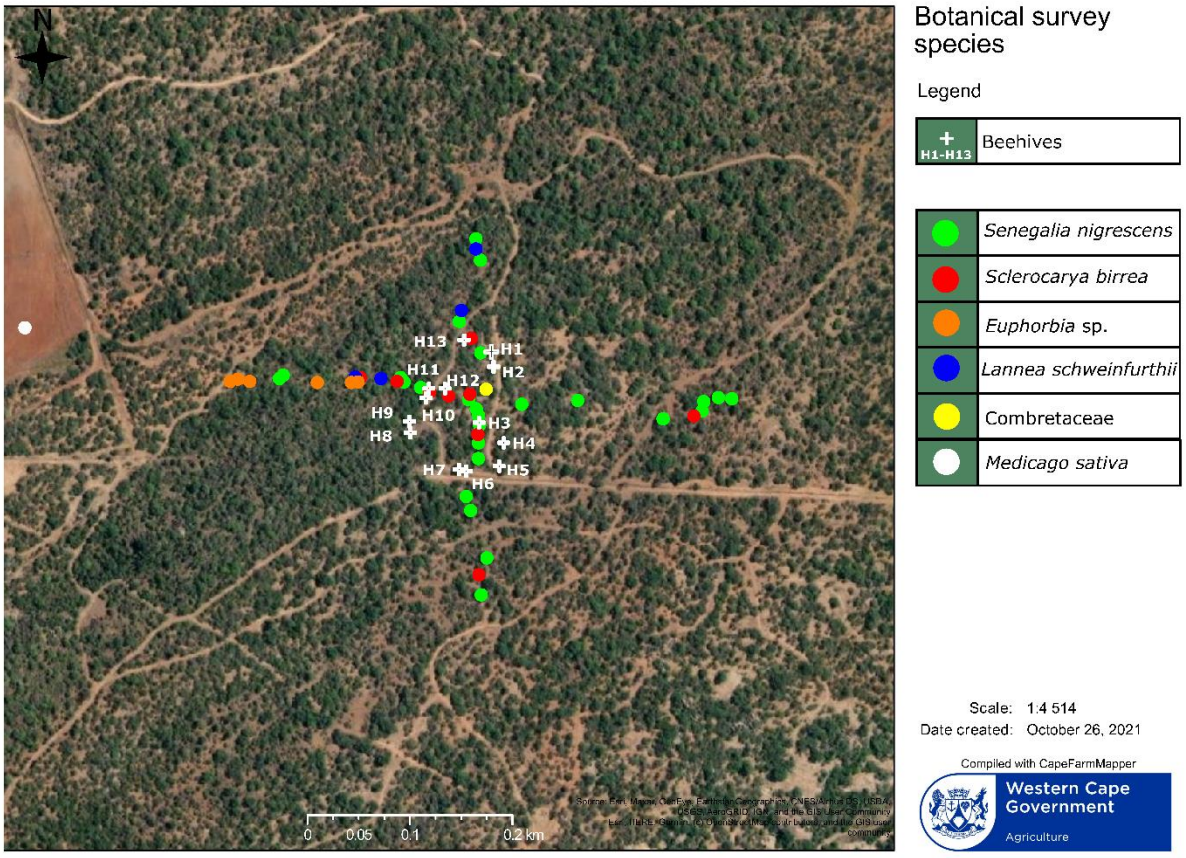

Figure 19. Species plot of plants identified from the preliminary botanical survey.

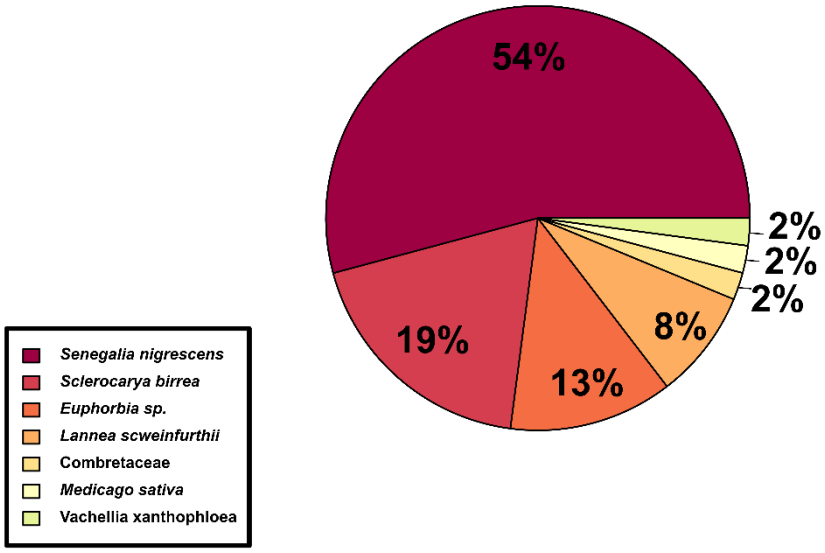

Figure 20. Pie chart plot displaying the plant species percentage composition for selective species that had a high relative abundance of pollen in the honey samples from the botanical survey (Figure 12). Figure 20 is a quantitative representation of Figure 19.

5. Discussion

5.1 Melissopalynological Investigation

5.1.1 Dominant Honey Pollen Types

The undiluted wild (lowveld) savanna honey pollen spectra are characterised by the presence of Combretaceae (*Combretum* spp.), Poaceae, *Harpephyllum caffrum*, *Sclerocarya birrea*, *Gerbera* sp., *Lannea* sp., *Euphorbia* sp., *Senegalia* type, *Vachellia* type and *Peltophorum africanum*. These species are some of the important bee plants identified from the Lowveld honey samples. According to Johannsmeier (2016), the known bee plants of the Mopane bushvelds (Lowveld) include *Combretum* spp., *Senegalia mellifera*, *Sclerocarya birrea*, *Vachellia tortilis* as well as *Grewia* spp. and *Dichrostachys cinera,* which are not dominant species, but minor pollen types found in all honey sample throughout the year.

Based on the percentages of each pollen type in all honey samples, *Combretum* spp. was the only predominant pollen type for all the honey samples (December, March, July and October) except the January harvest. Throughout the whole year the honey samples had an average of 56% *Combretum* spp. pollen and it is present in all honey seasonal samples. Individuals of *Combretum* spp. produce a large amount of pollen and nectar (N0-3, P0-3), (Johannsmeier 2016), and have attractive brightly coloured (white, yellow, or cream) flowers (Schulz and Lueke 1994). Bees have been observed to perform buzz pollination on *Combretum* spp. flowers (Schulz and Lueke 1994, Proenca 1992) by opening the anthers with the help of vibrations which results in a heavy pollen load that can be collected by bees or fall to the surface (Schulz and Lueke 1994). The high abundance of *Combretum* spp. (56% all year) from honey samples relative to its low (2%) abundance in the surrounding vegetation, signifies how highly favoured the tree is by bees of the Lowveld, for reasons such as its high production of pollen and nectar, as well as its bright flowers and the species reliance on stimulating and sustaining brood rearing (Johannsmeier 2016 and 2001). *Combretum* spp. is therefore a staple/principle food to bees in the Lowveld based on the reasons that it present in honey samples in high abundance for all seasons despite its low abundance in the surrounding area (Petrides 1975, Figure 19 and 20).

Combretum spp. is reported to be spread across the savanna in KNP (Table 4., Harley et al. 2003). However, according to the preliminary botanical survey conducted by the NGO Elephants Alive, *Combretum* spp. has only been located once close to the location of the beehives (Figure 19), which indicates that the tree is not abundant within 250m of the beehives. This further indicates that the bees are foraging further than 250m from the beehives to reach the *Combretum* spp. trees for their source of nutrients, possibly marking this plant as a highly preferred food source. Similar results were observed in the savanna biome from Gaya, southern Niger, where a melissopalynological study was conducted over two years and *Combretum* spp. was one of the dominant species selected for throughout the years (Schulz and Lueke 1994).

The second most dominant pollen type -also known as "Secondary pollen type"- is *Sclerocarya birrea,* with a 14% average presence all year and it was most abundant in the spring (October) harvests with 39.95% (Figure 7.1, 7.2 D). *Sclerocarya birrea* has not been recorded in any melissopalynological investigation known to the author of the current study, but its importance in this study highlights that it can be regarded as an essential bee plant of the savanna biome (see below). The tree is mainly pollinated by honeybees and its production of sticky pollen and secretion of nectar is one of the main reasons it is highly favoured by bees (Helm et al. 2011, Hall et al. 2002). *Sclerocarya birrea* is an important food source for bees of the Lowveld and consequently an important bee plant in South Africa (Johannsmeier 2005, Henderson 2001).

Additionally, *Sclerocarya birrea* has the widest distribution of all trees in the KNP (Hall et al. 2002, Van Wyk 1972). It is found in every ecological niche, it is seldomly dominant but it is one of the most important woody components of the savanna (Hall et al. 2002 and Van Wyk 1972). The trees wide distribution is supported by the botanical survey that revealed *Sclerocarya birrea* to be the third most abundant plants species (54%) within the 250m radius of the beehives (Figure 18), making it widely available as an abundant food source in the Lowveld for the bees. Relative to its high presence in vegetation and second highest abundance in the honey samples, *Sclerocarya birrea* is present in honey samples throughout the year and a reliable food source for the honeybees of the Lowveld similar to *Combretum* spp.

Lastly, the flowering seasons of *Sclerocarya birrea* ranges according to the literature from November to March (Shackleton 2002), respectively September to November (Schmidt et al. 2002) or August to November (Johannsmeier 2016). This is a typical example of varying recorded flowering seasons meaning that the reliance on one flowering season might be misleading. Based on several flowering refences for *Sclerocarya birrea* (Johannsmeier 2016, Schmidt et al. 2002 and Shackleton 2002), on average the tree seems to be flowering from August to March (spring to summer season) and as expected due to its flowering seasons the tree is therefore available and highly favoured by honeybees of the Lowveld during spring.

Important minor pollen types that were detected during this study include Poaceae (3%), *Harpephyllum caffrum* (3%) and *Lannea* sp. (1.5%), (Figure 7.1). Poaceae is only present in the autumn (March), winter (July) and spring (October) harvest, it is most abundant in the March (autumn) harvest (15.12%) and least abundant in the July and October samples. Generally, Poaceae pollen tend to be in abundant in honey comb edge samples (Adeonipekun 2012), pollen pellets and not commonly in the honey samples because it is foraged by bees for food during a specific time like the dry seasons (Adeonipekun 2012, Aina and Owonibi 2011). One record of its abundance in honey samples from the savanna biome of North Central Nigeria has been recorded. The reason for this exception was explained by the fact that grass tends to thrive in the savanna vegetation compared to other plant groups (Adeonipekun 2012, Ige and Modupe 2010). The wide abundance of grass within the hive location (Figure 19) means that it is available in abundance to bees. The current study found that the bees foarged from Poaceae mostly during autumn even though Poaceae flowers from December to June (Mashau and Muthanyi 2015, Russell et al. 1990). This implies that during the December and January months it is flowering but it is not reflected in the summer honey harvest instead it is reflected mostly in the autumn honey harvest. In summer there might be more favourable plants flowering like *Combretum* spp. and *Medicago sativa* (Table 3, Figure 7.2A).

Harpephyllum caffrum (3%) and *Lannea* sp. (1.5%) are important minor tree taxa which both belong to the Anacardiaceae (Table 3). Anacardiaceae (*Sclerocarya birrea*, *Harpephyllum caffrum* and *Lannea* sp.) harbours three species that belong to the most abundant 5 taxa (*Combretum* spp., *Sclerocarya birrea*, Poaceae, *Harpephyllum caffrum* and *Lannea* sp.). Anacardiaceae including *Lannea* sp. have been described as major sources for both nectar and pollen in several study sites in the savanna in Ghana (Sudan Savanna and Guinea Savannah agro-ecological zones) and Gaya in southern Niger, underlining its significance as

an important bee plant in the the biome (Schulz and Lueke 1994). *Harpephyllum caffrum* is a bee plant (N0-1, P0-1) with a poor pollen and nectar value but it an important minor bee plant of the Lowveld according to this study, and one record of bee visits from the southwestern Cape and Pretoria has been recorded (Johannsmeier 2016). *Harpephyllum caffrum* has not been recorded within the radius of 250m of the beehive's location implying that the tree is further than 250m distance from the beehives but the bees forage from them still (Figure 17, Figure 18). This implies that the *Harpephyllum caffrum* according to other studies has been underestimated as an important bee plant and therefore to this study only known study to reflect its high preference to honeybees of the Lowveld according to the author of this study.

5.1.2 Minor Honey Pollen Types

Other minor important pollen types of this study that are characteristic of the savanna biome are *Gerbera* sp. (1.2%), *Euphorbia* sp. (1.62%) *Senegalia* type (0.83%), *Vachellia* type (0.28%), and *Peltophorum africanum* (0.10%). *Senegalia* type, *Vachellia* type, and *Euphorbia* sp. have been identified and located by the preliminary botanical survey. *Vachellia* type and *Peltophorum africanum* belong to the Fabaceae, these trees have not been located within the 250m radius of the beehives, but they appear in the honey samples (Table 3, Figure 19).

Peltophorum africanum it is a typical savanna species and has been listed as a bee plant (Johannsmeier 2016, Mostert 2001). According to the study findings *Peltophorum africanum* pollen is only present in the winter harvest (July samples) even though the species flowers from September to March (Johannsmeier 2016, Schmidt et al. 2002). Since *Peltophorum africanum* is not detected within a 250m radius from the beehives and it is only represented in the winter harvest, the taxon might be a typical example of how bees increase their foraging range during the dry seasons (when resources are insufficient) to feed from other bee plants in the vicinity (Danner et al. 2017). This is supported by the relatively high average species richness in the winter samples compared to all honey harvest seasons (Figure 13 and 16). Otherwise, *Peltophorum africanum* is potentially a food to honeybees albeit very seasonal.

Vachellia type pollen detected in the current study might have been produced by *Vachellia xanthophloea* since this taxon was growing about 920m from the hive site (Figure 19). The absence of *Vachellia xanthophloea* and *Peltophorum africanum* within 250m radius implies that bees will forage further than 250m to feed from these trees. Apart from the influence of seasonality, the distance of the food source from the hives might be a contributing factor to their low abundance in the honey samples, especially since honeybees tend to compensate for a lower vegetation diversity by increasing their pollen foraging range to maintain pollen amounts and diversity of their pollen diet (Danner et al. 2017). Certainty regarding the influence of distance on the foraging choices of bees cannot be confirmed in the frame of the current study but only estimated since the botanical survey is preliminary and not extensive. Consequently, this excluded certain taxa that were far (further than 250m) and between the transact spaces were not included in the survey, despite the fact that the foraging maximum range is estimated to be 5983m (Hagler et al. 2011) to 16 km (Bekman and Ratnieks 2000, Schneider 1990, Figure 19).

Euphorbia spp. on the other hand is present within 250m of the hives and it appears in all the honey harvest all year except for samples collected in summer (Table 3 and 4, Figure17). Even though it flowers from October to March, (Schmidt et al. 2002) and or January to December (Johannsmeier 2016), it is present in most harvests throughout the year in low abundance (Table 3 and 4, Figure 7.1). Therefore, *Euphorbia* spp. is one of the species found in the honey samples which might be proportional in honey samples to its presence in the area.

According to the current study, Combretaceae and Anacardiaceae are the most important families, Fabaceae (*Euphorbia* sp., *Vachellia* type and *P. africanum*) are an important minor pollen family in this study. This is based on the dominant presence of Combretaceae and Anacardiaceae (especially *Sclerocarya birrea*) pollen in all honey harvest throughout the year and the Fabaceae family (especially *Euphorbia* sp. and *Vachellia* type) which has species that are constantly present in all honey harvest throughout year but in lower quantities. As hypothesised, melissopalynology is indeed a good indicator of pollen collected by bees and the plants they prefer in the Lowveld region of southern Africa.

5.2 Seasonality in Honey Samples and Foraging Behaviour of Bees

Seasonality is hypothesised to have an influence on the plants the bees forage from and this affects the pollen types that constitute the honey. The analysis of pollen throughout different harvest seasons has revealed the flowering results: Polygon shapes on the NMDS plot show an overlap of the sample groups throughout the dimensional space (Figure 8). This implies that there are similarities in all the honey seasonal groups despite the existence of meaningful variations between and within different seasonal groups (Figure 8). The similarities within different hives are supported by the fact that pollen of *Combretum* spp. dominate the honey samples of all the hives (except for the January sample (Table 3). Pollen of several taxa are present in all seasonal samples, they include *Combretum* spp., *Senegalia* type, *Vachellia* type and *Sclerocarya birrea*. These taxa might be the main reason for the seasonal overlap shown in the NMDS plots hence all the mentioned pollen taxa plot close or within the overlapping zone of the winter, autumn, and spring season (Figure 8), especially since the mentioned taxa are all important savanna biome bee plants (Johannsmeier 2016).

Despite the overlap of all the seasons as depicted in the NMDS, there are still variations in all seasons, and this is depicted by the polygon shapes (representing seasonality) stretching further into their different directions and varying distances from the common area of overlap (Figure 8, significant stress of NMDS is 0.0875). Therefore, according to literature, seasonal variation of pollen contained in honey samples is to a high degree attributed to the diversity of pollen collected by the foragers rather than the landscape level effects, such as the level of urban development in the surrounding landscape of a bee colony (Lau et al. 2019, Danner et al. 2017). Indeed, the species diversity and richness of all seasons varies over time (Figure 10 and 16), suggesting that the pollen spectra of honey vary from season to season and month to month, but the important bee plant species of the region are generally represented throughout the whole year.

Moreover, the highest average species diversity was observed in the spring harvest, then followed by the autumn harvest, winter harvest and eventually the summer harvest (Figure 16). Notably, the winter harvest had the widest range of species diversity of all samples (varied between 2.09 and 4.17) compared to all other seasons (Figure 13). The winter season also has the highest average species richness (5.00 and 23.00) compared to all other seasons (Figure 13 and 16). The lowest average species richness and diversity were observed from the summer harvests (Figure 13). These seasonal findings imply that bees forage from more

floral sources in drier seasons compared to the warmer season (December and January harvest for this study) (Laura and Cynthia 2018 and Aswini 2013), hence winter has the highest average pollen species richness.

Apart from seasonal variations in honey samples it was also noted that that there is variation between different hives from the same season. The species density of all the hives was found to vary within and throughout different seasons (Figure 10) hence the NMDS plot displays all the hives (honey samples) at varying distances from each other (Figure 8). Even though seasonality affects the density and richness of pollen found in honey (Laura and Cynthia 2018 and Aswini 2013), bees forage from similar sources in all hives but each different hive has its unique pollen spectra (Figure 10), suggesting that the foraging behaviour of all hives is similar, but it varies from hive to hive, and these differences have been explored by entomologists who have investigated the economics of foraging and foraging strategies (Carvalheiro et al. 2011, Núñez 1982).

A study was conducted to investigate the economics of foraging using an artificial food source (sugar solution) to attract bees and it was found that the proximity of the food source to the hive and the quantity of sugar solution determined whether the bees would visit the food source or not (Núñez 1982). The investigation revealed that the further the food source is and the lower the quantity of sugar available to bees, the less likely the bees were to return to the food source (Núñez 1982, Grüter and Ratnieks 2011). This is because foraging is an energy consuming effort for the bees, and it can be assumed that a shorter flight distance is advantageous for the health and development of the colony (Danner et al. 2016). The mean foraging distance in one study of honeybees was 1015m, in spring the increased number of flowering plants, resulted in a foraging range between 435m to 1324m, then in summer the foraging ranged reduced and ranged between 469m and 846m (Danner et al 2016). Similarly, in a study with African honeybees, foraging ranges increased during a shortage of resources (Danner et al. 2016, adapted from Schneider & McNally 1993). Therefore, foraging distances vary depending on landscape structure, resource availability, and season (Danner et al. 2016, Danner et al. 2017, Steffan-Dewenter & Kuhn 2003 and Visscher & Seeley 1982). A detailed botanical and landscape survey would have been crucial in exploring the factors that contribute to the variability of pollen within hives from the Lowveld and warrants further investigation.

5.3 Honey Samples Compared to Surface Sediment Samples

Surface sediment analyses are an important component of palaeo-ecological research (Li et al. 2005), especially since pollen preservation and the relationship between pollen and vegetation can influence the most precise interpretation of fossil pollen spectra (Li et al. 2005). Therefore, hypothetically both surface sediment samples and honey samples consist of pollen that can be used to understand the pollen-vegetation relationship within the study area. For the surface sediment samples, it is hypothesised that the sampling bias is influenced by the different pollination pathways within the ecosystem, for example the under-representation of entomophilous (insect-pollinated) taxa and the overrepresentation of wind transported taxa. In contrast, for the honey samples it is hypothesised that samples can expose the effect of the different pollination pathways by showing a higher representation of insect transported pollen than wind transported taxa.

According to the findings of the current study, the pollen composition in sediment samples were significantly different to all honey samples as expected (Table 5.1 and 5.2). *Morella* sp. (1.05), *Heteromorpha* sp. (1.71), *Pinus* sp. and *Vernonieae* type are the only pollen exclusively found in the surface sediment samples (Table 4 and Figure 9) whereas about nine pollen types (*Peltophorum africanum, Lannea* sp., *Commiphora* spp., *Tribulus* sp., *Plectranthus* sp., *Helianthus annuus, Triumeffetta* sp., *Salix mucronata* and *Podocarpus/Afrocarpus*) are exclusively detected in the honey samples. These differences in the pollen composition of the samples might play an important role in the significant difference between the samples. The differnces between the samples are also supported by the species richness and diveristy of the pollen composition. In comparison, the species richness and diversity were higher for sediment samples compared to the different seasonal honey samples (Figure 17), these differences can be attributed to the way in which the pollen is deposited which depends on the pollen's specific transportation pathways.

There is a large overlap regarding the pollen types in surface sediment samples and honey samples (Figure 18), which can be explained by the similarities between the samples. Apart from both sample sets being significantly different, both sample sets have several pollen taxa in common that are dominant like Poaceae (22.62%: sediment sample), *Combretum* sp. (20.44%: sediment sample) and *Seclerocarya birrea* (17.78%: sediment sample) (Table 4, Figure 9). These similarities suggest that the pollen that is available in the surrounding vegetation is more or less the same abundant pollen collected by bees and the same pollen

trapped in the sediemnt samples implying that honeybees are generalist (Hausmann et al. 2016, Garibaldi 2013).

How the sediment samples reflect the surrounding vegetation is discussed here in connection to the brief botanical survey. The botanical survey reflect the dominance and the relative wide distribution of Poaceae (widely spreadout around the hives sites), *Senegalia nigrescens* (54%), *Scelrocarya birrea* (19%), *Euphorbia* sp. (13%) and *Lannea schweinfurthii* (8%) around the beehives. *Senegalia nigrescens* and *Euphorbia* sp. were present in the sediment samples but not as dominant as they are distributed in the beehive location (Table 4 and Figure 9).

Senegalia nigrescens is mainly insect pollinated (often by *Apis mellifera*) but it is also wind-transported (Villanueva-Gutiérrez et al. 2015, Georginah and Maanda 2015, Melusi and Mojeremane, 2012). The *Senegalia nigrescen*'s pollen polyade is larger and heavier than most pollen found in this study with a diameter of c. 55-60µm (Table 3 and Figure 6.2 A-B). Despite its wide distribution in the KNP (Mills et al. 2021), the sediment samples do not reflect its predominance (Table 5). This may be due to the fall speed of the pollen that is dependent on the pollen grain density, which affects the pollen productivity and in doing so influences its deposition (Julier et al. 2018).

Therefore, pollen size/weight, the low pollen production, and the form of transportation of the tree's pollen are the main factors contributing to its scarcity in the surface sediment samples, since *Senegalia*/*Vachellia* trees only produce a few (about eight) large pollen grains in one anther and for this reason very few pollens are deposited (Duffin and Bunting 2008, Coetzee 1955). I conclude that the *Senegalia* type pollen detected during this study might have been produced by *Senegalia nigrescens* because it is the only abundant *Senegalia* species in the surrounding vegetation according to the preliminary botanical survey. *Senegalia nigrescens* is one of the important underrepresented pollen types in both the sediment samples and honey samples. Similar results were discovered from surface sediment samples palynology analysis conducted in the KNP (Julier et al. 2018).

Therefore, the hypothesised influence of the pollination pathways can be accepted because there is a slight significant observation pattern between some insect pollinated pollen and wind pollinated pollen. For example, taxa like *Lannea* sp. are insect pollinated and it is abundant in honey samples but not in sediment samples whereas *Pinus* sp. is wind pollinated and abundant in sediment samples and absent in honey samples. The significance of the

pollination pathways on pollen deposition is said to be slightly significant because pollen types like *Combretum* spp. which is both animal and insect pollinated ends up in abundant amounts in sediment samples because it produces high amounts of pollen. Another example is *Senegalia nigrescens*, pollinated by both insects and wind (Villanueva-Gutiérrez et al. 2015, Georginah and Maanda 2015, Melusi and Mojeremane, 2012). The taxon is well distributed in the location around the hive but it is underrepresented in both samples relative to its abundance in the surrounding vegetation. These examples imply that in addition to the influence of pollination pathways the above-mentioned factors like the pollen productivity additionally have a contributing effect on the representation of individual pollen types detected in either the surface sediment or honey samples (Duffin and Bunting 2008).

5.4 How the Surrounding Vegetation is Represented in Different Samples

Pollen grains are amongst the most abundant and well-dispersed fossil plant remains and as a result the analysis of sedimentary pollen records is one of the most effective methods to reconstruct past vegetations (Meltsov et al. 2011). The correlation of the sediment samples is indirectly proportional to the floristic diversity of the pollen catchment area (MacDonald at al. 2008). Several factors like the pollen production of specific species, dispersal strategies and spatial scale of the sample representation result in a nonlinear relationship between pollen and vegetation representation (Prentice 1985, Sugita et al. 1999). Pollen records from surface sediment samples have been used to interpret Holocene pollen records from the savanna biome in southern Africa (for example Tabares et al. 2021, Duffin and Bunting 2008, and Scott and Nyakale 2002). Honey on the other hand is a recognised proxy used by melissopalynologists to determine the geographical and botanical origins of the honey (Dustmann and Von der Ohe 1993, Johannsmeier 2016). It is hypothesised that honey samples can be used along with sediment samples to contribute to the combined representation of the surrounding vegetation as useful pollen archives.

When comparing these two environmental proxies in the current study, it was revealed that the total honey samples collected all year represents about 85% of pollen from both samples and possibly within the surrounding vegetation (Figure 19), in comparison to surface sediment samples (which yielded results only in the October collection) which represented 72% of pollen from both samples and possibly within the surrounding vegetation (Figure 19). These different deposits (honey versus surface sediment/to soil) accumulate pollen in specific forms: Pollen contained in honey consist mostly of pollen intentionally collected by bees (Ghosh et al. 2020) and pollen in surface sediment samples are deposited by the wind from the surrounding vegetation (compare Fall 1987). The results suggest an overlap in the pollen transportation pathways despite the main form of deposition for each sample type (were pollen in honey samples is mainly transported by bees and pollen in sediment samples is mainly transported by the wind) (Fall 1987, Ghosh et al. 2020). Therefore, about 24 pollen types (64.86%) out of a total of 37 total pollen types from both samples can be found both in the honey and the surface sediment samples (Figure 20).

For this study, three surface sediment samples from beneath each beehive were collected throughout the year and only the spring samples yielded satisfactory pollen counts above an average of 50 pollen counted per site (S. Figure 6), whereas during the year *Pinus* sp. was the only pollen type detected continuously. The inconsistencies in pollen accumulation in sediment samples all year suggest that the seasonal results are inconsistent and there are overlaps when compared to honey samples, which yielded satisfactory pollen counts (above an average of 333 pollen counted per hive) for all the harvest seasons. The inconsistencies in the surface sediment samples can be attributed to the relationship between the surrounding vegetation and the pollen diversity, pollen concentration, the sampling season, wind variation and direction, and the distance of the sampling location (Luo et al. 2016 and Luo et al. 2015) It might be useful to collect a large quantity (>15g) of sediment samples (own observation).

Therefore, the pollen concentration was calculated using *Lycopodium* spore tablets, for the autumn sediment samples and it was found to be 30 632 pollen grains per 10g of soil, whereas honey samples were 50 080 pollen grains per 10g of honey, resulting in a 61.16 % difference in the pollen concentration of the two samples. The limitations of comparing the honey samples to sediment samples is that only one season yielded satisfactory pollen counts and the pollen concentration for those seasons were not done because pollen concentration values were not proposed in the study, but later they were included to quantify the pollen concentration of both samples. Otherwise, the relatively low pollen concentration of the surface samples and the absence of satisfactory pollen count in other seasons except for autumn implies that the main factor contributing to the pollen deposition of surface sediment during the year is probably the low pollen concentration in the top soil. The ideal sediment sampling season for the Lowveld region, according to this study, is during autumn. However, this may be dependent on the varying extreme climate of northern South Africa (Kruger et al. 2016). Seasonality may affect the preservation of pollen since several factors like soil oxidation, soil pH above a 5 and aerobic microbiological processes are factors contributing to the corrosion of pollen and therefore the low pollen concentration in surface sediment samples (Li et al. 2005, Havinga 1967 and Dimbleby 1957).

Ultimately, these factors contribute to the relatively low pollen concentration deposition of pollen in surface sediment samples. Out of a total number of pollen types found in both samples, 72% pollen types were found in the sediment samples (meaning that out of the total/100% pollen types found in both samples, only 72% of those types were present in the sediment samples) (Figure 19). This is compared to 85% pollen types present in honey

samples, which is relatively higher compared to sediment samples. Therefore, honey samples are a better representation of the pollen found in the surrounding vegetation than sediment samples. Firstly, because honey pollen reflects seasonality better (Figure 8) than sediment samples and secondly honey samples account for comparably more pollen types within the vegetation that are not represented at all in the sediment samples. These pollen types include important savanna indicators like *Peltophorum africanum, Lannea* sp., *Commiphora* spp., which are also contained in the fossil pollen deposits from the late Quaternary Transvaal in South Africa (Scott 1982), and minor species like *Tribulus* sp., *Plectranthus, Helianthus* sp., *Triumeffetta* sp., *Salix mucronate* and *Podocarpus/Afrocarpus* (Figure 20). *Tribulus* sp. weed is a good source of pollen and has stimulative amounts of nectar which both promote brood production (Johannsmeier 2001). *Helianthus annua* is a neophyte that produces sufficient nutritious pollen to strengthen honeybee colonies (Johannsmeier 2001).

Moreover, from all the mentioned species only present in the honey, *Lannea* sp. is the most important taxa because it is absent in sediment samples but present in the winter honey harvest with an abundance of 7.1%. *Lannea* sp. can be identified as *Lannea schweinfurthii* which is the species found to be present in the preliminary botanical survey with 8%. *Lannea schweinfurthii* is the second most important underrepresented pollen type in the sediment samples following *Senegalia nigrescens*. The pollen types (*Peltophorum africanum, Commiphora* spp., *Tribulus sp., Plectranthus, Helianthus annua, Triumeffetta* sp., *Salix mucronata* and *Podocarpus/Afrocarpus*) present in the honey samples and absent in the sediment samples cannot be regarded as underrepresented or overrepresented because they do not appear in the botanical findings. This is due to the limitations of the botanical survey that was conducted within a 250m radius, so any plants beyond the 250m distance and between the four transacts might have been neglected.

In retrospect to all the data analysed, pollen assemblages found in honey samples and sediment samples can be used as a combined tool to reflect the surrounding vegetation and bridge the gap between the representation of certain pollen taxa. Pollen taxa that might be affected by the different sampling biases, seasonal influences affecting each individual sample and the different pollen production strategies of different plants. For example, the under- representation of taxa like *Senegalia nigrescens* and *Lannea schweinfurthii* can be accounted for by using honey samples and taxa like *Pinus* sp. can be accounted for by utilising sediment samples since it is rather unlikely that they are found in honey (as reflected in Figure 21 and 22).

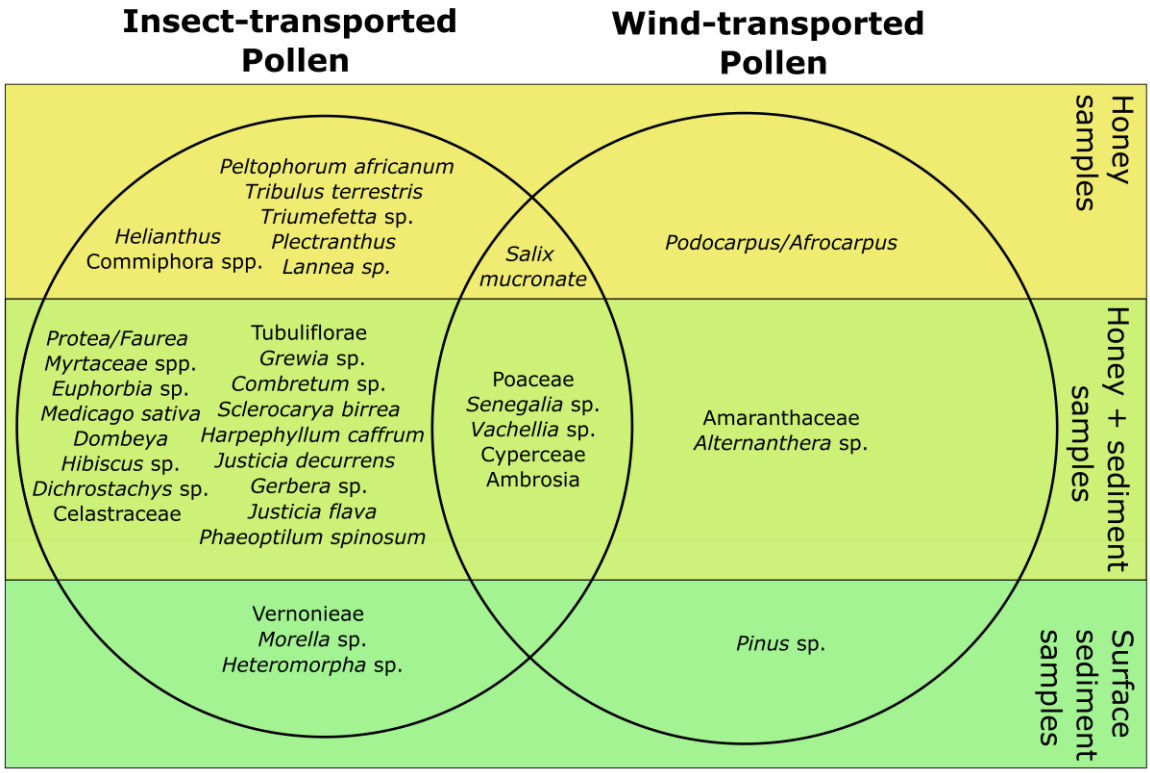

Figure 21. Summary of all pollen taxa found in the honey and sediment samples assorted according to the known pollination pathway used by the different plant species.

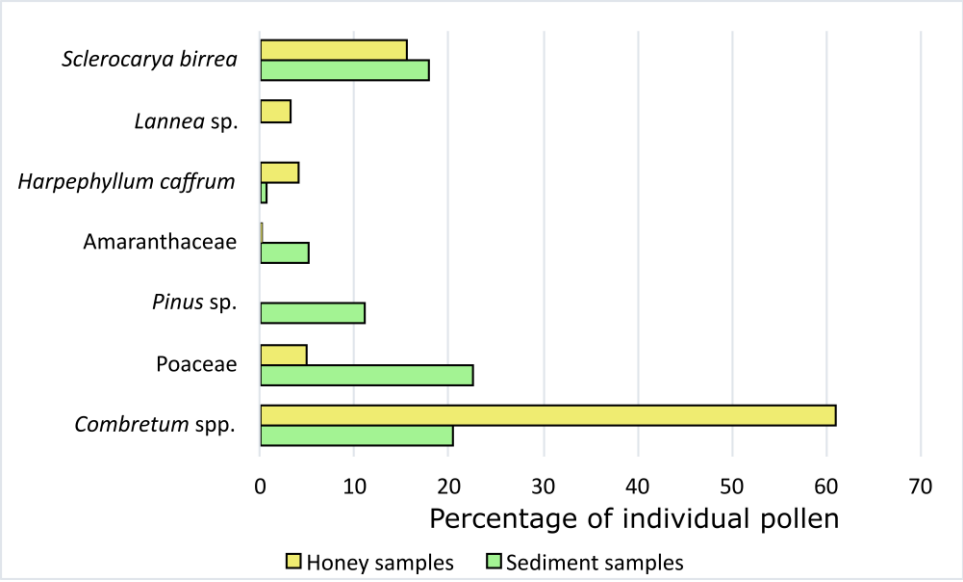

Figure 22. A bar plot of relative percentages of individual pollen types found in both sediment and honey samples, reflecting the abundance differences of each taxon in both sample sets. This plot is a quantitative expression of important taxa (dominant and distinct taxa) as displayed in Figure 21.

6. Conclusion

This study expands on the influence of seasonality and the behaviour of honeybees reflected in the pollen spectra from the Lowveld region (Ndlovu et al 2021). The pollen spectra are characterised by important taxa like *Combretum* spp., *Sclerocarya birrea*, Poaceae, *Harpephyllum caffrum, Gerbrea* sp., *Lannea schweinfurthii, Euphorbia* sp., *Senegalia nigrescens, Vachellia xanthophloea, Peltophorum africanum, Grewia* sp., *Dichrostachys* sp. and invasive species like *Medicago sativa*. Apart from the observation that honeybees are generalists who forage from the vegetation available in the ecosystem, seasonality contributes immensely to pollen types honeybees prefer during different seasons, for example during summer, bees forage generally from less floral sources like *Combretum* spp. and *Medicago sativa.* Consequently, the species diversity and richness are the lowest in the summer seasons compared to other seasons all year. During the drier seasons like autumn, winter and spring the species diversity and richness is relatively high compared to summer. Furthermore, the highest species richness is observed during the winter honey harvest meaning that bees forage from more floral sources during the driest season.

The foraging behaviour of honeybees have been observed from different hives having varying pollen taxa diversity throughout the year and within different seasons. This particular finding was unexpected, and it may be influenced by the relative distance of different hives to the foraging plants. An extensive botanical survey on each hive would potentially shed light on the proposed influence of distance to the pollen diversity variation in different hives from the same landscape, bioregion and biome and warrants further investigation.

Therefore, honey samples can be successfully used to determine the surrounding vegetation and the seasonal influence on the pollen spectra from the Lowveld region, whereas surface sediment samples can also be used to represent the surrounding vegetation, e.g., in the context of palynological analyses of Quaternary wetland deposits. However, the surface sediment samples have limitations like seasonal overlaps and low pollen concentrations. These two limitations can be accounted for by using honey samples along with sediment samples which can also be applied to palaeoecological studies which also investigate modern pollen-vegetation relationships. Additionally, the honey seasonal finding can be averaged over a year to replicate the sequence of fossil pollen records which tend to be recorded over more than one year's accumulation. These suggestions can bridge the gap between the pollen taxa deposited in honey and underrepresented in sediment samples for species such as

Lannea schweinfurthii (insect pollinated), and pollen taxa that is deposited in sediment samples and tends to be underrepresented in honey samples for example *Pinus* sp. (wind transported). The pollination pathways have an influence on the pollen found in different depositions and the plants specific pollen productivity also influences pollen deposition. *Senegalia nigrescens* is a typical example of the influence of pollen productivity on pollen deposition. *Senegalia nigrescens* is the most dominant and widespread species but its dominance is not reflected in both samples primarily because of its low pollen production. In summary, the study has shown the potential use of honey and surface sediment samples as a useful tool to contribute to the investigation of pollination pathways in the savanna biome of the KNP for the improved interpretation of Holocene fossil pollen archives.

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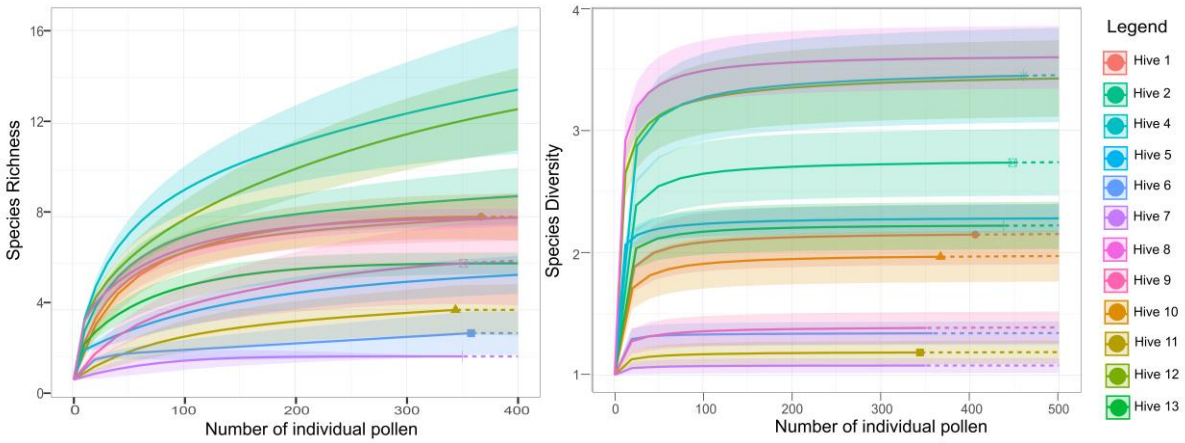
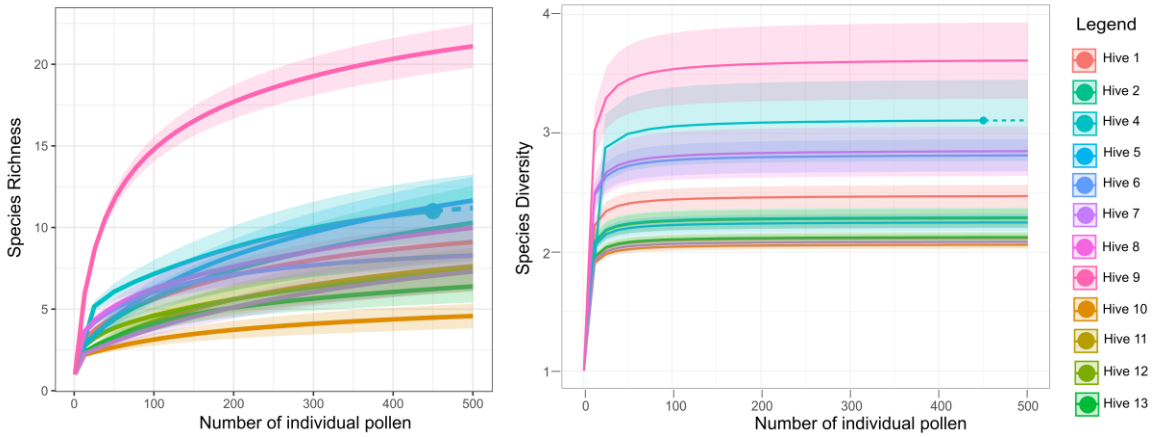
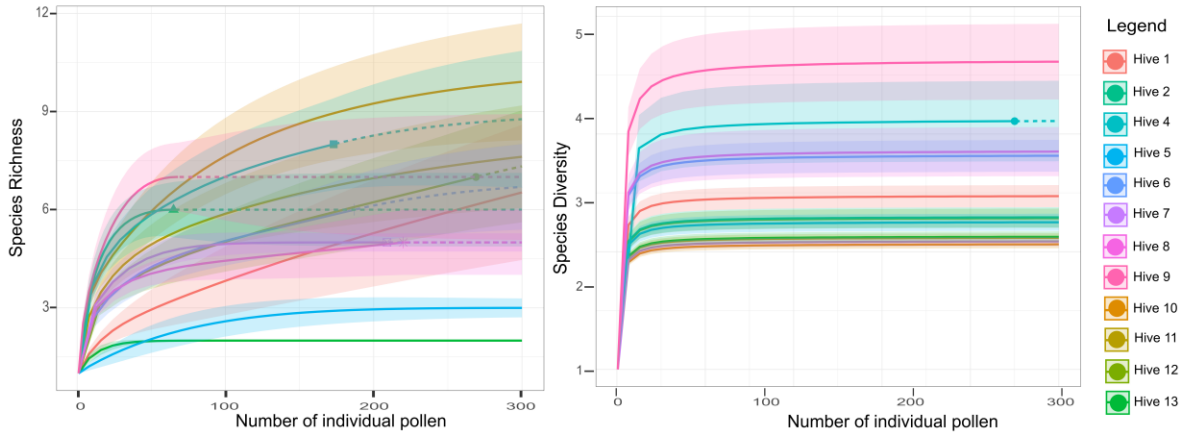
9. Supplementary Data



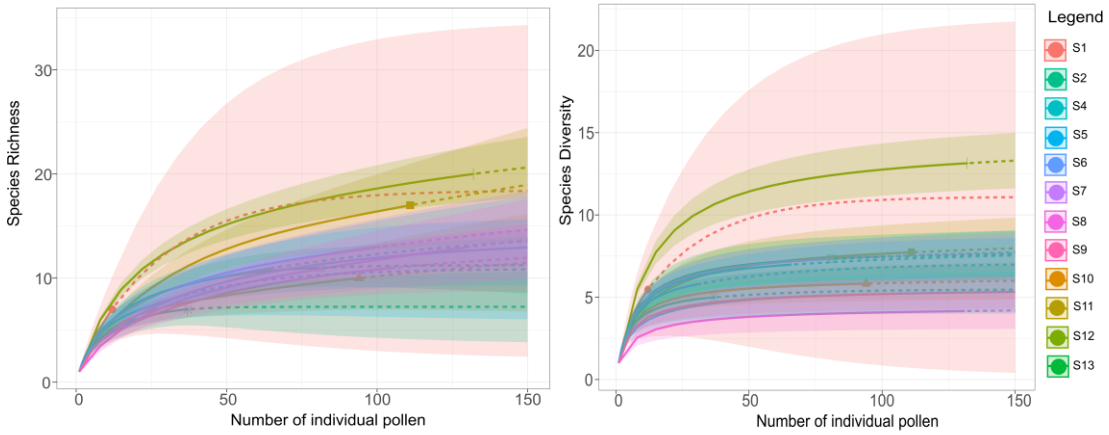
Supplementary Figure 1: Rarefaction curves (95% CI) displaying differences in richness and diversity between twelve of the honey samples collected in autumn (March samples). The left figure (q = 0) is comparing the season in terms of the Species Richness index and the right figure (q = 1) is comparing the season in terms of the Shannon diversity index. This graph (S. Figure 1) is a full extension of Figure 12 that contains two samples from this figure.



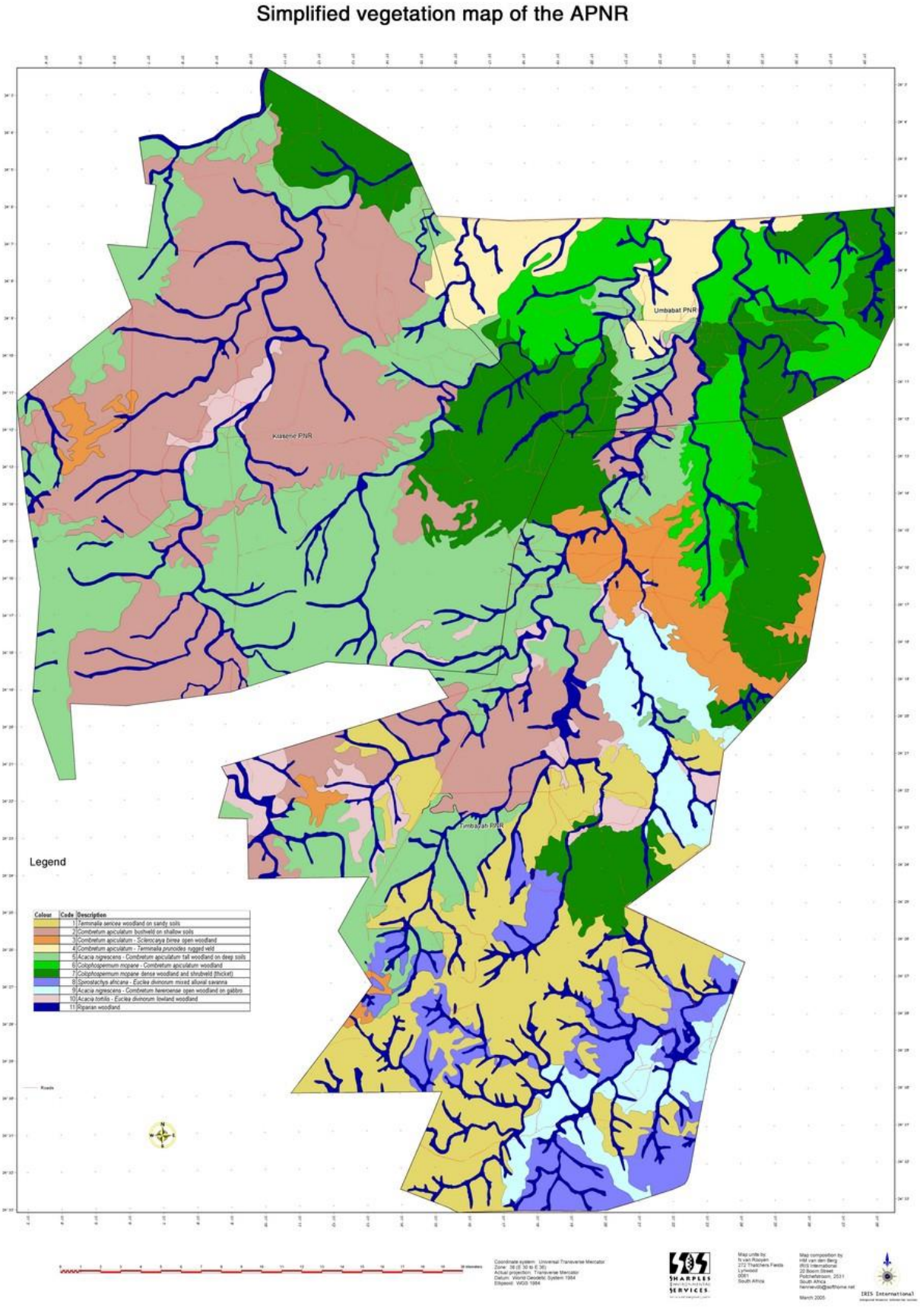
Supplementary Figure 2: Rarefaction curves (95% CI) displaying differences in richness and diversity between twelve of the honey samples collected in winter (July samples). The left figure (q = 0) is comparing the season in terms of the Species Richness index and the right figure (q = 1) is comparing the season in terms of the Shannon diversity index. This graph (S. Figure 2) is a full extension of Figure 13 that contains two samples from this figure.



Supplementary Figure 3: Rarefaction curves (95% CI) displaying differences in richness and diversity between twelve of the honey samples collected in spring (October samples). The left figure (q = 0) is comparing the season in terms of the Species Richness index and the right figure (q = 1) is comparing the season in terms of the Shannon diversity index. This graph (S. Figure 3) is a full extension of Figure 14 that contains two samples from this figure.



Supplementary Figure 4: Rarefaction curves (95% CI) displaying differences in richness and diversity between twelve of the sediment samples collected in spring (October sediment samples). The left figure (q = 0) is comparing the sites in terms of the Species Richness index and the right figure (q = 1) is comparing the sites in terms of the Shannon diversity index. This graph (S. Figure 4) is a full extension of Figure 15 that contains two samples from this figure.



Supplementary Figure 5a: Vegetation survey provided by Elephants Alive, conducted in the APNR. The survey is not specific to the location of the beehives.

	1	*Ficus abutilifolia - Ochna inermis* rocky outcrops and ridges
	2	*Combretum apiculatum - Sclerocarya birrea* open woodland
	3	*Terminalia sericea - Combretum zeyheri* woodland
	4	*Terminalia sericea - Combretum zeyheri - Pterocarpus rotundifolius* - open woodland
	5	*Combretum apiculatum - Sclerocarya birrea - Strychnos madagascariensis* open woodland
	6	*Combretum apiculatum - Xerophyta retinervis* low thicket
	7	*Combretum apiculatum - Grewia bicolor* low thicket
	8	*Combretum apiculatum - Terminalia prunioides* rugged veld
	9	*Acacia nigrescens - Combretum apiculatum* mixed woodland
	10	*Acacia nigrescens - Terminalia prunioides* woodland
	11	*Colophospermum mopane - Combretum apiculatum* woodland
	12	*Colophospermum mopane* dense woodland and shrubveld (thicket)
	13	*Spirostachys africana - Euclea undulata* mixed alluvial savanna
	14	*Acacia tortilis* lowland woodland
	15	*Euclea divinorum - Sporobolus ioclados* short woodland on saline lowlands and floodplains
	16	*Acacia luederitzii - Euclea divinorum* lowland woodland
	17	*Albizia harveyi - Combretum hereroense - Acacia gerrardii - Euclea divinorum* lowland woodland
	18	*Acacia nigrescens - Combretum hereroense* open woodland
	19	*Acacia gerrardii - Euclea divinorum - Sporobolus nitens* lowland savanna
	20	*Acacia gerrardii - Combretum hereroense* lowland savanna
	21	*Schotia brachypetala - Philenoptera violacea* riparian woodland
	22	*Phragmites australis* river beds
	23	**Disturbed areas (old fields, airfields, habitation)**

Supplementary Figure 5b: Legend of the vegetation survey provided by Elephants Alive, conducted in the APNR. The survey is not specific to the location of the beehives.

Hives	Hive 1	Hive 1	Hive 1	Hive 2	Hive 4	Hive 5	Hive 6	Hive 7	Hive 8	Hive 9	Hive 10	Hive 11	Hive 12	Hive 13
Harvest	**December**	**January**	**March**											
Combretaceae	295	25	260	206	198	168	22	300	340	20	306	257	322	154
Harpephyllum caffrum	0	2	0	0	11	10	1	1	0	186	0	2	0	19
Sclerocarya birrea	4	43	26	0	66	16	0	0	0	104	0	4	0	0
***Vachellia* type**	1	8	0	0	8	0	0	0	0	8	0	2	0	0
***Senegalia* type**	3	2	0	3	20	1	0	0	0	7	0	8	0	0
Poaceae	0	0	18	89	0	29	312	28	5	4	11	15	7	91
***Protea* sp.**	0	0	11	0	0	15	0	0	0	0	0	0	1	2
Myrtaceae	0	0	5	0	0	1	0	0	0	0	0	0	0	15
Helianthus sp.	0	0	0	0	6	0	0	0	0	0	2	7	0	1
Tubuliflorae	0	0	0	7	1	2	9	0	0	0	0	0	0	2
Ambrosia	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Podocarpus/ Afrocarpus	0	0	1	0	0	1	0	0	0	0	0	0	0	0
Dombeya	0	0	0	0	0	0	0	0	0	0	0	0	0	0
***Hibiscus* sp.**	0	0	0	4	0	3	0	0	0	0	0	0	0	1
***Euphorbia* sp.**	0	0	9	0	11	4	5	0	0	13	7	17	3	4
***Myriophyllum* sp.**	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Gerbera	0	0	3	13	2	59	0	0	0	0	2	0	0	77
Lannea sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
***Alternanthera* sp.**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tribulus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Justicia decurrens	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Justica flava	0	0	0	0	0	0	0	0	0	0	0	0	0	0
***Dichrostachys* sp.**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Plectranthus	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Celastraceae	0	5	0	0	0	0	0	0	0	0	0	0	0	0
Phaeoptilum spinosum	0	0	0	0	0	0	0	0	0	0	0	0	0	0
***Grewia* spp.**	0	0	0	0	0	0	0	0	0	22	0	0	0	2
Commiphora	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Peltophorum africanum	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Salix	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Medicago sativa	0	225	0	0	0	0	0	0	0	0	0	0	0	0
Amaranthaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyperaceae sp.	0	0	0	0	0	0	3	0	0	0	0	0	0	5
Plantanus	0	0	0	0	0	8	0	0	0	0	1	0	0	1
Total	303	313	333	322	323	310	352	329	345	364	328	312	333	376

Supplementary Table 1A: Pollen counts from the December, January, and March honey harvest, referenced as S. Table 1A.

Hives	Hive 1	Hive 2	Hive 4	Hive 5	Hive 6	Hive 7	Hive 8	Hive 9	Hive 10	Hive 11	Hive 12	Hive 13
Harvest	**July**											
Combretaceae	75	436	70	458	200	585	199	110	511	510	445	490
Harpephyllum caffrum	0	3	2	15	18	5	0	54	0	2	3	5
Sclerocarya birrea	6	10	90	17	330	0	128	51	0	8	51	12
Vachellia type	0	1	0	0	5	0	0	2	0	0	0	0
Senegalia type	0	2	2	1	33	0	0	6	0	1	12	0
Poaceae	0	0	31	0	0	0	0	27	0	0	0	0
Protea sp.	1	0	0	0	0	0	0	3	0	0	0	0
Myrtaceae	0	0	0	0	0	0	0	0	0	0	0	0
Helianthus sp.	0	1	3	1	0	2	0	4	0	0	0	0
Tubuliflorae	0	0	0	0	0	0	0	25	0	0	1	0
Ambrosia	0	0	0	0	0	0	0	3	0	0	0	0
Podocarpus/ Afrocarpus	0	0	0	0	0	0	0	0	0	0	0	0
Dombeya	0	0	0	2	0	0	0	11	0	0	0	0
***Hibiscus* sp.**	0	0	23	2	0	1	0	26	0	0	0	0
***Euphorbia* sp.**	4	5	0	4	6	4	24	10	3	12	3	1
***Myriophyllum* sp.**	0	0	0	0	0	3	0	0	0	0	0	0
Gerbera	0	0	0	0	0	0	1	0	0	0	0	0
***Lannea* sp.**	360	37	2	0	0	0	13	2	12	4	0	1
***Alternanthera* sp.**	3	1	1	2	0	2	0	96	0	3	2	0
Tribulus sp.	14	2	0	9	7	10	4	9	2	2	0	13
Justicia decurrens	0	1	1	2	0	0	2	97	0	0	1	1
Justica flava	0	0	0	4	0	0	1	60	0	0	0	0
***Dichrostachys* sp.**	2	9	0	0	0	0	0	0	0	0	0	0
Plectranthus	0	0	0	0	0	0	0	0	0	0	0	0
Celastraceae	0	0	0	0	0	0	2	0	0	0	0	0
Phaeoptilum spinosum	0	0	0	0	0	0	2	22	0	0	0	0
***Grewia* spp.**	0	0	0	0	1	0	0	2	0	0	0	0
Commiphora	0	0	0	0	0	0	0	0	0	0	0	0
Peltophorum africanum	4	0	0	4	0	0	0	5	0	0	0	0
Salix	0	0	0	0	0	0	0	0	0	0	0	0
Medicago sativa	0	0	0	0	0	0	0	0	0	0	0	0
Amaranthaceae	0	0	0	0	0	0	0	23	0	0	0	0
Cyperaceae sp.	0	0	0	0	0	0	0	0	0	0	0	0
Plantanus	0	0	0	0	0	0	0	0	0	0	0	0
Total	469	508	225	521	600	612	376	648	528	542	518	523

Supplementary Table 1B: Pollen counts from the July honey harvest, referenced as S. Table 1B.

Hives	Hive 1	Hive 2	Hive 3	Hive 4	Hive 5	Hive 6	Hive 8	Hive 9	Hive 10	Hive 11	Hive 12	Hive 13
Harvest	**October**											
Combretaceae	310	11	62	350	134	81	70	33	121	19	150	22
Harpephyllum caffrum	23	3	0	6	8	0	15	5	50	92	5	0
Sclerocarya birrea	1	39	73	0	38	105	127	20	130	261	93	280
Vachellia type	0	0	0	0	0	0	1	0	0	1	0	0
Senegalia type	1	0	1	0	0	0	7	2	0	2	0	0
Poaceae	1	0	0	0	0	0	0	0	2	0	0	0
Protea sp.	0	0	0	0	0	0	0	0	0	0	0	0
Myrtaceae	0	0	0	0	0	11	0	0	0	0	0	0
Helianthus sp.	0	0	0	0	0	0	0	0	0	0	0	0
Tubuliflorae	0	0	0	0	0	0	0	0	0	0	0	0
Ambrosia	0	0	0	0	0	0	0	0	0	0	0	0
Podocarpus/ Afrocarpus	0	0	0	0	0	0	0	0	0	0	0	0
Dombeya	0	0	0	0	0	0	0	0	0	0	0	0
***Hibiscus* sp.**	0	0	0	0	0	0	0	0	0	0	0	0
***Euphorbia* sp.**	1	2	15	0	5	7	0	0	3	20	18	0
***Myriophyllum* sp.**	0	0	0	0	0	0	0	0	0	0	0	0
Gerbera	0	0	0	0	0	0	0	0	0	0	0	0
***Lannea* sp.**	3	0	0	4	0	0	0	0	1	2	0	0
***Alternanthera* sp.**	0	0	0	0	0	0	0	0	0	0	0	0
Tribulus sp.	0	0	0	0	0	0	0	0	0	0	0	0
Justicia decurrens	0	0	0	0	0	0	0	0	0	0	0	0
Justica flava	0	0	0	0	0	0	0	0	0	0	0	0
***Dichrostachys* sp.**	0	0	0	0	0	0	0	0	0	0	0	0
Plectranthus	0	0	0	0	0	0	0	0	5	5	1	0
Celastraceae	0	0	3	0	0	5	0	2	2	0	0	0
Phaeoptilum spinosum	0	0	0	0	0	0	0	0	0	0	0	0
***Grewia* spp.**	0	5	15	0	1	0	0	0	0	0	1	0
Commiphora	0	0	3	0	0	0	0	3	6	0	0	0
Peltophorum africanum	0	0	0	0	0	0	0	0	0	0	0	0
Salix	0	5	1	0	1	0	0	3	6	0	1	0
Medicago sativa	0	0	0	0	0	0	0	0	0	0	0	0
Amaranthaceae	0	0	0	0	0	0	0	0	0	0	0	0
Cyperaceae sp.	0	0	0	0	0	0	0	0	0	0	0	0
Plantanus	0	0	0	0	0	0	0	0	0	0	0	0
Total	340	65	173	360	187	209	220	68	326	402	269	302

Supplementary Table 1C: Pollen counts from the October honey harvest, referenced as S. Table 1C.

Sediment Number	S2	S3	S5	S6	S7	S8	S10	S11	S12	S13	S14	S15
Collection Date	October											
Combretaceae	2	5	5	1	38	25	10	43	9	39	20	18
Sclerocarya birrea	5	10	7	0	73	26	3	4	9	30	14	6
Senegalia type	1	0	3	4	3	0	0	1	0	0	2	3
Poaceae	0	14	25	4	7	4	84	20	33	4	20	23
Morella sp.	0	1	0	0	1	0	1	0	1	3	2	2
Tubuliflorae	0	0	0	0	0	0	0	0	0	1	0	0
Ambrosia	0	0	0	1	0	1	0	0	0	1	0	2
Pinus	1	3	1	4	2	12	8	25	25	5	18	13
Dichrostachys sp.	0	0	5	0	0	0	0	0	0	0	0	7
Dombeya sp.	0	0	0	0	0	0	1	1	0	0	2	0
Hibiscus sp.	0	0	0	0	0	0	0	0	0	0	1	0
Euphorbia sp.	0	2	2	0	6	0	0	0	0	1	0	0
Protea sp.	0	0	0	1	0	0	0	0	0	0	0	0
Gerbera	0	0	0	0	2	0	0	0	0	0	1	0
Alternanthera sp.	1	0	1	0	0	1	6	0	1	1	1	0
Justicia decurrens	1	0	0	0	0	0	0	4	0	3	1	0
Justica flava	0	0	1	0	0	1	0	0	0	3	0	0
Dichrostachys sp.	0	0	0	0	0	0	0	0	0	4	1	0
Plectranthus	0	0	0	0	0	1	1	2	2	4	0	0
Celastraceae	0	0	0	0	0	0	0	0	1	0	5	0
Phaeoptilum spinosum	0	0	0	0	0	0	0	0	0	0	4	0
Grewia spp.	0	2	0	0	1	0	1	0	0	1	11	0
Commiphora	0	0	0	0	0	0	0	0	0	0	0	0
Peltophorum africanum	0	0	0	0	0	0	0	0	0	0	0	0
Medicago sativa	0	0	0	0	4	3	0	0	0	0	0	0
Amaranthaceae	0	0	9	5	7	3	5	3	8	4	6	3
Cyperaceae sp.	0	0	0	0	7	0	1	1	0	6	7	0
Harpephyllum caffrum	0	0	0	0	0	5	0	0	0	0	0	1
Ambrosia	0	0	7	0	0	0	0	0	5	0	7	0
Px19	0	0	0	12	0	0	0	0	0	0	0	0
Px18	1	0	0	0	0	0	0	0	0	0	0	0
Px20	0	0	0	0	0	16	8	0	0	1	4	0
Heteromorpha-type (Apiaceae)	0	0	0	0	3	3	2	2	0	0	5	3
Total	12	37	66	32	154	101	131	106	94	111	132	81
Lycopodium spores	11	15	14	17	35	40	27	44	25	13	81	11

Supplementary Table 1D: Pollen counts from the October surface sediment samples, referenced as S. Table 1D.

Acanthaceae	[Photo reference]

Species and author-name: *Justicia flava,* (Vahl) Vahl (House and Balkwill 2017) **[Figure 6.2 I-J]**

Morphological description: Tricolporate (Gosling et al. 2013), obtuse-triangular in polar view due to the 3-apertures and 3- sides with an equatorial diameter of 40-50 µm (own observation). The exine is formed by arching rays supported by columellae, the structure is distinct in this genus (House and Balkwill 2017).

Growth form: Slender shrub is up to 2.5m in height (Schmidt et al. 2002)

Flowering period: January to December (Johannsmeier 2016)

Ecology: *Justicia flava* is distributed mostly in soils or rocky outcrops of the grassland and woodland biomes, further distributed from coast to the bushveld in South Africa (Johannsmeier 2016). *Justicia flava* is also along drainage lines in Afrotemperate Forest, along forested riverbanks, riverine forest and thickets in the Mpumalanga and Limpopo Province (Schmidt et al. 2002).

Pollinator: The bees that mainly pollinate this plant family are the carpenter bee *Xylocopa* cf*. inconstans* and the honeybee *Apis mellifera* (Padysakova et al. 2013). *Justicia flava* is also pollinated by insects and various species of butterflies (Froneman 2008).

Additionally, Acanthaceae is visited by potential pollinators like the long-proboscis flies, some sunbirds, butterflies, flies, bees, and moths in a study area from Bamenda Highlands, Cameroon (Riegert et al. 2011, Bartos et al. 2011).

Bee plant value: N1-2, P0-2? (Johannsmeier 2016)

Species and author-name: *Justicia decurrens*, Imlay 1938 (Hansen 1989) **[Figure 6.4 F]**

Morphological description: Tricolporate pollen, bilaterally symmetrical, circular to oval shape in polar view and oval shape in the equatorial view (Rueangsawang et al. 2013). The equatorial diameter is c. 20 µm and the polar diameter is c. 30-35 µm (own observation).

Growth form: Slender shrub to 2.5m (Schmidt et al. 2002)

Flowering period: November to April (Schmidt et al. 2002)

Ecology: Along drainage lines in Afrotemperate Forest, along forested riverbanks, riverine forest and thickets in the Mpumalanga and Limpopo Province (Schmidt et al. 2002).

Pollinator: The bees that mainly pollinate this plant family are carpenter bee *Xylocopa* cf*. inconstans* and Apis *mellifera* (Padysakova et al. 2013).

Bee plant value: N1-2, P0-2? (Johannsmeier 2016)

Amaranthaceae

Species and author-name: Amaranthaceae, Erdtman 1952, also labelled as to Amaranthaceae in Scott et al 1982 **[Figure 6.4 L]**

Morphological description: The pollen grain is pantoporate with perforate (with holes) tectum, the pores are distinctly convex (Eliasson 1988). The apertures are small in size (1 µm) and numbers but are mostly more than 6 apertures for this type, 36 apertures where counted (own observation) and they are covered with granular structures of the sexine (compare Eliasson 1988).
Growth form: Green, leafy vegetable plant grows up to 20cm tall (Department of Agriculture, Forestry and Fisheries 2010).
Flowering period: October to June (Johannsmeier 2016)
Ecology: Distributed in the savanna and riverine forests of southern Africa (Johannsmeier 2016).
Pollinator: Amaranthaceae are wind pollinated (Müller and Borsch 2005, Rohwer et al 1993)
Bee plant value: P0-2?, N0-2?, most species of the family are seldomly visited by bees (Johannsmeier 2016).

Species and author-name: *Alternanthera* sp., Erdtman 1952 **[Figure 6.4 C]**
Morphological description: The pollen grain is pantoporate with perforate (with holes) tectum, the pores are distinctly convex (Eliasson 1988). The apertures vary in size (0.5 µm) and numbers but are mostly more than six (more or less 6 apertures for this type (own observation)) and they are covered with granular structures of the sexine (Eliasson 1988).
Growth form: Amaranthaceae herb grows up to 20cm tall (Department of Agriculture, Forestry and Fisheries 2010).
Ecology: *Alternanthera* sp. Is a neophyte from South and Central America is distributed throughout the central and eastern regions of South Africa (Johannsmeier 2016).
Pollinator: Amaranthaceae are wind pollinated (Müller and Borsch 2005, Rohwer et al 1993).
Bee plant value: P0-1, N0-2, the species is rarely visited by bees in hot and moist weather conditions (Johannsmeier 2016).

Apiaceae

Species and author-name: *Heteromorpha* sp. Cham and Schlechtd (Winter 1994) **[Figure 6.5 H]**
Morphological description: Tricolporate, shape is oval to oblong with a length to width ration of c. 1.8m to 2.2m (Winter 1994). with a slightly rugulose surface sculpturing (Winter 1994, Erdtman 1969), which look more reticulate of these samples (Own observation).
Growth form: Multi-stemmed shrub to medium sized tree (Johannsmeier 2016)
Flowering period: September to May (Johannsmeier 2016)
Ecology: Found in rocky bushveld of Southwestern Cape to KZN and Free State (FS) provinces of South Africa (J). South Africa is the origin of the predominantly herbaceous Apiaceae subfamily Apioideae (Calvino et al. 2006).

Pollinator: The species rely on self and cross pollination, Apiaceae species are monomorphic meaning that all flowers are staminate and other bisexual (Koul et al. 1993). The male flowers in andromonoecious species produce pollen of which only small part is used in pollinating bisexual flowers, the rest is used as reward to insect pollinators visiting the flowers (Koul et al. 1993).

Bee plant value: P1, N2 (Johannsmeier 2016)

Burseraceae

Species and author-name: *Commiphora* sp., Harley, and Hall 1999 (Harley et al. 2005)

[Figure 6.1 K-L]

Morphological description: Isopolar, tricolporate, the pollen shape is suboblate, spheroidal with a reticulate sculpture, coarsely reticulate with shallow lumina (Harley et al. 2005). Pollen size: polar length c. 17 – 46 µm, equatorial width c. 14 – 40 µm (Harley et al. 2005) and c. 30 µm diameter (own observation).

Growth form: Usually a shrub of 1m rarely a small tree up to 4m (Schmidt et al. 2002).

Flowering period: September to March (Schmidt et al. 2002)

Ecology: *Commiphora* is the only genus of the family Burseraceae in South Africa (Van Wyk 2013). There are about 12 taxa distributed in the KNP, the most dominant is *Commiphora africana* and *Commiphora harveyi* (Schmidt et al. 2002). Species are conspicuous in arid bushveld and semi-desert areas (Schmidt et al. 2002). Distributed in hot dry Lowveld on sandy and rocky outcrops throughout northern Botswana, Namibia, and extreme northern parts of South Africa (Schmidt et al. 2002).

Pollinator: Insect pollinated, including bees, wasps, and hover flies (Farwig et al. 2004).

Bee plant value: Bee plant value not provided but *Commiphora* sp. is mentioned as a bee plant of South Africa (Johannsmier 2005 and Henderson 2001).

Cyperaceae

Species and author-name: Cyperaceae

Morphological description: Monoporate (porus at the base, vaguely visible), Oblate spheroidal shape and has a diameter of 30 - 35 µm (own observation), lacunae, scabrate ornamentation (Nagels et al. 2009).

Growth form: Herbs (Browning 1989) about 42 cm in height (Bir et al. 1992)

Flowering period: October to April (Browning 1989), January to December (Johannsmeier 2016)

Ecology: In southern Africa the family it is not confined to coastal dunes, river mouths and estuaries, it is also distributed inland in rivers and streams beds, or marshes which are particularly in the northwestern region of the Cape Province (Browning 1989). Cyperaceae in southern Africa are commonly associated with wetlands and other temporarily moist areas (Gordon Gray 1995),

however, some species from the family occur in drier areas like grasslands and savanna biome (Stock et al. 2004).

Pollinator: Anemophilous and entomophilous (Ambophily), the tribe is believed to be predominantly wind-pollinated but contains a few species that appear to have made reversals to insect-pollinated through modification of the inflorescences (Buddenhagen et al. 2017).

Bee plant value: N0, P0-2 (Johannsmeier 2016)

Haloragaceae

Species and author-name: *Myriophyllum* sp.*,* Moar and McGlone 2011.

Morphological description: Spheroidal pollen grain that has 5 zonocolpi with a 25-30 µm long polar axis and an equatorial diameter of 25- 32 µm, reticulate sculpture (Al-Saadi and Al-Mayah 2012).

Growth form: Rooted aquatic plant with terminal leafy shoots emerging above the water surface (Henderson 2001). The emergent shoots are 0.2 to 0.5 m above the water surface and the plants stems can be 1.5 to 3 m long (Cilliers 1999).

Flowering period: September and February (Weyl 2015)

Ecology: Aquatic plant, wetland invasive species, neophyte from Europe, Asia, and North Africa (Mood and Les 2002). Spread across rivers in Gauteng (Bronkhorstspruit River) and KZN (Mooi River etc), including Zimbabwe (Zambezi River) and other South African provinces (Weyl and Coetzee 2014). Two species of the genus *Myriophyllum* occur in South Africa; *Myriophyllum aquaticum* (Vell.) (parrot's feather) is an emergent species, whereas *Myriophyllum spicatum* L. (*M. spicatum* L.) (spiked watermilfoil, Eurasian watermilfoil) is a submerged plant with the inflorescence only emergent for a short period when flowering (Cilliers 1999).

Pollinator: Water is the main vector of pollination (Harris et al. 1992, Sculthorpe 1967), but they are also transported by wind, insects, or gravity when their flowers grow above the water level (Harris et al. 1992).

Lamiaceae

Species and author-name: *Plectranthus* **[Figure 6.4 G-H]**

Morphological description: Spheroidal, hexa-zonocolpate with a rough reticulate surface. Polar diameter of c. 32µm and equatorial diameter of c. 34µm Doaigey et al. 2018), diameter of 35 µm (own observation) Erdtman 1945 (Doaigey et al. 2018).

Growth form: Upright, soft shrub to 3m (Schmidt et al. 2002)

Flowering period: January to June (Schmidt et al. 2002, Johannsmeier 2016)

Ecology: Found in the understorey of montane or riverine forest, or in scrub on rocky slopes. Distributed in the WC, EC, KZN, Swaziland to northern South Africa (Schmidt et al. 2002). Attractive plants for shady gardens (Schmidt et al. 2002).

Pollinator: Entomophilous, nemestrinid flies of the genus *Stenobasipteron*, acrocerid flies, tabanid flies and anthophorid bees (Potgieter et al. 1999).

Bee plant value: N0-3?, P0-2? (Johannsmeier 2016)

Species and author-name: *Triumfetta* sp. (Perveen et al. 2004)

Morphological description: Isopolar, polar diameter 37-52m and equatorial diameter 22-31, prolate and tricolpate with a coarsely reticulate sculpture (Perveen et al. 2004).

Growth form: Forb (Siebert *et al* 2020), shrub up to 2.5 m (Schmidt et al. 2002)

Flowering period: December to May (Johannsmeier 2016)

Ecology: Distributed along the semi-arid Lowveld savanna of the Gazankulu area (now Limpopo province) of South Africa (Siebert et al. 2020). On forest margins, disturbed roadsides and in grasslands of the EC, Gauteng, Limpopo Province into Zimbabwe (Schmidt et al. 2002).

Pollinator: Entomophilous, pollinated by honeybee (Johannsmeier 2016)

Bee plant value: N1-2?, EN, P1-3 (Johannsmeier 2016), according to Johannsmeier, only afternoon foraging on *Triumfetta* sp. by honeybees has been. Additionally, extrafloral nectar taken from young leaves of *T. pentandra* and pollen of *T. sonderi* constituted 27% of the total pollen trapped in extrafloral nectar for January in the bushveld north of Pretoria (Johannsmeier 2016).

Nyctaginaceae

Species and author-name: *Phaeoptilum spinosum,* Nair 1965 **[Figure 6.3 C-D]**

Morphological description: 3- zonicolpate, sub-oblate to spheroidal, reticulate sculpture (Tripathi et al. 2017). Large pollen grain with a diameter of 88 µm (own observation) (Tripathi et al. 2017).

Growth form: Large scrambling, spiny shrub, or tree 1-2m (Schmidt et al. 2002).

Flowering period: August to September (Schmidt et al. 2002), August to January (Johannsmeier 2016)

Ecology: In low-lying dry areas in the bushveld and along riverbanks. Typically, a Karoo species, widespread in the dry western regions of southern Africa and the northernmost parts of the KNP (Schmidt et al. 2002).

Pollinator: Entomophilous, pollinated by bees during the afternoon and morning but moths are the most effective pollinators during the night (Cruden 1973).

Bee plant value: N?, P? (Johannsmeier 2016), the plants small white/ yellow flowers are known to be visited by bees but the bee plant value is unknown (Johannsmeier 2016).

Pinaceae

Species and author-name: *Pinus* sp. **[Figure 6.5 C]**

Morphological description: Bisaccate monad pollen grain with a think exine, the exine patterns are regulate on the corpus (diameter of 20 - 35µm, own observation) but smooth on the sacci (diameter of 10-25µm from the corpus, own observation) (adapted from Nakagawa et al 2000).

Growth form: Tree that is 25 to 60m tall (Britannica 2020)

Flowering period: June to November (Johannsmeier 2016)

Ecology: The species was introduced into South Africa in the 1920's from Central America, and it is currently predominant moist summer rainfall areas in Mpumalanga and KZN, as well as on a smaller scale in EC province (Nyoka 2003). The genus was introduced earlier since c. 300 years in eastern South Africa (Neumann et al. 2011). The species thrives on a wide variety of soils including those derived from dolomite, dolerite, quartzite, granite and sandstone (Nyoka 2003).

Pollinator: *Pinus* species are wind-pollinated (Williams 2009)

Bee plant value: N0, P0-2 and HD0-2, pollen grains are released in masses from the male cones and honey dew is scarcely collected if there are more attractive winter-flowering eucalyptus nearby. Pollen is only collected if a nectar source is available simultaneously this might be due to the low (7-13%) crude protein content of the species (Johannsmeier 2016).

Podocarpaceae

Species and author-name: *Podocarpus*/*Afrocarpus*

Morphological description: Bisaccate monad pollen grain with a diameter ranging from 40 to 45 µm. The sculpture of the corpus (pollen body) is rugulate and the sacci (air sacs) have a reticulate surface sculpture (Song et al. 2012).

Growth form: Small to a large evergreen tree up to 33m tall (Schmidt et al. 2002)

Pollen production period: Conifer plant male cones produce pollen between July and September and female cones which do not produce pollen flower between December and February (Schmidt et al. 2002).

Ecology: In Afrotemperate forest and bush clumps on rocky outcrops. Distributed in the WC, northwards into northern provinces of South Africa (Schmidt et al. 2002). *Podocarpus latifolius* is the dominant type in the northern provinces of South Africa (Schmidt et al. 2002).

Pollinator: Anemophilous, wind and water transported pollen due to its saccus morphology that supports the pollen buoyancy (Runions et al. 1999).

Bee plant value: -

Proteaceae

Species and author-name: *Protea*/*Faurea* sp., Baker 1911 **[Figure 6.2 E-F]**

Morphological description: Triporate with a porus shape that is elliptic (tall), reticulate sculpture (Gosling et al. 2013, Scott 1982).

Growth form: small to large tree to 10m and even 25m (Schmidt et al. 2002)

Flowering period: Winter and spring (June - October), (Heelemann et al. 2008), April to September (Johannsmeier 2016). *Faurea* sp. flowers from August to January (Johannsmeier 2001).

Ecology: Proteaceae are widespread in the Bewaarkloof mountains, Transvaal and throughout the savanna bushveld and sandstone sour veld of the Limpopo province and KZN respectively (Rutherford et al. 2006). Proteaceae (which includes the genus *Faurea*) is distributed in the woodland and wooded grasslands but it is dominant in the fynbos biome of South Africa (Heelemann et al. 2008, Schmidt et al. 2002). *Fauea saligna* is found in the Bushveld and Lowveld at the northern provinces of South Africa (Johannsmeier 2001).

Pollinator: Entomophilous, insect-pollinated, long-proboscid flies and butterflies (Johnson et al. 2012).

Bee plant value: N0-3, P0-2 (Johannsmeier 2016), honey is light golden/dark in colour while the nectar is diluted by rain which may lead to fermentation (Johannsmeier 2016).

Salicaceae

Species and author-name: *Salix mucronata*, Thunb. (Shamso and Toshiyuki 2012) **[Figure 6.4 I]** (could also be *Salix babylonica* (invasive species) since the pollen morphology is very similar (pollen morphology ref: Qureshi et al. 2007)

Morphological description: Radially symmetrical tricolpate, prolate pollen grain with a diameter of c. 20 µm. The exine ornamentation is reticulate with lumina and muri which varied in shape and size (Maciejewska-Rutkowska et al. 2021).

Growth form: Shrub or tree up to 10m (Schmidt et al. 2002).

Flowering period: August to September (Schmidt et al. 2002), August to October (Johannsmeier 2016)

Ecology: This southern African native species is distributed along running streams and rivers in the highveld in Mpumalanga, Gauteng, KZN, Limpopo, FS and North-West Province (Schmidt et al. 2002).

Pollinator: Anemophilous and entomophilous, visited by flies and small bees (Andrenidae and Tenthredinidae) were observed on sunny and calm days. These insects seemed to be attracted by the nectar of both male and female catkins and pollen of male catkins (Tamura and Kudo 2000).

Bee plant value: N?, P1 (Johannsmeier 2016), the plant is usually not attractive to bees, but it has been reported by Mr J.R Dias of Oudshoorn 1924-2019, that during September the trees in the river are strongly worked for pollen and nectar (Johannsmeier 2016).

Supplementary Table 2: An extended list of Table 4 with all the rare pollen taxa found in the December, January, March, July, October honey samples and October surface sediment samples. The pollen morphology, flowering period, ecology, bee-plant values, and the pollinators of the plants have been documented for each taxon. S. Table 2 is a full extension of Table 4.