Pollination of *Plectranthus* L'Hér. (Lamiaceae) along the Eastern seaboard of southern Africa

by

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for the degree of Doctor of Philosophy
in the School of Biological & Conservation Sciences,
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ABSTRACT

Pollination data is provided for a third of the *Plectranthus* species in southern Africa. In the largest genus of Lamiaceae in the region (53 species), 18 were studied, plus two species of allied genera (*Pycnostachys urticifolia* and *Aeollanthus parvifolius*). Study of these 20 species aimed to describe the groups of pollinators that have driven pollinator specialisation. Case histories are provided upon which future studies of Lamiaceae pollination, breeding systems and speciation may be based. Bees (Apidae) and flies (Nemestrinidae, Acroceridae and Tabanidae) are the main pollinating insect groups. Seven straight-tubed *Plectranthus* species show a match between corolla tube- and proboscis length of nectar-feeding pollinators. Long-proboscid nemestrinid flies are specialised on long-tubed *Plectranthus* species (*P. ambiguus*, *P. hilliardiae*, *P. reflexus* and *P. saccatus*), while shorter-proboscid flies of all three families are important pollinators of straight-tubed species with medium- and short corolla tubes. Seven species with sigmoid corolla tubes are bee-pollinated, with fly-pollination prevalent in some. Bent corolla tubes, coupled with length, act as barriers to illegitimate nectar-feeders and ensures alignment of pollinators for effective pollen placement and carry-over. It is suggested that straight-tubed species may have evolved from sigmoid-tubed species. Long-tubed species with straight corollas in other Lamiaceae may show convergent pollination by long-proboscid flies, with the guild being dependent on habitat and distribution of plants and flies. Formal establishment of the *Stenobasipteron wiedemanni* pollination guild extends the study from *Plectranthus* to selected Acanthaceae, Orchidaceae, Balsaminaceae, Gesneriaceae and Iridaceae, occurring in forested habitat along the Eastern seaboard. Micro-distillation of essential oils confirmed parentage of a putative natural hybrid; once established, hybrid data allows studies of the importance of natural hybridisation events in explaining pollinator fidelity. Nectar sugar studies in *Plectranthus* mostly showed sucrose dominance; cases of hexose dominance are noted and discussed. Nectar volume and concentration proved variable and do not fit any trends. Pollination by medium-proboscid acrocerid flies has importance for ‘medium-tubed’ plants, since six of the *Plectranthus* species are solely or partially reliant on Acroceridae for pollination. An appendix with consolidated data describes the 20 study species i.t.o. morphology, habitat, study sites, field work, pollinator observations and insect vouchers.

STUDENT DECLARATION 1

Pollination of *Plectranthus* L'Hér. (Lamiaceae) along the Eastern seaboard of southern Africa

(Thesis Title)

I, **Christina Johanna Potgieter**

(Full name)

902409960

(Student Number)

declare that :

(i) The research reported in this dissertation, except where otherwise indicated, is the result of my own endeavours in the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg;

(ii) This dissertation has not been submitted for any degrees or examination at any other University;

(iii) This thesis does not contain data, figures or writing, unless specifically acknowledged, copied from other researchers; and

(iv) Where I have reproduced a publication of which I am an author or co-author, I have indicated which part of the publication was contributed by me.

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STUDENT DECLARATION 2: PUBLICATIONS

Publication 1:

Potgieter, C.J., Edwards, T.J., Miller, R.M., Van Staden, J., 1999. Pollination of seven *Plectranthus* spp. (Lamiaceae) in southern Natal, South Africa. Plant Systematics and Evolution 218, 99–112.

All field work and write-up was done by C. Potgieter, with assistance from Prof. T. Edwards (discussions and editing), Dr R. Miller (Diptera identification) and Prof. J. van Staden (discussions and editing).

Publication 2:

Potgieter, C.J., Edwards, T.J., 2001. The occurrence of long, narrow corolla tubes in southern African Lamiaceae. Systematics and Geography of Plants 71, 493–502.

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Viljoen, A.M., Demirci, B., Baser, K.H.C., Potgieter, C.J., Edwards, T.J., 2006. Microdistillation and essential oil chemistry - a useful tool for detecting hybridisation in *Plectranthus* (Lamiaceae). South African Journal of Botany 72, 99–104.

All field work and sample collection in South Africa was done by C. Potgieter; the idea of analyzing hybrids was put forward by C. Potgieter and Prof. T. Edwards. The laboratory-based work and analysis was arranged and executed by Prof. A Viljoen, in collaboration with Dr B. Demirci and Dr K. Baser (in Turkey).

Publication 5:

Potgieter, C.J., Edwards, T.J., Van Staden, J., 2009. Pollination of *Plectranthus* spp. (Lamiaceae) with sigmoid flowers in southern Africa. South African Journal of Botany 75: 646–659.

All field work and write-up was done by C. Potgieter, with assistance from Prof. T. Edwards (discussions and editing) and Prof. J. van Staden (discussions and editing).

Signed at Pietermaritzburg on the ……….…. day of ………………………………, 2009.

SIGNATURE

DECLARATION BY SUPERVISOR

We hereby declare that we acted as Supervisors for this MSc / PhD student:

Student's Full Name:	**Christina Johanna Potgieter**
Student Number:	**902409960**
Thesis Title:	**Pollination of *Plectranthus* L'Hér. (Lamiaceae) along the Eastern seaboard of southern Africa**

Regular consultation took place between the student and ourselves throughout the investigation. We advised the student to the best of our ability and approved the final document for submission to the Faculty of Science and Agriculture Higher Degrees Office for examination by the University appointed Examiners.

SUPERVISOR: PROFESSOR T.J. EDWARDS

CO-SUPERVISOR: PROFESSOR J. VAN STADEN

DECLARATION BY CO-SUPERVISOR

We hereby declare that we acted as Supervisors for this MSc / PhD student:

Student's Full Name:	**Christina Johanna Potgieter**
Student Number:	**902409960**
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CONTENTS

CHAPTER 1: INTRODUCTION

Plectranthus L'Hér. (Lamiaceae) is a horticulturally important genus of predominantly herbaceous plants that is increasingly popular in indigenous landscaping in South Africa (Van Jaarsveld 2006), and internationally in the potted plant trade (Brits *et al*. 2001, Brits & Ling Li 2008). Current horticultural research is focused on aspects of flowering in the genus (Ascough & Van Staden 2007, Ascough *et al*. 2008), while chemical research (Abdel-Mogib *et al*. 2002, Stavri *et al*. 2009) and ethno-botanical research (Rabe & Van Staden 1998, Lukhoba *et al*. 2006, Van Zyl *et al*. 2008) point to the biomedical potential of this ornamental genus.

The genus *Plectranthus* is diverse in terms of floral morphology, especially on the sandstone islands of southern KwaZulu-Natal (KZN) and the northern parts of the Eastern Cape (EC), i.e. the Pondoland Centre of Endemism *sensu* Van Wyk & Smith (2001) where a total of 29 described species of *Plectranthus* occur. Eleven of these species (or sub-species) are endemic or near-endemic to the region: *Plectranthus aliciae* (Codd) Van Jaars. & T.J.Edwards, *P. brevimentum* T.J.Edwards, *P. ernstii* Codd, *P. hilliardiae* Codd, *P. malvinus* Van Jaars. & T.J.Edwards, *P. oertendahlii* T.C.E.Fr., *P. oribiensis* Codd, *P. praetermissus* Codd, *P. reflexus* Van Jaars. & T.J.Edwards, *P. saccatus* Benth. subsp*. pondoensis* Van Jaarsv. & Milstein*, P. stylesii* T.J.Edwards (Van Jaarsveld & Edwards 1997; Van Wyk & Smith 2001). These endemic species probably form part of a natural group of species, found in Eastern and Southern Africa and in Madagascar. The details of relationships within this group are not clear. The Pondoland Centre is described as a "major centre of diversity and endemism for the genus" [*Plectranthus*] (Van Wyk & Smith 2001).

The general vegetation type of the Pondoland Centre is grassland plateaux, dissected by deep, narrow river gorges lined by patches of forest (Van Wyk & Smith 2001). It is in and around these forested gorges that most of the endemic *Plectranthus* species occur. The sandstone regions of KZN and Pondoland have been described as remarkable centres of endemism with several species which are uncommon or absent on surrounding substrates (Van Wyk 1990). Many of these species are palaeo-endemic forest and forest margin elements, and studies on reproductive biology and population dynamics have been encouraged for those species that exhibit poor seed-set and that are vulnerable to extinction (Van Wyk 1990). The area is also rich in apparent neo-endemics with strong affinities to the Cape and Afro-montane areas (Van Wyk & Smith 2001). *Plectranthus* does not fit well within either of these broad phyto-geographic groups. The genus is Tropical in origin (Paton *et al*. 2004) with relatively few species occurring in the Cape Floral Region and along montane corridors.

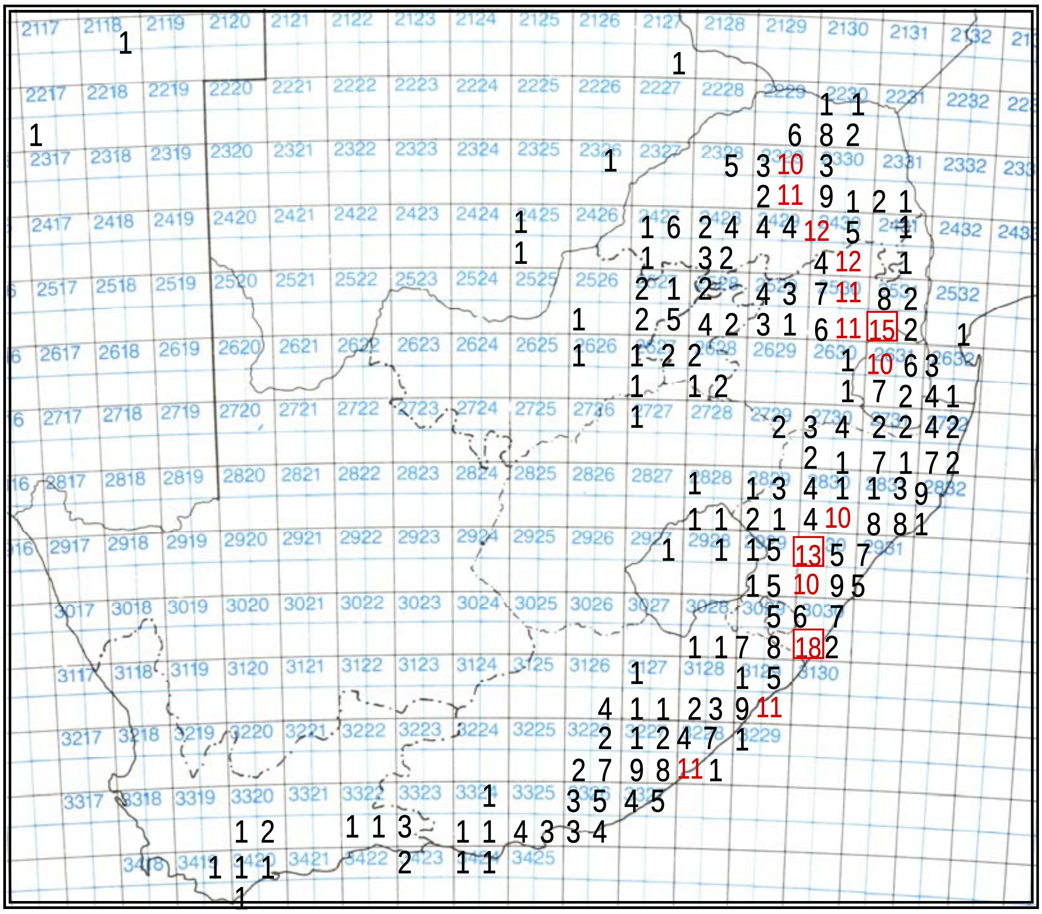

Figure 1: Map of southern Africa showing number of *Plectranthus* species per half degree square, indicating areas of high diversity; after Codd (1985a) and specimens from PRE and NU Herbaria. Areas with ten or more species are shown in red, with the three main areas of species diversity indicated with red boxes.

In southern Africa, *Plectranthus* exhibits its highest level of diversity in the Pondoland Centre of Endemism, where the main study areas for this research were located. Oribi Gorge (3030C) is home to 18 species and Umtamvuna Gorge (3030C and 3130A) has 12 species (Fig. 1). Several other regions of high *Plectranthus* diversity are indicated, with a prominent area being 2531C with 15 species (the area surrounding Barberton in Mpumalanga Province). The area north-west of Pietermaritzburg, in the KZN Midlands, also boasts a high species number, with 13 species of *Plectranthus* present in the half degree square that includes the Karkloof (2930A).

Taxonomy and Phylogeny

Plectranthus belongs to the subfamily Nepetoideae, tribe Ocimeae, subtribe Plectranthinae. The Ocimeae originated in Asia (Paton *et al*. 2004) and gave rise to the African Ociminae and Plectranthinae. With about 300 species world-wide, the genus covers a wide distribution in the tropical and warm regions of the Old World (Retief 2000). Its generic boundary is controversial, with Paton *et al*. (2004) showing that the current circumscription is paraphyletic and requires expansion to include allied genera such as *Pycnostachys* Hook., *Solenostemon* Thonn., *Aeollanthus* Mart. ex K.Spreng., *Thorncroftia* N.E.Br. and *Tetradenia* Benth. Even without these inclusions it is the largest genus of Lamiaceae in southern Africa, with 53 species described to date (Codd 1975, 1985a; Van Jaarsveld & Edwards 1991, 1997; Van Jaarsveld & Hanky 1997; Edwards *et al*. 2000; Van Jaarsveld & Van Wyk 2004; Edwards 2005; Winter & Van Jaarsveld 2005).

The taxonomy of *Plectranthus* is fairly well established (Codd 1975 & 1985a, b), but what has not been adequately resolved is a phylogeny for the genus. A phylogenetic study of the tribe Ocimeae, to which *Plectranthus* belongs, by Paton *et al*. (2004) included only five *Plectranthus* and one *Pycnostachys* species relevant to the current pollination study, with no long-tubed species included. In addition, it was found that *Plectranthus* is paraphyletic and that further sampling is needed to recognise monophyletic groups within the subtribe Plectranthinae (Paton *et al*. 2004). These samples will need to include more of the endemic species of the Pondoland Centre before conclusions can be drawn with respect to phylogeny and the evolution of their pollination systems.

Studies based on phylogenies are able to polarise characters and interpret pollinator shifts within a genus, for example Johnson *et al*. (1998) - *Disa* P.J.Bergius (Orchidaceae), Goldblatt *et al*. (2000) - *Sparaxis* Ker Gawl (Iridaceae), Goldblatt *et al*. (2001) - *Gladiolus* L. (Iridaceae), Johnson *et al*. (2002) - *Zaluzianskya* F.W.Schmidt (Scrophulariaceae), Beardsley *et al*. (2003) - *Mimulus* L. (Scrophulariaceae), Wilson *et al*. (2006) - *Penstemon* Mitch. and *Keckiella* Straw (Scrophulariaceae). Phylogenies enhance the understanding of floral character evolution (Kay *et al*. 2006); hence the lack of such a complete phylogeny in *Plectranthus* and its allies is unfortunate.

General importance of pollination studies

Pollination Biology is a growing field of study, complementing the natural history of “anthecology” by using rigorous analytical techniques, the quantification of observations and experimentation (Baker & Baker 1986). Many long-held theories regarding co-evolution, mimicry and plant-animal interactions are being tested experimentally, with some researchers suggesting that descriptive pollination biology studies, combined with theoretical mating system studies, will lead to a “new plant reproductive biology” (Morgan & Schoen 1997). The “traditional descriptive, natural-history approach” [to understanding floral evolution] is being broadened to incorporate various forms of experimentation, genetics, mathematical theory and pollinator behaviour, thus integrating plant reproductive biology into evolutionary ecology (Harder & Barrett 2006).

There is, however, still a great deal of base-line observational data that needs to be collected to elucidate the pollinators or pollination syndromes for most plant genera, which restricts the theoretical conclusions that can be made globally. In developing countries that are species rich, little is known about pollination systems (Johnson & Steiner 2000), which means that theories from northern hemisphere studies may not be appropriate for generalisations about pollination syndromes. In a review of angiosperm speciation, as driven by pollinators and as evidenced by floral diversity, Johnson (2006) concludes that the environmental factors behind this evolution need more attention, and that “a diversity of approaches from natural history to molecular biology” is necessary. In the editorial of a recent volume of the South African Journal of Botany, which is dedicated to pollination studies, Johnson *et al*. (2009) reiterate that “solid documentation of pollination systems” is the basis upon which the ecology and evolution of plants may be understood w.r.t. pollination systems.

Apart from adding to basic natural history information, pollination studies are critical in conservation management and agriculture. Conservation management plans need to incorporate studies on floral reproductive biology in their syntheses. The pollination component of any ecosystem is important for: (1) management of ecosystems; (2) servicing agricultural needs and (3) revealing the co- and contra-evolutionary development of plants and animals (Stirton 1981). Kevan (1975) discussed pollination with respect to the use of insecticides and concluded that the elucidation of critical pollination phenomena, which have far-reaching ramifications on whole ecosystems, is clearly needed. Johnson & Steiner (2000) mentioned the urgent need for pollination studies to assess the viability of plant populations i.t.o. conservation.

Baker & Hurd (1968) stressed that detailed studies of plant families should be complemented by intensive studies of a single species so that the mechanics of the pollination systems may be discovered, their operations placed on a meaningful quantitative basis and their modes of evolution inferred with some measure of certainty. For example, restrictions may be placed on the geographical distribution of plants by the lack of suitable pollinators and, conversely, the opportunity for anthophilous animals to live in an area may be limited by a lack of suitable flowers for them to visit (Baker & Hurd 1968). In an overview of anthecology in the Labiatae, Meeuse (1992) refers to the “limited number of case histories” [of pollination syndromes] in the Labiatae, pointing out that more case histories are required.

Previous studies on *Plectranthus* pollination

Scott Elliot (1891) reported the honeybee *Apis mellifera* L., 1758, a bombyliid fly and two lepidopterans as visitors to *P. ecklonii* Benth. in South Africa. Marloth (1932) did not record any insect visitors to two *Plectranthus* species from the Cape (South Africa), but noted that self-pollination would be unlikely as the stigma matures after the last anther has withered. Van der Pijl (1972) further mentioned butterflies and species of *Bombus* Latreille, 1802 and *Apis* L., 1758 bees as visitors to *Plectranthus* species in Nepal, Australia and Java. Gupta *et al*. (1984) studied the foraging activity of two *Apis* species on *Plectranthus rugosus* Wall. [=*Isodon rugosus* (Wall. ex Benth.) Codd] in India, and found that bumble bees and lepidopterans also visit the flowers. In Madagascar *Pachymelus limbatus* Saussure, 1890 bees (Anthophoridae) and a *Stylogaster* Macquart, 1835 fly species (Conopidae) are visitors to *Plectranthus vestitus* Benth., with the former species being the principal pollinator (Nilsson *et al*. 1985). *Pachymelus limbatus* was also shown to exhibit male patrolling and territoriality associated with plants of *Plectranthus* aff. *vestitus* Benth. and *P. madagascariensis* (Pers.) Benth. in Madagascar (Nilsson & Rabakondrianina 1988).

Stirton (1977) listed the following South African insect visitors to cultivated plants of *Plectranthus neochilus* Schltr.: Hymenoptera - five species of *Megachile* Latreille, 1802, three species of *Xylocopa* Latreille, 1802, one species of *Anthophora* Latreille, 1803, *Apis mellifera* (all Apidae); Diptera - unidentified bombyliids, *Asarkina* Macquart, 1842 (Syrphidae); Lepidoptera - *Macroglossum trochilus* Hubner, 1823 (Sphingidae). Two species of *Xylocopa* and *Macroglossum trochilus* also visited *Plectranthus barbatus* Andr. Only the bees were seen to consistently and effectively work the pollination mechanism (Stirton 1977).

Huck (1992) reviewed pollination in the Lamiaceae and added *Bombus diversus* Smith, 1869 bees (Apidae) and *Gurelca himachala* Butler, 1875 moths (Sphingidae) as pollination vectors of the Japanese species, *Plectranthus inflexus* Vahl ex Benth.

In summary the documented insect visitors to *Plectranthus* belong to the hymenopteran families Apidae, including the Anthophoridae and Megachilidae – now Anthophorinae and Megachilinae (Brothers 1999); the dipteran families Syrphidae, Bombyliidae and Conopidae; and Sphingidae and other Lepidoptera. This mirrors groups of pollinators in the Ocimeae in general, as Paton *et al*. (2004) noted that these include bees, butterflies and flies.

A recent study, also centred on southern African species of a genus of Lamiaceae, was conducted on five species of *Syncolostemon* E.Mey. (Ford & Johnson 2008) and over-lapped with one of the general study sites of the current study (grassland habitat at Umtamvuna Gorge). It showed that a variety of pollinator groups, ranging from sunbirds to long-proboscid flies, day-flying hawkmoths and bees, were active on species that have a range of corolla tube sizes. One major difference between the genus *Syncolostemon* and *Plectranthus* is that the former has more or less trumpet-shaped flowers with more open corolla mouths, while *Plectranthus* tends to have narrow entrances to the corolla or smaller, laterally compressed entrances than *Syncolostemon*, which restricts the possible suite of pollinators, especially sunbirds.

Rationale of the project

This study was initiated during 1994 in KZN, for various reasons. There was little baseline data available on pollination in *Plectranthus* (Nilsson *et al*. 1985), especially from South Africa. The main (Pondoland) Centre of endemism for the genus occurs in this province, making it an ideal study area. There has been increased interest in the cultivation of this genus, with new species discoveries and artificial hybrids becoming available in recent years. As the largest genus of Lamiaceae in southern Africa, with remarkable floral diversification, it makes the ideal subject for broad-based pollination studies. Species of *Plectranthus* associated with the Pondoland Centre in particular, show considerable diversification with respect to corolla tube length and shape, which suggests that pollinator syndromes are involved in the speciation process. Both endemic and non-endemic species were studied in the main study area, and the study was extended to provide a comparison of *Plectranthus* pollination in other sandstone areas further south and non-sandstone areas further north in KZN, thus extending the study to the greater Eastern seaboard.

In light of discussions surrounding the ‘generalisation’ versus ‘specialisation’ debate in pollination systems (Waser & Price 1993, Waser *et al*. 1996, Johnson & Steiner 2000), the study aimed to describe the syndromes or groups of pollinators that may have driven pollinator specialisation in *Plectranthus*, while adding base-line observational information to provide case histories for eighteen species of *Plectranthus* and two species of allied genera (*Pycnostachys urticifolia* Hook. and *Aeollanthus parvifolius* Benth.).

Thesis structure

Following this introductory chapter, five chapters are in the form of published papers, with an additional chapter on Nectar studies followed by a Discussion & Conclusions chapter. The study of *Plectranthus* has lead to further discussions on the Lamiaceae in general, as well as a newly described pollination syndrome that extends beyond *Plectranthus* to other plant families and genera.

Chapter 1: Introduction

This current chapter provides an introduction to the genus *Plectranthus* and the study of pollination in the family Lamiaceae, and introduces the project. The introductory sections of the five papers presented in Chapters 2 – 6 provide further general background information to the study and are not repeated here.

Chapter 2: Pollination of Straight-tubed species

Potgieter, C.J., Edwards, T.J., Miller, R.M., Van Staden, J., 1999. Pollination of seven *Plectranthus* spp. (Lamiaceae) in southern Natal, South Africa. Plant Systematics and Evolution 218: 99–112.

The pollination of seven straight-tubed *Plectranthus* species of varying tube length is described, showing that both bees (Hymenoptera) and flies (Diptera) with varying proboscid lengths are the main pollinator groups.

Chapter 3: Pollination of Sigmoid-tubed species

Potgieter, C.J., Edwards, T.J., Van Staden, J., 2009. Pollination of *Plectranthus* spp. (Lamiaceae) with sigmoid flowers in southern Africa. South African Journal of Botany 75: 646–659.

The pollination of five sigmoid-tubed *Plectranthus* (and two allied species with sigmoid corollas) is described. Bees are the main pollinators, but fly pollination is also prevalent in some species. The potential origin of the sigmoid corolla shape is discussed.

Chapter 4: Convergent pollination in southern African Lamiaceae

Potgieter, C.J., Edwards, T.J., 2001. The occurrence of long, narrow corolla tubes in southern African Lamiaceae. Systematics and Geography of Plants 71: 493–502.

This paper uses known *Plectranthus* pollination data to speculate on the possible pollinators of other Lamiaceae in the region that have long, straight corolla tubes.

Chapter 5: A new Pollination Guild

Potgieter, C.J., Edwards, T.J., 2005. The *Stenobasipteron wiedemanni* (Diptera, Nemestrinidae) pollination guild in eastern southern Africa. Annals of the Missouri Botanical Garden 92: 254–267.

The discovery of this exciting new pollination guild led to a discussion on *Plectranthus* and species of other genera and plant families that have representatives that conform to this long-proboscid fly guild.

Chapter 6: Natural Hybrids

> Viljoen, A.M., Demirci, B., Baser, K.H.C., Potgieter, C.J., Edwards, T.J., 2006. Microdistillation and essential oil chemistry - a useful tool for detecting hybridisation in *Plectranthus* (Lamiaceae). South African Journal of Botany 72: 99–104.

This paper tests a microdistillation technique to detect hybridization, using a putative *Plectranthus* hybrid for the study. The discussion relates the occurrence of natural hybrids to pollinator fidelity in long-tubed *Plectranthus*.

Chapter 7: Nectar studies

This is a general chapter on nectar, outlining data on *Plectranthus* nectar sugar composition and comparing it with trends in other Lamiaceae and long-proboscid fly-pollinated plants. Nectar concentration and volume were studied in selected *Plectranthus* species. The relevance of nectar studies is discussed.

Chapter 8: Discussion and Conclusions

The final chapter brings the various chapters and published papers together.

Appendix

This final section provides consolidated data on the 20 studied plant species, with brief plant and habitat descriptions, study site and field work information, and pollinator observations and vouchers. The appendix should be read in conjunction with the published papers, since it provides the most recent data for all studied species. It also contains unpublished records that have not been, or are in the process of being, compiled for publication.

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CHAPTER 2:
POLLINATION OF STRAIGHT-TUBED SPECIES

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Pollination of seven *Plectranthus* spp. (*Lamiaceae*) in southern Natal, South Africa

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Abstract: The genus *Plectranthus* (*Lamiaceae*) shows remarkable radiation on the sandstones of southern Natal and northern Transkei in South Africa, where six endemic species occur. Two of these endemic species, *P. hilliardiae* and *P. oribiensis*, are included in this study, as well as *P. reflexus*, for which only limited data are available. The other species that were studied are *P. ambiguus*, *P. ciliatus*, *P. ecklonii*, *P. madagascariensis* and *P. zuluensis*. Four of these taxa, *P. ambiguus*, *P. hilliardiae*, *P. reflexus* and *P. saccatus* var. *longitubus*, have uniquely long corolla-tubes (20–30 mm) and this is related to pollination by nemestrinid flies of the genus *Stenobasipteron* that have proboscides of similar length. Other nemestrinid species of the genus *Prosoeca* have shorter proboscides and pollinate two species of *Plectranthus* with shorter corolla-tube lengths (6–15 mm). Acrocerid flies, tabanid flies and anthophorid bees are also important visitors to these species. This study on the pollination of seven species of varying corolla-tube lengths shows a correlation between floral tube length and proboscis length of insect visitors, many of which are recorded for the first time as pollinators of *Plectranthus*.

Key words: *Lamiaceae*, *Plectranthus*, *Nemestrinidae*, *Stenobasipteron*, *Prosoeca*, *Acroceridae*, *Psilodera*, *Anthophoridae*, *Amegilla*. Pollination, long-tubed flowers, long-proboscid flies, Natal Group sandstone, adaptive radiation, speciation.

The genus *Plectranthus* (*Lamiaceae*) is represented by 45 species in southern Africa and shows a remarkable diversity in terms of floral morphology. This diversity is most prominent on the sandstones of southern Natal and northern Transkei where a total of 25 species occur, including six endemic species–*Plectranthus ernstii* Codd, *P. hilliardiae* Codd, *P. oertendahlii* Th. Fries jun., *P. oribiensis* Codd, *P. praetermissus* Codd and *P. reflexus* E. J. van Jaarsveld and T. J. Edwards. The endemics are distinctive species with no obvious relationships to each other or more widespread species (Codd 1985a). The sandstones of Pondoland form forest refugia in which allopatric speciation of *Plectranthus* has occurred. These sandstone regions have been described as remarkable center of endemism with several species which are uncommon or absent from surrounding substrates (Van Wyk 1990).

Plectranthus species are predominantly herbaceous varying from soft herbs (*P. ciliatus* E. Mey. ex Benth.) to erect soft shrubs [*P. oribiensis*, *P. zuluensis* T. Cooke and *P. ambiguus* (H. Bol.) Codd], that are occasionally woody below (*P. ecklonii* Benth.). *Plectranthus hilliardiae* is a short, erect semi-succulent herb and *P. madagascariensis* (Pers.) Benth. is a semi-succulent herb with trailing or erect stems (Codd 1985b). The latter is the only grassland species in the study, but it also grows along forest margins. The other six species grow in forest or forest margins, with *P. ciliatus* restricted to moist areas. *Plectranthus oribiensis*, *P. ecklonii* and *P. ambiguus* may also be found in semi-shade.

The inflorescences of *Lamiaceae* are typically decussate cymes that form verticillasters. Superimposed upon this basic pattern are structural elaborations to form branched indeterminate thyrses, e.g.

P. ecklonii and *P. ambiguus*. Inflorescence size varies from 40–80 mm in *P. zuluensis* to 120–250 mm tall in *P. ecklonii* (Codd 1985b).

Flower colour of the studied species varies from white (*P. ciliatus* and *P. madagascariensis*), to pale mauve (*P. hilliardiae*), mauve (*P. oribiensis*), pinkish purple (*P. ambiguus*), pale blue (*P. zuluensis*) and bluish purple (*P. ecklonii*). Nectar guides are present as purple speckles on the upper and lower corolla limbs of *P. ciliatus* and *P. hilliardiae*, as rows of mauve speckles on the upper lip of *P. zuluensis* and as a few speckles on the upper lip of *P. ecklonii*. Faint purple vertical lines occur on the upper lip of *P. ambiguus* and pale blue lines in *P. madagascariensis*.

An interesting phenomenon is the occurrence of species of *Plectranthus* with long corolla-tubes in the general study area. *Plectranthus hilliardiae* and *P. ambiguus* are included in the current study, but *P. reflexus* occurs south of the study area at Port St. Johns, and only limited time was available for observations on this species. *Plectranthus reflexus* has a pale mauve corolla-tube of 28–30 mm (Van Jaarsveld and Edwards 1991). Another species, *P. saccatus* Benth., has very variable corolla-tube lengths and one of the varieties that occur at Umtamvuna (*P. saccatus* var. *longitubus* Codd) has a tube length of 20–26 mm (Codd 1985b). These long-tubed species of *Plectranthus* are unique within the African members of the genus. It is hypothesised that these species have coevolved with a pollinator with a similar proboscis length.

The possibility of pollination by long-tongued flies exists for the long-tubed species of *Plectranthus* as some of their floral traits correspond to those listed by authors reporting this syndrome (Rebelo et al. 1985; Whitehead et al. 1987; Johnson and Steiner 1995, 1997; Manning and Goldblatt 1995, 1996, 1997). The floral tubes of *P. hilliardiae*, *P. ambiguus* and *P. reflexus*, as well as that of the long-tubed variety of *P. saccatus*, are long relative to other species in the genus, narrowly flattened laterally and straight. Flowers are zygomorphic with exserted stigmas and anthers and flowers are oriented horizontally. Nectar is hidden at the base of saccate corolla-tubes, except in *P. ambiguus* with its narrow base that raises the nectar level slightly. Flowers are not sweetly scented but inflorescences emit terpenoid-like scents from a variety of glandular trichomes.

Records of pollination by long-proboscid flies of the families *Nemestrinidae* and *Tabanidae* are scattered through older texts such as those of Marloth (1916–1932) and Vogel (1954) and in recent years the topic has received increasing attention. Whitehead et al. (1987) observed that general texts on pollination had not given much attention to bee flies and long-proboscid flies and that the importance of pollination by flies, particularly in South Africa, was not recognised. Reviews of insect pollination in the Cape flora of South Africa, done by Whitehead et al. (1987) and Johnson (1992), outline the syndrome of pollination by long-proboscid flies. Accounts of individual species or groups of species that rely on this syndrome are given by Rebelo et al. (1985) for *Ericaceae*; Goldblatt (1991) and Goldblatt et al. (1995) for *Iridaceae*; and Johnson and Johnson (1993) and Johnson and Steiner (1995, 1997) for *Orchidaceae*. Manning and Goldblatt (1995) mention a floral guild adapted to pollination by long-proboscid flies in the southern Cape (South Africa) and elaborate on two distinct guilds of long-tubed flowers specialised for this pollination syndrome that occur in winter rainfall areas along the west coast and near interior of southern Africa (Manning and Goldblatt 1996, 1997).

The genus *Plectranthus* contains around 350 species (Codd 1985b) that are distributed throughout the Old World tropics, with the majority of species occurring in eastern and southern Africa and Madagascar. Codd subdivides species on the subcontinent into six subgenera, with all of the species mentioned in this paper restricted to subgenus *Plectranthus*. *Plectranthus madagascariensis* falls within the section *Coleoides*, while the other species under study fall within the typical section (Codd 1975).

Although the species delimitation of the South African species is fairly well established (Codd 1975, 1985b), little is known about their pollination, especially in natural habitats (Nilsson et al. 1985). Marloth (1932) did not record any insect visitors to two *Plectranthus* species from the Cape (South Africa), but noted that self-pollination would be unlikely as the stigma matures after the last anther has withered.

Scott Elliot (1891) reported *Apis mellifera*, a bombyliid fly and two lepidopterans as visitors to *P. ecklonii* in South Africa. Van der Pijl (1972) further mentions a number of butterflies and species of *Bombus* and *Apis* as visitors to *Plectranthus* species in Nepal, Australia and Java. Gupta et al. (1984) studied the foraging activity of two *Apis* species on *P. rugosus* Wall. in India, and found that bumble bees and lepidopterans also visit the flowers. In Madagascar *Pachymelus limbatus* (*Hymenoptera*, *Anthophoridae*) and a *Stylogaster* species (*Diptera*, *Conopidae*) are visitors to *P. vestitus* Benth., with the former species being the principal pollinator (Nilsson et al. 1985). *Pachymelus limbatus* was also shown to exhibit male patrolling and territoriality associated with plants of *Plectranthus* aff. *vestitus* Benth. and *P. madagascar-*

iensis in Madagascar (Nilsson and Rabakondrianina 1988).

Stirton (1977) listed the following South African insect visitors to cultivated plants of *P. neochilus* Schltr.: *Hymenoptera* – five species of *Megachile*, three species of *Xylocopa*, one species of *Anthophora*, *Apis mellifera* (*Apidae*); *Diptera* – unidentified bombyliids, *Asarkina* (*Syrphidae*); *Lepidoptera* – *Macroglossum trochilus* (*Sphingidae*). Two species of *Xylocopa* and *Macroglossum trochilus* also visited *Plectranthus barbatus* Andr.. Only the bees were seen to work the pollination mechanism effectively every time.

Huck (1992) reviewed pollination in the *Lamiaceae* and added *Bombus diversus* (*Apidae*, *Hymenoptera*) and *Gurelca himachala* (*Sphingidae*, *Lepidoptera*) as pollinators of *Plectranthus inflexus* Vahl ex Benth..

In summary the documented insect visitors to *Plectranthus* belong to the families *Anthophoridae*, *Apidae* and *Megachilidae* (*Hymenoptera*); *Syrphidae*, *Bombyliidae* and *Conopidae* (*Diptera*) and *Sphingidae* and other *Lepidoptera*.

This study considers variation in corolla-tube length within seven species of *Plectranthus* and correlates these data to various insect pollinators that are recorded for the first time.

Materials and methods

Field observations and collections were made during the flowering seasons (December–May) of 1995, 1996 and 1997. Voucher specimens of insects are lodged at the Natal Museum Pietermaritzburg and names are listed in Appendix 1. Plant vouchers are lodged at the University of Natal herbarium (NU) and are listed in Appendix 2.

Study sites. Field work was conducted at Umtamvuna and Oribi Gorge Nature Reserves in southern Natal, South Africa (Fig. 1). These sandstone gorges are separated by about 35 km and four of the endemic species occur in the two reserves. Umtamvuna is closest to the coast with study sites ranging from 3 to 8 km inland, while Oribi Gorge is situated about 15 km inland. Additional observations were done at World's View and Ferncliffe Nature Reserve in Pietermaritzburg, 75 km inland and separated by 125 km and 160 km from Oribi Gorge and Umtamvuna respectively. Limited observations were made on *P. reflexus* at Port St. Johns (Fig. 1b).

Species studied. The following species were studied: *P. ambiguus* and *P. hilliardiae* at Umtamvuna, *P. oribiensis* and *P. zuluensis* at Oribi Gorge, and *P. ecklonii*, *P. ciliatus* and *P. madagascariensis* at all three study sites. *Plectranthus oribiensis* and *P. hilliardiae* are endemic to southern Natal. *Plectranthus reflexus* is endemic to forest along the Bulolwe River at Port St. Johns.

Observations. Populations of flowering *Plectranthus* species were observed during the daytime and notes were made of the types of insect visitors, type of floral reward utilised and insect behaviour on the flowers. Voucher insects were netted and killed in separate ethyl acetate-containing vials to prevent pollen contamination. Each specimen was set on a pin with its proboscis extended forward.

Length measurements. Measurements of proboscis length were done from the tip up to the point of attachment of the proboscis to the face of the insect. Corolla-tube lengths of the relevant *Plectranthus* species were measured from the base (at the junction to the calyx) to the mouth of the corolla (at the point where the upper and lower lips diverge). Style and filament lengths were also recorded; where the filaments are partially attached to the corolla the measurement included the length of the corolla. Both posterior (shorter) and anterior (longer) filaments were measured. These values were averaged and compared to proboscis length of insect visitors.

Visitation frequency. To give an indication of the importance of various insect visitors, an estimate of visitation frequency was made by calculating the proportion of observed visits made by each insect species. This estimation was only done for species with proboscis lengths that fall within a range that may promote outcrossing, i.e. nectar and pollen robbing species were excluded.

Pollen loads. Pollen loads of insects were examined under a Hitachi S570 scanning electron microscope to establish whether insects carried mixed pollen loads. Insects were examined under a dessecting microscope to establish where pollen grains were deposited on the insect body. Small pieces of double-sided tape were used to pick pollen off various parts of the insect body and these were placed on a stub, coated with gold-palladium and examined under the scanning electron microscope (SEM). The percentages of *Plectranthus* and foreign pollen were estimated.

Results

Distribution. Figures 1 and 2 show the distribution of the studied species, with longer-tubed species in Fig. 1 and shorter-tubed species in Fig. 2.

Phenology. Flowering times are indicated in Table 1. December to April comprises the main flowering season, with intermittent flowering during the rest of the year for some species. *Plectranthus reflexus* flowers from January to March. The flowering of *Plectranthus* within the gorges is strongly seasonal, with marked overlaps occurring between species.

Time of visit. Flowering populations are visited by insects between 8.00 and 17.00 with continuous visits throughout the day.

Insect behaviour during visits. All the studied *Plectranthus* species are herkogamous and dichogamous. Pollen is presented upon elongate filaments, and after a few days these curl downward and the style elongates and becomes receptive. While autogamy is avoided in this manner, geitonogamy can occur. Foraging behaviour of both dipteran and hymenop-

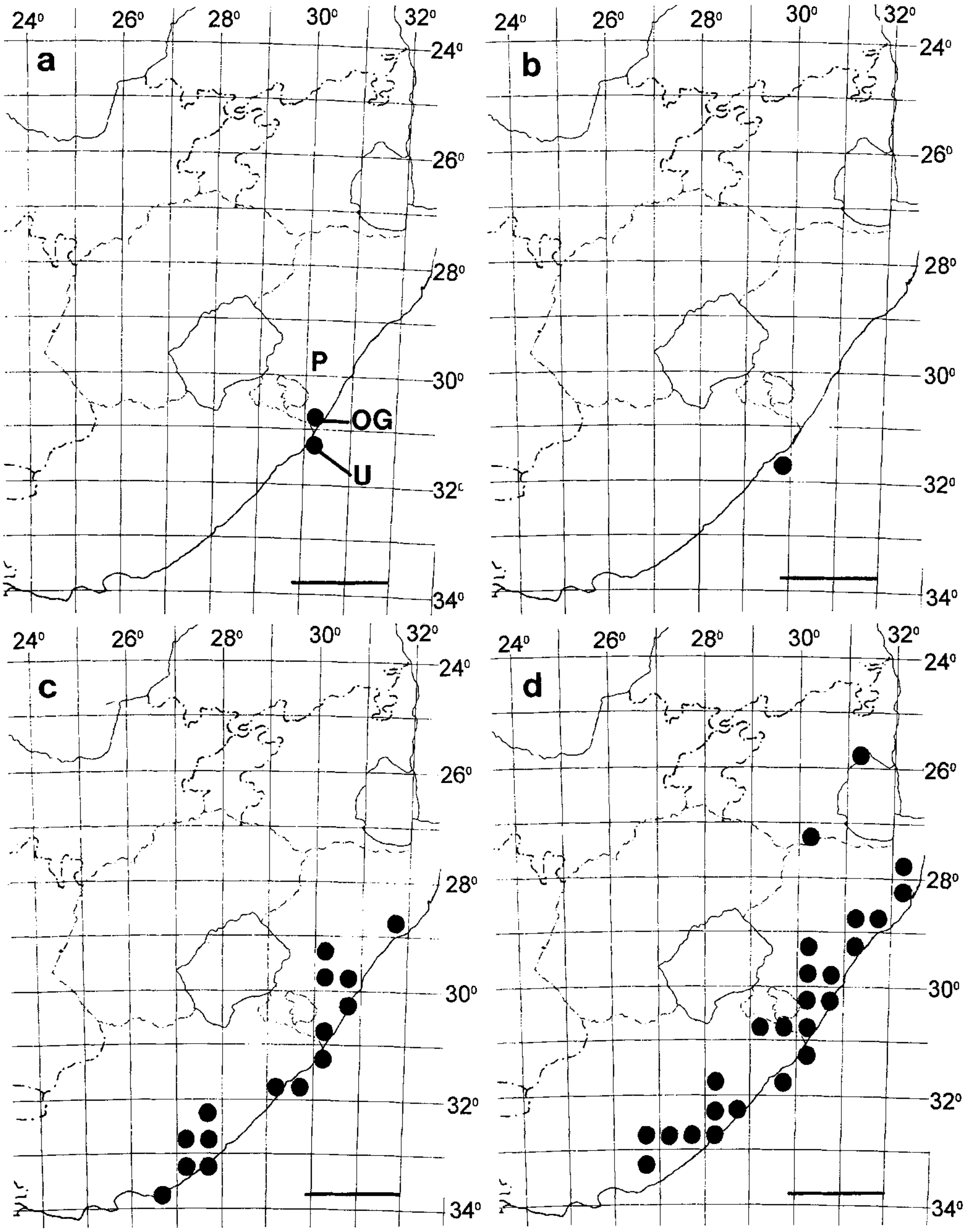

Fig. 1. Location of study sites and distribution of four longer-tubed species of *Plectranthus*. **a** *P. hilliardiae*, OG 3030C (Oribi Gorge), P 2930C (Pietermaritzburg), U 3130A (Umtamvuna). **b** *P. reflexus* (3129D, Port St. Johns). **c** *P. ambiguus*. **d** *P. ecklonii*. Bar: 200 km

teran visitors follows a typical pattern with insects moving up an inflorescence with basipetally maturing flowers, first depositing pollen on stigmas of female stage flowers and picking up pollen from male stage flowers before leaving the inflorescence. Thus geitonogamy within the inflorescences is minimised.

Dipteran and hymenopteran visits to individual flowers last between one and four seconds and result in sternotrobic pollen deposition. Anthophorid bees rest on the lower lip of the corolla while probing the flower for nectar, while nemestrinid and acrocerid flies hover in front of the flower while probing the tube horizontally to reach nectar at the base of the flower. Nemestrinid flies sometimes grasp the filaments and style while probing the flower. All the insects represented in Figs. 3 and 4 pick up pollen from *Plectranthus* ventrally on the abdomen, thorax, bases of the legs and wings or on the head and broad base

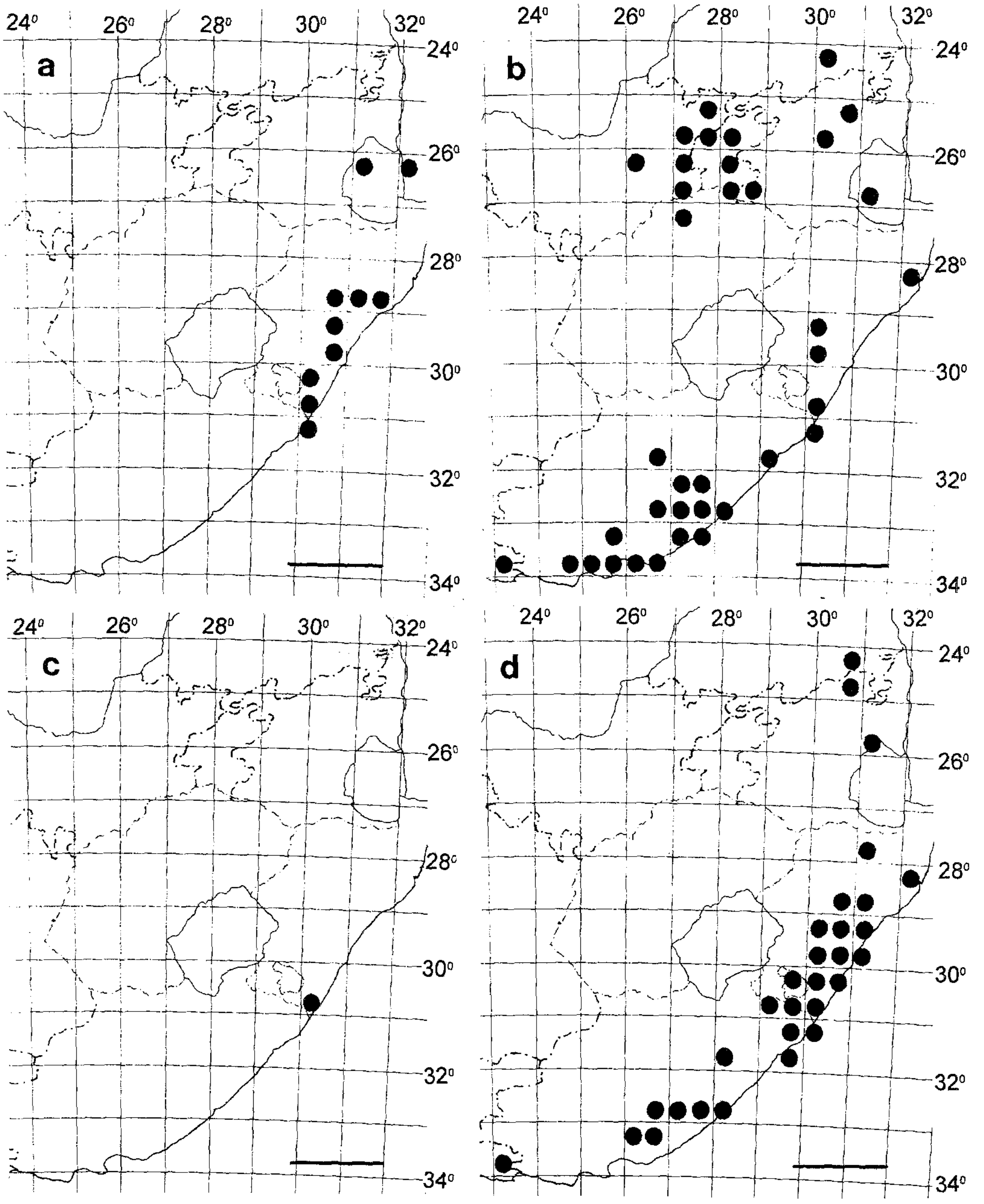

Fig. 2. Distribution of four shorter-tubed species of *Plectranthus*. **a** *P. zuluensis*. **b** *P. madagascariensis*. **c** *P. oribiensis*. **d** *P. ciliatus*. Bar: 200 km

Table 1. Phenology of the seven studied species of *Plectranthus* with months arranged from July to June. *F* flowering

	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	April	May	June
P. ambiguus							F	F	F			
P. hilliardiae						F	F	F	F			
P. ecklonii		F					F	F	F			
P. zuluensis			F	F	F		F	F	F		F	
P. ciliatus					F	F	F	F	F			
P. oribiensis								F	F			
P. madagascariensis	F				F			F	F	F	F	

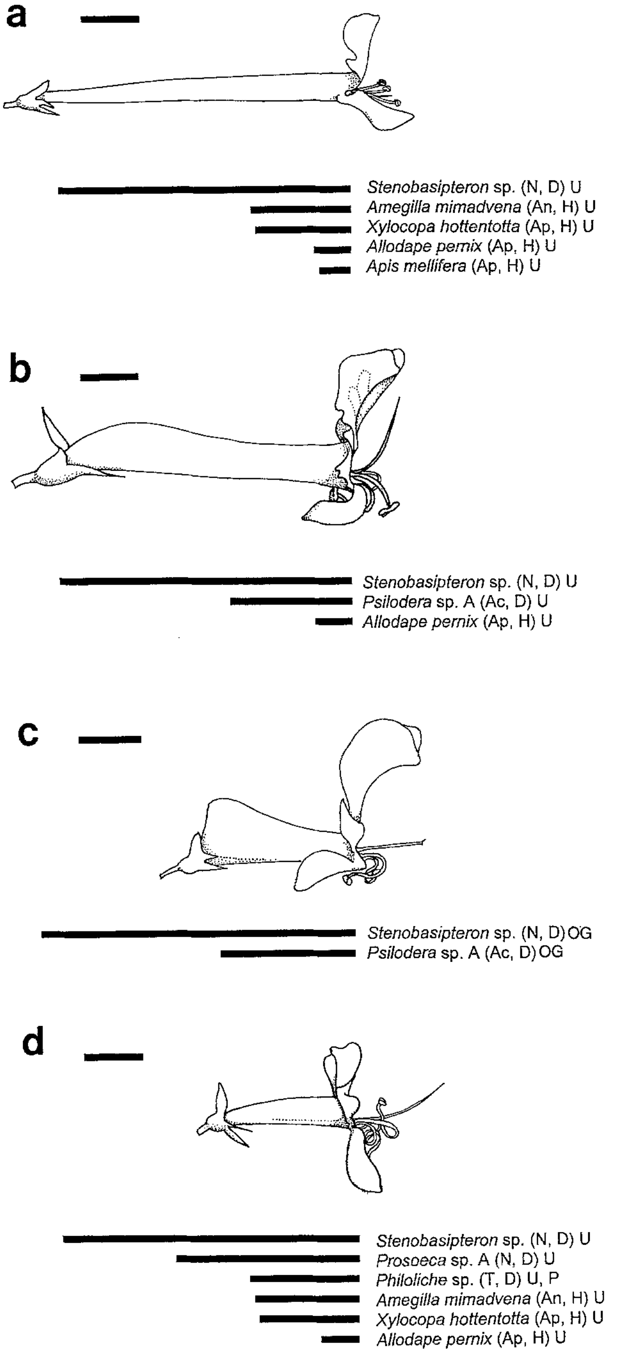

Fig. 3. Comparisons of floral tube length with proboscis lengths of insect visitors to the longer-tubed species of *Plectranthus*. Lines below flower indicate insect proboscis length in relation to floral tube length. **a** *P. ambiguus*, **b** *P. hilliardiae*, **c** *P. zuluensis*, **d** *P. ecklonii*, N *Nemestrinidae*, D *Diptera*, An *Anthophoridae*, H *Hymenoptera*, Ap *Apidae*, Ac *Acroceridae*, T *Tabanidae*; U at Umtamvuna, OG at Oribi Gorge, P at Pietermaritzburg. Bar above flower: 5 mm

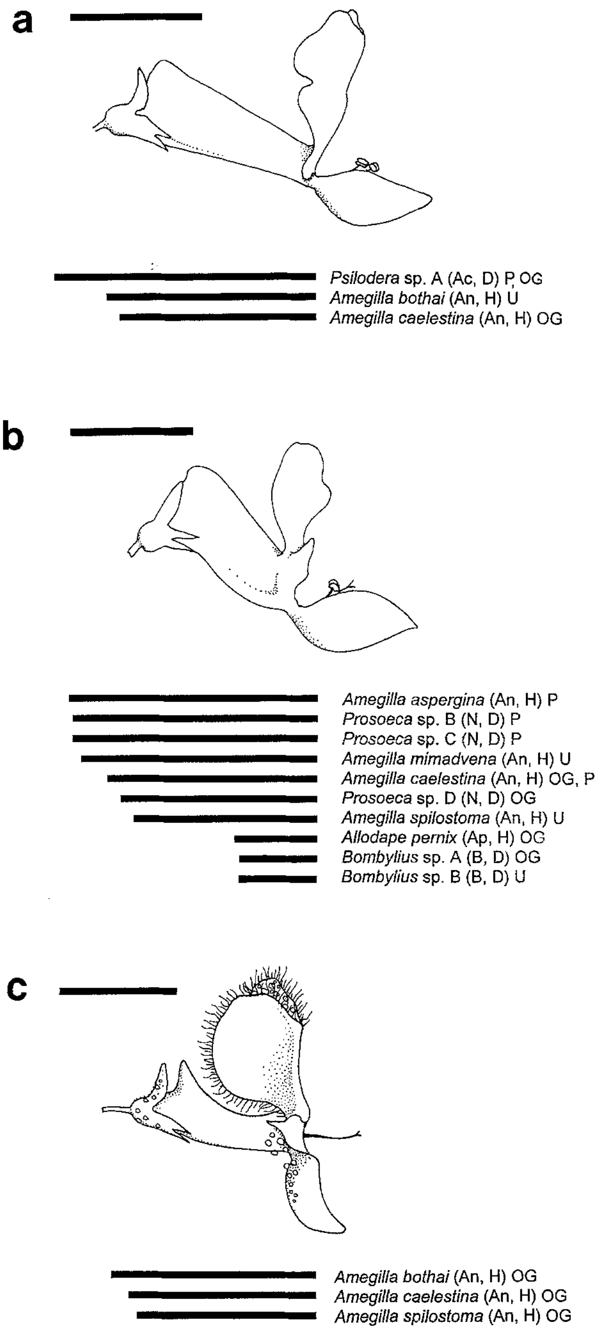

Fig. 4. Comparisons of floral tube length with proboscis lengths of insect visitors to the short-tubed species of *Plectranthus*. Lines below flower indicate insect proboscis length in relation to floral tube length. **a** *P. ciliatus*, **b** *P. madagascariensis*, **c** *P. oribiensis*. An *Anthophoridae*, H *Hymenoptera*, N *Nemestrinidae*, D *Diptera*, Ap *Apidae*, B *Bombyliidae*, Ac *Acroceridae*; U at Umtamvuna, OG at Oribi Gorge, P at Pietermaritzburg. Bar above flower: 5 mm

Table 2. Measurement values (in mm) for floral tube, filament and style lengths of seven species of *Plectranthus* (n = 22, except *P. ciliatus* and *P. zuluensis* where n = 20 and *P. oribiensis* where n = 8). *SD* standard deviation

Species	Corolla tube			Upper filament			Lower filament			Style		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
P. ambiguus	28.1	4.3	20–33	32.2	5.5	22–39	35.1	6.0	23–41	33.2	3.9	26–39
P. hilliardiae	25.7	2.7	21–29	31.2	4.1	25–38	32.7	4.2	27–39	31.9	3.6	25–39
P. ecklonii	12.5	1.5	10–15	20.8	5.4	14–36	23.7	5.8	16–38	27.3	4.3	17–34
P. zuluensis	12.5	0.6	12–13	13.3	0.6	12–15	18.2	0.9	16–20	16.6	1.8	15–20
P. oribiensis	7.4	0.9	7–9	9.4	1.0	8–11	10.7	1.1	9–12	9.6	0.6	9–10
P. ciliatus	7.1	0.7	6–8	10.5	1.2	10–13	11.9	1.1	8–12	11.0	2.3	7–14
P. madagascariensis	5.8	0.5	4–6	9.2	1.3	6–10	10.0	1.2	8–12	9.9	1.2	8–11

of the proboscis. This places pollen in an optimal position to be transferred to the receptive styles of flowers visited subsequently.

Floral tube, filament and style lengths. Values indicating the average and range of floral tube, filament and style lengths are represented in Table 2. *Plectranthus ambiguus* and *P. hilliardiae* have corolla-tubes longer than 25 mm and filaments and styles longer than 30 mm. *Plectranthus ecklonii* and *P. zuluensis* have corolla-tubes of 12.5 mm, but *P. zuluensis* has shorter filaments and styles than *P. ecklonii* where the style length approaches that of the previous two species. In *P. zuluensis* the upper pair of filaments is reduced to staminodes that protrude slightly from the mouth of the corolla. *Plectranthus oribiensis* and *P. ciliatus* have corolla-tubes longer than 7 mm and filaments and styles longer than 9 mm. *Plectranthus madagascariensis* has the shortest corolla-tube of the studied species, with correspondingly shorter filaments and styles.

Insect proboscis lengths. Table 3 presents measurements of proboscis lengths of insect visitors, some of which are represented in Figs. 3 and 4.

Insect identifications. Positive identifications were obtained for most of the *Hymenoptera*, but the *Diptera* (in particular the *Nemestrinidae*) proved problematic. The revision of South African *Nemestrinidae* (Bezzi 1924) appears to have excluded many Natal specimens, making identification below generic level unreliable (Stuckenberg, pers, comm.). The four species of *Prosoeca* (*Nemestrinidae*) will be referred to as species A–D; those of *Bombylius* (*Bombyliidae*), *Allobaccha* (*Syrphidae*) and *Psilodera* (*Acroceridae*) as species A and B respectively for each genus.

Insect visitors. Figures 3–7 show the range of dipteran and hymenopteran visitors and the similarity between corolla-tube and insect proboscis length for the seven species of *Plectranthus*. Observations on *P. reflexus* showed that a *Stenobasipteron* sp. visits this species, but more extensive observations may show that other insect species are also visitors.

Frequency. Nemestrinids (*Diptera*) and anthophorids (*Hymenoptera*) were the principle insect visitors of the studied species of *Plectranthus*. The proportion of visits made by each insect species, excluding pollen and nectar robbers, is represented in Table 4.

Table 3. Length measurements (in mm) of proboscis lengths of insect visitors to *Plectranthus*. *n* number of measurements, *SD* standard deviation

Diptera				*Hymenoptera*			
Taxa	Mean proboscis length (n)	SD	Range	Taxa	Mean proboscis length (n)	SD	Range
Psilodera sp. A	10.6 (7)	1.2	9–12	*Amegilla bothai*	8.6 (5)	0.4	8–9
Bombylius sp. A	3.0 (1)	0	3	*A. caelestina*	8.0 (8)	0.7	7–9
Bombylius sp. B	3.0 (1)	0	3	*A. mimadvena*	9.0 (4)	0	9
Prosoeca sp. A	15.5 (2)	0.7	15–16	*A. aspergina*	9.5 (1)	0	9.5
Prosoeca sp. B	9.3 (3)	1.2	8–10	*A. spilostoma*	7.5 (2)	0.7	7–8
Prosoeca sp. C	9.3 (3)	1.2	8–10	*Allodape pernix*	3.2 (8)	0.5	2.5–4
Prosoeca sp. D	7.0 (1)	0	7	*Apis mellifera*	2.7 (3)	0.3	2.5–3
Stenobasipteron sp.	25.1 (6)	6	22–29	*Xylocopa hottentotta*	8.3 (2)	0.6	8–9
Philoliche sp.	8.7 (3)	3	8–9	*Megachilidae*	3.0 (1)	0	3

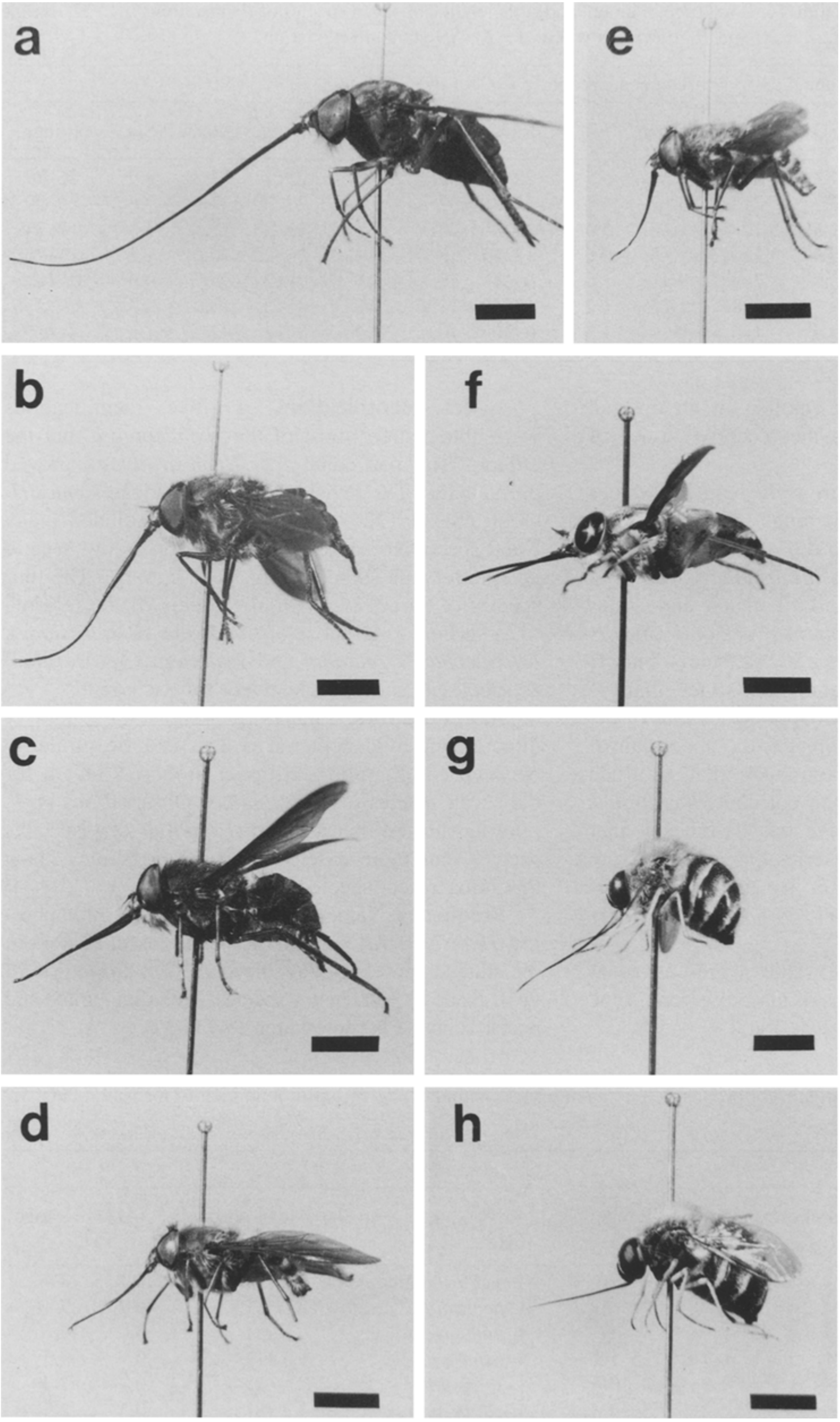

Fig. 5. Eight species of *Diptera* showing the range in body size and proboscis length of flies that visit *Plectranthus* spp. **a–e** *Nemestrinidae*: **a** *Stenobasipteron* sp., **b** *Prosoeca* sp. A, **c** *Prosoeca* sp. B, **d** *Prosoeca* sp. C, **e** *Prosoeca* sp. D; **f** *Tabanidae*: *Philoliche* sp.; **g–h** *Acroceridae*: *Psilodera* sp. A, note variability in proboscis length/body size ratio. Bars: 5 mm

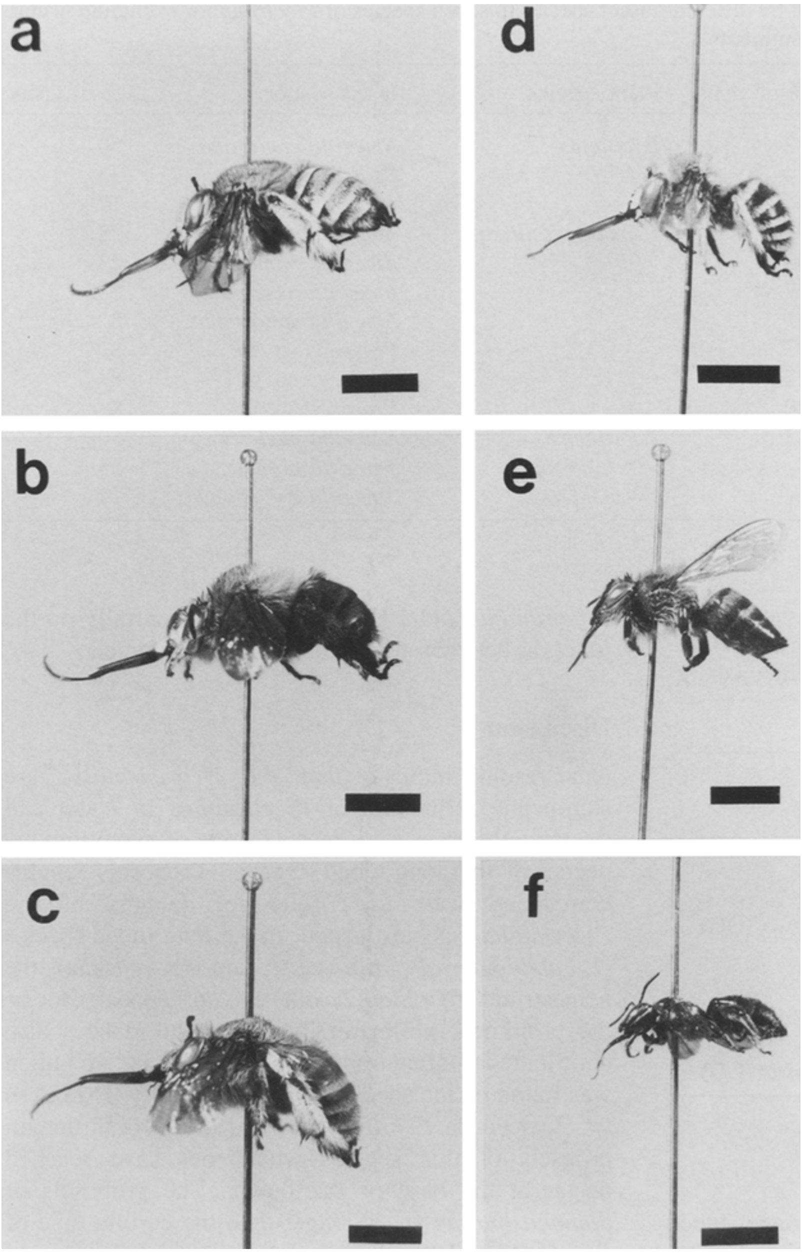

Fig. 6. Six species of *Hymenoptera* showing the range in body size and proboscis length of bees that visit *Plectranthus* spp. **a–d** *Anthophoridae*: **a** *Amegilla caelestina*, **b** *A. mimadvena*, **c** *A. bothai*, **d** *A. spilostoma*; **e–f** *Apidae*: **e** *Apis mellifera*, **f** Allodape pernix. Bars: 5 mm

Pollen loads. *Plectranthus* pollen grains are radially symmetrical and 6-colpate with double-reticulate exine patterns. Pollen grain diameters vary from 19 × 29 µm (*P. ciliatus*) and 23 × 24 µm (*P. madagascariensis*), to 27 × 37 µm (*P. hilliardiae*). The genus is stenopalynous, which makes it difficult to distinguish between pollen of different species, but it is possible to distinguish *Plectranthus* pollen from grains of other plant species.

Specimens of anthophorid bees and nemestrinid files that were caught visiting *P. ambiguus*, *P. ecklonii*, *P. ciliatus P. oribiensis* and *P. madagascariensis* were found to contain more than 90% *Plectranthus* pollen on their bodies. In cases where mixed pollen loads were found the majority of foreign pollen was restricted to the scopae (of female bees) or dorsally on the insect body (of flies). Three species of *Pieridae* and one of *Lycaenidae* were the observed butterfly visitors to *P. madagascariensis*, but no pollen was found on these specimens. The pierids were only abundant towards the end of the main flowering season (May) in 1996.

Table 4. Proportions (frequency) of visits made by different insect species to seven species of *Plectranthus*, excluding nectar- and pollen-robbing species with very short mouthparts

Plant species	Insect visitor	% of visits	Plant species	Insect visitor	% of visits
P. ambiguus	*Stenobasipteron* sp.	72	*P. ciliatus*	*Amegilla caelestina*	50
	Amegilla mimadyena	14		*Psilodera* sp. A	33
	Xylocopa hottentotta	14		*Amegilla bothai*	17
P. hilliardiae	*Stenobasipteron* sp.	67	*P. madagascariensis*	*Amegilla caelestina*	38
	Psilodera sp. A	33		*Amegilla mimadvena*	23
P. zuluensis	*Psilodera* sp. A	67		*Amegilla aspergina*	8
	Stenobasipteron sp.	33		*Amegilla spilostoma*	8
P. ecklonii	*Stenobasipteron* sp.	33		*Prosoeca* sp. B	8
	Amegilla mimadvena	27		*Prosoeca* sp. C	8
	Philoliche sp.	20		*Prosoeca* sp. D	8
	Xylocopa hottentotta	13	*P. oribiensis*	*Amegilla caelestina*	50
	Prosoeca sp. A	7		*Amegilla bothai*	33
				Amegilla spilostoma	17

Table 5. Pollen collecting insect visitors with short or non-sucking mouthparts that are not presented in Figs. 3 and 4. *U* at Umtamvuna, *OG* at Oribi Gorge, *P* at Pietermaritzburg

Insect species	Plant species
Diptera	
Syrphidae	
Allobaccha sp. A	*P. ambiguus* (U) *P. ecklonii* (U)
Allobaccha sp. B	*P. ambiguus* (U)
Episyrphus sp.	*P. madagascariensis* (OG)
Rhingia sp.	*P. ciliatus* (OG)
Acroceridae	
Psilodera sp. B	*P. madagascariensis* (P)
Hymenoptera	
Megachilidae (1 sp.)	*P. madagascariensis* (OG)

Nectar robbing. In three of the longer-tubed species (*P. ambiguus*, *P. hilliardiae* and *P. ecklonii*) a small bee, *Allodape pernix*, was observed to make a hole in the base of the corolla through which nectar is robbed. The proboscis of this bee is 3.2 mm long. These bees are fairly frequent visitors to the flowers and the holes left by the robbers are commonly found. In addition, individuals of *A. pernix* collect pollen from the dehisced anthers.

Pollen-collecting insects. Table 5 lists pollen-collecting *Diptera* and *Hymenoptera* with short mouthparts that are not represented in Figs. 3 and 4. The only specimen that yielded sufficient pollen to allow any assumptions about pollination was that of *Bombylius* sp. B, collected on *P. madagascariensis* form Umtamvuna (Fig. 4b). This specimen had 90% *Plectranthus* and 10% foreign pollen ventrally on the junction between the thorax and the abdomen.

Discussion

Our results indicate that the *Nemestrinidae* are significant pollinators of *Plectranthus* in Natal and are the only observed insects capable of exploiting the nectar in the long-tubed species. Proboscis lengths correspond well to corolla-tube lengths of the *Plectranthus* species visited. In the long-tubed species (*P. ambiguus*, *P. hilliardiae* and *P. reflexus*) the nemestrinid fly, *Stenobasipteron* sp. appears to be the prominent pollinator, but anthophorid bees also contribute to pollen transfer in *P. ambiguus*. No pollen was found on the specimen of acrocerid fly (*Psilodera* sp. A) seen on *P. hilliardiae*, and the medium-length probscis of this species would not have reached nectar at the base of the flower. The proboscis of *Stenobasipteron* sp. is longer than the corolla tube of *P. ecklonii* and *P. zuluensis*, but the extended style and filaments are grasped by the insect during feeding, enhancing sternotrobic pollen transfer. The shorter corolla tubes in the latter two species allow insects with shorter proboscides to effect pollination as well. Thus *Psilodera* sp. A accounts for the majority of visits to *P. zuluensis* (Figs. 3c, 7b) and *Prosoeca* sp. A frequently visits *P. ecklonii* (Fig. 3d). The tabanid fly and anthophorid bee species that visit *P. ecklonii* also have proboscis lengths comparable to that of the corolla-tube (Fig. 3d).

The shorter-tubed species rely predominantly on anthophorid bees and nemestrinid flies of the genus *Prosoeca* for pollination. In *P. ciliatus*, *P. oribiensis* and *P. madagascariensis* the proboscis lengths of

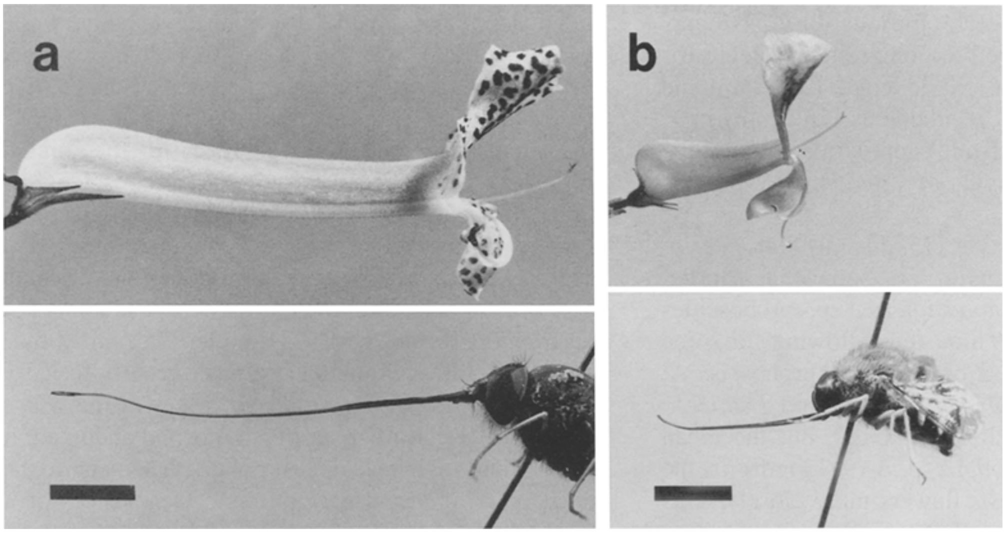

Fig. 7. Comparisons of floral tube length with proboscis length of visiting dipterans for two species of *Plectranthus*. **a** *P. hilliardiae* above with *Stenobasipteron* sp. below, **b** *P. zuluensis* above with *Psilodera* sp. A below; note the close correlation between proboscis and floral tube length. Bars: 5 mm

visiting bees and flies in most cases correspond well with that of the corolla-tube, style and filaments of the *Plectranthus* species in question (Fig. 4). Insects with shorter mouthparts tend to concentrate on pollen collection.

Pollen loads. Voucher specimens of anthophorid bees and nemestrinid and other flies that yielded a high percentage of *Plectranthus* pollen show that although pollen transfer is achieved for *Plectranthus*, these vectors are not exclusive to the genus. A few observations of vectors moving between *Plectranthus* flowers and those of other genera, e.g. *Lobelia* spp. (*Campanulaceae*) and *Isoglossa* spp. (*Acanthaceae*), have been made and this is supported by the placement of foreign pollen in patches inaccessible to *Plectranthus* stigmas on some insect specimens. It is difficult to distinguish between pollen grains from different species of *Plectranthus*, but it is seldom that more than two species grow in close proximity. Differences in filament and style lengths place pollen in different areas on the insect body, thus isolating pollen from different *Plectranthus* species. The lack of pollen on the voucher specimens of *Lepidoptera* suggests that butterflies are not important polinators of *P. madagascariensis*. The *Pieridae* were present of a limited period of the flowering season and were seldom encountered.

Nectar robbing. The proboscis of *Allodape pernix* is too short to reach nectar legitimately, hence the behaviour of robbing nectar through a hole pierced at the base of the corolla tube. This corresponds to primary nectar robbing according to the system suggested by Inouye (1980). Glandular trichomes are abundant on the calyces of *Plectranthus* and in other members of the *Lamiaceae* have been shown to secrete essential oils that have insecticidal properties (see Levin 1973 and Konstantopoulou et al. 1992). Individuals of *A. pernix* do not appear to be deterred by these oils, as it is the corolla tissue, containing fewer terpenoid-containing trichomes, that is pierced above the calyx and nectary.

The generally small body size, short proboscis length and pollen-collecting habit of the insects listed in Table 5 make them unlikely primary pollinators of *Plectranthus*. In his account on floral larceny Inouye (1980) refers to pollen theft which is the collection of pollen directly from the anthers by small bees, without contacting the stigma. This is probably the case for these species and that of the nectar robber *A. pernix*, but the possiblility that some contribution is made to intra-specific pollen transfer cannot be discounted.

Comparison of tube length with proboscis length. This study records several new groups of visitors to *Plectranthus*: flies of the families *Nemestrinidae*, *Acroceridae* and *Tabanidae*, and lepidopterans of the families *Lycaenidae* and *Pieridae*. The occurrence of pollination by long-tongued flies (primarily of the family *Nemestrinidae*, but also one species of *Tabanidae*) is confirmed for both longer- and shorter-tubed species of *Plectranthus*. Species with intermediate tube length (*P. ecklonii* and *P. zuluensis*) allow for visits by shorter-tongued insects –

anthophorid bees, acrocerid flies or *Prosoeca* app. (*Nemestrinidae*) – as well as long-tongued nemestrinids (*Stenobasipteron* sp.). Style length (27.3 mm) and that of the anterior pair of filaments (23.7 mm) in *P. ecklonii* (tube length 12.5 mm) approximate that of the average proboscis length of *Stenobasipteron* sp. (25.1 mm).

In *P. zuluensis* the style (15–20 mm) and anterior filament (16–20 mm) lengths are somewhat shorter, but the lengths of *Stenobasipteron* sp. proboscides range from 22 mm to 29 mm, thus allowing for some pollen carryover via the base of the proboscis. At Oribi Gorge both *Stenobasipteron* sp. and *Psilodera* sp. A (*Acroceridae*) visit *P. zuluensis*, but the mean proboscis length of *Psilodera* sp. A (9–12 mm) fits the dimensions of *P. zuluensis* flowers more closely. This species visits populations of *P. ciliatus* at Oribi Gorge as well as in Pietermaritzburg and observations of its behaviour suggest it to be an important pollinator of a number of other plant species. *Psilodera* sp. A tends to be restricted to forest and forest margins, while *Prosoeca* spp. prefer open grassland and patches bordering forest margins.

Evolution of long corollas. No specimens of *Stenobasipteron* sp. have been seen visiting *Plectranthus* in Pietermaritzburg, suggesting its replacement by shorter-tongued *Prosoeca* species in this region. One could speculate that corolla-tube length in the Natal species of *Plectranthus* has evolved in response to the proboscis length of visiting insects. A similar case was suggested by Johnson and Steiner (1997) for the adaptation of spur length in the *Disa draconis* (L. F.) Sw. species complex, except that the flowers of this species are non-rewarding, thus discounting evolution of fly proboscis length in response to corolla-tube length. For *Plectranthus* it is possible that there has been a coevolutionary process between flower and fly.

Through experimental testing of Darwin's hypothesis regarding the evolution of flower depth, Nilsson (1988) confirmed that long-tongued pollinating vectors can drive the evolution of longer floral tubes. This may be true in the cases of *P. ambiguus* (average tube length 28.1 mm) and *P. hilliardiae* (average tube length 25.7 mm) that are visited by individuals of *Stenobasipteron* sp. (average proboscis length 24.1 mm), as well as for *P. reflexus* (tube length 28–30 mm). Frequency of insect visits also shows that the long–tubed *P. hilliardiae* and *P. ambiguus* are visited most often by this long-tongued fly species, while this species only accounts for a third of insect visits to the 'medium-tubed' *P. zuluensis* and *P. ecklonii*. Slightly longer corolla tubes will encourage visiting insects to throust their heads deeper into the flower, thus ensuring pollen deposition on the insect head or body, while slightly shorter tubes still allow for pollen-carryover due to the exserted style and filaments (longer than 30 mm for three of these species).

Pollen placement. In contrast to the orchid pollinaria found attached to the proboscides of nemestrinids and tabanids in studies by Johnson and Steiner (1995, 1997), very few pollen grains were found on the thin parts of proboscides of the dipteran specimens examined in the current study. Members of the *Lamiaceae* do not shed pollen in units, thus individual pollen grains adhere more readily to hairy areas at the base of the proboscis, bases of the wings and legs and ventrally over the thorax and abdomen. It appears that, for *Plectranthus*, pollen placement on the actual body of the insect optimises the chances of it being transferred to the style of a subsequent species. Similar areas of pollen placement were found on the long-proboscid flies that visit *Pelargonium* (*Geraniaceae*), *Geissorhiza*, *Hesperantha* and *Ixia* (all *Iridaceae*) in studies by Manning and Goldblatt (1996, 1997) and Johnson and Steiner (1997).

Lamiaceae. The current study provides the first evidence of long-tongued fly pollination in the *Lamiaceae*. The floral features of the long-tubed species of *Plectranthus* correspond to many of those reported for this pollination syndrome, with some variations on the theme. Flower colours range from white to purple and contrasting nectar guides are positioned on the upper or both corolla limbs. The nectar guides on *P. ambiguus* are only feint lines and in *P. reflexus* and the long-tubed variety of *P. saccatus* there are no contrasting nectar guides visible to the human eye. Nevertheless the latter two species would fit in with the '*Stenobasipteron*' syndrome on account of flower colour and corolla-tube length.

In addition, the importance of flies with 'medium tongue lengths' should be emphasised. The observed species of *Prosoeca* (*Nemestrinidae*), *Philoliche* (*Tabanidae*) and *Psilodera* (*Acroceridae*) do not have exceptionally long proboscis lengths but, together with anthophorid bees, play a significant role in the pollination of shorter-tubed *Plectranthus* species. It is hypothesised that these insects, especially the acrocerid flies at Oribi Gorge, are the pollinators of shorter-tubed species of *Plectranthus* that occur in the same area, such as *P. oertendahlii* (tube length 8–13 mm), *P. ernstii* (tube length 4–8 mm), *P. fruticosus* L'Hérit. (tube length 5–13 mm) and the shorter-tubed variety of *P. saccatus* Benth. (tube length 8–16 mm) for which no insect visitors have yet been observed (tube length values from Codd 1985b).

Implications. This study and other documenting pollination by long-tongued flies highlight the importance of conserving sufficiently large areas of natural vegetation in order to support pollination systems. Little detail is known about the life cycles of these flies, except that their larvalstages are parasitic on egg pods of orthoperans (in *Nemestrinidae*) and arachnids (in *Acroceridae*). Those flower-feeding species with adults that have proboscides that are highly modified, elongate and specialised for feeding on nectar from long-tubed flowers, such as the nemestrinids documented in this study, require 'established complex biocoenoses' in order to survive (Bowden 1978). Bowden (1978) noted that the relations between flowers and *Diptera* merit more attention than they have received and that the distribution of these flies may be driven by adult biology where the adults are flower visitors. The documentation of these specialised flower-fly relationships in the Cape and Natal flora is addressing the need for these studies, but more information is needed on the life histories of the flies and the distribution of this syndrome.

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Appendix 1. List of insect visitors collected during the study (lodged at Natal Museum Pietermaritzburg).

- *Diptera*
  - *Acroceridae*
    - *Psilodera*-2 spp.
  - *Bombyliidae*
    - *Bombylius*-2 spp.
  - *Nemestrinidae*
    - *Prosoeca*-4 spp.
    - *Stenobasipteron* sp.
  - *Syrphidae*
    - *Allobaccha*-2 spp.
    - *Episyrphus* sp.
    - *Rhingia* sp.
  - *Tabanidae*
    - *Philoliche* sp.
- *Hymenoptera*
  - *Anthophoridae*
    - *Amegilla* (*Aframegilla*) *bothai*
    - *A.* (*Aframegilla*) *caelestina*
    - *A.* (*Aframegilla*) *mimadvena*
    - *A.* (*Zebramegilla*) *aspergina*
    - *A.* (*Zebramegilla*) *spilostoma*
  - *Apidae*
    - *Allodape pernix*
    - *Apis mellifera*
    - *Xylocopa hottentotta*
  - *Megachilidae*-1 sp.
- *Lepidoptera*
  - *Lycaenidae*-1 sp.
  - *Pieridae*-3 spp.

Appendix 2. List of plant voucher specimens (lodged at NU).

Plectranthus ambiguus Potgiester 86
P. ciliatus Potgieter 67
P. ciliatus Potgieter 68
P. ciliatus Potgieter 69
P. ciliatus Potgieter 116
P. ciliatus Potgieter 136
P. ecklonii Potgieter 65
P. ecklonii Potgieter 66
P. ecklonii Potgieter 70
P. ecklonii Potgieter 114
P. hilliardiae Potgieter 110
P. hilliardiae Potgieter 111
P. hilliardiae Potgieter 112
P. madagascariensis Potgieter 89
P. madagascariensis Potgieter 90
P. madagascariensis Potgieter 91
P. madagascariensis Potgieter 94
P. oribiensis Potgieter 102
P. saccatus var. *longitubus* Potgieter 107
P. saccatus var. *longitubus* Potgieter 120
P. saccatus var. *saccatus* Potgieter 58
P. saccatus var. *saccatus* Potgieter 103
P. saccatus var. *saccatus* Potgieter 106
P. saccatus var. *saccatus* Potgieter 108
P. saccatus var. *saccatus* Potgieter 131
P. zuluensis Potgieter 64
P. zuluensis Potgieter 118

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CHAPTER 3:
POLLINATION OF SIGMOID-TUBED SPECIES

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Pollination of *Plectranthus* spp. (Lamiaceae) with sigmoid flowers in southern Africa

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Abstract

Within the South African *Plectranthus* species two specialized lines of corolla adaptations have evolved. Long-proboscid flies (Nemestrinidae) appear to have driven the development of *Plectranthus* species with long corolla tubes that are limited to the sub-continent. *Plectranthus* s.l. (including *Coleus*) species with sigmoid corollas are far more widespread and evidence presented here supports the hypothesis that this floral type has evolved as a response to melittophily. Thirty percent of southern African *Plectranthus* species have corolla tubes that are bent to some degree. Pollination of the following four labiate species with sigmoid corollas was studied in detail: *Plectranthus petiolaris*, *P. laxiflorus*, *P. calycinus* and *Pycnostachys urticifolia*. The pollination of three other species was investigated to a lesser degree: *Plectranthus spicatus*, *P. rehmannii* and *Aeollanthus parvifolius*. Bee pollination is confirmed for *P. laxiflorus* and *Py. urticifolia* and is recorded here for the first time in *P. petiolaris* and *P. calycinus*. A new group of floral visitors comprising nemestrinid flies of the genus *Prosoeca* with moderately long proboscids is recorded for *P. laxiflorus* and *P. calycinus*, where the corolla tube shape allows visits by medium-proboscid floral visitors. The sigmoid corolla shape limits the type and size of insects that can access nectar and act as pollinators. Explanations for the existence and function of the sigmoid corolla shape are suggested.

Keywords: Apidae; Anthophorinae; Lamiaceae; Nemestrinidae; *Plectranthus*; Pollination; Sigmoid corolla; Southern Africa

1. Introduction

Plectranthus (Lamiaceae), a member of subfamily Nepetoideae, tribe Ocimeae, subtribe Plectranthinae (Paton et al., 2004), comprises ±300 species that occur in the tropical and warm regions of the Old World (Retief, 2000). It is the largest genus of the Lamiaceae in southern Africa, represented by ca. 53 species (Codd, 1975, 1985; Van Jaarsveld and Edwards, 1991, 1997; Van Jaarsveld and Hankey, 1997; Edwards et al., 2000; Van Jaarsveld and Van Wyk, 2004; Edwards, 2005; Winter and Van Jaarsveld, 2005). Recent evidence (Paton et al., 2004) suggests that the genus is paraphyletic as currently circumscribed.

In an overview of pollination biology in the Lamiaceae, Huck (1992) commented on the large gaps that exist in our knowledge and recommended that pollination studies focus on species *in situ*. In view of the large size of the genus we identified it as an ideal group for pollination studies. The pollination of long-tubed *Plectranthus* species by long-proboscid flies was first reported by Potgieter et al. (1999), along with the pollination of a number of medium- and short-tubed species of the genus by bees and flies with medium- to short proboscids. The discussion surrounding long-tubed species of *Plectranthus* was extended to the rest of the Lamiaceae in southern Africa by relating the distribution of long-tubed Lamiaceae to the biogeography of long-proboscid flies (Potgieter and Edwards, 2001). Pollination of Lamiaceae with corollas of intermediate length, by pollinators with intermediate length proboscids, may have predisposed the group to

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pollination by long-proboscid flies, with subsequent extension of corolla tubes. Elongation of corolla tubes leads to increased protection of nectar resources, which leads to increased pollinator fidelity (Potgieter and Edwards, 2001). Publication of the *Stenobasipteron wiedemanni* (Diptera, Nemestrinidae) Pollination Guild (Potgieter and Edwards, 2005) established the multi-family plant guild to which long-tubed species of *Plectranthus* (with tube lengths of 20–33 mm) belong.

Both the specialized long-proboscid fly pollinated and more generalized shorter-proboscid fly and bee pollination guilds that have been studied within southern African *Plectranthus* (Potgieter et al., 1999; Potgieter and Edwards, 2001, 2005) involve species with relatively straight corolla tubes. A new pollination guild is described here for species with sigmoid-shaped corollas.

Initial phylogenetic work by Paton et al. (2004), supplemented by Lukhoba et al. (2006), creates a framework for phylogenetic interpretation of *Plectranthus* and its allies. Subsequent work shows that there are three main clades in the genus *Plectranthus*, but the relationships between them are unresolved (A. Paton, pers. comm.). They are: 1) a sigmoid '*Coleus*' clade, including *P. rehmannii* Gürke and *P. calycinus* Benth., possibly *P. spicatus* E. Mey. ex Benth,. and *Pycnostachys urticifolia* Hook.; 2) a sigmoid *Plectranthus* clade, including *P. petiolaris* E.Mey. ex Benth. and *P. laxiflorus* Benth.; and 3) a straight *Plectranthus* clade. The genus *Aeollanthus* Mart. ex K.Spreng is placed near the base of the Plectranthinae (A. Paton, pers. comm.). The definition of sigmoid corollas by Codd (1985) included corollas with varying degrees of geniculation of the tube, and for the purpose of this paper the term 'sigmoid' is used in a loose sense. Within southern African *Plectranthus* 30% of species have evolved short to medium sigmoid corollas similar to those in *Solenostemon* Thonn., *Aeollanthus* and *Pycnostachys* Hook. These three genera all fall within the broader generic circumscription of *Plectranthus* proposed by Paton et al. (2004).

The bulk of this paper deals with four species that were studied in detail: *P. petiolaris* and *P. laxiflorus*, with sigmoid tubes bent to a similar degree; *P. calycinus* Benth. [=*Rabdosiella calycina* (Benth.) Codd], with weakly bent corollas, and *Pycnostachys urticifolia* Hook., with distinctly bent corollas. Three other species were studied in less detail, but are recorded and discussed since they fall within the same syndrome: *P. spicatus* (with distinctly bent corollas), *P. rehmannii* (with weakly bent corollas), and *Aeollanthus parvifolius* Benth. (with curved corolla tubes).

During the course of the study it was noted that a site in Pietermaritzburg with extensive stands of *P. laxiflorus* showed a temporal separation of pollinator classes during each flowering season. At first only bee pollinators were present, but nemestrinid flies emerged *en masse* at the end of March/early April each year, with bees and flies actively visiting flowers for the rest of the season. An investigation on the effectiveness of the two pollinator types was included in the study.

2. Literature on sigmoid Lamiaceae

In a discussion on corolla adaptations in the Lamiaceae, Meeuse (1992) noted two important aspects: the size of specialized corollas and the resistance offered to insect visitors attempting to access nectar. The upper corolla lip of *Plectranthus* is erect and does not hamper access, whereas the lower lip allows for easy landing, thus some kind of barrier in the tube would function as a selective device (Meeuse, 1992). Van der Pijl (1972) considered why some *Plectranthus* species, as a transition to *Coleus*, should have 'geniculate' corolla tubes. One suggestion was that the bend may be a mechanical necessity for a horizontal flag-type blossom (with an upright upper limb) to combine with the long vertical tube that already exists in this group (Van der Pijl, 1972). Another is that sigmoid corollas provide an effective shift from butterfly to bee pollination without major changes in tube dimensions (Van der Pijl, 1972), possibly since the sigmoid shape may allow bees better access to nectar than in a long-tubed, straight corolla.

There are no published records on the pollination of *P. petiolaris* and only one for *P. laxiflorus* (Scott Elliot, 1891). In *P. calycinus* the sides of the lower lip are bent upwards, giving it a boat-like appearance, enclosing the stamens and style. As an insect visitor forces the lower lip down the stamens are exposed, dusting pollen onto the insect. In both *P. calycinus* and *P. laxiflorus* this action requires considerable force from the insect (Scott Elliot, 1891) and the corolla shape of *P. petiolaris* suggests that a similar system operates in this species. Van der Pijl (1972) mentioned the bee-blossoms of *P. laxiflorus* and tried to explain their sigmoid corolla shape. It was first suggested that the bend in the corolla tube may have functioned in excluding bee visitors while fitting a lepidopteran proboscis, yet the fused filaments and hinged carina suggests bee visitors, or a regression to melittophily (bee pollination). Vogel (1954) listed *Plectranthus* under melittophily and sphingophily (moth pollination), while *Pycnostachys* was placed under melittophily. Percival (1965) discussed how bees with abdominal brushes (the Dasygastrae) exploit sternotrobic flowers, such as *Py. urticifolia*, by collecting pollen ventrally on the abdomen. She mentioned that the open corolla mouth of the labiates did not hinder bees from probing, but that visitors were stratified according to tube length.

Stirton (1977) described the insect visitors to cultivated plants of the South African species *Plectranthus neochilus* Schltr. The corolla of this species has a narrow tube (15–18 mm long) that ascends, then bends knee-like and expands about the middle (Codd, 1985). Although the corolla tube is not bent to the same extent as found in *P. petiolaris* and *P. laxiflorus*, *P. neochilus* makes for an interesting comparison. Stirton (1977) found five species of *Megachile*, three *Xylocopa* species, one species of *Anthophora* (now genus *Amegilla*) and *Apis mellifera* (all Hymenoptera, Apidae) to be effective pollinators. The bees landed on the boat-shaped lower lip, depressed it and exposed the stigma and stamens which transferred pollen ventrally onto the insects (Stirton, 1977). This study also listed unidentified bombyliids (Diptera: Bombyliidae), one syrphid species (Diptera: Syrphidae) and a sphingid moth, *Macroglossum trochilus* (Lepidoptera: Sphingidae), as ineffective visitors.

Paton et al. (2004) noted that the sigmoid tubes of the *Coleus* clade and the sigmoid *Plectranthus* clade (*P. laxiflorus* and *P. petiolaris*) always combine with a horizontal lower (anterior) corolla lobe, a combination which they believed would favour landing insects with flexible proboscids.

3. Materials and methods

All seven plant species were studied in KwaZulu-Natal (KZN), with some work done in the Eastern Cape Province of South Africa (Fig. 1a). Field work was conducted in various localities from 1995 to 2009 (see Appendix 1 for study site, year of study and voucher details). Plant species were identified using Codd (1985), as well as the texts listed in the introduction, for species described subsequent to Codd's (1985) revision.

Plant distributions were compiled from flora accounts (Codd, 1985), herbarium records (NU, NH and PRE) and field observations. Pollinator observations were made across a number

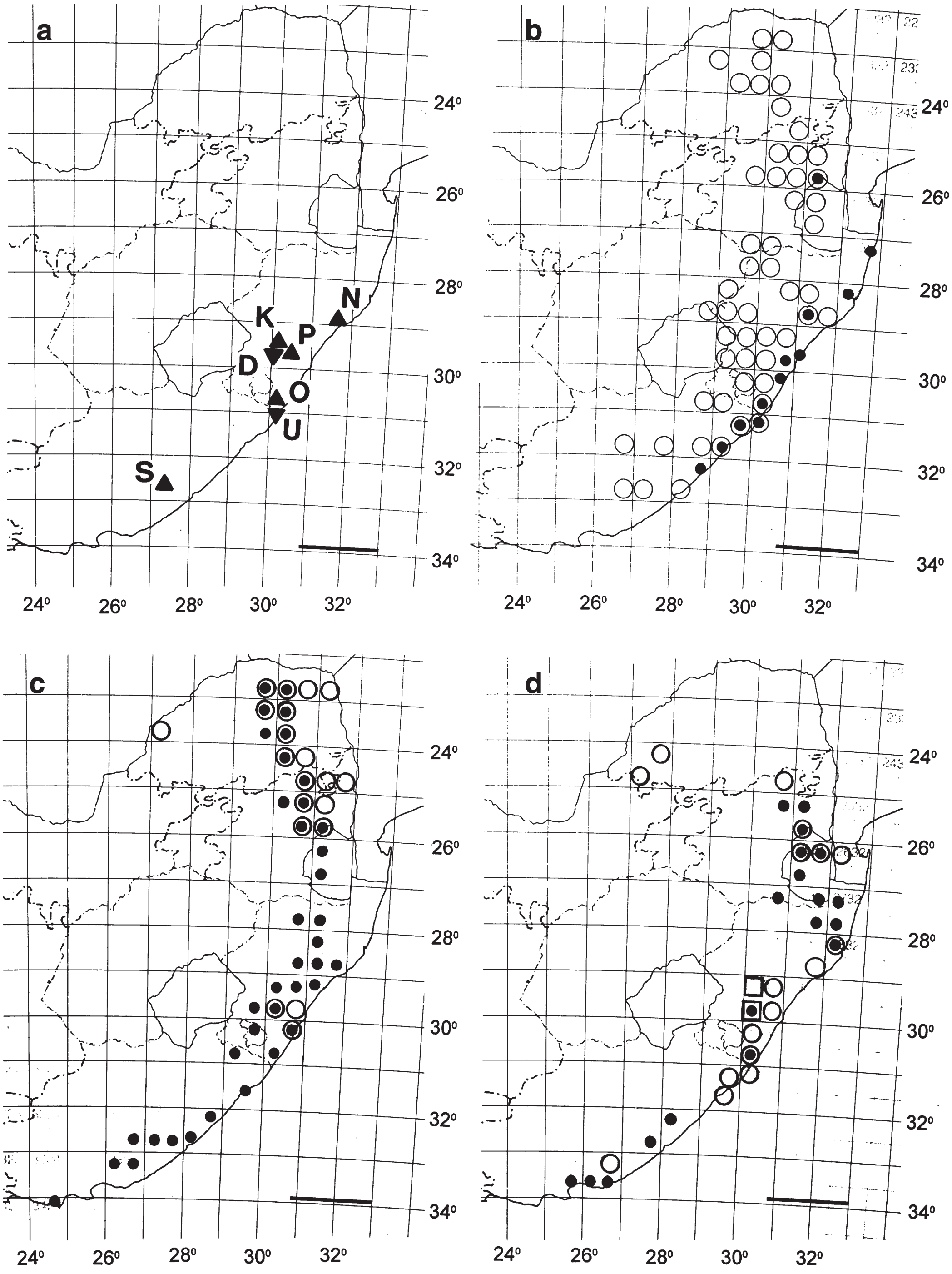


Fig. 1. Distribution maps for study species and field study sites: (a) Study sites in eastern South Africa; (b) distribution of *P. petiolaris* (solid circles) and *P. calycinus* (open circles); (c) distribution of *P. laxiflorus* (solid circles) and *Py. urticifolia* (open circles); (d) distribution of *P. spicatus* (closed circles), *A. parvifolius* (open circles) and *P. rehmanii* (open squares). S = Stutterheim–Kologha Forest, U = Umtamvuna N.R., O = Oribi Gorge N.R., D = Dargle, P = Pietermaritzburg, K = Karkloof–Leopards' Bush N.R., N = Ngoye Forest. Bar: 200 km.

of flowering seasons, between 7.00 am and 6.00 pm. Insects that visited study flowers were netted and asphyxiated in separate glass pill vials to prevent pollen transfer between specimens. Vials were prepared by compressing absorbent paper into the base of each vial and saturating it with ethyl-acetate. Bees and flies were pinned with proboscids extended forward.

Insect voucher specimens are lodged with the Natal Museum in Pietermaritzburg (Diptera) and with the Biosystematics Division, Plant Protection Research Institute, Pretoria (Hymenoptera). Hymenoptera were classified according to Brothers (1999). Plant vouchers are housed at the Bews Herbarium, University of KwaZulu-Natal (NU) (Appendix 1).

Areas of pollen deposition on insects were determined using a dissecting microscope. Initially pollen samples were removed with double-sided tape for Scanning Electron Microscopy (SEM). Stubs were coated with gold-palladium and examined under a Hitachi S570 scanning electron microscope at an accelerating voltage of 10 kV. Pollen grains of *Plectranthus* and *Pycnostachys* species are morphologically similar, with 6-colpate grains and reticulate exine patterns, and can be distinguished from pollen grains of other plant families.

Percentages of *Plectranthus* to total pollen loads were estimated. For *Pycnostachys*, representative voucher insects were killed and examined, but subsequent specimens were caught and cooled to allow handling. Tiny cubes of Fuchsin gel were used to remove pollen from different areas on the insect body, placed on separate slides and gently heated to make semi-permanent mounts. Insects were marked with tiny drops of white correction fluid on the thorax to avoid re-examination if captured subsequently. Slides were examined with a compound light microscope to determine the percentage *Pycnostachys* pollen present. The Fuchsin gel technique was followed in later years.

Proboscis length measurements were made from the tip of the proboscis to the point where the proboscis attaches to the head of the insect. In the case of bees this measurement was divided into the length of the solid base of the proboscis (galea) including the clypeus, and the protruding flexible part (glossa).

Corolla tube lengths were measured using a fine piece of wire bent in the shape of the sigmoid corolla, extending from the corolla base to the point where the upper and lower corolla lips diverge. The wire was then straightened and measured. Filament and style measurements include the entire, functional length of the corolla, although filaments attach to the corolla for some distance. Measurements were made from fresh, preserved and pressed plant specimens.

Nectar levels were recorded in *P. petiolaris* by comparing the height of the nectar column from the base of the corolla, with the distance from the base to the bend in the corolla. This was measured in unvisited flowers, early in the morning (at 9.00 am) and again at 2.00 pm (after many bee visits on a sunny day). The ratio of nectar column to 'base-to-bend' distance was compared between the morning and the afternoon.

To gauge the relative efficiency of apinid bees and nemestrinid flies in pollinating *P. laxiflorus* at Ferncliff Nature Reserve (NR), Pietermaritzburg, fruit set was recorded for two intervals: before (bees only) and after fly emergence (bees and flies). As soon as flies emerged in 2003, the position of the most recently opened verticil of flowers was marked on each of 16 inflorescences. At the end of the flowering season the resulting infructescences were collected. To ensure a suitable time lapse between observed and actual fly emergence, fruit set was only recorded from verticils three rows below the mark on each inflorescence (to represent 'bee only' visitation) and then from the verticil above the mark (to represent 'bee and fly' visitation). Fertilisation rate was calculated by comparing actual fruit set (no. of swollen calyces) with potential fruit set (no. of pedicels with and without calyces, representing no. of flowers).

By accessing unpublished data on other straight-tubed species of *Plectranthus*, combined with data on sigmoid species, twenty species were first grouped by corolla shape and length and then graded by reliance on shaded (forest) through to sunny habitat. The proportion of visits received by effective fly pollinators versus bee pollinators was estimated for each species, using pollinator observation and voucher data. Flies included the families Nemestrinidae, Tabanidae and Acroceridae; bees included the sub-families Apinae and Megachilinae of the family Apidae.

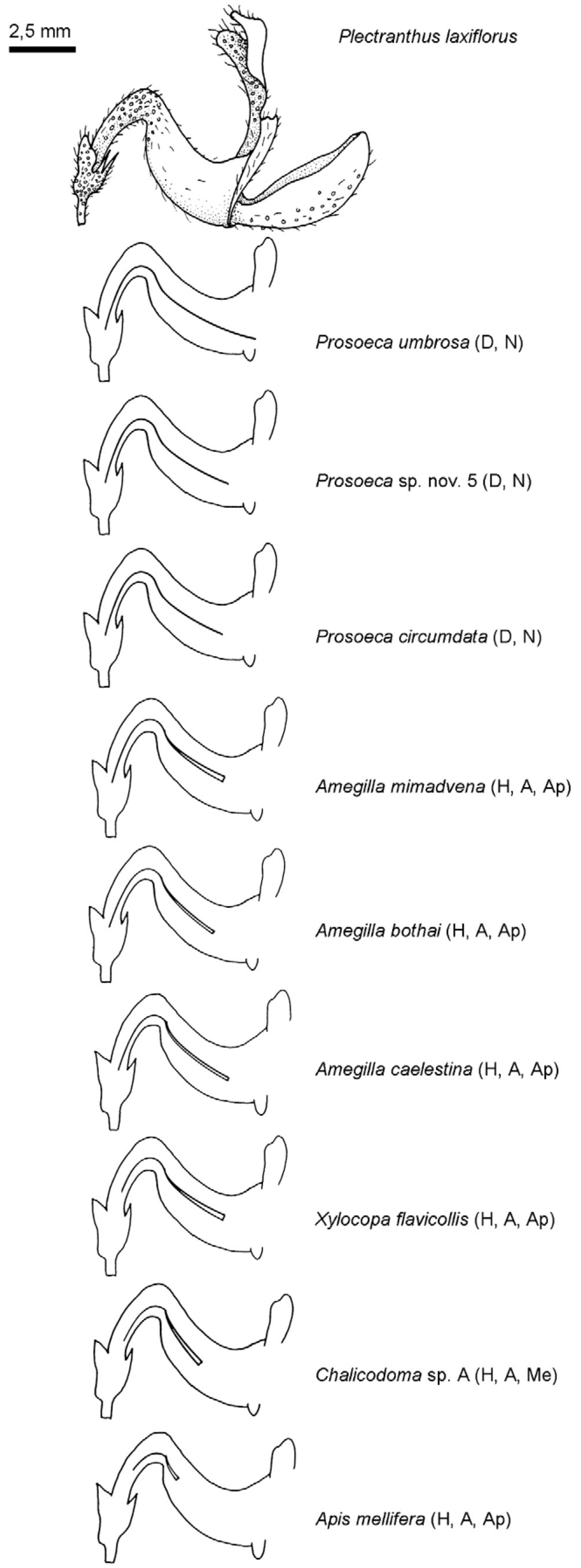
4. Results

The study species are more or less widely distributed along the eastern seaboard of southern Africa, with more than one species often co-occurring and one species (*P. rehmannii*) endemic to the KZN Midlands (Fig. 1b–d; Appendix 1). The habit, floral characteristics and habitat varies between species, but all flower in late summer to autumn (see Table 1).

Two floral subtypes were represented (see Table 2): flowers up to 11 mm long, with narrow corolla bases: *P. laxiflorus* (Figs. 2, 7c), *P. petiolaris* (Figs. 3, 7a) and *Py. urticifolia* (Figs. 5, 7b); and smaller flowers less than 7 mm long, with saccate bases: *P. calycinus* (Fig. 4). In *Py. urticifolia* the filaments are fused for a few millimetres beyond the corolla tube, enclosing the style in a rigid sheath that provides mechanical support once the bent filaments and style elongate and straighten after anthesis (Fig. 7b).

Sigmoid *Plectranthus* flowers have tubular corollas and are zygomorphic, with the stigma and four anthers enclosed by the lower lip (Fig. 7a–d). Flowers are protandrous, with anther dehiscence in the male phase followed by an extension of the style, bringing the stigma upwards into a position previously occupied by the anthers. The style is smooth and generally pin-like in appearance, with closely appressed bifid stigmatic lobes which open to reveal inner receptive surfaces during the female phase (Fig. 7c). The flowers are herkogamous, with the filaments and anthers dropping down into the boat-shaped lower lip of the flower during the female phase. In some of the non-sigmoid *Plectranthus* species with long filaments (e.g. *P. ecklonii* Benth.) the anthers and filaments curl away sideways after dehiscence (Potgieter et al., 1999).

The upright upper limbs of the bilabiate flowers are held vertically in all studied species and function in advertising nectar, which is secreted by a nectariferous disc around the ovary at the base of the corolla; nectar guides, when present, are most often located on the inner or adaxial surface of the upper corolla limb.



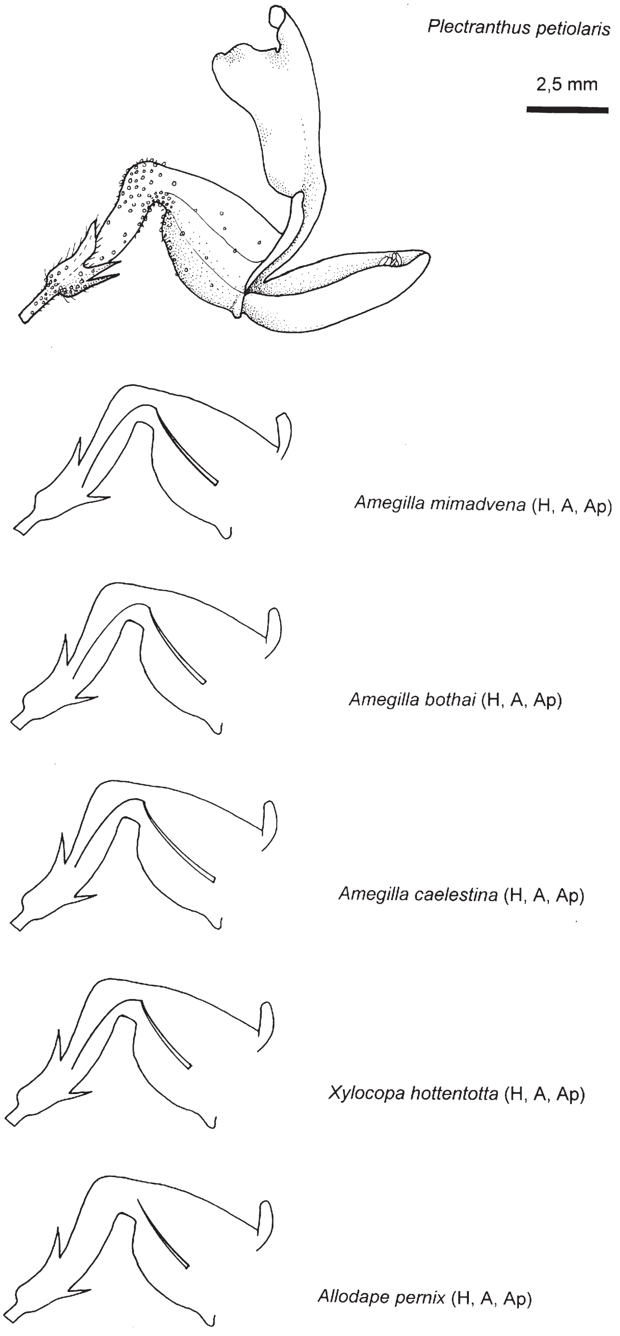


Fig. 3. Comparisons of floral tube shape and length with proboscids of insect visitors to *Plectranthus petiolaris*. Single lines show flexible parts of proboscis, double lines show solid parts. H = Hymenoptera, A = Apidae, Ap = Apinae. Bar: 2.5 mm.

Fig. 2. Comparisons of floral tube shape and length with proboscids of insect visitors to *Plectranthus laxiflorus*. In bees the single lines show the proximal flexible part of a proboscis (glossa) that extends along the whole length; double lines show the rigid basal part (clypeus and galea). D = Diptera, N = Nemestrinidae, H = Hymenoptera, A = Apidae, Ap = Apinae, Me = Megachilinae. Bar: 2.5 mm.

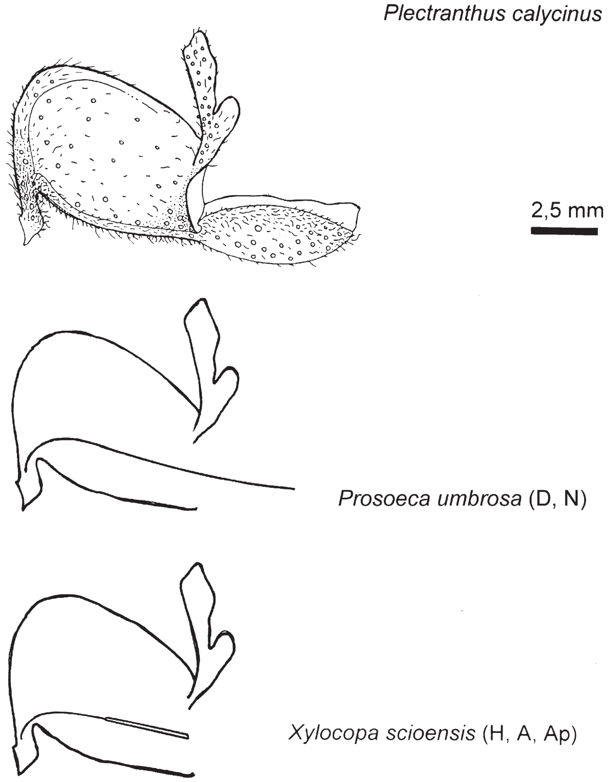


Fig. 4. Comparisons of floral tube shape and length with proboscids of insect visitors to *Plectranthus calycinus*. Single lines show flexible parts of proboscis, double lines show solid parts. D = Diptera, N = Nemestrinidae, H = Hymenoptera, A = Apidae, Ap = Apinae. Bar: 2.5 mm.

Table 1
Floral, plant and habitat characteristics of the four main study species.

Species	Habit	Habitat	Flowering time	Flower colour
P. petiolaris	Branched herb	Scree below cliffs covered by scarp forest; forest margins	December to April	Deep purple in northern KZN and Umtamvuna NR; pink at Oribi Gorge NR
P. laxiflorus	Freely branching soft shrub or herb, up to 1.5 m tall	Forest margins; damp open vegetation	Mid-February to April (sporadic in October and November)	White, with 4–5 thin purple linear nectar guides on flag-like upper lip
P. calycinus	Erect, branched shrub with slightly woody annual stems from woody rootstocks, stems up to 1.5 m tall	Grasslands	January to May (sporadically to July)	Creamy-white, tinged with mauve on edges of the corolla limbs
Py. urticifolia	Erect herb or shrub with a woody base, 1–2.5 m tall	Moist areas, such as forest margins and grassy stream banks	April to June	Deep blue

Nectar levels are generally confined to below the bend of the corolla. The base of the corolla tube is held at a near vertical or oblique angle upwards before the tube bends downwards; this presumably acts gravitationally to retain nectar. The nectar level fluctuation that was measured in *P. petiolaris* (by comparing the height of the nectar column from the base of the flower with the 'base-to-bend' corolla tube length), showed that unvisited flowers had an average nectar to tube bend ratio of 0.88 (SD 1.00, $n = 27$) and visited flowers had an average ratio of 0.41 (SD 1.26, $n = 31$). Thus in the morning newly secreted nectar was pushed up closer to the bend in the corolla tube, but later in the day it became more difficult for insects with shorter proboscids to extract nectar, as the nectar level dropped due to repeated insect visitation.

Flower colour varies from predominantly white (*P. laxiflorus*, *P. calycinus*) and creamy-white (*P. rehmannii*), to pale pink (*Aeollanthus parvifolius*), pink (*P. petiolaris* Oribi Gorge), deep purple (*P. petiolaris* Umtamvuna, *P. spicatus*) and deep blue (*Py. urticifolia*) (Table 1). In sigmoid species the darker coloured species tend to be pollinated by bees only, while the paler, whitish species appear to favour bee and fly pollinators. This pattern does not, however, extend to the rest of the genus when data from 20 species with a range of corolla shape is compared (C. Potgieter, unpubl. data).

Insect identifications were obtained for most bee species, excepting the megachilinid and halictinid bees, where generic identities are provided (Table 3; Appendix 2). Few KwaZulu-Natal specimens were included in the revision of nemestrinid flies done by Bezzi (1924), but subsequent work done by Barraclough (2006) allowed him to identify most *Prosoeca* (Diptera, Nemestrinidae) specimens to species-level for this study. Some species are new collections awaiting description (D. Barraclough, pers. com.).

The main daily period of activity of insect visitors was between 9.00 am and 4.00 pm. Apinid bees of the genera *Amegilla* and *Xylocopa* are the main pollinators of *Plectranthus petiolaris* (Fig. 3; Table 4). Bees of the genera *Amegilla* and *Xylocopa*, and pollen-collecting megachilinid bees of the genera *Megachile* and *Chalicodoma*, pollinate *Py. urticifolia* (Fig. 5; Table 4). *Plectranthus laxiflorus* is pollinated by species of *Chalicodoma* (Megachilinae), *Amegilla* and *Xylocopa* (both Apinae), as well as nemestrinid flies of the genus *Prosoeca*, of

Table 2
Mean floral measurements (in mm) for the four main studied sigmoid species: tube length measured from base of corolla to junction with lower lip; filament and style measurements include the full length of tube; *SD* standard deviation (in brackets after mean); *n* sample size.

Species (*n*)	Corolla tube (SD)	Style (SD)	Upper filament (SD)	Lower filament (SD)
P. petiolaris (22)	10.9 (0.7)	15.1 (4.2)	14.3 (0.6)	15.3 (2.3)
P. laxiflorus (18)	10.5 (0.9)	16.9 (1.8)	15.9 (1.3)	17.4 (1.4)
P. calycinus (29)	6.6 (0.6)	11.7 (1.1)	10.5 (0.8)	11.6 (0.9)
Py. urticifolia (26)	11.0 (0.7)	19.3 (1.0)	17.2 (1.1)	18.6 (1.0)

Table 3
Proboscis (and components of proboscis) length measurements of bee and fly visitors to all of the seven studied species.

Visitor	Average proboscis length (mm)			*N*
	Solid base (SD)	Flexible tip (SD)	Total length (SD)	
Prosoeca umbrosa			10.5 (0.8)	10
Prosoeca circumdata			9	1
Prosoeca sp. nov. 5			10	1
Amegilla mimadvena	5.1 (0.4)	3.9 (0.4)	9 (0.3)	6
Amegilla caelestina	4.3 (0.3)	4.2 (0.5)	8.5 (0.5)	12
Amegilla bothai	4.8 (0.3)	3.7 (0.3)	8.6 (0.4)	11
Amegilla fallax	4	3	7	1
Xylocopa hottentotta	4.2 (0.2)	3.3 (0.6)	7.5 (0.5)	3
Xylocopa scioensis	3.5	3.0	6.5	1
Xylocopa flavorufa	3.5	3.0	6.5	1
Xylocopa flavicollis	3.3 (0.4)	3.3 (0.4)	6.5 (0.7)	2
Thyreus sp.	4.3 (0.4)	2.0 (0)	6.3 (0.4)	2
Chalicodoma sp. A	3.0 (0)	2.8 (0.4)	5.8 (0.4)	2
Chalicodoma sp. B	2.0 (0.4)	2.8 (0.3)	4.8 (0.5)	4
Apis mellifera			3.3 (0.3)	5
Allodape pernix			3.2 (0.5)	8
Megachile sp. A			3	1
Megachile sp. B			3	1
Pseudoanthidium truncatum			2.8 (0.8)	2

SD standard deviation (in brackets after measurement); *n* sample size.

Table 4
Pollen placement and pollen loads (% *Plectranthus*/*Pycnostachys* pollen) on effective insect visitors of the four main study species.

Labiate species: with insect visitor	Pollen placement (ventrally)	Pollen load (%)
P. petiolaris		
Amegilla mimadvena	Thorax, abdomen.	25–95–100
	Scopae.	50
Amegilla caelestina	Thorax, abdomen.	100
Amegilla bothai	Thorax: between leg bases.	50–75
Xylocopa hottentotta	Thorax: between leg bases.	50
P. laxiflorus		
Amegilla mimadvena	Base of proboscis, head/thorax junction, abdomen, hind legs, scopae.	100
	Thorax: between leg bases.	95
Amegilla caelestina	Thorax, abdomen.	100
Amegilla bothai	Thorax, abdomen, scopae.	75–90
Prosoeca circumdata	Base of proboscis, head/thorax junction, thorax: leg bases, abdomen.	100
Prosoeca umbrosa	Head/thorax junction, thorax: leg bases	100
Prosoeca sp. nov. 5	Head/thorax junction, thorax, abdomen	100
Chalicodoma sp. A	Thorax, abdomen.	60–10
Xylocopa flavicollis	Head/thorax junction, thorax: between leg bases.	90
	Scopae.	75
P. calycinus		
Prosoeca umbrosa	Base of head, head/thorax junction, thorax, abdomen.	100
Py. urticifolia		
Amegilla mimadvena	Thorax, abdomen.	90
Apis mellifera	Thorax, abdomen, scopae.	100
Xylocopa scioensis	Head/thorax junction, base of proboscis.	100
	Thorax, abdomen.	95
Xylocopa flavorufa	Proboscis, head.	100
	Thorax, abdomen, hind legs.	75–100
	Thorax: bases of hind legs.	90
Megachile sp. A	Abdomen, hind legs	95–100
Megachile sp. B	Proboscis, base of head, thorax, thorax: leg bases, abdomen, hind legs	100
Chalicodoma sp. B	Abdomen	75 (few grains)

which *P. umbrosa* is the most abundant (Fig. 2; Table 4). *Prosoeca umbrosa* also pollinates *P. calycinus* at the Dargle, but is replaced by the apinid bee *Xylocopa scioensis* at Umtamvuna NR (Fig. 4; Table 4).

In all cases these insects were the most abundant visitors to the plants under study and all populations under study showed high levels of fruit set, as evidenced by the retention of swollen calyces. Under greenhouse conditions, where bees and flies are excluded, fruit set in the study species was found to be negligible, confirming that insects are necessary for pollination. Hand-pollination experiments done in a few non-sigmoid species of *Plectranthus* indicate that most species can set fruit from geitonogamous pollen transfer, but a few species do not (C. Potgieter, unpubl. data). Observations made on a single clone of the sigmoid *P. petiolaris*, grown in a garden, show that geitonogamous fruit set is possible.

In the four species that were studied in greater detail the edges of the lower corolla lip are folded inwards to partially conceal the anthers of unvisited flowers until a suitable insect visits the flower. Bees and flies with flexible proboscids of appropriate length can access nectar at the base of the corolla and, in the case of bees, the lengths of the galea and glossa in relation to the positioning of the corolla bend within the tube, also determine whether nectar can be reached (Table 3; Figs. 2–5). Before landing, bees swing their proboscids forward (the galea hinge does not bend beyond linear alignment with the bee's body). After landing on the lower lip the insect must angle its proboscis upwards into the corolla tube to access nectar at the base of the declined flower and since the proboscis is locked in linear alignment, the insect is forced to lower its body to raise the head and proboscis upwards. This action results in forced contact between the ventral surface of the insect's body and the anthers and style that are concealed in the lower lip, which facilitates pollen transfer. The lower lip does not return to its original position, and the anthers or stigma thus remain exposed for future visits.

Bees are covered with setae (hairs), especially along the groove between the legs on the ventral surface; these hairs generally point towards the posterior of the insect (Fig. 7g). As an insect arrives at the flower and lands on the lower lip (or contacts the sexual organs) the bifid stigma of a female phase flower dislodges pollen from between the hairs of the sternum onto the stigmatic surface. Pollen can only be picked up by the stigma as the insect moves into the flower and not as it retreats. Upon retreat from a male phase flower, pollen is passively loaded onto the hairs of the sternum, since the dehisced anthers brush close to the insect and force pollen to lodge between the hairs.

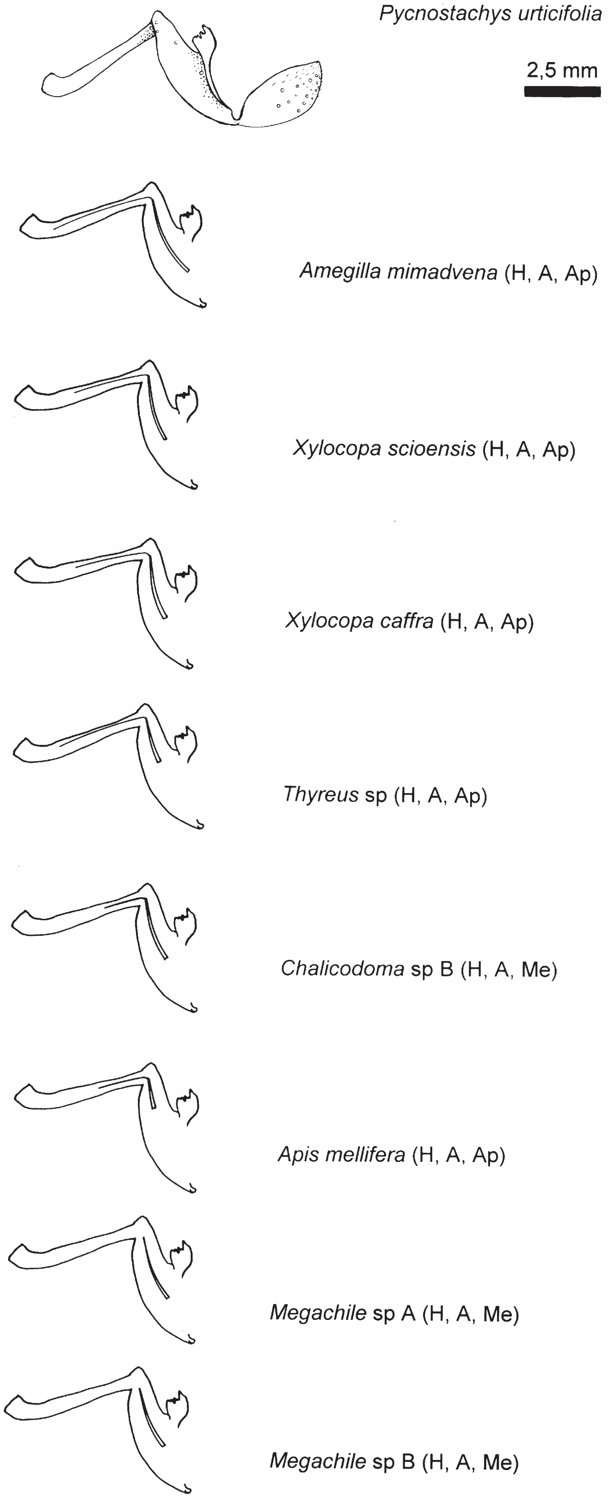


Fig. 5. Comparisons of floral tube shape and length with proboscids of insect visitors to *Pycnostachys urticifolia*. Single lines show flexible parts of proboscis, double lines show solid parts. H = Hymenoptera, A = Apidae, Ap = Apinae, Me = Megachilinae. Bar: 2.5 mm.

In all cases the pollinators of sigmoid species picked up and transported pollen on the ventral parts of their bodies (Table 4), with the thoracic area between the leg bases and the hairy area below the head being good sites for pollen carryover (Fig. 7g–h).

In the case of *P. calycinus* studied at Umtamvuna NR, no vouchers of *Xylocopa caffra* were caught, hence areas of pollen deposition could not be checked. Since bees crawl over the anthers and filaments to access nectar at the base of the floral tube, pollen placement is not always localised in discrete areas on the insect body. All visitors carried substantial amounts of *Plectranthus* pollen on their bodies, but the percentages of foreign pollen varied from 0 to 75% (Table 4).

Observations during the course of this study show that nemestrinid flies visit flowers for nectar; apinid bees visit flowers for nectar and sporadically for pollen collection, while megachilinid bees utilise nectar and/or load pollen onto the ventral abdominal scopae. With thoracic lengths of 13–18 mm and widths of 6–7 mm, apinid bees and nemestrinid flies comprise a class of large-bodied, nectar-feeding pollinators sufficiently powerful to depress the lower lip of a *Plectranthus* flower and pick up pollen from the anthers, but too broad to fully enter the mouth of a corolla tube (Table 5). Likewise, the smaller pollen-collecting megachilinid bees are also too large to fully enter into the laterally compressed corolla tubes (Table 5).

Results from the 2003 study on bee and fly visitor effectiveness on *P. laxiflorus* did not show a definitive increase or decrease as a result of fly emergence: average fruit set was 57.6% (SD = 19.5) before fly emergence (i.e. bees only), and 49.2% (SD = 22.8) after fly emergence (i.e. bees and flies). In seven inflorescences the fruit set was higher after fly emergence while in nine cases fruit set was lower. At a site in the Dargle it was noted that on an overcast morning flies were the only visitors to *P. laxiflorus* flowers, until about 11.30 am, when the sun came out and a few bees emerged. In this instance, *P. umbrosa* was the main floral visitor for the morning.

A few butterfly and one day-flying hawkmoth species feed on nectar of sigmoid *Plectranthus* species (Appendix 2: Lepidoptera); no pollen was found on the examined voucher specimens.

The pollination of three species with variously sigmoid corolla shapes was studied in less detail and is summarized as follows.

Plectranthus spicatus is a savanna species (Fig. 1d) with a succulent perennial habit, producing many decumbent stems from the base, with sub-spicate inflorescences that ascend up to 60 cm (Codd, 1985). The small flowers are blueish-purple in colour, with a sigmoid tube that is basally narrow, ascending at first and then curving sharply downwards, expanding at the throat (Figs. 6, 7d). Floral shape is similar to that of *Py. urticifolia*, but the corolla tube is shorter. *Xylocopa caffra* bees (proboscis length 6.5 mm) are the main pollinators of *P. spicatus* flowers at Oribi Gorge NR, with sporadic visits made by *Amegilla mimadvena* bees (proboscis length 9 mm).

Table 5
Range of body (thorax) size of insects that form the major pollinator groups of all seven studied plant species.

Insect group	Length of thorax (mm)	Width of thorax (mm)
Amegilla spp. (Apinid bee)	14–15	6–6.5
Xylocopa spp. (Apinid bee)	15–18	6–8
Prosoeca spp. (Nemestrinid fly)	13	7
Chalicodoma spp. (Megachilinid bee)	13–15	5–6

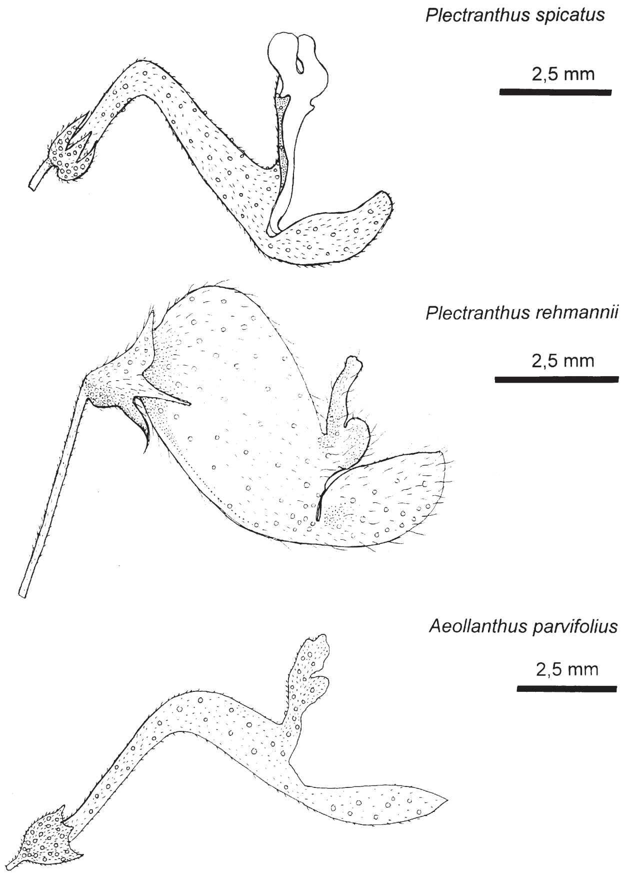


Fig. 6. Corollas of three additional *Plectranthus* and allied species studied: *Plectranthus spicatus*, *P. rehmanii*, *Aeollanthus parvifolius*. Bar: 2.5 mm.

Plectranthus rehmannii is endemic to the KZN Midlands (Fig. 1d), where it grows along or near forest margins. It is an erect, perennial sub-shrub reaching 1.2 m, with paniculate inflorescences up to 350 mm tall (Codd, 1985). The small flowers are creamy-white, with saccate bases and deflexed tubes (Fig. 6), similar to that of *P. calycinus*. At Leopard's Bush NR in the Karkloof, where *P. rehmannii* and *P. laxiflorus* grow in close proximity, the pollinator is a megachilinid bee, *Chalicodoma* sp. A (proboscis length 6 mm), which visits both species of *Plectranthus*. In a forest in the Dargle these two species also grow together, but none of the abundant *Prosoeca* flies at that site were seen to visit *P. rehmannii*. Honey bees (*Apis mellifera*) and *Allodape pernix* visited flowers of *P. rehmannii*, but only collected pollen from the anthers.

Aeollanthus parvifolius is a semi-succulent, perennial, herbaceous species that grows amongst rocks in grassland. The white to pinkish red flowers are borne on relatively lax, branched inflorescences. Cylindrical corolla tubes are 7–10 mm long, narrow at the base, expanding slightly towards the mouth (Codd, 1985), with a midway curve approaching the sigmoid shape. On the granite outcrops at Ongoye Forest (Fig. 1d), *Aeollanthus parvifolius* was pollinated by the apinid bees *Amegilla bothai*, *Amegilla mimadvena* and *Amegilla fallax*, all with proboscids ranging from 7 to 9 mm long. An acrocerid fly species (*Psilodera* sp.), with a flexible proboscis of similar length to those of the visiting bees, also probed flowers, but was not caught.

Estimations of proportional visitation by fly and bee pollinators to straight-tubed *Plectranthus* species showed that species that grow in or near shaded forest habitat tend to have more fly pollination visits, while species growing out in the open or in more sunny areas tend to have more bee visits (Table 6). This pattern is not clear in the sigmoid species, where most species are associated with sunlit patches in or near forest, or with sunny areas away from forest altogether.

5. Discussion

The observed modes of visitation by bees to sigmoid labiates generally correspond to that described by Scott Elliot (1891) and Stirton (1977). The nemestrinid flies (*Pr. umbrosa*, *Pr. circumdata* and *Prosoeca* sp. nov. 5, with proboscids 9–10.5 mm long) that visit *P. laxiflorus* and *P. calycinus*, are a new group of pollinators of sigmoid *Plectranthus* species. Nemestrinid flies with proboscids ranging from 8 to 30 mm also pollinate other, straight-tubed species of *Plectranthus* (Potgieter et al., 1999; Potgieter and Edwards, 2005).

There is an apparent close fit between the length of corolla tubes and the length of the proboscids of most apinid bees and nemestrinid flies that pollinate *P. laxiflorus* and *P. petiolaris* (as evidenced by visual comparisons in Figs. 2, 3). The flexible tips of bee proboscids accommodate the bend of the corolla tube, as does the flexibility of the nemestrinid proboscis along its whole length (Figs. 2, 3). These insects pick up pollen ventrally on the head, thorax and abdomen as the insect moves over the anthers. Species such as *Apis mellifera* (Fig. 2, *P. laxiflorus*) and *Allodape pernix* (Fig. 3, *P. petiolaris*) cannot reach nectar and rather collect pollen.

In the case of *P. calycinus* this fit by visual comparison only seems to hold for the apinid bee, *Xylocopa scioensis* (Fig. 4). Yet, despite its longer proboscis, the nemestrinid fly, *Prosoeca umbrosa*, picks up *P. calycinus* pollen on the hairy junction at the base of the head, as well as on the ventral surface of the thorax and abdomen (Table 4; Fig. 7h), when it appears as though only the base of the head should remove pollen from the anthers. The saccate base and inflated corolla of *P. calycinus* may be responsible for better contact between the anthers (and stigma) and the fly body, since the flexible proboscis may follow the curve of the upper part of the dorso-laterally flattened tube as it probes, which bends the proboscis and brings the fly body closer. Despite the weakly sigmoid shape of the corolla (it only bends close to the base) this design may function in excluding insects that do not have flexible proboscids in a way similar to other sigmoid corollas, while allowing for shorter-proboscid bees with flexible proboscids to probe directly towards the nectary by aligning with the lower part of the corolla (Fig. 4).

In *Py. urticifolia* the lengths and point of flexibility of proboscids of *Amegilla mimadvena*, *Xylocopa scioensis*, *X. flavorufa* and *Thyreus* sp. fit the corolla bend perfectly for nectar extraction and pollen removal (following visual comparison in Fig. 5). The recorded megachilinid bees cannot reach the corolla base for nectar. These bees actively collect pollen ventrally into abdominal scopae, in the same way as described by Percival (1965).

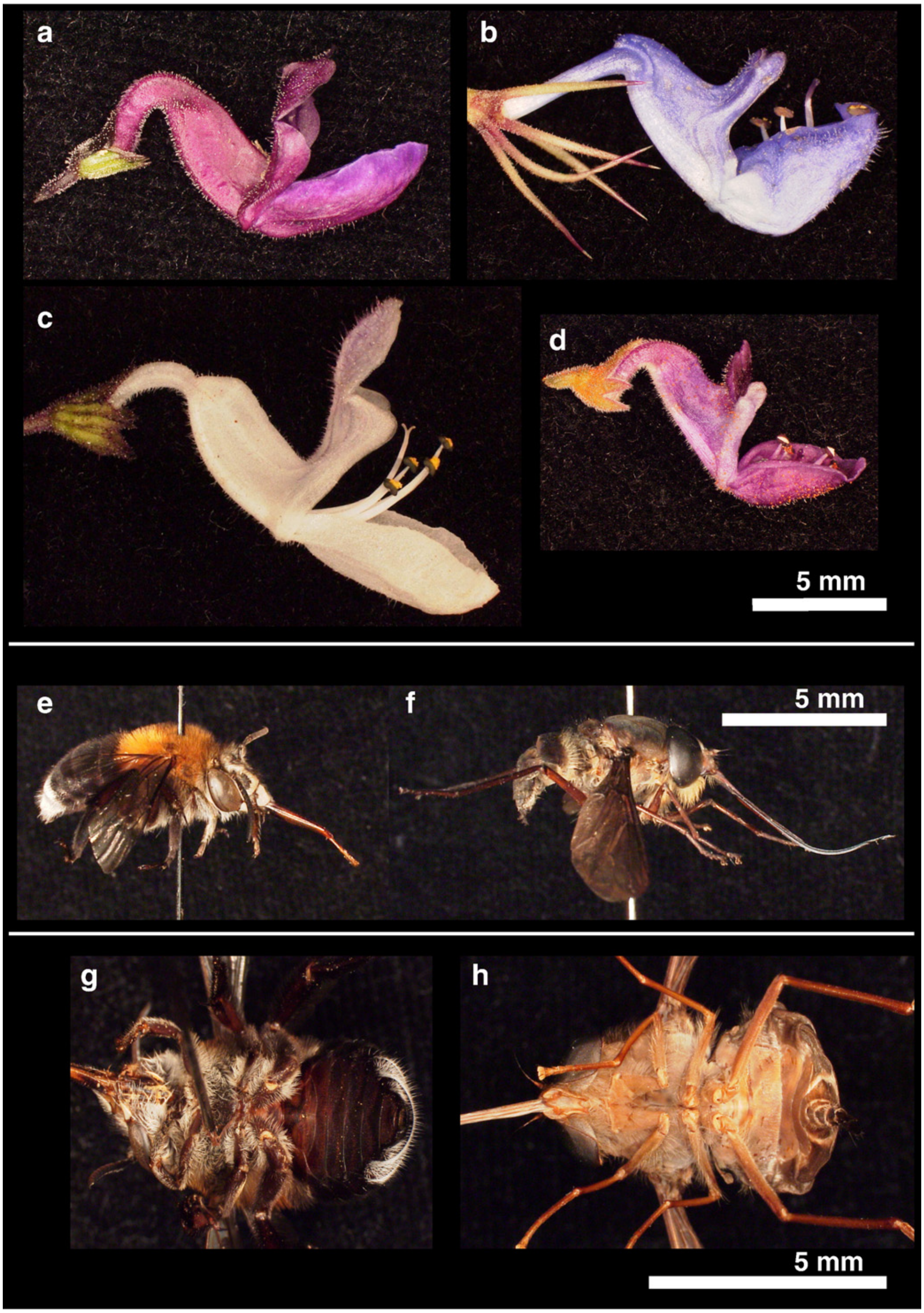


Fig. 7. A comparison of corollas of four sigmoid species (a–d), two insect pollinators (e–f) and undersides of an apinid bee and a nemestrinid fly (g–h). (a) *Plectranthus petiolaris*; (b) *Pycnostachys urticifolia*; (c) *Plectranthus laxiflorus*; (d) *Plectranthus spicatus*; (e) *Amegilla mimadvena* (Apinae, Hymenoptera); (f) *Prosoeca umbrosa* (Nemestrinidae, Diptera); (g) ventral surface of a typical apinid bee, *Amegilla mimadvena*, showing hairs on thoracic area and below the head; (h) ventral surface of a typical nemestrinid fly, *Stenobasipteron wiedemanni*, showing hairs below the head and groove between leg bases.

In general, the knee-like bend in the corolla tubes of *P. laxiflorus*, *P. petiolaris* and *Py. urticifolia* acts as a physical barrier in the corolla tube that only allows insect groups with flexible proboscids of sufficient length access to the nectar; the boat-shaped lower lip also limits the type of insect that can pick up pollen on its body. In *P. calycinus* this bend is less restrictive, sitting quite close to the corolla base, yet the two recorded visitors belong to bee and fly genera that also visit other sigmoid species. The same situation exists in *P. rehmanii*, while *A. parvifolius* has more gently curved corollas (Fig. 6). These act in much the same way as the knee-like bends discussed earlier, and that of *P. spicatus*, since a flexible proboscis is required to access nectar resources. The declined angle of the corolla tube forces insects into close contact with the reproductive parts of the flower, but this means that an ascending tube may be necessary at the base of the corolla as it may prevent the gravitational bleed of nectar—the combination of these two evolutionary forces are most likely responsible for the sigmoid shape of the corollas. In a study on the pollination of *Disa versicolor* (Orchidaceae) it was found that there was a good fit between orchid spur length and that of bee mouthparts, while the sharply decurved spur of this species functions to accommodate the 'long curved tongue of *Amegilla* or similar large long-tongued bees' (Johnson, 1995).

The effect of the sigmoid corolla shape on forcing contact between insect and floral reproductive parts (i.e. anthers and stigma) is more pronounced in bees than in flies. The flight of the approaching, probing bee is interrupted by the need to settle on the lower lip and/or reproductive parts of the flower, since the proboscis locks in linear alignment with the insect's body; to accommodate the angle in the sigmoid corolla the insect body is forced below the horizontal. By settling there is close contact between the ventral surface of the bee body and the anthers/stigma and any movement by the bee to probe into or retreat out of the corolla tube leads to further chances of pollen deposition onto the bee and removal from the anthers. In nemestrinid flies the proboscis is more flexible and can bend to some extent to accommodate the sigmoid shape of the corolla tube, hence flies can hover while feeding and often bypass the anthers/style in the process. This happens during some visits, but observations also showed that the flies may grip the floral reproductive parts as they settle on the lower lip during feeding.

The short proboscis of *Allodape pernix* cannot legitimately reach the nectar in *P. petiolaris* (Fig. 3), other than by observed instances of robbing, where the bee pierces holes into the corolla base. This is akin to the robbing mentioned by Scott Elliot (1891) for *P. calycinus*, where a small 'fly' [sic.] was implicated. Species of *Plectranthus* with straight corolla tubes are robbed in a similar way by *A. pernix* (Potgieter et al., 1999), where the bees act as "primary nectar robbers" and possibly as secondary robbers as well, where nectar is accessed through holes made by primary robbers (Inouye, 1980).

Honey bees, *Apis mellifera*, are only able to reach the nectar in *P. laxiflorus* and *Py. urticifolia* (Figs. 2, 5) if the bees force their way into the corolla and if nectar levels are very high (e.g. at the start of the day). In most cases honey bees were observed rather to collect pollen from the anthers of flowers, as in the case of *Allodape pernix*. Observations in *P. petiolaris* show that while nectar may be more accessible to shorter-proboscid insects early in the morning, residual nectar is available later in the day only to longer-proboscid insects, when the bend in the corolla tube functions as an effective exclusion mechanism.

The short proboscids of megachilinid bees prohibit nectar feeding altogether, although these species collect copious amounts of pollen on the modified hairs (abdominal scopae) on the ventral surfaces of their abdomens. Unlike the situation in other bee families where the insects groom pollen off the body into scopae located on the hind legs (and hence out of the reach of stigmatic surfaces), the abdominal scopae of megachilinid bees make pollen constantly available for subsequent deposition on stigmatic surfaces. While this ensures effective pollination, it may lead to increased levels of geitonogamous fruit set, compared to other bees where pollen is sporadically groomed out of the reach of stigmas.

Although Van der Pijl (1972) suggested that sigmoid corollas may provide a shift from butterfly to bee pollination, the current study records both bee and a few butterfly visitors, thus the corolla shape allows access to both groups. Lepidoptera are, however, not effective pollinators of *Plectranthus* since they tend to bypass the reproductive parts of the flowers by hovering or briefly settling on the lower lip of the flower without contacting stigma or anthers.

The sigmoid species that have both bee and fly pollinators, such as *P. laxiflorus* and *P. calycinus*, pose interesting questions with respect to which pollinator group is more efficient at pollen carryover. The experiment conducted at Ferncliff NR to test this in *P. laxiflorus* does not, however, answer these questions fully. The results show little or no increase in fruit set later in the season, despite a considerable increase in pollinator activity when the nemestrinid flies emerge and forage actively alongside apinid bees.

Table 6
Twenty species of *Plectranthus* with straight or sigmoid, long or shorter corolla tubes listed according to habitat, showing proportions of bee and fly visits (C. Potgieter, unpubl. data).

Species of *Plectranthus* (or allied genus)	Habitat	% Flies	% Bees
Long-tubed, straight (20–32 mm)			
P. saccatus Benth. (long-tubed variety)	FOR: deep shade	100	
P. reflexus E.J. Van Jaarsveld and T.J.Edwards	FOR: deep shade	100	
P. hilliardiae Codd	FOR: deep shade	100	
P. ambiguus (H.Bol.) Codd	FOR: sunlit patches	80	20
Shorter-tubed, straight (4–18 mm)			
P. oertendahlii Th.Fries jun.	FOR: deep shade	100	
P. ciliatus E.Mey ex Benth.	FOR: deep shade and sunlit	60	40
P. zuluensis T. Cooke	FOR: shade	100	
P. praetermissus Codd	FOR: shade and sunlit patches	100	
P. fruticosus L'Hérit.	FOR: shade and sunlit patches/ margins	100	
P. ecklonii Benth.	FOR: sunlit patches/margins	70	30
P. oribiensis Codd	FOR/SUN: forest margins and wooded areas		100
P. madagascariensis (Pers.) Benth.	SUN: grassland, woodland, forest margin	50	50
P. ernstii Codd	SUN: sunny cliffs		100
Sigmoid-tubed (5–11 mm)			
P. petiolaris E.Mey. ex Benth.	FOR: sunlit patches/margins	1	99
P. laxiflorus Benth.	FOR: sunlit margins (late season)	50	50
P. rehmannii Gürke	FOR: shade and sunlit patches/ margins		100
Py. urticifolia Hook.	SUN: stream banks, sunlit forest margin		100
P. spicatus E.Mey. ex Benth.	SUN: grassland and dry woodland		100
P. calycinus Benth.	SUN: grassland	100	
A. parvifolius Benth.	SUN: sunlit rocky areas	25	75

FOR: species associated with forest habitat (sometimes on sunny forest margins); SUN: species in sunny habitats.% Flies represents an estimate of the number of visits received by effective fly pollinators in the families Nemestrinidae, Tabanidae and Acroceridae; % Bees represents an estimate of the number of visits received by effective bee pollinators in the apid sub-families Apinae and Megachilinae.

One explanation for this could be the genetic constraint on ovule number in the Lamiaceae: flowers of this family have just four ovules per flower, setting four nutlets per fruit. The sequential maturation of fruit did not allow for individual nutlets to be counted at the end of the season, since some of the smooth seeds were shaken from the calyces before the last fruits matured, hence the number of nutlets per fruit could not be considered a reliable count and only fruit set was represented here. The limited number of ovules in *Plectranthus* may explain why bee visitors alone effected as much fruit set as bees combined with flies, since only four pollen grains are needed to give full fertilisation of a fruit; any subsequent visits would not increase fruit set. Bees alone may then be adequate to provide maximum fruit set, or, at least, as much fruit set as when flies and bees are visiting together. While the maximal fruit set was not established with the aid of hand pollinations, it would seem likely that heavy visitation by two pollinator groups later in the season would account for most of the potential fruit set in the population.

The distribution of *P. laxiflorus* extends beyond that of the most abundant fly visitor, *Pr. umbrosa*, but where the plants do co-occur with the flies it is heavily visited by these flies (in addition to bees); in overcast conditions where bee activity may be limited, the flies may be the dominant visitor (as was seen at the Dargle site). This suggests that flies could be more important as pollinators in years with predominantly overcast weather. Most of the sigmoid species discussed here are limited to sunny or grassland habitats (*P. calycinus*, *P. spicatus*, *Py. urticifolia*, *A. parvifolius*), sunlit forest margins (*P. laxiflorus*, *P. rehmannii*) or open patches in forests and along forest margins (*P. petiolaris*) (Table 6). In other, straight-tubed species of *Plectranthus* there is a general pattern of species in shaded forest habitat being associated with a greater proportion of fly pollinators, while species in sunny areas (away from forest and forest margins) favour more bee pollinators (Table 6). This is confirmed by a study on pollination in a South African grassland community, where it was found that long-proboscid solitary bees were the most important floral visitors (Johnson et al., 2009-this issue). This pattern is not as clear in the sigmoid species, none of which occur in deep forest shade, but it could point to a trend in increasing reliance on fly pollinators adjacent to forests (e.g. *P. laxiflorus* with a 50:50 split in the latter part of the season—after fly emergence). The possibility exists that, as sigmoid species became more shade-tolerant and moved closer to forest habitat, they became pollen-limited as a result of bees not being active in denser forest shade; nemestrinid flies would then be of greater importance as pollinators. Nemestrinid flies are, however, also active in some grassland habitats (e.g. *P. calycinus*).

It is possible that nemestrinid flies are more efficient at pollen carryover than bees since the latter group of insects groom pollen off their bodies more frequently than flies do (S. Morita, pers. com.) At one stage it was thought that nemestrinid flies do not groom, but observations during this study confirmed that limited grooming happens during hovering and whilst resting. This happens especially to clean the eyes (S. Morita, pers. com.).

The observation that a species of acrocerid fly, *Psilodera* sp., visited *A. parvifolius* at Ongoye, where *Amegilla* bees were the more abundant floral visitors, is another case of a fly species occupying the same niche as a bee species. In this case the fly looks and sounds like one of the resident anthophorine bees, *A. fallax*. A similar observation on Iridaceae prompted Goldblatt et al. (1997) to describe a related acrocerid fly as a bee mimic. Acrocerid flies are pollinators of a number of straight-tubed *Plectranthus* species that are also pollinated by various species of *Amegilla* bees with similar proboscis lengths (Potgieter et al., 1999; Viljoen et al., 2006).

The phylogenetic work by Paton et al. (2004 and pers. com.) does not adequately explain the relationships between the different floral types of *Plectranthus*, hence the question of whether sigmoid tubes evolved from straight corollas, or vice versa, is difficult to answer. We suggest the possibility that straight corollas have evolved from sigmoid ones, for the following reasons.

Our data from *P. laxiflorus* shows that a shift from sigmoid to straight corolla tubes could occur since nemestrinid flies can accommodate the bend in sigmoid tubes. Why then did corollas straighten? When nemestrinid flies visit sigmoid corollas they do not always contact the anthers, since direct observations show that their flexible proboscids can bypass the anthers and stigma if the fly is hovering. This reduced efficiency is not significant where visiting insects are plentiful. However data collected from forest species of *Plectranthus* show widespread pollen limitation and field observations confirm that insect visits are rare (C. Potgieter, unpubl. data). Under these circumstances increased efficiency of pollen transfer would be strongly selected. By straightening the corolla tube the anthers and stigma are shifted into the flight path of flies which makes it more difficult to bypass. Those grassland labiate species (e.g. *Orthosiphon*, *Thorncroftia*) that are pollinated by nemestrinid flies all have straight corolla tubes (Potgieter and Edwards, 2001).

A shift from sigmoid to straight corolla tubes could explain the saccate corolla bases found in many straight-tubed forest *Plectranthus* species (e.g. *P. saccatus*, *P. reflexus*), since the saccate base may represent the upper remnants of a sigmoid corolla. In this study the corolla shapes of *P. calycinus* and *P. rehmannii* represent a possible intermediate situation of a saccate corolla base combined with a bend near the base. Only two forest *Plectranthus* species, *P. ambiguus* and *P. ecklonii*, have straight corolla bases. These questions will only be answered fully once a comprehensive phylogeny of *Plectranthus* and its allies, including all the long-tubed species from southern Africa, has been constructed.

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Appendix 1

Study site details and plant vouchers for species studied at each site.
KZN = KwaZulu-Natal Province; EC = Eastern Cape Province; NR = Nature Reserve.

Study site	Province	Quarter deg. grid	Plant species studied	Year studied	Voucher
Umtamvuna NR	KZN	3030CC	*P. petiolaris*	1995–1998	C. Potgieter 115
			P. calycinus	2000	
Oribi Gorge NR	KZN	3030CB	*P. petiolaris*	1996–1998	C. Potgieter 100
			P. laxiflorus	1998	
			P. spicatus	1998	C. Potgieter 142
Ferncliff NR: Pietermaritzburg	KZN	2930CB	*P. laxiflorus*	1996–2003	C. Potgieter 135
Leopard's Bush NR: Karkloof	KZN	2930CB	*P. laxiflorus*	1999	C. Potgieter 145
			P. rehmannii	1999	C. Potgieter 150
Dargle: KZN Midlands	KZN	2930AC	*P. laxiflorus*	2009	
			P. calycinus	2000	C. Potgieter 154
			P. rehmannii	2009	T. Edwards 3518
Ngeli Forest: Weza	KZN	3029DA	*P. laxiflorus*	1998, 2001	
Garden, Pietermaritzburg	KZN	2930CB	*Py. urticifolia*	1998	C. Potgieter 1064
Ongoye Forest: Empangeni	KZN	2831DC	*A. parvifolius*	1998	
Kologha Forest: Stutterheim	EC	3227CB	*P. laxiflorus*	1998, 2000	

Appendix 2

All observed insect visitors to flowers of the four species studied in detail: *Plectranthus petiolaris*, *P. laxiflorus*, *P. calycinus* and *Pycnostachys urticifolia*.

Localities indicated; U, Umtamvuna Nature Reserve; O, Oribi Gorge Nature Reserve; P, Pietermaritzburg (Ferncliff Nature Reserve); K, Karkloof (Leopards Bush Nature Reserve); D, Dargle area; N, Ngeli area; S, Stutterheim (Kologha forest).

P. petiolaris	*P. laxiflorus*	*Py. urticifolia*	*P. calycinus*
Hymenoptera	Hymenoptera	Hymenoptera	Hymenoptera
Apidae	Apidae	Apidae	Apidae
Apinae	Apinae	Apinae	Apinae
Amegilla mimadvena U, O	*Amegilla mimadvena* P, S	*Amegilla mimadvena* P	
Amegilla bothai O	*Amegilla bothai* P, K		
Amegilla caelestina U, O, P	*Amegilla caelestina* O	*Thyreus* sp. P	
	Apis mellifera P	*Apis mellifera* P	*Apis mellifera* D
Xylocopa hottentotta U	*Xylocopa flavicollis* S	*Xylocopa scioensis* P	*Xylocopa scioensis* U
Allodape pernix U	*Allodape ceratinoides* P	*Xylocopa flavorufa* P	
Halictinae	Halictinae		
Lasioglossum sp. O	*Zonalictus* sp. P		
	Megachilinae	Megachilinae	
	Chalicodoma sp. A K	*Chalicodoma* sp. B P	
		Pseudoanthidium truncatum P	
		Megachile sp. A P	
		Megachile sp. B P	
Diptera	Diptera		Diptera
	Nemestrinidae		Nemestrinidae
	Prosoeca umbrosa P		*Prosoeca umbrosa* D
	Prosoeca circumdata P, N, S		
	Prosoeca sp. nov. 5 P		
Syrphidae	Syrphidae		
Asarkina sp. O	*Asarkina* sp. A N		
	Asarkina sp. B K		
Episyrphus sp. U	*Oniromyia* sp. P		
	Voria sp. P		
	Bombyliidae P		
Lepidoptera	Lepidoptera	Lepidoptera	
Pieridae U	Pieridae S		
Lycaenidae U, O, P	Hesperidae K, P	Lycaenidae P	
	Papilionidae		
	Papilio nireus lyaeus S		
	Sphingidae		
	Macroglossum trochilus P, S		

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CHAPTER 4:
CONVERGENT POLLINATION IN SOUTHERN AFRICAN LAMIACEAE

Potgieter, C.J., Edwards, T.J., 2001.
The occurrence of long, narrow corolla tubes in southern African Lamiaceae.
Systematics and Geography of Plants 71: 493–502.

Syst. Geogr. Pl. **71**: 493-502 (2001)

The occurrence of long, narrow corolla tubes in southern African Lamiaceae

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Abstract. *– Long, narrow corolla tubes have evolved several times in genera of southern African Lamiaceae, such as* Plectranthus, Thorncroftia, Orthosiphon, Hemizygia, Stachys *and* Salvia. *A study of the pollination of* Plectranthus *in KwaZulu-Natal and the Eastern Cape of South Africa showed a nemestrinid fly,* Stenobasipteron wiedemanni, *to be the pollinator of the four species that have long corolla tubes. The proboscis length of this fly (19 - 30 mm) corresponds to the corolla tube lengths of the flowers (20 - 33 mm). This fly is restricted to forest patches and woodlands along the eastern parts of southern Africa, while long-proboscid species of the nemestrinid genus* Prosoeca *occur mostly in grassland habitats. It is postulated that* S. wiedemanni *is the pollinator of* Stachys tubulosa, Salvia scabra, Salvia repens *var.* keiensis *and* Hemizygia ramosa *in forest and woodland habitats. Long-proboscid species of the nemestrinid genus* Prosoeca *are suggested as pollinators of* Orthosiphon tubiformis, Thorncroftia longiflora, T. succulenta, Hemizygia rugosifolia, H. gerrardii *and* Salvia repens *var.* keiensis, *in grassland habitats. Novel distribution maps for four long-proboscid fly species are provided. This paper aims to discuss the distribution of long-tubed members of the Lamiaceae in relation to that of long-proboscid flies by relating the biogeography of the flies to that of the plant species.*

Key words: *long corolla tubes, Lamiaceae,* Plectranthus, Orthosiphon, Thorncroftia, Salvia, Stachys, Hemizygia, *long-proboscid flies, Nemestrinidae, Stenobasipteron, Prosoeca.*

Abbreviations: *SEM, scanning electron microscopy; LM, light microscopy; PRECIS, Pretoria (PRE) Computerised Information System.*

Résumé. *–* **Présence de tubes corollins longs et étroits chez des Lamiaceae sud-africaines.** *Des tubes corollins longs et étroits se sont développés dans plusieurs genres sud-africains de Lamiaceae, comme* Plectranthus, Thorncroftia, Orthosiphon, Hemizygia, Stachys *et* Salvia. *Une étude de la pollinisation de* Plectranthus *au KwaZulu-Natal et dans l'Eastern Cape en Afrique du Sud ont montré qu'une mouche nemestrinide,* Stenobasipteron wiedemanni, *pollinisait quatre espèces à longs tubes corollins. La longueur du proboscis de cette mouche (19-30 mm) correspond à celle du tube corollin des fleurs (20-33 mm). La présence de cette mouche est limitée aux îlots forestiers et aux savanes arborées des régions situées à l'est de l'Afrique australe, alors que les espèces de nemestrides à long proboscis appartenant au genre* Prosoeca *se rencontrent principalement dans des milieux herbacés. Il est postulé que* S. wiedemanni *est le pollinisateur de* Stachys tubulosa, Salvia scabra, Salvia repens *var.* keiensis *et* Hemizygia ramosa *dans les habitats forestiers et en forêt claire. Les espèces de nemestrides à long proboscis du genre* Prosoeca *sont supposées être les pollinisatrices de* Orthosiphon

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tubiformis, Thorncroftia longiflora, T. succulenta, Hemizygia rugosifolia, H. gerrardii *et* Salvia repens *var.* keiensis *dans les milieux herbacés. De nouvelles cartes de distribution pour quatre espèces de mouches à long proboscis sont établies. Cet article a pour objectif de discuter la distribution de représentants à long tube de la famille des Lamiaceae en relation avec celle de mouches à long proboscis en comparant la biogéographie des mouches à celle des espèces végétales.* Traduit par le journal.

Introduction

Long, narrow corolla tubes have undoubtedly evolved several times in insect-pollinated genera of southern African Lamiaceae. Of the 49 species of *Plectranthus* in southern Africa, four have (or include forms with) corolla tubes that are substantially longer than those in the rest of the genus. These long-tubed species are pollinated by the long-proboscid fly, *Stenobasipteron wiedemanni* (Nemestrinidae, Diptera); cited as *Stenobasipteron* sp. in Potgieter & al. (1999).

Stenobasipteron wiedemanni has been recorded or inferred as a pollinator in few other studies (Goldblatt & Manning 1998, 2000; Manning & al. 1999) and is a relatively unknown pollinator that is restricted to forested or wooded areas along the eastern parts of southern Africa.

Other nemestrinid fly species are well-known as pollinators in southern Africa, particularly in the Cape flora and montane grasslands of the eastern seaboard. A number of studies in recent years have highlighted this long-proboscid fly pollination syndrome, which appears to be almost unique and highly evolved in southern Africa. These studies were conducted in the families Ericaceae (Rebelo & al. 1985), Iridaceae (Goldblatt & al. 1995; Goldblatt & Manning 1998, 1999), Orchidaceae (Johnson & Johnson 1993; Johnson & Steiner 1995, 1997) and Geraniaceae (Struck 1997), and a number of long-proboscid fly pollination guilds were identified by Manning & Goldblatt (1995, 1996, 1997) in the Cape flora. Goldblatt & Manning (2000) recently published a revision of this pollination syndrome in southern Africa.

During the course of our study on pollination in *Plectranthus* in KwaZulu-Natal and the Eastern Cape of southern Africa, it was shown that *S. wiedemanni* is the primary pollinator of the four species with long corolla tubes (*P. hilliardiae* Codd, *P. ambiguus* (H.Bol.) Codd, *P. reflexus* E.J.Van Jaarsveld & T.J.Edwards and *P. saccatus* Benth. – long-tubed forms). No other long-proboscid insect was seen to visit a long-tubed *Plectranthus*, except for one instance where a swallowtail butterfly (*Papilio* sp.) visited a flower of *P. reflexus* at Port St Johns (Potgieter & Edwards, unpubl. data). Other visits to long-tubed *Plectranthus* were either by *S. wiedemanni* or pollen-collecting bees.

This pattern of long corolla tubes is repeated in other genera of the Lamiaceae, such as *Thorncroftia*, *Orthosiphon*, *Stachys*, *Salvia* and *Hemizygia* (table 1).

Table 1. Occurrence of long and medium corolla tubes in southern African Lamiaceae.
Numbers are numbers of species. Long tube lengths fall in the range of 18 - 35 mm; medium tube lengths fall in the range of 10 - 17 mm. From Codd 1985; Van Jaarsveld & Edwards 1991, 1997.

Genus	Total	long tubes	medium tubes
Plectranthus	49	4	7
Thorncroftia	3	1	1
Orthosiphon	9	1	2
Hemizygia	28	3	21
Stachys	40	2	4
Salvia	22	3	15

Stenobasipteron wiedemanni visits *Gladiolus macneilii* Oberm. (Iridaceae) in woodland and grassland habitats in northern Mpumalanga and was also seen to visit *Orthosiphon tubiformis* R.Good in this area (M. Lotter pers com.; Goldblatt & Manning 1998, 1999, 2000).

In the same area another long-proboscid nemestrinid fly species, *Prosoeca robusta*, was seen to visit *Gladiolus calcaratus* G.J.Lewis, and a couple of the examined insect specimens carried pollen of a *Hemizygia* species (Goldblatt & Manning 1998, 1999). Apart from the above records, no observational data on insect pollination of long-tubed Lamiaceae in southern Africa is available. This excludes the genus *Leonotis*, which is primarily bird pollinated (Vos & al. 1994). The genus *Syncolostemon* is insect pollinated, but has flowers with a wide corolla throat which are more suited to bee-pollination.

Stachys tubulosa MacOwan, *Salvia scabra* L.f. and *Hemizygia ramosa* Codd occur in forests in KwaZulu-Natal and the Eastern Cape and have similar mauve, lilac or purple flowers with long, narrow corolla tubes. Other long-tubed labiate species that occur in grassland and woodland habitats in cool mist-belt areas include *Orthosiphon tubiformis*, *Thorncroftia longiflora* N.E.Br., *Thorncroftia succulenta* (Dyer & Bruce) Codd, *Hemizygia rugosifolia* Ashby and *Hemizygia gerrardii* (N.E.Br.) Ashby. *Salvia repens* Burch. ex Benth. var. *keiensis* Hedge occurs both in open woodland and grassland habitats (Codd 1985). In this paper we propose that these long-tubed labiates are also pollinated by long-proboscid flies such as *S. wiedemanni* (in forest and woodland) or *Prosoeca* spp. (in grassland).

Material and methods

Pollinator observations on the long-tubed members of *Plectranthus* were carried out during the flowering season (December - May) from 1995 - 2000. Field work was conducted at Stutterheim, Port St Johns, Umtamvuna Nature Reserve, Oribi Gorge Nature Reserve and Karkloof (fig. 1). Insect visitors to flowers were captured after observation, killed in separate ethyl-acetate containing vials, pinned and inspected under a dissecting microscope. Areas of pollen deposition on the insects were noted. Pollen grains were removed for SEM using small strips of double-sided tape, or LM using tiny blocks of fuchsin jelly.

Data on the occurrence and distribution of long-tubed species in *Plectranthus* and other genera of the Lamiaceae was obtained using Codd (1985), Van Jaarsveld & Edwards (1991,1997) and PRECIS records from 1984 until present. The latter records contributed to extended distributions for endemic species such as *P. hilliardiae*, *P. reflexus* and *T. longiflora*.

Proboscis length measurements of flies were done from vouchers collected during the study and specimens from the Natal Museum. Insect distributions were augmented using specimen data from the Natal Museum in Pietermaritzburg, Albany Museum in Grahamstown and South African Museum in Cape Town.

Additional distributional and proboscis length information was obtained from Goldblatt & Manning (1998, 1999).

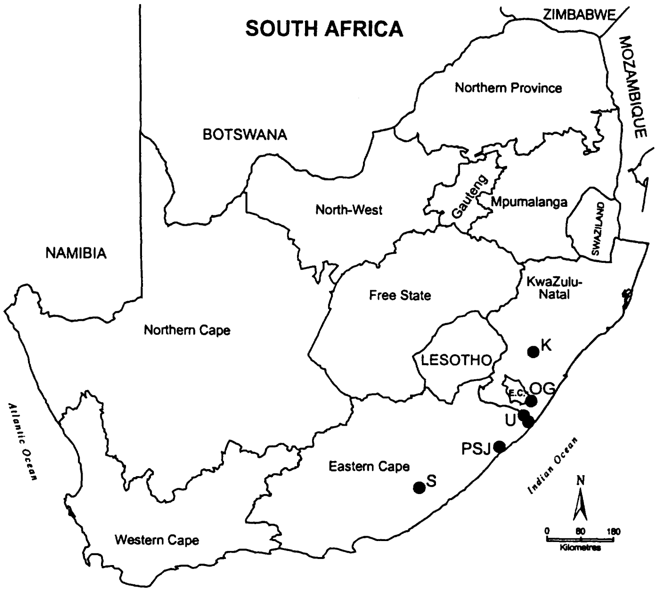

Figure 1.
Field study sites (●) for Plectranthus *pollination observations in KwaZulu-Natal and the Eastern Cape.*
K, Karkloof (2930AC);
OG, Oribi Gorge Nature Reserve (3030CA);
U, Umtamvuna Nature Reserve (3030CC & 3130AA);
PSJ, Port St Johns (3129 DA);
S, Stutterheim (3227CB).

Results and discussion

The long-tubed species of Lamiaceae that (potentially) belong to the long-proboscid fly pollination guild are listed in table 2. Tube length, habitat, flower colour and distribution are shown for each species in table 2. The long-tubed forms of *Plectranthus saccatus* have arisen convergently in both subspecies, *P. saccatus* subsp. *saccatus* and *P. saccatus* subsp. *pondoensis* E.J.Van Jaarsveld & S.Milstein (*sensu* Van Jaarsveld & Edwards 1997), thus only the long-tubed forms are listed and discussed.

Table 2. Floral tube length, habitat, general distribution and flower colour of fourteen long-tubed South African species of Lamiaceae.

Tube lengths in mm. See figs. 2 & 3 A for exact distributions. After Codd 1985; Van Jaarsveld & Edwards 1991, 1997; PRECIS; Potgieter unpubl. data. KZN, KwaZulu-Natal; EC, Eastern Cape; WC, Western Cape; Mp, Mpumalanga; NP, Northern Province.

Plant species	Tube length	Habitat	Distribution	Flower colour
Plectranthus				
P. ambiguus	20 - 33	Forest	KZN & EC	violet - purple
P. reflexus	24 - 30	Forest	EC (Pt St Johns)	pale blue
P. hilliardiae	21 - 32	Forest	S. KZN & EC	pale bluish
P. saccatus (long-tubed)	20 - 30	Forest	KZN	mauve
Stachys				
St. tubulosa	(12) 18 - 23	Forest	KZN & Swaziland	pinkish white
St. thunbergii	16 - 20	Forest margins	WC & EC	red/ purple
Hemizygia				
H. ramosa	20 - 22	Woodland	N. KZN	mauve
H. rugosifolia	ca. 18	Rocky grassland	Mp & NP	pale pink
H. gerrardii	17 - 20	Rocky grassland	Mp, Swaziland	mauve - pink
Thorncroftia				
T. longiflora	30 - 38	Rocky grassland	Mp & Swaziland	mauve pink
T. succulenta	15 - 20	Open woodland	NP & Mp	bluish mauve
Orthosiphon				
O. tubiformis	20 - 36	Open woodland	Mp	whitish - mauve
Salvia				
Sa. scabra	20 - 35	Forest margins	KZN & Swaziland	mauve/ lilac
Sa. repens var. *keiensis*	15 - 19	Grassland & woodland	EC	mauve - purple

Table 3. Proboscis length, habitat and general distribution of four long-proboscid fly species.
Proboscis lengths in mm. See fig. 3 B for exact distributions.
Flies are (potential) pollinators of long-tubed Lamiaceae in the eastern parts of South Africa.
After literature cited and measurements of Natal Museum (NM) and voucher specimens from this study. N, Nemestrinidae: Diptera; KZN, KwaZulu-Natal; EC, Eastern Cape; Mp, Mpumalanga; G & M, Goldblatt & Manning; J & S, Johnson & Steiner.

Name of dipteran	Proboscis length	Distribution	Habitat	Reference
Stenobasipteron wiedemanni (N)	19 - 30	EC & KZN	Forest	Potgieter & al. '99, NM, our vouchers.
	(23 - 29)	Mp (Abel Erasmus Pass)	Woodland	G & M '98, '99.
Stenobasipteron cf. *gracile* (N)	14 - 24	Mp (Barberton & Mariepskop)	Forest	NM
Prosoeca robusta (N)	20 - 23	Mp	Grassland	G & M '98, '99.
Prosoeca ganglbaueri (N)	17 - 42	Eastern areas	Grassland	
	(29 - 35)	Mp		G & M '98, '99; NM.
	(19 - 30)	KZN Drakensberg		G & M '98, '99; NM.
	(17 - 29)	EC (Naude's Nek)		J & S '95; NM.
	(25 - 42)	EC		G & M '98, '99; NM.

Distribution maps of long-tubed labiate species and subspecies are given in figs. 2 A, B & 3 A.

Four nectar-feeding nemestrinid fly species from the summer rainfall region have proboscides that fall within the range of tube lengths of long-tubed labiate species. Table 3 lists each species with proboscis length, habitat and general distribution. Fig. 3 B shows a distribution map of all four fly species.

While measuring proboscis lengths of *S. wiedemanni* specimens at the Natal Museum it became apparent that large collections from two localities in Mpumalanga looked different to the rest, i.e. from Barberton, De Kaap (in 1920) and Mariepskop (in 1932). These specimens are similar to *Stenobasipteron gracile* and will be referred to as *Stenobasipteron* cf. *gracile*, as the proboscis lengths place them in a functionally different pollinator category. It is likely that these flies pollinate flowers that occur in Mpumalanga and have corolla tubes slightly shorter than those visited by *S. wiedemanni.*

The nemestrinid fly, *Pr. ganglbaueri*, is restricted to grassland habitats at high altitudes and is the likely pollinator of a few grassland Lamiaceae with very long tubes (table 3 shows variation in proboscis length across its distribution).

Fig. 4 A, B, C & E shows the similarities in floral tube length for *Plectranthus*; the similarly long, narrow corolla tubes of *O. tubiformis* are shown in fig. 4 F, and fig. 4 D & G shows the long-proboscid nemestrinid flies *S. wiedemanni* and *Pr. ganglbaueri.*

Most of the labiate species listed in table 2 have flower colours in the purple/mauve/pale blue to white spectrum, coupled with long, constricted corolla tubes and odourless flowers, which conforms to the requirements of long-proboscid fly pollinators. In the case of *P. saccatus* the corolla tube is vertically broad, but the entrance is laterally compressed, thus restricting foraging to pollinators with long proboscides.

One species, *Stachys thunbergii* Benth., deviates from this colour range and it is the only long-tubed labiate that grows in the winter rainfall area of the Western and Eastern Cape. It has red, magenta or purple flowers and is probably pollinated by sympatric long-proboscid flies, e.g. *Philoliche rostrata* (Tabanidae) or *Prosoeca nitidula* (Nemestrinidae).

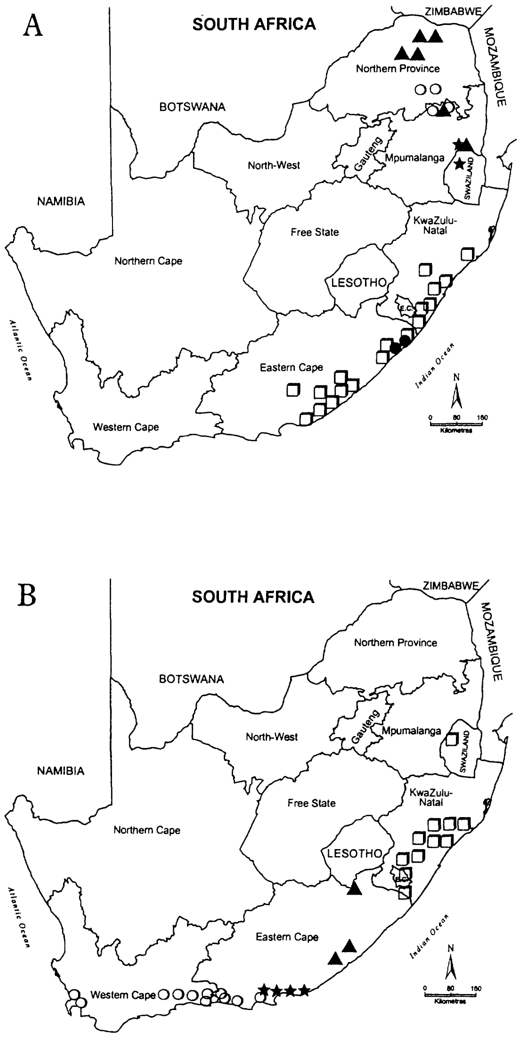

Figure 2.
Distribution of long-tubed Lamiaceae.

A: ★, Thorncroftia longiflora *(tube 30 - 38 mm);*
▲, T. succulenta *(tube 15 - 20 mm);*
○, Orthosiphon tubiformis *(tube 20 - 36 mm);*
❐, Plectranthus ambiguus *(tube 20 - 33 mm);*
●, P. reflexus *(tube 24 - 30 mm).*

B: ❐, Stachys tubulosa *(tube 18 - 23 mm);*
○, St. thunbergii *(tube 16 - 20 mm);*
★, Salvia scabra *(tube 20 - 35 mm);*
▲, Sa. repens *var.* keiensis *(tube 15 - 19 mm).*

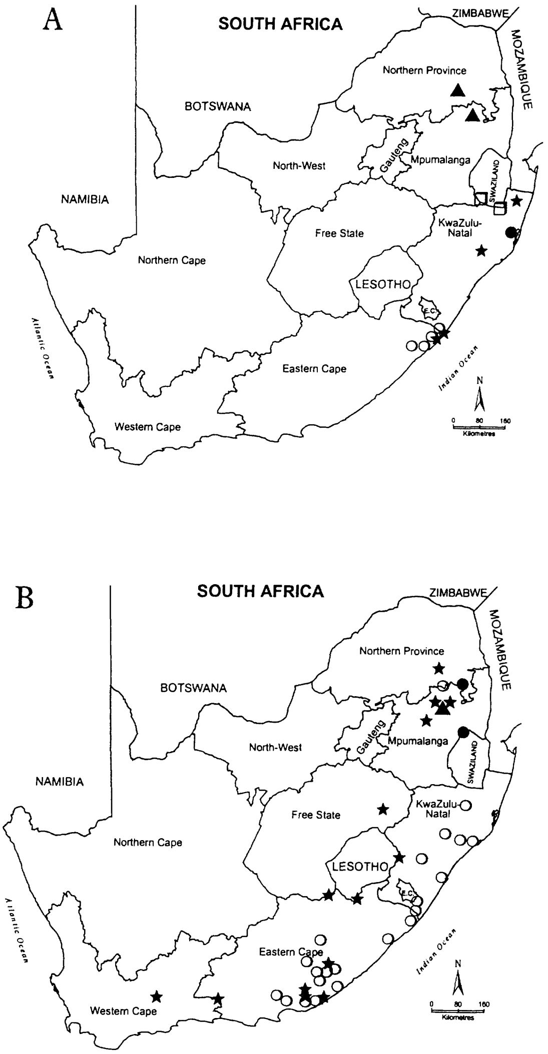

Figure 3. Distribution of long-tubed Lamiaceae and long-proboscid flies.

A, *Lamiaceae:*
▲, Hemizygia rugosifolia *(tube ca. 18 mm);*
❐, H. gerrardii *(tube 17 - 20 mm);*
●, H. ramosa *(tube 20 - 22 mm);*
○, Plectranthus hilliardiae *(tube 21 - 32 mm);*
★, P. saccatus *(long-tubed forms, 20 - 30 mm).*

B, *Flies:*
○, Stenobasipteron wiedemanni *(proboscis 19 - 30 mm);*
●, Stenobasipteron *cf.* gracile *(proboscis 14 - 24 mm);*
★, Prosoeca ganglbaueri *(proboscis 17 - 42 mm);*
▲, Prosoeca robusta *(proboscis 20 - 23 mm).*

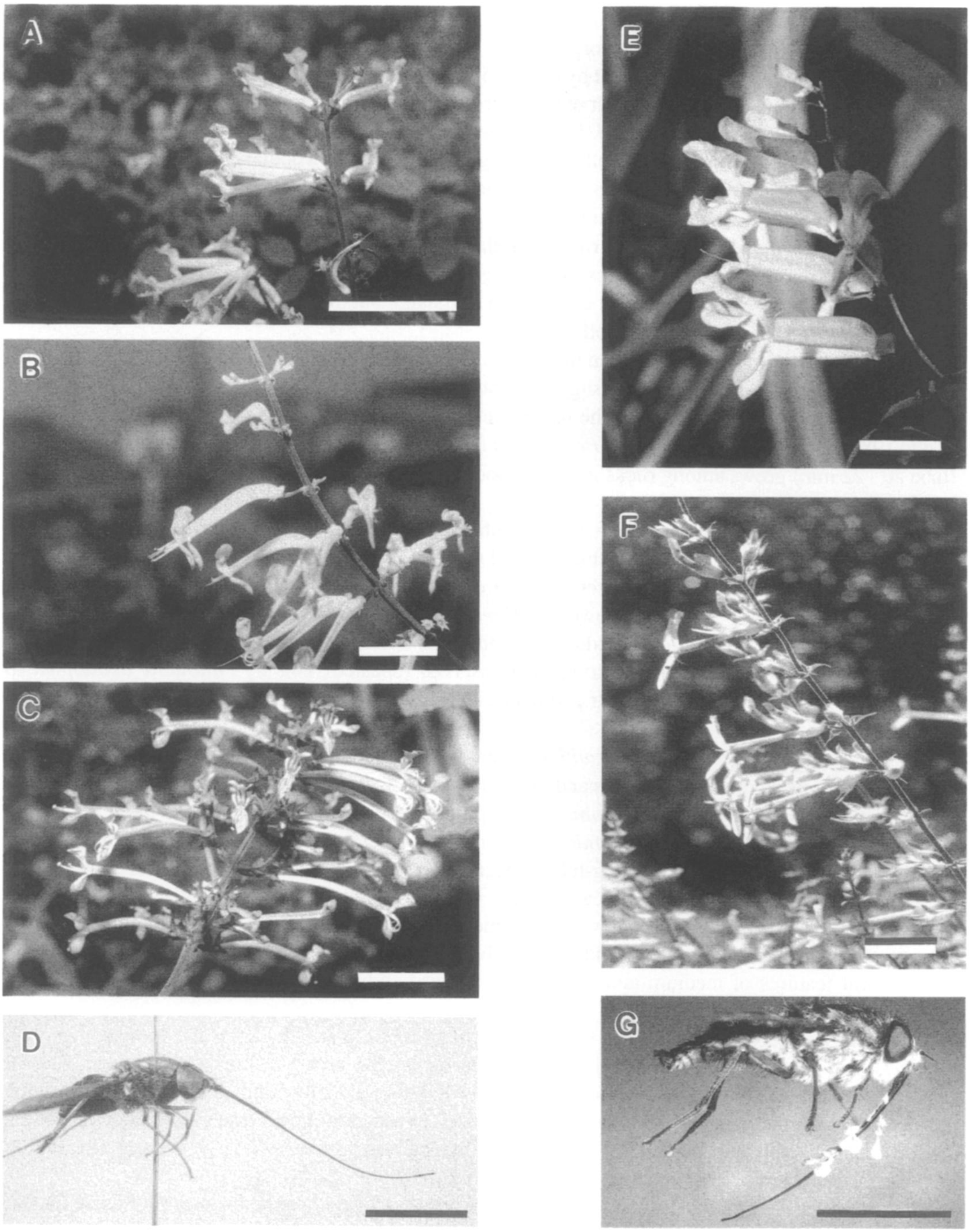

Figure 4. Long-tubed Lamiaceae and their pollinators.
A, Plectranthus hilliardiae; *B,* Plectranthus reflexus; *C,* Plectranthus ambiguus; *D,* Stenobasipteron wiedemanni; *E,* Plectranthus saccatus *(long-tubed form, Umtamvuna); F,* Orthosiphon tubiformis,; *G,* Prosoeca ganglbaueri *(with orchid pollinaria, photo: S. Johnson)*; *scale bars A, B, C, E & F = 2 cm, scale bars D & G = 1 cm.*

Stachys tubulosa is the only other long-tubed *Stachys*, but it has pinkish white flowers, a tube that is nearly straight and a deflexed lower lip; it also grows in moist forests and forest margins, thus *S. wiedemanni* is proposed as its pollinator.

Two other species may be pollinated by the fly, *S. wiedemanni*. *Salvia scabra* has a straight tube (in a genus where the corolla tube is often curved) and grows in forest margins and bush clumps at the lower end of the distribution of *S. wiedemanni*. *Salvia repens* var. *keiensis* is a variable species with a straight upper lip (often hooded in the genus) and occurs in grassland or open woodland in the Eastern Cape. At the coastal sites *S. wiedemanni* is proposed as the pollinator in woodland areas, but at Naude's Nek *Pr. ganglbaueri* may be the grassland pollinator.

Thorncroftia longiflora grows in rocky grassland and has a very narrow, long tube (30 - 38 mm) which closely matches the long proboscis of *Pr. ganglbaueri* in Mpumalanga (29 - 35 mm). *Thorncroftia succulenta* also occurs on rock outcrops in grassland, but the shorter tube length (15 - 20 mm) suggests that *Pr. robusta* may be its pollinator.

No nemestrinid flies are recorded in the area where *Hemizygia gerrardii* (tube 17 - 20 mm) grows in grass among rocks, but the habitat suggests that a species of *Prosoeca* (such as *Pr. robusta* or *Pr. ganglbaueri*) may be its pollinator. The narrow endemic *H. rugosifolia* with a tube ca. 18 mm long (Codd 1985), is probably pollinated by *Pr. robusta* (proboscis length 20 - 23 mm). *Hemizygia ramosa* (tube 20 - 22 mm) grows among rocks in open woodland towards the coast and may be pollinated by *S. wiedemanni*.

A number of the labiate genera that have long-tubed members, also have species that have 'medium-tubed' corollas in the range of 10 - 17 mm (table 1).

In northern KwaZulu-Natal we observed visits of a horsefly, *Philoliche aethiopica* (family Tabanidae, proboscis length 8 - 9 mm), to flowers of *Hemizygia pretoriae* (Gürke) Ashby. The corolla tubes are of medium length (10 - 12 mm; Codd 1985), but other floral features are similar to those visited by long-proboscid flies. In the same area we also observed repeated visits by *Philoliche aethiopica* to flowers of *Orthosiphon serratus* Schltr., which has a medium corolla tube length of 9 - 16 mm (Codd 1985).

A similar situation occurs in *Plectranthus* where medium-tubed species are pollinated by a corresponding suite of medium-proboscid flies and bees (Potgieter & al. 1999). *Plectranthus ecklonii* Benth., for example, is visited by *S. wiedemanni*, shorter-proboscid flies (such as *Philoliche aethiopica*) and bees (Potgieter & al. 1999). In *P. ecklonii* and a number of other cases the filament and style lengths of these species approach that of the long-tubed species, but the shortened corolla tube allows for nectar exploitation by shorter-proboscid insects as well.

The occurrence of a guild of medium-tubed Lamiaceae adapted for pollination by medium-proboscid flies may explain how long tubes could have evolved simply by elongation of the corolla tube, as the other floral features of medium-tubed species are already adapted to fly pollinators with extended proboscides.

Since four species of long-proboscid flies with varying proboscis lengths occur in Mpumalanga, with *Prosoeca* spp. limited to grasslands and *Stenobasipteron* spp. occurring in forest or woodland, we will concentrate future field studies in this area to confirm which flies actually pollinate the species for which observations are not available. Studies are also needed to ascertain how closely pollinator proboscis lengths and floral tube lengths are correlated in specific localities, as there is a large amount of variation in these measurements (see *Prosoeca ganglbaueri*, table 3).

It is, however, clear that long-tubed Lamiaceae are adapted for pollination by long-proboscid flies in the eastern parts of southern Africa. Furthermore, the habitat and distribution of the fly species determine which Lamiaceae are able to exploit this pollination syndrome.

The production of long corolla tubes is energetically expensive and results in elevated water loss (Potgieter & Edwards unpubl. data). However, the advantages of this syndrome lie in the protection of

Table 4. Recorded natural hybrids of *Plectranthus* from Oribi Gorge.
Natural hybrids are only derived from species with short or medium corolla tube lengths. Measurements are in mm. Standard deviation (SD) given after mean value. Sample size (n) = 20 for each species. Data from Potgieter & al. (2000).

Hybrid	**Parent *Plectranthus* spp**	**Tube length: range**	**Tube length: mean (SD)**
P. zuluensis x *P. ciliatus*	*P. zuluensis* T.Cooke *P. ciliatus* E.Mey. ex Benth.	10 - 12 6 - 8	11.5 (0.6) 6.8 (0.7)
P. oribiensis x *P. ernstii*	*P. oribiensis* Codd *P. ernstii* Codd	6.5 - 8 6 - 10 .5	7.2 (0.5) 7.6 (1.2)
P. ciliatus x *P. oertendahlii*	*P. ciliatus* E.Mey. ex Benth. *P. oertendahlii* Th.Fries jun.	6 - 8 8 - 11.5	6.8 (0.7) 9.6 (1.2)

nectar, which leads to heightened pollinator fidelity. Potgieter & al. (1999) outline the ability of pollinators to access nectar in seven species of *Plectranthus*. These results indicated that only *S. wiedemanni* is capable of reaching the nectar of long-tubed *Plectranthus* species. Extensive fieldwork a t Oribi Gorge N.R. has revealed natural hybrids between medium - and short-tubed *Plectranthus* species (table 4), but no natural hybrids have been recorded from species with long corolla tubes.

This system would break down if there were areas where many long-tubed species co-occur, but the long-tubed *Plectranthus* species are mostly endemics that seldom co-occur. Where the more widespread *P. ambiguus* grows in Umtamvuna N. R. with *P. hilliardiae* and long-tubed forms of *P. saccatus*, the populations are all ecologically separated along vertical gradients along the slopes of the gorge, or occur either along the Umtamvuna River or one of its tributaries.

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CHAPTER 5:
A NEW POLLINATION GUILD

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THE *STENOBASIPTERON WIEDEMANNI* (DIPTERA, NEMESTRINIDAE) POLLINATION GUILD IN EASTERN SOUTHERN AFRICA[1]

C. J. Potgieter[2] *and T. J. Edwards*[2]

Abstract

Stenobasipteron wiedemanni, a long-proboscid nemestrinid fly, services a Guild of flowers distinct from the *Prosoeca ganglbaueri* pollination Guild in which it has previously been placed. The former fly is the recorded pollinator of 19 plant species in six families: the Acanthaceae, Balsaminaceae, Gesneriaceae, Iridaceae, Lamiaceae, and Orchidaceae. *Stenobasipteron wiedemanni* is a mainly forest-dwelling fly with a proboscis length of 19–30 mm. The plant species that are pollinated by this fly are also restricted to forest (or woodland) habitat along the eastern parts of southern Africa. The flowers of plants in this pollination Guild tend to have long, narrow nectaries and corollas in shades of purple, pink, mauve, pale blue, and white. Nectar is present in all species, pollination is diurnal, and most species have zygomorphic flowers with nectar guides. This pollination Guild is separated on the basis of the limitation of the fly, *S. wiedemanni*—and hence the plant species—to forest or closed-canopy habitat. Biogeographically, the *S. wiedemanni* Guild also occurs at lower altitudes in subtropical regions of southern Africa.

Key words: Acanthaceae, *Asystasia*, Balsaminaceae, *Barleria*, *Brownleea*, forest, Gesneriaceae, *Hesperantha*, *Hypoestes*, *Impatiens*, Iridaceae, *Isoglossa*, Lamiaceae, long-proboscid flies, Nemestrinidae, Orchidaceae, *Plectranthus*, pollination, *Stenobasipteron wiedemanni*, *Streptocarpus*.

The study of long-proboscid fly pollination is a subject of increasing interest in southern Africa. This pollination syndrome is well represented on the sub-continent and has been reported for a number of plant families: Ericaceae (Rebelo et al., 1985), Iridaceae (Goldblatt et al., 1995; Goldblatt & Manning, 1999; Manning et al., 1999), Orchidaceae (Johnson & Steiner, 1995, 1997), Geraniaceae (Struck, 1997), and Lamiaceae (Potgieter et al., 1999; Potgieter & Edwards, 2001). This pollination syndrome was reviewed by Goldblatt and Manning (2000), and three discrete Guilds were identified. These are the *Prosoeca peringueyi* Guild, the *Moegistorhynchus–Philoliche* Guild, and the *Prosoeca ganglbaueri* Guild.

The *Prosoeca peringueyi* Guild is restricted to the western half of the winter-rainfall area in southern Africa and comprises two fly species that pollinate flowers with intense shades of violet, deep purple, and magenta, often with cream to yellow markings and areas of darker pigmentation (Goldblatt & Manning, 2000). The *Moegistorhynchus–Philoliche* Guild comprises six to seven species of nemestrinid and tabanid flies, the tabanids extending from southern Namibia to southeastern parts of the Western Cape (see Fig. 1 for location of provinces in South Africa), with the nemestrinids being restricted to narrow ranges in the southwestern parts of the Western Cape. Flowers in this second Guild are white to cream with pink undertones, or pale to deep pink; nectar guides are red or deep pink (Goldblatt & Manning, 2000). These two Guilds occur in the winter-rainfall areas of southern Africa, while the *Prosoeca ganglbaueri* Guild (in which *Stenobasipteron wiedemanni* has been placed) occurs mainly in summer-rainfall areas.

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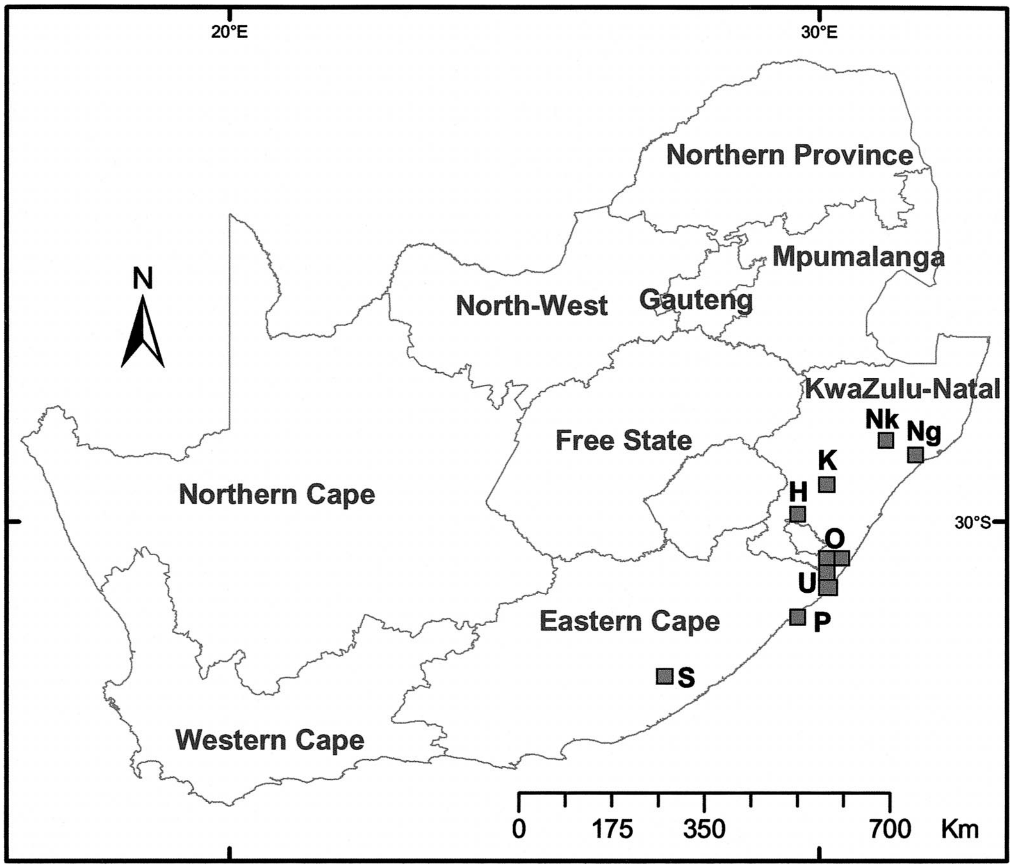

Figure 1. Map of South Africa showing provinces and study sites. Note that Northern Province is now called Limpopo Province. —Nk. Nkandla Forest. —Ng. Ongoye Forest. —K. Karkloof (Leopards Bush Nature Reserve). —H. Hlabeni Forest (Creighton). —O. Oribi Gorge Nature Reserve. —U. Umtamvuna Nature Reserve. —P. Port St. Johns. —S. Stutterheim (Kologha Forest).

The *Prosoeca ganglbaueri* Guild sensu Goldblatt and Manning (2000) included four nemestrinid fly species: *Prosoeca ganglbaueri* Lichtwardt, *Prosoeca longipennis* Loew, *Prosoeca robusta* Bezzi, and *Stenobasipteron wiedemanni* Lichtwardt. Of these species, the floral Guilds pollinated by the first three show considerable overlap, but no overlap has been shown with the Guild pollinated by *S. wiedemanni.* Flowers pollinated by the first three fly species are pink with dark pink markings, but some species are cream or white or deep blue. The fourth fly species, *S. wiedemanni,* visits flowers in shades of pink, pale blue, or mauve (Goldblatt & Manning, 2000). Goldblatt and Manning (2000) indicated that further research may show this latter species to constitute a separate Guild of pollinating flies, and in this paper we confirm the existence of this separate *S. wiedemanni* pollination Guild.

Stenobasipteron wiedemanni (cited as *Stenobasipteron* sp. in Potgieter et al., 1999) is a brown nemestrinid fly species with a proboscis length of 19–30 mm. The species is largely limited to subtropical and temperate forests along the eastern seaboard of southern Africa (Potgieter & Edwards, 2001), and adults have been collected from December to June, with activity in each locality restricted to a few months during summer (see Appendix 1). By contrast, the nemestrinid genera *Moegistorhynchus* and *Prosoeca* occur in temperate fynbos, montane grasslands, and other habitats without a closed canopy.

We proposed (Potgieter & Edwards, 2001) that there are a number of long-tubed species in genera of Lamiaceae in South Africa that are pollinated by either *Stenobasipteron* or *Prosoeca,* depending on habitat. Comparative plant and long-proboscid fly pollinator distributions are presented in Potgieter and Edwards (2001). These species are distributed over the eastern part of the country and include long-tubed members of *Plectranthus* (Potgieter et al., 1999), *Hemizygia, Salvia, Stachys, Orthosiphon*

(Goldblatt & Manning, 1999, 2000), and *Thorncroftia.* Goldblatt and Manning (2000) included species of *Thorncroftia* as inferred members of their *Prosoeca ganglbaueri* Guild.

In forests of the eastern seaboard of southern Africa a number of plant families have evolved long, narrow corolla tubes, spurs, or hypanthia (ca. 20–35 mm), and in our study area we suggest that *Stenobasipteron wiedemanni* may be the primary pollinator of such species. Most of these plant species are unscented and have flowers of mauve, pink, purple, pale blue, or white.

MATERIALS AND METHODS

Fieldwork was conducted during the main flowering season of *Plectranthus,* between December and May, from 1995 to 2002. Study sites for pollinator observations are shown in Figure 1. We avoided over-collecting fly specimens, since the species occurs at low densities and we did not want to impact on the pollination service it renders. Where appropriate, observations of insect visits were followed by capture of voucher specimens that were killed in separate ethyl-acetate-containing vials and pinned with proboscides extending forward for easy measurement. Areas of pollen deposition were identified under a dissecting microscope, and samples of pollen were collected using blocks of Fuchsin Jelly and mounted. Slides were studied with a compound light microscope, and pollen samples were identified by comparison with reference slides of pollen from plants flowering in the area.

Stenobasipteron wiedemanni is not easily confused with other insects in the study area, and thus only one (or rarely a few) vouchers were collected per plant species to ascertain areas of pollen deposition on the insect. After the first voucher per plant species was captured, careful observations of flies during subsequent flower visits allowed the observer to judge whether pollen would be deposited on certain areas of the insect.

In cases where observations were not made by the authors of this paper, we relied on personal communication with other field workers that obtained photographic records or insect vouchers where possible.

Insect vouchers for this study are lodged with the Natal Museum in Pietermaritzburg, South Africa, and plant vouchers are housed at NU, Natal University Herbarium (see Appendices 1 & 2).

Distributions of the fly species were compiled from field observation and by using data from the Natal Museum (Pietermaritzburg), Albany Museum (Grahamstown), and the South African Museum (Cape Town).

Distributions of Guild constituents were compiled from NU and PRECIS (National Herbarium, Pretoria (PRE) Computerised Information System), as well as published information (Codd, 1985; Pooley, 1998; Linder, 1981; Hilliard & Burtt, 1986; Edwards, 1988) and observations made during the course of this study. Distribution maps for shorter-tubed species of *Plectranthus* are available in Potgieter et al. (1999).

Distribution maps were generated using ArcGIS, combining data from MS Access. Data were collected at quarter-degree-grid level, and hence the forest land cover was re-sampled to quarter-degree-grid resolution.

Floral and other characters of plants shown to be effectively visited by *Stenobasipteron wiedemanni* were compared, and these data were used to suggest other possible members of the Guild.

Nectar volume measurements were made during mornings from cultivated plants in a greenhouse, and these represent the maximum amount of nectar available to insect visitors. Nectar concentrations (as percentage of sucrose equivalents) were measured using a Bellingham & Stanley Eclipse (0–50%) hand-held refractometer. Nectar samples were dried on Whatmans no. 1 filter paper and analyzed using a modified Gas Chromatographic nectar sugar analysis method based on Tanowitz and Smith (1984). Samples of 10 to 30 flowers were pooled to obtain sufficient nectar for analysis.

RESULTS

THE POLLINATOR

Stenobasipteron wiedemanni is a day-flying dipteran that visits flowers from about 9 A.M. to 4 P.M., extracting nectar as a reward. Specimens have been collected between December and July, but we found that in the Eastern Cape and KwaZulu-Natal activity continued to about May (depending on the year).

The behavior of these flies makes them ideal pollinators of long-tubed flower species. During flight the proboscis is folded underneath the body, but as a flower is approached the proboscis is brought forward to extend in front of the body (Fig. 2A). Flies hover in front of flowers while probing for nectar, but occasionally grasp protruding filaments or styles (in the case of Lamiaceae) or rest on landing platforms (e.g., in *Isoglossa hypoestiflora* Lindau, Acanthaceae). Visits to individual flowers last between one and four seconds, and a number of flowers on the same inflorescence or plant may be

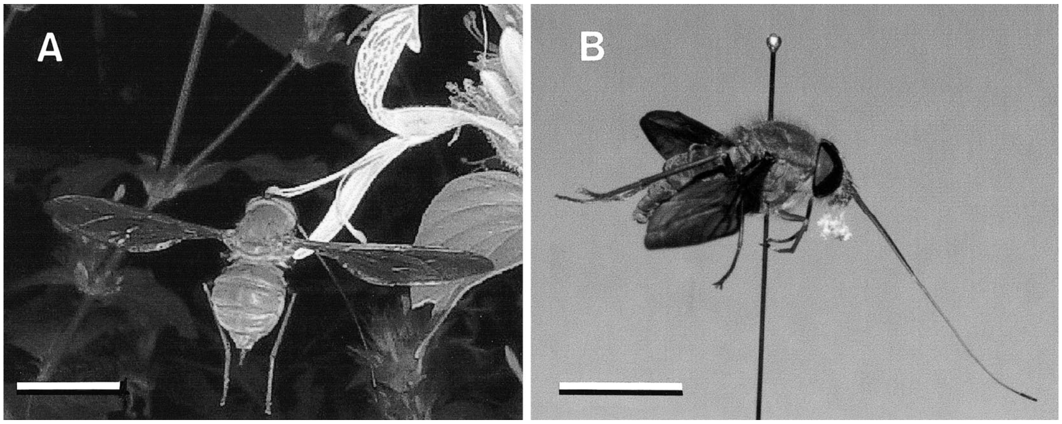

Figure 2. —A. *Stenobasipteron wiedemanni* visiting a flower of *Hypoestes aristata.* The proboscis is extended forward and upward on approach of the flower. —B. *S. wiedemanni,* with pollinaria of *Brownleea coerulea* attached to the base of the proboscis. This specimen also carried pollen from *Isoglossa hypoestiflora* on the dorsal surface, from *Hesperantha huttonii* on the face and from *Plectranthus* on the ventral surface. Scale bars 10 mm.

probed sequentially. The flies are highly mobile, strong fliers that can cover large distances in the forest understory. This is evident from the fact that we observed low frequency visitation at individual patches of studied plants. The relatively large body size (up to 20 mm long, excluding proboscis) offers many sites for pollen deposition (Fig. 2B).

THE FLORAL GUILD

Nineteen species from six plant families are currently included in the *Stenobasipteron wiedemanni* pollination Guild (Table 1). Members of this Guild have functional floral tube lengths that range from 16 to 39 mm (Table 1). This length coincides with that of the proboscis of *Stenobasipteron wiedemanni* (19–30 mm). Shorter-tubed species that also belong to the Guild have tube lengths of 6–21 mm, but often have filaments that approach the length of the long-tubed species (indicated by ^{1} in Table 1). *Plectranthus saccatus* Benth. contains forms with variable corolla tube length, hence the emphasis on the long-tubed forms relevant to this study.

Flowers of species that belong to this Guild are zygomorphic with flowers held horizontally, and where they tend to be more actinomorphic (e.g., *Hesperantha*), the floral tubes are held horizontally. Pollinators are thus directed to visit in a certain way. Nectar guides are present in many of the species.

DISTRIBUTION OF THE GUILD

The distribution of *Stenobasipteron wiedemanni* coincides with the Forest Biome along the eastern seaboard of South Africa (Fig. 3A). Similarly, members of the Guild coincide with forest and fly distribution (Figs. 3, 4). The presence of *S. wiedemanni* at Ongoye Forest, Oribi Gorge Nature Reserve (N.R.), Umtamvuna N.R., Ngeli Forest, Hlabeni Forest (Creighton), Hlatikhulu Forest, and Port St. Johns is based on new distribution records resulting from this study.

The maps only include South Africa (excluding Swaziland and Mozambique), but it is likely that the fly distributions, and that of some of the plant species involved in the Guild, extend into these neighboring countries.

FLOWER-POLLINATOR INTERACTION

A comparison of areas of pollen deposition on *Stenobasipteron wiedemanni* shows that few of the long-tubed plant species share the same site on the insect (Fig. 5 and listed in Table 1). In cases where this does happen, the plants do not co-occur (except in a case of two *Plectranthus* species at one of the study sites). Even though we did not study *Orthosiphon tubiformis* R. D. Good, we included it in Figure 5 to show where pollen would be deposited on the insect. It shares the same areas of deposition on the insect as long-tubed *Plectranthus* species, but is allopatric.

Nectar volumes range from 0.1 to 8.7 μl per flower with high maximum volumes found in the longer-tubed species (Table 2). Nectar sugar concentrations range from 24% to 33% sucrose equivalents (Table 2), which falls within the range reported by Goldblatt and Manning (2000). Nectar tends to be sucrose dominant, i.e., sucrose:hexose ratio > 1 (Table 2), with two species having sucrose

Table 1. Floral and other characters of plants pollinated exclusively or partially by *Stenobasipteron wiedemanni* (the "fly"). In five *Plectranthus* species with short and medium tubes, as well as in *Hypoestes aristata* (indicated with [1]), the style and stamen filament lengths approach that of the tube lengths of long-tubed species. F = forest, W = high-altitude open woodland/wooded savanna. [2] = narrow portion of floral tube, where tube length is longer, but sufficiently broad distally to allow access to a fly or bee body. See Appendix 2 for references for floral tube lengths and pollinator observations.

Plant species (arranged by family)	Habitat	Flower color	Length of floral tube spur (mm)	Area of pollen deposition on fly	Is the fly the only observed visitor to reach nectar?
Lamiaceae					
Plectranthus ambiguus	F	pinkish purple	20–33	ventral thorax & abdomen	yes
P. hilliardiae	F	pale mauve	21–32	ventral thorax & abdomen	yes
P. reflexus	F	pale blue	24–30	ventral thorax & abdomen	no
P. saccatus (long-tubed)	F	mauve to pale blue	20–30	ventral thorax & abdomen	yes
[1]*P. ecklonii*	F	bluish purple	10–15	ventral head & thorax	no
[1]*P. zuluensis*	F	pale/dark blue	12–13	ventral head & proboscis base	no
[1]*P. ciliatus*	F	white	6–8	proboscis	no
[1]*P. fruticosus*	F	bluish mauve	6–9	ventral head & thorax	no
[1]*P. praetermissus*	F	blue to purple	13–15	proboscis	no
Orthosiphon tubiformis	W	pale pink	20–36	ventral thorax & abdomen	yes
Acanthaceae					
Isoglossa hypoestiflora	F	mauve	24–27	dorsal thorax	yes
[1]*Hypoestes aristata*	F	bright pink	11–21	ventral thorax & abdomen	no
Barleria obtusa	F edge	blue to purple	[2]12–18	ventral & lateral thorax	no
Orchidaceae					
Brownleea coerulea	F	mauve	20–24	base of proboscis	yes
Balsaminaceae					
Impatiens hochstetteri subsp. *hochstetteri*	F	pink	(10–)16–24(–27)	proboscis	no
Gesneriaceae					
Streptocarpus formosus	F	pale violet	[2]31–36	dorsal body	yes
Iridaceae					
Hesperantha brevicaulis	—	pink	25–37	—	—
H. huttonii	F	purple	22–27; 33–39	ventral & lateral head & thorax	no
Gladiolus macneilii	W	pink	[2]32–37	dorsal thorax	yes

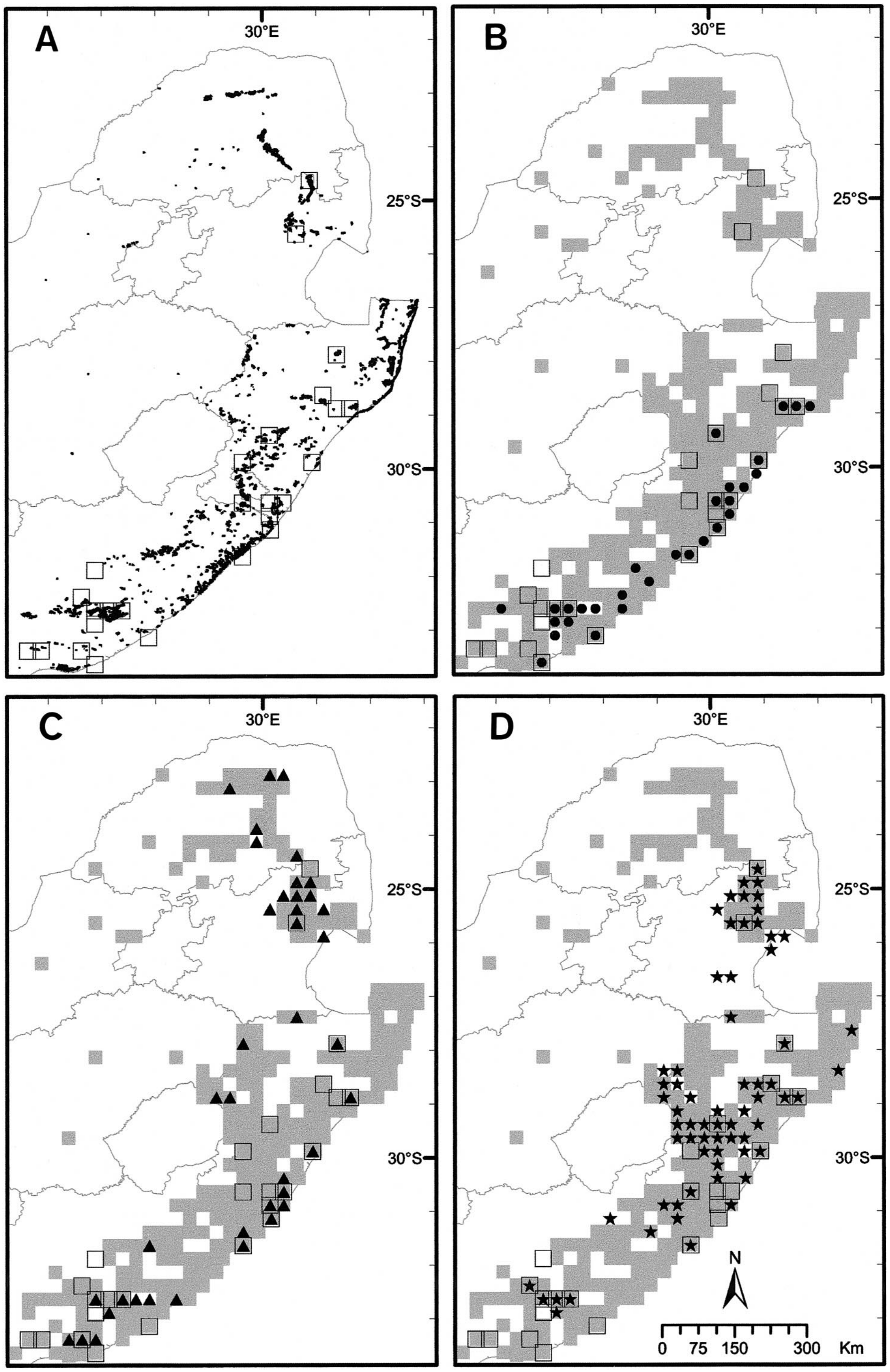

Figure 3. Distribution of forest biome, the fly (*Stenobasipteron wiedemanni*) and members of the *S. wiedemanni* pollination Guild in eastern South Africa. The forest biome is mapped at quarter-degree level for maps B–D (shaded squares). *Stenobasipteron wiedemanni* is shown in each map (□). Plant species are shown on different maps. —A. Actual extent of the forest biome (black spots). —B. *Plectranthus ambiguus* (●). —C. *Brownleea coerulea* (▲). —D. *Impatiens hochstetteri* subsp. *hochstetteri* (★).

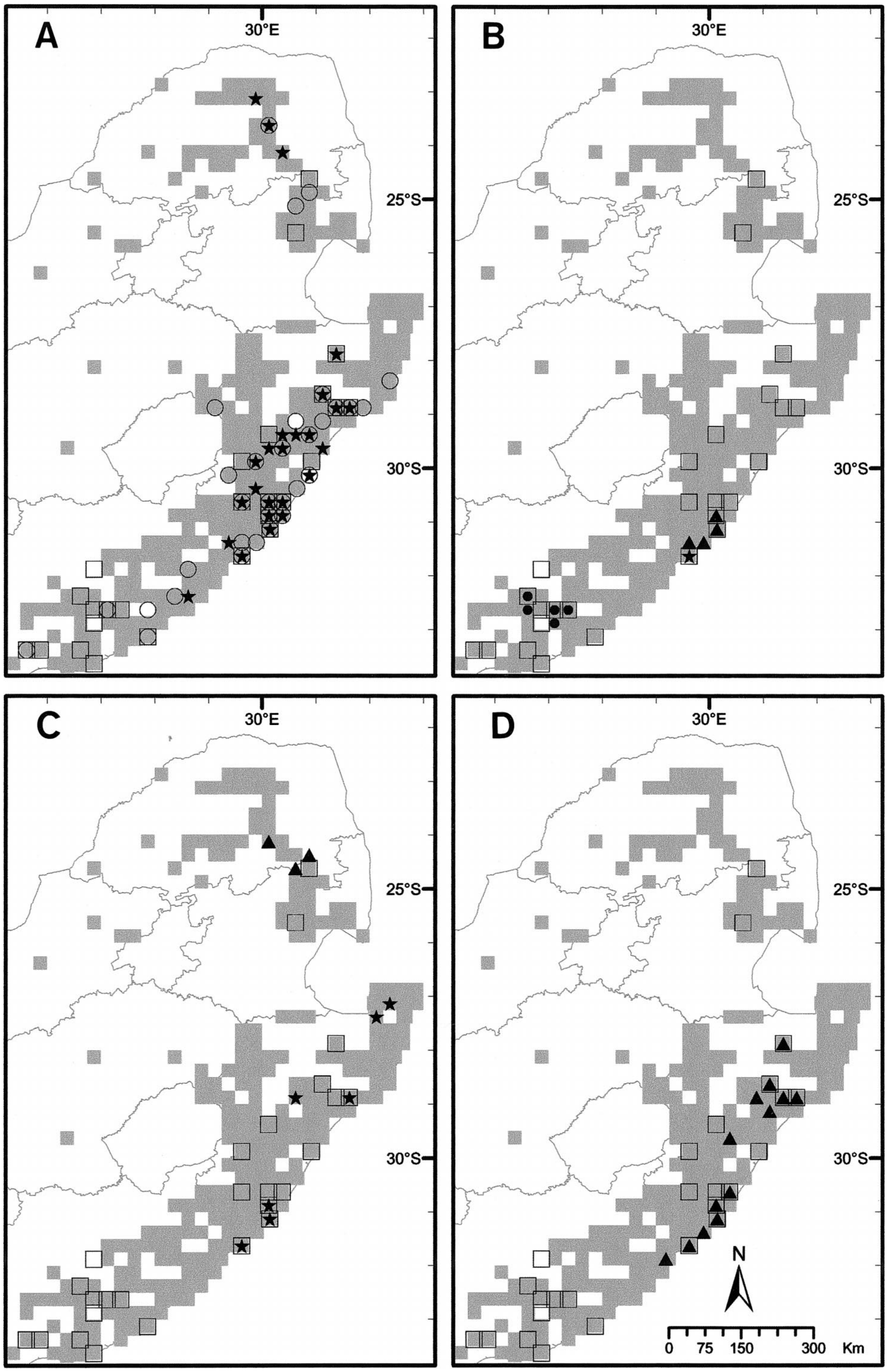

Figure 4. Distribution of forest biome, the fly (*Stenobasipteron wiedemanni*), members and inferred members of the *S. wiedemanni* pollination Guild in eastern South Africa. The forest biome is mapped at quarter-degree level for each map (shaded squares). *Stenobasipteron wiedemanni* is shown in each map (☐). Plant species are shown on different maps. —A. *Isoglossa hypoestiflora* (★), *Isoglossa cooperi* (○). —B. *Plectranthus hilliardiae* (▲), *P. reflexus* (★), *Hesperantha huttonii* (●). —C. *Orthosiphon tubiformis* (▲), *P. saccatus*—long-tubed forms (★). —D. *Asystasia varia* (▲).

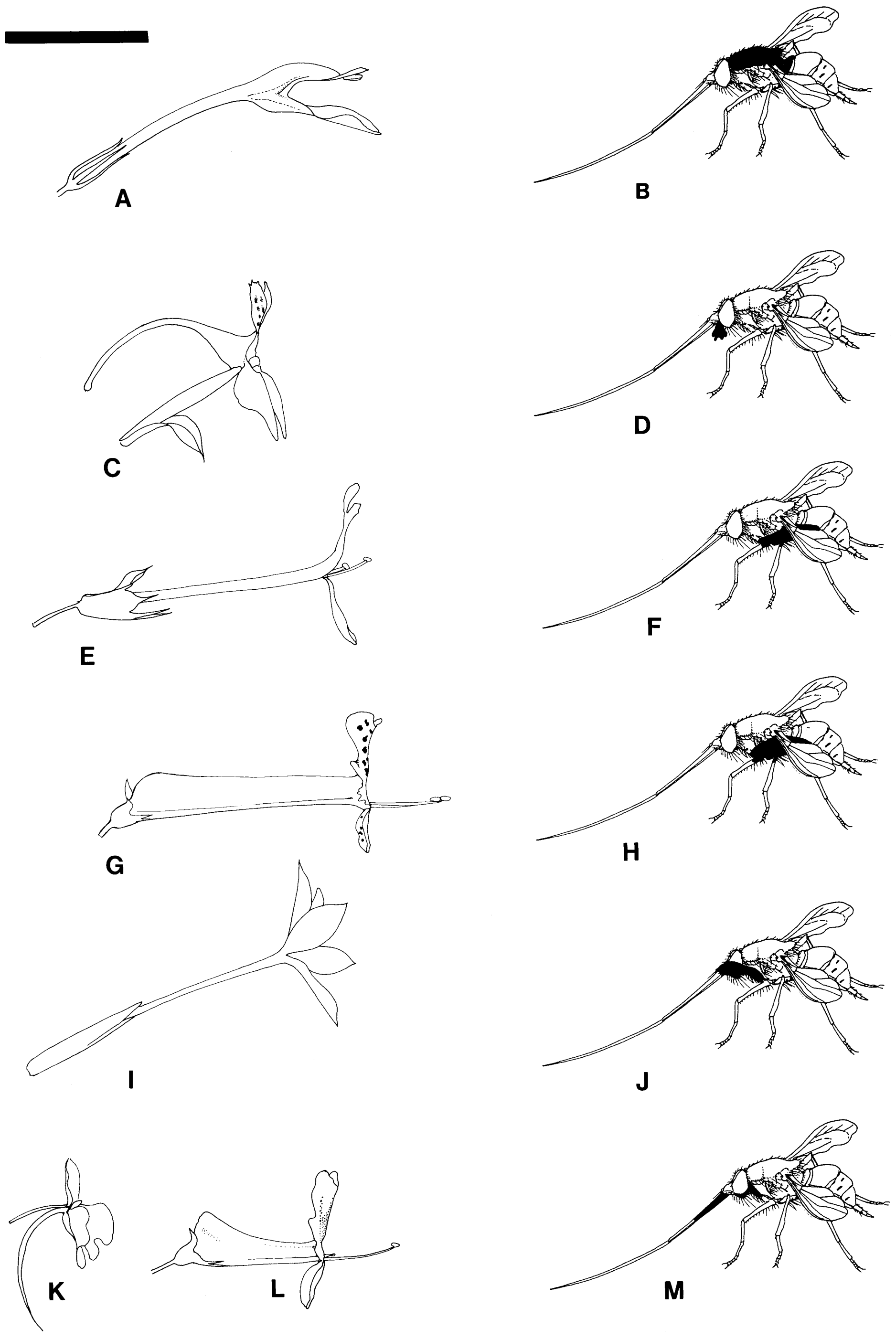

Figure 5. Areas of pollen deposition (indicated by black shading on insect) on *Stenobasipteron wiedemanni* for floral forms in the Guild. —A. *Isoglossa hypoestiflora* (Acanthaceae). —B. Pollen placed dorsally on thorax. —C. *Brownleea coerulea* (Orchidaceae). —D. Pollinaria attached at base of head and proboscis. —E. *Orthosiphon tubiformis* (Lamiaceae). —F. Pollen placed ventrally on thorax. —G. *Plectranthus hilliardiae* (Lamiaceae). —H. Pollen placed ventrally on thorax. —I. *Hesperantha huttonii* (Iridaceae). —J. Pollen placed ventrally and on lower sides of thorax and head. —K. *Impatiens hochstetteri* subsp. *hochstetteri* (Balsaminaceae). —L. *Plectranthus zuluensis* (Lamiaceae). —M. *Impatiens* (K) pollen placed on proboscis; *Plectranthus* (L) pollen placed ventrally on head and thorax. Scale bar 20 mm. Drawn by T. Edwards.

Table 2. Nectar sugars in species belonging to the *Stenobasipteron wiedemanni* pollination guild. G & M = data from Goldblatt and Manning (2000); otherwise data are from the present study. The four *Plectranthus* species with short or medium tubes are indicated by ^{1}.

Species	Nectar		Range of sugars %			Sucrose/	Reference
	Volume μl (n)	Conc. %	Fru	Glu	Suc	Fru + Glu	
Lamiaceae							
Orthosiphon tubiformis	2.7–4.1 (10)	24.5	0–1	0–5	94–100	25.2	G & M
Plectranthus reflexus	0.9–3.2 (8)	—	1	3	96	24.0	
P. hilliardiae	0.2–2.4 (40)	31	5	4	91	10.11	
P. ambiguus	—	—	24	22	54	1.17	
P. saccatus (long tube)	1.4–8.7 (10)	4–18	30	29	41	0.70	
^{1}*P. ecklonii*	—	—	9	16	75	3.0	
^{1}*P. ciliatus*	0.2–2.8 (25)	29–33	13	15	72	2.57	
^{1}*P. fruticosus*	—	24	19	25	56	1.27	
^{1}*P. zuluensis*	0.4–1.4 (11)	—	17–19	21–23	57–62	1.5	
Acanthaceae							
Isoglossa hypoestiflora	0.1–5.3 (27)	—	18	33	49	0.96	
Orchidaceae							
Brownleea coerulea	1.3–1.8 (2)	25–27	10	0–11	79–90	5.45	G & M
Iridaceae							
Gladiolus macneilii	4.5–5.8 (5)	26	—	—	—	—	G & M

rich nectar (sucrose:hexose ratio 0.5–0.99), using the categories of Baker and Baker (1990).

Notes on Pollination in Each Family

LAMIACEAE

In *Plectranthus* the deposition of pollen is sternotribic, since the bilabiate flowers have filaments and stamens that are ventrally aligned within the floral tube and lower lip. The long-tubed species force the fly, *Stenobasipteron wiedemanni*, to probe the floral tube fully, which maximizes pollen deposition and subsequent removal from the insect's body. Shorter-tubed species of *Plectranthus* are also visited by the fly, but it is not the only pollinator (Potgieter et al., 1999).

ACANTHACEAE

The flowers of *Isoglossa hypoestiflora* have long, narrow tubes (up to 27 mm, Table 1) that widen to form a raised palate with petal sculpturing on the lower lip, and a hooded upper lip where the anthers and stigma are positioned. Pollen transfer is nototribic on the thorax and abdomen of the fly, with a few grains placed on the proboscis. *Isoglossa cooperi* C. B. Cl. may be conspecific with *I. hypoestiflora*. The species were mapped separately in accordance with current taxonomy (Clarke, 1912).

Hypoestes aristata (Vahl) Sol. ex Roem. & Schult. has a distribution greater than that of the fly (extending into coastal parts of the Western Province and into Tropical Africa), and it is not exclusively pollinated by *Stenobasipteron wiedemanni*; xylocopid bees, tabanid flies, and papilionoid butterflies have been observed as floral visitors (G. Nicholls and G. Upfold, pers. comm.). Insects access nectar from the lilac, bilabiate, medium-tubed flowers (tubes up to 21 mm, Table 1), with filament and style lengths that reach 34 mm (measured at NU) that are crowded into verticillate inflorescences (Balkwill & Getliffe Norris, 1985). *Barleria obtusa* Nees was seen to be visited by *S. wiedemanni* at Oribi Gorge (F. Field, pers. comm.), but bees also visit this species.

ORCHIDACEAE

Brownleea coerulea Harv. ex Lindl. is the only orchid seen to be pollinated by *Stenobasipteron wiedemanni*. We studied it at Stutterheim, where the fly accesses nectar from the slightly recurved spur (20–24 mm long, Table 1) and picks up pollinaria at the base of the proboscis.

BALSAMINACEAE

Impatiens hochstetteri Warb. subsp. *hochstetteri* is a widespread African species ranging from northern Sudan to South Africa. It grows in shaded moist places—often in forests—and flowers throughout the year, with seasonal flowering in some areas

(Grey-Wilson, 1980). Corollas are pink with a curved calyx spur (10–)16–24 mm long (Grey-Wilson, 1980), but specimens from our study areas in KZN (measured at NU) have spur lengths 16–27 mm long. We have seen a number of visits by the fly, but never managed to collect a voucher. One was caught by M. Byrne at Nkandla Forest after observing visits to *I. hochstetteri* subsp. *hochstetteri* (McLellan, pers. comm.), but very little pollen remained on the voucher. *Impatiens* L. pollen adheres dorsally to the proboscis of the fly, since the short spur and sessile anthers prevent deposition elsewhere on the insect's body. The throat of the spur is exceedingly narrow, and the sessile anthers are situated directly above this aperture.

GESNERIACEAE

Streptocarpus formosus (Hilliard & B. L. Burtt) T. J. Edwards is pollinated by *Stenobasipteron wiedemanni* (J. Manning, pers. comm.). Flies enter the flower fully, since the 40–55 mm long corolla (Weigend & Edwards, 1994) is only narrow in the lower part (31–36 mm, Table 1). Pollen deposition is nototribic on the fly thorax. The pale violet corollas have dark stippling in the throat, which acts as a nectar guide, and yellow pigmentation on the floor of the corolla tube. It is a narrow endemic in forest habitats from Umtamvuna to the area just north of Oribi Gorge (NU records), and observations were made at Umtamvuna in January 2002.

IRIDACEAE

Hesperantha huttonii (Baker) Hilliard & Burtt is a day-flowering species with purple flowers and narrow, straight perianth tubes. Flowers have exserted anthers and perianth tube lengths that vary from 22 to 39 mm, which fall into two size classes (Table 1); those with longer tubes occur at the Katberg, but specimens collected outside of the Katberg (in the Amatole Mountains) have shorter tubes (Hilliard & Burtt, 1986), such as the plants studied at Stutterheim in this study (tubes 24–27 mm long). We found *Hesperantha* pollen on *Stenobasipteron wiedemanni* individuals caught in two different years at this site, but we never saw the visits. Our voucher plant specimen of *H. huttonii* had grains of *Plectranthus* pollen on the styles and anthers, which shows that pollinators that visited a nearby *Plectranthus* also visited *H. huttonii.* Subsequent to this observation, P. Goldblatt (pers. comm.) recorded *S. wiedemanni* visiting flowers of *H. huttonii,* confirming our indirect observations.

DISCUSSION

The suite of characteristic floral attributes associated with pollination by *Stenobasipteron wiedemanni* includes long, narrow floral tubes or spurs (Table 1); flower colors in shades of purple, mauve, pale blue, pink, and white; both subtropical and montane forest habitats; and the presence of nectar guides as darker spots or lines in certain species (e.g., *Brownleea coerulea, Hypoestes aristata, Plectranthus hilliardiae* Codd, *P. ecklonii* Benth., *P. zuluensis* T. Cooke, *P. ciliatus* E. Mey. ex Benth., *P. praeternissus* Codd, *P. fruticosus* L'Hér., *P. ambiguus* (Bolus) Codd, *Orthosiphon tubiformis,* and *Streptocarpus formosus*).

Nectar rewards are offered in all studied species and nectar is sucrose dominant or rich. Nectar concentrations are similar to those previously recorded for long-proboscid fly pollinated plants (Goldblatt & Manning, 2000).

Natural forest habitat in southern Africa is largely restricted to the eastern parts of the sub-continent, extending as far west as the eastern section of the Western Cape Province (not fully shown in Figs. 3, 4) (Low & Rebelo, 1996). Observations and museum records show that members of the *Stenobasipteron wiedemanni* Guild are largely limited to forested areas.

In Mpumalanga Province this Guild does not operate in the same kind of closed-canopy, moist forest. *Orthosiphon tubiformis* is visited by *S. wiedemanni* in rocky grassland that appears to have been recently transformed from more wooded to more open vegetation (Goldblatt, pers. comm.), and *Gladiolus macneilii* Oberm. occurs in more wooded savanna or forest margins (Manning et al., 1999). Nevertheless, there is still some tree cover and a similarity in temperature regimes. The KwaZulu-Natal and Eastern Cape forests where *S. wiedemanni* is found are warm coastal or cool mist-belt forests, while the Mpumalanga observations are from cooler, higher altitudes.

At least eight plant species within this pollination Guild appear to rely solely on *Stenobasipteron wiedemanni* for pollination (indicated in Table 1 as having the fly as the only observed visitor to reach nectar). In *Plectranthus reflexus* Van Jaarsv. & T. J. Edwards, an opportunist butterfly visitor was seen (Potgieter & Edwards, 2001), but it is likely that the corolla design is geared toward long-proboscid fly pollination. In *P. ambiguus* visits by two species of long-proboscid bees were seen (Potgieter et al., 1999), but they are unable to reach nectar in most flowers. Some specimens have shorter corolla tubes (20 mm is the lower end of the range, Table 1) and these may allow exploitation by bees. Other species in the Guild (with shorter corolla tubes) are not solely visited by *S. wiedemanni,* but still need to be included since the fly provides an efficient pollination service (Potgieter et al., 1999).

Discussion by Family

LAMIACEAE

Pollination of *Plectranthus hilliardiae, P. reflexus, P. ambiguus,* and long-tubed forms of *P. saccatus* has previously been discussed in Potgieter et al. (1999) and Potgieter and Edwards (2001). In most areas where *Stenobasipteron wiedemanni* has been observed, *Plectranthus* species (long- or short-tubed) tend to form a major component of the forest understory. *Orthosiphon tubiformis* occurs in different habitat to forest understory *Plectranthus* species and has been discussed by Goldblatt and Manning (2000).

ACANTHACEAE

Honeybees collect pollen from *Isoglossa hypoestiflora,* but the long, narrow corolla tube prohibits nectar access. Honeybees would not contribute to significant pollen carryover over large distances, since pollen is groomed into the scopae before the worker bees return to their hives. *Isoglossa cooperi* closely resembles *I. hypoestiflora* in floral shape and size, differing only in the presence of glandular hairs on the calyx and bracts. The species are often sympatric (Fig. 4A). From a pollination perspective, these two species are very similar and fit the *Stenobasipteron wiedemanni* pollination Guild. They occur in afromontane and scarp forest and are generically anomalous with respect to flower color and corolla morphology. Most *Isoglossa* Oerst. species in South Africa are hymenophilous (pers. obs.), with creamy white flowers and short corolla tubes (mostly less than 10 mm in length). These creamy white species are common in savanna and tropical and subtropical forests.

The observation of visits to *Hypoestes aristata* shows that *Stenobasipteron wiedemanni* may form part of a more generalized suite of pollinators in species with shorter floral tubes. As in shorter-tubed *Plectranthus* species, the fly still contributes to pollen carryover, since the elongate anther filaments deposit pollen on the body of the fly and the elongate style is able to remove pollen from this position. A similar situation exists in *Barleria obtusa,* where long-proboscid bees can also access nectar by crawling into the widened distal part of the corolla tube.

ORCHIDACEAE

The record for long-proboscid fly pollination in *Brownleea* Harv. ex Lindl. (Goldblatt & Manning, 2000) highlights the disjunction between grassland- and forest-growing species. *Brownleea macroceras* Sond. is pollinated by *Prosoeca ganglbaueri* (Johnson & Steiner, 1995), which occurs in montane grassland at high altitudes. By contrast, *B. coerulea* is a forest margin species of lower altitudes and is pollinated by *Stenobasipteron wiedemanni. Brownleea coerulea* is a widespread species (Fig. 3C) that also occurs in Madagascar, where its pollination has not been studied.

BALSAMINACEAE

The floral visits by *Stenobasipteron wiedemanni* to *Impatiens hochstetteri* subsp. *hochstetteri* are the first published records of nemestrinid fly pollination in the Balsaminaceae. This plant species is widespread across Africa, but the distribution of *S. wiedemanni* elsewhere in Africa is not known. Grey-Wilson (1980) recorded butterflies as pollinators of the “flat type” flowers in the section to which *I. hochstetteri* subsp. *hochstetteri* belongs, yet little information is available on the pollination of specific *Impatiens* L. (Grey-Wilson, 1980). We have observed papilionoid butterfly visits to *I. hochstetteri* subsp. *hochstetteri* at Ferncliffe Nature Reserve in Pietermaritzburg (KZN) confirming the above. The distribution of *Impatiens hochstetteri* subsp. *hochstetteri* in southern Africa (Fig. 3D) shows a number of plots outside forested areas. This species relies on moist forest habitats and may occur in scrub forest patches that are below the resolution of our Geographic Information System that was used for mapping.

GESNERIACEAE

Very little is known about pollination in *Streptocarpus* (Hilliard & Burtt, 1971), and it is likely that *Stenobasipteron wiedemanni* is the pollinator of a number of *Streptocarpus* species that conform to the floral morphology of *S. formosus.*

IRIDACEAE

The genus *Hesperantha* comprises many scented, pale species with crepuscular anthesis that are thought to be pollinated by moths (Goldblatt, 1984). Contrary to this pattern, *H. huttonii* is odorless, has diurnal anthesis and colored flowers—attributes suited to nemestrinid fly pollination. Long-proboscid fly pollination of day-flowering species of *Hesperantha* is not uncommon. *Hesperantha latifolia*

(Klatt) M. P. de Vos is pollinated by *Prosoeca peringueyi* in Namaqualand, while *H. grandiflora* G. J. Lewis, *H. scopulosa* Hilliard & B. L. Burtt, and *Hesperantha* cf. *woodii* Baker are pollinated by *P. ganglbaueri* in grasslands in the eastern parts of the country (Goldblatt & Manning, 2000). In Mpumalanga *Hesperantha brevicaulis* (Baker) G. J. Lewis is pollinated by *Stenobasipteron wiedemanni* (Goldblatt & Manning, 2000). The switch between moth and nemestrinid pollination was recently also recorded in *Zaluzianskya* F. W. Schmidt (Scrophulariaceae) by Johnson et al. (2002).

Inferred Members of the *Stenobasipteron wiedemanni* Guild

There are a number of other plant species that may belong to the *Stenobasipteron wiedemanni* pollination Guild. Labiate species such as *Stachys tubulosa* MacOwan, *Salvia repens* Burch. ex Benth. var. *keiensis* Hedge, *Salvia scabra* L.f., and *Hemizygia ramosa* Codd have been suggested as candidates (Potgieter & Edwards, 2001).

Manning et al. (1999) suggested that *Gladiolus sekukuniensis* P. Winter and *G. saxatalis* Goldblatt & J. C. Manning (both Iridaceae) are pollinated by *Stenobasipteron wiedemanni* in the highlands of Mpumalanga and Northern (Limpopo) Province, since they have similar morphology and phenology to *G. macneilii* and occur in forest margins and wooded savannas.

Streptocarpus species (Gesneriaceae) that may conform to this Guild include *S. rexii* (Bowie ex Hook.) Lindl. (forests of the Western and Eastern Cape Provinces, from Knysna to Kokstad), *S. primulifolius* Gand. (forests of the Eastern Cape Province and KwaZulu-Natal), and *S. cyaneus* S. Moore (forests of Mpumalanga Province).

Pelargonium transvaalensis (Geraniaceae) is a forest species of the eastern seaboard. It is likely that *Stenobasipteron wiedemanni* is the pollinator, since the hypanthial tubes are ca. 20 mm long and pink in color.

Rhinacanthus gracilis Klotzsch and *Asystasia varia* N. E. Br. (both Acanthaceae) are also likely to belong to this Guild; *R. gracilis* was inferred as a member of the *Prosoeca ganglbaueri* pollination system of Goldblatt and Manning (2000) that included *Stenobasipteron wiedemanni. Rhinacanthus gracilis* is a subtropical species of forest and woodland; it has mauve to white narrow floral tubes ca. 20 mm long. Similarly, *A. varia* has mauve flowers with tubes 35–40 mm long of which the basal half to two-thirds is narrowed, and the lower floral lobe has a raised palate with violet nectar guides (Edwards, 1988). It occurs in forest patches ranging from northern KwaZulu-Natal to the Eastern Cape, a distribution coinciding with that of *Stenobasipteron wiedemanni* (Fig. 4D). *Asystasia gangetica* T. Anderson and *A. pinguifolia* T. J. Edwards are bee-pollinated species of grasslands (Edwards, 1988) with shorter tubes and white flowers.

Asystasia varia may indicate a shift from bee to long-proboscid fly pollination associated with elongation of the floral tube and a flower color shift from creamy white to mauve. A similar shift may have happened in *Isoglossa* (also Acanthaceae), where most species have creamy white flowers with short corolla tubes (adapted to bee pollination), but *I. hypoestiflora* and *I. cooperi* have mauve flowers with longer tubes. Without cladistic analyses we can only speculate about such shifts. In the Iridaceae it has been shown that long-proboscid fly flowers are often closely related to ancestors pollinated by long-tongued bees (Goldblatt & Manning, 2000).

Conclusions

The *Stenobasipteron wiedemanni* pollination Guild should be recognized separately from the three Guilds suggested by Goldblatt and Manning (2000). There is no overlap in the plant species pollinated by this fly compared to the other three Guilds. On the basis of habitat there is no or very little overlap in the distribution of the fly species. Unlike the mostly pink flowers with dark pink to red markings pollinated by the *Prosoeca ganglbaueri* Guild (sensu Goldblatt & Manning, 2000), the *S. wiedemanni* Guild consists of flowers with shades of purple, mauve, pale blue, pink, and white. In cases where flower color is similar, e.g., the blue-flowered *Nivenia stenosiphon* Goldblatt and pale pink-flowered *Nerine* Herb. and *Brunsvigia* Heist. species that were included in the *Prosoeca ganglbaueri* Guild of Goldblatt and Manning (2000), there is no overlap in plant species at the microgeographic scale. The *S. wiedemanni* pollination Guild is thus limited by the (forest) distribution of the fly, rather than by floral color or shape.

We have confirmed the existence of long-proboscid fly pollination in the Acanthaceae, as suggested by Goldblatt and Manning (2000) and report it here for the first time in the families Balsaminaceae and Gesneriaceae.

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Appendix 1. Vouchers of *Stenobasipteron wiedemanni* (Diptera, Nemestrinidae) lodged at Natal Museum, Pietermaritzburg. KZN = KwaZulu-Natal Province, E Cape = Eastern Cape Province.

Collector's no.	Locality (all in South Africa)	Plant	Date
C. Potgieter 1	KZN: Umtamvuna	*Plectranthus ambiguus*	17 Mar. 1995
C. Potgieter 2	KZN: Umtamvuna	*P. ambiguus*	18 Mar. 1996
C. Potgieter 3	KZN: Umtamvuna	*P. ambiguus*	15 Mar. 1996
C. Potgieter 102	KZN: Oribi Gorge	*P. ciliatus*	25 Mar. 1997
C. Potgieter 103	KZN: Oribi Gorge	*P. zuluensis*	25 Mar. 1997
C. Potgieter 154	KZN: Karkloof, Leopards Bush	*P. fruticosus*	3 Mar. 1999
C. Potgieter 155	KZN: Karkloof, Leopards Bush	in spider web	3 Mar. 1999
C. Potgieter 170	KZN: Ongoye Forest	on ground (with *I. hypoestiflora* pollen)	22 Apr. 1999
C. Potgieter 191	E Cape: Port St Johns	*P. praetermissus*	9 Mar. 1998
C. Potgieter 192	E Cape: Stutterheim, Kologha Forest	*P. ciliatus*	5 Mar. 1998
C. Potgieter 193	E Cape: Stutterheim, Kologha Forest	*P. ciliatus*	5 Mar. 1998
C. Potgieter 194	E Cape: Stutterheim, Kologha Forest	*P. ecklonii*	5 Mar. 1998
C. Potgieter 195	E Cape: Stutterheim, Kologha Forest	*P. ecklonii*	5 Mar. 1998
C. Potgieter 197	E Cape: Stutterheim, Kologha Forest	*P. ecklonii*	29 Feb. 2000
C. Potgieter 198	E Cape: Stutterheim, Kologha Forest	*Brownleea coerulea*	29 Feb. 2000
F. Field s.n.	KZN: Oribi Gorge	*Barleria obtusa*	5 Apr. 1997
M. Byrne s.n.	KZN: Nkandla Forest	*Impatiens hochstetteri*	31 Jan. 2001

Appendix 2. Vouchers for studied plant species lodged at NU Herbarium, Pietermaritzburg. References for pollinator observations and/or floral tube measurements are given where this has been recorded in addition to observations in the current study. P & E = Potgieter & Edwards, P et al. = Potgieter et al., G & M = Goldblatt & Manning, G-W = Grey-Wilson, W & E = Weigend & Edwards. KZN = KwaZulu-Natal Province, E Cape = Eastern Cape Province, PMB = Pietermaritzburg.

Plant species (arranged by family)	Collector's no.	Locality (all in South Africa)	Reference of previous work
Lamiaceae			
Plectranthus ambiguus (Bolus) Codd	*C. Potgieter 86*	KZN: Umtamvuna	P & E (2001)
P. hilliardiae Codd	*C. Potgieter 110, 111, 112*	KZN: Umtamvuna	P & E (2001)
P. reflexus Van Jaarsv. & T. J. Edwards	*T. Edwards 1554*	E Cape: Port St Johns	P & E (2001)
P. saccatus Benth. (long-tubed)	*C. Potgieter 107, 120*	KZN: Umtamvuna	P & E (2001)
P. ecklonii Benth.	*C. Potgieter 65, 114*	KZN: Oribi Gorge	P et al. (1999)
	C. Potgieter 70	KZN: Umtamvuna	
P. praetermissus Codd	*T. Edwards 1556*	E Cape: Port St Johns	
P. zuluensis T. Cooke	*C. Potgieter 64, 118*	KZN: Oribi Gorge	P et al. (1999)
P. ciliatus E. Mey. ex Benth.	*C. Potgieter 67*	KZN: Oribi Gorge	P et al. (1999)
	C. Potgieter 68, 69, 116	KZN: Umtamvuna	
	C. Potgieter 136	KZN: PMB, Ferncliffe	
P. fruticosus L'Hér.	*C. Potgieter 66*	KZN: Oribi Gorge	
	C. Potgieter 134	KZN: PMB, Ferncliffe	
	C. Potgieter 144	KZN: Karkloof, Leopards Bush	
Orthosiphon tubiformis R. D. Good			G & M (2000); P & E (2001)
Acanthaceae			
Isoglossa hypoestiflora Lindau	*C. Potgieter s.n.*	KZN: Oribi Gorge	G. Nicholls (pers. comm.)
	C. Potgieter & D. Thompson 735	KZN: Creighton, Hlabeni Forest	
Hypoestes aristata (Vahl) Sol. ex Roem. & Schult.			G. Nicholls (pers. comm.)
Barleria obtusa Nees			F. Field (pers. comm.)
Orchidaceae			
Brownleea coerulea Harv. ex Lindl.	*Edwards & Potgieter 1841*	E Cape: Stutterheim, Kologha Forest	G & M (2000)
Balsaminaceae			
Impatiens hochstetteri Warb. subsp. *hochstetteri*	*C. Potgieter & D. Thompson 744*	KZN: Creighton, Hlabeni Forest	McLellan (pers. comm.); G-W (1980)
Gesneriaceae			
Streptocarpus formosus (Hilliard & B. L. Burtt) T. J. Edwards			W & E (1994); Manning (pers. comm.)
Iridaceae			
Hesperantha brevicaulis (Baker) G. J. Lewis			G & M (2000)
H. huttonii (Baker) Hilliard & B. L. Burtt	*Edwards & Potgieter 1841a*	E Cape: Stutterheim, Kologha Forest	Goldblatt (pers. comm.)
Gladiolus macneilii Oberm.			G & M (1999)

CHAPTER 6:

NATURAL HYBRIDS

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Microdistillation and essential oil chemistry—a useful tool for detecting hybridisation in *Plectranthus* (Lamiaceae)

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Abstract

The essential oil composition is reported for *Plectranthus ciliatus*, *Plectranthus zuluensis* and their putative hybrid. The essential oil chemistry is in support of morphological data and pollination studies, which have indicated a natural hybrid between *P. ciliatus* and *P. zuluensis*. The hybrid plant contains terpenoids from both putative parents together with 'hybrid compounds,' which are not present in any of the two parents. The composition of the essential oil obtained through microdistillation is virtually identical to the analysis of the hydrodistilled essential oil.

Keywords: *Plectranthus*; Essential oil; Chemotaxonomy; Hybridisation

1. Introduction

In southern Africa, the Lamiaceae are most abundantly represented by the genus *Plectranthus* with 48 indigenous species (Hankey, 1999; Van Jaarsveld and Edwards, 1997; Edwards et al., 2000). This group of aromatic Labiates have been the subject of various taxonomic studies (Codd, 1975, 1985; Van Jaarsveld and Edwards, 1991, 1997; Edwards et al., 2000). The species in some complexes are notoriously difficult to identify. Pollination studies in the genus have revealed a number of natural hybrids amongst the medium-tubed species of *Plectranthus*, particularly at Oribi Gorge Nature Reserve (3030CA) in southern KwaZulu-Natal, South Africa. Potgieter et al. (2000) illustrated that the leaf and flower morphology of the putative *Plectranthus ciliatus* × *Plectranthus zuluensis* hybrid appear to be intermediate to that of the sympatric putative parents. *P. ciliatus* E. Mey. ex Benth is the only species in the study area that has nectar guides on both upper and lower corolla lips; thus, the presence of this character in the hybrid plant indicates putative parentage in *P. ciliatus* × *P. zuluensis*. Hybrids of *P. ciliatus* × *P. zuluensis* show a general habit and purple colouration on the lower leaf surface that is intermediate to that of the putative parent species. Corolla shape, size and colouration are also intermediate to that of the putative parents. Furthermore, two different dipteran species were seen to visit and move between the inflorescences of sympatric populations of *P. zuluensis* T. Cooke and *P. ciliatus* at Oribi Gorge; these are *Stenobasipteron wiedemanni*, a long-proboscid fly (family Nemestrinidae), and *Psilodera confusa*, a medium-proboscid fly (family Acroceridae) illustrated in Potgieter et al. (1999).

Hybrids are not unknown in this genus of horticulturally interesting plants and many crosses have been made by plant breeders (E. van Jaarsveld, personal communication). Natural hybridisation has a pronounced impact on the understanding of taxonomic relationships (reticulate vs. divergent evolution). As hybrids could often defy detection by morphology alone, additional information is required to detect and/or confirm possible hybridisation events. The aromatic character of *Plectranthus* leaves allows for the extraction and analysis of the essential oils. The aim of this investigation was to determine if essential oil chemistry could be used to confirm hybridisation in *Plectranthus*. As hybrid plants are often scarce and the essential oil yields for *Plectranthus* species is generally very low, microdistillation was investigated as an alternative method to extract the essential oils and compare it to the

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Table 1
Essential oil composition for (1) *P. ciliatus* (ex hort Witwatersrand Botanical Garden), (2) *P. ciliatus* (Ferncliff), (3A) *P. ciliatus* × *P.* zuluensis (ex Oribi Gorge), hydrodistilled oil, (3B) *P. ciliatus* × *P. zuluensis* (ex Oribi Gorge), microdistilled oil, (4) *P. zuluensis* (ex hort Witwatersrand Botanical Garden), (5) *P. zuluensis* (ex Oribi Gorge)

RRI : Relative Retention Indices

RRI	Compound	1	2	3A	3B	4	5
1032	α-Pinene	0.26	–	0.08	1.06	0.02	0.02
1035	α-Thujene	–	–	–	0.08	–	–
1076	Camphene	–	–	–	0.04	–	–
1118	β-Pinene	0.01	0.01	2.44	18.01	0.01	0.01
1132	Sabinene	0.01	0.03	0.08	0.45	0.01	–
1159	δ-3-Carene	0.12	–	0.47	2.49	–	–
1174	Myrcene	0.09	–	–	–	0.01	–
1176	α-Phellandrene	–	–	0.08	0.62	–	–
1187	*o*-Cymene	–	–	0.01	0.13	–	–
1188	α-Terpinene	–	–	0.01	0.48	–	–
1195	Dehydro-1,8-cineole	–	–	–	0.03	–	–
1203	Limonene	3.80	0.04	0.11	0.56	0.01	0.01
1205	Sylvestrene	0.02	–	–	–	–	–
1213	1,8-Cineole	–	0.01	–	–	0.01	0.01
1218	β-Phellandrene	–	–	0.07	0.66	–	–
1224	*o*-Mentha-1(7),5,8-triene	–	–	–	0.05	–	–
1225	(*Z*)-3-Hexenal	–	–	–	0.04	–	–
1232	(*E*)-2-Hexenal	–	–	–	–	–	0.02
1244	Amyl furan (=2-Pentyl furan)	–	–	–	0.02	–	–
1246	(*Z*)-β-Ocimene	1.27	–	0.02	0.09	–	–
1255	γ-Terpinene	0.02	–	0.13	3.58	–	–
1266	(*E*)-β-Ocimene	0.42	–	0.01	0.05	–	–
1278	*m*-Cymene	0.01	–	0.10	0.31	–	–
1280	*p*-Cymene	0.12	0.02	1.18	2.01	–	–
1286	Isoterpinolene	–	–	0.02	0.13	–	–
1290	Terpinolene	0.07	–	0.02	0.28	–	–
1319	(*E*)-2,6-Dimethyl-1,3,7-nonatriene	0.01	–	–	0.01	0.01	–
1345	3-Octyl acetate	–	0.07	0.02	–	–	0.04
1360	Hexanol	–	–	–	–	–	0.02
1382	*cis*-Alloocimene	0.02	–	–	–	–	–
1386	Octenyl acetate	0.76	4.25	0.06	0.09	–	–
1391	(*Z*)-3-Hexenol	–	–	–	–	–	0.02
1393	3-Octanol	–	0.03	0.02	0.01	0.05	0.05
1400	Nonanal				0.01	–	–
1406	α-Fenchone	–	–	–	–	0.01	–
1412	(*E*)-2-Hexenol	0.01	–	–	–	–	–
1415	Rose furan	0.02	–	–	–	–	–
1452	α,*p*-Dimethylstyrene	0.01	–	0.03	0.14	–	–
1452	1-Octen-3-ol	1.11	1.83	0.71	0.13	0.07	0.13
1466	α-Cubebene	0.15	0.10	0.05	0.04	0.04	0.11
1468	*trans*-1,2-Limonene epoxide	0.07	–	–	–	–	–
1474	*trans*-Sabinene hydrate	0.16	0.02	0.02	0.03	–	–
1479	δ-Elemene	–	0.09	–	–	–	–
1476	(*Z*)-β-Ocimene epoxide	0.01	–	–	–	–	–
1478	*cis*-Linalool oxide (*Furanoid*)	–	–	–	0.01	–	–
1495	Bicycloelemene	0.07	0.43	0.04	0.03	0.21	0.03
1492	Cyclosativene	0.90	–	0.08	0.04	–	–
1493	α-Ylangene	–	1.23	–	–	–	–
1497	α-Copaene	2.25	0.48	1.53	0.76	1.16	2.97
1528	α-Bourbonene	0.01	0.22	0.03	–	0.29	0.22
1532	Camphor	–	–	–	0.02	–	–
1535	β-Bourbonene	0.21	3.08	0.36	0.05	3.85	2.65
1544	α-Gurjunene	1.00	0.08	0.07	0.04	0.03	–
1545	*cis*-α-Bergamotene	–	0.08	0.06	–	–	–
1548	(*E*)-2-Nonenal	–	–	–	–	0.02	–
1549	β-Cubebene	0.26	0.28	0.44	0.18	0.60	1.25
1553	Linalool	0.21	0.30	0.98	2.14	1.24	6.21
1565	Linalyl acetate	–	–	–	–	–	0.06
1568	1-Methyl-4-acetylcyclohex-1-ene	–	0.04	0.03	–	–	–
1571	*trans*-*p*-Menth-2-en-1-ol	0.03	0.01	0.01	0.01	–	–

Table 1 (*continued*)

RRI : Relative Retention Indices

RRI	Compound	1	2	3A	3B	4	5
1572	β-Ylangene	0.06	0.51	0.09	0.03	–	0.39
1586	Pinocarvone	–	–	0.03	0.09	–	–
1594	*trans*-β-Bergamotene	–	0.34	0.21	0.09	–	–
1597	Bornyl acetate	0.10	5.48	0.02	0.09	–	–
1598	Thymol methyl ether (=Methyl thymol)	–	–	–	0.04	–	–
1599	(*E*,*Z*)-2,6-Nonadienal	–	–	–	–	0.01	–
1600	β-Elemene	0.81	1.16	0.56	0.25	0.38	0.36
1611	Terpinen-4-ol	–	–	–	0.01	–	–
1612	β-Caryophyllene	9.29	2.64	3.35	3.90	6.19	7.45
1614	Carvacrol methyl ether (=Methyl carvacrol)	–	–	–	0.06	–	–
1617	6,9-Guaiadiene	0.12	0.61	–	–	–	–
1638	*cis*-*p*-Menth-2-en-1-ol	–	–	0.02	0.04	–	–
1648	Myrtenal	–	–	0.04	0.11	–	–
1628	Aromadendrene	0.05	0.20	–	–	–	–
1639	*trans*-*p*-Mentha-2,8-dien-1-ol	0.06	–	–	–	–	–
1650	γ-Elemene	0.02	0.48	–	–	–	–
1658	Sabinyl acetate	–	–	1.44	2.51	–	–
1661	Alloaromadendrene	3.82	1.12	–	–	0.40	–
1664	*trans*-Pinocarveol	–	–	0.08	0.11	–	–
1668	(*Z*)-β-Farnesene	–	0.03	0.12	0.04	0.02	0.02
1674	γ-Gurjunene	–	0.20	–	–	0.54	0.23
1677	*epi*-Zonarene	0.16	–	–	–	–	–
1684	β-Guaiene	–	0.20	–	–	–	–
1687	α-Humulene	0.86	0.35	8.19	6.99	21.40	9.24
1698	Myrtenyl acetate	–	–	0.53	0.88	–	–
1700	*p*-Mentha-1,8-dien-4-ol (=Limonen-4-ol)	–	–	–	0.06	–	–
1704	γ-Muurolene	0.26	0.40	–	–	–	–
1706	α-Terpineol	0.17	–	0.08	0.51	–	0.11
1708	Ledene	2.09	–	–	–	–	–
1709	α-Terpinyl acetate	–	–	–	0.06	–	–
1726	Germacrene D	3.10	9.37	6.41	5.52	7.11	6.53
1740	α-Muurolene	0.38	–	–	–	–	–
1741	β-Bisabolene	–	1.32	0.74	0.29	–	–
1743	Eremophilene	–	–	–	–	0.06	–
1747	*trans*-Carvyl acetate	2.54	–	0.10	0.13	–	–
1755	Bicyclogermacrene	3.54	16.82	1.73	1.30	7.28	1.41
1773	δ-Cadinene	11.77	0.33	1.28	0.88	0.17	0.28
1776	γ-Cadinene	–	0.25	0.06	–	0.02	0.02
1782	*cis*-Carvyl acetate	0.34	–	–	2.00	–	–
1784	(*E*)-α-Bisabolene	–	5.97	8.10	2.56	–	–
1786	Kessane	–	–	–	0.13	–	–
1798	Methyl salicylate	–	–	–	0.16	–	–
1799	Cadina-1,4-diene (=Cubenene)	0.15	–	–	–	–	–
1804	Myrtenol	–	–	0.24	0.07	–	–
1808	Nerol	–	–	–	–	–	0.03
1810	3,7-Guaiadiene	0.08	–	–	–	–	
1830	2,6-Dimethyl-3(*E*),5(*E*),7-octatriene-2-ol	0.05	–	–	–	–	–
1838	β-Damascenone	–	–	–	0.03	–	–
1845	*trans*-Carveol	0.22	–	0.18	0.09	–	–
1853	*cis*-Calamenene	0.39	–	–	–	–	–
1853	Dehydrocostuslactone	–	–	10.61	6.86	–	–
1854	Germacrene-B	–	2.60	–	–	–	–
1857	Geraniol	–	–	–	–	0.01	0.08
1864	*p*-Cymen-8-ol	0.10	–	0.91	0.95	–	–
1871	*p*-Mentha-1,8-dien-10-yl acetate	0.01	–	–	–	–	–
1882	*cis*-Carveol	0.04	–	–	0.02	–	–
1900	*epi*-Cubebol	6.19	0.10	0.05	–	0.02	0.05
1941	α-Calacorene	0.26	0.03	–	–	–	–
1945	1,5-Epoxy-salvial-4-14-ene	–	0.16	–	–	0.06	0.02
1953	Palustrol	1.07	0.04	–	0.01	–	–
1957	Cubebol	1.95	0.27	0.07	–	–	0.05

(continued on next page)

Table 1 (*continued*)

RRI : Relative Retention Indices

RRI	Compound	1	2	3A	3B	4	5
1958	β-Ionone	–	–	–	0.02	–	–
1981	(*Z*)-Methyl cinnamate	–	–	–	–	0.01	–
1984	γ-Calacorene	0.27	–	–	–	–	–
2001	Isocaryophyllene oxide	–	0.14	0.10	–	0.05	0.03
2008	Caryophyllene oxide	1.50	1.13	0.55	0.37	0.19	0.17
2030	Methyl eugenol	–	–	0.13	–	8.56	2.93
2033	Epiglobulol	–	0.80	–	–	–	–
2037	Salvial-4(14)-en-1-one	–	0.12	0.04	–	–	–
2045	Humulene epoxide-I	–	–	–	–	0.10	0.02
2050	(*E*)-Nerolidol	–	–	0.32	0.14	0.16	0.06
2057	Ledol	7.42	0.11	0.21	0.12	–	–
2069	1,6-Germacradien-5β-ol (=Germacrene *D*-4β-ol), (=1(10),5-Germacradien-4β-ol)	2.24	0.46	–	–	–	–
2071	Humulene epoxide-II	–	–	0.79	0.42	0.71	0.16
2080	Cubenol	0.93	–	–	–	–	0.02
2081	Humulene epoxide-III	–	–	–	–	0.05	0.01
2088	1-*epi*-Cubenol	1.18	–	–	–	–	–
2096	Elemol	–	1.52	2.37	1.23	–	–
2096	(*E*)-Methyl cinnamate	–	–	–	–	0.02	–
2103	Guaiol	–	–	0.23	0.17	–	–
2104	Viridiflorol	0.55	0.27	–	–	0.03	–
2109	*cis*-Methylisoeugenol	–	–	–	–	0.51	0.78
2127	10-*epi*-γ-Eudesmol	–	–	9.00	6.50	–	–
2144	Spathulenol	2.23	15.52	1.11	0.36	0.60	0.09
2185	γ-Eudesmol	–	–	0.15	0.51	–	–
2187	T-Cadinol	3.38	–	–	–	–	–
2200	*trans*-Methylisoeugenol	–	–	–	–	0.62	2.79
2202	1,6-Germacradien-5α-ol (=Germacrene *D*-4α-ol), (=1(10),5-Germacradien-4α-ol)	0.15	–	–	–	–	–
2219	δ-Cadinol	0.68	–	–	–	–	–
2209	T-Muurolol	1.26	0.22	–	–	–	–
2232	α-Bisabolol	–	1.33	1.33	0.46	–	–
2245	Elemicine	–	–	4.30	2.16	10.56	0.63
2247	*trans*-α-Bergamotol	0.16	0.86	–	–	–	–
2255	α-Cadinol	2.99	0.66	–	–	–	0.04
2257	β-Eudesmol	–	–	0.40	0.38	–	–
2282	γ-Asarone	–	–	0.74	0.20	14.93	35.76
2361	(*Z*)-Asarone	–	–	–	–	–	2.48
2403	*trans*-Isoelemicine	–	–	–	0.02	2.79	1.54
2478	(*E*)-Asarone	–	–	–	–	0.15	3.66
2324	Caryophylla-2(12),6(13)-dien-5α-ol (=Caryophylladienol-II)	0.06	–	–	–	–	–
2438	Kaur-16-ene	0.07	–	–	–	–	–
2607	14-Hydroxy-δ-cadinene	0.02	–	–	–	–	–
2676	*epi*-13-Manool	0.37	–	1.32	0.03	–	–
	Total (%)	89.00	86.85	77.70	85.72	91.36	91.27

composition of hydrodistilled oils. Although the essential oil composition has been reported for some species of *Plectranthus* (Ascensão et al., 1998; Buchbauer et al., 1993; Smith et al., 1996), research on the South African representatives of this genus has been neglected. This paper forms part of a broader investigation on the composition, chemotaxonomy and biological activity of the essential oil of South African *Plectranthus* species.

2. Materials and methods

Leaf material of *P. ciliatus*, *P. zuluensis* and their putative hybrid were collected from various localities (Table 1). Only one clone of the putative hybrid was present; hence, only one collection could be made. Voucher specimens of *P. ciliatus* (Ferncliff) C. Potgieter 745, *P. ciliatus* × *P. zuluensis* (Oribi Gorge) C. Potgieter 556 and *P. zuluensis* (Oribi Gorge) C. Potgieter 64 are deposited at the University of KwaZulu–Natal Herbarium (UN). Vouchers of *P. ciliatus* (WBG) AV 78 and *P. zuluensis* (WBG) AV 23 are housed in the Department of Pharmacy and Pharmacology, University of the Witwatersrand. The essential oils were obtained through hydrodistillation (3 h) using a Clevenger apparatus. In addition, leaves collected from the hybrid plant (*P. ciliatus* × *P. zuluensis*) were air dried and subjected to microdistillation using the following method; crushed leaves (~500 mg) were placed in a sample vial together with 10 ml of water. NaCl (2.5 g) and water (0.5 ml) were placed in the collecting vial. *n*-Hexane (300 μl) was added into the collecting vial to trap volatile components. Sample vials were heated to 108 °C at a rate of 20 °C/min and then kept at 108 °C for 90 min, heated to 112 °C at a rate of 20 °C/min and kept at this temperature for 30 min. Finally the samples were subjected to post-run for 6 min under the same conditions. Collecting vials were cooled to −1 °C during distillation. After distillation was completed, the organic layer in the collection vial was analyzed by GC/MS.

The essential oils were analysed using a Hewlett-Packard G1800A GCD system. Innowax FSC column (60 m × 0.25 mm Ø, with 0.25 μm film thickness). Helium (0.8 ml/min) was used as carrier gas. GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min and then kept constant at 220 °C for 10 min to 240 °C at a rate of 1 °C/min. Mass range was recorded from *m*/*z* 35 to 425. Split ratio was 50:1 and the splitless mode was used for essential oil samples obtained from micro-distillation. Injection port temperature was at 250 °C. MS were taken at 70 eV. Relative percentage amounts of the separated compounds were calculated automatically from peak areas of the total ion chromatogram. Library searches were carried out using the Wiley GC/MS Library and the Başer Library of Essential Oil Constituents. Cluster analysis was carried out with the NTSYS-PC package version 2.00 (Rohlf, 1997). The entire data set as presented in Table 1 was converted to qualitative data (presence/absence) and a hierarchical cluster analysis was performed using the UPGMA clustering algorithm.

3. Results and discussion

The compounds identified in the essential oil for all the taxa analyzed are summarized in Table 1. Eighty-three compounds were identified in the essential oil of *P. ciliatus* (89%) from the Witwatersrand Botanical Garden and sixty-two compounds (86.9%) were identified in the essential oil obtained from *P. ciliatus* from Ferncliff. Although quantitative variations are apparent, the essential oil composition from the two plants growing some ca. 600 km apart is similar as indicated in the dendrogram presented (Fig. 1). Both samples contained 6,9-guaiadiene, aromadendrene, γ-elemene, γ-muurolene, α-calacorene, 1,6-germacradien-5β-ol, T-muurolol, *trans*-α-bergamotol and α-cadinol. These compounds are absent in both *P. zuluensis* and the putative *P. ciliatus* × *P. zuluensis* hybrid. The high correlation coefficient (Fig. 1) suggests that the oil composition of the two samples obtained for *P. zuluensis* are congruent. In both instances, α-humulene, germacrene D and γ-asarone are the major compounds.

The morphological data and field observations presented in the Introduction prompted us to investigate the essential oil as an independent test to confirm hybridisation events in *Plectranthus*. Demarne and Van der Walt (1989) and others (Emboden and Lewis, 1967; Kokkini, 1992; Viljoen and Van Wyk, 2001) have illustrated the value of using phytochemical data to confirm the origin of natural hybrids. Certain compounds found only in *P. ciliatus* are present in the hybrid (e.g., ledol) while some terpenoids found only in *P. zuluensis* are present in the hybrid (e.g., γ-asarone). A chemotaxonomic survey of the essential oils of South African species of *Plectranthus* shows that γ-asarone is restricted to *P. zuluensis* (Maistry, 2001). Compounds found in both parents are present in the hybrid (e.g., linalool). 'Hybrid compounds' are absent in both parents and only found in the hybrid, e.g., 10-epi-γ-eudesmol. It could be hypothesised that new compounds form when enzymes which were previously mutually exclusive in the two parents combine in the hybrid forming 'new hybrid phytochemicals'. Recently Viljoen and Van Wyk (2001) and Viljoen (1999) demonstrated very similar chemical patterns for phenolic compounds in artificial and natural hybrids of the genus *Aloe*. The cluster analysis (Fig. 1) shows the hybrid plant nested between the putative parents which is indicative of chemical 'characters' shared with both *P. ciliatus* and *P. zuluensis*. Fig. 1 also clearly indicates that the composition of the hydrodistilled oils and the oil obtained through micro-distillation are virtually identical. Microdistillation is a very quick and accurate method to extract the oils from small amounts of low-yielding leaf material such as *Plectranthus* hybrids.

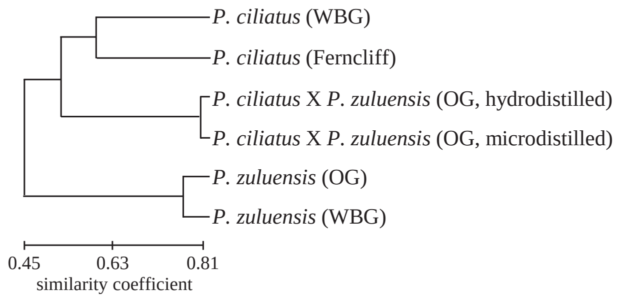

Fig. 1. Dendrogram constructed on qualitative data (absence/presence) using all compounds in Table 1. WBG = Witwatersrand Botanical Garden, Ferncliff = Ferncliff Nature Reserve, Pietermaritzburg, OG = Oribi Gorge Nature Reserve.

The importance of hybridisation was noticed by Linnaeus who proposed a model of speciation through hybridisation. Lotsy (1916, 1931) identified hybridisation as the most important factor in evolutionary change. Hybrids do, however, pose a problem to systematics as it is often believed that 'good species' do not hybridise. Many species concepts consider the process of natural hybridisation at best to be nonexistent and that they make taxonomic treatments difficult (Arnold, 1997). Since the cladistic method is based on the assumption of divergence, it is imperative to determine the role of hybridisation in any taxonomic study as this could lead to reticulate phylogenies. The example presented here provides evidence that natural hybridisation occurs in *Plectranthus*, which is instrumental in unraveling the evolutionary history and taxonomy of this genus.

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CHAPTER 7: NECTAR STUDIES

Nectar is primarily composed of water and sugars, containing lesser amounts of a variety of possible compounds, e.g. amino acids, lipids, antioxidants, alkaloids, proteins, vitamins, inorganic ions, organic acids, phenolics and terpenoids (Nicolson & Thornburg 2007). It constitutes the main calorific reward to almost all known pollinators (Dafni 1992). Nectar is a reward that is not part of the sexual system of the plant, thus a tight correlation between pollinator demands and behaviour, and the composition, amount and rhythm of secretion of nectar, is not surprising. It is widely accepted that nectar plays an important role in plant-pollinator interactions and this reflects the co-evolution between plants and pollinators (Dafni 1992). Since nectar characteristics tend to be similar for plants that are visited by the same groups of animals, nectar may show differences between related plants that have different pollinators (Kearns & Inouye 1993).

The nectary of *Plectranthus*, as in the rest of the Lamiaceae, is gynobasic, with an asymmetrical disc at the base of the four-lobed ovary (Dafni *et al*. 1988, Petanidou *et al*. 1999). Nectar is secreted at the base of a tubular corolla and visiting insects require proboscides of sufficient length to access it.

In the current study, (A) nectar sugar composition was analysed, and (B) nectar sugar concentration and nectar volume were measured, for a selection of species.

A. Nectar Sugar Composition

Nectar sugars are derived from sucrose that is translocated in phloem sap. The enzyme invertase (located in nectaries) determines the final composition of nectar by hydrolysing sucrose to glucose and fructose; the extent of this reaction determines the nectar sugar ratio (Nicolson & Fleming 2003). Sucrose and the hexose sugars fructose and glucose are the most common nectar sugars, the occurrence and relative proportions of which tend to remain constant in any one species (Percival 1961), but which may show wide inter-specific differences (Baker & Baker 1983b). The ratios in which these sugars occur have traditionally been correlated with the type of pollinator involved (Baker & Baker 1983a, 1990; Percival 1961). Percival (1961, 1965) showed that sucrose-dominated nectars are common in long-tubed flowers pollinated by bumble- and honey bees (i.e. long-tongued bees), as well as butterflies and moths. In contrast, shallow flowers with relatively unprotected nectar tend to produce hexose-dominated nectars.

The following nectar classes were identified by Baker & Baker (1983a) on the basis of sucrose to hexose ratios, with corresponding % sucrose indicated as per Nicolson & Thornburg (2007):

‘Sucrose-dominant’: sucrose:hexose ratio > 1; sucrose 51 – 100 %
‘Sucrose-rich’: sucrose:hexose ratio 0.5 – 1; sucrose 34 – 50 %
‘Hexose-rich’: sucrose:hexose ratio 0.1 – 0.5; sucrose 10 – 33 %
‘Hexose-dominant’: sucrose:hexose ratio < 0.1; sucrose 0 – 9 %

Baker & Baker (1983a) tabulated insect pollinator preference for the four nectar classes and showed that hawkmoths prefer ‘sucrose-dominant’ and ‘sucrose-rich nectars’, while other Lepidoptera such as settling moths, butterflies and skippers tolerate a greater range from ‘sucrose-dominant and -rich’, to ‘hexose-rich’ nectar. The latter is also true for long-tongued bees, with ‘sucrose-dominant’ nectar being most popular. Short-tongued bees and butterflies, on the other hand, show a strong preference for ‘hexose-dominant and -rich’ nectar. Wasps and beetles show a more or less equal preference for ‘sucrose- or hexose-dominant’ nectar (observation based on a low sample size), while flies seem to prefer ‘hexose-dominant and -rich nectar’, with a few preferring higher sucrose ratios (Baker & Baker 1983a).

Percival (1961) warned that the observed pattern of certain pollinator classes preferring certain types of nectar may be overridden by the plant family to which the species belongs, hence a phylogenetic effect may operate. This is echoed by Dafni (1992), Van Wyk (1993) and Nicolson & Van Wyk (1998) who caution that, in addition to considering pollinator demands, one should also consider plant phylogeny when studying the components of nectar, since genetic constraints limit the base on which selection can act.

The recent review on nectar chemistry by Nicolson & Thornburg (2007) cautions against using the terminology (outlined above) suggested by Baker & Baker (1983a), since it over-emphasises sucrose; describing nectar sugar ratios as the percentage sugar composition is preferable (Nicolson & Thornburg 2007). Both practises are followed in this chapter, with new results presented as % sucrose and interpreted w.r.t.

Baker & Baker's (1983a) categories, while discussions involving past studies will utilise the nectar sugar categories in inverted commas, for ease of comparison.

B. Nectar Volume and Concentration

Knowledge of nectar volume, concentration, composition and spatial distribution is considered important for the interpretation of pollinator behaviour, as well as understanding pollinator energetics and nutrient requirements (Kearns & Inouye 1993). A study of honeybee attraction in nine species of Lamiaceae was conducted by Dafni *et al*. (1988) with the intention of correlating a number of floral traits (including nectar volume and concentration) and the rate of attraction of honeybees, but the results failed to establish any clear relationships. Experimental manipulations of irrigation and fertilisation showed some surprising results in a study on three species of Lamiaceae in the phrygana, with volume and sugar content varying considerably between species and treatments (Petanidou *et al*. 1999). Nectar production was studied under natural and experimental conditions in three species of Lamiaceae by Macukanovic-Jocic *et al*. (2004), and results showed variation in volume and concentration in response to microclimate (habitat) and other factors.

The techniques used to collect nectar, and the limitations imposed by small flowers with small nectar volumes in particular, introduce possible sources of variation in nectar volume and concentration estimates. It is for this reason that Nicolson & Thornburg (2007) caution against the practise of attributing ecological significance to nectar concentration, especially in cases where averages are used.

Materials and Methods

A. Nectar Sugar Analysis

The nectar sugar ratios of 14 species of *Plectranthus* were analysed, as well as an additional seven varieties or forms of species. Two species that fall within the broader concept of *Plectranthus* (Paton *et al*. 2004) were included: one *Pycnostachy*s (*Py. urticifolia* Hook.) and one *Thorncroftia* N.E.Br. (*T. longiflora* N.E.Br), as well as five other species of Lamiaceae for comparison: three *Orthosiphon* Benth. (*O. tubiformis* R.D.Good [=*Ocimum tubiforme* (R.D.Good) A.J.Paton], *O. labiatus* N.E.Br. [=*Ocimum labiatum* (N.E.Br.) A.J.Paton], *O. serratus* Schltr. [=*Ocimum serratum* (Schltr.) A.J.Paton]), and two *Hemizygia* (Benth.) Briq. (*H. albiflora* (N.E.Br.) M.Ashby

[=*Syncolostemon albiflorus* (N.E.Br.) D.F.Otieno], *H. incana* Codd [=*Syncolostemon incanus* (Codd) D.F.Otieno]). One member of the Acanthaceae, *Isoglossa hypoestiflora* Lindau, was also analysed since it falls within the nemestrinid fly (*Stenobasipteron wiedemanni* Lichtwardt, 1910) pollination syndrome.

Sample preparation and analysis followed that outlined by Tanowitz & Smith (1984), with some modifications. Nectar was sampled by pulling the corolla tubes from the calyces of freshly harvested flowers and squeezing the tubes gently to force nectar from their bases. These droplets were spotted onto Whatman's No. 1 filter paper and a circle was drawn in pencil to mark the spots before they dried. Nectar was allowed to air-dry and filter paper was then stored in separate stamp collector's envelopes in a container with silica gel to keep it dry until analysis.

Nectar spots were carefully cut from filter paper discs and collected in pill vials. In species with very little nectar per flower, a number of nectar spots had to be pooled to obtain a sufficient sample for Gas Chromatographic (GC) analysis. This amount was determined by trial and error and was recorded for each species (it varied from 4 to 30 flowers per sample, according to nectar volume). Usually several flowers were sampled from the same plant, to reduce potential variation.

Nectar was dissolved by adding 1 ml of 80% re-distilled ethanol to each vial with the nectar sample disc, closing it and sonicating for 15 min., then heating it for 10 min. at 40^{o}C. Samples were dried down under a nitrogen stream at 40^{o}C. Sugars were converted to their oxime forms by adding 0.5 ml of a solution containing 25 mg.ml^{-1} of hydroxylamine hydrochloride and 6 mg.ml^{-1} of phenyl-β-D-glucoside in dry, silylation grade pyridine and heating at 40^{o}C for 20 min. Samples were cooled to room temperature and 0.1 ml of each was removed and placed in another pill vial and reduced to dryness under a nitrogen stream at 40^{o}C. Trimethylsilyl derivatisation of sugars was achieved by adding 0.2 ml Sylan BTZ to each sample and leaving it to react at room temperature for 15 min. Samples were stored in Eppendorf tubes and refrigerated until GC analysis. Care was taken to analyse samples within two days of preparation.

Sugar standards of sucrose, glucose and fructose were prepared in the same way by dissolving 5 mg of sugar standard in 5 ml of 80% re-distilled ethanol in a pill vial, heating it at 40^{o}C until completely dissolved (and sonicating if necessary). The technique outlined above was followed to convert sugars to their oxime forms and for trimethylsilyl derivatisation.

Nectar sugars were analysed by injecting a 1 µl sample into a Varian 3700 Gas Chromatograph equipped with a 1.8 m x 6 mm O.D. glass column packed with OV-17 on Chromosorb HP 80/100, with a flame ionising detector. Helium gas was used as a carrier and chromatographic conditions were: injector temperature 200^{o}C, ion detector temperature 300 ^{o}C, with program conditions: 3 min. at 125^{o}C, raising temperature at 4^{o}C.min.$^{-1}$ to 270^{o}C and holding at 270^{o}C for 10 min. Each run took about one hour to complete and allow the machine to cool down to the starting temperature.

Peak integration was performed using an HP 3800A integrator and sample peaks were compared to those of standards. In cases of very low sugar concentration in samples (i.e. low nectar yield), attenuation threshold was dropped to its lowest limit for detection. The phenyl-β-D-glucoside that was included with the solvent during sample preparation acted as an internal standard to provide consistency for each run. In samples with very low sugar concentration (and hence small peaks) this internal standard confirmed that the correct amount of sample had been injected and that the run was successful.

Nectar sugar percentages were calculated from integrated areas for sucrose, glucose and fructose, which were the major sugar peaks detected.

Variability in nectar sugar composition was tested using a number of samples of *P. oertendahlii* T.C.E.Fr., a species that is endemic to the Oribi Gorge area and that showed initial results contrary to expectation. Samples of pooled flowers, analysed by GC, were compared to pooled and single-flower samples sent away for High Performance Liquid Chromatography (HPLC) analysis by B.-E. van Wyk at the University of Johannesburg. A number of duplicate samples of *P. ecklonii* Benth. were also analysed, using GC and compared with HPLC performed by Tracy Odendaal at the University of KwaZulu-Natal (UKZN), Pietermaritzburg campus. Duplicate samples of *P. oribiensis* Codd and *T. longiflora* were also analysed.

B. Nectar Volume and Concentration

Nectar volume ranges were recorded for 19 species or varieties of *Plectranthus*, and *Py. urticifolia*.

Nectar volumes were measured by using calibrated micro-capillaries as well as the spot-size technique recorded in Dafni (1992). In the latter, nectar was collected from detached corollas by squeezing the corolla gently and spotting the nectar onto Whatman No. 1 filter paper; the diameter of the spot was measured and then a circle was drawn around the spot so that this nectar could be used for later sugar analysis. The diameter of each spot was converted to a volume measurement using known nectar volumes from Dafni (1992: Table 3, Chapter 5, p 140). In the first method corollas were detached and the micro-capillary was held at the base of the corolla to take up nectar for a direct reading.

In most cases nectar volume and concentration were measured mid-morning, using cultivated plants in greenhouses where insects do not enter, or garden plants that were covered by netting at dawn, which meant that no nectar had yet been taken by insects. Volume results reflect the maximum amount of nectar available to insects.

Nectar concentrations were initially recorded for ten species of *Plectranthus*, using an Atago hand-held refractometer (type N1). Some species of *Plectranthus* have small nectar volumes, thus a number of flowers were used to give pooled samples since the refractometer was not capable of measuring small samples. Another refractometer (Bellingham and Stanley Eclipse hand-held refractometer), capable of measuring smaller samples, was subsequently used (in 2005) to measure nectar concentration. Consequently, individual flower nectar concentrations are known for 8 species of *Plectranthus*.

Results

A. Nectar Sugar Analysis

Examples of GC traces are shown in Fig. 1. The hexose peaks appear first (fructose, then glucose), followed by the internal standard (phenyl-β-D-glucoside), with sucrose appearing last.

The majority of studied *Plectranthus* species have (truly) sucrose-dominant nectar, ranging from 50 – 96% sucrose (Table 1). All three species of *Orthosiphon* (71 – 81% sucrose) and *Py. urticifolia* (87% sucrose) had sucrose-dominant nectar, while the two species of *Hemizygia* (both 0 % sucrose) as well as *T. longiflora* (6% sucrose) had hexose-dominant nectar.

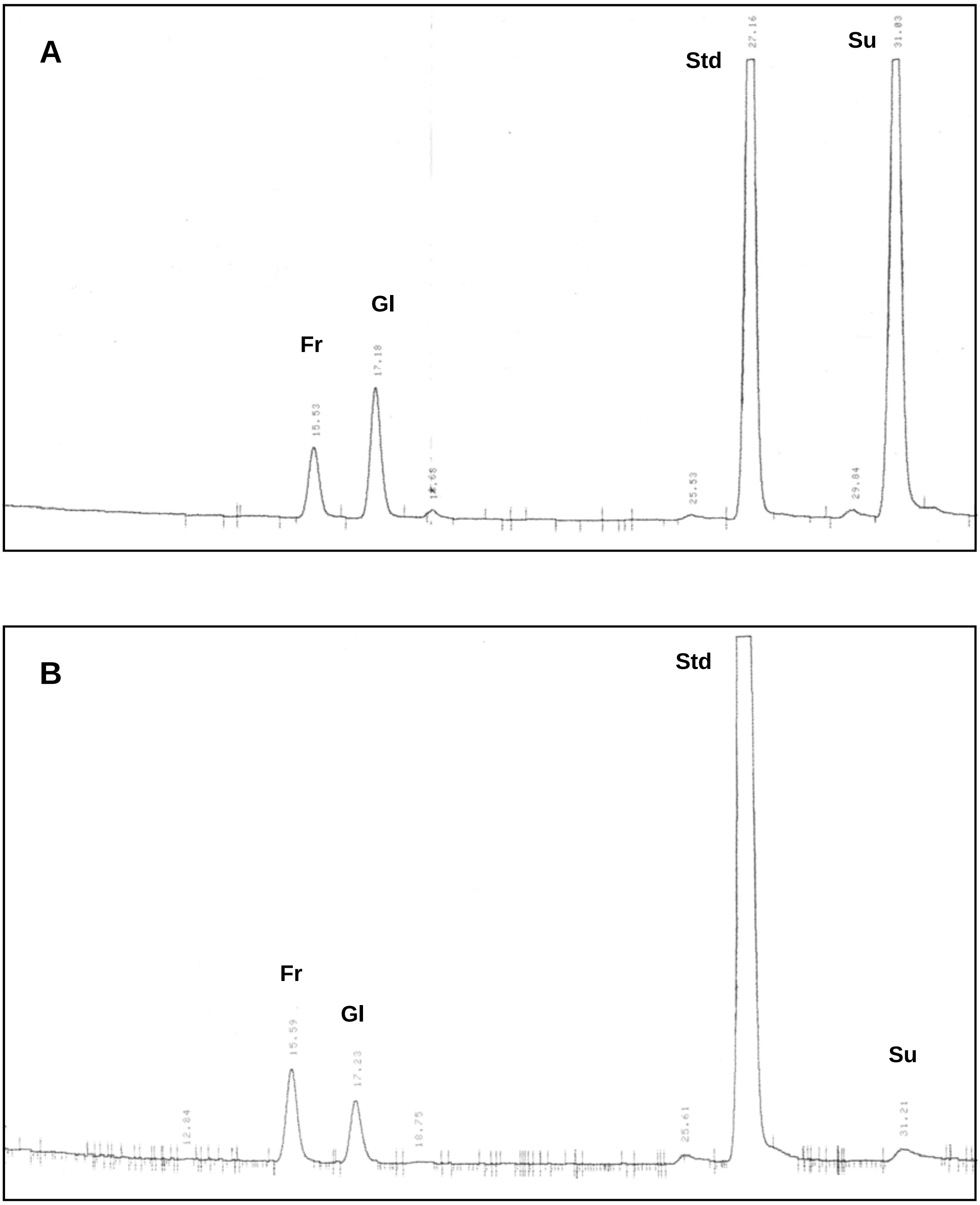

Figure 1: Examples of gas chromatographic (GC) traces of nectar sugars for two species of *Plectranthus*. A: *P. ecklonii* (sucrose-dominant), B: *P. oertendahlii* (hexose-dominant sample). Fr: Fructose, Gl: Glucose, Su: Sucrose, Std: Internal standard – phenyl-β-D-glucoside.

Table 1: Nectar sugar properties of *Plectranthus* and other species analysed in this study, using Gas Chromatography (GC), with an indication of main pollinator class. Plant vouchers are lodged at Bews Herbarium (NU).
CP: C. Potgieter, NV: no voucher; Fr: Fructose, Gl: Glucose, Su: Sucrose; S/H nectar: 'True' nectar sugar class dominance; S: more than 50% sucrose, H: more than 50% hexose; B&B nectar: Nectar sugars classified according to Baker & Baker (1983a); HR: 'hexose-rich', HD: 'hexose-dominant', SR: 'sucrose-rich', SD: 'sucrose-dominant'; Apin: Bee (Apidae, Apinae), Mega: Bee (Apidae, Megachilinae), Nem: Fly (Nemestrinidae), Acro: Fly (Acroceridae), Tab: Fly (Tabanidae). Species marked with an asterisk***** are visited for nectar by the nemestrinid fly, *S. wiedemanni*, or expected to be visited by a *Stenobasipteron* species.

Species	**% Fr**	**% Gl**	**% Su**	**S/H nectar**	**B&B nectar**	**Voucher**	**Pollinator class**
***Plectranthus* spp.**							
P. reflexus *****	1	3	**96**	S	SD	CP 95	Nem
P. hilliardiae *****	5	4	**91**	S	SD	CP 112	Nem
P. petiolaris (purple)	3	7	**90**	S	SD	CP 115	Apin
P. ernstii	5	5	**90**	S	SD	CP 85	Apin
P. oribiensis	2	13	**85**	S	SD	CP 102	Apin
P. hadiensis	4	11	**85**	S	SD	CP 92	Apin, Nem
P. ecklonii *****	9	16	**75**	S	SD	CP 114	Nem, Apin, Tab
P. ciliatus *****	13	15	**72**	S	SD	CP 116	Acro, Apin
P. zuluensis (pale blue) *****	17	21	**62**	S	SD	CP 118	Acro
P. zuluensis (dark blue) *****	20	23	**57**	S	SD	CP N12	Acro
P. fruticosus (long spur) *****	19	25	**56**	S	SD	CP 126	Nem
P. ambiguus *****	24	22	**54**	S	SD	CP 86	Nem
P. laxiflorus	17	33	**50**	50:50	SR	CP 927	Apin, Nem
P. oertendahlii	53	36	**11**	H	HR	CP N13	Acro
Varieties of *P. saccatus*							
short, white	22	21	**57**	S	SD	CP 131	Acro?/Apin?
long tube *****	30	29	**41**	H	SR	CP 120	Nem
medium, fine spots	30	28	**42**	H	SR	NV	Nem?
medium, speckled *****	33	31	**36**	H	SR	CP 109	Nem
medium, no spots	41	37	**22**	H	HR	NV	Nem?
medium, blotched	45	37	**18**	H	HR	CP 108	Nem?
medium, pale spots	50	43	**7**	H	HD	NV	Nem?
Other Lamiaceae							
Pycnostachys urticifolia	5	8	**87**	S	SD	CP 1064	Meg
Orthosiphon labiatus	4	16	**80**	S	SD	CP N7	?
Orthosiphon serratus	7	15	**78**	S	SD	CP N8	?
Orthosiphon tubiformis *****	7	22	**71**	S	SD	CP N6	Nem
Stachys tubulosa *****	19	23	**58**	S	SD	CP N4	?
Stachys grandifolia	29	34	**37**	H	SR	CP N5	?
Thorncroftia longiflora	12	60	**28**	H	HR	CP N3	Nem?
Hemizygia albiflora	69	31	**0**	H	HD	NV	?
Hemizygia incana	60	40	**0**	H	HD	NV	?
Acanthaceae							
Isoglossa hypoestiflora *****	16	34	**50**	50:50	SR	CP N10	Nem

Of the seven forms of *P. saccatus* that were analysed, two medium-tubed forms and one long-tubed form had ‘sucrose-rich’ nectar (36 – 42% sucrose), three medium-tubed forms had ‘hexose-rich or -dominant’ nectar (7 – 22 % sucrose) and the short-tubed succulent form from Umtamvuna was ‘sucrose-dominant’ (57% sucrose). In reality, six of the forms had truly hexose-rich nectars, while only the short-tubed form had nectar with more than 50% sucrose.

Initial GC results, analysed in Pietermaritzburg in 2003, showed *P. oertendahlii* to have ‘hexose-dominant’ to ‘hexose-rich’ nectar (0–1% sucrose), with the small percentage of sucrose in the latter case only detected when integrator attenuation was set at the lowest setting (Table 2). Subsequent samples, analysed by HPLC at the University of Johannesburg, showed varying results; pooled flower samples had sucrose percentages of 54 – 59%, while individual flowers varied from 38 – 60% sucrose.

Table 2: Variability in nectar sugar properties of samples of *Plectranthus oertendahlii*, a species endemic to Oribi Gorge (OG). All samples were collected in the field, at OG, except for sample 1, which was from material cultivated in a greenhouse. Some samples represent pooled nectar from different flowers; some are from individual flowers. Plant vouchers are lodged at Bews Herbarium (NU).
CP: C. Potgieter, NV: no voucher; GC: Gas Chromatography, done by C. Potgieter; HPLC: High Performance Liquid Chromatography, done by B.-E. van Wyk; Fr: Fructose, Gl: Glucose, Su: Sucrose; S/H nectar: ‘True’ nectar sugar class dominance, S: more than 50% sucrose, H: more than 50% hexose; B&B nectar: Nectar sugars classified according to Baker & Baker (1983a), HR: ‘hexose-rich’, HD: ‘hexose-dominant’, SR: ‘sucrose-rich’, SD: ‘sucrose-dominant’.

Plant no.	**Sample details**	**Sample collection date**	**Method & date**	**Voucher**	**% Fr**	**% Gl**	**% Su**	**S/H nectar**	**B&B nectar**
1	Pooled	Feb ‘99	GC, June ‘03	CP 96	58	42	**0**	H	HD
2a	Pooled	March ‘03	“	CP N13	59	41	**0**	H	HD
2 b	Above: Re-run, low attenuation	“	“	CP N13	53	36	**11**	H	HR
3a	Single flower	Feb ‘04	HPLC, June ‘04	CP N11	36	26	**38**	H	SR
3b	Single flower	“	“	CP N11	20	20	**60**	S	SD
3c	Single flower	“	“	CP N11	22	23	**55**	S	SD
3d…	Pooled; same plant as above	“	“	CP N11	20	22	**58**	S	SD
4	Pooled; plants of same patch	“	“	NV	22	24	**54**	S	SD
5	Pooled; plants of different patches	“	“	NV	24	17	**59**	S	SD

To test whether these results could be attributed to the GC technique that was followed for the majority of species shown in Table 1, samples of *P. ecklonii* were re-analysed using HPLC in Pietermaritzburg. Samples from the same plant, collected on the same day, showed 65% sucrose with GC and 77 – 89% sucrose with HPLC (Table 3). Repeat analyses of the same sample by HPLC showed, in three cases, the same or very similar results (Table 3). A repeat sample of *P. oribiensis*, analysed with GC in both cases, showed the same result (85% sucrose), while repeat samples of *T. longiflora*, using GC, showed slightly different results (6% and 28% sucrose), but both were truly hexose-dominant (Table 3).

Table 3: Duplicate samples and nectar sugar analysis technique checks (GC / HPLC), for three species of Lamiaceae. Plant vouchers are lodged at Bews Herbarium (NU). Sub-samples a & b of samples 2 – 4 & 7 are duplicate analyses of the same nectar sample.
GC: Gas Chromatography, HPLC: High Performance Liquid Chromatography; Fr: Fructose, Gl: Glucose, Su: Sucrose; S/H nectar: 'True' nectar sugar class dominance, S: more than 50% sucrose, H: more than 50% hexose; B&B nectar: Nectar sugars classified according to Baker & Baker (1983a); HR: 'hexose-rich', HD: 'hexose-dominant', SD: 'sucrose-dominant'.

Species & sample collection date	**Sample no.**	**Analysis method & date**	**% Fr**	**% Gl**	**% Su**	**S/H nectar**	**B&B nectar**
P. ecklonii C. Potgieter N9 (all from same plant, cultivated in UKZN botanical garden)							
April 2008	1	GC: C. Potgieter April 2008	14	21	**65**	S	SD
June 2008	2a	HPLC: T. Odendaal June 2008	6	14	**80**	S	SD
"	2b	"	6	14	**80**	S	SD
"	3a	"	3	8	**89**	S	SD
"	3b	"	4	10	**86**	S	SD
"	4a	"	8	16	**76**	S	SD
"	4b	"	8	15	**77**	S	SD
P. oribiensis C. Potgieter 102 (from same cultivated cutting, at UKZN botanical garden)							
March 1999	5	GC: C. Potgieter July 2003	2	13	**85**	S	SD
February 2005	6	GC: C. Carbutt February 2005	2	13	**85**	S	SD
Thorncroftia longiflora C. Potgieter N3 (same sample, re-run)							
March 1999	7a	GC: C. Potgieter July 2003	12	60	**28**	H	HR
March 1999	7b	"	16	78	**6**	H	HD

B. Nectar Volume and Concentration

Nectar volume ranges for 19 studied taxa are shown in Table 4, arranged according to corolla tube length and with main nectar-feeding pollinator class indicated. The highest volume of nectar was found in a flower of the long-tubed form of *P. saccatus* (8.7 µl), with the other three long-tubed species of *Plectranthus* (pollinated by *S. wiedemanni*) showing moderate maximum nectar levels (2.4 – 3.2 µl). The bee-pollinated *P. ernstii*, which has a relatively short corolla tube, showed a maximum of 6.8 µl, while the bee-pollinated *P. petiolaris* E.Mey. ex Benth. had a maximum of 3.2 µl.

Table 4: Nectar volume range and corolla tube length of 19 species or forms of *Plectranthus*, and *Py. urticifolia*. Pollinator class represents the main nectar-feeding class of pollinators; Apin: Bee (Apidae, Apinae), Nem: Fly (Nemestrinidae), Acro: Fly (Acroceridae), Tab: Fly (Tabanidae); Shape of corolla base and presence of spur indicated; Nar: Narrow corolla base, Sac: Saccate corolla base. Species marked with an asterisk* are visited for nectar by the nemestrinid fly, *S. wiedemanni*.

Species	Volume range (µl)	n	Corolla tube length (mm)	Corolla base shape	Pollinator class	Voucher
Floral visitors excluded						
P. ambiguus *	0 – 2.7	32	20 – 33	Nar	Nem	CP 86
P. hilliardiae *	0.2 – 2.4	40	21 – 32	Sac	Nem	CP 112
P. reflexus *	0.9 – 3.2	8	24 – 30	Sac	Nem	CP 95
P. saccatus (long) *	1.4 – 8.7	10	20 – 30	Sac	Nem	CP 120
P. zuluensis (dark)*	0.4 – 1.4	11	10 – 16	Sac	Acro	CP N12
P. zuluensis (pale)*	0 – 1.1	33	10 – 16	Sac	Acro	CP 118
P. ecklonii *	0.1 – 1.4	28	10 – 15	Nar	Nem, Apin, Tab	CP 114
P. saccatus (med.)*	0.1 – 1.8	17	9 – 11	Sac	Nem	CP 108
P. petiolaris	0 – 3.2	61	7 – 11	Nar	Apin	CP 115
P. oribiensis	0.2 – 0.6	7	6 – 12	Sac, Spur	Apin	CP 102
P. hadiensis	0 – 2.4	82	6 – 8	Sac	Apin, Nem	CP 92
P. ciliatus	0.2 – 2.8	25	6 – 8	Sac	Acro, Apin	CP 116
P. fruticosus *	0.1 – 0.9	23	5 – 8	Sac	Nem	CP 126
P. saccatus (short)	0.2 – 1.1	24	5 – 7	Sac	Acro? / Apin?	CP 131
P. ernstii	0.3 – 6.8	25	4 – 8	Sac	Apin	CP 85
Floral visitors not excluded						
Py. urticifolia	0 – 1.4	74	11	Nar	Apin	CP 1064
P. laxiflorus	0.2 – 1.7	40	10.5	Nar	Apin, Nem	CP 135
P. oertendahlii	0 – 0.4	33	8 – 13	Sac	Acro	CP 97
P. petiolaris (pink)	0 – 1.8	31	7 – 11	Nar	Apin	CP 100

Nectar sugar concentration results are presented (as % sucrose equivalents) in Tables 5 & 6. Table 5 shows measurements for individual flowers, taken with a more recent model of refractometer, while Table 6 shows measurements where flowers were pooled to obtain sufficient nectar for a reading with a less modern Atago handheld refractometer. In the latter case the ranges of pooled readings are indicated, while range and average are presented for individual readings in Table 5. Both tables show corolla tube length and major class of pollinator.

Table 5: Mean and range of nectar concentration (as % sucrose equivalents) for individual flowers, taken in 2005 with a sensitive Bellingham and Stanley Eclipse hand-held refractometer. Readings were taken from cultivated plants from which floral visitors were excluded, except for *P. hilliardiae.* Pollinator class represents the main nectar-feeding class of pollinators; Apin: Bee (Apidae, Apinae), Nem: Fly (Nemestrinidae), Acro: Fly (Acroceridae), Tab: Fly (Tabanidae).

***Plectranthus* species**	**Mean nectar concentration**	**Concentration range**	**SD**	**n**	**Corolla tube length (mm)**	**Pollinator class**	**Voucher**
Floral visitors not excluded							
P. hilliardiae	17 %	13–20 %	2.6	5	21–32	Nem	CP 112
Floral visitors excluded							
P. ambiguus	17 %	13–22 %	2.0	23	20–33	Nem	CP 927
P. zuluensis	29 %	15–36 %	5.6	11	10–16	Acro	CP 118
P. ecklonii	24 %	13–30 %	3.8	22	10–15	Nem, Apin, Tab	CP 114
P. saccatus (medium)	34 %	4– 49 %	11.3	13	9–11	Nem	CP 108
P. petiolaris	30 %	25–36 %	2.6	21	7–11	Apin	CP 100
P. hadiensis	28 %	10–49 %	11.8	17	6–8	Apin, Nem	CP 92
P. fruticosus (short)	30 %	22– 7 %	3.3	20	5–8	Nem	CP 131

Plectranthus nectar concentrations ranged from 4 – 55% and results were highly variable (Tables 5 & 6). The short-tubed forms of *P. saccatus* showed highly variable concentrations, from 7 – 55% (Table 6), with individual flower measurements of a medium-tubed species of *P. saccatus* also being highly variable, from 4 – 49% (Table 5).

Table 6: Mean and range of nectar concentration (as % sucrose equivalents) of pooled flower samples, measured using an Atago hand-held refractometer (type N1), prior to 2005.
All were cultivated plants from which floral visitors were excluded.
Pollinator class represents the main nectar-feeding class of pollinators; Apin: Bee (Apidae, Apinae), Mega: Bee (Apidae, Megachilinae), Nem: Fly (Nemestrinidae), Acro: Fly (Acroceridae), Tab: Fly (Tabanidae).

Species	**Nectar concen-tration (%)**	**No. pooled flowers**	**Corolla tube length (mm)**	**Conc. range (%)**	**Pollinator class**	**Voucher**
P. hilliardiae	7	20	21–32	**7–8**	Nem	CP 112
	8	2	21–32			
	8	15	21–32			
P. saccatus (long)	18	5	20–23	**4–18**	Nem	CP 120
	4	2	23–25			
	7	3	23–25			
	4	2	23–25			
P. saccatus (med.)	26	18	13–15	**26–51**	Nem	CP 108
	36	21	13–15			
	33	17	10–11			
	51	13	9–11			
P. zuluensis	49	15	10–16	**49–52**	Acro	CP 118
	52	20	10–16			
P. saccatus (short)	7	25	7–9	**7–55**	Acro? / Apin?	CP 131
	6	11	6–8			
	55	10	6–8			
	54	10	6–8			
P. ciliatus	33	41	6–8	**29– 33**	Acro, Apin	CP 116
	29	28	6–8			
P. fruticosus	24	35	5–8	**4–34**	Nem	CP 131
	34	30	5–8			
P. hadiensis	44	20	6–8	**30– 4**	Apin, Nem	CP 92
P. madagascariensis	30	18	5–6			CP 90
P. ernstii	21	5	4–8	**21–23**	Apin	CP 85
	23	3	4–8			

Discussion

A. Nectar Sugars

Very few studies have reported on nectar sugar ratios in the genus *Plectranthus*, but surveys have revealed the general condition for the Lamiaceae (Percival 1961, Baker & Baker 1983a).

A semi-quantitative paper chromatographic technique was used by Percival (1961) to describe the nectar sugars of about 900 species from wide-ranging plant families. Descriptions of the abundance of sugars were made subjectively, based on the size and depth of spot colour on the chromatogram (Percival 1961). *Plectranthus oertendahlii* nectar was found to fall in class **S**FG, the capitals indicating plenty of each sugar and the bold **S** indicating "a strong preponderance of" sucrose (Percival 1961). This result was based on one sample of 20 flowers from 1 locality, probably a cultivated plant. *Pycnostachys urticifolia*, a relative of *Plectranthus*, showed a very similar nectar sugar profile (Percival 1961).

The Lamiaceae in general have long-tubed flowers with nectar in which sucrose dominates (Percival 1961). This observation was confirmed by Baker & Baker (1983a) by studying 765 species with more modern methods. Certain families show conservatism in the proportions of major nectar sugars and in the Lamiaceae and Ranunculaceae, for example, nectars are characteristically 'sucrose-dominant' or 'sucrose-rich'. In contrast, nectars from the Asteraceae and Brassicaceae were found to be dominated by hexose sugars (Baker & Baker 1983b). This pattern breaks down in the alpine zone of the Rocky Mountains (Colorado), where Baker & Baker (1983b) found that most of the plant species have 'hexose-rich' nectar; this energy-economical nectar type is thought to be in response to the "less than optimum environment for sugar production by photosynthesis" (Baker & Baker 1983b).

The data presented in Table 1 confirms that most of the Lamiaceae analysed in this study have truly sucrose-dominant nectar that also fall in the 'sucrose-rich' and 'sucrose-dominant' categories of Baker & Baker (1983a). In a few cases the categories of Baker & Baker (1983a) were found to be misleading, for example three of the varieties of *P. saccatus* have truly hexose-dominant nectar that would previously have classified as 'sucrose-rich' (Table 1). This general trend of sucrose dominance in a lamiaceous genus, as well as the re-analysis of samples of *P. ecklonii* with HPLC

(Table 3), indicate that the GC technique followed for the majority of samples is acceptable.

The nectar data for *Py. urticifolia* (see Table 1) corresponds to the ‘sucrose dominance’ noted by Percival (1961), but some of the samples did not correspond to her result for *P. oertendahlii*. Two sets of independent nectar samples of *P. oertendahlii* were initially analysed in this study: one collected from the field in the same year as analysis, and one collected from cultivated material four years prior to analysis. Initial results showed no sucrose, only fructose and glucose, with the former slightly dominant. One sample was re-run with the lowest possible integrator threshold and then a very small peak of sucrose was detected (values in Table 2). A possible explanation is that the cultivated specimen that Percival would have sampled could have been misidentified (possibly confused with *P. ciliatus* E.Mey. ex Benth., another common container plant). Another is that Percival’s technique depended on visual estimation of sugar abundance, which by the author’s admittance was subjective. This is a concern also raised by Van Wyk (1993). Subsequent analyses of *P. oertendahlii* nectar showed variability in samples from individual flowers taken from the same plant, with values varying from hexose-dominant to sucrose-dominant (Table 2).

The nutrient-poor Natal Group Sandstones on which the endemic *P. oertendahlii* grows, linked with its dim understorey habitat, could lead to low levels of photosynthesis which may favour energy-economical hexose-dominant nectar. However, sampling of cultivated material under good light and nutrient regimes showed that the nectar composition was similar (hexose-dominant) in three samples collected from the greenhouse and the field, but different from sucrose-dominant samples collected in the field at another time. A number of other species in this study, such as *P. hilliardiae* and *P. reflexus*, are also sandstone endemics that grow on the forest floor, yet these species show highly dominant levels of sucrose in their nectar. It is possible that some species simply display large variance in nectar sugar characteristics and since most studies do not analyse multiple samples of the same species, such variability most probably goes unnoticed.

The pooling of nectar from flowers collected from the same plant, and even from the same population, for sugar analysis, was shown to be an acceptable practise by Lanza *et al*. (1995). In their study they investigated various nectar characteristics of *Impatiens capensis* Meerb. at the level of the individual flowers, plant and population. Significant differences in sucrose concentration were detected only in between-population comparisons (Lanza *et al*. 1995). Since pollinating insects visit more than one flower in a foraging bout, pooling of nectar from various flowers in a population was considered to be acceptable for the determination of average nectar sugar composition in the current study.

The methods of Freeman *et al*. (1991) could be used as a guideline whereby a species needs to show two or more samples with consistent nectar sugar composition, before inclusion into the results, with exemptions being single samples of species where conspecific or congeneric taxa are included. Interestingly, Freeman *et al*. (1991) included two Lamiaceae in their survey of South and South East Asian taxa. *Leucas zeylanica* R.Br. was shown to have nectar with 86% sucrose, but *Orthosiphon aristatus* (Blume) Miq. had only 53% sucrose. In the present study *O. tubiformis* [= *Ocimum tubiforme* (R.D.Good) A.J.Paton] was found to have 71% sucrose (based on one sample), and Goldblatt & Manning (2000) found the latter species to have nectar with 94 – 100% sucrose, based on three samples. In light of such variation, even the inclusion of two or more samples could be questioned, let alone congeneric comparisons.

Since one of the most interesting findings during the course of this project has been the discovery and description of the *S. wiedemanni* (Nemestrinidae) pollination guild, it makes sense to establish whether long-proboscid flies have a particular preference for a certain class of nectar. In a pollination study of *Lapeirousia* Pourr. subgenus *Lapeirousia* (Iridaceae), Goldblatt *et al*. (1995) found that most species have ‘sucrose-dominant or -rich’ nectar and their pollinators include long-proboscid nemestrinid and tabanid flies with proboscis lengths of 30 – 60 mm. The authors noted that this adds a new dimension to the categorisation of nectar and pollinators by Baker & Baker (1983a, 1990), since the hexose-loving flies in their studies were short-tongued species from families such as the Syrphidae, Muscidae and Phoridae. Goldblatt *et al*. (1995) pointed out that in the same way that long-tongued bees prefer sucrose in nectar, the flowers visited by long-proboscid flies also produce sucrose. They suggested that large-bodied insects that are physically active and that maintain wing movement during feeding, may require more sucrose rather than hexose. This holds for the nemestrinids, tabanids such as *Philoliche* Wiedemann, 1820, hawkmoths and some anthophorine bees (Goldblatt *et al*. 1995).

A study on the pollination of *Sparaxis* (also Iridaceae) by Goldblatt *et al*. (2000) shows that a species of *Mesomyia* Macquart, 1850 (Tabanidae) and the nemestrinid fly *Prosoeca peringueyi* Lichtwardt, 1920 both visit flowers with 'sucrose-dominant' nectar. Subsequent work showed that the nectar sugar characteristics in long-proboscid fly-pollinated species of Iridaceae, Orchidaceae and Lamiaceae are 'sucrose-rich or -dominant' (Goldblatt & Manning 2000). However, surveyed *Pelargonium* L'Hér. ex Aiton species (Geraniaceae), with one exception, have 'hexose-rich or -dominant' nectar (Goldblatt & Manning 2000). From such divergent nectar properties, Manning & Goldblatt (1996) concluded that nectar sugar composition is not a significant factor in the *Prosoeca peringueyi* (Nemestrinidae) pollination guild. This observation was confirmed for other long-proboscid species by Goldblatt & Manning (2000) in their review on long-proboscid fly pollination in southern Africa.

Data from the current study show that samples of long-tubed Lamiaceae such as *P. reflexus*, *P. hilliardiae*, *P. ambiguus*, *O. tubiformis* and *S. tubulosa* all had truly sucrose-dominant nectar (54 – 96% sucrose), while long-tubed species such as *P. saccatus* (long-tubed form) and *T. longiflora* had truly hexose-dominant nectar (Table 1). *Plectranthus* species that are not long-tubed, but that are visited for nectar by the long-proboscid *S. wiedemanni* (e.g. *P. ecklonii*, *P. ciliatus*, *P. zuluensis* and *P. fruticosus*) all have truly sucrose-dominant nectar, as do *O. tubiformis* and *S. tubulosa*, that have been inferred to be visited by this nemestrinid fly species (Potgieter & Edwards 2001). The sample from the long-tubed *I. hypoestiflora* (Acanthaceae), which also belongs to the *S. wiedemanni* pollination guild, showed equal amounts of sucrose and hexose (Table 1). While most of the species correspond to a sucrose-dominant pattern, there are exceptions.

The question then, is whether *Plectranthus* species that are visited by different types of pollinator have different nectar classes as a result. Most *Plectranthus* species recorded in Table 1 have sucrose-dominant nectar, with *P. saccatus* and some samples of *P. oertendahlii* being the exceptions with hexose-dominant nectar. *Plectranthus oertendahlii* is pollinated by medium-proboscid acrocerid flies (see Appendix), but then, so is *P. ciliatus* and *P. zuluensis* T.Cooke, which have sucrose-dominant nectar. *Plectranthus saccatus* happens to be the one variable species for which limited pollinator data was evident during this study: the long-tubed form of *P. saccatus* is long-proboscid fly-pollinated and, from garden-based observation, at least one medium-tubed form is pollinated by *S. wiedemanni* (see Appendix). A further exception is that the shortest-tubed *P. saccatus*, which possibly has an acrocerid, tabanid or apinid pollinator (see Appendix), has sucrose-dominant nectar, unlike the other long-tubed and medium-tubed forms of this species.

Species of *Orthosiphon* and *Pycnostachys* (recorded in Table 1) showed sucrose dominance, with *Thorncroftia* and *Hemizygia* having hexose-dominant nectar. In the case of *Stachys* one species was hexose-dominant and another was sucrose-dominant. Nectar from *P. laxiflorus* showed an equal split between hexose and sucrose, which is interesting in a species where both bees and medium-proboscid nemestrinid flies are frequent floral visitors (Potgieter *et al*. 2009). No definite pattern can at present be seen in the comparison of nectar sugars and pollinator type in a range of *Plectranthus* species and their relatives.

In a study of bees (Anthophoridae) and bee-mimicking flies (Acroceridae) that pollinate *Gladiolus brevifolius* Jacq. (Iridaceae), Goldblatt *et al*. (1997) noted that 'sucrose-dominant' nectar is characteristic of most of the insect-pollinated *Gladiolus* species (and of the subfamily Ixioideae). This nectar class for Iridaceae is expected, but what is of interest is the range of pollinators that visit these flowers for 'sucrose-dominant' nectar. Long-tongued anthophorine bees (referred to as apinid bees in the current study), short-tongued bees (species of *Allodape* Lepeletier & Serville, 1825) and interestingly, a species of *Psilodera* Gray, 1832 (a medium-proboscid acrocerid fly similar to the species that visits *P. oertendahlii*) all pollinate this *Gladiolus*.

Does this mean that acrocerid flies prefer 'sucrose-dominant' nectar? The data presented here for three medium-tubed *Plectranthus* species that are, amongst others, pollinated by acrocerid flies of the genus *Psilodera* (Table 1), show sucrose-dominant nectar (regardless of nectar classification system), but *P. ciliatus* is also visited by long-tongued bees that 'prefer' sucrose (according to Baker & Baker 1983a). *Plectranthus zuluensis* is only visited by acrocerid and nemestrinid flies and has sucrose-rich nectar, while *P. oertendahlii* only has acrocerid visitors and may have either sucrose- or hexose-rich nectar (Table 2).

The *Lapeirousia* study by Goldblatt *et al*. (1995) shows a similar range of pollinators in plants with mostly 'sucrose-dominant' nectar. Apart from the long-proboscid nemestrinids and tabanids, these included hawkmoths (22 mm long proboscides), bombyliid flies and honey bees (6 – 8 mm long proboscides) and noctuid moths and anthophorine bees (7 – 8 mm long proboscides) (Goldblatt *et al*. 1995).

Some of the conclusions drawn by Baker & Baker (1983a) summarise the debate around phylogenetic constraints versus the adaptation of nectar sugar ratios (nectar classes) to pollinator preference. They point out that there does seem to be a tendency for intra-familial resemblances in the ratio between sucrose and hexoses in nectar. They mention that some nectar sugar ratio modification can take place when new flower-pollinator partnerships are established, and they suggest a tendency for nectar chemistry to predispose members of a particular plant family to pollination by certain pollinator classes on the basis of nectar chemistry; however they caution that this could be outweighed by morphological and phenological adaptations in the features of flowers and inflorescences (Baker & Baker 1983a).

The recent review on nectar chemistry by Nicolson & Thornburg (2007) concludes that the phylogenetic history of a plant group is likely to be the primary determinant of nectar sugar composition, but that pollinator class may have a secondary effect.

For the *Plectranthus* and other species of Lamiaceae studied here, with a few exceptions, the occurrence of sucrose- or hexose-dominance appears to relate more to phylogeny, rather than the preference of a certain class of pollinator. A more diverse range of plant families will have to be sampled locally for nectar sugars and studied with respect to pollination to tease this issue apart. It is clear that some of the previously held ideas regarding the nectar preferences of long-tongued bees and other pollinator classes (such as those held by Baker & Baker 1983a) may be a case of inferring patterns from circumstantial evidence, rather than stringent testing for preferences.

B. Nectar Volume and Concentration

Nectar volume and concentration was highly variable in the studied species of *Plectranthus*. This is not uncommon, since volume measurements for a range of long-proboscid fly-pollinated families in Goldblatt & Manning (2000: Table 3, pp 163 – 167) often show a doubling or tripling of the lowest measured volume (and occasionally even more) to reach the upper range.

Nectar volume and concentration data is sparsely recorded for other members of the Lamiaceae. One record is given in Goldblatt & Manning (2000) for the long-tubed *O. tubiformis* which had nectar volumes of 2.7 – 4.1 µl and concentration of 24.5% (SD 2.2). Nectar volumes in three bird-pollinated species of *Leonotis* (Pers.) R.Br. were measured by Vos *et al*. (1994), and ranged from 5.3 – 11.3 µl per flower with concentrations of 19 – 28%. These volumes reflect the nectar-feeding capacity of sunbirds, but the nectar concentration falls within the range of insect-pollinated species. Nine species of the Lamiaceae were studied by Dafni *et al*. (1988) and nectar volume per flower was found to be small, ranging from 0.14 – 6.3 µl, with most of the species having less than 1 µl per flower. Nectar sugar concentration was generally high, from 25 – 52%, and no correlation was found between honeybee preference for different plants and nectar volumes or sugar concentrations (Dafni *et al*. 1998). A subsequent study, measuring floral parameters in thirteen species of Labiatae in Israel (Dafni 1991), presents nectar concentration values of 17 – 60% sucrose equivalents, with nectar volumes of 0.4 – 18.2µl per flower over 24 hours. Larger flowers were found to offer more calorific reward per flower, and large flowers lasted longer. Nectar rewards in large flowers, with longer floral tubes, were also better protected from short-tongued insects and general climatic conditions (Dafni 1991). The study by Ford & Johnson (2008) showed four species of *Syncolostemon* (Lamiaceae) with tube lengths varying from 9.6 – 28.0 mm to have corresponding nectar volumes of 0.7 – 3.2 µl and variable nectar concentrations of 21.6 – 29.3 %.

In the current study there does not appear to be a relationship between corolla tube length and maximum volume of nectar (Table 4), since short-tubed species such as *P. hadiensis* (Forssk.) Schweinf. ex Spreng. and *P. ciliatus* had nectar volumes similar to those of the long-tubed *P. ambiguus* and *P. hilliardiae*. This is contrary to results in *Syncolostemon*, where Ford & Johnson (2008) found a positive correlation between nectar volume and corolla tube length. The occurrence of saccate corolla bases in *Plectranthus* shows no obvious relationship to nectar volume either (Table 4).

There is, however, a general trend for lower concentrations of nectar to be found in longer-tubed flowers of *Plectranthus* that are pollinated by *S. wiedemanni*, but no other trend is evident with respect to pollinator class (Tables 5 & 6). The nectar concentration values for *Plectranthus* species pollinated by *S. wiedemanni* vary from 4 – 55%, which extends the 20 – 32 % range suggested by Goldblatt & Manning (2000) for long-proboscid flies. The limited nectar concentration data presented in Potgieter *et al*.

(2005) was based on preliminary results and more recent data are contained here, in Table 5. In *Syncolostemon* nectar concentration was shown to vary among species, but was not correlated to corolla tube length (Ford & Johnson 2008).

The way in which nectar volume is presented has been extended in the present study to include the range, since the practice of only giving averages does not reflect what is experienced at individual flowers by a visiting insect (Table 4). For the most recent volume measurements in this study zero readings for nectar (i.e. no nectar in the flower) were included, but in other cases not, which would make a comparison of averages meaningless.

A survey of pollination literature shows that most often only averages and/or ranges, without zero measurements, are given, which makes it difficult to gauge whether the researcher(s) included data for flowers containing no nectar. Southwick *et al*. (1981) recorded volume averages and ranges of floral nectar production, but none with zero at the lower limit; likewise, the long-proboscid fly pollination review by Goldblatt & Manning (2000) showed ranges, but none with zero values. Many studies (e.g. Spira 1980, Whitten 1981, Johnson 2000, Luyt & Johnson 2001, Potts *et al*. 2001, Johnson *et al*. 2002, Johnson *et al*. 2003, Macukanovic-Jocic 2004, Goldblatt *et al*. 2005, Manning & Goldblatt 2005, Larsen *et al*. 2008) only reflect averages and standard deviation/error, but not range. However, data on diurnal nectar secretion dynamics, by Macukanovic-Jocic (2004), show cases where nectar volume is zero at certain times of day; these authors also ascribe high values of standard error to absence, or very low levels, of nectar in some flowers.

In the current study most measurements were made on unvisited plants, hence the zero readings (see Table 4) indicate that the variability in nectar volume extends to some flowers not producing any nectar at all at any one time. This is important with respect to pollinator movement, since there is unpredictability in whether an insect will find nectar in any one flower, which forces movement between flowers, inflorescences and plants. In a study on bumble-bee foraging movements in *Monarda fistulosa* L. (Lamiaceae), Cresswell (1990) suggests that bees initiate movements between inflorescences on the basis of the amount of nectar found at the most recently probed flower, with the number of flowers already probed at an inflorescence only exerting a weak influence on such choices.

Complete rewardlessness is rarely recorded in plant families other than orchids, although many plant populations are suggested to contain a proportion of rewardless flowers because individual plants can produce both nectar-bearing and empty flowers. Some species are known to be polymorphic for the presence or absence of nectar production (Smithson & Gigord 2003). A few authors draw attention to the phenomenon of occasional nectarless flowers in their results. Petanidou *et al*. (1999), whilst presenting average values for nectar volume in three species of Lamiaceae in an experimental array, recorded some nectarless flowers in 10 out of 15 cases in pot treatments. Petanidou *et al*. (1999) also found that increasing the nutrient status of three species of Lamiaceae, increased the number of empty flowers in experimental treatments. A study by Real & Rathke (1991) showed that two out of 32 studied flowers of *Kalmia latifolia* L. (Ericaceae) did not accumulate any nectar over a 24 hour period, over the life span of the flower.

This chapter provides new records of nectar sugar composition and measurements of nectar volume and concentration for species of *Plectranthus* and other Lamiaceae that have not had such data recorded previously. While general trends may be seen in the data, it is clear that exceptions occur, that results can be variable and that the types of conclusions that may be drawn from nectar studies may have limited value, other than showing that variation may be inherent in the interactions between plants and insect pollinators.

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CHAPTER 8: DISCUSSION AND CONCLUSIONS

This thesis contributes a number of new findings to the field of Lamiaceae pollination and pollination studies in southern Africa in general.

Chapter 2 represents a selection of the initial data gathered during the course of this study, showing that bees and flies are the main pollinators of seven straight-tubed species of *Plectranthus* with varying tube lengths (Potgieter *et al.* 1999). It goes on to show a correlation of corolla tube length with the proboscis lengths of the main pollinator groups. This paper presents the first record of long-proboscid (nemestrinid) fly pollination in the Lamiaceae family, while pointing to the importance of flies with medium proboscid lengths (nemestrinid, tabanid and acrocerid flies) in the pollination ecology of *Plectranthus* with medium- and short corolla tubes.

Since the publication of this paper, a number of specific identifications for the dipteran vouchers have been made. The nemestrinid fly, *Stenobasipteron* sp., is now known to be *S. wiedemanni*, and the four species of *Prosoeca* (Nemestrinidae) are actually representatives of two species of variable size and appearance (D. Barraclough pers. comm.): *Prosoeca umbrosa* is represented by *Prosoeca* spp. A and B, while *Pr. circumdata* incorporates *Prosoeca* spp. C and D. The tabanid fly *Philoliche* sp. is *Ph. aethiopica.* Acrocerid flies have been identified as *Psilodera confusa* and *Psilodera* aff. *confusa*; hence the photographs of '*Psilodera* sp. A' in Fig. 5 g & h (p 106, Potgieter *et al.* 1999) are in fact two species, with Fig. 5g representing *Ps. confusa* and Fig. 5h *Psilodera* aff. *confusa*.

These positive identifications are largely due to the efforts of Dr David Barraclough who, during the course of this study, undertook a revision of type material of the southern African genus *Stenobasipteron* (Barraclough 2005), followed by the publication of an overview of the South African Nemestrinidae (Barraclough 2006). This was done in response to the need created by various pollination studies, including the current *Plectranthus* study, and the overview was specifically intended for inter-disciplinary use by botanists, entomologists and pollination biologists, with the aim of stimulating interest in the conservation and taxonomic research on South African Nemestrinidae (Barraclough 2006). One of the reasons stated for not sinking the genus *Stenobasipteron* as a synonym of *Prosoeca* is the use of this generic name in the pollination biology literature (Barraclough 2005), which points to the synergy that is developing between insect taxonomists and pollination biologists in South Africa.

Dr Barraclough identified the vouchers of Acroceridae collected during the study and pointed out that the species collected at Umtamvuna NR represents a new taxon. Likewise, five of the species of *Prosoeca* collected during the study as a whole, are new taxa that need further study by insect taxonomists. A number of the bee species collected during the study are new range extensions when compared to distributions given in revisions of the genera *Amegilla* (Eardley 1994) and *Xylocopa* (Eardley 1983).

In addition to taxonomic clarifications of insect vouchers cited in Potgieter *et al.* (1999), some new pollinator observations were also made since publication of this paper. Most of these observations expand the class of pollinator to other species of the same genus already recorded, but in *P. ambiguus* the nemestrinid fly *Prosoeca umbrosa* was a new observation and in *P. madagascariensis* the acrocerid fly *Psilodera valida* was added. In *P. ciliatus* the original observation of one species of acrocerid fly, '*Psilodera* sp. A', was expanded to include four species of *Psilodera*. Three additional apinid bee species were added to the list of valid floral visitors in *P. oribiensis*, with the genera *Xylocopa* and *Thyreus* now represented. The Appendix to the thesis should be consulted for updated pollinator data for the seven species that are the main subjects of Potgieter *et al.* (1999): *P. ambiguus*, *P. hilliardiae*, *P. ecklonii*, *P. zuluensis*, *P. ciliatus*, *P. madagascariensis* and *P. oribiensis*.

Chapter 3 is the most recently published paper (Potgieter *et al.* 2009), outlining the pollination of five species of *Plectranthus* (plus two allied species) with sigmoid-shaped corolla tubes. In addition to showing that corolla tube length is related to the proboscis length of pollinating groups (as is the case with the straight-tubed species), this paper also shows that the bend in the corolla tube acts as a barrier that protects nectar resources from illegitimate visitations, as well as ensuring that the bodies of bee visitors are aligned in a way that enhances effective pollen placement and carry-over.

Sigmoid species with corollas bent or declined to some degree, account for a third of southern Africa *Plectranthus* species and this functional group is shown to be geared towards melitophily. Two of the three clades in *Plectranthus*, the '*Coleus*' clade and the 'sigmoid *Plectranthus*' clade (Paton *et al.* 2004, Paton pers. comm.) show this syndrome and are bee-pollinated, with a few species also receiving nectar-feeding visits from medium-proboscid nemestrinid flies (Potgieter *et al.* 2009). This is a new discovery, not previously recorded or suggested for sigmoid *Plectranthus* and allied species. While straight corolla tubes may be considered pleisiomorphic due to its presence in the Ociminae (sister group to the Plectranthinae), the discussion in Chapter 3 suggests the possibility that straight corolla tubes may have evolved from the sigmoid condition.

As a result of its recent publication, basic data contained in Chapter 3, such as the number of species of *Plectranthus* that occur in southern Africa (ca. 53), is updated compared to the 45 *Plectranthus* species originally noted in Chapter 2. This highlights new species discoveries in the intervening decade, systematic work in the genus, and a generally increased interest in *Plectranthus*. As our knowledge of insect taxonomy and systematics improves, we can also draw better conclusions from data linking floral features to insect morphology.

The paper on convergent pollination syndromes in southern African Lamiaceae (Potgieter & Edwards 2001) is a case in point. Chapter 4 aims to describe what is known about pollination in long-tubed Lamiaceae and speculates that most of these long, straight-tubed species are adapted to pollination by long-proboscid flies. With the overview of nemestrinid flies (Barraclough 2006) and the review of *Stenobasipteron* (Barraclough 2005) not yet available in 2001, some of the species concepts of long-proboscid flies in the summer rainfall region of the eastern part of southern Africa were not clear.

It is now known that the ‘Abel Erasmus Pass form’ of *S. wiedemanni* (cited by Goldblatt & Manning 1998, 1999) is not *S. wiedemanni*, but rather an undescribed species, which accounts for its different habitat requirements to the *S. wiedemanni* guild (described in Potgieter & Edwards 2005). True *S. wiedemanni* only occurs in forest habitat in the Eastern Cape and KwaZulu-Natal (Barraclough 2005), while a number of undescribed species of *Stenobasipteron* are recorded from savanna and grassland habitats in the Limpopo and Mpumalanga Provinces (Barraclough 2006).

Similarly, the various forms of *Prosoeca ganglbaueri* (with varying proboscid lengths) listed in Table 3 of Potgieter & Edwards (2001, Chapter 4) may represent a species complex, with the Drakensberg populations comprising at least two species (Barraclough 2006), while the identification of *Pr. robusta* from Mpumalanga Province (by Goldblatt & Manning 1998, 1999) is incorrect and probably represents a different species (Barraclough 2006).

Such details do not change the notion that these plant species are adapted for long-proboscid fly pollination, but the details of the possible interactions may be slightly different. It is, however, clear that there are guilds based on the habitat preferences of the flies, with *S. wiedemanni* being limited to forest or adjacent areas, while *Pr. ganglbaueri* tends to occur in open habitats such as high-altitude grassland. The mechanism by which floral extension was possible in long-tubed species may be explained by the presence of shorter-tubed species with straight corollas in the same genera, that are also pollinated by medium-proboscid flies and/or long-tongued bees (see Discussion in Potgieter & Edwards 2001).

The genus *Syncolostemon* was not considered by Potgieter & Edwards (2001) as potentially being pollinated by long-proboscid flies, but rather by bees, on account of the wide corolla entrance. The subsequent study by Ford & Johnson (2008) showed that the long-tubed (22 – 28 mm) species, *S. macranthus*, *S. rotundifolius* and *S. densiflorus*, are pollinated, at least in part, by medium-proboscid nemestrinid (*Prosoeca* sp.) and tabanid (*Philoliche aethiopica*) flies.

One of the most exciting aspects of this project was the discovery (in *Plectranthus*) and description of the *Stenobasipteron wiedemanni* pollination guild, which constitutes Chapter 5 (Potgieter & Edwards 2005). Having tentatively been included in the *Prosoeca ganglbaueri–Pr. robusta* pollination system by Goldblatt & Manning (2000), it was evident that this nemestrinid fly species serviced a completely different guild of plants, largely in forest habitat, which is quite distinct from *Pr. ganglbaueri*. Based on the work done by Barraclough (2005) it is now clear that the Mpumalanga species that was identified as *S. wiedemanni* by Goldblatt & Manning (2000), is a different, unidentified species of *Stenobasipteron* that was found to pollinate *Gladiolus macneilii* (Iridaceae) and *Orthosiphon tubiformis* (Lamiaceae). This anomaly in habitat type in Mpumalanga was pointed out in the discussion of Potgieter & Edwards (2005), since it did not fit the pattern seen along the rest of the distribution of *S. wiedemanni*.

During the current study *S. wiedemanni* was first observed pollinating the long-tubed *Plectranthus* species, *P. hilliardiae* and *P. ambiguus*, at Umtamvuna NR in 1995, with subsequent observations extending the species list to seven more species of

Plectranthus (see Appendix). Anecdotal evidence, publications by Goldblatt & Manning (1999, 2000) and confirmation by pollen collected from voucher specimens of *S. wiedemanni*, extended the Guild to members of other plant families (Acanthaceae, Orchidaceae, Balsaminaceae, Gesneriaceae and Iridaceae) that occur in forested habitat along the Eastern seaboard of southern Africa.

A study on pollination systems in *Brownleea* (Larsen *et al.* 2008) confirmed that in a Grahamstown population (EC, South Africa), *B. coerulea* is pollinated by *S. wiedemanni*, with flies at this site also visiting stands of *Hypoestes aristata* (Vahl) Sol. ex. Roem & Schult. (Acanthaceae) which may act as a magnet species; this species was included in the *S. wiedemanni* guild by Potgieter & Edwards (2005). Interestingly, *B. coerulea* at Umtamvuna was rather found to be pollinated by a tabanid fly, *Ph. aethiopica*, with *P. ciliatus* creating a possible magnet effect at the study site which was a forest patch situated next to grassland (Larsen *et al.* 2008).

This type of habitat is similar to that occupied by succulent forms of *P. saccatus* with short, white flowers that have blue speckling on the corolla limbs, which superficially resemble the speckled nectar guides of *P. ciliatus* (and that of *B. coerulea*). A few plants of *B. coerulea* were seen amongst the population of *P. saccatus* studied at Beacon Hill, Umtamvuna NR, but for which no pollinator data was recorded. It is possible that *Ph. aethiopica* pollinates this form of *P. saccatus* at Umtamvuna NR, since the average proboscis length (8.9 mm) listed in Table 2 of Larsen *et al.* (2008) corresponds favourably to the 5 – 7 mm tube length of *P. saccatus* at this site. It is, however, also possible for acrocerid flies or anthophorine bees to be the pollinators of this *Plectranthus* species (see Appendix, *P. saccatus*). The month of observation of *B. coerulea* at Umtamvuna NR is not given by Larsen *et al.* (2008), but it is likely that *S. wiedemanni* had not yet emerged at that site at the time of the study, since the fly species has been recorded from forests at that site (C. Potgieter unpubl. data). At the Kologha Forest study site where *P. ciliatus* was observed for pollinators in the current *Plectranthus* study (near Stutterheim, EC), *B. coerulea* was seen to be pollinated by *S. wiedemanni*, with a voucher carrying pollinaria of the orchid (Potgieter & Edwards 2005).

Stenobasipteron wiedemanni is responsible for the evolution of specialised long-tubed corollas in four species (or forms) of *Plectranthus*, three of which are endemic to the Pondoland Centre of Endemism. The long-tubed form of *P. saccatus* that occurs on steep forest slopes of the Umtamvuna Gorge does not have the exact floral morphology as the long-tubed *P. saccatus* endemic to the Hlatikulu Reserve area (Kaliweni forest) in far northern KZN; the latter form has a vertically narrower corolla, but with similar or shorter tube length, and was presumed to be pollinated by *S. wiedemanni* as well. A search for the pollinator in early February 2008 at Hlatikulu Reserve did not reveal any *Stenobasipteron* activity, but visits by *Philoliche aethiopica* (with shorter proboscis than *S. wiedemanni*) were noted to long-tubed *P. saccatus*, as well as some visits by apinid bees that tended to be brief. It is possible that *Stenobasipteron* had not yet emerged so early in the flowering season, since specimens of a fly species of that genus have been reported from that site (Barraclough pers. comm.). This species appears to be distinct from *S. wiedemanni* and more collections are needed to confirm its identity.

As is the case for all the varieties of *P. saccatus* (see Appendix), more field work is needed to tease out the diversity of floral forms in this species, some of which are pollinated by *S. wiedemanni* and possibly other long-proboscid flies.

The topic of Chapter 6 is not centred on pollination, but has relevance to understanding the importance of natural hybrids and hybridisation events in the group. The paper by Viljoen *et al.* (2006) tests microdistillation as a method for analysing small samples of essential oil-containing material and succeeds in showing that this technique, which may be used to analyse very small samples, compares well with hydro-distillation. It also shows that for a case study of a putative hybrid, *P. zuluensis* x *P. ciliatus* at Oribi Gorge, the essential oil profile of the confirmed hybrid plant is intermediate to those of the parent species. It is worth noting that flowers of the putative hybrid had four functional stamens, despite the fact that *P. zuluensis* only has two functional stamens.

A number of cases of suspected natural hybridisation were found during the course of the study and in most cases it was possible to speculate on the parentage of the hybrids, based on plant morphology, with nectar guide and other floral characteristics proving particularly useful. All the cases of suspected hybridisation were between species with medium- and/or short corolla tube lengths, with no hybridisation events recorded between long- and shorter-tubed species (as discussed in Potgieter & Edwards 2001, Chapter 4). This shows that long corolla tubes offer greater pollinator fidelity while shorter tubes tend to be visited by a larger array of insect pollinators that also visit other related species (see Appendix, and Potgieter *et al.* 1999, Chapter 2). Most hybrids observed during the study were not fertile (no fruits or remaining calyces were seen on older inflorescences), even in the case of *P. ciliatus* x *P. zuluensis* where a large, unidentified species of *Prosoeca* was seen to visit the hybrid inflorescence (see Appendix, *P. zuluensis*).

In at least one case the pollinator of a species could be inferred from the presence of a hybrid plant, even when pollinators were never observed for the species, as was the case in *P. ernstii* (see Appendix, *P. ernstii*).

In some *Plectranthus* populations (e.g. at Ngeli and Oribi Gorge) extensive hybrid stands were present due to vegetative propagation, since the genus grows very easily from cuttings and even small bits of broken-off stem will grow under favourable conditions. In some of these populations hybrid swarms were evident, with one or both species apparently crossing back onto parent clones, e.g. stands of *P. ciliatus* x *P. fruticosus* at Magwa (see Appendix, *P. ciliatus*); in such cases some hybrids would have had to be fertile in order to cross back.

More studies involving essential oil composition will allow the identity of more hybrids to be revealed, especially in contentious cases such as *Plectranthus brevimentum* T.J.Edwards from Lupatana River Gorge near Port St Johns, EC (Edwards 2005). The author of the species presents it as a distinct species, while Van Jaarsveld (2006) suggests it to be of hybrid origin. In his predominantly horticultural review of *Plectranthus* in southern Africa, Van Jaarsveld (2006, p 104) maintains that *P. brevimentum* (incorrectly referred to as “*P. brevilabrum* Edwards”) represents a hybrid of *P. hilliardiae* and *P. strigosus* Benth. (a very short-tubed species that occurs in the current study area, but was not studied). If this is the case it would be the first recorded natural hybrid between a long-tubed and short-tubed species. A number of very attractive horticultural hybrids appear to involve *P. hilliardiae* and shorter-tubed species (e.g. *Plectranthus* Mona Lavender), hence crosses between long-and short-tubed species are possible.

The nectar studies of Chapter 7 represent the most complete set of nectar data for the genus. As is the case in most genera of the Lamiaceae, *Plectranthus* nectar tends to be dominated by sucrose, with a few notable exceptions.

One aspect of this study that has not yet been fully published is pollination of a number of *Plectranthus* species by acrocerid flies of the genus *Psilodera*. The pollinator data contained in the Appendix (see *P. ciliatus*, *P. zuluensis*, *P. oertendahlii*, *P.*

praetermissus, *P. madagascariensis* and *Aeollanthus parvifolius*) is being compiled into a paper on this topic (Potgieter *et al.* in prep.), since limited mention has been made of this pollinator group in other papers published from this thesis. There is a growing appreciation of the importance of this group of medium-proboscid flies in the pollination literature, with Borkent & Schlinger (2008a, 2008b) describing aspects of the floral visitation behaviour and pollen-carrying ability of *Eulonchus* spp. (Acroceridae) in North America. They point out that this family of flies has potential to form an important part of the pollinator fauna, but that more research is needed on the topic (Borkent & Schlinger 2008a). This mirrors the opinion expressed in this thesis, with Potgieter *et al.* (1999) suggesting that the behaviour of acrocerid flies would make them important pollinators of 'medium-tubed' plant species, including several *Plectranthus* species, while Potgieter *et al.* (2009) mentions that these flies fill a similar niche, w.r.t. proboscis length, to apinid bee pollinators.

A total of five different species of Acroceridae, representing two sub-genera of *Psilodera* (C. Conway & D. Barraclough pers. comm.) were observed as potential pollinators of *Plectranthus*. Some species show wide distribution ranges, e.g. *Ps. valida* collected from Karkloof to Stutterheim (see Appendix, *P. ciliatus*), while others are potentially new species with apparent restricted distribution (*Psilodera* aff. *confusa* from Umtamvuna NR). Most acrocerid species were nectar-feeding floral visitors to straight-tubed species of *Plectranthus* that matched the proboscis length of the pollinator. In species such as *P. oertendahlii* and *P. praetermissus*, both of which are narrow Pondoland endemics occurring in forest habitat, the corolla shape is basally saccate with a distinct narrowing at the mouth of the corolla, which restricts the range of floral visitors to insect species with thin proboscides, capable of hovering, such as *Psilodera* spp. (see Appendix, *P. oertendahlii* & *P. praetermissus*).

This study provides a basis for future studies of Lamiaceae pollination, breeding systems and speciation. In addition to conducting more field studies to elucidate the pollinators for *Plectranthus* species that have not yet been recorded, a number of questions beg to be answered.

Field-based experimental studies could investigate whether *Plectranthus* species are pollen-limited, as was found in insect-pollinated *Syncolostemon* (Ford & Johnson 2008), and whether apinid bees or nemestrinid flies are more efficient at pollen carry-over. Following on from the study on *P. laxiflorus*, conducted at Ferncliff in 2003

(Potgieter *et al.* 2009), developing inflorescences could be bagged and, once matured, opened to allow one flower to receive one visit from either pollinating group, and closed up again to record seed set.

Studies that track gene flow in *Plectranthus*, such as the one using microsatellite and RFLP markers in two species of *Streptocarpus* (Gesneriaceae), by Hughes *et al.* (2007), would be useful in understanding the Pondoland *Plectranthus* distribution patterns and diversity. The forest-dwelling species of *Plectranthus* are trapped in deep gorges by aspects of their life history, such as the inability to disperse, and intolerance of desiccation and fire (on surrounding grassland plateaux), which favours allopatric speciation (Edwards 2005). *Streptocarpus* presents a similar situation and the forest-dwelling species, *S. primulifolius*, which is pollinated by the nemestrinid fly *S. wiedemanni*, shows patterns of infrequent seed-dispersal coupled with limited movement of pollen between populations (Hughes *et al.* 2007).

The inclusion of *Tetradenia* in future pollination studies will broaden the scope for analyses of floral diversification in the *Plectranthus* clade. *Tetradenia* is embedded within the *Plectranthus* clade and is the likely sister-group to *Thorncroftia* (Edwards 2006). Unlike *Plectranthus* and most of its allies, *Tetradenia* has small, unisexual flowers that appear almost actinomorphic. A study of its pollination syndrome would highlight the ability of clades to diverge i.t.o. floral morphology, and may explain its anomalous floral design.

Finally, by building on the work done by Paton *et al*. (2004), an extended phylogeny that incorporates all of the southern African endemic and specialised species of *Plectranthus*, would provide the ideal platform for testing theories w.r.t. pollinator shifts and convergence in this large genus.

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APPENDIX:

Descriptive and pollinator accounts for twenty study species

This appendix provides a summary of the main results from pollinator field observations for the twenty studied plant species, as a complementary reference to the published and nectar chapters. Each account includes a short description of the habitat, habit, distribution and phenology of the species, with a description of inflorescence design and floral morphology. The basic inflorescence structure in *Plectranthus* is cymose. Each cyme is subtended by a bract and these are arranged in a decussate synflorescence, resembling a raceme or a panicle. The species accounts also include study sites and observation details, with corresponding pollinator and insect voucher information.

Descriptions are based on observations and measurements made during the study, with additions from the revision by Codd (1985a), as well as the following publications: Dyer (1934), Verdoorn (1949), Dyer & Bruce (1951a), Dyer & Bruce (1951b), Lewis (1951), Codd (1957), Codd (1970), Codd (1975), Codd (1977), Codd (1979), Codd (1980), Codd (1982a), Codd (1982b), Codd (1985b), Codd (1985c), Van Jaarsveld & Edwards (1991), Ryding (1993), Codd (1994), Van Jaarsveld & Edwards (1997), Van Jaarsveld & Van Wyk (2001).

Insect vouchers are lodged at the Natal Museum, Pietermaritzburg (Diptera), or with the Biosystematics Division, Plant Protection Research Institute, Pretoria (Hymenoptera). Family classification for Hymenoptera follows Brothers (1999). Voucher details are followed by date of collection (or observation where no voucher was collected), and locality.

Plant vouchers collected during the study, cited in the various publications and chapters, are lodged at the Bews Herbarium (NU).

The following abbreviations are used:

NV: No voucher collected;
NR: Nature Reserve;
Localities
DV: Dargle Valley, KwaZulu-Natal Province (KZN) Midlands;
FC: Ferncliff Nature Reserve, Pietermaritzburg, KZN Midlands;
HF: Hlatikulu NR, Kaliweni forest, northern KZN;
KF: Kologha forest, Stutterheim, Eastern Cape Province (EC);
KK: Karkloof, Leopard’s Bush Nature Reserve, KZN Midlands;
MF: Magwa Forest, EC Coast;
NG: Ngeli Forest, Weza, southern KZN;
OG: Oribi Gorge Nature Reserve, KZN South Coast;
ON: Ongoye Nature Reserve, near Empangeni, KZN North Coast;
PMB Gdn: Pietermaritzburg, in a garden, KZN Midlands;
PSJ: Port St Johns, Bulolwe River, EC Coast;
UNR: Umtamvuna Nature Reserve, KZN South Coast;
UV: Umgeni Valley Nature Reserve, Howick, KZN Midlands;
WV: World’s View, near Ferncliff, Pietermaritzburg, KZN Midlands.

Study species, grouped according to corolla tube shape and length

A. Long-tubed species with straight corollas; corolla tube lengths 20 – 33 mm

Species from this group are included in Potgieter *et al*. (1999) and Potgieter & Edwards (2005).

1. *Plectranthus ambiguus* (Bolus) Codd	Tube 20 – 33 mm
2. *Plectranthus hilliardiae* Codd	Tube 21 – 32 mm
3. *Plectranthus reflexus* E.J. van Jaarsv. & T.J.Edwards	Tube 24 – 30 mm
4. *Plectranthus saccatus* Benth.	Tube 20 – 28 mm

B. Shorter-tubed species with straight corolla; corolla tube lengths 4 – 18 mm

Five species from this group, indicated with ***,** are included in Potgieter *et al*. (1999).

5. *Plectranthus ecklonii* Benth. *****	Tube 10 – 15 mm
6. *Plectranthus zuluensis* T.Cooke *****	Tube 10 – 16 mm
7. *Plectranthus ciliatus* E.Mey ex Benth. *****	Tube 6 – 8 mm
8. *Plectranthus ernstii* Codd	Tube 4 – 8 mm
9. *Plectranthus oribiensis* Codd *****	Tube 6 – 12 mm
10. *Plectranthus fruticosus* L'Hér.	Tube 5 – 13 mm
11. *Plectranthus oertendahlii* T.C.E.Fr.	Tube 8 – 13 mm
12. *Plectranthus praetermissus* Codd	Tube 12 – 15 mm
13a. *Plectranthus madagascariensis* (Pers.) Benth. *****	Tube 5 – 6 mm
13b. *Plectranthus hadiensis* (Forssk.) Schweinf. ex Spreng.	Tube 7 –18 mm

C. Sigmoid-tubed species; with corolla tube lengths 5 – 11.5 mm

Species from this group are included in Potgieter *et al*. (2009).

14. *Plectranthus petiolaris* E.Mey. ex Benth.	Tube 7– 11 mm
15. *Plectranthus laxiflorus* Benth.	Tube 10 – 11 mm
16. *Plectranthus calycinus* Benth.	Tube 6 – 7 mm
17. *Plectranthus rehmannii* Gürke	Tube 5 mm
18. *Plectranthus spicatus* E.Mey. ex Benth.	Tube 5 mm
19. *Pycnostachys urticifolia* Hook.	Tube 10.5 – 11.5 mm
20. *Aeollanthus parvifolius* Benth.	Tube 7 – 10 mm

1. *Plectranthus ambiguus* (H.Bol.) Codd

Habit, habitat, distribution and phenology

Plectranthus ambiguus is a soft herb with erect or decumbent stems, growing 0.4 – 1.2 m in height, in clearings in forest, on forest margins and on shaded rocky slopes. It occurs along semi-coastal and coastal areas of the Eastern Cape towards Ongoye Forest in KZN. It flowers from January to April and produces a showy mass of purple to violet flowers in the forest understorey.

Inflorescence and floral morphology

Synflorescences are simple (or sparingly branched), congested panicles, 40 – 170 mm in height. Flowers are purple to violet, with narrow, straight, long corolla tubes (20 – 33 mm) that expand slightly towards the mouth to 2 mm deep. The upper lip is 4 – 5 mm long with fine vertical dark lines as nectar guides; the concave lower lip is 3 – 5 mm long. Four free stamens and the style extend 6 – 7 mm and 6mm respectively from the corolla mouth.

Study sites and observations

Most pollinator observations for this species were done at Umtamvuna NR where large patches occurred along the forested slopes of the Bulolo River Gorge. One large population was mixed with *Plectranthus ecklonii* and insects frequently moved from the one species to the other. Less dense populations were studied at Oribi Gorge NR. Observation time on *P. ambiguus* was over a number of days in different years for at least 20 hours, during the main period of insect activity from 9.00 am to 4.00 pm.

Pollinators

The long-proboscid nemestrinid fly, *Stenobasipteron wiedemanni*, is the main pollinator for this species; few other insect visitors can reach nectar at the base of the long corolla tube. Visits by *S. wiedemanni* lasted from 1 – 4 seconds per flower, with the fly hovering into position, then settling on the exserted stamens and style while probing for nectar. Pollen was deposited ventrally on the thorax, especially in the hairy area between the bases of the legs, and on the abdomen. The length of each visit appeared to be dictated by the amount of nectar at the corolla base where the nectary is positioned. Flies tended to forage from the base to the top of the inflorescence, often visiting a few flowers per inflorescence before moving on to the next.

Other visitors included the apinid bees *Amegilla mimadvena* and *Xylocopa hottentotta*, both with proboscis lengths too short to extract nectar (mean length 9 and 8 mm respectively), unless flowers on the short end of the range were visited while nectar levels were very high. Syrphid flies (two species, one being *Allobaccha* sp.), apinid honeybees (*Apis mellifera*) and *Allodape* pernix collected pollen from the anthers; the latter also robbed nectar from holes made in the base of the corolla tissue near the nectary.

Insect vouchers

Stenobasipteron wiedemanni (Nemestrinidae)

Potgieter 1	UNR, 17-3-95
Potgieter 2	UNR, 18-3-96
Potgieter 3	UNR, 15-3-96

Prosoeca umbrosa (Nemestrinidae)

Potgieter 4	UNR, 19-3-96

Allobaccha sp. 2 (Syrphidae)

Potgieter 8	UNR, 17-3-95

Syrphidae sp. 1

Potgieter 27	UNR, 17-3-97

Apis mellifera (Apidae)

Potgieter 10	UNR, 19-3-96

Allodape pernix (Apidae)

Potgieter 12	UNR, 18-3-96
Potgieter 19	UNR, 18-3-96
Potgieter 20	UNR, 18-3-96
Potgieter 21	UNR, 19-4-95
Potgieter 22	UNR, 17-3-95

Amegilla mimadvena (Apidae)

NV

Xylocopa hottentotta (Apidae)

NV

2. *Plectranthus hilliardiae* Codd

Habit, habitat, distribution and phenology

Plectranthus hilliardiae is an erect, short herb growing 300 – 650 mm tall. It is semi-succulent with a branched habit. It grows among rocks – often in sandy soil near rivers – in forest areas. The species is endemic to coastal forests from the Umtamvuna area in KZN, southwards to the Mbotyi/Magwa area in the Eastern Cape. It is locally sparse to abundant in a narrow niche, but in some years formed large displays, especially along paths in the Umtamvuna NR. It flowers from December to April.

Inflorescence and floral morphology

Synflorescences are simple racemes / panicles, 80 – 150 mm in height, with one or two branch pairs at the base. The corolla tube is slightly deflexed at the base, straight and long (21 – 32 mm) with a saccate base; the tube is parallel-sided or slightly narrowed towards the mouth (from 5 – 3 mm deep). Flowers are pale blue with nectar guides as dark purple flecks on the inside of the upper and lower lips. The upper lip is 5 – 6 mm long and the concave lower lip is 4 mm; the latter may sit horizontally or be deflexed, depending on floral age. The four, free stamens are didynamous, with the lower pair extending up to 8 mm from the corolla mouth. The style extends 8 – 10 mm from the corolla mouth.

Study sites and observations

Pollinator observations were mainly conducted at Umtamvuna NR, along the Bulolo River, which is a tributary to the Umtamvuna River where Codd (1985a) recorded the species as being endemic. Subsequent collections (Van Jaarsveld & Van Wyk 2001) extended the distribution south to the northern Eastern Cape (Transkei) where populations were found at Mbotyi Forest, Magwa Falls, Fraser Falls, Myokane Gorge and Noyokaan Gorge (subsp. *australis sensu* Van Jaarsveld & Van Wyk), as well as Lupatana Gorge and Mkambati Gorge (which, with Umtamvuna, represents the distribution of subsp. *hilliardiae sensu* Van Jaarsveld & Van Wyk). We conducted shorter periods of pollinator observations at Lupatana Gorge and Magwa Gorge. Total observation time was at least 20 hours over a number of days in different years, between 9.00 am and 4.00 pm.

Our observations suggest that the glossy-leafed population at Lupatana Gorge is closer to subspecies *australis* than *hilliardiae*. This subspecies or form is much easier to cultivate than the typical subspecies.

Pollinators

The main pollinator of *P. hilliardiae* is the long-proboscid nemestrinid fly, *Stenobasipteron wiedemanni*. The fly hovered in front of the flower, briefly settling on the exserted stamens and style, while probing for nectar. Pollen is deposited ventrally on the thorax and abdomen. Pollinator sightings for this plant species were much less frequent than observations on other species that occur in the same locality, such as *P. ambiguus*. During 15 hours of observation on *P. hilliardiae*, on sunny days, only three observations of visits by *S. wiedemanni* were made, compared to 25 visits to *P. ambiguus* over the same amount of time.

The only other recorded visit to *P. hilliardiae* was by an acrocerid fly (*Psilodera* aff. *confusa*) which probed a flower briefly. This fly could not reach nectar with its medium-length proboscis (16 mm), even if it extended the proboscis slightly, but may have attempted a visit since other medium-tubed species of *Plectranthus* look superficially similar.

Nectar robbing visits by the apinid bee, *Allodape pernix,* were observed on *P. hilliardiae* flowers, with about 30% of flowers showing robbing holes on the dorsal side of the saccate corolla base, near the nectary

Insect vouchers

Stenobasipteron wiedemanni (Nemestrinidae)
    Potgieter 3   UNR, 15-3-96
Psilodera aff. *confusa* (Acroceridae)
    Potgieter s.n.   UNR, 17-3-95
Patellapsis sp. (Apidae, Halictinae)
    Potgieter 53   UNR, 19-3-96
Allodape pernix (Apidae)
    NV

3. *Plectranthus reflexus* Van Jaarsv. & T.J.Edwards

Habit, habitat, distribution and phenology

Plectranthus reflexus is a semi-succulent, soft herb with an erect habit. It grows 1 – 1.5 m tall and has few, ascending branches. It is a narrow endemic of densely shaded coastal forest along the Bulolwe River at Port St Johns in the Eastern Cape (Transkei), and further south at Mkambati (D. Styles pers. comm.). Flowering occurs between January and March.

Inflorescence and floral morphology

Synflorescences are lax, terminal racemes (150 – 200 mm long), with occasional subtending lateral branches. Flowers are pale blue without any markings. Floral tubes are long (24 – 30 mm) with a slightly saccate base 3 mm deep, tapering to 1 mm in the corolla mouth. The upper lip is reflexed to the extent that it may touch the corolla tube, as is the boat-shaped lower lip. Stamens are four, free and didynamous, with the lower pair exserted by 12 mm and the upper pair by 6 mm from the corolla mouth. Stamens coil away in older flowers and the style extends 8 – 9 mm from the corolla mouth.

Study sites and observations

The population of *P. reflexus* was visited once in March 1998 at Port St Johns. About five hours of observation time was spent with the population, from mid-morning to mid-afternoon.

Pollinators

The nemestrinid fly, *Stenobasipteron wiedemanni*, is the main pollinator, but a brief visit to one inflorescence by a Citrus Swallowtail butterfly (Papilionidae) was also seen. Due to the long proboscis of this butterfly it is unlikely that pollen was deposited on the insect's body. The reflexed upper and lower corolla lips make it difficult for insects to settle on the flowers, hence hovering insects with long proboscides are at an advantage for reaching nectar. Nemestrinid flies grasped the extended stamens and style during visitation, thus ensuring pollen carry-over on the fly body. Pollen was deposited ventrally on the thorax and abdomen of *S. wiedemanni*.

No bees were seen to visit this species, other than a pollen-collecting apinid *Allodape pernix*. A syrphid fly was observed eating pollen.

Insect vouchers

Stenobasipteron wiedemanni (Nemestrinidae)

NV on *P. reflexus* (but voucher caught soon after on *P. praetermissus*, at PSJ: Potgieter 191)

Allodape pernix (Apidae)

Potgieter 119 PSJ, 9-3-98

Allobaccha sp. 2 (Syrphidae)

Potgieter 138 PSJ, 9-3-98

4. *Plectranthus saccatus* Benth.

Habit, habitat, distribution and phenology

Plectranthus saccatus is a freely branched, erect to spreading soft shrub with semi-succulent stems. It grows 0,5 – 1,2 m tall in coastal or semi-coastal forests or areas of semi-shade. It is distributed from the southern Transkei area of the Eastern Cape, northwards to northern KZN. It flowers from December to April (and sporadically in June).

Inflorescence and floral morphology

Synflorescences are simple racemes (occasionally branched near the base), 50 – 120 mm in height, with relatively few, deflexed flowers per inflorescence, of which only a couple are open at any time. The large mauve to pale blue or white flowers make a striking display in the forest understorey. Floral tubes are markedly saccate at the base (up to 6 mm), with laterally compressed parallel sides that may narrow slightly towards the mouth. The upper lip is upright and the boat-shaped lower lip starts off horizontal and deflexes with floral age. The upper lip may be unmarked or heavily blotched with purple around the edges; forms occur with fine purple speckling across upper and lower lips. The four stamens are free and exserted from the corolla mouth by 8 – 10 mm; the style is exserted by ca. 10 mm.

This is a highly variable species and two varieties were recognized by Codd (1985a). *P. saccatus* var. *saccatus* has a shorter corolla tube (8 – 16 mm long) with broader vertical sides (5 – 6 mm deep at the base), while *P. saccatus* var. *longitubus* has a longer corolla tube (20 – 28 mm), but narrower vertical sides (4 – 5 mm deep at the base). The latter variety occurs at the northernmost range in the Kaliweni Forest near Ingwavuma, with some long-tubed populations further south at Umtamvuna NR.

On the basis of succulence two subspecies were recognized by Van Jaarsveld & Edwards (1997). *Plectranthus saccatus* Benth. subsp. *pondoensis* Van Jaarsv. & Milstein has succulent leaves, a procumbent to decumbent habit and flexible stems that may reach 4 m in length. This subspecies occurs in scrub near forest along the lips of gorges, while *P. saccatus* subsp. *saccatus* occurs in forest understorey. The variation in corolla tube length in this species presents an apparent continuum, which questions the validity of recognizing the variety *P. saccatus* var. *longitubus* Codd (Van Jaarsveld

& Edwards 1997). For the purposes of this study we refer to populations only, referring to long-tubed, medium-tubed and short-tubed forms for convenience.

Study sites and observations

Pollinator observation was attempted at four populations of this species. At Umtamvuna NR the mauve, long-tubed population (with no nectar guides and tubes 20 – 30 mm long) was studied in the forest understorey along the steep slopes of the Umtamvuna Gorge. One study visit was made to Hlatikulu NR at Kaliweni forest in northern KZN in early February 2008, with ca. eight hours spent at two different populations of the long-tubed form of *P. saccatus*. Another, short-tubed (5 – 7 mm), white- to pale mauve-flowered variety (with dark blue speckles as nectar guides) with succulent stems, was studied on the forest edge above the Bulolo River Gorge. At Oribi Gorge NR a medium-tubed population (9 – 11 mm) with pale mauve flowers, speckled upper lips and succulent stems was studied. At least 23 hours of observation time included observations from early morning to dusk over a number of days, in different years.

Pollinators

Pollinators of this species proved to be the most elusive at the Pondoland study sites, despite many hours of observation time. A long-proboscid nemestrinid fly (*S. wiedemanni*) was recorded on the long-tubed population at Umtamvuna NR. This population also showed evidence of nectar-robbing at the bases of most of the open flowers on each plant, as well as on fallen corollas. The apinid bee, *Allodape pernix*, was even seen making holes in the bases of unopened flowers in search of nectar.

No observations were made on the short-tubed variety at Umtamvuna, but based on corolla tube dimensions it is highly likely that an acrocerid fly of the genus *Psilodera*, or an apinid bee of the genus *Amegilla*, is the pollinator.

At Oribi Gorge one brief visit to a flower of the medium-tubed population was seen at dusk (6.15 pm in April 1998), but the identity of the visitor remains unclear. It looked like a brown moth, which would make sense considering the time of day (and nemestrinids had not been seen out at that time before), but *Plectranthus* has no scent typical of that which would attract moths at night. It is likely that *S. wiedemanni*, or one of the shorter-proboscid *Prosoeca* species, is the pollinator at this site. Repeated visitations of the long-proboscid *S. wiedemanni* were seen to a medium-tubed form of

P. saccatus (12 – 14 mm) in cultivation in a garden in New Germany, Durban area (A. Beaumont pers. comm.).

At the northern KZN site, where *P. saccatus* with long corolla tubes were prominent in certain parts of the forest understorey, growing alongside a species of *Justicia* L. (Acanthaceae), no nemestrinid flies were active at the start of the flowering season. A few tabanid flies *(Ph. aethiopica*), were seen to visit the flowers, and appeared to reach nectar. Apinid bee species such as *Amegilla mimadvena* were frequent visitors to *P. saccatus* flowers, but spent more time on the adjacent *Justicia* species. Fewer visits were noted by *A. bothai* bees which attempted visits to *P. saccatus* but then moved on the more accessible *Justicia* flowers. Individuals of *Xylocopa hottentotta* appeared to occasionally reach nectar in *P. saccatus* flowers, but often relocated to the base of the flower to pierce holes for robbing nectar.

The paucity of pollinator observations for *P. saccatus* in the Pondoland area is unfortunate, since the range in floral tube length is the most extensive in the genus. This aspect of the project will be extended in future years to ascertain why there are so many different floral varieties in this species.

Insect vouchers

Stenobasipteron wiedemanni (Nemestrinidae)

NV	UNR, 18-3-96

Philoliche aethiopica (Tabanidae)

Potgieter 300	HF, 5-2-08

Xylocopa hottentotta (Apidae)

Potgieter 301	HF, 5-2-08
Potgieter 307	HF, 6-2-08

Amegilla mimadvena (Apidae)

Potgieter 303	HF, 5-2-08
Potgieter 304	HF, 5-2-08

Amegilla bothai (Apidae)

Potgieter 302	HF, 5-2-08

5. *Plectranthus ecklonii* Benth.

Habit, habitat, distribution and phenology

Plectranthus ecklonii is a tall, erect, robust sub-shrub that grows 0.7 – 2.5 m tall; stems are succulent below with ascending branches. It is locally common forming populations along forest margins, clearings and stream banks. It has a coastal, semi-coastal and midlands distribution from the Eastern Cape, through KZN into Mpumalanga Province. Flowering time extends from January to April (and sporadically in August).

Inflorescence design and floral morphology

Plants produce a showy display of dark blue-purple or mauve flowers on large, terminal, dense panicles (120 – 250 mm in height). Populations of pale blue, white and pink-flowered individuals have been recorded, but the populations studied in this project were of the commonest colour morph (purple). The whole inflorescence, including calyces, pedicels, peduncles and bracts, are similar in colour to the flowers. Corolla tubes are straight, laterally compressed and expanding from a narrow base (1 mm deep) towards the mouth (3 mm deep) to form a trumpet shape. The tube is of medium length (10 – 15 mm); the upper lip is 5 – 6 mm and the concave lower lip is 4 – 5 mm long. Nectar guides of darker purple irregularly shaped blotches occur on the upper lip. The four stamens are free and didynamous, extending up to 15 mm from the corolla mouth. Stamens coil away from the style as the flower ages; the style extends 12 – 16 mm from the corolla mouth.

Study sites and observations

Populations of *P. ecklonii* were studied at Oribi Gorge NR, Umtamvuna NR, Kologha Forest at Stutterheim and in cultivation at Pietermaritzburg. Observation time was at least 25 hours between 9.00 am and 4.00 pm over a number of days in different years, with similar amounts of time spent at Oribi Gorge and Umtamvuna, and one visit to Stutterheim. Mixed populations with *P. ambiguus* occurred at Umtamvuna NR.

Pollinators

The long-proboscid nemestrinid fly, *Stenobasipteron wiedemanni*, was a common visitor at the Stutterheim population, and was also seen at Oribi Gorge and Umtamvuna NR. Large-bodied, medium-long proboscid nemestrinid flies of the genus *Prosoeca* (*P. umbrosa*) were frequent visitors at Oribi Gorge and Umtamvuna NR; these flies ‘fit’ the corolla tube very well with a proboscis length of 15 – 16 mm.

Nemestrinid flies hovered while probing the tubes, briefly settling on the stamens and style while extracting nectar. Visits lasted from 1 – 5 seconds. The proboscis was folded beneath the fly body during flight and was raised into a horizontal position on approach of a flower. Pollen was deposited ventrally on the head and thorax in the case of *S. wiedemanni*, and on the thorax and abdomen in *P. umbrosa*.

Medium-proboscid tabanid flies (*Philoliche aethiopica*) and apinid bees of the genus *Amegilla* (*A. caelestina* and *A. mimadvena*), as well as *Xylocopa hottentotta*, were frequent visitors. These four species had proboscides 8 – 9 mm long and were able to access nectar if the body was forced into the trumpet-shaped corolla. This action brushed the ventral surface of the insect body over the exserted anthers and deposited pollen on the abdomen of the insect. In the female phase, when the anthers were coiled away, the pin-like style picked up pollen off the insect in the same way.

Hummingbird hawkmoths (*Macroglossus trochilus*) were occasional nectar feeders, while syrphid flies and the apinid bee, *Allodape pernix*, collected pollen. The latter also robbed nectar by making holes in the corolla bases and buds.

The flowers of *P. ecklonii* straddle the long- and medium-tubed guilds in that the corolla is of medium length and dilated at the mouth, which allows visits by medium-proboscid insects, as well as long-proboscid insects that pick up pollen on the body from the long anthers that approximate the length of long-tubed species. As a result more insect species visit *P. ecklonii* effectively than the four preceding long-tubed species.

Insect vouchers

Stenobasipteron wiedemanni (Nemestrinidae)

Potgieter 194	KF, 5-3-98
Potgieter 195	KF, 5-3-98
Potgieter 197	KF, 29-2-00

Prosoeca umbrosa (Nemestrinidae)

Potgieter 4	UNR, 19-3-96
Potgieter 5	UNR, 18-3-96 (= *Prosoeca* sp. A, Potgieter *et al*. 1999)

Prosoeca sp. nov. 1 (Nemestrinidae) – close to Potgieter 171

Potgieter 190	OG, 7-4-98

Philoliche aethiopica (Tabanidae)

Potgieter 6	UNR, 15-3-96
Potgieter 39	PMB Gdn, 7-4-94

Amegilla caelestina (Apidae)

Potgieter 176	OG, 3-4-98

Amegilla mimadvena (Apidae)

NV

Xylocopa hottentotta (Apidae)

NV

Macroglossus trochilus (Sphingidae)

NV

Allobaccha sp. 1 (Syrphidae)

Potgieter 7	UNR, 15-3-96

Allodape pernix (Apidae)

Potgieter 11	UNR, 18-3-96

6. *Plectranthus zuluensis* T. Cooke

Habit, habitat, distribution and phenology

Plectranthus zuluensis is an erect, soft sub-shrub that grows 1 – 2 m tall, with ascending branches, along streams and margins of the forest. It has a coastal and semi-coastal distribution from southern KZN northwards to central KZN, occurring again in Swaziland. There is an extended flowering season for this species: September to November and January to May.

Inflorescence and floral morphology

It is a showy species when in flower, with tightly packed, cylindrical blue synflorescences. These are racemose (occasionally branched near the base), 40 – 80 mm in height. Flower colour in the study area was pale blue (sometimes appearing white), but dark blue forms also exist. Six rows of darker blue dots act as nectar guides on the upright upper lip. The corolla tube is of medium length (10 – 16 mm), slightly deflexed and laterally compressed, with a large saccate base that narrows towards the corolla mouth. Both the upper lip and concave lower lip are 5 – 6 mm long; the latter reflexes as the lower stamens fold away when the style elongates. The stamens are free with only the lower pair fertile, extending 5 – 7 mm from the corolla mouth. The upper pair is reduced to small staminodes that only extend 1 – 2 mm from the mouth. This is the only species with only two functional anthers. The style exerts 5 mm from the corolla mouth.

Study sites and observations

Pollinator observations on *P. zuluensis* were only made at Oribi Gorge, where an easily accessible, large population occurred along the upper end of the gorge. At least 15 hours were spent on observations over a number of days in different years (mostly 1997 – 1998), from early morning (8 am) to late afternoon (5.30 pm).

Pollinators

The acrocerid fly, *Psilodera confusa*, was the commonest visitor. These flies have proboscis lengths (9.5 – 11 mm) matching or slightly shorter than that of the corolla tubes, which forced close contact between the fly bodies and the anthers or styles. The flies never occurred in large numbers, but one or two were seen visiting the population at any one time. The flies hovered in front of the flower and briefly settled on the sexual organs while probing for nectar. Pollen was deposited ventrally on the head and hairy thorax. These flies moved between *P. zuluensis* and an adjacent population of *P. ciliatus* and a hybrid between these two species was found close by.

A less frequent visitor was the nemestrinid fly, *Stenobasipteron wiedemanni*. Despite the long proboscis of this fly, it could contribute to pollen carryover by brushing against the anthers with its proboscis, or in individuals at the short end of the proboscis range pollen may be carried on the hairy patch below the head. These flies also moved between *P. zuluensis* and the shorter-tubed *P. ciliatus*. One observation was made of a very large-bodied black fly (probably *Prosoeca*) visiting *P. zuluensis* and subsequently visiting the hybrid plant nearby, but this specimen could not be captured to verify identity. This fly was not observed again, but a similar-looking species was seen at Ongoye Forest.

Insect vouchers

Psilodera confusa (Acroceridae)

Potgieter 105	OG, 25-3-97
Potgieter 106	OG, 25-3-97
Potgieter 134	OG, 12-3-98
Potgieter 136	OG, 23-4-98

Stenobasipteron wiedemanni (Nemestrinidae)

Potgieter 103	OG, 25-3-97

7. ***Plectranthus ciliatus*** **E.Mey ex Benth.**

Habit, habitat, distribution and phenology

Plectranthus ciliatus is a low-growing soft, branching herb with decumbent to ascending stems, growing up to 0.6 m tall. It occurs in moist areas in forests and other shady places, with a coastal to midlands distribution from Knysna in the Western Cape, through the Eastern Cape and KZN and into Mpumalanga Province. It flowers from November to April.

Inflorescence and floral morphology

Synflorescences are fairly lax, simple racemes (sometimes with a pair of branches near the base), 50 – 200 mm in height. Flowers are white with dense purple speckles over the inside surfaces of the upper and lower lips. The white flowers contrast well with the dark green foliage (with purple undersides) in shaded, dark places. The corolla tube is medium to short (6 – 8 mm), slightly deflexed, with a saccate base, narrowing slightly towards the corolla mouth. The upper lip is 5 – 8 mm and the boat-shaped lower lip is 3 – 7 mm long; the latter may be held horizontally or deflex as the flower ages. The four anthers are free and extend beyond the corolla mouth by 5 mm (upper pair) and 7 mm (lower pair).

Study sites and observations

Pollinator observations were made at Oribi Gorge and Umtamvuna NR in southern KZN, Kologha Forest near Stutterheim and Magwa area in the Eastern Cape, Leopard's Bush in the Karkloof and Ferncliff in Pietermaritzburg. At least 25 hours of observations were made over a number of days in different years, between early morning (8.00 am) and late afternoon (5.30 pm).

Pollinators

At Oribi Gorge the medium-proboscid acrocerid fly, *Psilodera confusa* (proboscis 9.5 – 11 mm), was a common visitor to *P. ciliatus*. In Pietermaritzburg the acrocerid flies, *Psilodera hessei* (proboscis 8.5 – 9.5 mm) and *Psilodera nhluzane* (proboscis 12 mm), were common at Ferncliff, with *Psilodera valida* visiting *P. ciliatus* at a nearby population (World's View). In the Karkloof and at Umgeni Valley, and further south at Stutterheim, this species (a yellow fly of a different sub-genus to *Ps. confusa*, with proboscis 8 mm), was observed visiting *P. ciliatus*. A similar yellow acrocerid species was seen visiting *P. ciliatus* and *P. fruticosus* at Magwa in an area where putative *P.*

ciliatus x *P. fruticosus* hybrids occurred. It is clear that acrocerid flies are important pollinators of *P. ciliatus* over a large extent of its range. The different acrocerid species that visited *P. ciliatus* have proboscides in a common range (8 – 12 mm), which matched or were longer than the corolla tubes of *P. ciliatus*, but corresponded to the length of the stamens and style, which facilitated pollen carry-over on the ventral head surface and base of the proboscis of the fly.

The long-proboscid nemestrinid fly, *Stenobasipteron wiedemanni*, also visited *P. ciliatus* populations in Stutterheim and at Oribi Gorge. The fly reached nectar without contacting the sexual organs of the plant with its body. While some pollen may be carried over on the proboscis, this is more a case of the fly exploiting the flowers for nectar.

The apinid bee species *Amegilla caelestina* and *A. bothai* visited *P. ciliatus* at Umtamvuna NR. No acrocerid flies were observed on *P. ciliatus* at this a site, but *Psilodera* aff. *confusa* did occur at this site and may also pollinate the species. Likewise, species of *Amegilla* bees occur at the other study sites and may also visit *P. ciliatus* there.

At Magwa both the apinid bees and acrocerid flies were seen visiting flowers of the same population of the tentative hybrid *P. ciliatus* x *P. fruticosus*. The bees were *Amegilla mimadvena* and *Zebramegilla* sp. These bees may function as effectively as pollinators as acrocerid flies, since they are similar in body shape and size and the proboscis lengths match that of the *P. ciliatus* corolla tube. In most of the cases where *Amegilla* bees and *Psilodera* flies visited the same species, they fulfilled a similar role in terms of body sizes and proboscis lengths.

Other visitors to this tentative hybrid included a medium-proboscid nemestrinid fly (*Prosoeca* sp.) and three lepidopterans: the hummingbird hawkmoth *Macroglossus trochilus*, the papilionoid butterfly *Papilio dardanus* and a species of hesperid butterfly. These Lepidoptera were unlikely to be major pollinators, since the proboscides were much longer than the floral parts, which prevented bodily contact. Syrphid flies visited flowers at Oribi Gorge and Pietermaritzburg, and the short-proboscid halictinid bee, *Lasioglossum* sp., was observed at Pietermaritzburg. These species collected pollen, but are unlikely to reach nectar from the corolla base.

Plectranthus ciliatus is a relatively common and widespread species of *Plectranthus*; this, coupled with its shorter corolla tube, allows the species to have many insect visitors, of which the various species of Acroceridae and Apinae are effective pollinators in different localities.

Insect vouchers

Psilodera confusa (Acroceridae)

Potgieter 107	OG, 15-3-97
Potgieter 134	OG, 12-3-98

Psilodera hessei (Acroceridae)

Potgieter 108	FC, 7-3-97
Potgieter 109	FC, 7-3-97
Potgieter 131	FC, 2-3-98
Potgieter 132	FC, 2-3-98

Psilodera nhluzane (Acroceridae)

Potgieter 110	FC, 7-3-97

Psilodera valida (Acroceridae)

Potgieter 31	WV, 11-4-96
Potgieter 130	KF, 5-3-98
Potgieter 156	KK, 3-3-99
Potgieter 221	MF, 19-4-02
D. Martins s.n.	UV, 18-3-05

Stenobasipteron wiedemanni (Nemestrinidae)

Potgieter 102	OG, 25-3-97
Potgieter 192	KF, 5-3-98
Potgieter 193	KF, 5-3-98
Potgieter 198	KF, 29-2-00

Prosoeca umbrosa (Nemestrinidae)

Potgieter 215	MF, 19-4-02	(on *P. ciliatus* hybrid)

Amegilla caelestina (Apidae)

Potgieter 56	UNR, 18-3-96
Potgieter 94	UNR, 2-3-97

Amegilla bothai

NV

Amegilla mimadvena

NV

Macroglossus trochilus (Sphingidae)

Potgieter 206	MF, 19-4-02

Papilio dardanus (Lepidoptera)

NV

Hesperidae (Lepidoptera)

Potgieter 98	FC, 7-3-97

Asarkina sp. (Syrphidae)

Potgieter 100	FC, 7-3-97

Rhingia sp. (Syrphidae)

Potgieter 104	OG, 25-3-97

Zonalictus sp. (Apidae, Halictinae)

Potgieter 99	FC, 7-3-97

8. *Plectranthus ernstii* Codd

Habit, habitat, distribution and phenology

Plectranthus ernstii is a semi-succulent herb that branches from thickened stem bases; it reaches 250 mm in height and survives dry conditions with the potato-like stem bases. The species escapes fire by growing in pockets of humus-rich soil between rocks and on south-facing cliffs. It is an endemic species confined to the area from Oribi Gorge to Umtamvuna and Mkambati further south. Flowering time extends from February to April, but in cultivation the species flowers over a longer period of time.

Inflorescence and floral morphology

The pale bluish-mauve flowers are produced in relatively lax, often secund synflorescences, 30 – 120 mm in height. Corolla tubes are straight and of medium length (4 – 8 mm) with a saccate base 4 – 5 mm wide, narrowing to 2 mm wide at the corolla mouth. The upper lip is 4 – 5 mm, with fine vertical dark blue lines as nectar guides; the boat-shaped lower is lip 3 – 4 mm long. Four free, didynamous stamens extend by 1.5 mm (upper pair) and 3 mm (lower pair) from the corolla mouth. The style exerts by 2 mm.

Study sites and observations

A population at Oribi Gorge was observed for a whole morning, but the rocky cliff face where the plants were growing was inaccessible and plants were observed from some distance. No pollinators were seen. A subsequent visit to another, slightly more accessible, population on a neighbouring farm was disappointing, since it was not in flower when expected. Observations made in Pietermaritzburg on cultivated plants in a garden showed the apinid bee *Amegilla caelestina* to be a constant visitor. This bee species occurs at Oribi Gorge and may prove to be the natural pollinator. It has the correct proboscis length to match the corolla tube of *P. ernstii,* as do other *Amegilla* species recorded at the site. The bees have also been observed in the kind of exposed areas where this species grows.

A putative hybrid between *P. ernstii* and the endemic *P. oribiensis* was found amongst the *P. ernstii* population. In cultivation the hybrid showed vegetative features of *P. ernstii* and floral features of *P. oribiensis*. The latter is pollinated by four species of *Amegilla* at Oribi Gorge – evidence that at least one of these bee species must have visited the rocky *P. ernstii* site to transfer pollen.

9. *Plectranthus oribiensis* Codd

Habit, habitat, distribution and phenology

Plectranthus oribiensis is a tall, erect, branched soft shrub with ascending stems, reaching up to 1.5 m in height. It grows along forest margins and in wooded kloofs on steep slopes, especially south-facing ones. According to Codd (1985a) this species is endemic to Oribi Gorge and the Umtamvuna River, but during the course of this study it was only found at Oribi Gorge. Flowering time extends from February to May.

Inflorescence and floral morphology

Mauve flowers are produced in lax racemose synflorescences with one or two pairs of branches near the base, up to 200 mm tall. The floral display is not striking, with inflorescences scattered at low density throughout the population. The upper and lower corolla lips have mauve hairs and white gland-dots on the outer surface. The compressed corolla tube is deflexed, straight and of medium length (6 – 12 mm). The base is saccate and extends into a short spur; the tube narrows slightly towards the corolla mouth. The upper lip is 5 – 6 mm and the boat-shaped lower lip 5 – 7 mm long; the latter deflexes as the flower ages. The four stamens are free, extending 2 – 3 mm from the corolla mouth, as does the style.

Study sites and observations

Pollinators were observed at Oribi Gorge, mostly along the road that passes through the mid- to upper part of the gorge, where plants were abundant. Twelve hours of observations were made from February to April, during 1995, 1997 and 1998.

Pollinators

Mostly bees visit this species, with apinid bees of the genus *Amegilla* as the most abundant pollinator. Four species of *Amegilla* were recorded: *A. caelestina*, *A. mimadvena*, *A. bothai* and *A. spilostoma*. The proboscis lengths of these species range from 7 – 9 mm, which fits the range of *P. oribiensis* corolla tubes (7 – 9 mm). A bee would inserted the whole length of its proboscis into the corolla, and pollen would be deposited ventrally on the thorax. An apinid *Thyreus* sp. was a frequent visitor and *Xylocopa flavorufa* (proboscis 9 mm long) was also recorded. One visitation of a small lycaenid butterfly was observed.

As discussed for *P. ernstii*, a tentative hybrid of *P. oribiensis* x *P. ernstii* was found near a population of *P. ernstii*. The range of corolla tube length of both overlap, and even though no bee visits were observed for *P. ernstii* at Oribi Gorge, at least one of the bees that pollinate *P. oribiensis* must visit *P. ernstii* as well.

Insect vouchers

Amegilla caelestina (Apidae)		
	Potgieter 57	OG, 15-3-95
	Potgieter 88	OG, 22-2-97
	Potgieter 172	OG, 7-4-98
Amegilla mimadvena (Apidae)		
	Potgieter 180	OG, 7-4-98
Amegilla bothai (Apidae)		
	Potgieter 87	OG, 22-2-97
Amegilla fallax (Apidae)		
	Potgieter 58	OG, 15-3-98
Thyreus vachali (Apidae)		
	Potgieter 117	OG, 7-4-98
Xylocopa flavorufa (Apidae)		
	NV	
Lycaenidae (Lepidoptera)		
	Potgieter 89	OG, 22-2-97

10. *Plectranthus fruticosus* L'Hér.

Habit, habitat, distribution and phenology

Plectranthus fruticosus is a variable soft suffrutex, growing 0.6 – 2 m tall. It branches freely, with branches ascending or rarely decumbent. The different varieties differ in terms of growth forms and/or floral shape, colour and size. The species occurs in forest and scrub forest, or in shaded places between rocks. It has the widest distribution of all the studied species, extending from the southern parts of the Western Cape, along the eastern seaboard through the Eastern Cape, KZN, Swaziland, Mpumalanga and the Northern Province. Flowering time extends from December to June.

Inflorescence design and floral morphology

The branched paniculate synflorescences, 80 – 300 mm tall, are relatively dense, with large flowers, tapering towards the apex. Plants produce showy displays of bluish mauve flowers. Pink and pale blue forms also occur, but not in the study sites of this project. Corolla tube lengths vary from 5 – 13 mm, with the broad upper lip 2.5 – 8 mm and the boat-shaped lower lip 2 – 8 mm long. The latter deflexes as the flower ages; the upper lip reflexes. Purple blotches or speckles mark the upper lip. The corolla tube is deflexed and saccate at the base, extending upwards and backwards into a spur. The tube narrows slightly towards the mouth, giving a 2 mm vertical aperture. Four free, didynamous stamens extend 8 – 14 (upper pair) and 10 – 17 mm (lower pair) from the corolla mouth. These coil away as the flower ages and the style elongates to a similar length as the lower stamens. Some populations have reduced floral spurs, while others have spurs up to 5 mm long (Ongoye Forest).

Study sites and observations

Populations were studied at Oribi Gorge, Leopard's Bush in the Karkloof, Ferncliff in Pietermaritzburg, Ngeli Forest, and Magwa area. At least 18 hours were spent observing this species for pollinators, on various occasions over a number of years, between 9.00 am and 4.00 pm. At both the Magwa and Ngeli sites hybrid swarms involving *P. fruticosus* were observed.

Pollinators

Nemestrinid flies were the most commonly observed pollinators of *P. fruticosus.* These included the long-proboscid *Stenobasipteron wiedemanni*, and medium and shorter-proboscid species of *Prosoeca* with proboscides from 7 – 11mm long. At Magwa acrocerid flies of the genus *Psilodera* (*P. valida*) visited a mixed stand of *P. fruticosus*, *P. ciliatus* and putative *P. fruticosus* x *P. ciliatus* hybrids. Medium-proboscid species of *Prosoeca* also visited these hybrids. The proboscis of *S. wiedemanni* exceeds the length of the *P. fruticosus* corolla tube, but some varieties have stamen and style lengths that approach the length of the *S. wiedemanni* proboscis and facilitate pollen deposition at the base of the proboscis. Medium-proboscid *Prosoeca* species carry pollen ventrally on the thorax and abdomen.

No bee visits were seen to *P. fruticosus*, except for *Allodape pernix* (Apidae, Apinae) that collected pollen form the anthers on the exserted stamens. One visit of a pierid butterfly was seen at Oribi Gorge.

Insect vouchers

Stenobasipteron wiedemanni (Nemestrinidae)
    Potgieter 154   KK, 3-3-99
Prosoeca sp. nov. 4 (Nemestrinidae) – near Potgieter 34
    Potgieter 34   FC, 11-4-96
Prosoeca circumdata (Nemestrinidae)
    Potgieter 148   OG, 3-4-98
Psilodera valida (Acroceridae)
    Potgieter 222   MF, 19-4-02
    Potgieter 223   MF, 16-4-02
Episyrphus sp. (Syrphidae)
    Potgieter 213   MF, 16-4-02
Zonalictus sp. (Apidae, Halictinae)
    Potgieter 41   FC, 24-4-96
Allodape pernix (Apidae)
    NV
Pieridae (Lepidoptera)
    NV

11. *Plectranthus oertendahlii* T.C.E.Fr.

Habit, habitat, distribution and phenology

Plectranthus oertendahlii is a short semi-succulent herb with decumbent branches, reaching 200 mm in height. It grows in open forest understorey near rivers and is endemic to the coastal areas of Oribi Gorge and the Uvongo River near Port Shepstone. Flowering time extends from March to April.

Inflorescence and floral morphology

White flowers are borne on 70 – 200 mm tall synflorescences of relatively lax, simple or branched racemes. The white flowers are sometimes suffused with pale mauve, and nectar guides of four thin, purple, vertical lines occur on the upper lip. Straight, laterally compressed corolla tubes of medium length (8 – 13 mm) with saccate bases 4 mm wide, narrow gradually to 1.5 mm wide at the corolla mouth. The upper lip is 5 mm and the boat-shaped lower lip 4 – 5 mm long; the latter may deflex slightly from a horizontal position. Four free stamens extend slightly (2 – 3 mm) beyond the corolla mouth, with the style extending by 3 mm.

Study sites and observations

Observations were conducted at Oribi Gorge only, since no populations were found at the Uvongo River. After initial attempts yielded no observations of floral visits at the scattered plants at the base of the gorge, a population was studied at dusk, but with no success. At least 20 hours of observations were made on various days in different years, between 9.00 am and 6.30 pm. Finally, in March 2004, pollinator visits were recorded to a large plant in a patch situated at a more elevated level along the gorge.

Pollinators

The acrocerid fly, *Psilodera confusa*, pollinates the species. Even on a rainy day the fly visited 30 – 40 flowers out of the ca. 60 on the plant, before moving on. A year later the same patch was observed and *Psilodera confusa* was once again seen pollinating the flowers. The proboscis length of this fly species (9.5 – 11 mm) matches the length of the corolla tube and sexual organs of *P. oertendahlii* closely. The floral tube is constricted at the mouth to the extent that only insects with suitably long, thin proboscides can probe for nectar. Pollen is deposited on the base of the proboscis and ventrally on the head and hairy thorax of the fly, between the bases of the legs.

This fly species also pollinates white *P. ciliatus* and pale blue forms of *P. zuluensis* at Oribi Gorge. It appears to seek out white or pale coloured flowers, since visits to a white *Lobelia* sp. were also seen in the same area.

Insect vouchers

Psilodera confusa (Acroceridae)

NV (but same species as Potgieter 105, 106, 134, 136, collected at OG)

Allodape pernix (Apidae)

Potgieter 101	OG, 25-3-97

12. *Plectranthus praetermissus* Codd

Habit, habitat, distribution and phenology

Plectranthus praetermissus is a freely branched herb with decumbent stems growing 20 – 50 cm tall. It occurs in open areas in coastal forest and is endemic to the Port St Johns area of the Eastern Cape (Transkei). It was studied along the Bulolwe River, not far from populations of another Pondoland endemic, *P. reflexus*. This species flowers from January to March.

Inflorescence and floral morphology

Racemose synflorescences are 120 – 200 mm tall, occasionally branching near the base. Flowers are violet to mauve with darker blotches as nectar guides on the inside of the upper and lower lips. Corolla tubes are straight and of medium length (12 – 15 mm) with saccate base 4 mm deep, narrowing to 1.5 – 2 mm at the corolla mouth. The upper lip is 5 mm and the concave lower lip is 4 mm long, deflexing with age. The four free, didynamous stamens extend beyond the corolla mouth by 1.5 – 2 mm (upper pair) and 4 – 5 mm (lower pair). The style exerts from the corolla mouth by 4 mm.

Study sites and observations

Two populations of *P. praetermissus* were studied in March 1998 at Port St Johns. Observations were made in the morning and the afternoon, for four hours in total.

Pollinators

The main pollinator was the acrocerid fly, *Psilodera nhluzane*, with a proboscis length (at this site) of 9 mm, which, together with the small head size of the fly, allows the fly to reach into the constricted floral tube for nectar. This facilitates pollen deposition ventrally on the head and thorax of the fly. The basic floral shape and size of *P. praetermissus* is very similar to that of *P. oertendahlii* and both species are pollinated by acrocerid flies.

One visit of the nemestrinid fly, *Stenobasipteron wiedemanni*, was observed at a population near the other endemic, *P. reflexus*. This fly species may not pick up pollen on its body, since its proboscis is longer than the corolla tube, but the narrow opening of the corolla mouth and the short extension of the upper pair of anthers facilitated some pollen deposition on the fly proboscis. Other floral visitors included a species of syrphid fly, a hesperid butterfly and the apinid bee, *Allodape pernix*, which collected pollen.

Insect vouchers

Psilodera nhluzane (Acroceridae)
Potgieter 133 PSJ, 9-3-98
Stenobasipteron wiedemanni (Nemestrinidae)
Potgieter 191 PSJ, 9-3-98
Allobaccha sp. 2 (Syrphidae)
Potgieter 214 PSJ, 7-3-98
Hesperidae (Lepidoptera)
NV
Allodape pernix (Apidae)
NV

13. *Plectranthus madagascariensis* (Pers.) Benth. (and *Plectranthus hadiensis* (Forssk.) Schweinf. ex Sprenger.)

Plectranthus madagascariensis and *P. hadiensis* will both be treated under *P. madagascariensis*, since these widespread, variable species are not always easy to distinguish in KZN (Codd 1985a) – the three varieties of each tend to inter-grade. The floral shape is functionally very similar and there is an overlap in pollinators. Most observations were made on *P. madagascariensis*, with a few *P. hadiensis* records included.

Habit, habitat, distribution and phenology

Plectranthus madagascariensis is a relatively low-growing herb with erect to procumbent or decumbent stems, which may be semi-succulent. It is tolerant of a variety of habitats, growing along forest margins, in rocky grasslands or dry woodlands. The distribution of the varieties of *P. madagascariensis* and *P. hadiensis* overlap to a large extent, being widespread from the eastern part of the Western Cape, through the Eastern Cape, KZN, Swaziland, Mpumalanga, Northwest and the Northern Province, extending further into Mozambique and the Mascarenes (*P. madagascariensis*) and Tropical East Africa to Somalia and beyond (*P. hadiensis*). Flowering time extends from February to May, with sporadic flowering in July and November.

Inflorescence and floral morphology

Synflorescences are produced terminally on the main stem and on side branches, as simple racemes or with 1 – 2 pairs of branches at the base (90 – 250 mm in height). In *P. hadiensis* the synflorescences may be taller (up to 500 mm) with flowering verticils further apart. Flowers are white or shades of mauve or purple, often with red glands on the lips. Corolla length is relatively short, varying from 5 – 6 to 7 – 18 mm long; the variety studied at Oribi Gorge had corolla tubes 4 – 6 mm long. The tube expands gradually from the base and is bent (downwards) at about a third of the distance from the base, after which the sides are more or less parallel. The upper lip is relatively short and the concave lower lip may be longer than the corolla tube. The four stamens are free to the base and extend more or less as long as the lower lip. The variety at Oribi Gorge has didynamous stamens, extending 2 – 4 mm (upper pair) and 4 – 6 mm (lower pair) from the corolla mouth.

Study sites and observations

Observations on *P. madagascariensis* were made at Oribi Gorge, Umtamvuna and World's View (near Ferncliff in Pietermaritzburg); a population of *P. hadiensis* was studied once at Ongoye Forest. A total of at least 20 hours was spent observing these species, on various days over a number of years, from 9.00 am to 4.00 pm.

Pollinators

The population at World's View occurred in rocky grassland and was pollinated by medium-proboscid nemestrinid fly species (*Prosoeca circumdata* & *P. umbrosa*) and *Psilodera valida* (Acroceridae). The apinid bee, *Amegilla aspergina*, also visited flowers. These observations were made in April after the mass emergence of *Prosoeca* spp. Both the fly and the bee species have proboscides that reach nectar easily in the short corolla tubes, while still permitting pollen carryover on the ventral head and thoracic surfaces.

More time was spent making observations at Oribi Gorge, which resulted in more floral visitors being recorded. Populations at this study site were most often in rocky areas near the river. The apinid bees *Amegilla caelestina*, *A. bothai*, *A. mimadvena* and *A. aspergina*, *Xylocopa caffra* and *X. hottentotta* were common visitors to *P. madagascariensis*; the proboscis lengths of this group are slightly longer than that of the floral tube, but as the bee settles on the boat-shaped lower lip while probing for nectar, the head rubs over the sexual organs contained in the lip. The bend in the corolla tube angles the mouth of the tube downwards, which forces large-bodied floral visitors to depress the lower lip that contains the stamens and style, as it angles upwards to access nectar which sits at a slightly higher level at the base of the tube.

Medium- and short-proboscid nemestrinid flies of the genus *Prosoeca* were frequent visitors at this study site, with the medium-proboscid flies functioning in the same way as the apinid bees discussed earlier, while the short-proboscid species match the corolla tube lengths so as to pick up pollen ventrally on the thorax and abdomen. The tabanid fly, *Philoliche aethiopica*, visited occasionally; it had similar proboscis lengths to that of the apinid bees and medium-proboscid *Prosoeca* species.

Less frequent visits by shorter-proboscid apinid bees at this site included *Thyreus* sp. and *Apis mellifera*, both of which probed for nectar, while a megachilinid bee species collected pollen ventrally onto the abdomen. The apinid bee, *Allodape pernix*, reached nectar by crawling into the corolla tube, but also collected pollen from the anthers of *P. madagascariensis*. The halictinid bee *Lasioglossum* sp. accessed nectar legitimately by crawling into the corolla tube, as well as by robbing it from a hole pierced at the base of the corolla. Four different species of syrphid fly were also occasionally seen probing for nectar with their relatively short proboscides; these flies are also known to collect pollen. Small lycaenid butterflies were occasional visitors to flowers and larvae of this species were also found feeding on the inflorescences.

At Umtamvuna similar bee species were visitors in open patches in the forest, as well as a number of lycaenid butterflies, a pierid butterfly species, a bombyliid fly with a short proboscis and a syrphid fly species.

At Ongoye Forest one set of observations at midday yielded a number of visitors to a form of *P. hadiensis* with blue corollas that occurred in an open, rocky area beyond the forest margin. Two species of the nemestrinid genus *Prosoeca* were very active visitors; *P. umbrosa* specimens had medium proboscides and the other, unidentified, species had shorter proboscides. The apinid bee *Amegilla bothai* was also observed, as well as a small halictinid bee and a bombyliid fly. All these species were capable of pollinating *P. hadiensis* and were also found on *P. madagascariensis* in other localities.

Plectranthus madagascariensis and *P. hadiensis* had the shortest corolla tubes of the ca. 20 species studied in this project and they showed the greatest number of visiting insects that were capable of facilitating pollination.

Insect vouchers

Prosoeca circumdata (Nemestrinidae)

Potgieter 28	WV, 11-4-96	
Potgieter 30	WV, 11-4-96	(= *Prosoeca* sp. D, Potgieter *et al*. 1999)
Potgieter 64	OG, 12-5-96	
Potgieter 111	OG, 20-4-97	
Potgieter 114	OG, 20-4-97	(= *Prosoeca* sp. C, Potgieter *et al*. 1999)
Potgieter 146	OG, 3-4-98	
Potgieter 148	OG, 3-4-98	
Potgieter 188	OG, 3-4-98	
Potgieter 189	OG, 3-4-98	

Prosoeca sp. nov. 3 (Nemestrinidae) – near Potgieter 34

Potgieter 147	OG, 3-4-98	
Prosoeca umbrosa (Nemestrinidae)		
Potgieter 29	WV, 11-4-96	(= *Prosoeca* sp. B, Potgieter *et al*. 1999)
Prosoeca umbrosa (Nemestrinidae)		
Potgieter 166	ON, 22-4-99	(on *P. hadiensis*)
Potgieter 167	ON, 22-4-99	(on *P. hadiensis*)
Psilodera valida (Acroceridae)		
Potgieter 32	WV, 11-4-96	
Amegilla aspergina (Apidae)		
Potgieter 33	WV, 11-4-96	
Amegilla caelestina (Apidae)		
Potgieter 67	OG, 14-3-95	
Potgieter 175	OG, 3-4-98	
Amegilla bothai (Apidae)		
Potgieter 178	OG, 3-4-98	
Amegilla mimadvena (Apidae)		
Potgieter 61	OG, 12-5-96	
Potgieter 116	OG, 19-4-97	
Potgieter 182	OG, 25-4-98	
Amegilla fallax (Apidae)		
Potgieter 90	UNR, 2-3-97	
Xylocopa caffra (Apidae)		
Potgieter 184	OG, 25-4-98	
Potgieter 184a	OG, 25-4-98	
Potgieter 184b	OG, 25-4-98	
Xylocopa hottentotta (Apidae)		
Potgieter 185	OG, 25-4-98	
Philoliche aethiopica (Tabanidae)		
Potgieter 113	OG, 20-4-97	
Potgieter 137	OG, 4-4-98	
Thyreus vachali (Apidae)		
Potgieter 118	OG, 25-4-98	
Apis mellifera (Apidae)		
Potgieter 187	OG, 3-4-98	
Pseudoanthidium truncatum (Apidae, Megachilinae)		

	Potgieter 65	OG, 12-5-96	
Chalicodoma sp. B (Apidae, Megachilinae)			
	Potgieter 120	OG, 25-4-98	
	Potgieter 121	OG, 25-4-98	
	Potgieter 122	OG, 25-4-98	
Allodape pernix (Apidae)			
	Potgieter 69	OG, 12-5-96	
	Potgieter 70	OG, 12-5-96	
	Potgieter 151	OG, 3-4-98	
	Potgieter 152	OG, 3-4-98	
	Potgieter 153	OG, 25-4-98	
Allodape ceratinoides (Apidae)			
	Potgieter 66	OG, 12-5-96	
	Potgieter 149	OG, 3-4-98	
	Potgieter 150	OG, 3-4-98	
Braunsapis (Apidae)			
	Potgieter 93	UNR, 2-3-97	
Lasioglossum sp. (Apidae, Halictinae)			
	Potgieter 68	UNR, 11-5-96	
Zonalictus sp. (Apidae, Halictinae)			
	Potgieter 164	ON, 22-4-99	
Bombylius (Bombyliidae)			
	Potgieter 55	UNR, 19-4-95	
	Potgieter 63	OG, 12-5-96	
	Potgieter 165	ON, 22-4-99	(on *P. hadiensis*)
Lycaenidae (Lepidoptera)			
	Potgieter 78	OG, 12-5-96	
	Potgieter 207	OG, 3-4-98	
	Potgieter 208	ON, 22-4-99	(on *P. hadiensis*)
Pieridae (Lepidoptera)			
	Potgieter 91	UNR, 2-3-97	
Episyrphus sp. (Syrphidae)			
	Potgieter 62	OG, 12-5-96	

14. *Plectranthus petiolaris* E.Mey. ex Benth.

Habit, habitat, distribution and phenology

Plectranthus petiolaris is a sprawling branched herb with ascending and descending stems, reaching 1 m in height. It grows on forest margins and in scree below cliffs covered by scarp forest. It occurs in the Eastern Cape (Transkei) northwards along the coast through KZN and inland to Mpumalanga. It flowers from December to May.

Inflorescence and floral morphology

Flowers are produced in lax, racemose synflorescences (that occasionally branch at the base), 100 – 250 mm in height. At Umtamvuna the flowers were deep purple (violet), with the corolla lips often tinged with blue; at Oribi Gorge the flowers were pale pink. The corolla tube is laterally compressed, 7 – 11 mm long and sigmoid in shape; from the narrow base it ascends for 3 mm, then deflexes and expands to 3 mm wide at the corolla mouth. The upper lip is 6 – 8 mm and the shallowly boat-shaped lower lip is 7 – 9 mm long. The four free stamens extend 4.5 mm (upper pair) and 5.5 mm (lower pair) from the corolla mouth.

Study sites and observations

Pollinator observations were made at Umtamvuna and Oribi Gorge, where at least 18 hours were spent with different populations on various days over a number of years, between 9.00 am and 5.30 pm. Some observations were also made on cultivated plants in Pietermaritzburg.

Pollinators

The main pollinators of *P. petiolaris* were apinid bees of the genera *Amegilla* and *Xylocopa*. At Umtamvuna *Amegilla caelestina*, *A. mimadvena* and *Xylocopa hottentotta* were common visitors. At Oribi Gorge *Amegilla bothai* and *A. caelestina* were commonly seen to visit the pink populations of *P. petiolaris*. At Pietermaritzburg *Amegilla caelestina* was a frequent floral visitor to cultivated plants. These bees picked up pollen ventrally on the thorax and abdomen. The flexible tip of the bee proboscis in most cases corresponds to the bend in the corolla of *P. petiolaris*, and the full length of the proboscis corresponds to the full tube length.

At Oribi Gorge one visit by the long-proboscid fly, *Stenobasipteron wiedemanni*, was seen on *P. petiolaris*. The hovering fly bent its proboscis around the corolla bend to reach nectar. It is unlikely that pollen was deposited on the body, but some may have rubbed off onto the proboscis; no voucher was collected.

Two syrphid fly species were seen collecting pollen at Oribi Gorge and another syrphid species did the same at Umtamvuna. The apinid bee *Allodape pernix* was regularly seen robbing nectar at the base of the corolla of *P. petiolaris* at Umtamvuna, with about 25% of corollas pierced in this way. These bees crawled onto the lower lip and around the outside to the base of the flower. The small halictinid bee *Lasioglossum* sp. crawled into the corolla tube to access nectar at Oribi Gorge. A few individuals of a pierid butterfly species were seen probing for nectar at Umtamvuna, but these made no contact with the style or anthers of the flowers.

Insect vouchers

Amegilla caelestina (Apidae)

Potgieter 13	UNR, 15-2-95
Potgieter 47	PMB Gdn, 7-4-94
Potgieter 84	OG, 10-1-97
Potgieter 115	OG, 20-4-97
Potgieter 174	OG, 2-4-98

A. mimadvena (Apidae)

Potgieter 14	UNR, 14-3-96
Potgieter 15	UNR, 16-3-96
Potgieter 61	OG, 12-5-96
Potgieter 112	OG, 20-4-97

Xylocopa hottentotta (Apidae)

Potgieter 16	UNR, 15-2-96
Potgieter 26	UNR, 11-5-96

Amegilla bothai (Apidae)

Potgieter 81	OG, 10-1-97
Potgieter 82	OG, 10-1-97

Amegilla caelestina

NV

Stenobasipteron wiedemanni

NV

Asarkina sp. (Syrphidae)

Potgieter 60	UNR, 11-5-96

Episyrphus sp. (Syrphidae)	
Potgieter 9	UNR, 11-5-96
Syrphidae	
Potgieter 86	OG, 10-1-97
Allodape pernix (Apidae)	
Potgieter 23	UNR, 15-2-95
Potgieter 24	UNR, 14-2-95
Potgieter 25	UNR, 14-2-95
Lasioglossum sp. (Apidae)	
Potgieter 59	OG, 14-3-96
Pieridae (Lepidoptera)	
Potgieter 71	UNR, 11-5-96

15. *Plectranthus laxiflorus* Benth.

Habit, habitat, distribution and phenology

Plectranthus laxiflorus is a soft suffrutex or freely-branched herb with spreading or ascending stems, growing 0.7 – 1.5 m tall. Populations occur on forest margins and shaded stream banks – seldom under forest canopy – and may form extensive stands that emit a characteristic citronella-like scent. The species is widely distributed, extending from the eastern part of the Western Cape, along the coast and midlands northwards through the Eastern Cape, KZN, Swaziland, Mpumalanga and the Northern Province, continuing into tropical Africa. This species flowers from mid-February to May and sporadically in October/November.

Inflorescence and floral morphology

Many synflorescences are produced, creating a striking show. These are lax racemes or panicles, 100 – 300 mm in height. The white flowers may be tinged with mauve, and 4 – 5 vertical, thin purple lines are positioned on the upright upper lip. The corolla tube is sigmoid in shape (mean length 10.5 mm long), narrow at the base and ascending for 2.5 mm, then bending downwards, expanding to 2.5 mm wide and laterally compressed at the corolla mouth. The upper lip is 6 – 7 mm and the boat-shaped lower lip 5 – 7 mm long. The four free, didynamous stamens extend 6 mm (upper pair) and 7.5 mm (lower pair) from the corolla mouth.

Study sites and observations

Pollinator observations were done at a small population in Oribi Gorge and extensive populations at Ferncliff in Pietermaritzburg, Leopard's Bush in the Karkloof, the Dargle Valley in the KZN Midlands, Ngeli Forest in southern KZN, and Kologha Forest near Stutterheim. At least 22 hours were spent observing at the various sites, over a number of days in different years, from 9.00 am to 4.00 pm. Additional observations were made at Long Tom Pass, Mpumalanga, where nectar collection was done for nectar studies.

Pollinators

Apinid bees of the genus *Amegilla* (*A. bothai* and *A. mimadvena*) were common pollinators throughout the flowering time at Ferncliff. However, a seasonal change in pollinators was noted late in each season (late March/early April) when medium-proboscid flies of the genus *Prosoeca* emerged in large numbers and joined the bees. These flies were also common at Kologha Forest in early March and Ngeli forest and

Dargle Valley in late March. On a overcast day at Dargle Valley, mostly nemestrinid flies (*Prosoeca umbrosa*) were seen to visit flowers during mid-morning, with only occasional bee visits later in the morning.

At Kologha Forest the apinid bees *Amegilla mimadvena* and *Xylocopa flavicollis* were also abundant pollinators. At Leopard's Bush the apinid bee *Amegilla bothai* was a common pollinator in early March, while the megachilinid bee *Chalicodoma* sp. A collected pollen in abdominal scopae. At Oribi Gorge the apinid bee *Amegilla caelestina* was a frequent pollinator in early April.

The apinid bees have proboscides that match the length of the corolla tube, with flexible tips that allow the bees to reach nectar beyond the bend near the base of the tube. Likewise, the flexible proboscides of the medium-proboscid nemestrinid flies also match the corolla tube length. These bees and flies pick up pollen ventrally on their thorax, abdomen and head.

Other floral visitors included *Apis mellifera* and two syrphid fly species that collected pollen at Ferncliff; two syrphid species collected pollen at Ngeli and one at Leopard's Bush. A few lepidopterans visited flowers: a hesperid butterfly at Leopard's Bush, a pierid butterfly and papilionoid butterfly (*Papilio nireus lyaens*) at Kologha Forest and the diurnal sphingid moth species, *Macroglossus trochilus*, at the latter site and at Ferncliff.

Insect vouchers

Amegilla bothai (Apidae)

Potgieter 95	FC, 7-3-97
Potgieter 177	FC, 4-3-98
Potgieter 158	KK, 3-3-99
Potgieter 179	FC, 4-3-98
Potgieter 212	FC, 6-4-03
Potgieter 201	KF, 29-2-00
Potgieter 46	FC, 11-4-96
Potgieter 45	FC, 11-4-96
Potgieter 96	FC, 7-3-97

Amegilla mimadvena (Apidae)	
Potgieter 181	FC, 4-3-98
Amegilla caelestina (Apidae)	
Potgieter 173	OG, 2-4-98
Xylocopa flavicollis (Apidae)	
Potgieter 202	KF, 29-2-00
Chalicodoma sp. A (Apidae)	
Potgieter 162	KK, 3-3-99
Potgieter 163	KK, 3-3-99
Apis mellifera (Apidae)	
Potgieter 43	FC, 11-4-96
Potgieter 44	FC, 24-4-96
Allodape ceratinoides (Apidae)	
Potgieter 211	FC, 4-3-98
Zonalictus sp. (Apidae, Halictinae)	
Potgieter 41	FC, 24-4-96
Potgieter 42	FC, 24-4-96
Potgieter 209	FC, 4-3-98
Potgieter 210	FC, 6-4-03
Prosoeca circumdata (Nemestrinidae)	
Potgieter 216	FC, 6-4-03
Prosoeca sp. nov. 5 (Nemestrinidae)	
Potgieter 37	FC, 24-4-96
Prosoeca umbrosa (Nemestrinidae)	
Potgieter 35	FC, 11-4-96
Potgieter 36	FC, 24-4-96
Potgieter 141	FC, 4-3-98
Potgieter 142	FC, 4-3-98
Potgieter 143	FC, 4-3-98
Potgieter 144	NG, 20-3-98
Potgieter 145	NG, 20-3-98
Potgieter 203	KF, 29-2-00
Potgieter 217	FC, 6-4-03
Potgieter 220	NG, 03-01
NV	DV, 03-09

Bombyliidae (Diptera)	
Potgieter 38	FC, 11-4-96
Asarkina sp. A (Syrphidae)	
Potgieter 140	NG, 3-5-98
Asarkina sp. B (Syrphidae)	
Potgieter 160	KK, 3-3-99
Syrphidae (Diptera)	
Potgieter 139	NG, 3-5-98
Hesperidae (Lepidoptera)	
Potgieter 97	FC, 7-3-97
Potgieter 101	KK, 3-3-99
Pieridae (Lepidoptera)	
NV	
Papilio nireus lyaens (Papilionidae)	
Potgieter 205	KF, 29-2-00
Macroglossus trochilus (Sphingidae)	
Potgieter 204	KF, 29-2-00

16. *Plectranthus calycinus* Benth. [= *Rabdosiella calycina* (Benth.) Codd]

This species was placed in a new genus, *Rabdosiella*, by Codd (1984), which meant that it was known as *Rabdosiella calycina* (Benth.) Codd until 1993 when it was placed back into *Plectranthus* (Ryding, 1993). The phylogeny by Paton *et al*. (2004) shows it to cluster with other species of *Plectranthus*, *Pycnostachys* and *Holostylon* within the *Coleus* clade of the Plectranthinae.

Habit, habitat, distribution and phenology

Plectranthus calycinus is a tall, erect, rigid sub-shrub with branches arising annually from a perennial woody rootstock, growing 0.6 – 1.5 m tall. It occurs in grasslands from the Eastern Cape northwards to KZN, the eastern Free State, Swaziland, Mpumalanga and the Northern Province. It flowers from January to May.

Inflorescence design and floral morphology

The dense synflorescences are terminal panicles of scorpioid cymes, 100 – 300 mm in height. The flowers are creamy-white with mauve edging on the upper and lower corolla lips. The short compressed corolla tube (6.5 mm long) is saccate at the base (4 mm wide), narrowing to 3 mm wide at the mouth. The erect upper lip is 2 mm and the spreading, shallowly boat-shaped lower lip 4 – 5 mm long. The four stamens are declinate with free filaments, enclosed in the lower lip, extending 4 mm (upper pair) and 5 mm (lower pair) from the corolla mouth.

Study sites and observations

Pollinator observations were made at a grassland site in the Dargle area (KZN Midlands), as well as on a grassland plateau at Umtamvuna. A total of ten hours was spent making observations at the three sites.

Pollinators

The Dargle population was pollinated exclusively by a medium-proboscid nemestrinid fly, *Prosoeca umbrosa*, with pollen loads deposited ventrally on the head, thorax and abdomen. At Umtamvuna the population was visited by the apinid bee *Xylocopa scioensis*, but no voucher was collected. This bee species has a short proboscis length (6.5 mm) that fits the corolla tube length well.

Insect vouchers

Prosoeca umbrosa (Nemestrinidae)

Potgieter 218	DV, 1-5-00
Potgieter 219	DV, 1-5-00

Xylocopa scioensis (Apidae)

NV	UNR, 05-00

17. *Plectranthus rehmannii* Gürke

Habit, habitat, distribution and phenology

Plectranthus rehmannii is an erect subshrub or branched herb with ascending stems, growing 0.6 – 1.2 m tall, in or near forest margins. It is endemic to the KZN Midlands and may be locally abundant. It flowers from January to April.

Inflorescence design and floral morphology

Synflorescences are paniculate, 250 – 350 mm in height, bearing small, creamy-white flowers that are densely tomentose. The deflexed, laterally compressed, short corolla tube (5 mm long) is saccate at the base and narrows slightly towards the mouth. The upper lip is very short (2 mm) and the boat-shaped lower lip is 4 mm long, curving upwards. The four free stamens extend about 2.5 mm from the corolla mouth.

Study sites and observations

Pollinator observations were made at Leopard's Bush in the Karkloof in 1999, where at least four hours were spent with the population, as well as at a forested site in the Dargle, in 2009. At both sites *P. rehmannii* grows alongside *P. laxiflorus*.

Pollinators

The megachilinid bee *Chalicodoma* sp. A visited both *P. rehmannii* and *P. laxiflorus* in the Karkloof. This bee has a short proboscis (ca. 6 mm) which could not reach nectar in *P. laxiflorus* (it only collected pollen), but it accessed nectar from the short corolla tubes of *P. rehmannii*. This was the main pollinator of *P. rehmannii* at the Karkloof site, and actively collected pollen in its abdominal scopae.

At the Dargle site there were no megachilinid bees on an overcast day, when general bee activity was limited. Interestingly, none of the *Prosoeca umbrosa* flies that visited flowers of adjacent *P. laxiflorus* plants, were seen on *P. rehmannii*.

At the Karkloof site *Apis mellifera* was another, less frequent, floral visitor that accessed nectar and collected pollen, while many *Allodape pernix* bees visited flowers to collect pollen.

Insect vouchers

Chalicodoma sp. A (Apidae)

Potgieter 162	KK, 3-3-99
Potgieter 163	KK, 3-3-99

Apis mellifera (Apidae)

Potgieter 159	KK, 3-3-99

Allodape ceratinoides (Apidae)

Potgieter 157	KK, 3-3-99

18. *Plectranthus spicatus* E.Mey. ex Benth.

Habit, habitat, distribution and phenology

Plectranthus spicatus is a succulent herb producing several annual stems from a perennial rootstock. The stems are decumbent, reaching 0.6 m in height. It grows in dry woodland or rocky grassland (where it was studied), extending coastally from the Eastern Cape to KZN, and inland to Swaziland and Mpumalanga. The flowering time extends from March to July.

Inflorescence and floral morphology

Purple flowers are produced in compact, simple, subspicate synflorescences (occasionally with a pair of branches near the base), 90 – 300 mm in height. Corolla tubes are sigmoid in shape, short (5 mm) and narrow at the base, ascending at first, then curving sharply downwards, expanding towards the mouth. The upper lip is 2.5 mm and the boat-shaped lower lip 2.5 – 3 mm long. Four free stamens extend 2.5 – 3 mm from the corolla mouth.

Study sites and observations

This species was studied at Oribi Gorge, in a dry, steep, rocky grassland, where four hours were spent on one occasion on a hot afternoon.

Pollinators

The main pollinator (about 80% of nectar-feeding floral visitors) was the apinid bee *Xylocopa caffra*, with these large-bodied bees picking up pollen ventrally on the thorax. The relatively short proboscis (6.5 mm) fitted the short tube of the flower, and the flexible tip to the proboscis allowed it access to nectar at the base of the sigmoid tube. About 20% of nectar-feeding floral visits were by the apinid bee *Amegilla mimadvena*. *Allodape pernix* was an infrequent apinid bee visitor, collecting pollen from the anthers in the lower lip.

Insect vouchers

Xylocopa caffra (Apidae)	
Potgieter 183	OG, 25-4-98
Potgieter 183A	OG, 25-4-98
Amegilla mimadvena (Apidae)	NV
Allodape pernix (Apidae)	NV

19. *Pycnostachys urticifolia* Hook.

Habit, habitat, distribution and phenology

Pycnostachys urticifolia is an erect soft shrub or herb that branches from a woody base, growing 1 – 2.5 m in height. It occurs in moist areas such as grassy stream banks and forest margins and is widely spread in South Africa, Zimbabwe, Mozambique, Malawi and Tanzania. In South Africa it was originally restricted to the Northern Province and Mpumalanga, but records from Bews Herbarium (NU) show it to have spread into KZN from the 1970's. It is now common in roadside vegetation in subtropical areas. It has flowers of similar dimensions to *Pycnostachys reticulata* (E.Mey.) Benth. which occurs naturally in KZN, hence similar pollinators should be found on both. It flowers from April to June.

Inflorescence and floral morphology

Synflorescences are dense, terminal, spicate panicles of helicoid cymes on the ends of upright branches, with the largest, central one 70 – 100 mm in height. Unlike those in *Plectranthus*, the sepal lobes of the calyces are elongate, rigid and spinescent, protecting the basal nectaries. Flowers are deep, bright blue (occasionally whitish-blue) with no visible nectar guides. The deflexed corolla tube is sigmoid in shape and of medium length (11 mm), with a narrow base. It ascends for 8 mm, then bends downwards, enlarging near the corolla mouth. The upper lip is short (3 mm) and the boat-shaped lower lip is 9 mm long. The four didynamous stamens are fused for a few mm outside the corolla mouth, providing mechanical support for the style that is enclosed in this rigid sheath. The upper pair extends 4 – 6 mm and the lower pair 6 – 7.5 mm from the corolla mouth; stamens are housed in the lower lip and are separated laterally by an apical fold of the lower lip. The style extends slightly further than the stamens.

Study sites and observations

Pollination observations were conducted on cultivated material in Pietermaritzburg, for five hours on a sunny day, from late morning onwards.

Pollinators

The main pollinators of *Py. urticifolia* were nectar-seeking apinid bees of the genera *Amegilla* (*A. mimadvena*) and *Xylocopa* (*X. caffra* and *X. scioensis*), as well as pollen-collecting megachilinid bees of the genera *Chalicodoma* (*Chalicodoma* sp. B) and *Megachile* (*Megachile* spp. A & B).

The apinid bees probed corollas for nectar and the flexible tips of their proboscides allowed them to negotiate the sigmoid corolla bend; the longer proboscis of *A. mimadvena* allowed pollen deposition ventrally on the bee thorax and abdomen, while the shorter proboscis of *Xylocopa* resulted in pollen placement on the base of the head as well. The downwards angle of the distal limb of the corolla tube forced the bees to probe upwards, and ensured body contact with the anthers and stigma in the lower lip.

The megachilinid bees had large ventral pollen loads. These bees have abdominal scopae into which pollen is gathered, which places it in a perfect position for stigma deposition.

Other floral visitors were the apinid bee *Apis mellifera*, collecting pollen, a lycaenid butterfly, a megachilinid bee, *Pseudoanthidium truncatum*, on which no pollen was found, and the apinid bee *Thyreus* sp., on which no pollen was found.

Insect vouchers

Amegilla mimadvena (Apidae)	NV
Xylocopa scioensis (Apidae)	
Potgieter 128	PMB Gdn, 28-4-98
Xylocopa flavorufa (Apidae)	
Potgieter 129	PMB Gdn, 28-4-98
Chalicodoma sp. B (Apidae)	
Potgieter 126	PMB Gdn, 28-4-98
Megachile sp. A (Apidae)	
Potgieter 123	PMB Gdn, 28-4-98
Megachile sp. B (Apidae)	
Potgieter 127	PMB Gdn, 28-4-98
Apis mellifera (Apidae)	
Potgieter 124	PMB Gdn, 28-4-98
Pseudoanthidium truncatum (Apidae)	
Potgieter 125	PMB Gdn, 28-4-98
Thyreus sp. (Apidae)	NV
Lycaenidae (Lepidoptera)	NV

20. *Aeollanthus parvifolius* Benth.

Habit, habitat, distribution and phenology

Aeollanthus parvifolius is a semi-succulent herb or sub-shrub with ascending branches that spread out from a woody base, growing 0.2 – 0.5 m tall. It usually occurs among rocks, coastally in the Eastern Cape and KZN, to Swaziland and at higher altitudes in Mpumalanga, North West and the Northern Province. It flowers from December to June.

Inflorescence and floral morphology

Relatively lax panicoid synflorescences that are often branched, reach 50 – 200 mm in height. Flowers are white, sometimes with a pinkish tinge, and the upper lip has nectar guides in the form of reddish purple spots. The corolla tube is 7 – 10 mm long, curving downwards from a cylindrical, narrow base, expanding towards the mouth. The upper lip is 5 mm and the concave lower lip 8 mm long. The four didynamous stamens are attached at the mouth of the corolla, with free filaments lying inside and exserted slightly beyond the lower lip, bearing one-celled anthers.

Study sites and observations

Pollinator observations were made at Umtamvuna and Ongoye Forest, with about equal amount of time spent at each locality – a total of six hours from early to late afternoons on sunny days.

Pollinators

At Umtamvuna *Apis mellifera* and a few syrphid flies were seen collecting pollen, but the apinid bee, *Amegilla fallax*, visited flowers for nectar and appeared to be a pollinator. At Ongoye Forest three apinid bee species (*Amegilla mimadvena*, *A. bothai* and *A. fallax*) were pollinators. All these bees could reach nectar at the base of the corolla and picked up pollen passively on their ventral abdominal surfaces. An acrocerid fly, *Psilodera* sp.**,** visited flowers in a similar way as the apinid bees, with its black and white striped abdomen looking superficially like that of *A. fallax* (subgenus *Zebramegilla*).

Insect vouchers

Amegilla mimadvena (Apidae)	
NV	
Amegilla bothai (Apidae)	
NV	
Amegilla fallax (Apidae)	
Potgieter 49	UNR, 11-5-96
Potgieter 169	ON, 22-4-98
Psilodera sp. (Acroceridae)	
NV	ON, 22-4-98

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