Dietary shifts in pollen-feeding lacewings (Nemopteridae) in relation to vegetation, biome and phylogeny.

Yolanda Chirango

CHRYOL001

Supervised by: Associate Professors Lindsey Gillson and Mike Picker

Presented for BSc(Honours) 2014

Biological Sciences

Faculty of Science

University of Cape Town

Abstract

The spoon-winged and threadwinged lacewings are a diverse nemopterid family that have flourished in the winter rainfall region of South Africa. Their diversity is thought to be linked to the radiation of Aizoaceae, a member of the Ruchioideae subfamily that radiated at the same time as the recently diversified nemopterids in the Succulent Karoo. Phylogenetic data supported this assertion, and seperated the family into two distinct lineages, a recently diversified Aizoaceae-dependent Succulent Karoo lineage and a basal Aizoaceae-independent Fynbos lineage. This study aimed to determine if the nemopterid diet is phylogenetically contrained or if diet was mearely a reflection of plant availability. This was investigated by carrying out a dietary analysis of the gut contents of nemopterids from different biomes, vegetation and localities. It was hypothesised that diet would be phylogenetically constrained and dietary grouping would reflect phylogeny. In addition, the derived nemopterid lineage thought to have co-evolved with Aizoaceae, (*Palmipenna*, *Nemopterella*, *Nemia,* and *Knersvlaktia*) would reflect this in its diet. The older lineage (*Nemeura, Sicyoptera*, *Semirhynchia*, and *Derhynchia*) was hypothesised to have its phylogenetic grouping reflected in its diet by having very little to no Aizoaceae in its diet. The results showed that dietary groupings did not reflect phylogenetic groupings and dietary similarities were shared across most genera, including subfamilies, with *Lauhervasia*, a member of the Crocinae subfamily sharing 80% of its pollen sources with *Semirhynchia*, of the Nemopterinae subfamily. Aizoaceae reliance was only consistantly present in *Nemia*, which belonged to the phylogenetic lineage expected to rely on Aizoacea. All the members of the phylogenetic lineage not expected to rely on Aizoaceae, did not have any Aizoaceae in their diet. The diet of the nemopterids was very diverse across all vegetation types and biomes. The study suggests that spoon-winged and thread-winged lacewings are generalist pollinators and recent diversification was most likely linked to their ability to utilise the large range of available resources and not linked to a single plant family that radiated around the same time.

Introduction

Insects are the most diverse group of organisms on Earth with an estimated diversity of 30 million species (Erwin 1982). Their success is mainly associated with their incredibly broad diet which has evolved to adapt to a wide range of habitats and niches (Janz *et al*. 2006). Insects are made up of carnivores, herbivores and scavengers, among these are generalists as well as a wide range of specialist guilds (Levins & MacArthur 1969; Jaenike 1990; Gripenberg *et al.* 2007; Craig & Itami 2008). A large proportion of insects are plant feeders (Craig and Itami 2008). Within this group alone lies a variety of specialisations including leaf-miners, fructivores, leaf-chewers, sap-suckers, xylophages, root-feeders and pollenophages (Novotny and Basset 2005). This diversity provides a reflection of plant-insect associations which account for 40% of the global terrestrial biodiversity (Price 2002). The sources of nourishment and their broadness or restrictiveness give insights into the evolutionary history of the insects, and is also influenced by environment (Fox and Lalonde 1993; Craig and Itami 2008). However, it is a challenge to determine if a diet is phylogenetically constrained or if it is merely a reflection of food availability.

Deciphering the diet of insects can involve direct field observations, insect gut dissections and more recently DNA analysis of insect gut contents (Monserrat 1985; Picker 1987; Sunderland *et al.* 1987; Basset 2001; Hoogendoorn and Heimpel 2001; Matheson *et al.* 2008; Jurado-Rivera *et al.* 2009). The diet can then be used to extrapolate whether insects are specialists or generalists, whether they rely on food sources that are only available at a certain time or place and how that limits or facilitates their ability to disperse into other areas (Bernays and Chapman 1994). As a result, insects may adopt ways to coincide their emergence or breeding with their main food source (Chew 1977; Crocin *et al*. 2001). Dietary studies therefore provide a diverse array of information including aspects of range limitation, phenology and phylogeny of the study species.

Insect diversity can occur in equally diverse localities (Janz *et al*. 2006). Such is the case in the Greater Cape Floristic Region (GCFR) (Vernon 1999). The GCFR is an internationally renowned biodiversity hotspot (Myers *et al.* 2000). The area shows exceptional endemism and species richness in both flora and fauna (Cowling & Hilton-Taylor 1997; Goldblatt *et al.* 2002; Born *et al.* 2007). Consisting of the Cape flora in the south-west and the succulent karoo in the north-west and east, the region is bounded by the Atlantic and Indian Oceans in the west, south and east (Cowling *et al.* 1999). The northern and north-eastern borders are formed by the transition from winter-rainfall to summer-rainfall climate as well as a decline in rainfall amount. The region, with an estimated species endemism of 77.9%, comprises of Fynbos, Renosterveld, Thicket, Succulent Karoo and Afrotemperate Forest, within which there are 11 423 species (Bergh *et al.* 2014). The faunal diversity, which has received much less attention than the plants, includes the largest group of endemic snakes and lizards in southern Africa (Bauer 1999), with the mesic winter rainfall area of the GCFR being globally renowned for its amphibian diversity. It has two endemic amphibian genera, and 16 endemic species out of the 40 in the area (Poynton & Broadley1978). The CFR is relatively poor in terms of bird diversity but has seven endemic species. However when combined with the succulent and Nama karoo, the GCFR boasts the largest regional concentration of endemic birds in Africa (Siegfried 1992). On par with the plant diversity is the immense invertebrate richness and endemism, dominated by insects (Wright & Samways 1998; Proches & Cowling 2006). The causes of this exceptional diversity in all forms are highly disputed and are the centre of several studies (Levyns 1964; Axelrod & Raven 1978; Linder *et al.* 1992; Linder 2003, 2005; Cowling *et al.* 2009).

The richness of the Cape Flora is largely the result of radiations (Linder and Hardy 2004). Much of the extant diversity of the Cape flora is thought to have evolved during the Late Miocene and Pliocene (Goldblatt & Manning, 2002; Verboom *et al.* 2009; Dupont *et al.* 2011). The extreme radiation during the Late Miocene is linked to the development of a winter-rainfall climate along the west coast of southern Africa as well as aridification (Levyns, 1964; Linder *et al.* 2010). Aridification has been linked to climatic changes in Antarctica, the aridity of the Namib desert and the development of the Benguela Current and Benguela Upwelling System (Siesser 1980; van Zinderen Bakker 1984; Linder *et al*. 2010,). These climatic changes in the presence of the region’s topographical complexities and wildfires are thought to have led to the extinction of a previous tropical flora, which allowed the Cape floral elements to radiate into these newly vacated niches (Linder *et al*., 1992, 2010; Verboom *et al*. 2009; Wilson *et al*. 2010; Dupot *et al* 2011; Schnitzler *et al*. 2001). It is thought that the vertebrate and invertebrate diversity followed. This is highly apparent in the insect diversity, particularly the pollinators (Johnson 1996, 2010; Vernon 1999).

Studies suggest that the plants and pollinators co-evolved in the GCFR, hence the matching diversity, however it is unclear whether plants or insects (or both) were the driver (Goldblatt and Bernhardt 1990; Steiner 1991; Johnson 1991, 1996, 2010). Pollinator-driven differentiation is reflected by the diversity of flower forms in the Cape. Such floral diversity is a prominent feature within large genera such as Erica (Ericaceae), Pelargonium L'Her. (Geraniaceae), Geissorhiza Ker Gawl., Gladiolus L., Lapeirousia Pourr., Watsonia Mill., Tritoniopsis L. Bolus (all Iridaceae), Disa P. J. Bergius and Satyrium Sw. (Orchidaceae) (Vogel 1954; Linder 1981; Goldblatt, 1991; Oliver, 1991). This is further supported by intraspecific variation in floral characters (Johnson 1996). For example; *Disa ferruginea* flowers have varying spur lengths and colour, allopatric *Disa tripetaloides* exist in yellow, white and red forms, *Hesperantha falcata* is made up of white-scented and yellow-unscented forms, while *Gladiolus maculatus* comes in brown-scented and reddish-unscented forms (Johnson 1996, Goldblatt 1991, Linder 1981). Artificial breeding of plants has demonstrated that floral characters can be rapidly modified through selective pollination (Gill 1989). Johnson (2010) suggests that adaptation to pollinators has contributed to the diversity in the Cape flora.

The Cape flora, particularly the Fynbos biome, was considered to be insect poor (Johnson 1996; Johnson & Bond 1997). However, Proches and Cowling (2006) found that fynbos has similar insect diversity values as grassland, subtropic thicket and higher values than the Nama-karoo. Several studies highlight that the region is a major centre of radiation for some insect groups, and in some cases contains the highest diversity within groups globally such as monkey beetles (Scarabaeidae: Hopliini), oil collecting bees(Melittidae), bee flies (Bombyliidae), tangle-winged flies (Nemestrinidae) and masarid wasps (Masaridae) (Picker & Midgley 1996, Steiner and Whitehead 1991, Hesse 1938; Struck 1994, Manning & Goldblatt 1996, Gess & Gess 1989, Mansell 1996, Sole *et al.* 2013). Lacewings, which have received much less attention in Africa, are also concentrated here with several endemic genera in the GCFR (Sole *et al*. 2013)

The lacewing family, Nemopteridae, has an extraordinary appearance, easily distinguished by remarkably specialised hindwings which are thin and very elongated The Nemopteridae include about 150 species (Aspöck & Aspöck 1999; Aspöck *et al*. 2001) in the Mediterranean, arid, semi-arid and desert regions of the world (Mansell 1996; Monserrat 1996). South Africa alone contains 72 of these species (Sole *et al*.

2012). The species feed exclusively on pollen and nectar using their elongated chewing mouthparts (Tjeder, 1967; Popov 1973). The family has been divided into two subfamilies, the Nemopterinae (spoon-winged lacewings), have ribbon-like hind wings which are thought to have an aerodynamic function by providing stability during flight as well as camouflage when at rest (Mansell 1996). Leon and Picker (1990) showed that the hindwings deter predators. This subfamily is diurnal and has elongated mouthparts with a tearing function evolved in response to its specialist pollen diet (Popov 1967, 1973; Tjeder 1967; Mansell 1986). However the diet has been poorly studied with the exception of the European *Nemoptera sinuata* (Popov 1973). This study showed that the species feeds exclusively on pollen by tearing off pollen bags in *Achillea* flowers and directly feeding on the pollen with its rostrum or collecting pollen with its tarsi and inserting the pollen filled tarsi into its mouth. Popov also confirmed its pollen diet through gut and excrement assessments in his study. The foregut was filled with whole pollen grains while the hindgut and excrement contained the shells of the pollen grains highlighting the lacewings' ability to digest the interior pollen grain. Tjeder (1967) also found pollen in some nemoptrid species' guts, however his studies focused on taxonomic descriptions of lacewings and not on diet.

The other subfamily is the Crocinae which was initially thought to be less advanced (Tjeder 1967) but is now considered to be the more derived subfamily (Holzel 1975, Mansell 1986, Sole *et al.* 2013). The Crocinae have distinct apomorphies such as reduced body size, filamentous hindwings in adults and their larvae have elongated prothoraxes suggesting that they are more advanced and therefore evolved from the Nemopterinae (Holzel 1975, Mansell 1986). The thread-like hindwings are thought to have a sensory function in their rock overhang and shallow cave habitat. This sub-family's diet is unknown although it is suggested that they are also pollen feeders due to the similarities between their mouthparts and those of the Nemopterinae (Tjeder 1967). Nemopterinae have a few species in the South African summer rainfall regions but most occur in the Fynbos and Succulent Karoo biomes dominated by Aizoaceae and Asteraceae (Cowling and Hilton-Taylor, 1997). Of the 72 species found in South Africa, 52 are confined to the Western and Northern Cape Provinces of South Africa (Sole *et al.* 2013).

According to a phylogenetic study carried out on southern African spoon-winged lacewings, Nemopterinae is estimated to be about 145.6 My old (Sole *et al*. 2013). However most of the genera appear to have diversified 44 – 11 Mya with recent rapid divergence of several genera occuring in the late Mioene (4.5 Mya). The authors suggested that the most recent radiation of Nemopterinae may be linked to Ruchioideae (Aizoaceae) which also diversified around this time (3.8 – 7.8 Ma) (Sole e*t al.* 2013) and have their highest degree of diversity concentrated in the same area as the derived nemopterids. Sole *et al*. (2013) hypothesised that the derived nemopterids in the Succulent Karoo should have a diet that is rich in Aizoaceae if the two co-evolved. The phylogeny used *Lauhervasia* of the Crocinae subfamily as well as Neomelambrotis as outgroups and showed the Nemopterinae as a monophyletic group with two distinct lineages. The first lineage (1) consisted of *Palmipenna*, which was the sister group of *Nemopterella* and *Nemia, Barbibucca, Knersvlaktia,* and *Halterina*. They made up the Succulent Karoo lineage expected to have a high reliance on Aizoaceae. While lineage (2) was made up of *Nemeura, SicyopteraSemirhynchia, Derhynchia,* the newly discovered *Gen. & sp. nov.*, as well as the Australian *Chasmoptera*. The second Nemopterinae lineage was described as the more basal Fynbos group expected to have less reliance on Aizoaceae. In this second lineage the species constituting *Nemeura, Sicyoptera* and *Semirhynchia* formed a polyphyletic complex showing variation within the individual genera. Although no morphological characters were used in the Sole *et al*. phylogeny, it was noted that the distinguishing feature between the two Nemopterinae lineages was the size of the abdomens. The genera in the lineage (1) all had short and stout abdomens while lineage (2) was made up of genera with long slender abdomens.

However, the diet of the African Nemopterids has not been directly studied. The aim of my study was to determine if diet is phylogenetically conserved and investigate the notion that certain nemopterid lineages are linked to Aizoaceae radiation. In the current study, pollen in the guts of lacewings from different localities, mainly from the fynbos and succulent karoo was studied and related to the phylogenetic clades presented in Sole *et al*. (2013). The following hypotheses were tested:

- Dietary similarity reflects phylogeny (i.e. diet is phylogenetically constrained).
- The Succulent Karoo clade diversification is linked to Aizoaceae radiation and Aizoaceae will therefore be reflected as a dominant item in their diet. Assuming that diet is phylogenetically contrained, Aizoaceae pollen should be more prevalent in their

guts compared to other pollen types. If diet is not phylogenetically constrained.

- In addition, the basal Fynbos clade which evolved before Aizoaceae diversification will retain the ancestral diet which had no reliance on Aizoaceae. Less Aizoaceae pollen should be present in their guts and instead fynbos elements should be more abundant.
- As the derived Crocinae are widespread they will have a wide dietary range that is also not restricted to Aizoaceae.
- Alternatively, if diet is not phylogenetically constrained it is determined by availability/ locality, and dietary similarity will reflect locality / vegetation type more strongly than phylogenetic relatedness. This can be tested by examining diet variation of one taxon across biomes/vegetation types.

Pollen analysis is a powerful tool for analysing diet as pollen morphology is specific to a particular plant family and sometimes even plant species (Matheson *et al.* 2008). Furthermore, gut analyses have the added advantages of allowing pollen to be quantified and are less biased compared to tackling such questions through direct field observations.

Methods

Study sites and samples:

Samples consisting of 14 lacewing species were previously collected from 20 locations in three biomes over the period of 2004 to 2013 by M. Picker (Appendix 1)

Lacewing identification and preparation

Lacewings were identified through morphological features by M. Picker using the key provided in Tjeder 1967. The biome and vegetation type of each specimen was determined using The Vegetation of South Africa, Lesotho and Swaziland (Mucina and Rutherford 2006). For pollen extraction the wings of each lacewing were then removed using a pair of fine scissors and each individual was placed in a vial containing 10% aqueous solution of potassium hydroxide. Potassium hydroxide dissolves the soft tissues of the lacewing, leaving the pollen and harder exoskeleton. The 10% aqueous solution of potassium hydroxide was heated on a hotplate in a glass container until all the powder dissolved. An equal amount of the dissolved potassium hydroxide solution was placed in the vials containing the lacewings. The vials were then placed in a waterbath at 80^{o}C. Each vial was removed once the lacewing body was transparent and all soft tissue had dissolved, leaving the pollen within the gut visible. The cleared lacewings were placed in stepped alcohol solutions of 8%, 35% and 70% respectively to ensure that the specimen did not burst as they were moved from an aqueous potassuim hydroxide solution into the final 70% alcohol solution for storage. The specimens were then placed in labelled eppendorf tubes in 70% alcohol.

Slide preparation

The prepared specimens were individually removed from the alcohol solution into a petri dish. A few drops of water were added to the specimen to increase visibility and they were then assessed for pollen presence using a light microscope. If pollen was present the specimen was dissected using two fine forceps. Pollen extraction involved isolating the gut of the specimen, removing the pollen and placing it in a different petri dish with forceps. The pollen was separated through maceration then stained with a drop of safranin in a glycerol solution. The stained pollen was transferred onto a microscope slide, a cover slip added and sealed using clear nail polish.

Pollen counts and identification

The pollen types were photographed using a Leica DFC295 light microscope. All photographs were taken at a 400x total magnification. Pollen was then identified using online pollen image databases which included the African Pollen Database and the Australasian Pollen and Spore Atlas (Weng *et al.* 2007) with the help of L. Gillson. Pollen was identitfied to species level, and morphotypes for a fine scale comparison and then grouped into families for an overview of the pollen represented in each genera. The pollen types on each slide were counted with a minimum count of 200 pollen grains in accordance with Maher (1972)'s method. The proportion of each pollen type was calculated for each specimen , these porportions were averaged for each species to represent the average diet of that individual species. For lacewing genera level analysese the average diet of all the species within that genus were used.

Data analyses

A comparison of the pollen composition amongst the different taxa was assessed at various taxonomic levels using multivariate analyses in PRIMER 6. 0 (Clarke and Gorley 2006). The dataset was untransformed and used to produce a Bray-Curtis resemblence matrix that calculated similarities between the lacewing diets. The matrix was then used in a group average cluster analysis to show how similar the lacewing diets were based on each lacewing genus' pollen family proportions. A multidimensional (MDS) plot was constructed to show dietary similarities across biomes. Analysis of Similarity (ANOSIM) tests were conducted on the specimens from different biomes to determine if the dietary similarities across biomes were stastically significant. A SIMPER analysis was carried out to determine the most influencial pollen type driving the biome cluster groupings on a pollen family and genus level along with a measure of how unsimilar the biomes were.m Additional group average cluster analyses were also carried out to show dietary similarities between; lacewing genera in different fynbos vegetation types, the same genus in different locations as well as the same genus in a single location.

Results

Pollen composition across species and genera

Of the 11 lacewing genera examined *Nemopterella* had the most diverse diet with 24 different pollen types (Table 1), followed by the two *Nemeura* species with a combined 13 pollen types, and *Palmipenna* and *Nemia* with 12 types each . *Derhynchia* and the *Concroce* specimens contained no pollen while the rest of the genera contained 2-4 pollen types.

At the pollen family level, Asteraceae was the most abundant pollen type, occurring in seven out of the 11 genera and eight out of the 13 species (Figure 1). *Knersvlaktia nigroptera* (Succulent Karoo) had a diet that was entirely made up of Asteraceae, which also made up the bulk of *Palmipenna aeoleoptera* (99%), *Palmipenna pilicornis* (90%) (Succulent Karoo), and the Nemopterella (57%) (Succulent and Nama Karoo, Fynbos) diet. Oxalidaceae was abundant in five taxa, namely, Semirhynchia spp., *Lauhervasia setacea*, *Nemeura gracilis* and *Nemia contumax* with a small presence in the Nemopterella species. This was followed by Aizoaceae pollen which was also present in five species and Fabaceae in four species. Unknown pollen families were present in six species, with *Nemuera tipularia* containing the largest amount of unidentified pollen.

The diverse *Nemopteralla* diet consisted mainly of Asteraceae (57%), while Aizoaceae (10%), Unknowns (10%), Scrophulariaceae (9%), and minor families

(Euphorbiaceae, Myrtaceae, Oxalidaceae, , Malvaceae, Fabaceae, Umbellifereae and Combretaceae) made up the rest of the diet. Scrophulariaceae, Malvaceae, Umbelliferae and Combretaceae were unique to this group. The *Nemeura gracilis* (sand fynbos vegetation) diet was also very broad and included Asteraceae, Juncaceae, Poaceae, Euphorbiaceae, Myrtaceae, Oxalidaceae, Liliaceae, Rhamnaceae and unknown pollen (1%). In addition, Liliaceae was only found in this species. Other unique pollen occurrences included Acanthaceae in *Nemopistha contumax* (savanna lowveld vegetation), Capparidaceae in *Nemia costalis* (sandstone fynbos vegetation) and Chenopodiaceae in *Palmipenna pilicornis* (sand fynbos vegetation).

Table 1. Nemopterinae samples examined and amount of pollen types found per genus.

Genera	Species examined	Individuals examined		Number of pollen types
Palmipenna	2	20	*P.pilicornis*	12
		20	*P.aeoleoptera*	
Nemeura	2	15	*N.gracilis*	13
		3	*N.tipularia*	
Nemia	2	8	*N.costalis*	12
		1	*Nemia* spp.	
Sicyoptera	1	10	*S.dilatata*	10
Nemopterella	_[*]	35	*Nemopterella* spp.	24
Derhynchia	1	3	*D.vansoni*	0
Nemopistha	1	4	*N.contumax*	4
Knersvlaktia	1	3	*K.nigroptera*	2
Semirhynchia	_[*]	2	*Semirhynchia* spp.	2
Concroce	1	1	*C.capensis*	0
Lauhervasia	1	18	*L.setacea*	4

[*] samples only identified to genus level

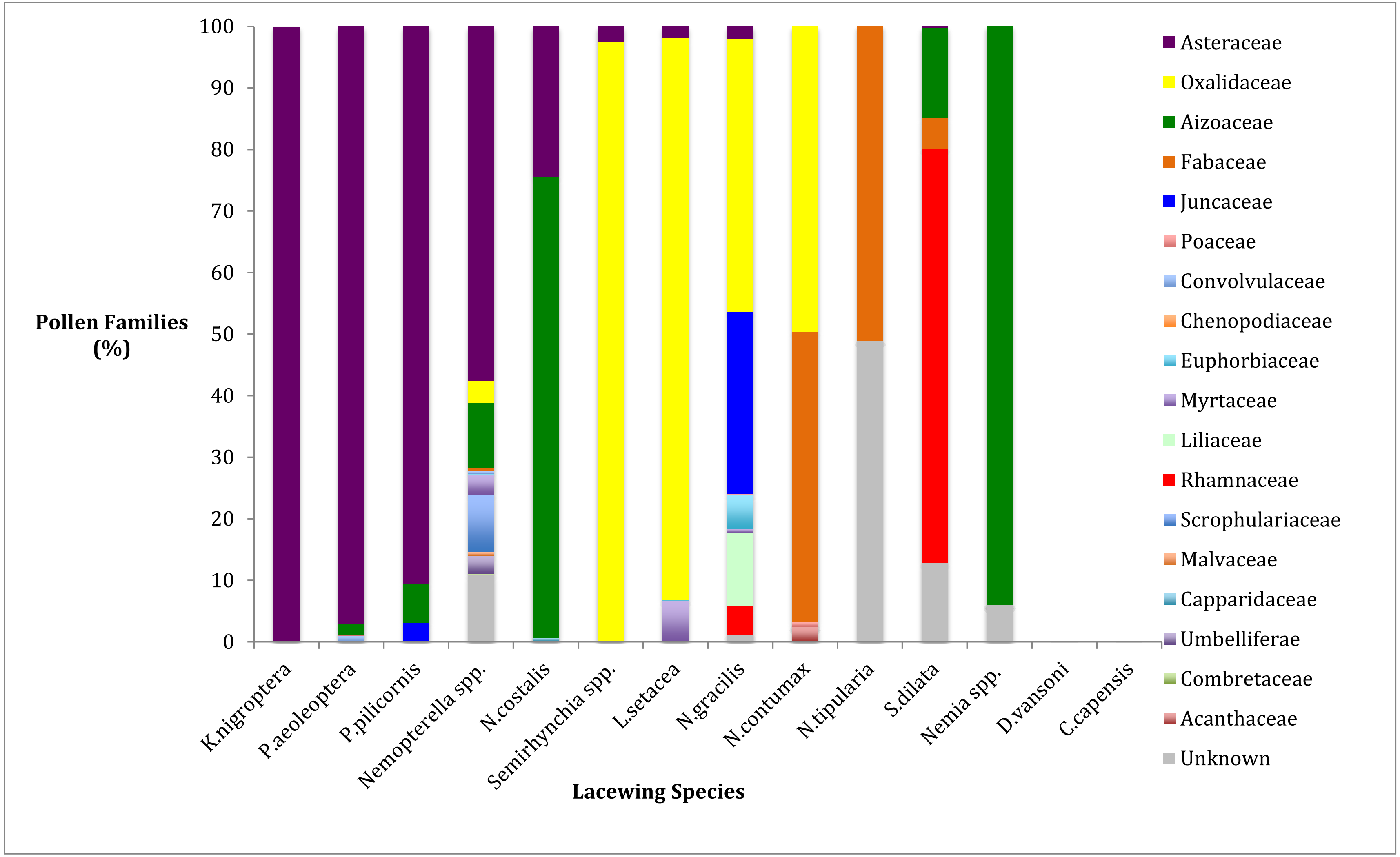


Figure 1. Percentage of pollen families present in Nemopteridae. *K.nigroptera – Knersvlaktia nigroptera, P.aeoleoptera – Palmipenna aeoleoptera, P.pilicornis – Palmipenna pilicornis, N.costalis – Nemia costalis, L.setacea – Lauhervasia setacea, N.gracilis –Nemeura gracilis, S.dilata – Sicyoptera dilata, N.tipularia - Nemeura tipularia, N.contumax - Nemopistha contumax, D.vansoni – Derhynchia vansoni, C.capensis – Concroce capensis.*

Comparison of diet across nemopterid genera and biomes

Two distinct groups clustered at the 20% similarity level on the basis of their diet. *Lauhervasia, Nemopterella, Nemeura, Semirhynchia and Knersvlaktia* formed the largest group (Figure 2), *Palmipenna*, *Nemia* and *Sicyoptera* formed the other group. Among all groups, *Lauhervasia* and *Semirhynchia* diets were the most similar (80%), while *Nemopistha* and *Sicyoptera* had the most unique diets.

An MDS plot of the nemopterid genera showed three groupings that corresponded to dietary similarities across different vegetation types and biomes at the 5% similarity level (Figure 3). The first and largest group consisted of all the lacewing genera associated with Fynbos vegetation types, as well as two taxa from the Succulent Karoo. Within this group the species with the most similar pollen diet were, the *Semirhynchia* species found in the sandstone fynbos vegetation and *Nemopterella* species in the alluvium fynbos. The two clustered very tightly, highlighting their almost identical pollen diet, as did *L.setacea* in the sandstone fynbos with *N.gracilis* in alluvium fynbos. Unrelated genera in similar vegetation types had similar diets, shown by clustering between *Semirhynchia spp*. and *L.setacea* in sand fynbos, *P.aeoleoptera* and *P.pilicornis* in quartzite fynbos and *Nemopterella* and *N.gracilis* in sandstone fynbos vegetation. *K.nigroptera* found in Knersvlatktia Succulent Karoo vegetation was nested within the cluster of Fynbos vegetation lacewing species, highlighting their similarity in diet. While the other Succulent Karoo *Nemopterella* from Rosh Pinah Namibia is isolated from the rest of the group indicating its dietary difference. The second group seen on the MDS was made up of two smaller karoo subgroups, *Nemopterella* species in upper karoo biome vegetation which makes up part of the Nama Karoo and *Nemia* species from the Rosh Pinah Succulent Karoo. *Nemeura tipularia* in tree and shrub savanna and *Nemia contumax* in the savanna lowveld biome form the third group with pollen more similar to each other (mainly Fabaceae pollen) than to the rest of the species from other biomes. The diet of lacewings in the fynbos biome was found to be significantly different ($p = 0.001$)from that of lacewings in the savanna and succulent karoo (Global $R = 0.467$ at a 0.004 significance level). SIMPER showed that the difference was due to the presence of *Acacia* in the savanna biome species' diets.

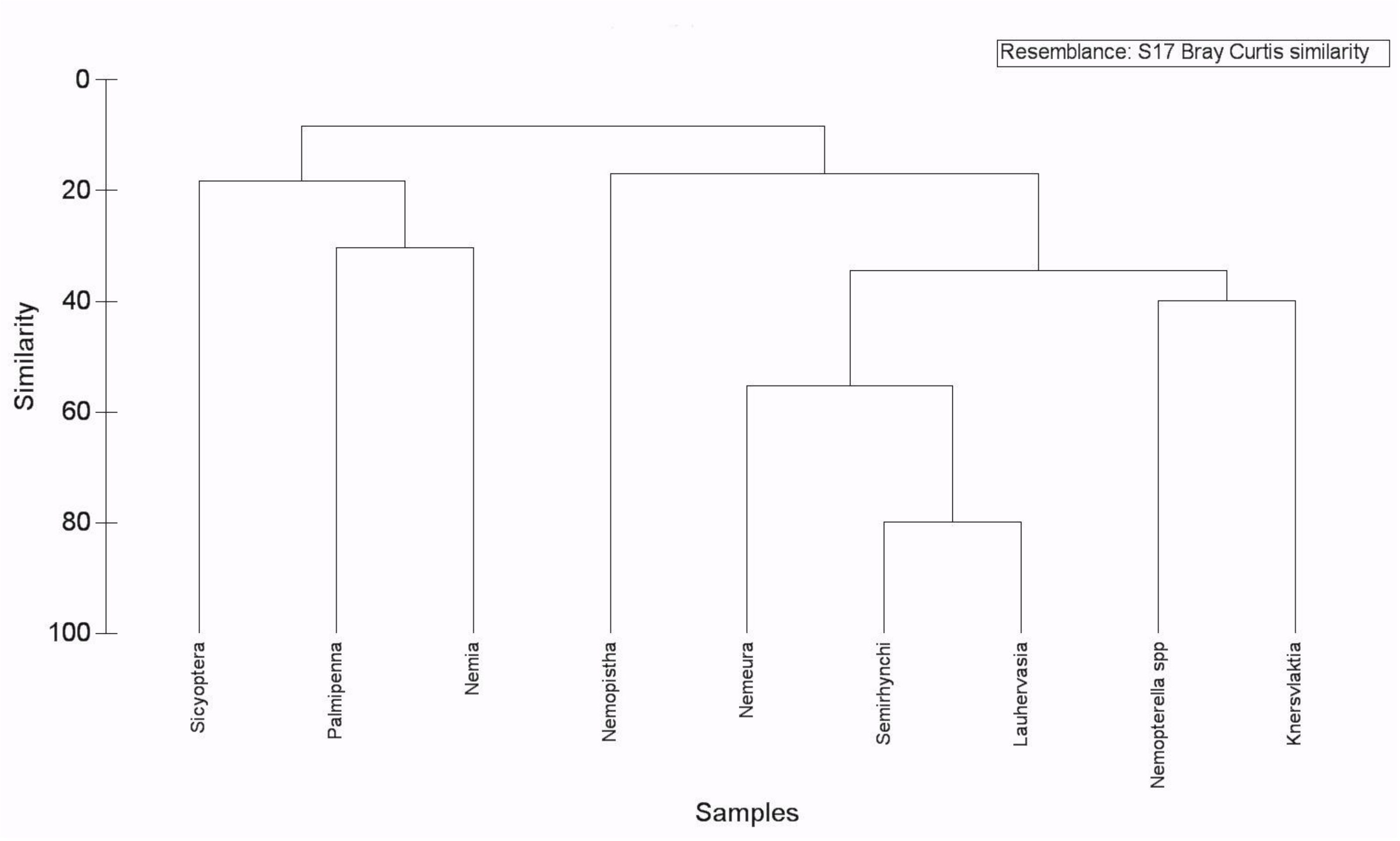


Figure 2. Cluster analysis of nemopterid genera based on pollen diet at family level.

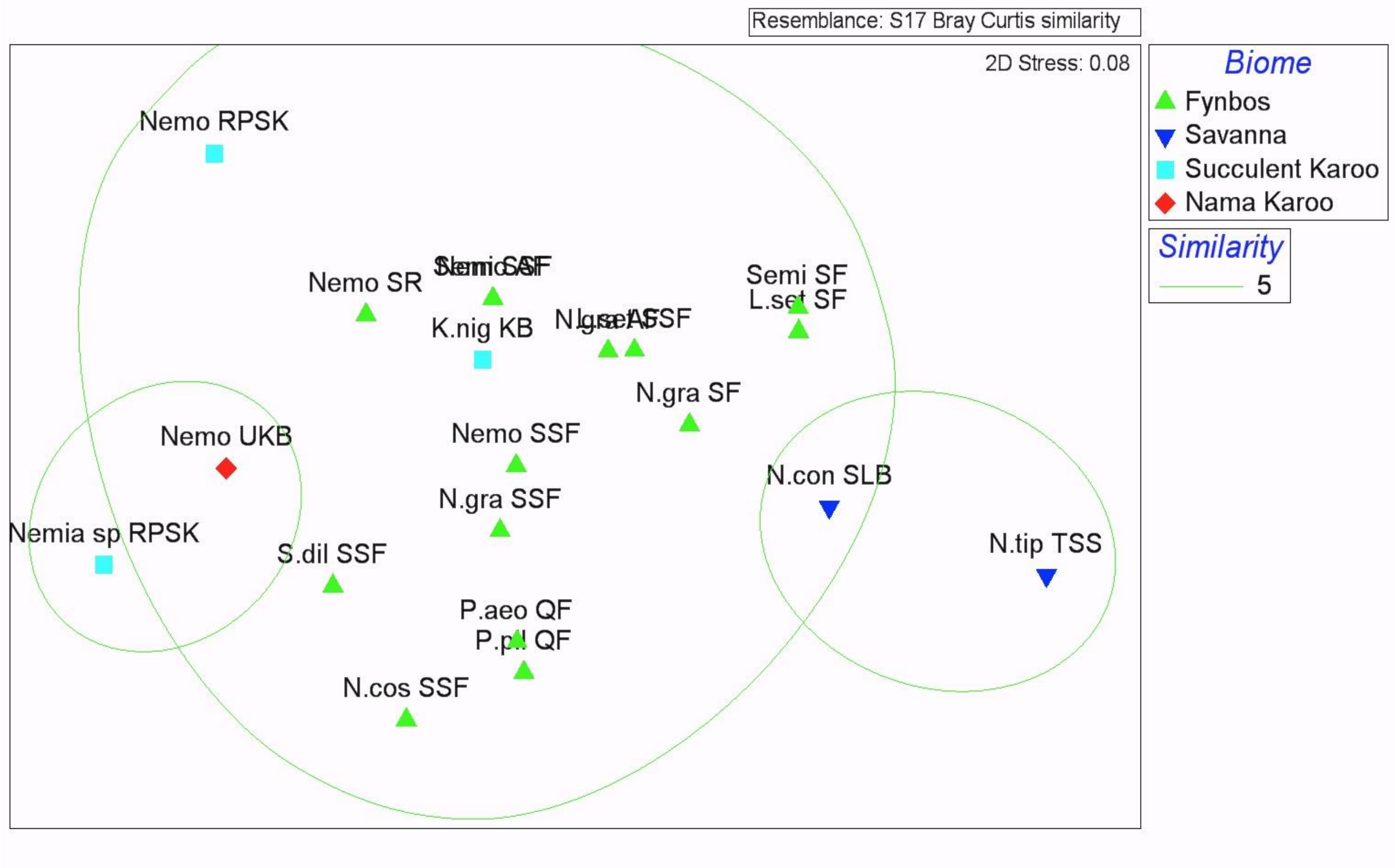


Figure 3. Similarities between lacewing species in different vegetation types and biomes based on pollen diet. Species: K.nig – *Knersvlaktia nigroptera*, Nemo – *Nemopteralla* spp., N.gra – *Nemeura gracilis*, N.tip – *Nemeura tipularia*, N.con – *Nemopistha contumax*, N. cos – *Nemia costalis*, P.aeo – *Palmipenna aeoleoptera*, P.pil – *Palmipenna pilicornis*, Semi – *Semirhynchia* spp, S.dil – *Sicyoptera dilata*, L.set – *Lauhervasia setacae*. Vegetation types: SF-sandstone fynbos, SSF- sandstone fynbos, QF –quartzite fynbos, SR-shale renosterveld, KB-Knersvlakte Bioregion, RPSK – Rosh Pinah succulent Karoo vegetation, SLB-savanna lowveld biome, TSS-tree and shrub savanna, UKB – upper karoo biome. ANOSIM significance of 0.001 between fynbos and savanna.

Comparison of diet of nemopterids in different fynbos vegetation types

Within the fynbos biome, there are three distinct clusters of lacewing genera based on diet (Fig. 4). The generic nemopterid clusters are generally a reflection of vegetation type with lacewings found in similar vegetation having a similar diet. However the diet of *L.setacea* from sandstone fynbos was most similar to that of *Nemeura* from alluvium fynbos vegetation. While *Nemopterella spp*. from shale renosterveld vegetation, *Semirhynchia spp.* from sand stone fynbos and *Nemopterella* from alluvium fynbos have similar diets despite occuring in different vegetation types.

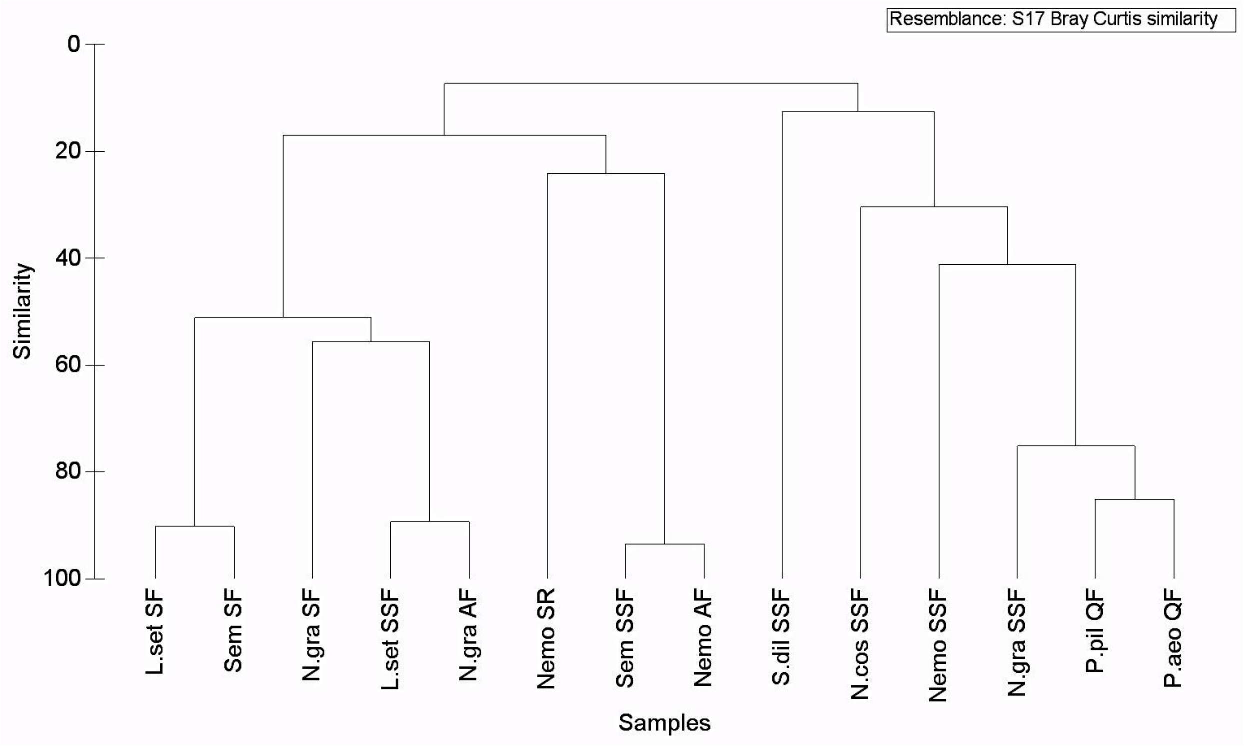


Figure 4. Similarities between lacewing species in different fynbos vegetation types. SF – sand fynbos, SSF – sandstone fynbos, AF – alluvium fynbos, QF- quartzite fynbos, SR – shale renosterveld. Species: Nemo – *Nemopteralla spp.*, N.gra – *Nemeura gracilis*, N. cos – *Nemia costalis*, P.aeo – *Palmipenna aeoleoptera*, P.pil – *Palmipenna pilicornis*, Semi – *Semirhynchia spp*, S.dil – *Sicyoptera dilata*, L.set – *Lauhervasia setacae*.

Diet of same genus of nemopterid in different vegetation types

Nemopterella showed great variation in dietary pollen composition across sites (Figure 5) and no clusters reflecting a match with vegetation type. *Nemopterella* 21, 23, 24, 8 and 16 from Algeria had an identical diet, as did *Nemopterella* 14 and 15 from nearby Krom River. Other individuals from Algeria had a more unique pollen composition, particularly *Nemopterella* 20 and 27. *Nemopterella* 11 from Rosh Pinah Namibia was more similar to samples from Bosluiskloof than to the other *Nemopterella* from Rosh Pinah which each had a unique diet. Matjiesrivier *Nemopterella* specimens were also different from one other as they are spread out across the cluster chart.

Diet of two closely related species in the same location, but in different soil types

The two *Palmipenna* species in Biedouw Valley, Clanwilliam had a very similar diet (Figure 6). Most of the individuals have an almost identical diet, with nine *P.pilicornis* sharing 100% of their diet with seven *P.aeoleoptera*. *P.pilicornis* 1 and 16 have the most unique diet. But overall the similarities between these two species in terms of their diet exceeds 80%.

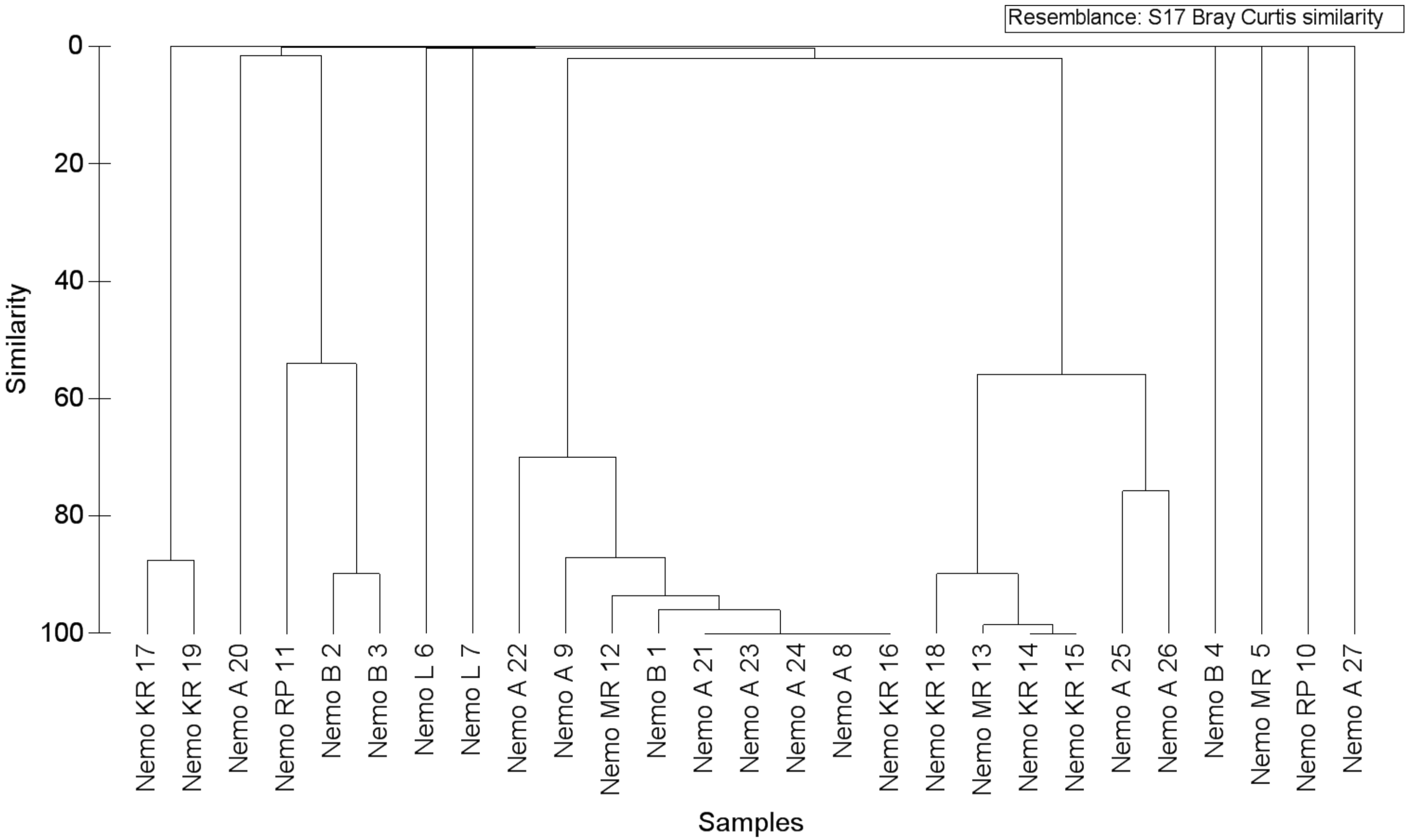


Figure 5. Pollen composition similarities of *Nemopterella* species from different localities. Locations: KR – Krom River, Cederberg; A-Algeria, Western Cape; RP – Rosh Pinah, Namibia; B – Bosluiskloof, Gampoorta Dam; L – Loxton, Northern Cape; MR – Matjiesrivier

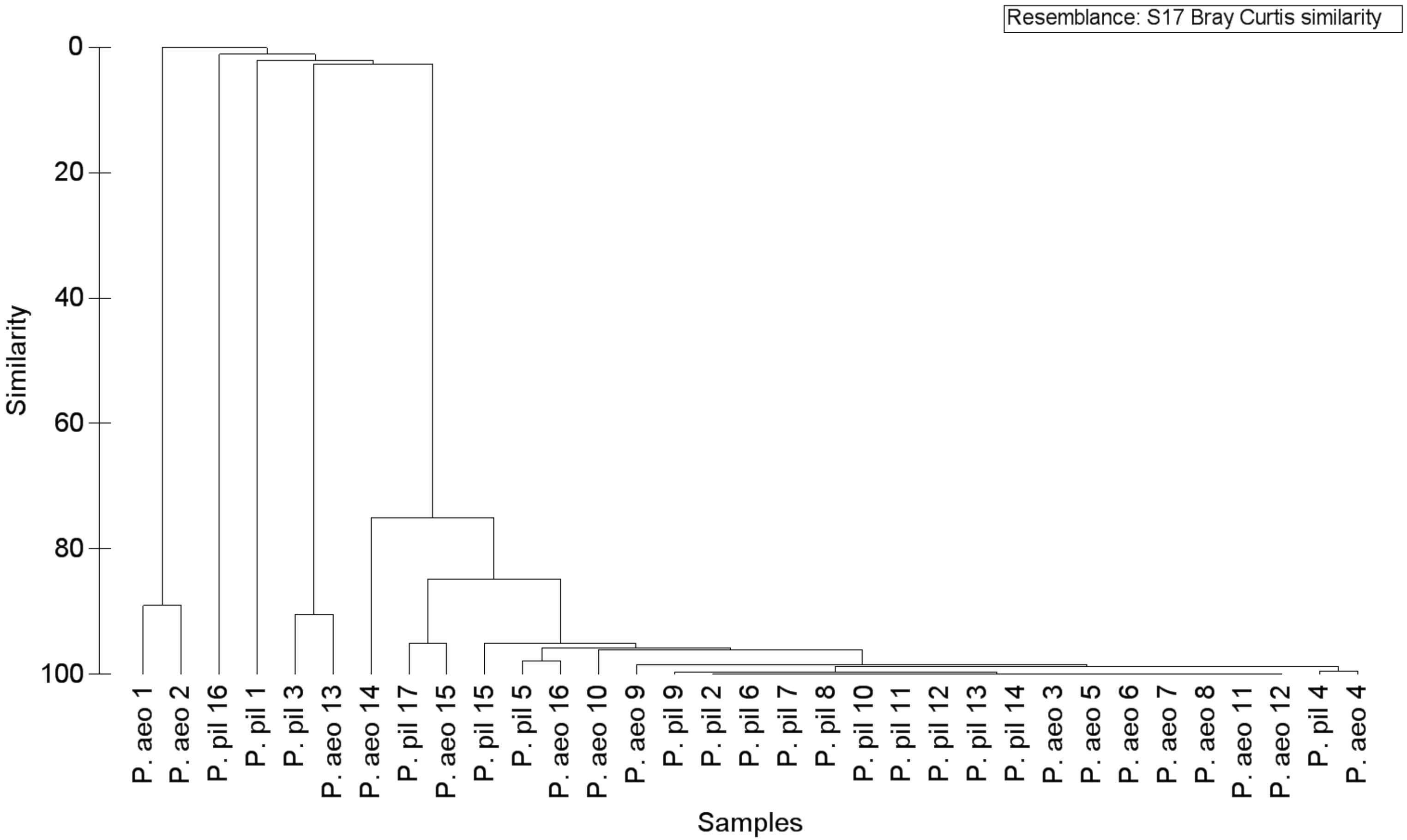


Figure 6. Similarities amongst *Palmipenna pilicornis* and *Palmipenna aeoleoptera* individuals from Biedouw Valley, Western Cape Province.

Discussion

Phylogenetic constraints on diet

The Nemopteridae comprises two subfamilies, the Crocinae and Nemopterinae (Tjeder 1967, Mansell 1996). The phylogeny of Sole *et al*.(2013) showed a deep phylogenetic node separating the two sub-families. If nemopterid diet is phylogenetically constrained then we would expect to see some phylogenetic signal in the diets, especially if diet is generally a conservative trait (linked to morphological specialisation in mouthparts and phenology). However the dietary grouping does not reflect this dichotomy in diet between the two subfamilies. Instead, *Lauhervasia,* the Crocinae representative in my study, is nested within the Nemopterinae dietary group (Figure 2). It shared an 80% dietary similarity with *Semirhynchia* which is in the Nemopterinae sub-family. This suggests that diet is not phylogenetically constrained and there is little distinction between the diets of two sub-families. This result is surprising considering the two subfamilies have a large body size difference. The Crocinae are much smaller than the Nemopterinae (approximately 50% smaller) and the expectation was that they may have different food sources related to this size difference and their phylogenetic distance (Sole *et al.* 2013).

The phylogeny showed the Nemopterinae as monophyletic with two distinct lineages. The genera included in my study were spread between these two lineages.The first lineage recovered by Sole *et al.* (2013) (1) consisted of *Palmipenna, Knersvlaktia, Nemopterella* and *Nemia,* the so-called Succulent Karoo lineage which is expected to have a high reliance on Aizoaceae. While lineage (2) was made up of *Nemeura, Sicyoptera, Semirhynchia* and *Derhynchia* which formed the more basal fynbos group with less reliance on Aizoaceae. It was hypothesised that diet would reflect these two phylogenetic groupings.

The results show that dietary patterns were considerably different from the proposed phylogenetic relationships. As in the phylogeny, the genera separated into two distinct dietary groups (Figure 2), but the group members differed from those proposed by the phylogeny of Sole *et al*. (2013) with only two members of each group remaining the same. *Sicyoptera, Palmipenna* and *Nemia* made up the first dietary group. *Palmipenna* and *Nemia* stayed together as in the phylogeny however *Knersvlaktia* and *Nemopterella* are no longer part of this group while *Sicyoptera* is a new member. *Knersvlaktia* and *Nemopterella* shared dietary similarities with less related genera and grouped with unrelated *Semirhynchia*, *Nemeura* and Nemopistha along with the *Lauhervasia* (a respresentative of the other subfamily Crocinae).

Nemuera and *Semirhynchia* have stayed together in this dietary group as in the phylogeny. These results suggest that diet does not reflect a phylogenetic signal as unrelated genera have similar diets and this dietary similarity is even prevalent across the two subfamilies. Thus the first hypothesis is not supported.

There a few taxa whose diet cannot be related to the nemopterid phylogeny. *Nemopistha* was not included in Sole *et al.*'s study therefore a comparison cannot be made. *Nemopistha* is a rare savanna taxon and the only South African Nemopterinae genus not included in Sole *et al.*'s (2013) study. The other genus collected in the savanna biome was *Derhynchia*. *Concroce* is part of the Crocinae sub-family (Tjeder 1967) and would have been most closely related to the *Lauhervasia* outgroup in the phylogeny of Sole *et al*. My single *Concroce* specimen did not contain any pollen (Table 1 and Figure 2) therefore a dietary analysis was not possible. *Concroce* is found in a mountain fynbos habitat so it may have a different diet to *Lauhervasia* (Tjeder 1967). *Derhynchia* which was included in the phylogenetic study as well as my own, is a very distinct monotypic genus with its own unique features (Tjeder 1967, Sole *et al*. 2013). but specimens examined did not have any pollen present in their gut either (Figure 2). In Sole's phylogeny *Derhynchia* was included in the second (fynbos) lineage along with *Nemeura*, *Sicyoptera* and *Semirhynchia*. *Derhynchia* was the most distantly related member of this fynbos lineage and this was attributed to autapomorphies such as its reduced mouthparts and rostrum. It is postulated to feed on dune grass pollen in its Kalahari habitat and therefore does not need a long rostrum (Mansell 1973). Sole *et al.*(2013) suggest that its mouthparts are atrophied. My study is the first to directly inspect the gut contents of this species therefore the absence of any pollen in their guts could indicate that *Derhynchia* adults might not feed at all. Further studies with more samples would verify this.

Ecological determinants of diet

Aizoaceae was hypothesised to be an important dietary component of the Succulent Karoo lineage (Sole *et al*. 2003). However the genera in this lineage had widely differing pollen diets (host plants) and were divided between the two dietary groups (Figure 2). Despite this, the first dietary group's only shared pollen source is Aizoaceae. The prominence of Aizoaceae in the diet however, differs amongst the dietary group members. It forms a major part of the *Nemia* diet (Figure 1). Aizoaceae makes up 75% of the Fynbos *Nemia costalis*' diet and 94% of the Rosh Pinah Succulent Karoo *Nemia* spp's diet. While it is a much smaller part of the *Sicyoptera* (15%) and *Palmipenna* (less than 10%) diet which both occur in the Fynbos biome. In general, this first dietary group has very little similary (only 18 – 30%) due to the differences in Aizoaceae representation.

The vegetation types where the lacewings occur do not differ considerably since *Sicyoptera* and *Nemia costalis* both occur in sand stone fynbos but still have very different diets, although they are from two different localities (Boesmanskloof Die Galg and Oudam Farm east of Clanwilliam (Appendix I)). This suggests that despite having similar vegetation types (Figure 3), different localities support different plant families, likely in different abundances. The lacewings might simply be feeding on whatever is readily available within their habitat, supporting the alternative hypothesis that diet is determined by plant availability. However some similarities point towards a genus specific diet. This is certainly the case for *Nemia* which occur in different biomes and vegetation types but maintain the same diet, dominated by Aizoaceae. *Nemia* is in the lineage that is thought to have co-evolved with Aizoaceae and its diet supports this (Sole *et al.* 2013). The other members of this dietary group have a much reduced reliance on Aizoaceae. *Palmipenna* was also expected to rely heavily on Aizoaceae. Instead, the major pollen sources were Asteraceae for *Palmipenna* (90% for *P.pilicornis* and 97% for *P.aeoleoptera*). *Palmipenna* therefore does not support the proposed second hypothesis that Succulent Karoo nemopterids have a large reliance on Aizoaceae. *Sicyoptera* forms part of the Fynbos lineage and the low Aizoaceae content in its diet is in accordance with the third hypothesis that Fynbos genera do not rely on Aizoaceae and their diet will reflect this. This first dietary group consisting of *Sicyoptera*, *Palmipenna* and *Nemia* shows mixed results with one member of Sole's Succulent Karoo lineage (*Nemia*) showing reliance on Aizoaceae as proposed in the hypothesis while the other member (*Palmipenna*) is not. *Sicyoptera,* of the Fynbos lineage does not show reliance on Aizoaceae in their diet as predicted.

In the second and much larger dietary group Nemopterella is the only genus that had Aizoaceae in its diet (Figure 1 and 2). Aizoaceae only formed 10% of its diet while Asteraceae (57%) formed the bulk of its diet. Nemopterella, was expected to have an Aizoaceae rich diet based on phylogeny however the results do not support this. Knersvlatktia, the final member of the proposed Aizoaceae dependent lineage did not contain any Aizoaceae at all, but its diet was completely made up of Asteareaceae. Therefore the two do not support the second hypothesis. The presence of Oxalidaceae, Fabaceae and Asteraceae in most of the genera is what is driving this dietary grouping (Figure 1).

The similarity between the diet of Lauhervasia to Semirhynchia in this group is due to the large amount of Oxalidaceae pollen present in both (97% in Semirhynchia and 91% in Lauhervasia). As predicted in the fourth hypothesis that stated that the derived Crocinae are widespread and will have a wide dietary range that is not restricted to Aizoaceae, *Lauhervasia* did not rely on Aizoaceae, there was none in its diet at all. The similarities in the diet of this Crocinae genus and *Semirhynchia* of the Nemopterinae sub-family may be caused by the fact that they both occur in the Fynbos biome and come from sand fynbos vegetation in the same localities (Figure 3 and Appendix 1), with the exception of the three *Lauhervasia* from Clanwilliam Dam. This further implies that plant availability has a much bigger influence on diet than phylogeny, supporting the fifth hypothesis of availability determining diet.

The rare Savanna *Nemopistha* has one of the most unique diets, sharing only 20% of its diet with the rest of the group. Its diet consists of Fabaceae pollen (47%) belonging to *Acacia* trees, whereas the other nemopterids only fed on forbs and shrubs. The rest of its diet consisted of Oxalidaceae (50%) as well as Acanthaceae which is absent in all other groups (although it only forms 2% of the diet). This difference is expected as it is one of only two genera collected from the savanna biome (Figure 3). It is also the only genus from the savanna lowveld vegetation. Fabaceae is also abundant in the savanna nemopterid *Nemeura tipularia* (57%) from tree and shrub vegetation in Okahandja Namibia. The similarity between the savanna specimens is not fully reflected in the lacewing genera diet cluster (Figure 2) as the genera level analysis of *Nemeura* diet includes the Fynbos *Nemeura gracilis* therefore diluting the similarity of *Nemeura tipularia* to its fellow savanna taxa *Nemopistha*. A species level analysis of *Nemeura* highlights the close dietary similarity as *Nemeura tipularia* and *Nemopistha contumax* are both dominated by Fabaceae pollen (Figure 1). The Fabaceae pollen consists of the genus *Acacia* in both cases, which is characteristic of Savanna biomes. ANOSIM showed that the dietary difference between the savanna nemopterids and those in the Fynbos biome is statistically significant and SIMPER showed that *Acacia* is responsible for this. The presence of a wide range of pollen families in the *Nemeura gracilis* (Fynbos species) diet distorts this similarity as it does not share any dietary similarities with *Nemeura tipularia* (tree and bush species), implicating the role of locality (food plant availability) rather than support for phylogenetic constraints on diet in support of the plant availability hypothesis.

The diet of *Nemeura gracilis* is mostly made up of Oxalidaceae (Figure 1). Oxalidaceae is also very abundant in all the members of the second dietary group based on dietary similarities (Figure 2). A SIMPER analysis showed that Oxalidaceae was the most influencial pollen family in this dietary group and is therefore responsible for most of the group's similarity. The presence of Oxalisdaceae in the Fynbos *Nemeura* species causes the diet of the *Nemeura* genus to be more similar to *Semirhynchia* and *Lauhervasia*. The last two genera in this dietary group are *Nemopterella* and *Knersvlaktia* whose 40% dietary simialarity is due to the large amount Asteraceae pollen (100% in *Knersvlaktia* and 57% in *Nemopterella*). This may be due to both genera's occurrence in the Succulent Karoo. This second dietary grouping highlights that Aizoaceae is not an important dietary element in the genera represented here, instead Oxalidaceae and Fabaceae are more prominent. In addition genera from the same biomes are more similar to each other than to the genera in other biomes and this over-rides phylogeny, further supporting the notion that diet is not phylogenetically constrained.

Sources of dietary variation:

Nemopterella shows variation within the genus' diet across different localities, vegetation types and biomes (Figure 3, 4 and 5). This could be due to several species being represented within this genus, however it is impossible to identify Nemopterella to species level based on morphology alone (Sole *et al*. 2013). The results show that Nemopterellas in sand stone fynbos vegetation in Algeria were spread out throughout the cluster diagram despite occuring in the same area (Figure 5). Some of the individuals from this area had an identical diet, while half of them had a diet more similar to Nemopterellas from other locations, vegetation types and biomes. This was a very diverse genus with individuals from Fynbos, Rosh Pinah Succulent Karoo as well as the Nama Karoo vegetation types and the dietary similarities of the individuals are spread throughout these biomes with no specific grouping (Figure 4). As such the diet was the most diverse among the genera and indicates that diet is not phylogenetically constrained but depends on availability and can vary even within the same locality.

Within a single biome many vegetation types exist – each with the potential to support a diverse range of plant families. The nemopterid taxa that occurred in various fynbos vegetation types had an diverse diet and showed no distinct grouping (Figure 4). With the exception of a few, the lacewing species share dietary similarities with species from different vegetation types within the fynbos biome. While some from the same vegetation types are very different from each other in diet, such as *Sicyoptera dilatata* from other species that occur in the sand stone fynbos. The unique shale renosterveld Nemopterella is also different from species in other fynbos vegetation types, as it is the only nemopterid from this vegetation type. This interspecific dietary variation both within the same vegetation type and between vegetation types is most likely a reflection of the different plant families supported in different localities. For example sandstone fynbos vegetation in two different locations may be exposed to very different environmental and edaphic conditions, therefore supporting different plant families. Fynbos is well known for its heterogenous soil profiles that change along a gradient leading to very different plant families occuppying the same niche in different locations (Verboom *et al.* 2004, Mummenhoff *et al.* 2005). Thus the dietary differences reflect plant availability and diversity across the fynbos biome.

In a single locality, within the same vegetation type, diet is expected to be the same amongst individuals of the same species or genera. However this was not entirely the case (Figure 5). The two sympatric Palmipenna species are very similar to each other, half of them had an identical diet, while the diet of the rest differed. *P. pilicornis* occurs about 1 km in very sandy soils from *P. aeoleoptera* which is restricted to rocky slopes (M. Picker:pers. obs.). The vegetation in the areas is the same though, and is classified as quartzite vegetation. The unique diets of P.pili 16 and P.pili 1 are due to the presence of Juncaceae and Chenopodiaceae which were only present in those two individuals in the genera. These would normally be considered contaminants since there are many replicates from the area that did not have those pollen types, and this is most likely the case for Chenopodiaceae as its presence was miniscule. However Juncaceae formed a very prominent part of the diet of P.pili 1 with well over 200 pollen grains found in the gut so it can be assumed that the insect was in fact feeding on it. The presence of this particularly unique dietary component which is not shared in the other samples for this species highlights the importance of increasing the number of individuals sampled. Increasing the number of sampled individuals in a species from the same area ensure that the samples truly reflect the diet of that particular lacewing species in that area. The one P.pili sample that showed that Juncaceae is a major food source in the Palmipenna diet provided insights that the other 32 Palmipenna samples did as this was completely absent from in their guts. This shows that one sample alone is not representative of the diet, and a larger sample size is more informative and representative of the true diet.

The findings also suggest that intraspecific variation is present even within the same vegetation type and locality.

The study would have benefited from more intense sampling of all genera as unequal sampling of some genera may have resulted in some dietary bias when the pollen families were converted into proportions. A greater number of individual insects, denser sampling of vegetation types across the range of a species, and the inclusion of the genus Barbibucca would have refined the results and conclusions. However such issues are inevitable in studies involving organisms that are hard to catch, particularly when samples are not easily accessible since some are known to only occur in one remote location.

In spite of the few limitations the study succeeded in confirmin that Nemopterinae and Crocinae lacewing families are indeed pollinators. Their generalist diet shows that they are able to utilise the pollen of a diverse range of open flowers despite their tubular rostrum formally thought to be a specific adaptation for accessing pollen from tubular flowers (Popov 1967). Their radiation and concentrated diversity in the winter rainfall area is not apparently linked to Aizoaceae as previously suggested (Sole *et al.* 2013), although this might not apply to Nemias which have an Aizoaceae rich diet across biomes. The diet of nemopterids vary both within and across vegetation types and biomes, further emphasising their generalist diet. The lacewings appear to consume the pollen of plants that are readily available and this has little phylogenetic signal. Their success in the winter rainfall area was most likely due to their flexible diet, like many other succulent karoo pollinators (Struck 1994). Their broad diet suggest that it is unlikely that any single plant genus solely depended on nemopterids for its radiation.

Though Popov (1967) first suggested that the mouthpart structure of the Nemopteridae proves that they are exclusive pollen feeders, to date, very few studies have directly focused on the Nemopteridae diet. Field observations of the Spanish *Nemoptera bipennis* and the South African *Palmipenna aeoleoptera* have confirmed this (Monserrat 1985, Picker 1967). However an indepth dietary study was yet to be conducted. This study therefore adds substantial grounding for the pollenophagous diet of adult Nemopteridae as well as the previously unknown diet of the Crocinae subfamily, at least for *Lauhervasia*. It also disputes the assertion that nemopterids in the Succulent Karoo are specialist pollinators with a strong link to Aizoaceae (Sole *et al.* 2013). However this may not apply to the *Nemia* as the genus heavily relied on Aizoacea regardless of biome or location. There was no clear dietary shift in the nemopterids across biomes, with the exception of the the Savanna nemopterids which appear to have a unique diet but this was purely based on plants that are unique to that biome. This emphasises the notion that nemopterids are feeding on whatever is available and easily accessible. Diet therefore appears to be a reflection of available vegetation and not phylogeny.

These findings show that there is still much to learn about these often neglected insects. Their diverse diet raises questions about their importance as pollinators and contribution to the diversity of the winter rainfall area where they have flourished. Their concentrated diversity and endemism in the Greater Cape Flouristic region adds to the uniqueness of this biodiversity hotspot. Increasing our understanding of this often neglected insect family is therefore essential to ensure its continued survival and diversitfication, and could possibly lead to further insights about the diversity of the region itself.

References

Aspöck, U. & Aspöck, H. 1999 Kamelhälse, Schlammfliegen, Ameisenlöwen … Wer sind. (Insecta: Neuropterida: Raphidioptera, Megaloptera, Neuroptera). *Stapfia*, 60 (Neue Folge Nr, 138):1 – 34.

Aspöck, U., Plant, J. D., & Nemeschkal, H. L. 2001. Cladistic analysis of Neuroptera and their systematic position within Neuropterida (Insecta: Holometabola: Neuropterida: Neuroptera). *Systematic Entomology*, *26*(1):73-86.

Axelrod, D.I., Raven, P.H., 1978. Late Cretaceous and Tertiary vegetation history of Africa. In: Werger, M.J.A. Ed., *Biogeography and Ecology of Southern Africa.* Junk, The Hague.

Basset, Y. 2001 Invertebrates in the canopy of tropical rain forests—how much do we really know? *Plant Ecology*. 153:87–107.

Bauer, A. M. 1999. Evolutionary scenarios in the Pachydactylus Group geckos of southern Africa: new hypotheses. *African Journal of Herpetology*, *48*(1-2):53-62.

Bergh, N. G., anthony Verboom, G., Rouget, M., & Cowling, R. M. 2014. Vegetation types of the Greater Cape Floristic Region. *Fynbos: Ecology, Evolution, and Conservation of a Megadiverse Region*, 1. Oxford University Press. Oxford.

Bernays, E.A. & Chapman, R.F. 1994. *Host–Plant Selection Behaviour of Phytophagous Insects*. Chapman & Hall, New York.

Born, J., Linder, H. P., & Desmet, P. 2007. The greater cape floristic region.*Journal of Biogeography, 34*1:147-162.

Chew, F.S. 1977. Coevolution of pierid butterflies and their cruciferous foodplants. 2. Distribution of eggs on potential foodplants. *Evolution*, 31:568–579.

Cowling, R. M., & Hilton-Taylor, C. 1997. Phytogeography, flora and endemism. *Vegetation of southern Africa*, 43-61.

Cowling, R. M., Proches, S., & Partridge, T. C. 2009. Explaining the uniqueness of the Cape flora: incorporating geomorphic evolution as a factor for explaining its diversification. *Molecular Phylogenetics and Evolution*, 51(1):64-74.

Cowling, R. M., Esler, K. J., & Rundel, P. W. 1999. Namaqualand, South Africa–an overview of a unique winter-rainfall desert ecosystem. *Plant Ecology*,*142*(1-2):3-21.

Craig, T.P. & Itami, J.K. 2008. Evolution of preference and performance relationships. In: *Specialization, Speciation, and Radiation. The Evolutionary Biology of Herbivorous Insects* ed. Tilmon, K.J.. University of California Press, Berkeley.
Cronin, J.T., Abrahamson, W.G. & Craig, T.P. 2001. Temporal variation in herbivore host–plant preference and performance: constraints on host-plant adaptation. *Oikos*, **93**, 312–320.

Dupont, L. M., Linder, H. P., Rommerskirchen, F., & Schefuß, E. 2011. Climate-driven rampant speciation of the Cape flora. *Journal of Biogeography*, *38*6:1059-1068.

Erwin, T. L. 1982. Tropical forests: their richness in Coleoptera and other arthropod species. *Coleopterists Bulletin*. *36*1: 74-75.

Fox, C.W. & Lalonde, R.G. 1993. Host confusion and the evolution of insect diet breadths. *Oikos*. 67:577–581.

Gess, K.S. & Gess, F.W. 1989. Flower visisting by masarid wasps in southern Africa Hymenoptera: Vespoidea: Masaridae. *Annals of the Cape Provincial Museum Natural History* 1850:95–133.

Gill, D. E. 1989. Fruiting failure, pollinator inefficiency and speciation in orchids. Pp. 456-481 in: Otte, D. & Endler, J. A. ed., *Speciation and its Consequences*. Sunderland.

Goldblatt, P. 1991. A overview of the systematics, phylogeny and biology of the AfricanIridaceae. Contr. Bolus Herb. 13: 1-74.

Goldblatt, P., & Bernhardt, P. 1990. Pollination biology of Nivenia Iridaceae and the presence of heterostylous self-compatibility. *Israel Journal of Botany*, 391-2:93-111.

Goldblatt, P., Bernhardt, P. & Manning, J.C. 1998 Pollination of petaloid geophytes by monkey beetles Scarabaeidae: Rutelinae: Hopliini in Southern Africa. *Annals of the Missouri Botanical Gardens* 852: 215–230

Goldblatt, P., & Manning, J. C. 2002. Plant diversity of the Cape region of southern Africa. *Annals of the Missouri Botanical Garden*, 281-302.

Gripenberg, S., Morriën, E., Cudmore, A., Salminen, J.P. & Roslin, T. 2007. Resource selection by female moths in a heterogeneous environment: what is a poor girl to do? *J. Anim. Ecol.* 76:854–865.

Hesse, A.J. 1938 A revision of the Bombyliidae Diptera of Southern Africa. Part 1. *Annuals of the South African Museum*. 34:1–1053.

Holzel, H. 1975. Revision der Netzflugler-Unterfamilie Crocinae (Neuroptera: Nemopteridae). *Entomologica germanica*.

Hoogendoorn, M., & Heimpel, G. E. 2001. PCR‐based gut content analysis of insect predators: using ribosomal ITS‐1 fragments from prey to estimate predation frequency. *Molecular Ecology*. *10*8:2059-2067.

Jaenike, J. 1990. Host specialization in phytophagous insects. *Annu. Rev. Ecol. Syst.* 21: 243–273.

Janz, N., Nylin, S., & Wahlberg, N. 2006. Diversity begets diversity: host expansions and the diversification of plant-feeding insects. *BioMedical Central Evolutionary Biology*, *6*1, 4.

Johnson, M. O. 1991. Natural selection on floral traits in two species of Lobelia with different pollinators. *Evolution*, 1468-1479.

Johnson, S. D. 1994. Pollination and the evolution of floral traits - selected studies in the Cape flora. University of Cape Town. Cape Town. Pp 388

Johnson, S. D. 1996. Pollination, adaptation and speciation models in the Cape flora of South Africa. *Taxon*, 59-66.

Johnson, S. D., & Bond, W. J. 1997. Evidence for widespread pollen limitation of fruiting success in Cape wildflowers. *Oecologia*, *109*(4):530-534.

Johnson, S.A., Nicolson, S.W., 2001. Pollen digestion by flower-feeding Scarabaeidae: protea beetles Cetoniini and monkey beetles Hopliini. *Journal of Insect Physiology*. 47:725–733.

Johnson, S. D. 2010. The pollination niche and its role in the diversification and maintenance of the southern African flora. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1539):499-516.

Jurado-Rivera, J. A., Vogler, A. P., Reid, C. A., Petitpierre, E., & Gómez-Zurita, J. 2009. DNA barcoding insect–host plant associations. *Proceedings of the Royal Society B: Biological Sciences*, *276*1657:639-648.

Legendre, P., D. Borcard, and P. R. Peres-Neto. 2008. Analyzing or explaining beta diversity: Comment. Ecology 89: 3238-3244.

Legendre, P., and L. Legendre. 2012. Numerical ecology, 3rd English edition. Developments in Environmental Modelling, Vol. 24. Elsevier Science BV, Amsterdam. xiv + 990 pp.

Leon, B., & Picker, M. 1990. Behavioral thermoregulation inpalmipenna aeoleoptera neuroptera: Do the hypertrophied hindwings play a role?. *Journal of Insect Behavior, 3*3, 381-393.

Levyns, M.R., 1964. Migrations and origins of the Cape flora. *Trans. Roy. Soc. S. Afr.* 37:85–107.

Levins, R. & MacArthur, R. 1969. A hypothesis to explain the incidence of monophagy. *Ecology*. 50:910–911.

Linder, H. P. 1981. Taxonomic studies on the Disinae III. A revision of Disa Berg. excluding sect. Micranthae. *Contr. Bolus Herb*. 9:1-370.

Linder, H.P., 2003. The radiation of the Cape flora, southern Africa. *Biological. Reviews.* 78:597–638.

Linder, H.P., 2005. Evolution of diversity: the Cape flora. *Trends Plant Science*. 10:536–541.

Linder, H. P., & Hardy, C. R. 2004. Evolution of the species-rich Cape flora.*Philosophical Transactions of the Royal Society B: Biological Sciences,359*(1450):1623.

Linder, H.P., Meadows, M.E., Cowling, R.M., 1992. History of the Cape flora. In:

Cowling, R.M. Ed., *The Ecology of Fynbos: Nutrients, Fire and Diversity*. Oxford University Press, Cape Town, pp. 113–134.

Linder, H. P., Johnson, S. D., Kuhlmann, M., Matthee, C. A., Nyffeler, R., & Swartz, E. R. 2010. Biotic diversity in the Southern African winter-rainfall region. *Current opinion in environmental sustainability, 2*1:109-116.

Maher, L. 1972, Nomograms for computing 0.95 confidence limits of pollen data. Review Palaeobot. *Palynology,* 13: 85-93.

Manning, J.C. & Goldblatt, P. 1996. The *Prosoeca peringuyei* Diptera: Nemestrinidae pollination guild in southern Africa: long-tongued flies and their tubular flowers. *Annals of the Missouri Botanical Gardens.* 83:67–86.

Mansell, M.W., 1973. The first record of a larval nemopterid from southern Africa

Neuroptera: Nemopteridae: Nemopterinae. *J. Entomol. Soc. S. Afr.* 36, 133–137.

Mansell, M.W., 1976. The larva of Laurhervasia setacea Klug, Neuroptera:

Nemopteridae: Crocinae from southern Africa. *J. Entomol. Soc. S. Afr*. 39, 153–158.

Mansell, M. W. 1986. *Biogeography and phylogeny of the Crocinae Neuroptera: Nemopteridae*. na.

Mansell, M. 1996. Unique morphological and biological attributes: the keys to success in Nemopteridae Insecta: Neuroptera. In: Canard M, Aspöck H and Mansell MW eds Pure and Applied Research in Neuropterology. *Proceedings of the Fifth International Symposium on Neuropterology, Cairo, Egypt. Toulouse, France.*

Mansell, M. W. 2002. Monitoring lacewings Insecta: Neuroptera in Southern Africa. *Acta Zoologica Academiae Scientiarum Hungaricae, 48*2, 165-173.

Matheson, C. D., Muller, G. C., Junnila, A., Vernon, K., Hausmann, A., Miller, M. A., & Schlein, Y. 2008. A PCR method for detection of plant meals from the guts of insects. *Organisms Diversity & Evolution, 7*4, 294-303.

Monserrat, V. 1985. Nuevos datos sobre los Myrmeleontoidea ibéricos (Neur., Plan., Myrmeleonidae, Ascalaphidae, Nemopteridae). *Boletim Soc. portug. Ent.* Suppl. 1 (2): 475–489.

Mummenhoff, K., Al-Shehbaz, I. A., Bakker, F. T., Linder, H. P., & Mühlhausen, A. 2005. Phylogeny, morphological evolution, and speciation of endemic Brassicaceae genera in the Cape flora of southern Africa. *Annals of the Missouri Botanical Garden,* 400-424.

Mucina, L., & Rutherford, M. C. 2006. *The vegetation of South Africa, Lesotho and Swaziland*. South African National Biodiversity Institute.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B. & Kent, J. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403:853–858.

Novotny, V., & Basset, Y. 2005. Host specificity of insect herbivores in tropical forests. *Proceedings of the Royal Society B: Biological Sciences*. 2721568:1083-1090.

Oliver, E. G. H. 1991. The Ericoideae Ericaceae - a review. *Contr. Bolus Herb*. 13:158-208.

Picker, M. D. 1987. An unusual species of spoon-wing lacewing (Neuroptera: Nemopteridae) from South Africa, with notes on its biology. *Systematic entomology*. *12*(2):239-248.

Picker, M.D. & Midgley, J.J. 1996. Pollination by monkey beetles Coleoptera: Scarabaeidae: Hopliini:flower and colour preferences. *African Entomology.* 4:7–14.

Popov, A. 1967. Investigations on the ecology, biology and distribution of Nemoptera sinuata Oliv.Insecta, Neuroptera. Sofia Univ. “Kl. Ohridski”, Biol. Fac., Sofia, 143 pp. [In Bulgarian]

Popov, A. 1973. Über die präimaginalen Stadien paläarktischer Vertreter der Ordnung Neuroptera und Versuch einer neuen systematischen Gruppierung der Familien mit Rücksicht auf ihre morphologischen und ökologischen Besonderheiten. Bull. Inst. zool. mus. 37: 79–101.

Poynton, J. C., & Broadley, D. G. 1978. The herpetofauna. In *Biogeography and ecology of southern Africa* (pp. 925-948). Springer Netherlands.

Price, P. W. 2002. Resource-driven terrestrial interaction webs. *Ecol. Res*. 17:241–247.

Procheş, Ş., & Cowling, R. M. 2006. Insect diversity in Cape fynbos and neighbouring South African vegetation. *Global ecology and biogeography*, *15*5:445-451.

Procheş, Ş., Forest, F., Veldtman, R., Chown, S. L., Cowling, R. M., Johnson, S. D., & Savolainen, V. 2009. Dissecting the plant–insect diversity relationship in the Cape. *Molecular phylogenetics and evolution*, *51*1, 94-99.

Roulston, T.H., Cane, J.H., 2000. Pollen nutritional content and digestibility for animals. Plant Systematics and Evolution 222, 187–209.
Science and Plants for Schools UK

Schnitzler, J., Barraclough, T. G., Boatwright, J. S., Goldblatt, P., Manning, J. C., Powell, M. P., & Savolainen, V. 2011. Causes of plant diversification in the Cape biodiversity hotspot of South Africa. *Systematic Biology*, *60*3:343-357.

Siegfried, W. R. 1992. Conservation status of the South African endemic avifauna. *South African Journal of Wildlife Research*, *22*(3):61-64.

Siesser, W.G. 1980. Late Miocene origin of the Benguela upswelling system off northern Namibia. *Science,* 208:283 –285.

Sole, C. L., Scholtz, C. H., Ball, J. B., & Mansell, M. W. 2013. Phylogeny and biogeography of southern African spoon-winged lacewings Neuroptera: Nemopteridae: Nemopterinae. *Molecular phylogenetics and evolution*, *66*1:360-368.

Steiner, K.E. & Whithead, V.B. 1991. Oil flowers and oil bees: further evidence for pollinator adaptation. *Evolution,* 45:1493–1501

Struck M. 1994 Flowers and their insect visitors in the arid winter rainfall region of Southern Africa: observations on permanent plots. Insect visitation behaviour. Journal of Arid Environments 28: 51–74.

Sunderland, K. D., Crook, N. E., Stacey, D. L., & Fuller, B. J. 1987. A study of feeding by polyphagous predators on cereal aphids using ELISA and gut dissection. *Journal of Applied Ecology*. 907-933.

Tjeder, B. 1967 Neuroptera – Planipennia. The lace-wings of Southern Africa. 6. Family Nemopteridae. *South African Animal Life* 13: 290–501.

Van Zinderen Bakker, E. M. 1984. Aridity along the Namibian coast.*Palaeoecology of Africa*, *16*(1984):149-160.

Verboom, G. A., Linder, H. P., & Stock, W. D. 2004. Testing the adaptive nature of radiation: growth form and life history divergence in the African grass genus Ehrharta Poaceae: Ehrhartoideae. *American Journal of Botany*, *91*9, 1364-1370.

Verboom, G. A., Archibald, J. K., Bakker, F. T., Bellstedt, D. U., Conrad, F., Dreyer, L. L., and Nowell, T. L. 2009. Origin and diversification of the Greater Cape flora: ancient species repository, hot-bed of recent radiation, or both?.*Molecular Phylogenetics and Evolution*, 511:44-53.

Vernon, C.J. 1999. Biogeography, endemism and diversity of animals in the Karoo. In: Dean WRJ and Milton SJ eds *The Karoo: Ecological Patterns and Processes.* Cambridge University Press, Cambridge.

Vogel, S. 1954. *Blütenbiologische Typen als Elemente der Sippengliederung: dargestellt anhand der Flora Südafrikas*. W. Troll, & H. von Guttenberg Eds.. G. Fischer.

Weng, C., Shimeld, P., Bowman, D., Van der Kaars, S., & Sniderman, K. 2007 The Australasian Pollen and Spore Atlas V10. Australian National University, Canberra.

Wilson, A. M., Latimer, A. M., Silander Jr, J. A., Gelfand, A. E., & De Klerk, H. 2010. A hierarchical Bayesian model of wildfire in a Mediterranean biodiversity hotspot: implications of weather variability and global circulation. *Ecological Modelling*, 2211: 106-112.

Wright, M. G., & Samways, M. J. 1998. Insect species richness tracking plant species richness in a diverse flora: gall-insects in the Cape Floristic Region, South Africa. *Oecologia*, *115*3:427-433.

Appendix I.

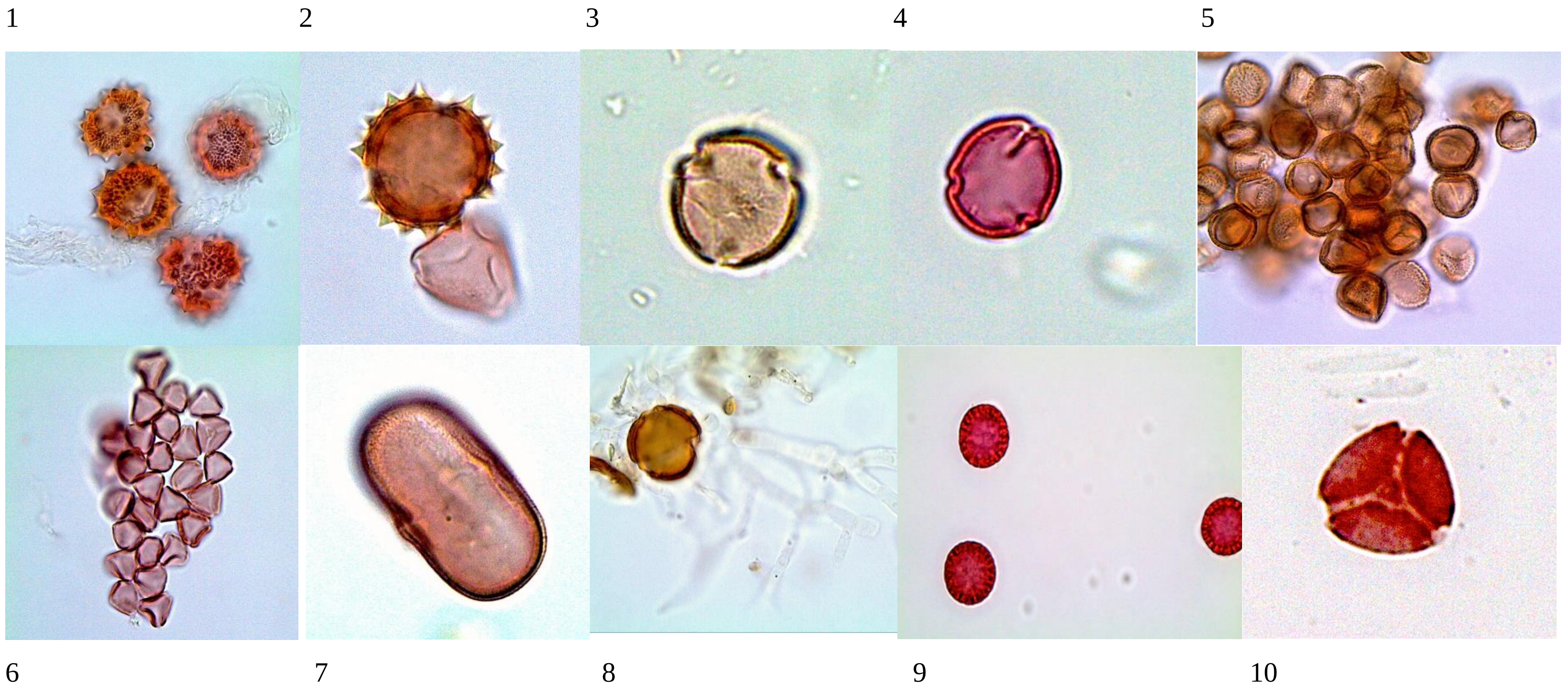
Lacewing specimens collected from 20 localities.

Taxon	Locality	Province	GPS Coordinates	Individuals
Palmipenna pilicornis	Biedouw Valley, Clanwilliam	Western Cape	-32.140352, 19.298176	19
Palmipenna aeoleoptera	Biedouw Valley, Clanwilliam	Western Cape	-32.140352, 19.298176	20
Nemeura gracilis	Algeria, Cederberg	Western Cape	-32.353737, 18.898978	2
	Bastiaanskloof	Western Cape	-33.543009, 19.157887	7
	Cederberg Wilderness Area	Western Cape	-32.332477, 19.116689	1
	Matjiesrivier	Western Cape	-32.505496, 19.342246	1
	Simonsberg	Western Cape	-34.205253, 18.438568	2
	Rondegat River, Cederberg	Western Cape	-32.193060, 18.900534	2
Nemeura tipularia	Okahandja	Otjozondjupa Region, Namibia	-21.980062, 16.919848	3
Nemia costalis	Oudam Farm, East of Clanwilliam	Western Cape	-32.054640, 18.715212	8
Nemia spp	Rosh Pinah	Karas Region, Namibia	-27.965370, 16.749043	1
Nemopterella spp.	Bosluiskloof, Gamkapoort Dam	Western Cape	-33.286625, 21.646787	6
	Matjiesrivier	Western Cape	-32.505496, 19.342246	1
	Loxton	Northern Cape	-31.458666, 22.345411	2

	Algeria, Cederberg, Western Cape	Western Cape	-32.353737, 18.898978	16
	Kunje Farm, Middledeur River	Western Cape	-32.670850, 19.240005	1
	Krom River, Cederberg	Western Cape	-32.542014, 19.302908	7
	Rosh Pinah	Karas Region, Namibia	-27.965370, 16.749043	2
Sicyoptera dilatata	Die Galg, Boesmanskloof McGregor	Western Cape	-34.009913, 19.716253	10
Derhynchia vansoni	Kuruman Nature Reserve	Northern Cape	-26.673237, 22.085422	3
Semirhynchia spp.	Matjiesrivier	Western Cape	-32.505496, 19.342246	7
	Jamaka Farm, Cederberg, Rondegat River	Western Cape	-32.310086, 19.022222	1
Concroce capensis	Jamaka Farm, Cederberg, Rondegat River	Western Cape	-32.310086, 19.022222	1
Nemopistha contumax	Jejane Game Farm, Hoedspruit	Limpopo	-24.306352, 30.977064	4
Knersvlaktia nigroptera	Rooiberg	Limpopo	-24.774159, 27.737601	3
Lauhervasia setacea	Matjiesrivier	Western Cape	-32.505496, 19.342246	14
	Clanwilliam Dam	Western Cape	32.230623, 18.912320	3
	Jamaka Farm, Cederberg, Rondegat River	Western Cape	-32.310086, 19.022222	1

Appendix II.

Light microscope images of some dietary elements



From left to right: Asteraceae, Aizoaceae (3-5), Oxalidaceae (6), Acanthaceae (7), Euphorbiaceae (8), Croton-type (9), and Eucalyptus (10).