

# **Taxonomy and Life History of Gall Midges (Diptera, Cecidomyiidae) on *Drosanthemum* and *Malephora* (Aizoaceae) in South Africa**

**Stephany van Munster**



Dissertation presented for the degree of Master of Science

Department of Biological Sciences

University of Cape Town

2020

**Supervisors: Dr Netta Dorchin, Dr Jonathan Colville and Emeritus  
Associate Professor John Hoffmann**

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

## Plagiarism Declaration

1. I know that plagiarism is wrong. Plagiarism is to use another's work and pretend that it is one's own.
2. I have used the Harvard convention for citation and referencing. Each contribution to, and quotation in this essay/report/project from the work(s) of other people has been attributed, and has been cited and referenced.
3. This thesis is my own work.
4. I have not allowed, and will not allow, anyone to copy my work with the intention of passing it off as his or her own work.

Signature:

Date: 31/10/20

## **Acknowledgements**

I would like to thank my supervisors, Dr Netta Dorchin, Dr Jonathan Colville and Assoc. Prof. John Hoffmann, for not only the opportunity to work on this project, but also for their guidance throughout my studies.

I am also grateful to Dr Cornelia Klak (Bolus Herbarium) for the huge amount of knowledge shared on fieldtrips, as well as for the identification of host plants. I would like to thank Dr Anthony Magee, Dr Stephen Boatwright and all the staff at the Compton Herbarium for all of the assistance and for providing me with lab space at the herbarium.

Thank you to Petra Muller for the huge amount of help and patience with the microscopes at the University of Cape Town. I would also like to thank Dr Annalie Melin for many gall collections and assistance with imaging adult midges.

A huge thank you to my aunt, Nina Carr, for her assistance with drawing GIS distribution maps.

Thank you to my fellow students, Christian Barnard, Randall Josephs, Shakirah Tregonning and Thuli Makhoba for their assistance with gall collecting and fieldwork. We visited various nature reserves and farms and I would like to thank all the landowners and reserve rangers for allowing us access to these. In particular, I would like to thank the Karoo Desert Botanical Garden for all their help.

I am grateful to the South African National Biodiversity Institute, the National Research Foundation (grant number 91442, awarded to Dr Jonathan Colville) and to the University of Cape Town for the financial support throughout my studies.

I am particularly grateful to my parents and family for the incredible amount of love and support.

## Table of Contents

Abstract .....	1
List of figures .....	2

### 1. Introduction

1.1 The Greater Cape Floristic Region .....	5
1.2 Diversity and importance of Aizoaceae in South Africa .....	7
1.3 Insect diversity in South Africa .....	9
1.4 Gall-inducing insects .....	10
1.5 Gall-midge systematics and ecology .....	11
1.6 Gall-midge diversity in South Africa .....	14
1.7 Study Objectives .....	15

### 2. Methods and Materials

2.1 Collecting and rearing of insects .....	17
2.1.1 Fieldwork and collecting .....	17
2.1.2 Rearing of insects .....	19
2.1.3 Gall dissection .....	21
2.2 Morphological study and taxonomy .....	21
2.2.1 Slide mounting .....	21
2.2.2 Drawing, image capture and measurements .....	24

### 3. *Asphondylia* species on *Drosanthemum* and *Malephora* in South Africa

<i>Asphondylia</i> Loew - introduction .....	25
3.1 <i>Asphondylia</i> n. sp. 1 .....	27

3.2	<i>Asphondylia</i> n. sp. 2 .....	39
3.3	<i>Asphondylia</i> n. sp. 3 .....	43
<b>4. <i>Lasioptera</i> species on <i>Drosanthemum</i> and <i>Malephora</i> in South Africa</b>		
	<i>Lasioptera</i> Meigen - introduction .....	50
4.1	<i>Lasioptera</i> n. sp. 1 .....	52
4.2	<i>Lasioptera</i> n. sp. 2 .....	63
<b>5. Discussion .....</b>		<b>71</b>
<b>6. References .....</b>		<b>74</b>

## Abstract

South Africa is known for its highly endemic and remarkably species-rich fauna and flora. The Greater Cape Floristic Region, consisting of the Cape Floristic Region and the Succulent Karoo Region, is home to South Africa's second most diverse plant family, the Aizoaceae. While the Aizoaceae are relatively well studied, there has been little work done on their associated insect fauna. Preliminary observations in recent years revealed a diverse community of gall midges on these plants. This study aimed to investigate the gall midges associated with two speciose genera of Aizoaceae and it is the first of its kind in South Africa. I report five new species of gall midges, three belonging to *Asphondylia* Loew and two to *Lasioptera* Meigen. These species are described from several species within the Aizoaceae genera *Drosanthemum* Schwantes and *Malephora* N.E.Br., and additional host records were recorded from *Lampranthus* N.E.Br., *Carpobrotus* N.E.br., *Acrodon* N.E.Br., *Cephalophyllum* Haw. and *Jordaaniella* H.E.K. Hartmann. The gall midges are described from adults, pupae and larvae and information is provided on their galls, life history and distribution. Morphological attributes of the gall midges support the description of five distinct species. Furthermore, morphological characters of the *Lasioptera* species described here do not fit entirely with those of the genus, suggesting that a new genus should be established for them. These results provide a mere snapshot of the gall midge diversity that is to be found on Aizoaceae in southern Africa, and much work is still to be done on the Cecidomyiidae of South Africa as a whole. Further targeted sampling may reveal greater distribution ranges and additional host plants for the five species described here, as well as many more undescribed species across the Aizoaceae.

## List of Figures:

<b>Figure 1:</b>	Map of South Africa showing collecting sites and gall-midge species collected at each site. ....	<b>18</b>
<b>Figure 2:</b>	Map of southern Africa indicating Aizoaceae generic richness per quarter degree square (taken from Valente <i>et al.</i> 2014) .....	<b>19</b>
<b>Figures 3-6:</b>	Gall-midge rearing and dissection: (3) Ventilated rearing cage; (4) Rearing cages in the laboratory; (5) Removal of adult gall midges from the rearing cages using an aspirator; (6) Dissected gall showing a single chamber containing a pupa. ....	<b>20</b>
<b>Figures 7-9:</b>	Specimen mounting: (7) Diagram showing the placement of chemicals in a pitted plate for microscope slide-mounting; (8) Layout of a mounted slide; (9) Heating plate with a double boiler setup with vials containing KOH. ....	<b>22</b>
<b>Figures 10-13:</b>	<i>Asphondylia</i> n. sp. 1: (10) Adult female; (11) Bud galls on <i>Malephora crassa</i> ; (12-13) Bud galls on <i>Malephora purpureo-crocea</i> with pupal exuviae. ....	<b>29</b>
<b>Figures 14-19:</b>	<i>Asphondylia</i> n. sp. 1: (14) Female head; (15) Palp; (16) Labella; (17) Female and male antennae (flg = flagellomere); (18) Female and male 5 <sup>th</sup> flagellomeres; (19) Acropod. ....	<b>30</b>
<b>Figures 20-22:</b>	<i>Asphondylia</i> female abdomen. (20) <i>Asphondylia</i> n. sp. 1; (21) <i>Asphondylia</i> n. sp. 2; (22) <i>Asphondylia</i> n. sp. 3. ....	<b>31</b>
<b>Figures 23-25:</b>	<i>Asphondylia</i> n. sp. 1; (23) Tip of female ovipositor; (24) Male Terminalia, dorsal; (25) Larval head. ....	<b>33</b>
<b>Figures 26-28:</b>	Larval spatula. (26) <i>Asphondylia</i> n. sp. 1; (27) <i>Asphondylia</i> n. sp. 2; (28) <i>Asphondylia</i> n. sp. 3. ....	<b>33</b>
<b>Figures 29-34:</b>	Pupa frontal (top) and lateral (bottom) views. (29-30) <i>Asphondylia</i> n. sp. 1; (31-32) <i>Asphondylia</i> n. sp. 2; (33-34) <i>Asphondylia</i> n. sp. 3. ....	<b>34</b>

<b>Figures 35-40:</b>	Pupal head, frontal (left) and lateral (right) views. (35-36) <i>Asphondylia</i> n. sp. 1; (37-38) <i>Asphondylia</i> n. sp. 2; (39-40) <i>Asphondylia</i> n. sp. 3. ....	<b>35</b>
<b>Figures 41-42:</b>	<i>Asphondylia</i> n. sp. 1. (41) Pupal head and associated papillae; (42) Pupal prothoracic spiracle. ....	<b>36</b>
<b>Figures 43-45:</b>	Pupal abdominal segments. (43) <i>Asphondylia</i> n. sp. 1; (44) <i>Asphondylia</i> n. sp. 2; (45) <i>Asphondylia</i> n. sp. 3. ....	<b>36</b>
<b>Figures 46-49:</b>	<i>Asphondylia</i> n. sp. 2: (46) Adult male; (47-49) Bud galls on <i>Malephora latipetala</i> . ....	<b>39</b>
<b>Figures 50-55:</b>	<i>Asphondylia</i> n. sp. 2: (50) Acromere; (51) Wing; (52) Male terminalia, dorsal; (53) Larval head; (54) Pupal head with associated papillae; (55) Pupal prothoracic spiracle. ....	<b>41</b>
<b>Figures 56-59:</b>	<i>Asphondylia</i> n. sp. 3: (56) Adult female; (57) Bud galls on <i>Drosanthemum comptonii</i> ; (58-59) Bud galls on <i>Drosanthemum</i> <i>oculatum</i> . ....	<b>44</b>
<b>Figures 60-62:</b>	<i>Asphondylia</i> n. sp. 3; (60) Acromere; (61) Male terminalia, dorsal; (62) Pupal head, frontal. ....	<b>45</b>
<b>Figures 63-66:</b>	<i>Lasioptera</i> n. sp. 1: (63) Adult female; (64) Leaf galls with exuviae on <i>Malephora purpurea-crocea</i> ; (65) Leaf galls on <i>Malephora</i> <i>latipetala</i> ; (66) Leaf gall on <i>Lampranthus uniflorus</i> . ....	<b>53</b>
<b>Figures 67-72:</b>	<i>Lasioptera</i> n. sp. 1: (67) Male head; (68) Variation in number of flagellomeres in male and female antennae; (69) Three apical flagellomeres; (70) Female and male palps; (71) Acropod; (72) Wing. ....	<b>55</b>
<b>Figures 73-76:</b>	<i>Lasioptera</i> n. sp. 1: (73) Female abdomen, lateral; (74) Variation in female 8 <sup>th</sup> tergite; (75) Female ovipositor, lateral; (76) Male terminalia, dorsal. ....	<b>58</b>
<b>Figures 77-78:</b>	<i>Lasioptera</i> n. sp. 1 Larva: (77) head; (78) spatula. ....	<b>59</b>

<b>Figures 79-84:</b>	<i>Lasioptera</i> pupae. (79) <i>Lasioptera</i> n. sp. 1, Antennal horns; (80) <i>Lasioptera</i> n. sp. 1, frontal; (81) <i>Lasioptera</i> n. sp. 1, lateral; (82) <i>Lasioptera</i> n. sp. 2, Antennal horns; (83) <i>Lasioptera</i> n. sp. 2, frontal; (84) <i>Lasioptera</i> n. sp. 2, lateral. ....	<b>60</b>
<b>Figure 85:</b>	<i>Lasioptera</i> n. sp. 1, pupal prothoracic spiracle. ....	<b>61</b>
<b>Figures 86-91:</b>	<i>Lasioptera</i> n. sp. 2: (86) Male adult; (87) Leaf galls <i>Drosanthemum</i> <i>crassum</i> ; (88-89) Leaf galls on <i>Drosanthemum karrooense</i> ; (90) Leaf galls on <i>Drosanthemum cf. roridum</i> ; (91) Leaf galls on <i>Drosanthemum parvifolium</i> . ....	<b>64</b>
<b>Figures 90-93:</b>	<i>Lasioptera</i> n. sp. 2: (92) Variation in male and female antennae; (93) Variation in palps; (94) Variation in the female 8 <sup>th</sup> tergite; (95) Larval spatula with associated papillae. ....	<b>66</b>

# 1. General Introduction

## 1.1. The Greater Cape Floristic Region

South Africa is known for its highly endemic and remarkably species-rich fauna and flora and is home to three global biodiversity hotspots, the Cape Floristic Region, the Succulent Karoo and the Maputuland-Pondoland-Albany corridor (Myers *et al.*, 2000; Bazelet *et al.*, 2016). The Greater Cape Floristic Region, which is situated around the southern tip of Africa, is of particular interest, combining the Cape Floristic Region – the world's smallest hotspot - and the Succulent Karoo Region – the world's most speciose succulent desert (Jurgens, 1991; Born *et al.*, 2007; Manning & Goldblatt, 2012; Snijman, 2013).

The Succulent Karoo is an arid winter rainfall succulent shrubland that borders the xeric fynbos and renosterveld vegetation biomes (Snijman, 2013; Bergh *et al.*, 2014), characterized by summer aridity and low winter rainfall (Low & Rebelo, 1996). A characteristic feature of the region is the adaptation of its many plants to the xeric environmental conditions (Von Willert *et al.*, 1992; Rouault & Richard, 2003; Hoffman *et al.*, 2009). It is the most species rich semi-arid winter rainfall region in the world (Cowling *et al.*, 1999) and holds one third of the world's succulent plant species (Myers *et al.*, 2000). Endemism levels are high, with around 26% of the species being strict endemics and 14% being near endemic (Hilton-Taylor, 1996; Desmet & Cowling, 1999; Driver *et al.*, 2003; Mucina *et al.*, 2006; Young & Desmet, 2016). The vegetation is comprised of, and dominated by, succulent dwarf shrubs, predominantly from two families, the Aizoaceae (vygies) and Crassulaceae (stonecrops) (Low & Rebelo, 1996; Hilton-Taylor, 1996; Cowling *et al.*, 1998; Mucina *et al.*, 2006; Bergh *et al.*, 2014).

The biome also exhibits mass displays of annual Asteraceae and Aizoaceae flowers in spring that draw tourists to the area (Low & Rebelo, 1996; Cowling *et al.*, 1999; Mucina *et al.*, 2006) and is estimated to host 6,356 plant species from about 1000 genera and 168 families (Driver *et al.*, 2003; Snijman, 2013). While it shares many species with the Fynbos biome, it is quite distinct from it (Bergh *et al.*, 2014) and occupies two regions: the Southern Karoo domain and the Namaqualand-Namib region (Jurgens, 1991).

Multiple studies have been undertaken in an attempt to understand when and how the remarkable diversity of the Cape Floristic Region came about (Cowling *et al.*, 1992; Goldblatt *et al.*, 2002; Linder, 2003; Schnitzler *et al.*, 2011), but similar insight about the Succulent Karoo is lacking (Cowling *et al.*, 1998). It has been suggested that the diversity in this biome is the result of remarkable environmental and climate conditions that drove high levels of diversification and resulted in low levels of extinction in the late Miocene (Klak *et al.*, 2004; Cowling *et al.*, 2009; Verboom *et al.*, 2009, 2014).

The Aizoaceae in this region display morphological diversity and specialization due to strong soil nutrient gradients and substrate characteristics, as well as limited gene flow and short generation times (Cowling *et al.*, 1998; Ellis *et al.*, 2006). The oscillating dry and wet climate of the Succulent Karoo coupled with its history of moderate climate have also been suggested as a driver of high plant diversity (Midgley *et al.*, 2001; Midgley & Thuiller, 2007). Thus, the plants in this region have adapted to cope in areas with both nutrient and water shortages (Klak *et al.*, 2004; Hoffman *et al.*, 2009; Ripley *et al.*, 2013).

## 1.2. Diversity and importance of Aizoaceae in South Africa

The Aizoaceae of the Succulent Karoo currently comprises of 1800 species in 145 genera and five subfamilies (Liede-Schumann *et al.*, 2020) and the family has been named a top priority for taxonomic research (von Staden *et al.*, 2013; Victor *et al.*, 2015), with 52% of the taxa requiring revision (von Staden *et al.*, 2013). This is South Africa's second most diverse plant family, and two of the subfamilies, Mesembryanthemoideae and Ruschioideae, are almost entirely endemic to South Africa (Van Wyk & Smith, 2001; von Staden *et al.*, 2013). It ranks second in the Greater Cape Floristic Region in the number of endemic genera and fifth in the number of endemic species (Goldblatt, 1978; Goldblatt & Manning, 2002; Manning & Goldblatt, 2012). The family is made up of five subfamilies, namely the Acrosanthoideae, Aizooideae, Mesembryanthemoideae, Ruschioideae, and Sesuvioideae (Klak *et al.*, 2017), of which Ruschioideae is the most diverse, with 1600 described species (Klak *et al.*, 2013). Of particular interest within the Ruschioideae is the largest tribe, Ruschieae, which contains 1560 species of shrubby succulents (Hartmann, 2001; Klak, 2009).

The Ruschieae show rapid diversification and are of considerable interest for evolutionary studies (Klak *et al.*, 2004, 2013, 2017; Valente *et al.*, 2014). Such diversification in plants has been hypothesized as a driver of diversification in some insect groups (Gess, 1992; Kuhlmann, 2009); for example, the Cape Floristic Region (CFR) is considered a centre of bee diversity (Kuhlmann, 2009), and a positive relationship has been found between plant species richness and insect species richness (Procheş *et al.*, 2009). Furthermore, Wright and Samways (1998) found a positive relationship between plant species richness and gall-insect richness in this region.

While the Aizoaceae of the Succulent Karoo have been relatively well studied and their phylogeny is well-resolved (Klak *et al.*, 2004; Klak *et al.*, 2007; Klak *et al.*, 2011; Valente *et al.*, 2014), the insect fauna associated with these plants has so far received little to no attention (Jurgens, 2002; Gess & Gess, 2014; Colville *et al.*, 2002, 2014). Several studies were undertaken on the insect pollinators associated with Aizoaceae (Peter *et al.*, 2004; Mayer *et al.*, 2006; Jurgens & Witt, 2014) and one study involved herbivorous insects associated with the family (Louw, 1998). Pollen wasps (Vespidae: Masarinae) were found to be the main visitors of diurnal *Conophytum* species, while various moth species were most frequently found visiting nocturnal flowering species (Jurgens & Witt, 2014). The weevil *Urodontus scholtzi* Louw (Anthribidae) induces very common stem galls on *Galenia africana* (Aizoaceae), which are also inhabited by *Baris* sp. (Curculionidae) (Louw, 1998).

Insect-plant interactions are generally considered to promote insect biodiversity (Novotný *et al.*, 2006; Procheş *et al.*, 2009; Wiens *et al.*, 2015). While it has previously been thought that insect diversity in the Cape Floristic Region does not match the high diversity of plants (Giliomee, 2003), it was later found that a strong positive relationship between them does exist (Procheş & Cowling, 2006). A more comprehensive phylogenetic tree for the Cape angiosperms (Forest *et al.*, 2007) and the availability of a spatial dataset for insect and plant occurrences (Procheş and Cowling, 2006) have made it possible to analyse how phylogenetic diversity of plants influences insect diversity and explore how this relationship is influenced over a spatial scale (Procheş *et al.*, 2009). These studies found that the number of plant genera is a better predictor of the diversity of insect species than plant species diversity is (Procheş *et al.*, 2009).

Further research on the insect fauna associated with the Succulent Karoo Aizoaceae will enable the scientific community and decision makers to deal with the challenges and threats posed by the demands for land for agriculture and the problems associated with climate change in this region (Mucina *et al.*, 2006; Desmet, 2007).

### **1.3. Insect diversity in South Africa**

Insects constitute at least 80% of all living organisms and are keystone components in the world's biological diversity (Scholtz & Chown, 1996; Grimaldi & Engel, 2005; Fonseca, 2009) due to their importance at all trophic levels of terrestrial biodiversity, their global occurrence and their vast numbers (Conrad *et al.*, 2006; Clausnitzer *et al.*, 2009; Hallmann *et al.*, 2017; Scudder, 2017). Global threats to insect diversity that contribute to the biodiversity crisis include the rapid rates of land transformation and habitat loss as well as invasive flora and fauna (McGeoch, 2002; Dunn, 2005; Samways, 2007; Rutherford & Powrie, 2010; Wagner & Van Driesche, 2010; Samways, 2019; Sánchez-Bayo & Wyckhuys, 2019; Wagner, 2020). The number of undescribed insects in the world is estimated at between 5-20 million (May, 1988; Mora *et al.*, 2011; Stork, 2018), and the discovery and description of these insects is vital for improving our knowledge of biodiversity.

South Africa has a rich insect fauna with about 44,000 described species in 7,700 genera and 570 families (Scholtz, 2010). While the rate of faunal descriptions in the country has been the highest in the last decade, scientists have probably only scratched the surface in this respect, and thousands of species still await discovery (Scholtz, 2010; Hamer, 2013; Melin & Colville, 2019). It is widely recognized that there is a great need for training and development of more taxonomists (in all

disciplines) in order to keep up with the number of taxa that still await discovery and description in the country (Smith *et al.*, 2008). So far, there has been a bias towards vertebrates and plants by scientists studying the diversity of South Africa and the patterns and processes thereof (Hamer & Slotow, 2002). However, in recent years insects have been named as an important group for taxonomic research (Hamer & Slotow, 2002; Scholtz, 2010; Hamer, 2013), as it is understood that our capacity for understanding insect diversity and how environmental change could affect it would be limited without such research (Smith *et al.*, 2008). Conservation strategies and management decisions cannot effectively be made without knowledge on species numbers and their distributions (Scholtz & Chown, 1996).

#### **1.4. Gall-inducing insects**

Galls are defined as “*abnormal plant growths caused by a stimulus of another organism, in which the regulation of developmental and physiological processes is controlled by the causing organism*” (Rohfritsch & Shorthouse, 1982). They constitute one of the most complex types of relationships between plants and insects (Shorthouse *et al.*, 2005) and offer model systems for studying insect-plant interactions and the role of plants in insect diversification (Shorthouse *et al.*, 2005; Joy & Crespi, 2007; Fernandes *et al.*, 2014; Dorchin *et al.*, 2015b). Gall inducing insects are known in five insect orders: Hemiptera, Diptera, Hymenoptera, Thysanoptera and Coleoptera (Felt, 1940; Mani, 1964; Gagné, 1989; Shorthouse *et al.*, 2005; de Souza Mendonça *et al.*, 2014). The galls induced by these specialist plant feeders provide nourishment, shelter and protection for both adult insects and their progeny (Shorthouse *et al.*, 2005). Galls can be considered as abnormal “plant organs” as it is the plant itself that produces the gall as a result of stimulus from the

insect (Gagné, 1989; Shorthouse *et al.*, 2005). The plant gains no known benefit from galls, which may be detrimental to it, although not always severely so (West & Shorthouse, 1982).

The biggest and most widely distributed group of gall inducing insects are the gall midges (Cecidomyiidae), and galls induced by these tiny flies are incredibly diverse and found on leaves, flowers, stems, shoots, roots and buds of plants from many families (Felt, 1940; Mani, 1964; Gagné, 1989; Fernandes *et al.*, 1996).

### **1.5. Gall-midge systematics and ecology**

The Cecidomyiidae are a large family, currently with 812 genera and approximately 6,600 described species (Gagné & Jaschhof, 2017; Dorchin *et al.*, 2019). The actual number of cecidomyiid species in the world is unknown (Gagné & Jaschhof, 2017) but recent studies suggested that many thousands await discovery and that the Cecidomyiidae may be the biggest family of Diptera (Hebert *et al.*, 2016; Borkent *et al.*, 2018). While the five basal subfamilies include only fungus-feeding species, the largest and youngest subfamily, the Cecidomyiinae, is the most diverse, and includes all herbivorous and predatory species (Gagné & Jaschhof, 2017). Contrary to the study of the fungus-feeding groups in the family, which is based almost exclusively on adult morphology, the systematics of the Cecidomyiinae has been traditionally based on the study of immature and adult stages, as well as on feeding habits and host plant associations (Gagné, 1989, 1994; Gagné & Jaschhof, 2017). Dorchin *et al.* (2019) undertook the first comprehensive phylogenetic analysis of Cecidomyiinae, which validated the currently accepted systematic division of the subfamily and provided insight into the evolution of feeding modes and diversification patterns.

While most herbivorous Cecidomyiinae are gall inducers, some species do not induce galls but develop as inquilines, inhabiting galls induced by other species, whereas others live in plant tissues without gall formation (Gagné, 1989, 1994). Herbivorous gall midges are generally considered to be host specific, with many species restricted to certain host plant species or occurring within one plant genus or family (Gagné, 1989; Raman *et al.*, 2005; Carneiro *et al.*, 2009; Gagné & Jaschhof, 2017). Many species can thus be regarded as highly specialized plant “taxonomists” (Sylven, 1979). However, a few species are known to be polyphagous (e.g., *A. gennadii* Marchal (Gagné & Orphanides, 1992), *A. websteri* Felt (Gagné & Wuensche, 1986) and *A. yushimai* Yukawa & Uechi (Yukawa *et al.*, 2003)).

Several cecidomyiid species are known to be of high economic importance (Barnes, 1946-1956), and some have been reported as agricultural pests of vegetables, fruits and grains (Gagné *et al.*, 2018; Gagné & Orphanides, 1992). For example, *Asphondylia yushimai* Yukawa & Uechi is a major pest of soybean crops in Japan (Yukawa *et al.*, 2003), *Contarinia sorghicola* Coq. attacks sorghum (Harris, 1961) and *Orseolia oryzae* (Wood-Mason) is a major pest of rice (Bentur & Kalode, 1996). In contrast, some gall midges are used as important biocontrol agents, including two species that are currently used in South Africa against alien invasive Acacias: *Dasineura rubiformis* Kolesik used against *Acacia mearnsii* De Wild (Impson *et al.*, 2008, 2013) and *Dasineura dielsi* Rübsaamen used against *Acacia cyclops* A.Cunn. ex G.Don (Post *et al.*, 2010). The Cecidomyiinae also include numerous predatory species whose larvae feed on pests such as aphids, mites, and scale insects. For example, the aphid midge *Aphidoletes aphidimyza* (Rond.) is used against aphids on vegetable crops in Europe (Markkula *et al.*, 1979) and *Feltiella*

*acarisuga* (Vallot) is used against spider mites on greenhouse vegetable crops in Canada and elsewhere (Gillespie *et al.*, 1998).

Taxonomic descriptions of gall-inducing species should ideally be based on larvae, pupae and adults of both sexes and include information on the galls and life history. Most adult Cecidomyiidae are tiny, delicate flies that have reduced wing venation and long antennae but the family is morphologically diverse, as species have adapted to various ways of life (Gagné, 1989). They are notoriously difficult to study mainly due to their small size and fragility, and the laborious process required for their preparation (Gagné, 1994; Dorchin *et al.*, 2017). Specimens for morphological study should be stored in ethanol and later mounted on microscope slides using an intricate process (Gagné, 1989, 1994) that allows taxonomists to study diagnostic characters. Identification often relies predominantly on the detailed structures of male and female terminalia (Dorchin *et al.*, 2017). Many genera include dozens or even hundreds of species that are very similar morphologically, making their identification very challenging and often requiring a combination of morphological, life history and molecular data in order to distinguish among species (Dorchin *et al.*, 2015a, b, 2019a).

The structure and shape of the galls are considered as extended phenotypes of the insects (Harris *et al.*, 2003; Tokuda, 2012); hence the gall provides valuable details for taxonomists about the insect's life history (Yukawa & Rohfritsch, 2005). Adult gall midges live for only a few days; typically long enough to mate and lay eggs (Gagné, 1989). All feeding is done by the larval stage inside the gall, a stage that may last between two weeks and several years for different species (Gagné, 1989). During this time, the larva manipulates the plant tissue physiologically and morphologically in a remarkably elaborate way such that it is beneficial for the

insects in terms of providing nutrition and possible protection from harsh environmental conditions and predators (Price *et al.*, 1987; Gagné, 1989, 1994; Rohfritsh & Shorthouse, 1982).

Most gall midges induce the formation of nutritive tissues within their galls, which are vital for the survival of the larvae (Rohfritsch, 2008). However, galls of many species in the tribes Alycaulini, Lasiopterini and Asphondyliini do not contain such nutritive tissues and constitute “ambrosia galls”, in which fungal mycelia line the walls of the gall chambers that the larvae are hypothesized to feed on (Rohfritsch, 2008). The present work focuses on the genera *Lasioptera* Meigen and *Asphondylia* Loew, which belong to different clades of “ambrosia gall midges”. Knowledge of these genera in the southern hemisphere, particularly in Africa, is greatly lacking, highlighting the need for taxonomic studies in this region (Wright & Samways, 1998; Dorchin *et al.*, 2015b; Dorchin *et al.*, 2017).

## **1.6. Gall-midge diversity in South Africa**

The Cecidomyiidae of North America and Europe are far better known than those in other regions, where the fauna is still mostly unstudied (Wright & Samways, 1998; Gagné & Jaschhof, 2017; Dorchin *et al.*, 2019). There is no doubt that the Afrotropical fauna of gall midges comprises numerous undiscovered and undescribed species (Dorchin & Gullan, 2007; Dorchin *et al.*, 2017).

The Greater Cape Floristic Region (GCFR) was shown to host an interesting and diverse gall midge fauna (Wright & Samways, 1998; Dorchin *et al.*, 2017).

However, only 29 species of Cecidomyiidae have been described from South Africa (Gagné & Jaschhof, 2017), the great majority of which were recorded before 1930 (Dorchin *et al.*, 2017), and with only one species, *Asphondylia mesembrianthemis*

Schiner, known from the Aizoaceae. The subjects of the present study – the speciose genera *Asphondylia* and *Lasioptera* – currently include six and four described species, respectively, from southern Africa, all of which were described before 1947 (Gagné & Jaschhof, 2017). The six *Asphondylia* species were described from Aizoaceae, Asteraceae, Euphorbiaceae, Solanaceae and Vitaceae (Gagné & Jaschhof, 2017), and the four *Lasioptera* species were described from Thymelaceae, Polygalaceae, Lamiaceae and Combretaceae (Gagné & Jaschhof, 2017). The most recently described cecidomyiid from South Africa is *Afrolasioptera tumida* Dorchin, described in 2007 from leafy bud galls on *Elytropappus rhinocerotis* (Linn.) (Renosterbos) in the Western and Northern Cape (Dorchin & Gullan, 2007).

This small number is clearly due to the lack of taxonomic work and collecting efforts undertaken in the country rather than a reflection of the actual situation. The number of taxonomists currently working on insects in South Africa is very small (Scholtz, 2010; Melin & Colville, 2019). There appear to be very few students who register for postgraduate study on insect systematics in South Africa, which is attributed to poor funding and a change in national research priorities within the fields of biodiversity science (Scholtz, 2010).

### **1.7. Study objectives**

This study is the first of its kind in South Africa, as no systematic study of any cecidomyiid group (on Aizoaceae or other plant families) has been conducted in the country. Therefore, the aim of this project was to investigate the gall midges associated with Aizoaceae, with a focus on the two speciose genera *Malephora* and *Drosanthemum*. The species described here form only a small portion of the gall-

midge fauna occurring on succulent Aizoaceae in South Africa, which is currently under study.

The specific objectives of this thesis were:

1. To survey and document gall midges from two succulent Aizoaceae genera throughout their distributional ranges and their activity seasons in South Africa.
2. To identify and describe the gall midges based on all developmental stages.
3. To define the host plant range of surveyed gall-midges and provide information on their gall types, life histories, phenologies and distribution patterns.

## 2. Materials and Methods

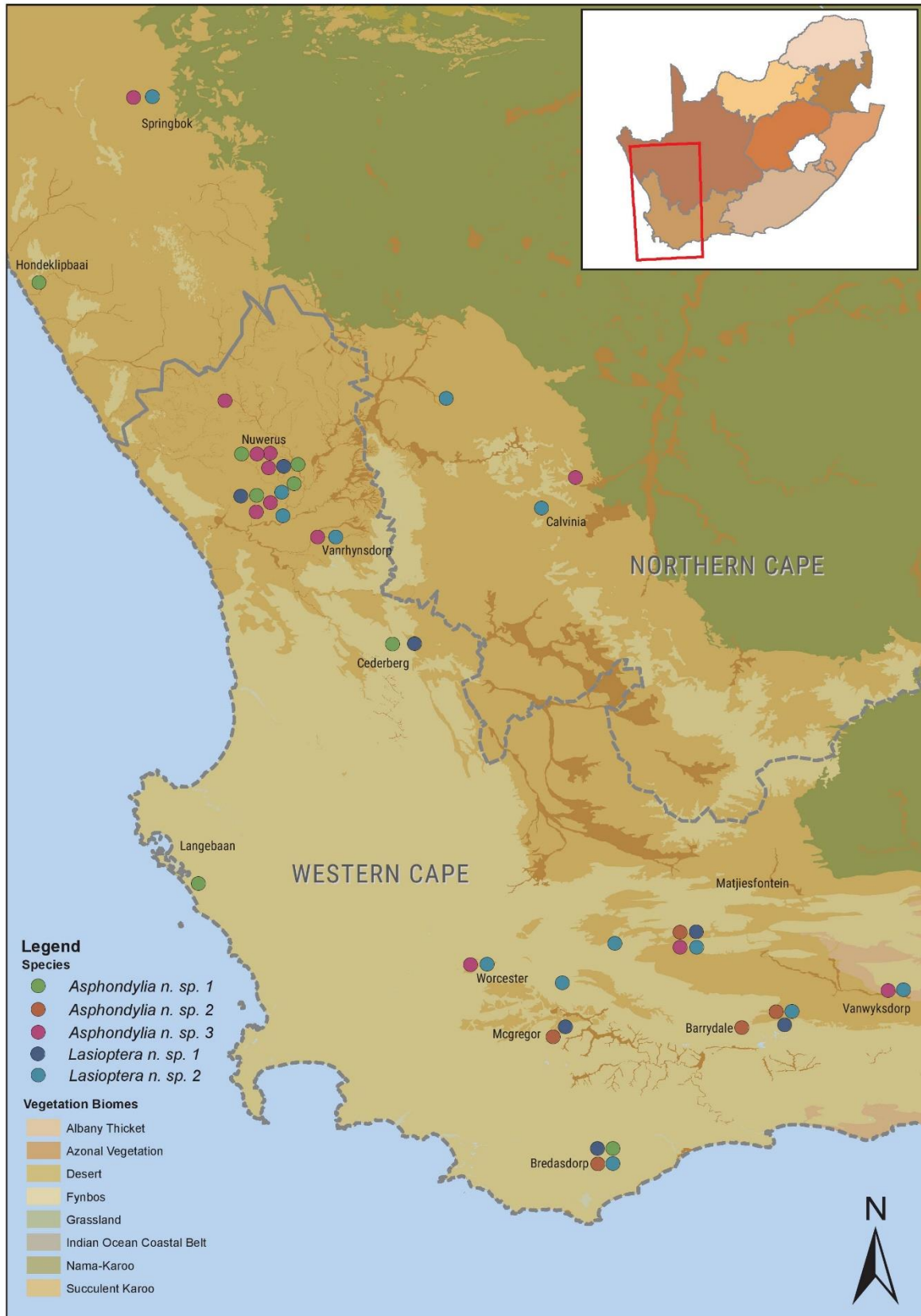
### 2.1. Collecting and rearing of gall midges

#### 2.1.1. Fieldwork and collecting

Fieldwork was conducted between July 2017 and December 2019, mostly during winter and spring (July-September) at numerous field sites in the Western and Northern Cape Provinces of South Africa (Fig. 1). Field sites were visited multiple times during the sampling period to ensure sufficient midge and gall samples were collected for taxonomic descriptions, and to obtain data on phenological patterns. Sites were chosen within areas of high Aizoaceae abundance and diversity (Fig.2), spanning the Fynbos and Succulent Karoo Biomes (Fig. 1).

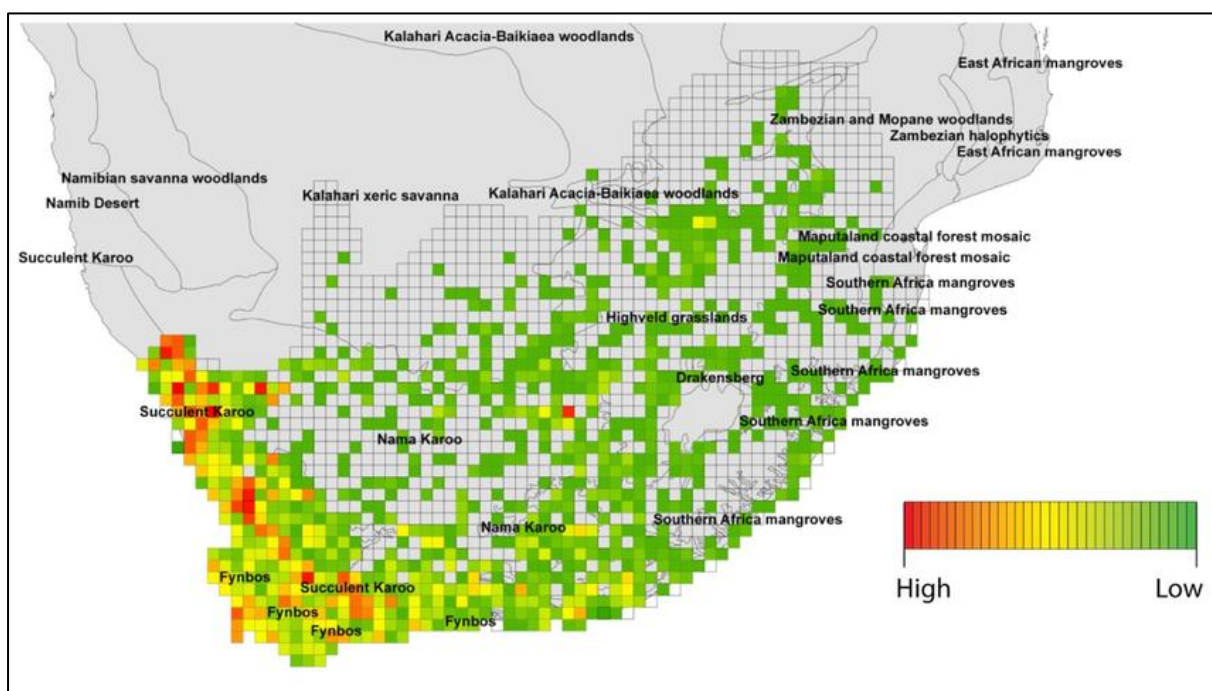
The map by Valente *et al.* (2014) shows the Aizoaceae generis richness per quarter degree square (QDS) (Fig.2). The quarter degree square Data for this map was obtained from the South African National Biodiversity Institute through their PRECIS database (Valente *et al.*, 2014).

Gall midges are known to suffer high parasitism levels by parasitoid Hymenoptera (Dorchin *et al.*, 2014), hence large numbers of galls need to be collected to increase the chances of sufficient adult midge emergence. Not all cecidomyiid species associated with Aizoaceae induce gall formation, some feed as tunnelling or mining endophages. The presence of these gall midges in a plant was only evident through meticulous searching for the exuviae of emerged adults left on the surface of the plants. In these cases, a large amount of plant material was collected and transported to the laboratory for inspection in order to increase the chances of obtaining sufficient numbers of insects. If exuviae were present, the plant material was dissected in order to obtain more information about the immature stages.



**Fig. 1.** Map of South Africa showing collecting sites and gall-midge species collected at each site.

Galls and other plant material were collected into mesh bags in the field for transportation to the laboratory where they were transferred to rearing cages (Fig. 3). For each new collection, a herbarium voucher specimen of the host plant was taken for identification and records were made of plant height, lithology, slope aspect, habitat, substrate and soil type in order to aid plant identification. The galled plant structure was also recorded, and photos were taken of the galls, host plant and habitat.



**Fig. 2.** Map of Southern Africa indicating Aizoaceae generic richness per quarter degree square (taken from Valente *et al.*, 2014).

### 2.1.2. Rearing of gall midges

Rearing cages (Fig. 3) were kept at room temperature (~23 °C) in the laboratory (Fig. 4) and adult gall midges emerged after 1-30 days. Cages were constructed using plastic containers of 5, 10 or 15 liters, with two mesh-covered windows for ventilation. Another larger hole was cut on the side to which a fabric sleeve was attached to allow handling and extraction of insects from the cage.

Cages were checked daily, once in the morning and again in the afternoon. Adult gall midges were extracted with the use of an aspirator connected to a scintillation vial (Fig. 5). The vial was then inserted into a cyanide killing jar for a few minutes, after which the insects were transferred into Eppendorf vials containing ethanol and stored in the freezer (-20°C). Specimens to be used for morphological study were stored in 70% ethanol and samples for future molecular study were stored in 99% ethanol.



**Figs. 3-6.** Gall-midge rearing and dissection: (3) Ventilated rearing cage; (4) Rearing cages in the laboratory; (5) Removal of adult gall midges from the rearing cages using an aspirator; (6) Dissected gall showing a single chamber containing a pupa.

### **2.1.3. Gall dissection**

Galls were dissected in the laboratory in order to document information on gall characteristics and cecidomyiid life history (Fig. 6). Both the pupal and larval stages were excised and stored in the same vial with the adults collected from the respective cage. Dissection of galls was carried out once adults began to emerge from the galls. Notes were taken on the number of chambers, gall size, shape, discolouration, abundance, texture and presence of parasitoids.

## **2.2. Morphological study and taxonomy**

Terminology used for general adult morphology follows McAlpine *et al.* (1981), that for wing venation follows Kirk-Spriggs & Sinclair (2017), and terminology for morphological characters of immature stages follows Gagné (1989). Terminology for characters of the female ovipositor follows Dorchin (2001). The new species are not named here officially to avoid nomenclatural problems prior to their official description in a scientific journal.

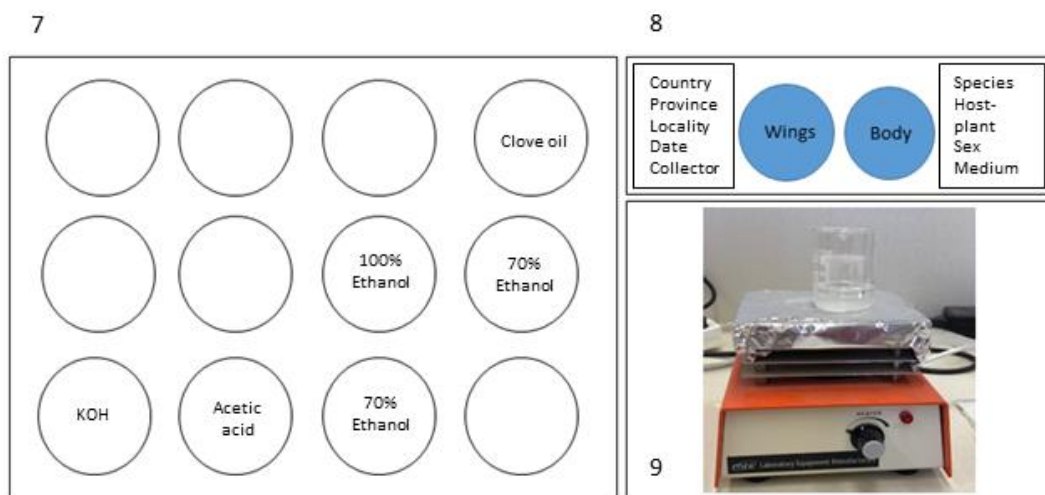
### **2.2.1. Slide mounting**

Gall-midge specimens were mounted onto microscope slides following the process used by Gagné (1989) and outlined in detail below. Adult gall midges are extremely fragile and great care was taken not to damage them in the slide-mounting process. On completion of the project the types will be deposited in the Iziko South African Museum, 25 Queen Victoria St, Gardens, Cape Town, South Africa (SAM), the Steinhardt Museum of Natural History, 12 Klausner St, Tel Aviv, Israel (SMNHTAU), and other relevant institutions as will be specified in the formal species descriptions.

Mounting process:

1. The specimens were transferred to a petri dish with 70% alcohol and individuals were selected under a Wild Heerbrugg M3C stereomicroscope.

- Specimens were transferred into a 70% ethanol pit on a pitted plate (Fig. 7). The chemicals were arranged in the pitted plate based on personal preference (Fig. 7).
- Wings were gently removed from the thorax by carefully grabbing their bases with fine tipped forceps while the thorax was held down with a pin. Wings were then gently lifted out of the ethanol using a pin and placed in clove oil.



**Figs. 7-9.** Specimen mounting: (7) Diagram showing the placement of chemicals in a pitted plate for microscope slide mounting; (8) Layout of a mounted slide; (9) Heating plate with a double boiler set up with vials containing KOH.

- The abdomen was perforated with an insect pin and the body was transferred to a heated solution of 10% KOH (potassium hydroxide) using a double boiler system. A 10ml measuring beaker containing 5mls of 10% KOH was placed inside a 50ml measuring beaker containing 20ml of water and put on a hotplate (Cenco Instrumenten B.V.: pyro mag stir, Fig. 9). The specimen was left in the solution for approximately eight minutes and examined periodically in order to ensure that it did reach boiling point, as that would damage the specimen.
- Specimens were then transferred to a ceramic-pitted plate using a pin or pipette and pressure was applied to the abdomen using two pins in order to clear the

specimen for slide mounting. In the case of female *Lasioptera*, whose telescopic ovipositor had to be exerted, the specimen was placed in the heated KOH solution before piercing the abdomen and heated for 1-2 minutes, which enabled pressure to be placed on the abdomen to push the ovipositor out without damaging the specimen.

6. The wings were mounted onto a microscope slide while the remainder of the body was in the heated KOH solution and covered by a separate round coverslip in Euparal (Fig. 8).
7. Cleared specimens were removed from the KOH on the pitted plate and placed in a few drops of acetic acid for 1-2 minutes, which provided further clearing.
8. The specimen was transferred from the acetic acid to 70% ethanol and then 99% ethanol for 1 minute each in order to discard any excess water.
9. The specimen was then placed in clove oil for approximately 5 minutes.
10. A small drop of Euparal was placed onto the slide next to the wings of the respective specimen and the body was gently placed into it. The head was separated from the body using a pin and positioned dorsoventrally. In male specimens, the terminalia were also separated from the body and placed dorsoventrally, and the specimen was covered by a round coverslip.
11. The slide was viewed under a Zeiss Axioskop compound microscope to determine if the specimen was positioned properly. Gently manoeuvring the coverslip with pins or by adding more Euparal to the sides of the coverslip enabled some adjustment of the specimen, if necessary.
12. Slides were stored in a flat position for about 2 months, or until the Euparal had dried fully. They were checked for position and shifts in orientation of the

specimen under a compound microscope every few days and adjustments were made if necessary. Thereafter the slides were stored in microscope slide boxes.

### **2.2.2. Drawing, image capture and measurements**

Drawings of morphological structures were made using a Zeiss Axioskop compound microscope with a mounted drawing tube. Specimens chosen for drawing and image capture displayed characters that were representative of the species as a whole. Morphological characters were measured using an eyepiece graticule. *Lasioptera* ovipositors were measured from the anterior margin of tergite 8 of the abdomen to the apex of the fused cerci and their length was expressed relative to the 7<sup>th</sup> abdominal sternite. In *Asphondylia*, the length of the needle-like part of the ovipositor was measured and expressed relative to the length of the 7<sup>th</sup> sternite. Wing length was measured for both sexes of both genera and expressed in millimeters. In both genera, flagellomere 5 was measured and its length expressed relative to that of flagellomere 1. Body length of pupae and larvae was measured and expressed in millimeters.

For capturing images of live gall midges, adults were placed in scintillation vials and set on ice packs for several minutes before capturing images with an Olympus SC50 camera mounted on an Olympus S261 stereomicroscope or a Leica Z16 APO stereoscope. A few adults were pinned in order to preserve the colour pattern created by scales and hair-like setae. Images of intact pupae and of slide-mounted specimens were taken using a Nikon SMZ1500 Stereoscopic Zoom Microscope and a Nikon DS Camera Control Unit DS-U3 & DS-5M camera head.

### **3. *Asphondylia* species on *Drosanthemum* and *Malephora* in South Africa**

#### ***Asphondylia* Loew 1850**

*Asphondylia* belongs in the Asphondyliini, a monophyletic tribe of 521 described species (Dorchin *et al.*, 2015b; Gagné & Jaschhof, 2017), all of which induce galls on their host plants (Gagné, 1989). *Asphondylia* is one of the largest Cecidomyiidae genera with more than 320 described species (Tokuda, 2012; Gagné & Jaschhof, 2017) from a wide range of host plants across the world, but many more still await discovery and description (Dorchin *et al.*, 2015, 2019). As with all gall midges, knowledge on *Asphondylia* species from the southern hemisphere is greatly lacking and there is still much work to be done in this region (Dorchin *et al.*, 2015). Life histories of species in this genus differ greatly across host plant phenologies and zoogeographical regions (Tokuda, 2012; Uechi & Yukawa, 2006).

*Asphondylia* galls are always associated with a fungus that lines the larval chambers inside the galls, but the nature of this association is not completely known. It has, however, been proposed that the fungus is important for gall formation and that larvae feed on it rather than on the plant tissue inside the gall (Bissett & Borkent, 1988; Rohfritsch, 2008; Heath & Stireman, 2010). It has also been hypothesized that cecidomyiid species with such fungal association have a broader host range than those that are not associated with fungi and that the fungus is important in facilitating new host associations and could be a driver of diversification (Bissett & Borkent, 1988; Joy, 2013). However, a recent robust phylogeny for Cecidomyiidae (Dorchin *et al.*, 2019) showed that currently available data do not support the hypothesis that the host ranges of symbiotic species are broader than those of non-symbiotic species or

that the symbiotic species associated with the fungi diversify faster than the non-symbiotic species.

*Asphondylia* is a morphologically uniform genus with very few diagnostic attributes, making its taxonomy particularly challenging (Dorchin *et al.*, 2014; Gagné *et al.*, 2018). Several characters are uniform across the genus and the tribe, and it is considered unnecessary to repeat them in new species descriptions (Gagné *et al.*, 2018). The number of antennal flagellomeres is always 12 and the three apical flagellomeres in females are consecutively shorter, with flagellomere 12 almost spherical. Male flagellomeres bear twisting circumfila - sensilla unique to gall midges that consist of several units fused into a single structure (Boddum *et al.*, 2010) - whereas female flagellomeres bear only two bands of horizontal circumfila with two longitudinal connections. Wing venation is also uniform throughout the genus, with R<sub>4+5</sub> joining C posterior to wing apex. While in many gall midge genera the male and female terminalia are used for distinguishing among species, in *Asphondylia* these are fairly uniform across the genus and are generally not used in species identification. *Asphondylia* females always have a needle-like ovipositor and an elongated 7<sup>th</sup> sternite, but the relative length of the ovipositor may differ considerably between species. Therefore, ovipositor length is often measured relative to the length of the 7<sup>th</sup> sternite when reported in descriptions (Gagné *et al.*, 2018). The male terminalia are compact with spherical gonostyli in a dorsal rather than apical position on the gonocoxites.

The best distinguishing characters among *Asphondylia* species are found in the immature stages, including the shape and number of pupal facial horns, antennal horns, facial papillae, and the organization of spines along the dorsal part of the abdominal segments (Gagné *et al.*, 2018). The pupa uses the modified facial

projections to move through plant tissue towards the surface when the adult is ready to emerge. The strong dorsal spines dig into the gall tissue and allow the pupa to wriggle through the gall towards the surface and exit (Gagné, 1989). Distinguishing characters in larvae include the shape of the spatula and the number and arrangement of the associated papillae.

In this chapter, I describe three new species of *Asphondylia* from the succulent Aizoaceae genera *Malephora* and *Drosanthemum* based on adult and immature stages. I also provide information on their life history and distribution as well as relevant taxonomic comments.

### **3.1. *Asphondylia* n. sp. 1**

**Host plants:** *Malephora purpureo-crocea* (Haw.) Schwantes.

**Gall and biology:** *Asphondylia* n. sp. 1 (Fig. 10) induces one of the most conspicuous galls on Aizoaceae (Figs 11-13). The bases of the very succulent leaves are conspicuously inflated, forming large, succulent, amorphous galls, up to 3 cm in diameter. Each gall contains 5-10 larval chambers, each with a single larva. The walls of the larval chambers are more rigid and dry than the surrounding gall tissue. Galls can be extremely abundant covering almost the entire plant in some instances. The species is univoltine: young galls become apparent around June and July and adults emerge in August-October. Hymenoptera parasitism rates in these galls appear to be very high based on the numbers of endo and ectoparasitoids that emerged in the rearing cages.

**Adult:** (Fig. 10) general colour brownish-orange, covered in greyish-white scales.

**Head:** (Fig. 14) eye facets round. Frons with numerous strong, dark setae. Palp (Fig. 15) three-segmented, second and third segments with numerous strong setae,

otherwise covered in microtrichia; segments successively longer; first segment as long as wide; second segment about three times as long as wide, wider distally than at base, club-shaped; third segment at least seven times as long as wide, tapered at apex. Labella (Fig. 16) rounded along lateral margins, wide anteriorly, with 6-8 strong, long, evenly scattered setae. **Antenna** (Fig. 17): scape wider distally than at base, pedicel spherical, both with long, strong setae. Male flagellomeres cylindrical; flagellomere 1 slightly longer than succeeding, apical flagellomere slightly pointed at apex, all covered by anastomosing circumfila loops (Fig. 18), microtrichia, and numerous long, fine setae. Flagellomere 5, 1.2-1.6 times as long as flagellomere 1 (n=29). Female flagellomeres 1-10 cylindrical with simple circumfila connected by two transverse sections (Fig. 18), numerous long, fine setae, otherwise covered by microtrichia; flagellomere 1 conspicuously longer than succeeding, flagellomere 5, 1.3-1.8 times as long as flagellomere 1 (n=43); flagellomeres 10-12 with two whorls of circumfila and several longitudinal connections, numerous long, fine setae and otherwise covered by microtrichia.

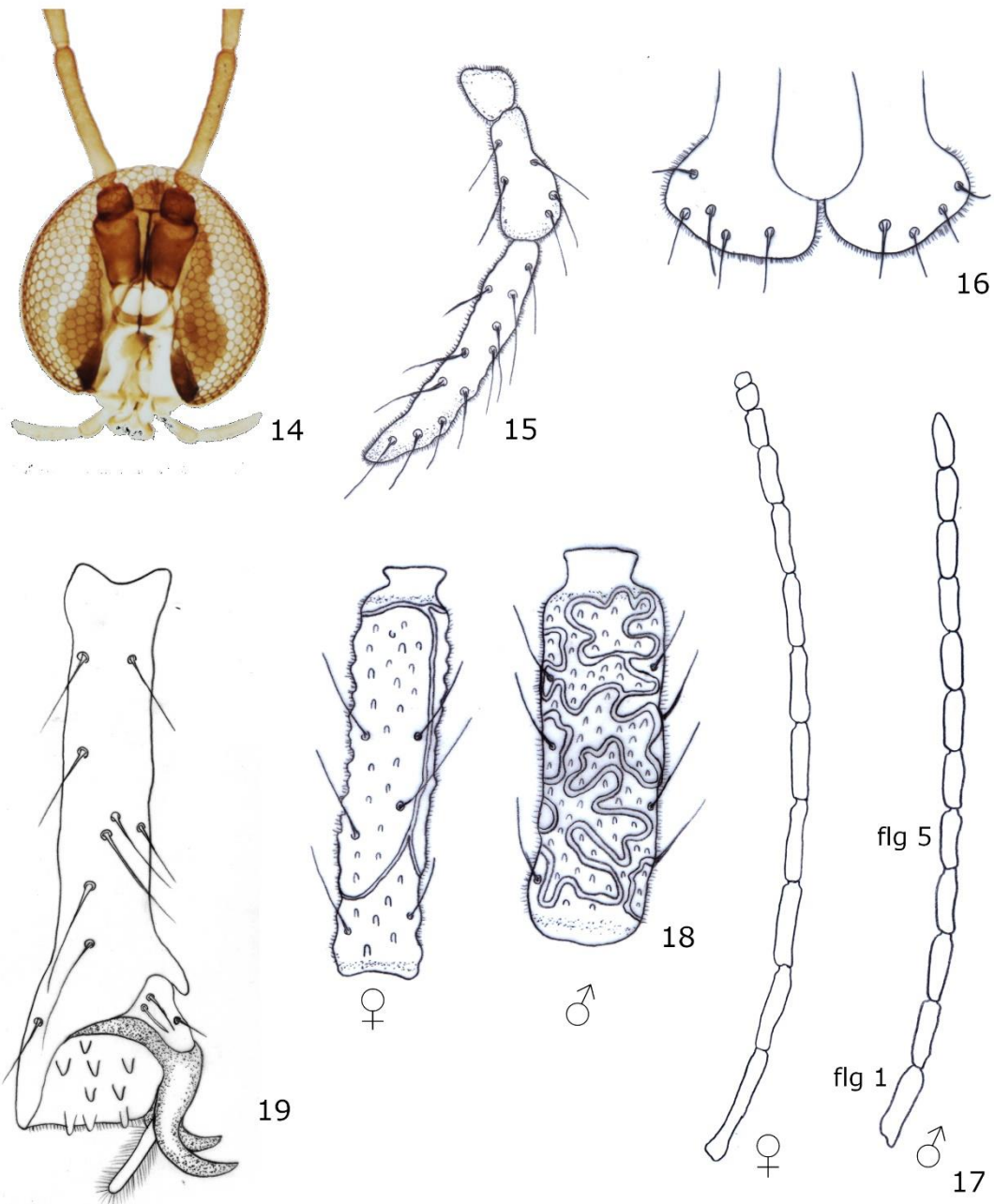


**Figs 10-13:** *Asphondylia* n. sp. 1: (10) Adult female; (11) Bud galls on *Malephora crassa*; (12-13) Bud galls on *Malephora purpureo-crocea* with pupal exuviae.

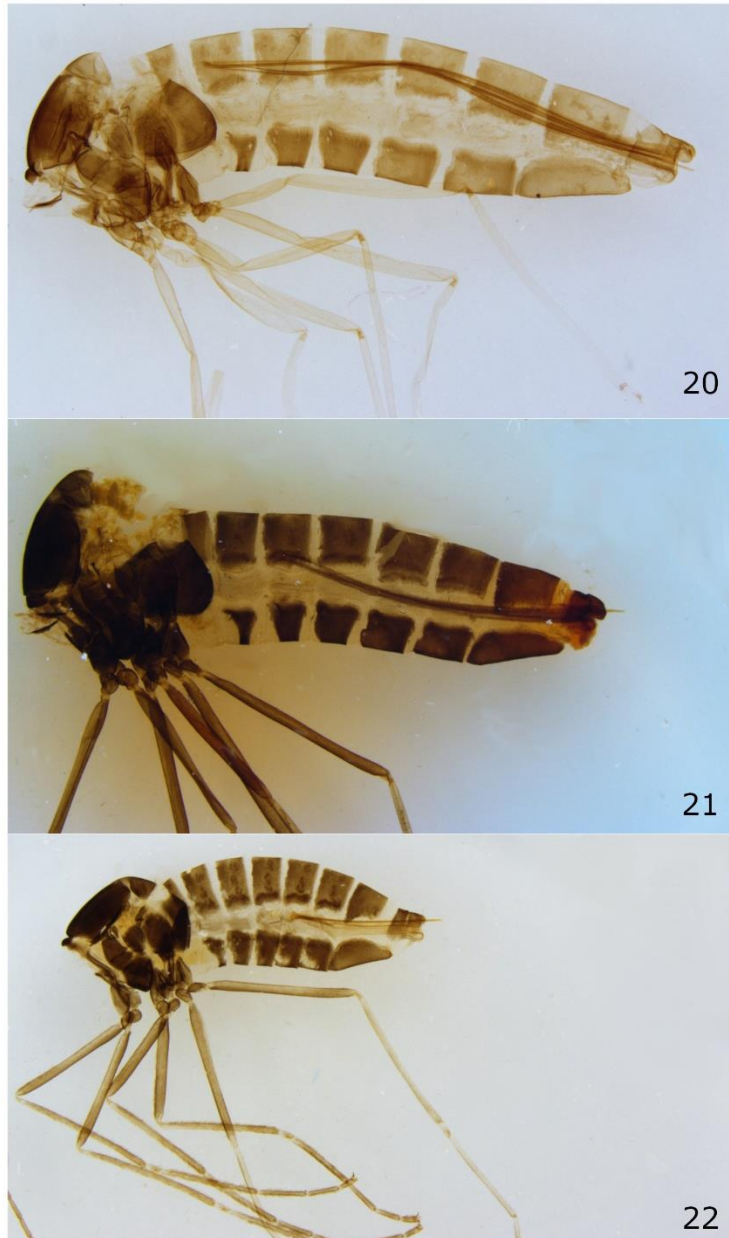
**Thorax: Legs:** brownish-orange, densely covered by white and brown scales and setae. Coxae with long, strong black setae. Tarsal claws evenly curved, empodia robust, densely setulose, longer than claw (Fig. 19). **Wing:** greyish, covered by dark hair-like microtrichia and fringed with long black setae; 2.62-3.46 mm in males (n=29), 3.92-5.12 mm in females (n=43);  $R_1$  joins C just before wing mid-length,  $R_{4+5}$  curved slightly towards apex, joining C beyond wing apex, CuA and  $M_4$  weak, forming a fork.

**Female abdomen** (Figs. 20, 23): long and slender; dorsum covered in grey scales, pleura and venter with silvery hair-like scales. Tergites 1-7 with two posterior rows of strong setae and evenly covered by setae on mid-part, otherwise covered by scales;

tergite 8 shorter than preceding, saddle-shaped, without setae. Sternites 2-6 roughly rectangular with posterior row of fine setae; sternite 7 much longer than preceding, slightly narrowed posteriorly, covered with fine setae. Ovipositor needle like with sharp point (Fig. 23). Sclerotized part of ovipositor 3.02-4.80 times as long as sternite 7 (n=42).



**Figs 14-19:** *Asphondylia n. sp. 1*: (14) Female head; (15) Palp; (16) Labella; (17) Female and male antennae (flg = flagellomere); (18) Female and male 5<sup>th</sup> flagellomeres; (19) Acropod.



**Figs 20-22:** *Asphondylia* female abdomen. (20) *Asphondylia* n. sp. 1; (21) *Asphondylia* n. sp. 2; (22) *Asphondylia* n. sp. 3.

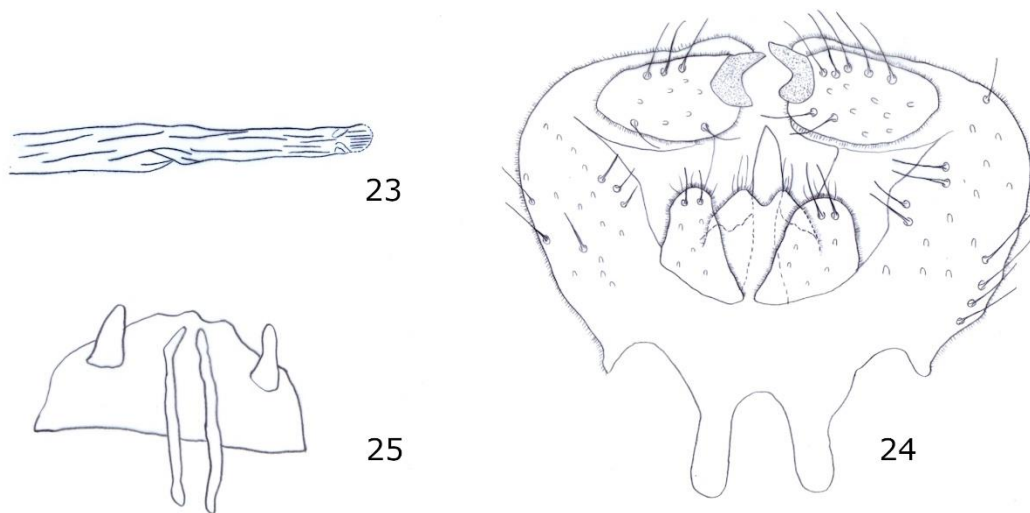
**Male abdomen:** colour pattern as in female. Tergites with posterior row of setae, a few scattered setae on basal area and otherwise covered in scales; tergite 1 shorter than succeeding, tergites 2-7 rectangular, with posterior row of setae, tergite 7 more setose posteriorly than preceding; tergite 8 a narrow band, smaller than preceding, without setae. Sternites 2-7 with posterior row of strong setae otherwise evenly covered in fine setae and scales; sternite 8 narrow, evenly covered by strong setae.

**Terminalia** (Fig. 24): gonocoxite longer than wide, with slight apical projection, bearing numerous strong setae apically and laterally, and otherwise evenly setulose. Gonostylus ovoid, wider than long, with numerous strong setae and otherwise evenly setulose, bearing strong, bidentate apical tooth. Aedeagus cylindrical, tapered towards apex, considerably longer than hypoproct. Hypoproct divided apically into two lobes by shallow notch, with few long setae apically on each lobe, otherwise evenly covered by fine setae. Cerci separated to base, evenly rounded apically, bulbous, with several strong setae apically and setulose throughout.

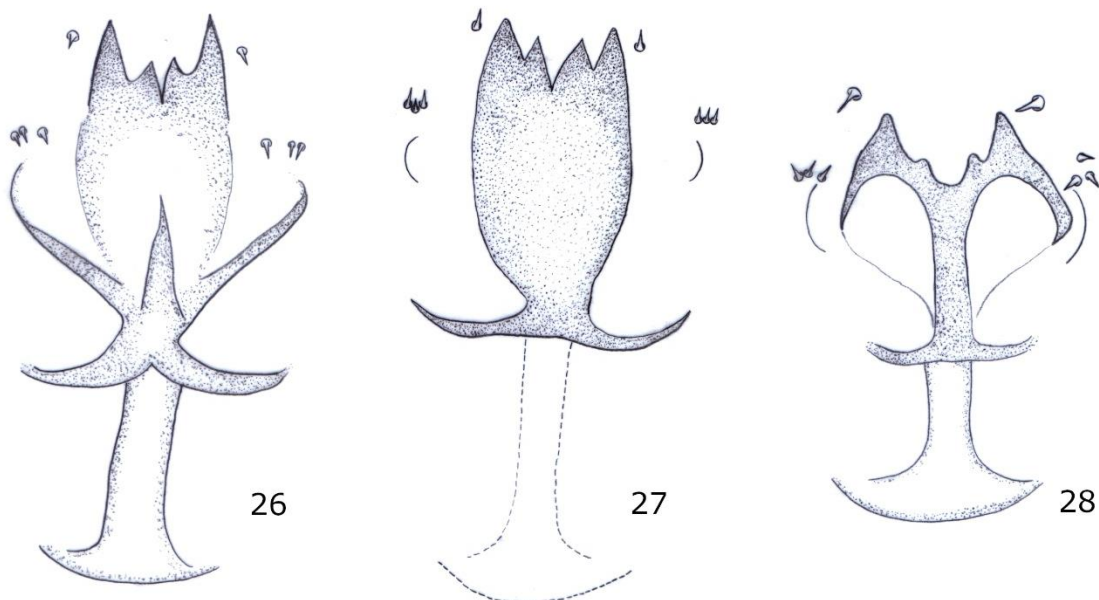
**Larva:** yellow to orange, body often arched backwards when alive, integument covered by spicules. Length 2.8-5.1 mm (n=6). Antennae (Fig. 25) about twice as long as wide; cephalic apodeme twice the length of head capsule. Spatula (Fig. 26) quadridentate, strongly sclerotized, longer than wide, lateral teeth three times as long as median teeth, gap between median teeth clearly deeper than gaps between lateral and median teeth, shaft long and widened at base with four to five strongly sclerotized splayed arms at mid-length, weakly sclerotized elsewhere. On each side of spatula three setose lateral papillae, one slightly separated from other two, with longer seta. Sternal papillae with strong setae.

**Pupa** (Figs. 29-30): dark brown when mature. Antennal horns (Figs. 35-36) wide at base, slightly arched, pointed, serrate along medial margins, with clear separation between smoothly pointed apices and medial serration. Cephalic setae short. Upper facial horn not divided, slightly curved anteriorly at apex. Lower facial horn not divided, slightly curved anteriorly at apex, on each side with two papillae (Fig. 41), one bearing long strong seta, the other asetose. Posterolateral area of frons on each side with two lateral papillae, one of which setose. Prothoracic spiracle (Fig. 42) long and slender, widened at base, featherlike apically. Abdominal segments (Fig. 43)

except for first and last each with straight, posterior row of spikes and 3-4 less ordered rows anteriorly. Spikes 4-5 times as long as wide. Last abdominal segment with 2-3 less ordered rows of spikes of varying lengths, posteriorly with strong spikes extending laterally.



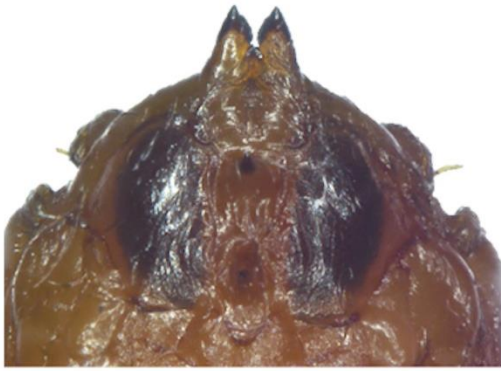
**Figs. 23-25:** *Asphondylia* n. sp. 1; (23) Tip of female ovipositor; (24) Male Terminalia, dorsal; (25) Larval head.



**Figs 26-28:** Larval spatula. (26) *Asphondylia* n. sp. 1; (27) *Asphondylia* n. sp. 2; (28) *Asphondylia* n. sp. 3.



**Figs. 29-34:** Pupa frontal (top) and lateral (bottom) views. (29-30) *Asphondylia* n. sp. 1; (31-32) *Asphondylia* n. sp. 2; (33-34) *Asphondylia* n. sp. 3.



35



36



37



38

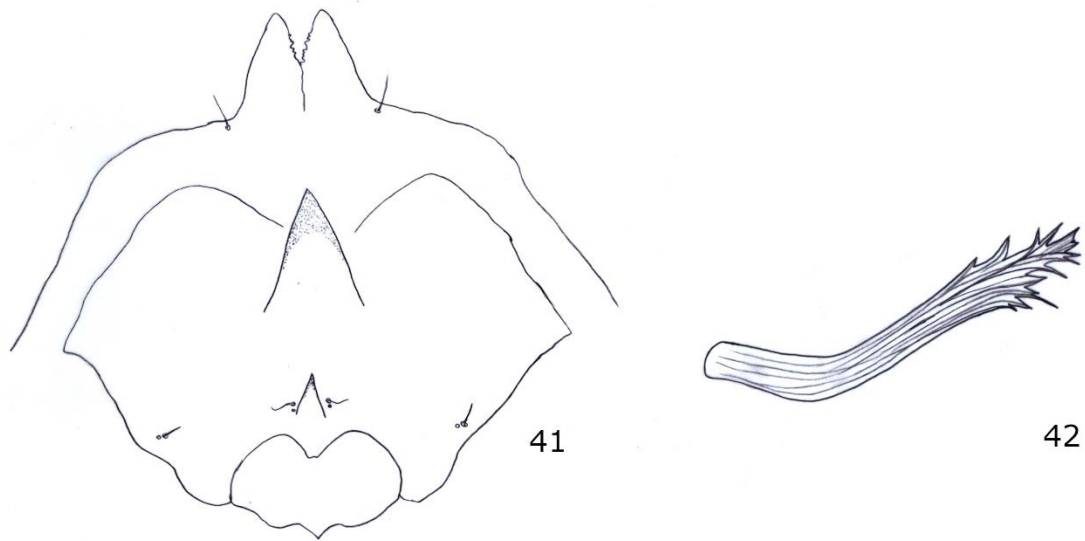


39



40

**Figs. 35-40:** Pupal head, frontal (left) and lateral (right) views. (35-36) *Asphondylia* n. sp. 1; (37-38) *Asphondylia* n. sp. 2; (39-40) *Asphondylia* n. sp. 3.



**Figs. 41-42:** *Asphondylia* n. sp. 1. (41) Pupal head and associated papillae; (42) Pupal prothoracic spiracle.



**Figs 43-45:** Pupal abdominal segments. (43) *Asphondylia* n. sp. 1; (44) *Asphondylia* n. sp. 2; (45) *Asphondylia* n. sp. 3.

**Distribution:** (Fig. 1) The species is found from Vanrhynsdorp in the northern part of the Western Cape to Hondeklipbaai in the north western part of the Northern Cape.

**Comments:** Of the three new *Asphondylia* species described here, *Asphondylia* n. sp. 1 is the most conspicuous due to the large size of its adults and galls. The needle-like section of the female ovipositor is almost as long as the entire abdomen compared to the same section in *Asphondylia* n. sp. 2 and n. sp. 3. Gagné (2018), in

his diagnosis of the genus, stated that *Asphondylia* larvae have five setose lateral papillae on each side of the spatula, but in all three species described here there are only three lateral papillae.

Towards the end of the study, this species was reared from several other mat-forming perennial Aizoaceae with large succulent leaves (5-10cm long): *Jordaaniella dubia* (Haw.) H.E.K. Hartmann and *Carpobrotus quadrifidus* L.Bolus in the West Coast national park, *C. acinaciformis* L. (L.Bolus) near Bredasdorp, *Malephora crassa* (L.Bolus) H.Jacobsen & Schwantes near Botterkloof pass, *Acrodon parvifolius* du Plessis in Botrivier, and *Cephalophyllum tricolorum* N.E.Br. near Vanrhynsdorp (all in the Western Cape). Galls on these species generally resemble those on *Malephora purpureo-crocea*. The galls on *Malephora* and *Carpobrotus* are bigger than those on other host plants but no morphological differences were found among the midges from these hosts. Preliminary molecular results confirm that individuals from all host plants belong to the same species (Dorchin *et al.*, unpublished).

**Material examined: Holotype:** ♀ South Africa: Ouplaas [31°12'33"S, 18°26'35"E], Northern Cape, 11.viii.2017, S. van Munster, N. Dorchin and C. Klak, ex bud gall on *Malephora purpureo-crocea*. On permanent microscope slide in Euparal.

**Paratypes:** From *Malephora purpureo-crocea*: 14♀, 10♂, 5 larvae (on three slides), same data as holotype; 7♀, 3♂, 2 larvae (on one slide), 4 exuviae (on two slides), Namaqua National Park [31°33'14"S, 18°24'55"E], Northern Cape, 10.viii.17, S. van Munster, N. Dorchin and C. Klak; 11♀, 9♂, 4 exuviae (on two slides), Grootgraafwater, Vanrhynsdorp [31°16'04"S, 18°32'32"E], Western Cape, 11.ix.2017, S. van Munster, N. Dorchin and C. Klak; 1♂, 9 exuviae (on 7 slides), 5km

N of Vanrhynsdorp [31°16'04"S, 18°32'32"E], Western Cape, 15.viii.2018, S. van Munster, N. Dorchin and C. Klak.

**Other material examined:**

From *Malephora purpureo-crocea*: 4♀, 2♂ Ouplaas [31°12'33"S, 18°26'35"E], Northern Cape, 11.viii.2017, S. van Munster, N. Dorchin and C. Klak; 2♀, 1♂, Namaqua National Park [31°33'14"S, 18°24'55"E], Northern Cape, 10.viii.17, S. van Munster, N. Dorchin and C. Klak; 2♀, 2♂, Grootgraafwater, Vanrhynsdorp [31°16'04"S, 18°32'32"E], Western Cape, 11.ix.2017, S. van Munster, N. Dorchin and C. Klak.

### 3.2. *Asphondylia* n. sp. 2

**Host plant:** *Malephora latipetala* (L.Bolus) H.Jacobsen & Schwantes.

**Gall and Biology:** *Asphondylia* n. sp. 2 (Fig 46) induces large, green, fusiform to amorphous bud galls (Figs 47-49) up to 2.2 cm in diameter that incorporate leaf bases on *Malephora latipetala*. Usually one leaf in a pair on the same node is affected. Galls are firm to the touch and juicy, each containing one or two chambers, occasionally more. The walls of the larval chambers are drier and slightly more rigid than the surrounding gall tissue. The walls of older chambers turn dark. This species completes several generations between August and November. Parasitism levels are relatively low compared to those in *Asphondylia* n. sp. 1 and *Asphondylia* n. sp. 3.



**Figs. 46-49:** *Asphondylia* n. sp. 2: (46) Adult male; (47-49) Bud galls on *Malephora latipetala*.

**Adult:** characters as in *Asphondylia* n. sp. 1, except for the following.

**Head:** frons with numerous strong white setae. **Antenna:** Male flagellomere 5, 1.1-1.4 times as long as flagellomere 1 (n=10). Female flagellomere 5, 1.3-1.5 times as long as flagellomere 1 (n=7).

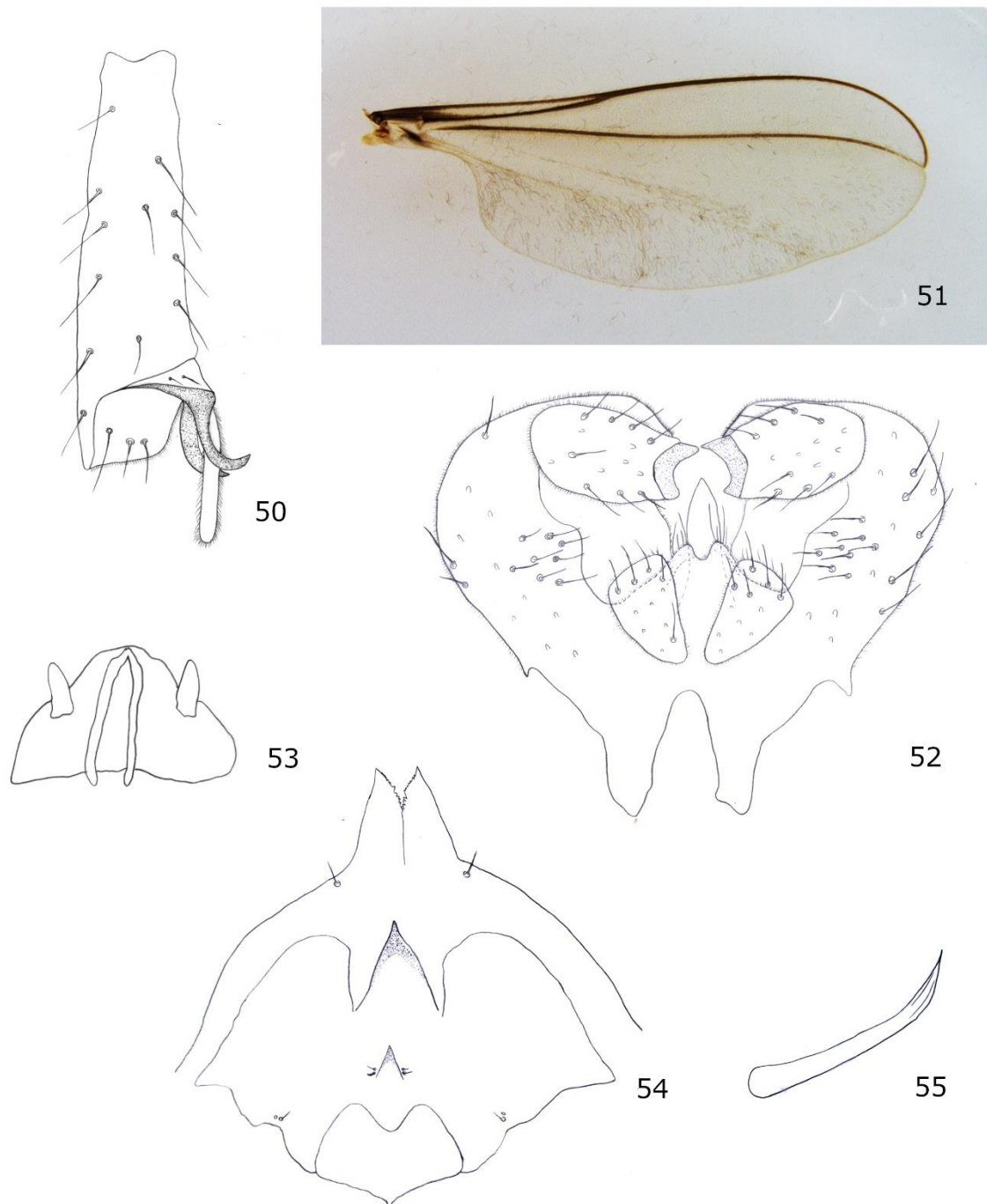
**Thorax: Legs:** brownish-orange, covered in greyish-white scales and setae. Coxae evenly covered by strong white setae. Tibia and tarsi covered in greyish white scales and setae. Empodia significantly longer than tarsal claws (Fig. 50). **Wing** (Fig. 51): 2.46-2.88 mm in males (n=10), 4.48-4.80 mm in females (n=7).

**Female abdomen** (Fig. 21): sclerotized part of ovipositor 3.57-3.91 times as long as sternite 7 (n=6).

**Male abdomen: Terminalia** (Fig. 52): aedeagus longer than hypoproct. Cerci truncate slightly at apex, setose and strongly setulose throughout.

**Larva:** length 2.5-4.4 mm (n=6). Cephalic apodeme as long as head capsule (Fig. 53). Spatula (Fig. 27): lateral teeth slightly longer than median teeth, gap between median teeth slightly deeper than gaps between lateral and median teeth, strongly sclerotized; sclerotized section of spatula longer than wide. Shaft weakly sclerotized with two sclerotized arms before mid-length. Three equally setose lateral papillae closely grouped on each side of spatula.

**Pupa** (Figs. 31-32): antennal horns wide-based (Figs. 37-38), apices pointed, lateral margins straight to apex, medial margins serrated from just below apex to base. Lower facial horn on each side with two papillae (Fig. 54), one bearing longer seta than other. Prothoracic spiracle (Fig. 55) long, slender with widened base and smoothly tapered to apex. Abdominal segments (Fig. 44) except for first and last, each with straight, posterior row of spikes and 3-4 less ordered, closely grouped rows anteriorly. Dorsal spines 3-4 times as long as wide.



**Figs. 50-55:** *Asphondylia* n. sp. 2: (50) Acromere; (51) Wing; (52) Male terminalia, dorsal; (53) Larval head; (54) Pupal head with associated papillae; (55) Pupal prothoracic spiracle.

**Distribution:** This species is found in the eastern regions of the Western Cape (Fig. 1). It was collected in Vrolijkheid Nature Reserve, Van Wyksdorp, Anysberg Nature Reserve, near Matjiesfontein and Bredasdorp.

**Comments:** *Asphondylia* n. sp. 2 is smaller than *Asphondylia* n. sp. 1 but larger than *Asphondylia* n. sp. 3. Its distribution only overlaps that of *Asphondylia* n. sp. 1 in the Overberg, where *Asphondylia* n. sp. 1 was found on *Carpobrotus acinaciformis* near Bredasdorp (Fig. 1). This species is morphologically very similar to *Asphondylia* n. sp. 1 but is found only in the south western parts of the GCFR, whereas the distribution range of *Asphondylia* n. sp. 1 extends into the north western parts of the GCFR as far as the Namaqua National Park (Fig. 1). The two species also differ in their host species: *Asphondylia* n. sp. 2 is restricted to *Malephora latipetala*, whereas *Asphondylia* n. sp. 1 has multiple host plants, contributing to its wider distribution range.

The most obvious morphological differences between the two *Asphondylia* species found on *Malephora* are seen in the immature stages. The pupal antennal horns in *Asphondylia* n. sp. 2 are shorter and the lateral papillae next to the lower facial horn are both setose, as opposed to one setose and one aetose papilla in *Asphondylia* n. sp. 1. In the adult males of *Asphondylia* n. sp. 2, the cerci are truncated slightly at the apex, unlike the rounded cerci of *Asphondylia* n. sp. 1. Preliminary molecular data confirm that these species are distinct (Dorchin *et al.*, unpublished).

**Material examined: Holotype:** ♀ South Africa: Vrolijkheid Nature Reserve [33°55'4"S, 19°52'39"E], Western Cape, 14.viii.2019, S. van Munster and N. Dorchin, ex bud gall on *Malephora latipetala*. On permanent microscope slide in Euparal.

**Paratypes:** From *Malephora latipetala*: 1♀, 3♂, Anysberg Nature Reserve [33°25'58"S, 20°47'47"E], Western Cape, 3.ix.2017, S. van Munster and N. Dorchin; 6♀, 7♂, 6 larvae (on six slides), 10 exuviae (on 10 slides), same data as holotype.

### 3.3 *Asphondylia* n. sp. 3

**Host plants:** *Drosanthemum comptonii* L.Bolus, *D. curtophyllum* L.Bolus, *D. desciduum* H.E.K.Hartmann & C.Bruckmann, *D. delicatulum* Schwantes, *D. karrooense* L.Bolus, *D. leipoldtii* L.Bolus, *D. oculatum* L.Bolus, *D. schoenlandianum* L.Bolus, *D. subclausum* L.Bolus, *D. subplanum* L.Bolus and several other *Drosanthemum* species (to be identified).

**Gall and Biology:** *Asphondylia* n. sp. 3 (Fig. 56) induces typical bud galls on numerous *Drosanthemum* species (Figs. 57-59). Gall shapes vary slightly, but are commonly amorphous, nearly spherical at the base and sometimes taper apically to form small projections. Galls are 0.5-1.0 cm wide, mostly green, often partly red or yellow. Despite the small size of the gall, it contains 3-6 chambers, the walls of which are drier and more rigid than the surrounding plant tissue. The galls are heavily parasitized by polyembryonic parasitoids (Hymenoptera, Platygasteridae). This species has multiple generations a year and galls were found from August to April. Some host plants were sampled only once, and more intensive sampling is needed in order to determine if they support multiple generations per year.

**Adult:** characters as in *Asphondylia* n. sp. 1 except for the following.

**Head: Antennae:** male flagellomere 5, 1.1-1.7 times as long as flagellomere 1 (n=50). Female flagellomere 5, 1.2-1.8 times as long as flagellomere 1 (n=79).

**Thorax: Legs:** empodia as long as tarsal claws (Fig. 60). **Wing:** 2.00-3.30 mm in males (n=50), 2.70-3.80 mm in females (n=79).

**Female abdomen** (Fig. 22): sclerotized part of ovipositor 2.00-3.33 times as long as sternite 7 (n=78).

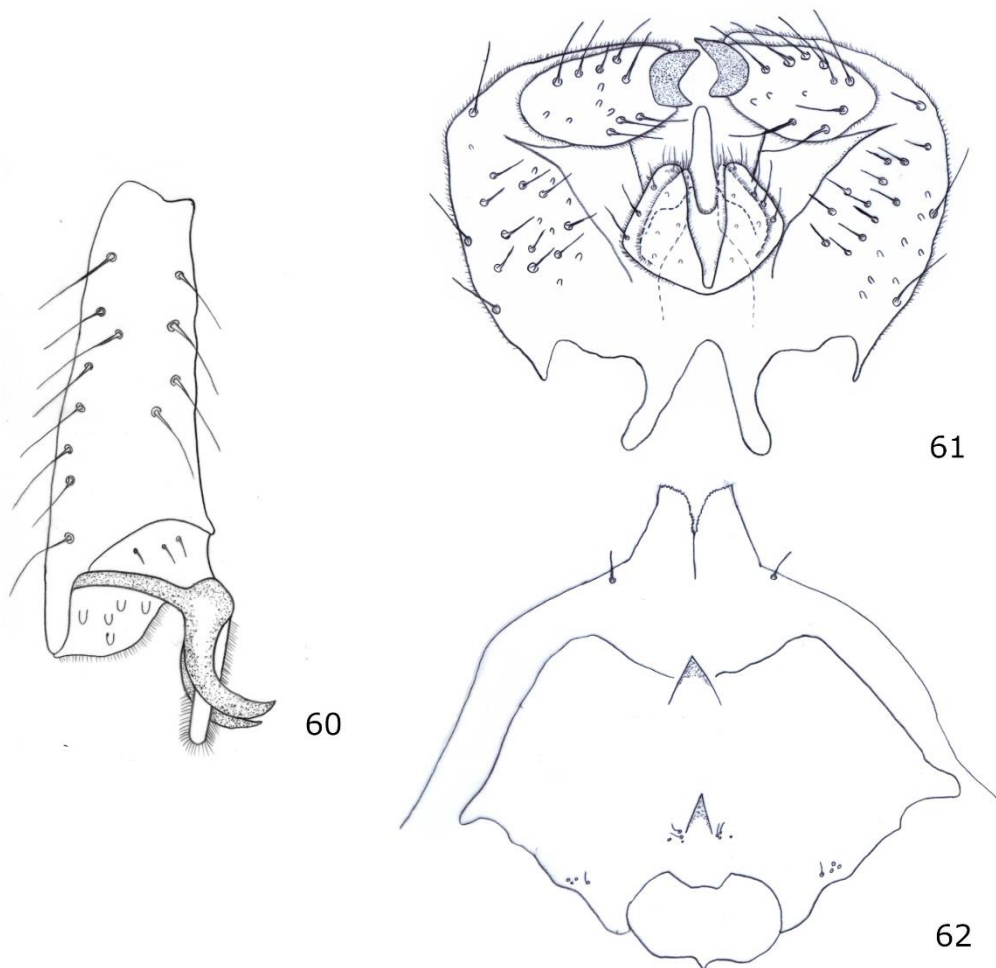
**Male abdomen: Terminalia** (Fig. 61): gap between hypoproct lobes about half as deep as hypoproct length. Cerci separated almost to base, tapering towards apex, setose and strongly setulose throughout.



**Figs. 56-59:** *Asphondylia* n. sp. 3: (56) Adult female; (57) Bud galls on *Drosanthemum comptonii*; (58-59) Bud galls on *Drosanthemum oculatum*.

**Larva:** length 1.8-3.4 mm (n=14). Antennae as long as wide; cephalic apodeme slightly longer than head capsule. Lateral teeth of spatula more robust than rudimentary median teeth (Fig. 28), about 5 times as long; gap between median teeth much deeper and wider than gaps between lateral and median teeth, strongly sclerotized; sclerotized section of spatula wider than long. Shaft widened and sclerotized at base, with two weakly sclerotized arms at mid-length and otherwise weakly sclerotized. Lateral papillae evenly spaced, and setose.

**Pupa** (Figs. 33-34): antennal horns (Figs. 39-40) short, wide at base, slightly splayed apically, medial margins serrated to apex. Upper facial horn slightly curved posteriorly. Lower facial horn slightly curved anteriorly, on each side with four papillae (Fig. 62), two setose, two asetose, one of which slightly separate from other three. Frons on each side with four lateral papillae, one of which setose, three asetose. Prothoracic spiracle long and slender, widened at base, evenly tapered to apex. Abdominal segments (Fig. 45) except for first and last, each with straight, posterior row of spikes and 1-2 less ordered, closely adjacent rows anteriorly, with large gap between anterior and posterior rows. Spikes about twice as long as wide.



**Figs 60-62:** *Asphondylia* n. sp. 3; (60) Acromere; (61) Male terminalia, dorsal; (62) Pupal head, frontal.

**Distribution:** (Fig. 1) This species is found from Springbok in the Northern Cape to De Hoop Nature reserve in the Western Cape and extending east as far as the Swartberg Mountains. Sampling from more *Drosanthemum* species may extend the distribution range of this species even further.

**Comments:** The galls and adults of this species from *Drosanthemum* are markedly smaller than those from the two gall midge species that develop on *Malephora*. In the male terminalia, the hypoproct is much more deeply notched than in the other two species. The larval spatula of *Asphondylia* n. sp. 3 is more compact, with a shorter shaft than those of the species from *Malephora*. The pupae have four papillae on each side of the lower facial horn and four papillae on each side of the frons, as opposed to two papillae in the species from *Malephora*. The serration on the antennal horns extends to the horn apex, whereas in the species from *Malephora* it ends before the apex. *Asphondylia* n. sp. 3 was reared from more than ten species of *Drosanthemum* and has a broader host range and distribution (Fig. 1) than those of the other two *Asphondylia* species described here.

**Material examined: Holotype:** ♀ South Africa: Soetwater [31°30'53"S, 19°24'22"E], Northern Cape, 11.viii.2017, S. van Munster, N. Dorchin and C. Klak, ex bud gall on *Drosanthemum subplanum*. On permanent microscope slide in Euparal.

**Paratypes:** From *Drosanthemum comptonii*: 13♀, 2♂, 2 exuviae (on one slide), Anysberg Nature Reserve [33°25'58"S, 20°47'47"E], Western Cape, 1.ix.2017, S. van Munster and N. Dorchin; 1♀, Van Wyksdorp [33°43'50"S, 21°28'39"E], Western Cape, 8.ix.2018, J. Colville and A. Melin.

From *Drosanthemum curtophyllum*: 2♀, 1♂, Moedveloor [31°29'21"S, 18°26'18"E], Western Cape, 12.ix.2017, S. van Munster and N. Dorchin.

From *Drosanthemum delicatulum*: 3♀, 5♂, Anysberg Nature Reserve [33°25'58"S, 20°47'47"E], Western Cape, 3.ix.2017, S. van Munster and N. Dorchin.

From *Drosanthemum karrooense*: 1♂, Anysberg Nature Reserve [33°25'58"S, 20°47'47"E], Western Cape, 4.ix.2017, S. van Munster and N. Dorchin.

From *Drosanthemum leipoldtii*: 10♀, 7♂, 8 larvae (on four slides), Karoo Botanical Garden Worcester [33°36'33"S 19°27'01"E], Western Cape, 20.viii.2017, S. van Munster and N. Dorchin.

From *Drosanthemum oculatum*: 2♀, 2♂, 8 larvae (on 4 slides), 4 exuviae, 70km N of Springbok [29°32'36"S, 17°51'51"E], Northern Cape, 16.viii.2018, S. van Munster and N. Dorchin.

From *Drosanthemum schoenlandianum*: 3♀, 1♂, Vanrhynsdorp [31°16'04"S, 18°32'32"E], Western Cape, 11.ix.2017, S. van Munster and N. Dorchin; 2 exuviae (on one slide), Nuwerus [31°08'14"S, 18°21'18"E], Western Cape, 25.viii.2018, S. van Munster and N. Dorchin.

From *Drosanthemum subclausum*: 3♀, 1♂, Moedveloor [31°29'21"S, 18°26'18"E], Western Cape, 12.ix.2017, S. van Munster and N. Dorchin.

From *Drosanthemum subplanum*: 1♀, 9♂, 2 exuviae (on one slide), Soetwater [31°30'53"S, 19°24'22"E], Northern Cape, 11.viii.2017, S. van Munster and N. Dorchin.

From *Drosanthemum* sp.: 5♀, 1♂, Bitterfontein [30°58'01"S, 18°17'26"E], Western Cape, 11.viii.2017, S. van Munster, N. Dorchin and C. Klak; 5♀, 5♂, Downes Farm Calvinia [31°29'52"S, 19°56'39"E], Northern Cape, 12.viii.2017, S. van Munster, N. Dorchin and C. Klak; 1♀, 20km N of Calvinia [31°36'21"S, 18°44'00"], Northern Cape, 7.ix.2018, S. van Munster, N. Dorchin and C. Klak; 1♀, 1♂, 3 larvae

(on three slides), 4 exuviae (on four slides), Van Wyksdorp [33°43'50"S, 21°28'39"E], Western Cape, 8.ix.2018, J. Colville and A. Melin.

**Other material examined:** From *Drosanthemum comptonii*: 4♀, Anysberg Nature Reserve [33°25'58"S, 20°47'47"E], Western Cape, 1.ix.2017, S. van Munster and N. Dorchin; 1♂, Van Wyksdorp [33°43'50"S, 21°28'39"E], Western Cape, 8.ix.2018, J. Colville and A. Melin.

From *Drosanthemum curtophyllum*: 2♀, Moedveloor [31°29'21"S, 18°26'18"E], Western Cape, 12.ix.2017, S. van Munster and N. Dorchin.

From *Drosanthemum delicatulum*: 1♀, 1♂, Anysberg Nature Reserve [33°25'58"S, 20°47'47"E], Western Cape, 3.ix.2017, S. van Munster and N. Dorchin.

From *Drosanthemum desciduum*: 2♀, Moedveloor [31°29'21"S, 18°26'18"E], Western Cape, 25.viii.2018, S. van Munster and N. Dorchin.

From *Drosanthemum leipoldtii*: 3♀, 3♂, Karoo Botanical Garden Worcester [33°36'33"S 19°27'01"E], Western Cape, 20.viii.2017, S. van Munster and N. Dorchin.

From *Drosanthemum schoenlandianum*: 1♂, Vanrhynsdorp [31°16'04"S, 18°32'32"E], Western Cape, 11.ix.2017, S. van Munster and N. Dorchin; 2♀, 2♂, Nuwerus [31°08'14"S, 18°21'18"E], Western Cape, 25.viii.2018, S. van Munster and N. Dorchin.

From *Drosanthemum subplanum*: 1♀, 4♂, Soetwater [31°30'53"S, 19°24'22"E], Northern Cape, 11.viii.2017, S. van Munster and N. Dorchin.

From *Drosanthemum* sp.: 1♂, Bitterfontein [30°58'01"S, 18°17'26"E], Western Cape, 11.viii.2017, S. van Munster, N. Dorchin and C. Klak; 1♀, Moedveloor [31°29'21"S, 18°26'18"E], Western Cape, 25.viii.2018, S. van Munster, N. Dorchin and C. Klak; 1♀, Van Wyksdorp [33°43'50"S, 21°28'39"E], Western Cape, 8.ix.2018,

J. Colville and A. Melin; 1♀, 1♂, Rooiberg Pass [33°44'14"S, 21°36'05"E], Western Cape, 9.ix.18, J. Colville and A. Melin.

## 4. *Lasioptera* species on *Drosanthemum* and *Malephora* in South Africa

### *Lasioptera* Meigen 1818

*Lasioptera* currently holds 129 species belonging to the tribe Lasiopterini, a large, predominantly Old World tribe consisting of 329 described species (Gagné & Jaschhof, 2017). Of the 29 gall-midge species that have been described in South Africa, only four belong to the Lasiopterini, one of which is the endemic genus, *Afrolasiopetra*, described from *Dicerotheramnus rhinocerotis* (Renosterbos) (Dorchin & Gullan, 2009). The other three were described from *Salvia* sp. (Lamiaceae Martinov), *Muraltia* sp. (Polygalaceae Hoffmanns. & Link) and *Passerina* sp. (Thymelaeaceae Juss.) (Gagné & Jaschhof, 2017). An additional species was described from Combretaceae in Mozambique (Gagné & Jaschhof, 2017).

*Lasioptera* species are known to form galls mostly on leaves and stems (Gagné, 1989; Gagné & Jaschhof, 2017), and are associated with a symbiotic fungus (Rohfritsch, 1992, 2008). There are also reports of a small number of species that develop as inquilines, or as successors, which inhabit galls that have been vacated by the gall inducers (Gagné & Jaschhof, 2017; Yukawa *et al.*, 2014). Several *Lasioptera* species have been reported as agricultural pests (Muthukumar *et al.*, 2017; Perdikis *et al.*, 2011; Tanasković & Milenković, 2012). These include *L. rubi* Schrank as a pest of raspberry crops (Milenković & Tanasković, 2008) and *Lasioptera tomaticola* Yukawa & Harris as a pest of tomato and cucumber glasshouse crops (Perdikis *et al.*, 2011; Yukawa *et al.* 2019).

Lasiopterini have a number of synapomorphic morphological characters, many of which pertain to the ovipositor. All species have a group of enlarged and

flattened setae just posterior to the 8<sup>th</sup> tergite on each side of the abdomen as well as hooked setae and a glabrous and sclerotized area at the base of the fused cerci (Gagné, 1994; Dorchin, 2001; Gagné & Jaschhof, 2017). *Lasioptera* accommodates lasiopterine species with 3-4 segmented palps and a short R<sub>4+5</sub> vein in the wing (Gagné, 1994; Dorchin & Freidberg, 2011). Antennal flagellomeres are similar in both sexes, having very short necks, but are often more numerous in females than in males, and their number may vary within species and sometimes even between antennae of the same individual (Dorchin, 2001; Dorchin *et al.*, 2004; 2019a).

The larvae in this tribe usually have four lateral papillae on each side of the spatula as well as 3-4 setose terminal papillae (Dorchin & Freidberg, 2011). Möhn (1966-1971) revised several Lasiopterini genera based only on the larval stage, despite the fact that this is generally uninformative apart from the presence or lack of a spatula (Dorchin, 2001), rendering his keys difficult, even impossible, to use. By contrast, Lasiopterini pupae show distinct characters that can be used to distinguish species, especially the shape and number of cephalic horns, which are usually well developed in the tribe (Dorchin, 2001).

In this work, two new species that were reared from ten known species of *Drosanthemum*, four species of *Malephora* and one species of *Lampranthus* are described below. The two species are tentatively placed in *Lasioptera* although they have a longer R<sub>4+5</sub> vein and palps with fewer segments than typical *Lasioptera* species, suggesting that they may require to be placed in a new genus.

#### 4.1. *Lasioptera* n. sp. 1

**Host plants:** *Malephora purpureo-crocea* (Haw.) Schwantes, *M. crassa* (L.Bolus) H. Jacobsen & Schwantes, *M. latipetala* (L.Bolus) H. Jacobsen & Schwantes, *M. uitenhagensis* and *Lampranthus uniflorus* (L.Bolus) L.Bolus.

**Gall and biology:** this species (Fig. 63) induces common leaf galls on *Malephora purpurea-crocea* (Fig. 64), *Malephora crassa*, *Malephora latipetala* (Fig. 65) and *Lampranthus uniflorus* (Fig. 66). In *M. latipetala* and *M. uitenhagensis*, the galled leaves are hard to the touch, swollen and reddish, occupying the entire leaf or only a section of it. If only part of the leaf is galled, the remaining part remains green and the galled section is harder to the touch than the ungalled section. Galls on *M. latipetala* are up to 3 cm long and 1 cm wide. Galls on *M. purpurea-crocea* and *M. crassa* are swollen and succulent rather than rigid, green to red, and much larger, up to 4 cm long and 2 cm wide. Galls on *Lampranthus uniflorus* are similar to those on *M. latipetala* but are widest at the apex of the leaf. In all four plants, the galls are succulent, with 5-10 well-defined larval chambers. Pupation takes place within the gall and adult emergence occurs from August to November. The galls are frequently parasitized by polyembryonic parasitoids (Hymenoptera, Platygasteridae).

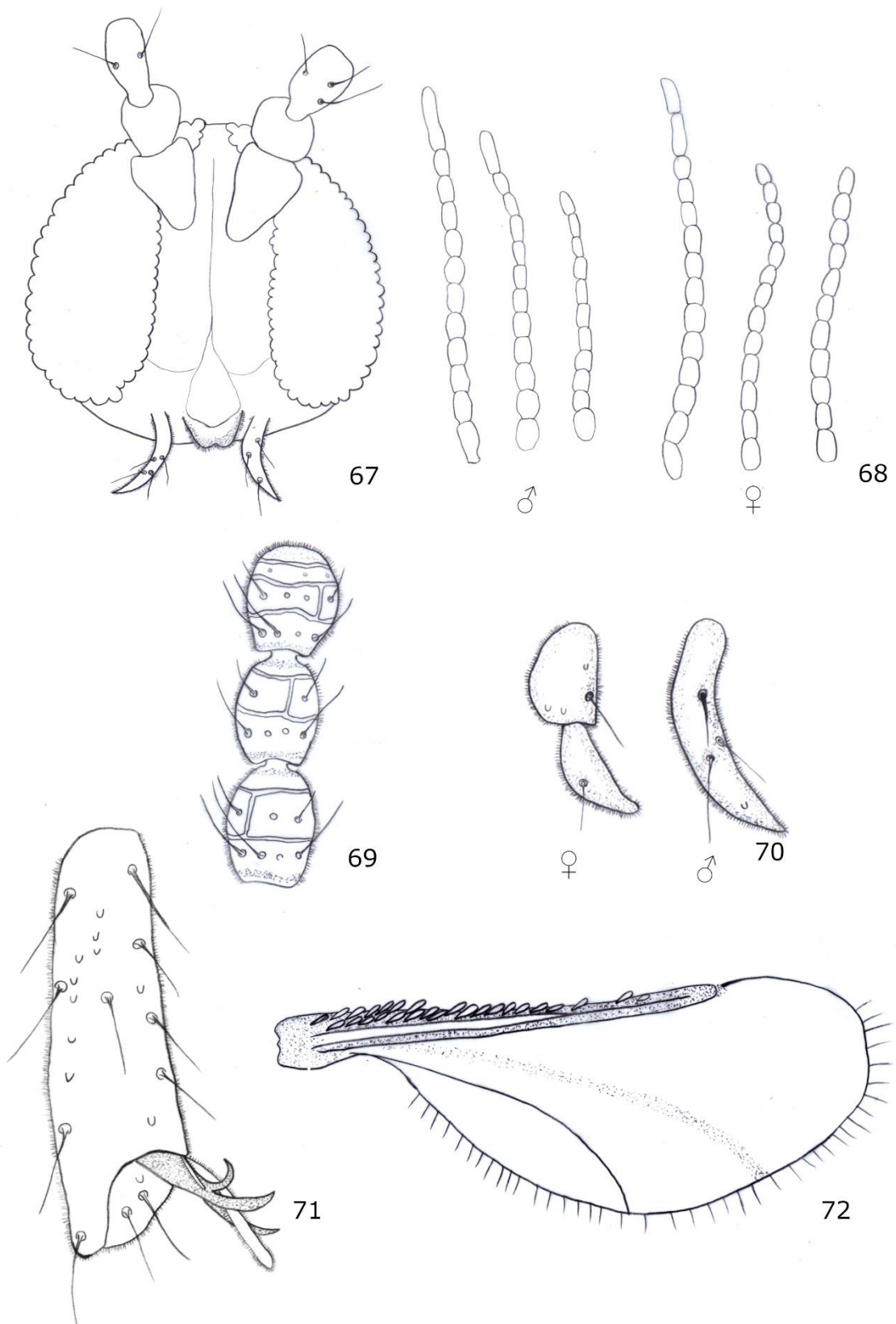


**Figs. 63-66:** *Lasioptera* n. sp. 1: (63) Adult female; (64) Leaf galls with exuviae on *Malephora purpurea-crocea*; (65) Leaf galls on *Malephora latipetala*; (66) Leaf gall on *Lampranthus uniflorus*.

**Adult** (Fig. 63): general colour orange to brown, covered in dense black setae and scales. **Head** (Fig. 67): frons covered in white setae. Eye facets round; gap between eyes on vertex 1.5-2 facets wide. **Antenna** (Fig. 68): scape wider distally than at base, pedicel spherical, both covered by long, strong setae, number of flagellomeres 11-15 in female (n=27), 11-13 in male, (n=25). Flagellomeres barrel shaped in both sexes, each with two simple whorls of appressed circumfila connected by longitudinal sections (Fig. 69); each flagellomere with 2 whorls of setae, the first composed of strong setae proximal to circumfila whorls, the second composed of shorter setae between two circumfila whorls; flagellomere otherwise evenly covered by microtrichia. Apical flagellomere sometimes composed of 2-3 fused flagellomeres,

rounded apically with 2-3 whorls of appressed circumfila and few strong setae, otherwise evenly setulose. Palp (Fig. 70) 1 segmented in males (n=21), 2 segmented in females (n=19), first segment ovoid to spherical, second segment elongate and tapered with few long setae, otherwise evenly setulose. Labella about as long as wide, evenly setulose.

**Thorax:** general colour orange to brown, covered in dark scales and setae. **Legs:** densely covered by black setae and scales. Tarsal claws (Fig. 71) slightly arched with strongly curved basal tooth, empodia clearly longer than bend in claw; pulvilli about 0.4 times as long as claw. **Wing** (Fig. 72): hyaline, evenly covered by delicate setae on entire surface with longer setae along posterior margin; length 1.68-2.20 mm in females (n=27), 1.40-2.18 mm in males (n=25); R<sub>4+5</sub> joining C around three quarters length of wing; C and R<sub>4+5</sub> densely covered by black scales to meeting point, C broken at meeting point; M<sub>4</sub> weak, CuA curved posteriorly at mid-length of wing.



**Figs. 67-72:** *Lasioptera* n. sp. 1: (67) Male head; (68) Variation in number of flagellomeres in male and female antennae; (69) Three apical flagellomeres; (70) Female and male palps; (71) Acropod; (72) Wing.

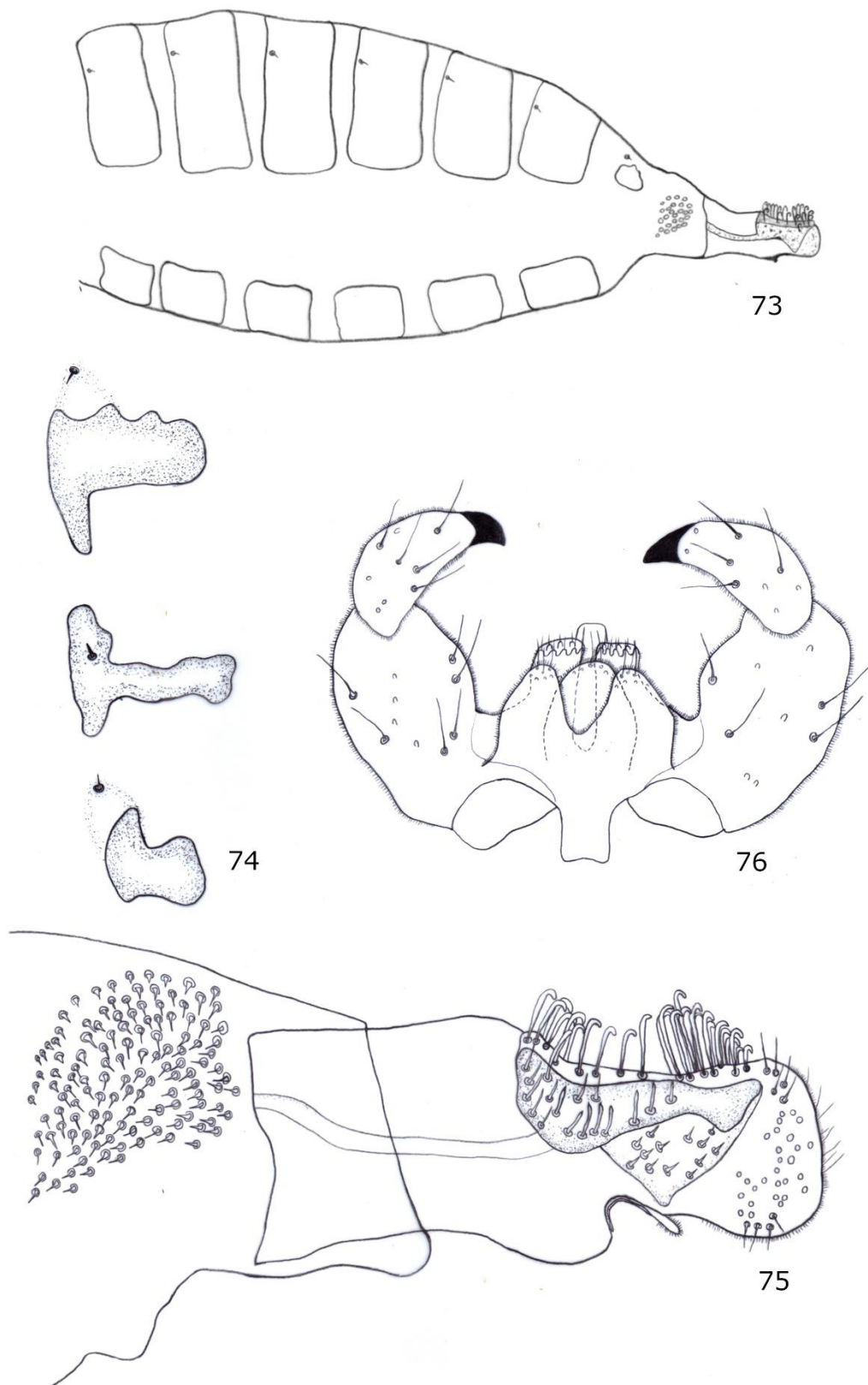
**Female abdomen** (Figs. 73-75): tergites 1–7 each with a pair of sensory setae anteriorly, posterior row of strong setae and otherwise evenly covered by scales and fine setae; tergite 8 (Fig. 74) much smaller than tergite 7, variably sclerotized, with anterior pair of sensory setae, evenly covered by fine setae. Sternites 2–7 without anterior sensory setae, evenly covered in long strong setae from mid-length to posterior margin, setae denser posteriorly, otherwise covered in evenly distributed scales. Sternite 8 indistinguishable from surrounding membrane. **Ovipositor** (Fig. 75): protrusible, 3.14-4.46 times as long as sternite 7 (n=25); basal part of segment 8 with lateral group of strong, straight setae stemming from prominent sockets, pointing ventrally. Cercal segment in straight angle relative to segment 9. Lateral plate sheathing dorsal half of apical lamella at base, extending to about three quarters length of apical lamella, more strongly sclerotized on dorsolateral area, laterally with 15-20 strong, straight setae. Aculeus slightly shorter than apical lamella, with about 15 evenly, sparsely spaced hooked setae on proximal half and about 15 densely packed hooked setae distally. Apical lamella ovoid, evenly setulose.

**Male abdomen:** tergites 1-7 each with anterior pair of sensory setae, 1-2 rows of strong setae posteriorly and otherwise evenly covered in fine setae and scales. Tergite 8 about half width of tergite 7, less sclerotized, with anterior pair of sensory setae, with posterior row of setae. Sternites 1-7 each with 3-5 unorganised rows of strong setae posteriorly and otherwise evenly covered in fine setae and scales. Sternite 8 narrower and shorter than sternite 7, similarly setose. **Terminalia** (Fig. 76): gonocoxite cylindrical, widest at mid-section, with numerous strong setae; mediobasal lobe rectangular, sheathing aedeagus to distal quarter, with deep longitudinal grooves, truncate at apex, with several strong setae apically and otherwise evenly setulose. Gonostylus slightly arched, widest at mid-length, tapering

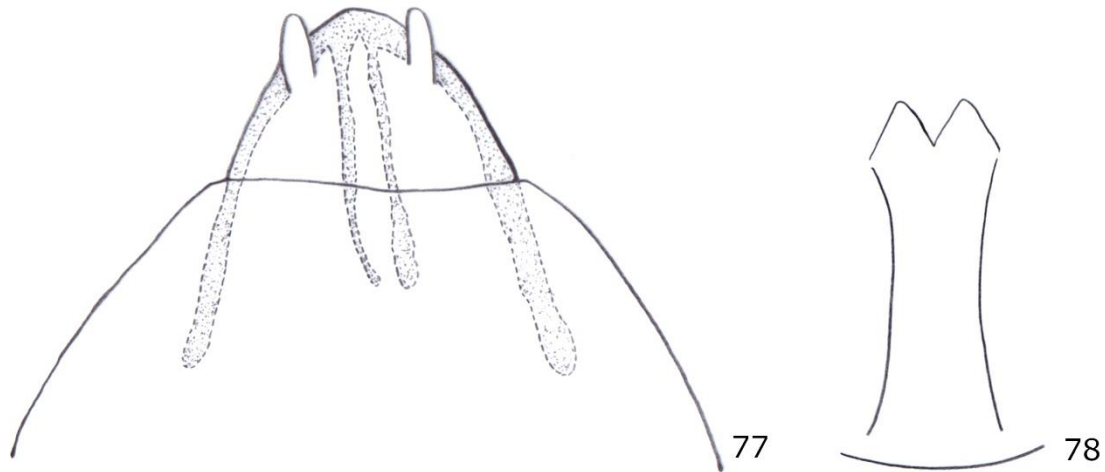
to strong pointed apical tooth; with few strong evenly spread setae, otherwise evenly setulose. Aedeagus wide, truncate apically, slightly longer than mediobasal lobes. Hypoproct evenly rounded, entire, widest at mid-length, setose and setulose. Cerci separated by deep rounded gap along distal half, each rounded apically with few strong apical setae and otherwise evenly setulose.

**Larva** (Figs. 77-78): light yellow to bright orange. Body elongate, tapered anteriorly, rounded posteriorly. Integument evenly covered by tiny bumps. Antennae about 2.5-3 times as long as wide (Fig. 77). Cephalic apodeme approximately 1-2 times as long as head capsule. Spatula bidentate (Fig. 78), long shaft narrowed at mid-length. Associated papillae indiscernible. Terminal papillae indiscernible.

**Pupa:** dark orange to brown. Antennal horns (Figs. 79-81) short and compact, bases wide, tapering to ventrally pointed apices. Horns smooth along lateral margin, smooth to slightly serrated along medial margin. Cephalic setae minute, originating from slightly elevated base. Prothoracic spiracle (Fig. 85) ovoid, looped dorsoventrally flattened. Frons without facial horns or apparent papillae. Abdominal segments completely and evenly covered by short, tapered spicules.



**Figs. 73-76:** *Lasioptera* n. sp. 1: (73) Female abdomen, lateral; (74) Variation in female 8<sup>th</sup> tergite; (75) Female ovipositor, lateral; (76) Male terminalia, dorsal.



**Figs. 77-78:** *Lasioptera* n. sp. 1 Larva: (77) Head; (78) Spatula.

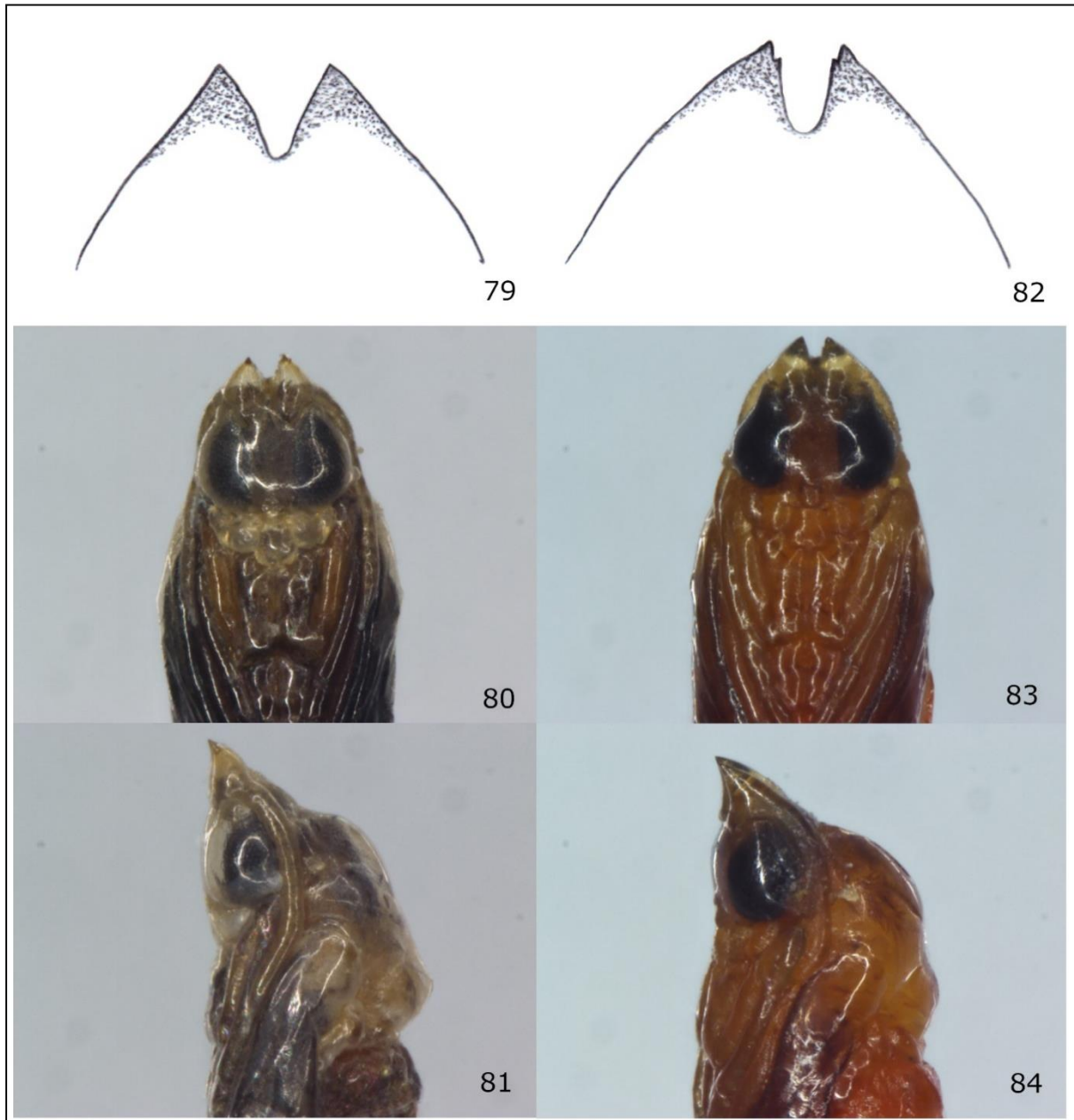
**Distribution:** This species is found in the Succulent Karoo and Nama Karoo biomes from Bredasdorp to Vanrhynsdorp in the Western Cape (Fig. 1).

**Comments:** *Lasioptera* n. sp. 1 is the larger of the two species described here.

Uniquely the palps in this species are consistently one segmented in males and two segmented in females. Tergite 8 of the female abdomen is variably sclerotized, but its width is consistently about half that of tergite 7. *Lasioptera* larvae usually have four lateral papillae on each side of the spatula (Dorchin & Freidberg, 2011) but those are indiscernible in *Lasioptera* n. sp. 1, as are the terminal papillae. The pupal prothoracic spiracle is unusually circular and dorsoventrally flattened rather than elongate and tapered (Fig. 85). Preliminary molecular results confirm that the individuals from all five host plants belong to the same species (Dorchin *et al.*, unpublished).

The galls of *Lasioptera* n. sp. 1 are robust and multi-chambered and are often found in large numbers (Fig. 65). It was found on four species of *Malephora* and one species of *Lampranthus*, and has a narrower host range than that of *Lasioptera* n. sp. 2 from *Drosanthemum*. During the first sampling season in the Cederberg, only

bud galls belonging to *Asphondylia* n. sp. 1 were found on *Malephora crassa*. However, only leaf galls belonging to *Asphondylia* n. sp. 2 were found from the same site the following sampling season.



**Figs. 79-84:** *Lasioptera* pupae. (79) *Lasioptera* n. sp. 1, Antennal horns. (80) *Lasioptera* n. sp. 1, frontal. (81) *Lasioptera* n. sp. 1, lateral; (82) *Lasioptera* n. sp. 2, Antennal horns. (83) *Lasioptera* n. sp. 2, frontal. (84) *Lasioptera* n. sp. 2, lateral.



**Fig. 85:** *Lasioptera* n. sp. 1, pupal prothoracic spiracle.

**Material examined: Holotype: (143(3))** ♀ South Africa: Botterkloof Pass Cederberg [31°54'49.5"S, 19°14'50.8"E], Western Cape, 12.ix.2018, S. van Munster and N. Dorchin, ex leaf gall on *Malephora crassa*. On permanent microscope slide in Euparal.

**Paratypes:** From *Malephora crassa*: 5♀, 1 larvae, same data as holotype.

From *Malephora latipetala*: 1♀, Anysberg Nature Reserve [33°25'58"S, 20°47'47"E], Western Cape, 3.ix.2017, S. van Munster, N. Dorchin and C. Klak; 1♀, 4 larvae (on two slides), 1 exuviae, Garcias Pass [33°50'47"S, 20°53'08"E], Western Cape, 10.x.2018, S. van Munster, R. Josephs & C. Barnard; 4♀, 4♂, 3 exuviae, between Ladismith and Anysberg Nature Reserve [33°25'58"S, 20°47'47"E], Western Cape, 13.x.2018, S. van Munster; 2♀, 3♂, 1 exuviae, Anysberg Nature Reserve [33°25'58"S, 20°47'47"E], Western Cape, 14.x.2018, S. van Munster, R. Josephs, C. Barnard; 4♀, 2♂, 1 larva, Vrolijkheid Nature Reserve [33°55'4"S, 19°52'39"E], Western Cape, 26.x.18, S. van Munster, N. Dorchin and J. Hoffmann; 1♀, 1♂, Beyersdal Farm [34°22'12"S, 20°16'53"E], Western Cape, 31.i.2019, S. van Munster, N. Dorchin and L. Friedman; 2♀, near Overberg Renosterveld Conservation Centre

[34°30'28"S, 20°04'45"E], Western Cape, 31.i.2019, S. van Munster, N. Dorchin and L. Friedman; 2 larvae (on two slides), 6 exuviae (on three slides), near Overberg Renosterveld Conservation Centre [34°30'28"S, 20°04'45"E], Western Cape, 20.viii.2019, S. van Munster and N. Dorchin.

From *Malephora purpureo-crocea*: 1♀, Grootgraafwater, Vanrhynsdorp [31°16'04"S, 18°32'32"E], Western Cape, 11.ix.2017, S. van Munster and N. Dorchin; 5♀, 9♂, 6 larvae (on 3 slides), Moedveloor [31°29'21"S, 18°26'18"E], Vanrhynsdorp, Western Cape, 26.viii.2018, S. van Munster, N. Dorchin and C. Klak.

**Other Material examined:** From *Lampranthus uniflorus*: 2♀, 2♂, Anysberg Nature Reserve [33°25'58"S, 20°47'47"E], Western Cape, 2.ix.2017, S. van Munster, N. Dorchin and C. Klak.

From *Malephora latipetala*: 1♀, 1♂, Garcias Pass [33°50'47"S, 20°53'08"E], Western Cape, 10.x.2018, S. van Munster, R. Josephs & C. Barnard; 1♂, between Ladismith and Anysberg Nature Reserve [33°25'58"S, 20°47'47"E], Western Cape, 13.x.2018, S. van Munster, R. Josephs & C. Barnard; 1♂, Anysberg Nature Reserve [33°25'58"S, 20°47'47"E], Western Cape, 14.x.2018, S. van Munster, R. Josephs, C. Barnard; 1♂, 1 larva, Vrolijkheid Nature Reserve [33°55'4"S, 19°52'39"E], Western Cape, 26.x.18, S. van Munster, N. Dorchin and J. Hoffmann; 1♀, 1♂, Beyersdal Farm [34°22'12"S, 20°16'53"E], Western Cape, 31.i.2019, S. van Munster, N. Dorchin and L. Friedman.

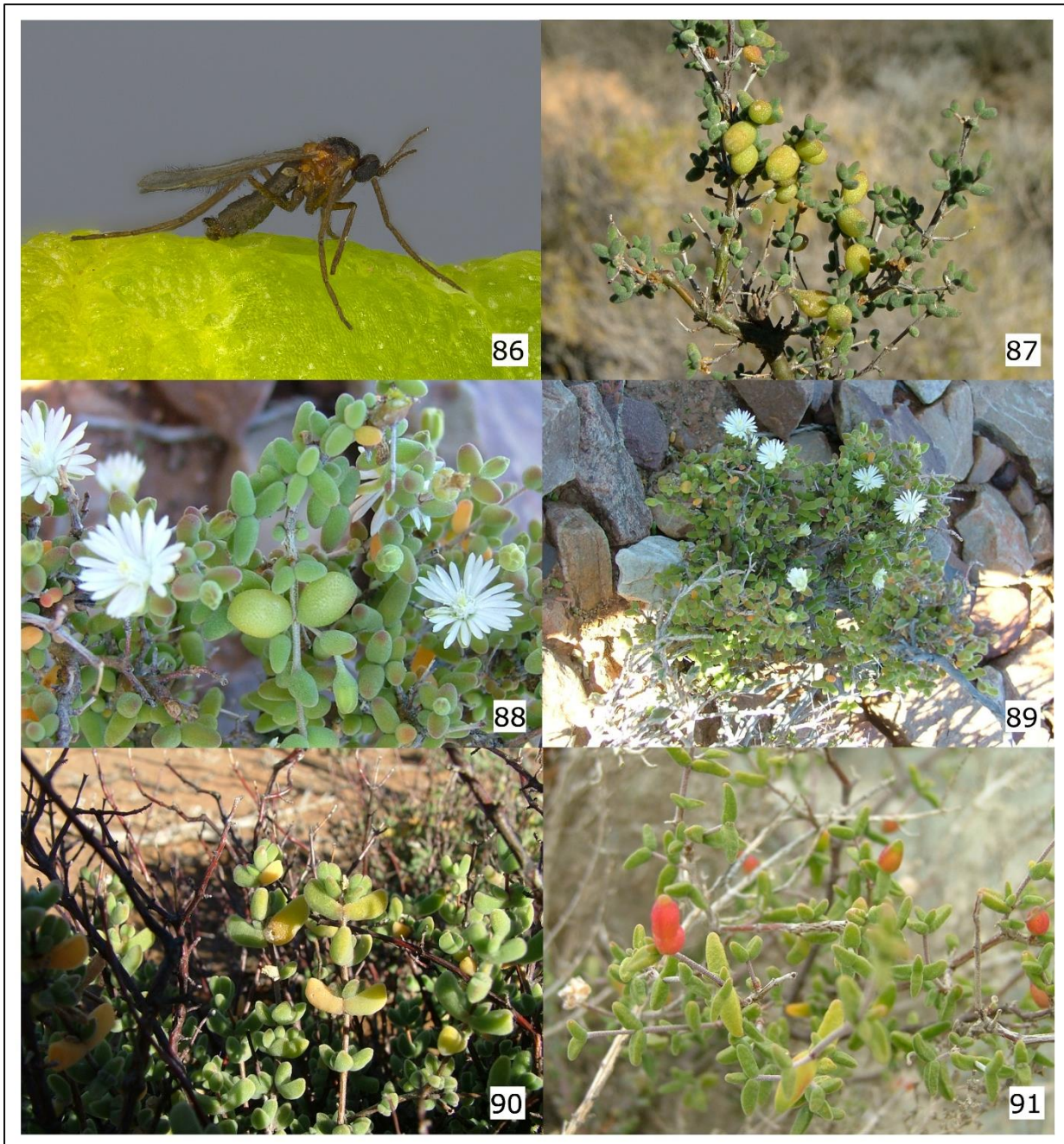
From *Malephora purpureo-crocea*: 1♂, 2 larvae (on 1 slide), Moedveloor [31°29'21"S, 18°26'18"E], Vanrhynsdorp, Western Cape, 26.viii.2018, S. van Munster, N. Dorchin and C. Klak.

#### 4.2. *Lasioptera* n. sp. 2

Characters as in *Lasioptera* n. sp. 1 except for the following.

**Host plants:** *Drosanthemum barkerae* L.Bolus, *D. crassum* L.Bolus, *D. comptonii* L.Bolus, *D. curtophyllum* L.Bolus, *D. karrooense* L.Bolus, *D. leipoldtii* L.Bolus, *D. muirii* L.Bolus, *D. oculatum* L.Bolus, *D. parvifolium* Schwantes, *D. cf roridum* L.Bolus and several other *Drosanthemum* species (to be identified).

**Gall and biology:** *Lasioptera* n. sp. 2 (Fig. 86) induces leaf galls in multiple *Drosanthemum* species. The galls (Figs. 87-89) vary somewhat between different plant species and are either slightly or conspicuously inflated with some degree of red or yellow discolouration. They are about 0.8 cm long and 0.4 cm wide. Often both leaves in a leaf pair are affected. Each gall contains 1-3 tiny larval chambers, the walls of which are slightly drier than the succulent surrounding tissues. Galls are frequently parasitized by endo- and ectoparasitoids from several families. In *D. barkerae*, leaves are inflated in an hourglass shape with a chamber in each bulge and both leaves in a pair affected. In *D. parvifolium* (Fig. 91), the galls are bright red and exceptionally common. In *D. crassum* (Fig. 87), the galls are usually prominent and showy with yellow discolouration. Galls of this species were collected from August to May with peak emergence of midges between August and October, suggesting that the species completes multiple generations throughout the year.



**Figs. 86-91:** *Lasioptera* n. sp. 2: (86) Male adult; (87) Leaf galls on *Drosanthemum crassum*; (88-89) Leaf galls on *Drosanthemum karroense*; (90) Leaf galls on *Drosanthemum cf. roridum*; (91) Leaf galls on *Drosanthemum parvifolium*.

**Adult** (Fig. 86): **Head:** antennae (Fig. 90): number of flagellomeres 11-14 in female (n=28), 8-12 in male (n=26). Palps (Fig. 91) 1-2 segmented in both male (n=14) and female (n=21), with few long strong setae and otherwise evenly setulose.

**Thorax: Wing:** length 1.20-1.90 mm in females (n=28), 1.14-1.78 mm in males (n=26);

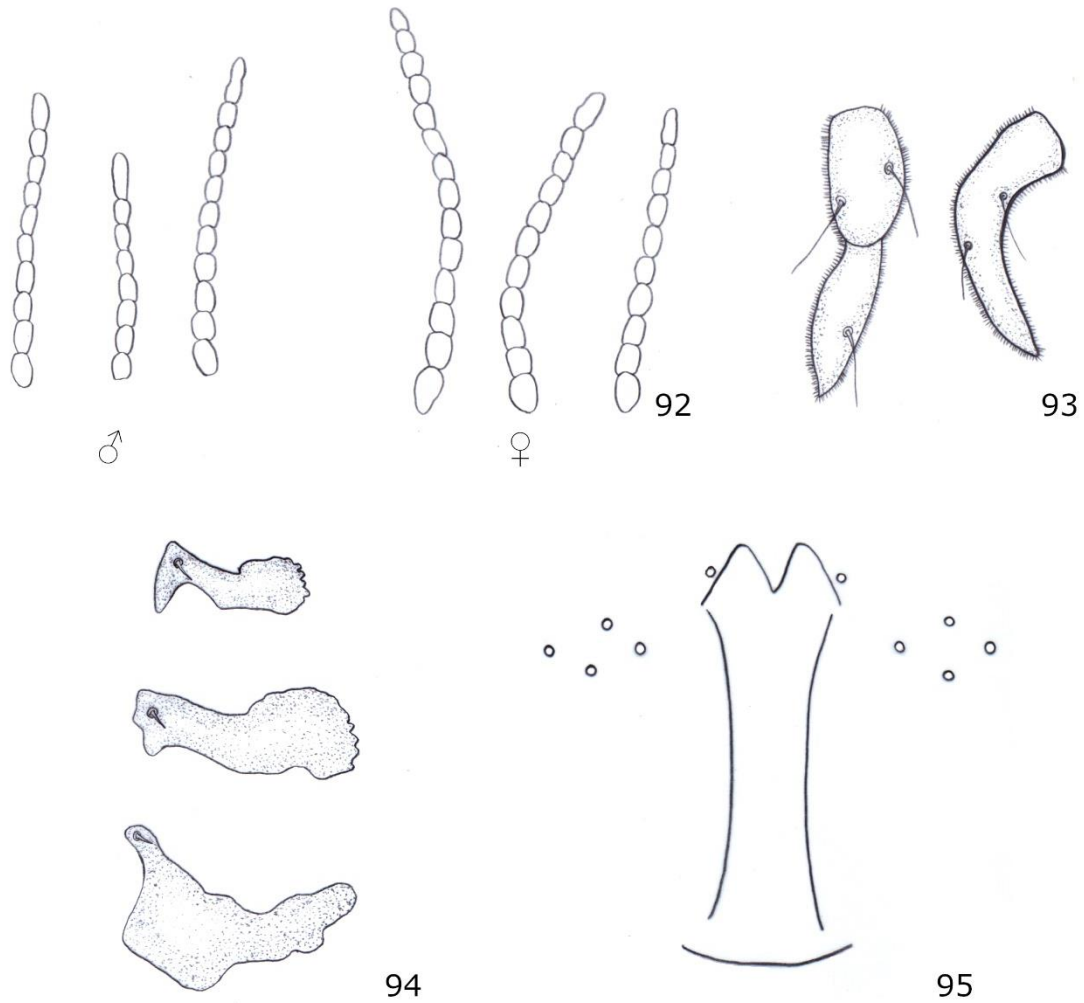
**Female abdomen:** tergites with posterior row of strong setae. Tergite 8 (Fig. 92) strongly sclerotized. Sternites 2–7 covered in long strong setae throughout.

**Ovipositor:** 2.73-4.25 times as long as sternite 7 (n=25).

**Larva:** antennae about twice as long as wide. Spatula (Fig. 93) on either side with one aetose sternal papilla and four aetose lateral papillae evenly spaced.

**Pupa** (Figs. 82-84): antennal bases wide, tapering to apex, divided apically into two points, one much smaller than the other (Fig. 82), forming robust, ventrally pointed horns.

**Distribution:** *Lasioptera* n. sp. 2 was collected from Springbok in the Northern Cape to the Swartberg Mountains in the Western Cape (Fig. 1). Further sampling is necessary to determine the full extent of this species distribution range.



**Figs. 90-93:** *Lasioptera* n. sp. 2: (92) Variation in male and female antennae; (93) Variation in palps; (94) Variation in the female 8<sup>th</sup> tergite; (95) Larval spatula with associated papillae.

**Comments:** The palps in this species are 1-2 segmented in both males and females contrary to the palps in *Lasioptera* n. sp. 1 where males have one-segmented palps and females have two segmented palps.

Tergite 8 of the female abdomen is similar to that in *Lasioptera* n. sp. 1, but in *Lasioptera* n. sp. 2 it is always strongly sclerotized as opposed to the variable degree of sclerotization in *Lasioptera* n. sp. 1. Characters of the adult terminalia can sometimes be used to distinguish species of *Lasiopterini* (Dorchin *et al.*, 2004) but this is not the case in the two species described here.

*Lasioptera* larvae usually have four lateral papillae next to the spatula, as seen in *Lasioptera* n. sp. 2, but the lateral papillae of *Lasioptera* n. sp. 1 are indiscernible. The terminal papillae in larvae of both species are indiscernible so it not possible to determine if their arrangement is consistent with that of other species in the genus. The pupal antennal horns are more robust in *Lasioptera* n. sp. 2 than in *Lasioptera* n. sp. 1. however, both species lack facial horns. The two species also differ in that the antennal horns of *Lasioptera* n. sp. 2 are divided apically into two points whereas in *Lasioptera* n. sp. 1 there is no such division.

This species is smaller than *Lasioptera* n. sp. 1 and its galls are also much smaller and contain fewer chambers, which may be because they develop in the tiny leaves of *Drosanthemum*. This species has a wide host range as it was reared from multiple species of *Drosanthemum*, whereas *Lasioptera* n. sp. 1 was reared only from three *Malephora* and one *Lampranthus* species. Consequently, the distribution range of *Lasioptera* n. sp. 2 is much wider than that of *Lasioptera* n. sp. 1, which was found only in the Western Cape (Fig. 1). Preliminary molecular data corroborate the morphological differences and suggest that *Lasioptera* n. sp. 2 is distinct from *Lasioptera* n. sp. 1.

**Material examined: Holotype:** ♀ South Africa: Karoo Botanical Garden, Worcester [33°36'33"S 19°27'01"E], Western Cape, 20.viii.2017, S. van Munster, N. Dorchin and C. Klak, ex leaf gall on *Drosanthemum karrooense*. On permanent microscope slide in Euparal.

**Paratypes:** From *Drosanthemum barkerae*: 2♀, 1♂, Anysberg Nature Reserve [33°25'58"S, 20°47'47"E], Western Cape, 3.ix.2017, S. van Munster, N. Dorchin and C. Klak.

From *Drosanthemum comptonii*: 2♂, Van Wyksdorp [33°43'50"S, 21°28'39"E], Western Cape, 8.ix.2018, J. Colville and A. Melin; 2♀, 3♂, Garcias Pass [33°50'47"S, 20°53'08"E], Western Cape, 10.x.18, S. van Munster, R. Josephs and C. Barnard.

From *Drosanthemum crassum*: 5♀, Anysberg Nature Reserve [33°25'58"S, 20°47'47"E], Western Cape, 3.ix.2017, S. van Munster, N. Dorchin and C. Klak; 1♀, 3♂, Gecko Rock Private Nature Reserve [33°30'51"S, 20°07'17"E], Western Cape, 24.iv.2019, S. van Munster, N. Dorchin and C. Klak.

From *Drosanthemum curtophyllum*: 1♀, Moedveloor [31°29'21"S, 18°26'18"E], Western Cape, 12.ix.2017, S. van Munster, N. Dorchin and C. Klak; 1♀, Quaggaskop [31°24'59"S, 18°35'43"E], Western Cape, 26.viii.18, S. van Munster, N. Dorchin and C. Klak.

From *Drosanthemum karrooense*: 3♀, 4♂, 2 larvae (on one slide), Karoo Botanical Garden Worcester [33°36'33"S 19°27'01"E], Western Cape, 20.viii.2017, S. van Munster, N. Dorchin and C. Klak; 1♀, Karoo Botanical Garden, Worcester [33°36'33"S 19°27'01"E], Western Cape, 20.ix.2017, S. van Munster, N. Dorchin and C. Klak.

From *Drosanthemum leipoldtii*: 1♀, Karoo Botanical Garden, Worcester [33°36'33"S 19°27'01"E], Western Cape, 20.viii.2017, S. van Munster, N. Dorchin and C. Klak; 1♀, Karoo Botanical Garden, Worcester [33°36'33"S 19°27'01"E], Western Cape, 20.ix.2017, S. van Munster, N. Dorchin and C. Klak; 1♂, Karoo Botanical Garden, Worcester [33°36'33"S 19°27'01"E], Western Cape, 4.ix.2018, S. van Munster, N. Dorchin and C. Klak.

From *Drosanthemum oculatum*: 4♀, 2♂, 70km N of Springbok [29°32'36"S, 17°51'51"E], Northern Cape, 16.viii.2018, S. van Munster and N. Dorchin.

From *Drosanthemum parvifolium*: 2 larvae (on two slides), Beyersdal Farm [34°22'12"S, 20°16'53"E], Western Cape, 31.i.2019, S. van Munster, N. Dorchin and L. Friedman.

From *Drosanthemum* sp.: 3♀, 1♂, Downes Farm Calvinia [31°29'52"S, 19°56'39"E], Northern Cape, 12.viii.2017, S. van Munster, N. Dorchin and C. Klak; 1♀, 20km N of Calvinia [31°36'21"S, 18°44'00"E], Northern Cape, 7.ix.2018, S. van Munster, N. Dorchin and C. Klak; 1♀, Loeriesfontein [30°57'21"S, 19°19'42"E], Northern Cape, 6.ix.18, S. van Munster and N. Dorchin; 1♂, 4 exuviae (on four slides), Van Wyksdorp [33°43'50"S, 21°28'39"E], Western Cape, 8.ix.2018, J. Colville and A. Melin.

**Other material examined:** From *Drosanthemum comptonii*: 2♂, Van Wyksdorp [33°43'50"S, 21°28'39"E], Western Cape, 8.ix.2018, J. Colville and A. Melin; 2♂, Garcias Pass [33°50'47"S, 20°53'08"E], Western Cape, 10.x.18, S. van Munster, R. Josephs and C. Barnard.

From *Drosanthemum crassum*: 1♂, Gecko Rock Private Nature Reserve [33°30'51"S, 20°07'17"E], Western Cape, 24.iv.2019, S. van Munster, N. Dorchin and C. Klak.

From *Drosanthemum karrooense*: 2♀, 1♂, Karoo Botanical Garden Worcester [33°36'33"S 19°27'01"E], Western Cape, 20.viii.2017, S. van Munster, N. Dorchin and C. Klak.

From *Drosanthemum muirii*: 1♂, Populiersbos farm [33°41'35"S, 19°53'40"E], Western Cape, 28.ix.2017, J. Colville and A. Melin.

From *Drosanthemum parvifolium*: 1 larva, Beyersdal Farm [34°22'12"S, 20°16'53"E], Western Cape, 31.i.2019, S. van Munster, N. Dorchin and L. Friedman.

From *Drosanthemum* sp.: 1 ♀, 1 exuviae, Downes Farm Calvinia [31°29'52"S, 19°56'39"E], Northern Cape, 12.viii.2017, S. van Munster, N. Dorchin and C. Klak; 1 ♀, 1 ♂, Van Wyksdorp [33°43'50"S, 21°28'39"E], Western Cape, 8.ix.2018, J. Colville and A. Melin.

## 5. Discussion

The aim of this thesis was to investigate the gall midge fauna associated with the Aizoaceae of the Greater Cape Floristic Region. This thesis forms part of a larger project focusing on the gall midge fauna of Aizoaceae in South Africa and contributes to this project by providing descriptions for several of the species found. In particular, the two Aizoaceae genera *Malephora* and *Drosanthemum* were studied in detail.

The thesis includes the description of five new species of gall midges from the Aizoaceae of the Greater Cape Floristic Region with notes on their biology, host plants and distributions. Preliminary molecular data support their taxonomic distinctness (Dorchin *et al.*, unpublished). Sampling of these taxa was carried out over three field seasons and a considerable amount of material was collected. However, further sampling is necessary to determine whether the host ranges, distributions and emergence periods of the new species are broader. For example, although I focussed on *Malephora* and *Drosanthemum*, I found that the species from *Malephora* also develops in the Aizoaceae genera *Lampranthus*, *Carpobrotus*, *Jordaaniella* and *Acrodon*. This raises questions concerning the host specificity of gall midges on Aizoaceae, in comparison to the high degree of host specificity generally seen in midge species associated with other plant families (Dorchin & Freidberg, 2011; Dorchin *et al.*, 2019b).

Three new species of *Asphondylia* and two new species of *Lasioptera* are described. However, some of the characters of the *Lasioptera* species, namely the longer R<sub>4+5</sub> vein and the palps with fewer segments, do not fit entirely with those of the genus and therefore they may merit a description of a new genus. While morphological characters of the species described here enable clear separation

among them, the findings of molecular studies that are currently underway could provide further support for the species delimitations (Dorchin & Gullan, 2007; Dorchin *et al.*, 2015, 2019b).

Host-plant material was sampled from numerous field sites across the western and northern parts of the Succulent Karoo in South Africa, which were selected as they are known areas of high Aizoaceae diversity (Valente *et al.*, 2014) and endemism (Goldblatt, 1978; Goldblatt & Manning, 2002; Manning & Goldblatt, 2012). It would be beneficial to extend collection efforts into areas that I did not sample, including the arid northern Namibian regions of the Succulent Karoo, and the summer rainfall arid areas of the Nama Karoo. Exploration of the Aizoaceae of the mesic winter rainfall Fynbos Biome may also yield new and interesting gall midge species, particularly as the Fynbos and Succulent Karoo Biomes share phylogenetically related insect taxa of different ages (Predel *et al.*, 2012; Colville *et al.*, 2014). This will not only determine whether the five new species described here have a wider distribution and more extensive host plant range but will also likely reveal many new species and a far better understanding of the evolution of the gall midge-Aizoaceae association.

Better taxonomic resolution of the host plants may determine more accurately the host plant ranges of the species described here. *Drosanthemum* is a large genus that displays morphological diversity but also contains several morphologically similar species (Liede-Schumann *et al.*, 2020), often making it difficult to identify them. *Asphondylia* n. sp. 3 and *Lasioptera* n. sp. 2 were both found on different but morphologically similar species of *Drosanthemum* and future work on host plant taxonomy and phylogeny (Liede-Schumann *et al.*, 2020) may reveal a wider or narrower host range of these two midge species.

During this project, there were many cases in which the gall midges did not induce galls and the only evidence of their presence in the plants was the exuviae left behind after emergence. It is possible that there are many more cecidomyiid species that do not induce galls, which means that collecting large amounts of un-galled plant material will be necessary to ensure a more accurate sampling of the gall midge fauna associated with Aizoaceae.

Overall, the descriptions of these new gall midge species, together with data on their distributions and life-history, adds to our knowledge on biodiversity, particularly of the entomofauna of the Greater Cape Floristic Region, and this sort of data can be useful to supplement conservation planning in this area (Colville *et al.*, 2002, 2014). There is still much taxonomic, phylogenetic and ecological work to be done on the gall-midge fauna of the Aizoaceae of South Africa's arid biomes and the species described here and the biological information given provide a mere snapshot of this fascinating insect-plant association.

## 6. References

- Barnes, H. F. 1946-1956. *Gall Midges of Economic Importance*. London: Crosby Lockwood & Son Ltd.
- Bazelet, C.S., Thompson, A.C. & Naskrecki, P. 2016. Testing the efficacy of global biodiversity hotspots for insect conservation: The case of South African katydids. *PLoS ONE*. 11(9): e0160630.
- Bentur, J.S. & Kalode, M.B. 1996. Hypersensitive reaction and induced resistance in rice against the Asian rice gall midge *Orseolia oryzae*. *Entomologia experimentalis et applicata*. 78(1): 77-81.
- Bergh, N.G., Verboom, A., Rouget, M. & Cowling, R.M. 2014. Vegetation types of the Greater Cape Floristic Region. In Allsopp, N., Colville, J.F., Verboom, G.A. (eds). *Fynbos: Ecology, Evolution and Conservation of a Megadiverse Region*. New York: Oxford University Press.
- Bissett, J. & Borkent, A. 1988. Ambrosia galls: the significance of fungal nutrition in the evolution of the Cecidomyiidae (Diptera). In K.A. Pirozynki & D.L. Hawksworth (eds). *Coevolution of fungi with plants and animals*. London: Academic Press. 203–205.
- Boddum, T., Skals, N., Hill, S.R., Hansson, B.S. & Hillbur, Y. 2010. Gall midge olfaction: pheromone sensitive olfactory neurons in *Contarinia nasturtii* and *Mayetiola destructor*. *Journal of insect physiology*. 56(9):1306–1314.
- Born, J., Linder, H.P. & Desmet, P. 2007. The Greater Cape Floristic Region. *Journal of Biogeography*. 34: 147-162.
- Carneiro, M.A.A., Branco, C.S., Braga, C.E., Almada, E.D., Costa, M., Maia, V.C. & Fernandes, G.W. 2009. Are gall midge species (Diptera, Cecidomyiidae) host-plant specialists?. *Revista Brasileira de Entomologia*. 53(3): 365-378.

- Clausnitzer, V., Kalkman, V., Ram, M., Collen, B., Baillie, J., Bedjanič, M., Darwall, W., Dijkstra, K., Dow, R., Hawking, J., Karube, H., Malikova, E., Paulson, D., Schütte, K., Suhling, F., Villanueva, R., Von Ellenrieder, N. & Wilson, K. 2009. Odonata enter the biodiversity crisis debate: The first global assessment of an insect group. *Biological Conservation*. 142(8): 1864–1869.
- Colville, J., Picker, M.D. & Cowling, R.M. 2002. Species turnover of monkey beetles (Scarabaeidae: Hopliini) along environmental and disturbance gradients in the Namaqualand region of the succulent Karoo, South Africa. *Biodiversity & Conservation*. 11(2): 243-264.
- Colville, J.F., Potts, A.J., Bradshaw, P.L., Measey, G.J., Snijman, D., Picker, M.D., Procheş, S., Bowie, R.C.K. & Manning, J.C. 2014. Floristic and faunal Cape biochoria: do they exist? In N. Allsopp, J.F. Colville, & G.A. Verboom (eds). *Fynbos: Ecology, Evolution, and Conservation of a Megadiverse Region*. Oxford University Press. 73–93.
- Conrad, K.F., Warren, M.S., Fox, R., Parsons, M.S. & Woiwod, I.P. 2006. Rapid declines of common, widespread British moths provide evidence of an insect biodiversity crisis. *Biological conservation*. 132(3): 279-291.
- Cowling, R.M., Holmes, P.M. & Rebelo, A.G. 1992. Plant diversity and endemism. In Cowling, R.M. (ed.). *The ecology of fynbos. Nutrients, fire and diversity*. Cape Town: Oxford University Press. 62-112.
- Cowling, R.M., Rundel, P.W., Desmet, P.G. & Esler, K.J. 1998. Extraordinary high regional-scale plant diversity in southern African arid lands: subcontinental and global comparisons. *Diversity and Distributions*. 27-36.
- Cowling, R., Esler, K. & Rundel, P. 1999. Namaqualand, South Africa: An Overview of a unique winter-rainfall desert ecosystem. *Plant Ecology*. 142(1/2): 3-21.

- Cowling, R.M., Procheş, Ş. & 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.
- De Souza Mendonça, M., Toma, T.S.P. & da Silva, J.S. 2014. Galls and galling arthropods of southern Brazil. In Fernandes, G.W. and Santos, J.C. (eds). *Neotropical Insect Galls*. Dordrecht: Springer. 221-256.
- Desmet, P. G. & Cowling, R. M. 1999. Biodiversity, habitat and range-size aspects of a flora from a winter-rainfall desert in north-western Namaqualand, South Africa. *Plant Ecology*. 142(1): 23–33.
- Desmet, P. 2007. Namaqualand—A brief overview of the physical and floristic environment. *Journal of Arid Environments*. 70: 570–587.
- Dorchin, N. 2001. Gall Midges (Diptera: Cecidomyiidae) Infesting *Suaeda monoica* (Chenopodiaceae) in Israel. *Proceedings of the Entomological Society of Washington*. 103:561–581.
- Dorchin, N., Freidberg, A. & Mokady, O. 2004. Phylogeny of the Baldratiina (Diptera: Cecidomyiidae) inferred from morphological, ecological and molecular data sources, and evolutionary patterns in plant–galler relationships. *Molecular Phylogenetics and Evolution*. 30(3): 503–515.
- Dorchin, N. & Gullan, P. J. 2007. A new genus and species of a lasiopterine gall midge (Diptera: Cecidomyiidae) from bud galls on renosterbos, *Elytropappus rhinocerotis* (Asteraceae), in South Africa. *African Entomology*. 15(2): 233–240.  
<https://doi.org/10.4001/1021-3589-15.2.233>
- Dorchin, N. & Freidberg, A. 2011. The gall midges (Diptera: Cecidomyiidae) of Apiaceae in Israel. *Zootaxa*. 48(3044): 28–48.
- Dorchin, N., Mifsud, D. & Askew, R. 2014. Saltbush-associated *Asphondylia* species

- (Diptera: Cecidomyiidae) in the Mediterranean Basin and their chalcidoid parasitoids (Hymenoptera: Chalcidoidea). *Zootaxa*. 3869(4): 383–396.
- Dorchin, N., Astrin, J.J., Bodner, L. & Harris, K.M. 2015a. Morphological and molecular revision of the genus *Ozirhincus* (Diptera: Cecidomyiidae) - Long-snouted seed-feeding gall midges on Asteraceae. *PLoS ONE*. 10(7):1–29.
- Dorchin, N., Joy, J.B., Hilke, L.K., Wise, M.J. & Abrahamson, W.G. 2015b. Taxonomy and phylogeny of the *Asphondylia* species (Diptera: Cecidomyiidae) of North American goldenrods: challenging morphology, complex host associations, and cryptic speciation. *Zoological Journal of the Linnean Society*. 174(2): 265-304.
- Dorchin N., Harris K.M. & Jaschhof, M. 2017. Cecidomyiidae (Chapter 22). in: Kirk-Spriggs A. and Sinclair B. (eds), *Manual of Afrotropical Diptera*. South African National Biodiversity Institute (SANBI) publications. 107-125
- Dorchin, N., Danon, G. & Dor, R. 2019a. Gall midges ( Diptera : Cecidomyiidae ) associated with *Suaeda* ( Chenopodiaceae ) in Israel and the Mediterranean Basin. *Israel Journal of Entomology*. 49(August):99–134.
- Dorchin, N., Harris, K.M. & Stireman, J.O. 2019b. Phylogeny of the gall midges (Diptera, Cecidomyiidae, Cecidomyiinae): Systematics, evolution of feeding modes and diversification rates. *Molecular Phylogenetics and Evolution*. 140(June):106602.
- Driver, A., Desmet, P., Rouget, M., Cowling, R.M. & Maze, K. 2003. Succulent Karoo Ecosystem Plan: biodiversity component. Technical Report CCU 1/03, Cape Conservation Unit, Botanical Society of South Africa, Kirstenbosch.
- Dunn, R.R. 2005. Modern insect extinctions, the neglected majority. *Conservation biology*. 19(4): 1030-1036.

- Ellis, A.G., Weis, A.E., & Gaut, B.S. 2006. Evolutionary radiation of 'stone plants' in the genus *Argyroderma* (Aizoaceae): unraveling the effects of landscape, habitat and flowering time. *Evolution*. 60: 39–55
- Felt, E.P. 1940. *Plant galls and gall makers*. Ithaca, New York.: Comstock Publishing Company, Inc.
- Fernandes, G.W., Carneiro, M.A.A., Lara, A.C.F., Allain, L.R., Andrade, G.I., Julião, G.R., Reis, T.R. & Silva, I.M. 1996. Gallling insects on neotropical species of *Baccharis* (Asteraceae). *Tropical Zoology*. 9(2): 315-332.
- Fernandes, G.W., Silva, J.O., Espírito-Santo, M.M., Fagundes, M., Oki, Y. & Carneiro, M.A.A. 2014. *Baccharis*: a neotropical model system to study insect plant interactions. In *Neotropical Insect Galls*. Dordrecht: Springer. 193-219.
- Fonseca, C. 2009. The Silent Mass Extinction of Insect Herbivores in Biodiversity Hotspots. *Conservation Biology*. 23(6): 1507–1515.
- Forest, F., Grenyer, R., Rouget, M., Davies, T.J., Cowling, R.M., Faith, D.P., Balmford, A., Manning, J.C., Procheş, Ş., van der Bank, M. & Reeves, G. 2007. Preserving the evolutionary potential of floras in biodiversity hotspots. *Nature*. 445(7129): 757-760.
- Gagné, R.J. & Wuensche, A.L. 1986. Identity of the *Asphondylia* (Diptera: Cecidomyiidae) on Guar, *Cyamopsis tetragonoloba* (Fabaceae), in the Southwestern United States. *Annals of the Entomological Society of America*. 79(1): 246–250.
- Gagné, R.J. 1989. *The Plant-Feeding Gall Midges of North America*. Ithaca, New York: Cornell University Press. 356
- Gagné, R.J. & Orphanides, G.M. 1992. The pupa and larva of *Asphondylia gennadii* (Diptera: Cecidomyiidae) and taxonomic implications. *Bulletin of Entomological*

*Research*. 82:313–316.

Gagné, R.J. 1994. *The gall midges of the Neotropical region*. Cornell University Press, Ithaca and London.

Gagné, R.J. & Jaschhof, M. 2017. *A Catalogue of Cecidomyiidae (Diptera) of the World*. Fourth Edition ed. Washington. [Online], Available: [http://www.ars.usda.gov/ARSTUserFiles/%0A80420580/Gagné\\_2017\\_World\\_Cat\\_4th\\_ed.pdf](http://www.ars.usda.gov/ARSTUserFiles/%0A80420580/Gagné_2017_World_Cat_4th_ed.pdf).

Gagné, R.J., Kim, J.W., Uechi, N. & Yukawa, J. 2018. A New Pest *Asphondylia* (Diptera: Cecidomyiidae) on Grape Berries (Vitaceae) in Southwestern North America with Descriptive Notes on the Genus . *Proceedings of the Entomological Society of Washington*. 120(4): 779–790.

Gess, S.K. 1992. Biogeography of the masarine wasps (Hymenoptera: Vespidae: Masarinae), with particular emphasis on the southern African taxa and on correlations between masarine and forage plant distributions. *Journal of Biogeography*. 19(5): 491-503.

Gess, S.K. & Gess, F.W. 2014. *Wasps and bees in southern Africa*. Pretoria: South African National Biodiversity Institute. 320.

Giliomee, J.H. 2003. Insect diversity in the cape floristic region. *African Journal of Ecology*. 41(3): 237-244.

Gillespie, D.R., Roitberg, B., Basalyga, E., Johnstone, M., Opit, G., Rodgers, J. & Sawyer, N. 1998. Biology and application of *Feltiella Acarisuga* (Vellot)(Diptera: Cecidomyiidae) for biological control of twospotted spider mites on greenhouse vegetable crops. *Agriculture & Agri-Food Canada*, Pacific Agri-Food Research Centre.

Goldblatt, P. 1978. An analysis of the flora of Southern Africa: Its characteristics,

- relationships, and origins. *Annals of the Missouri Botanical Garden*. 65(2): 369-436. doi:10.2307/2398858
- Goldblatt, P. & Manning, J.C. 2002. Plant diversity of the Cape region of southern Africa. *Annals of the Missouri Botanical Garden*. 281-302.
- Goldblatt, P., Savolainen, V., Porteous, O., Sostaric, I., Powell, M., Reeves, G., Manning, J.C., Barraclough, T.G. & Chase, M.W. 2002. Radiation in the Cape flora and the phylogeny of peacock irises *Moraea* (Iridaceae) based on four plastid DNA regions. *Molecular phylogenetics and evolution*. 25(2): 341-360.
- Grimaldi, D. & Engel, M. 2005. *Evolution of the Insects*. (Cambridge Evolution). Cambridge University Press.
- Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T. & Goulson, D. 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PloS one*. 12(10) e0185809.
- Hamer, M.L. & Slotow, R.H. 2002. Conservation application of existing data for South African millipedes (Diplopoda). *African Entomology*. 10(1): 29-42.
- Hamer, M. 2013. A National Strategy for Zoological Taxonomy (2013-2020). [Online], Available: <https://www.sanbi.org/wp-content/uploads/2018/04/national-strategy-zoological-taxonomy.pdf>.
- Harris, K.M. 1961. The sorghum midge, *Contarinia sorghicola* (Coq.), in Nigeria. *Bulletin of Entomological Research*. 52(1): 129-146.
- Harris, M.O., Stuart, J.J., Mohan, M., Nair, S., Lamb, R.J. & Rohfritsch, O. 2003. Grasses and gall midges: plant defense and insect adaptation. *Annual Review of Entomology*. 48(1): 549-577.
- Hartmann, H.E.K. 2001. Illustrated Handbook of Succulent Plants: Aizoaceae A-E;

- Aizoaceae F-Z. Berlin, Germany: Springer.
- Heath, J.J. & Stireman, J.O. 2010. Dissecting the association between a gall midge, *Asteromyia carbonifera*, and its symbiotic fungus, *Botryosphaeria dothidea*. *Entomologia Experimentalis et Applicata*. 137: 36–49.
- Hebert, P.D., Ratnasingham, S., Zakharov, E.V., Telfer, A.C., Levesque-Beaudin, V., Milton, M.A., Pedersen, S., Jannetta, P. & DeWaard, J.R. 2016. Counting animal species with DNA barcodes: Canadian insects. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 371(1702): 20150333.
- Hilton-Taylor, C. 1996. Patterns and characteristics of the flora of the Succulent Karoo Biome, southern Africa. *In The biodiversity of African plants*. Dordrecht: Springer. 58-72
- Hoffman, M.T., Carrick, P.J., Gillson, L. & West, A.G. 2009. Drought, climate change and vegetation response in the Succulent karoo, South Africa. *South African Journal of Science*. 105(1-2): 54-60.
- Impson, F.A.C., Kleinjan, C.A., Hoffmann, J.H. & Post, J.A. 2008. *Dasineura rubiformis* (Diptera: Cecidomyiidae), a new biological control agent for *Acacia mearnsii* in South Africa. *South African Journal of Science*. 104(7-8): 247-249.
- Impson, F.A.C., Post, J.A., & Hoffmann, J.H. 2013. Impact of the flower-galling midge, *Dasineura rubiformis* Kolesik, on the growth of its host plant, *Acacia mearnsii* De Wild, in South Africa. *South African Journal of Botany*. 87: 118–121. <https://doi.org/10.1016/j.sajb.2013.04.006>
- Joy, J.B. & Crespi, B.J. 2007. Adaptive radiation of gall-inducing insects within a single host-plant species. *Evolution: International Journal of Organic Evolution*. 61(4): 784-795.

- Joy, J.B. 2013. Symbiosis catalyses niche expansion and diversification. *Proceedings of the Royal Society B: Biological Sciences*. 280:20122820.
- Jurgens, A. 2002. Floral scent compounds in *Conophytum* species: chemical composition and its relevance to taxonomy and pollination biology. In Hammer, S. (ed.). *Dumpling and His Wife: New View of the Genus Conophytum*. Norwich: EAE Creative Colour.
- Jurgens, A. & Witt, T. 2014. Pollen-ovule ratios and flower visitors of day-flowering and night-flowering *Conophytum* (Aizoaceae) species in South Africa. *Journal of Arid Environments*. 109: 44-53.
- Jurgens, N. 1991. A new approach to the Namib Region. *Vegetation*. 97: 21-38.
- Kirk-Spriggs A.H. & Sinclair B.J. (eds). 2017. Manual of Afrotropical Diptera. SANBI Graphics & Editing, Pretoria, South Africa.
- Klak, C., Reeves, G. & Hedderson, T.A.J. 2004. Unmatched tempo of evolution in Southern African semi-desert ice plants. *Nature*. 427: 63-65.
- Klak, C., Bruyns, P.V. & Hedderson, T.A.J. 2007. A phylogeny and new classification for Mesembryanthemoideae (Aizoaceae). *Taxon*. 56(3):737-756.
- Klak, C. 2009. Three new species and two new combinations in the Aizoaceae from the Western and Northern Cape of South Africa. *South African Journal of Botany*. 76:299-307.
- Klak, C., Bruyns, P.V. & Hanáček, P. 2013. A phylogenetic hypothesis for the recently diversified Ruschieae (Aizoaceae) in southern Africa. *Molecular Phylogenetics and Evolution*. 69(3): 1005-1020.
- Klak, C., Hanáček, P. & Bruyns, P.V. 2017. Out of southern Africa: Origin, biogeography and age of the Aizooideae (Aizoaceae). *Molecular Phylogenetics and Evolution*. 109: 203-216.

- Kuhlmann, M. 2009. Patterns of diversity, endemism and distribution of bees (Insecta: Hymenoptera: Anthophila) in southern Africa. *South African Journal of Botany*. 75(4):726-738.
- Liede-Schumann, S., Grimm, G.W., Nürk, N.M., Potts, A.J., Meve, U. & Hartmann, H.E.K. 2020. Phylogenetic relationships in the southern African genus *Drosanthemum* (Ruschioideae, Aizoaceae). *PeerJ*. 8:e8999  
<http://doi.org/10.7717/peerj.8999>
- Linder, H. 2003. The radiation of the Cape flora, southern Africa. *Biological reviews of the Cambridge Philosophical Society*. 78: 597–638.
- Loew, H. 1850. Dipterologische Beiträge. Posen :W. Decker,. [Online], Available: <https://www.biodiversitylibrary.org/item/103276>.
- Louw, S. 1998. The gall-inhabiting weevil (Coleoptera) community on *Galenia africana* (Aizoaceae): co-existence or competition. In Csoka, G., Mattson, W.J., Stone, G.N., Price, P.W. (eds). *The Biology of Gall-inducing Arthropods*. Minnesota: U.S. Department of Agriculture. 122-126
- Low, A.B. & Rebelo, T. 1996 *Vegetation of South Africa, Lesotho and Swaziland : a companion to the vegetation map of South Africa, Lesotho and Swaziland* . Pretoria: Department of Environmental Affairs & Tourism.
- Mani, M.S. 1964. *Ecology of plant galls*. W. Den Haag: Junk Publishers. 434
- Manning J.C. & Goldblatt P. 2012. *Plants of the greater cape floristic region. 1: the Core Cape Flora*. *Strelitzia* 29. Pretoria: South African National Biodiversity Institute.
- Markkula, M., Tiitanen, K., Hamalainen, M. & Forsberg, A. 1979. The aphid midge *Aphidoletes aphidimyza* (Diptera, Cecidomyiidae) and its use in biological control of aphids. In *Annales Entomologici Fennici*. *Entomological Society of*

- Finland*. 45(4): 89-98.
- May, R.M., 1988. How many species are there on earth?. *Science*. 241(4872): 1441-1449.
- Mayer, C., Soka, G. & Picker, M. 2006. The importance of monkey beetle (Scarabaeidae: Hopliini) pollination for Aizoaceae and Asteraceae in grazed and ungrazed areas at Paulshoek, Succulent Karoo, South Africa. *Journal of Insect conservation*. 10(4): 323.
- McAlpine, J.F., Peterson, B.V., Shewell, G.E., Teskey, H.J., Vockeroth, J.R. & Wood, D.M. (eds). 1981. *Manual of Nearctic Diptera*. Ottawa: Research Branch, Agriculture Canada.
- McGeoch, M.A. 2002. Insect conservation in South Africa: an overview. *African Entomology*. 10:1–10.
- Meigen, J.W. 1818. Systematische Beschreibung der bekannten europäischen zweiflügeligen Insekten. Vol. 1. *Aachen*. xxxvi & 333 pp. [Online], Available: <https://www.biodiversitylibrary.org/item/45833>.
- Melin, A. & Colville, J.F. 2019. A review of 250 years of south African bee taxonomy and exploration (Hymenoptera: Apoidea: Anthophila). *Transactions of the Royal Society of South Africa*. 74(1): 86-96.
- Midgley, G., Hannah, L., Roberts, R., MacDonald, D.J. & Allsopp, J. 2001. Have Pleistocene climatic cycles influenced species richness patterns in the greater Cape Mediterranean Region? *Journal of Mediterranean Ecology*. 2:137–144.
- Midgley, G. & Thuiller, W. 2007. Potential vulnerability of Namaqualand plant diversity to anthropogenic climate change. *Journal of Arid Environments*. 70: 615–628.

- Milenković, S. & Tanasković, S. 2008. Harmfulness of raspberry gall midge, *Lasioptera rubi* Schrank (Diptera, Cecidomyiidae), to some raspberry cultivars. *IOBC/wprs Bulletin*. 39:71-75.
- Möhn, E. 1966-1971. *Cecidomyiidae = (Itonididae)*. Vol. 2 (2) In: Linder, E. (ed.), *Die Fliegen der Palaearktischen Region*. Schweizerbart'sche, Stuttgart. 1-248.
- Mora, C., Tittensor, D.P., Adl, S., Simpson, A.G.B. & Worm, B. 2011. How many species are there on earth and in the ocean? *PLoS Biology*. 9(8): e1001127. doi:10.1371/journal.pbio.1001127
- Mucina, L., Jürgens, N., Le Roux, A., Rutherford, M.C., Schmiedel, U., Esler, K.J., Powrie, L.W., Desmet, P.G., Milton, S.J., Boucher, C. & Ellis, F. 2006. Succulent karoo biome. the vegetation of south Africa, lesotho and swaziland. *Strelitzia*. 19: 221-299.
- Muthukumar, M., Kennedy, J.S., Jeyakumar, P., Sridharan, S. & Arumugam, T. 2017. Biology and natural parasitization of Gall Fly *Lasioptera falcata* Felt and *Lasioptera bryoniae* Schiner infesting bitter gourd. *Journal of Entomology and Zoology Studies*. 5(3): 1635-1639
- Myers, N., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A. & Kent, J. 2000. Biodiversity hotspots for conservation priorities. *Nature*. 403: 853-858.
- Novotny, V., Drozd, P., Miller, S.E., Kulfan, M., Janda, M., Basset, Y. & Weiblen, G.D. 2006. Why are there so many species of herbivorous insects in tropical rainforests? *Science*. 313(5790):1115-1118.
- Perdikis, D., Lykouressis, D., Paraskevopoulos, A. & Harris, K.M. 2011. A new insect pest, *Lasioptera* sp. (Diptera: Cecidomyiidae), on tomato and cucumber crops in glasshouses in Greece. *EPPO Bulletin*. 41(3):442-444.
- Peter, C.I., Dold, A.P., Barker, N.P. & Ripley, B.S. 2004. Pollination biology of

- Bergeranthus multiceps* (Aizoaceae) with preliminary observations of repeated flower opening and closure. *South African Journal of Science*. 100(11-12): 624-629.
- Post, J.A., Kleinjan, C.A., Hoffmann, J.H. & Impson, F.A.C. 2010. Biological control of *Acacia cyclops* in South Africa: the fundamental and realized host range of *Dasineura dielsi* (Diptera: Cecidomyiidae). *Biological Control*. 53(1): 68-75.
- Predel, R., Neupert, S., Huetteroth, W., Kahnt, J., Waidelich, D. & Roth, S. 2012. Peptidomics-based phylogeny and biogeography of Mantophasmatodea (Hexapoda). *Systematic Biology*. 61(4): 609-629.
- Price, P.W., Fernandes, G.W. & Waring, G.L. 1987. Adaptive nature of insect galls. *Environmental entomology*. 16(1): 15-24.
- 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., Richardson, D.M. & Savolainen, V. 2009. Dissecting the plant–insect diversity relationship in the Cape. *Molecular phylogenetics and evolution*. 51(1): 94-99.
- Raman, A., Schafer, C.W. & Withers, T.M. 2005. Galls and gall-inducing arthropods: an overview of their biology, ecology, and evolution. In *Biology, ecology, and evolution of gall-inducing arthropods*. New Hampshire: Science Publishers. 1-33.
- Ripley, B.S., Abraham, T., Klak, C., & Cramer, M.D. 2013. How succulent leaves of Aizoaceae avoid mesophyll conductance limitations of photosynthesis and survive drought. *Journal of Experimental Botany*. 64: 5485–5496.
- Rohfritsch, O. & Shorthouse, J.D. 1982. Insect galls. In *Molecular biology of plant tumors*. Academic Press. 131-152

- Rohfritsch, O. 1992. A fungus associated gall midge, *Lasioptera arundinis* (Schiner), on *Phragmites australis* (cav.) trin. *Bulletin de la Societe Botanique de France. Lettres Botaniques*. 45–59.
- Rohfritsch, O. 2008. Plants, gall midges, and fungi: A three-component system. In *Entomologia Experimentalis et Applicata*. 128: 208-216.
- Rouault, M. & Richard, Y. 2003. Intensity and spatial extension of drought in South Africa at different time scales. *Water SA*. 29: 489–500.
- Rutherford, M.C. & Powrie, L.W. 2010. Severely degraded rangeland: implications for plant diversity from a case study in Succulent Karoo, South Africa. *Journal of arid environments*. 74(6): 692-701.
- Samways, M.J. 2007. Insect conservation: a synthetic management approach. *Annual Review of Entomology*. 52: 465-487.
- Samways, M.J. 2019. *Insect Conservation: a global synthesis*. Boston: CABI
- Sánchez-Bayo, F. & Wyckhuys, K.A. 2019. Worldwide decline of the entomofauna: A review of its drivers. *Biological conservation*. 232:8-27.
- Schnitzler, J., Barraclough, T.G., Boatwright, J.S., Goldblatt, P., Manning, J.C., Powell, M.P., Rebelo, T. & Savolainen, V. 2011. Causes of plant diversification in the Cape biodiversity hotspot of South Africa. *Systematic biology*. 60(3): 343–357. <https://doi.org/10.1093/sysbio/syr006>
- Scholtz, C.H. & Chown, S.L. 1996. Insects in southern Africa: how many species are there? *South African Journal of Science*, 91: 124–126.
- Scholtz, C.H. 2010. Review of insect systematics research in South Africa. *Transactions of the Royal Society of South Africa*. 54: 53–63.
- Scudder, G. 2017. The importance of insects: Science and Society. In Footitt, R.G. & Adler, P.H. (eds). *Insect Biodiversity: Science and Society*. 9–43.

- Shorthouse, J.D., Wool, D. & Raman, A. 2005. Gall-inducing insects—Nature's most sophisticated herbivores. *Basic and Applied Ecology*. 6(5): 407-411.
- Smith, G.F., Buys, M., Walters, M., Herbert, D. & Hamer, M. 2008. Taxonomic research in South Africa: the state of the discipline. *South African Journal of Science*. 104(7-8): 254-256.
- Snijman, D.A. 2013. *Plants of the Greater Cape Floristic Region 2: the Extra Cape Flora*. *Strelitzia* 30. Pretoria: South African National Biodiversity Institute.
- Stork, N.E. 2018. How many species of insects and other terrestrial arthropods are there on Earth? *Annual review of entomology*. 61: 31-45.
- Sylven, E. 1979. Gall midges (Diptera, Cecidomyiidae) as plant taxonomists. *Symbolae Botanicae Upsalienses*. 22: 62-69.
- Tanasković, S.T. & Milenković, S.N. 2012. Open field surveys to evaluate the susceptibility of red raspberry genotypes to raspberry gall midge, *Lasioptera rubi* Schrank (Diptera, Cecidomyiidae) - 4 year results. *Acta Horticulturae*. 946: 247–251.
- Tokuda, M. 2012. Biology of Asphondyliini (Diptera: Cecidomyiidae). *Entomological Science*. 15(4):361–383.
- Uechi, N. & Yukawa, J. 2006. Host range and life history of *Asphondylia sphaera* (Diptera: Cecidomyiidae): Use of short-term alternate hosts. *Annals of the Entomological Society of America*. 99: 1166–1171.
- Valente, L.M., Britton, A.W., Powell, M.P., Papadopoulos, A.S.T., Burgoyne, P.M. & Savolainen, V. 2014. Correlates of hyperdiversity in southern African ice plants (Aizoaceae). *Botanical Journal of the Linnean Society*. 174(1): 110–129.
- Van Wyk, A.E. & Smith, G.F. 2001. *Regions of floristic endemism in southern Africa: a review with emphasis on succulents*. Hatfield: Umdaus press.

- Verboom, G.A., Archibald, J.K., Bakker, F.T., Bellstedt, D.U., Conrad, F., Dreyer, L.L., Forest, F., Galley, C., Goldblatt, P., Henning, J.F. & Mummenhoff, K. 2009. Origin and diversification of the Greater Cape flora: ancient species repository, hot-bed of recent radiation, or both?. *Molecular Phylogenetics and Evolution*. 51(1): 44-53.
- Verboom, G.A., Linder, H.P., Forest, F., Hoffmann, V., Bergh, N.G. & Cowling, R.M. 2014. Cenozoic assembly of the Greater Cape Flora. In N. Allsopp, J.F. Colville, & G.A. Verboom (eds). Oxford University Press *Fynbos: Ecology, Evolution, and Conservation of a Megadiverse Region*. 93-119.
- Victor, J.E., Smith, G.F. & Van Wyk, A.E. 2015. A method for establishing taxonomic research priorities in a megadiverse country. *Phytotaxa*. 203: 55–62.
- Von Staden, L., Raimondo, D. & Dayaram, A. 2013. Taxonomic research priorities for the conservation of the South African flora. *South African Journal of Science*. 109(3-4): 1-10.
- Von Willert D.J., Werger M.J.A., Brinckmann E., Ihlenfeldt H.D. & Eller B.M. 1992. *Life Strategies of Succulents in Deserts: With Special Reference to the Namib Desert*. Cambridge University Press.
- Wagner, D.L. & Van Driesche, R.G. 2010. Threats posed to rare or endangered insects by invasions of nonnative species. *Annual Review of Entomology*. 55: 547-568.
- Wagner, D.L. 2020. Insect declines in the Anthropocene. *Annual Review of Entomology*. 65: 457-480.
- West, R.J. & Shorthouse, J.D. 1982. Morphology of the balsam fir needle gall induced by the midge *Paradiplosis tumifex* (Diptera: Cecidomyiidae). *Canadian Journal of Botany*. 60(2): 131-140.

- Wiens, J.J., Lapoint, R.T. & Whiteman, N.K. 2015. Herbivory increases diversification across insect clades. *Nature communications*.6(1): 1-7.
- 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.
- Young, A.J. & Desmet, P.G. 2016. The distribution of the dwarf succulent genus *Conophytum* NE Br.(Aizoaceae) in southern Africa. *Bothalia-African Biodiversity & Conservation*. 46(1): 1-13.
- Yukawa, J., Uechi, N., Horikiri, M. & Tuda, M. 2003. Description of the soybean pod gall midge, *Asphondylia yushimai* sp. n. (Diptera: Cecidomyiidae), a major pest of soybean and findings of host alternation. *Bulletin of Entomological Research*. 93(1):73–86.
- Yukawa J. & Rohfritsch O. 2005. Biology and ecology of gall-inducing Cecidomyiidae (Diptera: Cecidomyiidae). In: Raman A., Schaefer C.W., Withers T.M. (eds) *Biology, Ecology, and Evolution of Gall-Inducing Arthropods*. Enfield: Science Publishers, Inc. 273–304.
- Yukawa, J., Tokuda, M. & Yamagishi, K. 2014. Host plant ranges and distribution records of identified and unidentified species of the genus *Lasioptera* (Diptera: Cecidomyiidae) in Japan. *ESAKIA*. (54):1–15.