Specialization for hawkmoth and long-proboscid fly pollination in *Zaluzianskya* section *Nycterinia* (Scrophulariaceae)

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The evolution of pollination systems has been investigated in *Zaluzianskya* section *Nycterinia* (Scrophulariaceae), a group characterized by very long-tubed flowers (up to 60 mm) that open either only at night (19 species) or during the day (one species). Field observations of three species: *Z. natalensis*, *Z. elongata* and *Z. pulvinata*, revealed that their flowers are pollinated shortly after dusk by hawkmoths. Further observations showed that *Z. microsiphon*, the sole species to have flowers that open only during the day, is pollinated exclusively by long-proboscid flies belonging to the family Nemestrinidae. A phylogenetic analysis of *Z.* sect. *Nycterinia* based on morphological characters indicates that *Z. microsiphon* evolved within a clade of night-flowering species. The shift from hawkmoth to long-proboscid fly pollination in *Zaluzianskya* was probably facilitated because long corolla tubes, white petals and dilute nectar in hawkmoth-pollinated flowers are pre-adaptations for pollination by long-proboscid flies. However, the autapomorphic features of *Z. microsiphon*, such as zygomorphy, diurnal flower anthesis and lack of scent, can be regarded as adaptations for long-proboscid fly pollination. Breeding system experiments on *Z. natalensis* and *Z. microsiphon* revealed the existence of genetic self-incompatibility. © 2002 The Linnean Society of London, *Botanical Journal of the Linnean Society*, **138**, 17–27.

ADDITIONAL KEYWORDS: adaptive radiation – flora of southern Africa – nectar – Nemestrinidae – phylogeny – self-incompatibility – Sphingidae.

INTRODUCTION

Repeated evolutionary shifts between pollination systems have long been thought to be one of the factors responsible for the tremendous radiation of the angiosperms (Stebbins, 1970). However, several authors have argued that the majority of plants have pollination systems that are generalized and diffuse, thus casting doubt on the traditional notion of an adaptive 'fit' between most flowers and their pollinators (cf. Ollerton, 1996). In the context of this debate (Johnson & Steiner, 2000, and refs therein), there is a need for studies that take a more critical look at floral adaptations in relation to pollinators. Experimental studies of pollinator-mediated selection on floral traits (cf. Johnston, 1991) provide one way forward, but do not reveal the actual consequences of selection for evolutionary divergence. Mapping of pollination systems on to phylogenies is a powerful way of determining how floral traits evolve (or, as importantly, don't evolve) when plants undergo transitions between pollination systems (Armbruster, 1992, 1993; Goldblatt & Manning, 1996; Bruneau, 1997; Johnson *et al.*, 1998; Weller & Sakai, 1999).

Phylogenies that include most or all taxa within groups (within the limits posed by natural extinctions) are useful for enabling researchers to draw conclusions about shifts in pollination systems during cladogenesis, and to infer ancestral states of pollination systems. For example, McDade (1992) used a cladogram based on morphology to arrive at her conclusion that pollination by short-billed trochiline hummingbirds was derived from pollination by long-billed hermit hummingbirds in *Aphelandra* (Acanthaceae). Less well sampled phylogenies have limitations for making inferences about evolutionary transitions,

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but may nevertheless reveal the minimum number of independent origins of pollination systems (cf. Johnson *et al.*, 1998).

The genus *Zaluzianskya* F.W. Schmidt (Scrophulariaceae; Manuleae) consists of about 55 species, the majority of which occur in southern Africa. Hilliard (1994) drew attention to the radiation of floral forms within the genus and provided a working classification, but there have been no published studies on pollination biology, compatibility systems or phylogeny in the genus. Kampny (1995) noted that the Manuleae is the only tribe of Scrophulariaceae for which there were no published data on pollinators. In their review of long-proboscid fly pollination in southern Africa, Goldblatt & Manning (2000) list *Zaluzianskya microsiphon* (O. Kuntze) K. Schum. in a table of plant species pollinated by the long-proboscid fly *Prosoeca ganglbauri*, but no further details are given.

Zaluzianskya section *Nycterinia* (D. Don) Hilliard is the most distinctive group in the genus, on account of a unique placenta structure, a largely perennial habit, and contrasting red and white petals (Hilliard, 1994). The deep red colour of the outside of the petal lobes renders the flowers inconspicuous when closed. Most of the species in *Z.* sect. *Nycterinia* have long-tubed actinomorphic flowers that open in the evening and produce a strong sweet fragrance, features which are consistent with hawkmoth pollination, as was first pointed out by Vogel (1954). However, *Z. microsiphon*, which is vegetatively very similar to other species in the section, differs in having zygomorphic flowers that open in the morning and lack a discernable scent (Hilliard & Burtt, 1983; Hilliard, 1994).

The aims of this study were to document the differences in pollination biology and floral morphology that exist between night- and day-opening flowers in *Zaluzianskya* section *Nycterinia* and to construct a phylogeny that would allow the evolutionary pathway of pollination systems to be established.

MATERIAL AND METHODS

POLLINATOR OBSERVATIONS

The pollination biology of four members of *Zaluzianskya* section *Nycterinia* were studied: *Z. elongata* Hilliard & Burtt, *Z. natalensis* (Bernh. ex) Krauss and *Z. pulvinata* Killick that are typical night-flowering species, while *Z microsiphon* is the only member of the group to have flowers that open during the day. Vouchers of plants from the study sites are deposited in the Natal University Herbarium (NU), while insect vouchers are deposited in the Natal Museum, Pietermaritzburg.

Floral visitors were captured with handnets. A dimmed flashlight was used when capturing and observing moths. In addition, moths were captured by means of a light trap equipped with a 250 W mercury-vapour lamp. Pollen was removed from insects using the fuschin gel technique described by Beattie (1971), and identified from a reference collection.

Observations on *Z. natalensis* were carried out in a large population of several thousand plants growing on the summit plateau of Mt. Gilboa in the Karkloof mountain range. Plants were observed from *c.* 18.00 h, when flowers open to 21.00 h, each evening on 21, 22 February 1999; 5, 6, 12 February 2000 and 7 February 2001. Observations on *Z. elongata* were carried out in a population of *c.* 200 plants at the Oribi Gorge nature reserve in Kwazulu-Natal, from the time that the flowers began to open at *c.* 17.30 h until 20.00 h on 21, 22 February 1997; 11 March 1998 and 2, 21, 24, 25 April 1998. The population is situated in open grasslands on the lip of the gorge. Observations on *Z. pulvinata* were carried out on the evenings of 2 and 3 December 2000 at the Mt. Gilboa site. Observations on *Z. microsiphon* were carried out at two sites. A population of *c.* 500 plants growing at *c.* 2500 m in short stony grassland on the slopes of Sentinel Peak in the Drakensberg Mountains was observed between 10.00 h and 15.00 h on 7, 8, 20 and 21 February 1998 and 21 February 2000. Observations were also carried out in a population of *c.* 300 plants of *Z. microsiphon* on the summit of Mt. Gilboa on 5, 6, 12 February 2000.

FLORAL MORPHOLOGY AND NECTAR PROPERTIES

The length of the corolla tube in flowers from at least 20 individuals per species was measured with a steel ruler to the nearest 0.5 mm. Additional measurements of floral tube lengths and corolla indumentum were made of all the specimens of *Zaluzianskya* section *Nycterinia* in the Natal University Herbarium (NU). Details of the corolla micromorphology were studied using critical point dried material, sputter coated with gold palladium and viewed with a Hitachi S-570 SEM.

Timing of floral anthesis in *c.* 20 plants of *Z. elongata* was recorded at 10-min intervals from 18.00 h to 20.00 h on 12 March 1998. The timing of scent production was assessed qualitatively by sniffing flowers at 10-min intervals on the same evening.

Nectar volume in flowers was measured with calibrated 0–5 μL micropipettes at 18.00 h, in the case of the night-flowering species, and 12.00 h in the case of the day-flowering species. Nectar sugar concentrations were determined with a pocket temperature-compensated refractometer.

BREEDING SYSTEMS

Flowers of *Z. natalensis* and *Z. microsiphon* on Mt. Gilboa were bagged in the bud stage and then subjected to the following treatments after anthesis: (1) unma-

Table 1. Characters used in the cladistic analysis of *Zaluzianskya* section *Nycterinia*

1. Plants annual (0); weakly perennial (1); strongly perennial (2).
2. Taproot: slender, not woody (0); slender, woody (1); thick (> 5 mm), woody (2).
3. Subterranean buds: absent (0); present (1).
4. Thick clump of buds on crown: absent (0); present (1).
5. Stem: highly branched (0); occasionally branched (1); simple (2).
6. Stem: prostrate (0); erect (1).
7. Stems: branching at base (0); solitary (1); several (2).
8. Stems: eglandular (0); with glandular trichomes (1)
9. Leaves: cauline (0); rosulate (1).
10. Leaves: ovate/elliptical (0) lanceolate (1); flabellate (2).
11. Leaves: broad at base (0); petiolate (1).
12. Leaves: slightly hairy/glabrous (0); densely hairy (1).
13. Leaves: glandular hairs absent (0); present (1).
14. Leaves: spreading (0); vertical and appressed to stem (1).
15. Bracts similar to leaves (0); not similar to leaves (1).
16. Calyx: glabrous or slightly hairy (0); densely hairy (1).
17. Calyx: glandular hairs absent (0); present (1).
18. Inflorescence: elongates in fruit (0); remains congested (1).
19. Corolla: opening in sunshine (0); opening in evening (1).
20. Corolla: actinomorphic (0); zygomorphic (1).
21. Corolla: uniform in colour (0); white/cream above and red below (1).
22. Corolla: orange marking around mouth absent (0); circular orange marking present (1); star-shaped orange marking present (2).
23. Corolla lobes: glabrous (0); glandular (1)
24. Circlet of hairs around corolla mouth: complete (0); asymmetric (1); vestigial (2)

nipulated, to test for autogamy, (2) self-pollinated by hand, and (3) cross-pollinated with pollen from another plant at least 5 m away. Hand-pollinations were performed from *c.* 18.00 h–19.00 h in *Z. natalensis*, and from *c.* 14.00 h–15.00 h in *Z. microsiphon*, on the presumption that maximum stigmatic receptivity is likely to coincide with flower opening and pollinator activity.

CLADISTIC ANALYSIS

A cladistic analysis of *Zaluzianskya* section *Nycterinia* was carried out to establish the relationships among the 20 species in this group. *Zaluzianskya pumila* (Benth.) Walp. (section *Macrocalyx* Hilliard) was chosen as an outgroup in order to root the tree. The analysis was based on 24 morphological characters, of which 14 were vegetative, and performed using the ie* procedure in Hennig86 (Farris, 1988) which produces a complete set of most parsimonious trees. No assumptions were made about the primitive vs. derived state of each character (cf. Nixon & Carpenter, 1993). Phylogenetic interpretations were based on the topology of a consensus cladogram (using the 'nelsen' procedure in Hennig86). Support for nodes was investigated using the bootstrap procedure (Felsenstein, 1985) set to 500 replicates with random replacement in PAUP version 3.1. To determine the tree topology for *Z.* sect *Nycterinia* when floral characters are excluded from the data set, we ran additional parsimony analyses (ie* routine) using only the 14 vegetative characters.

RESULTS

POLLINATOR OBSERVATIONS

Long-tubed night-flowering *Zaluzianksya* species in this study were pollinated by hawkmoths shortly after dusk. Observations of *Z. natalensis* revealed hawkmoths to be frequent visitors on each evening of the study (Figs 1,2). Activity was confined to a period of about 20 min shortly after dusk. Photographs of moths foraging at flowers revealed thick deposits of pollen along the length of the proboscis (Figs 1,2). This was confirmed by palynological analysis, which showed that the majority of captured hawkmoths carried large *Zaluzianksya* pollen loads (100–3000 grains) on their proboscides and head (Table 3). The hawkmoth fauna
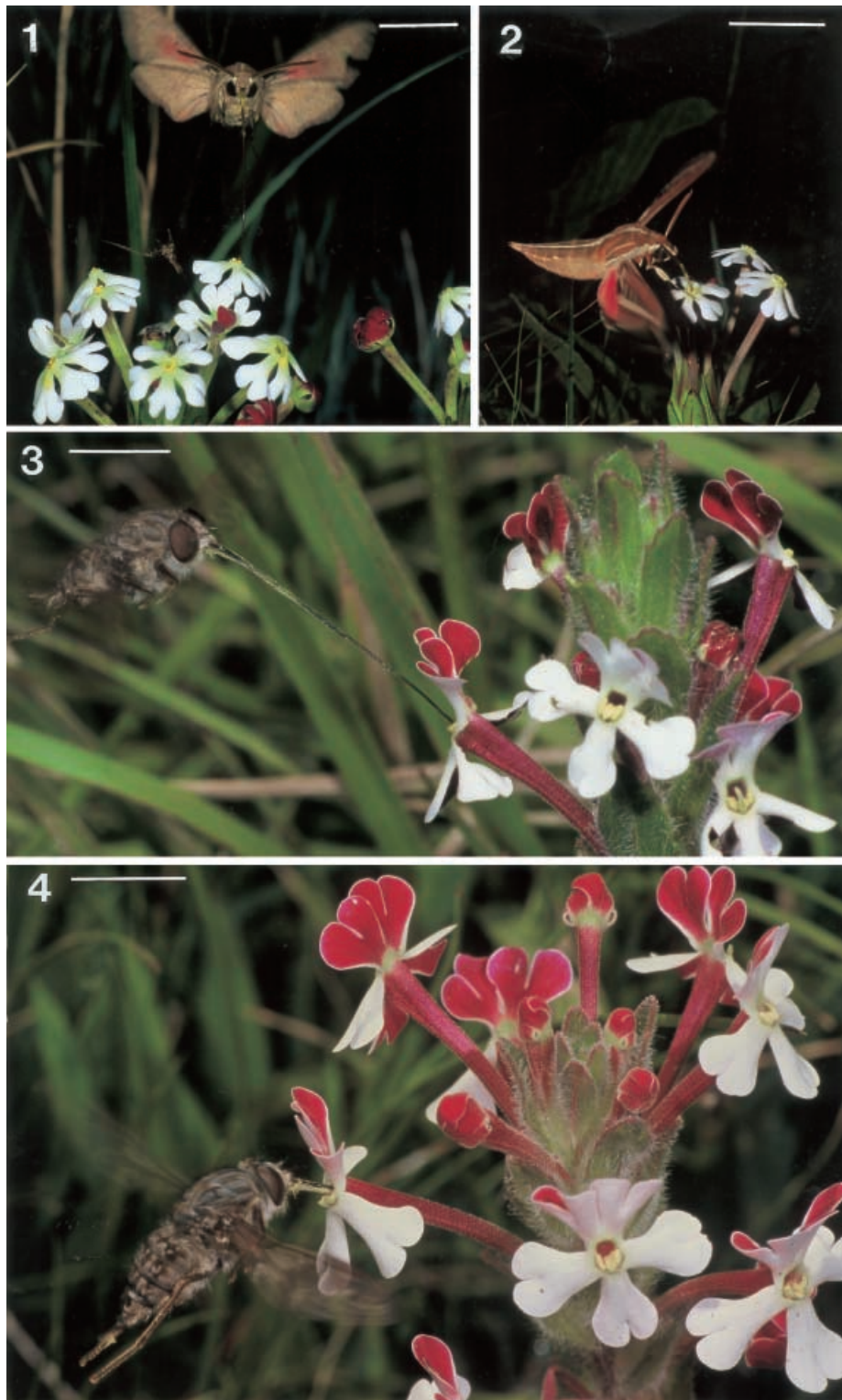
Figures 1–4. Insect pollinators foraging on *Zaluzianskya* species. Figs 1,2. Hawkmoths (*Basiothia schenki*) inserting their proboscides (visibly coated in pollen) into flowers of *Z. natalensis*. Scale bars = 20 mm. Figs 3,4. Long-proboscid flies (*Prosoeca ganglbauri*) probing flowers of *Z. microsiphon* for nectar. Thick deposits of pollen are visible on the underside of the proboscis and face. Scale bars = 10 mm.

at this site was dominated by *Hippotion celerio*, which has a proboscis that closely matches the floral tube of *Z. natalensis* in length (Tables 3 and 4).

Two hawkmoths (both *Basiothia charis*) were netted while feeding on the flowers of *Z. elongata*, and many other hawkmoths were seen without being captured. Both captured moths carried large loads of *Zaluzianskya* pollen along the length of the proboscis (Table 3). Observations of *Z. pulvinata* were restricted to two evenings, during which four visits by hawkmoths were observed in the small population. One hawkmoth, *Theretra cajus*, was netted on the flowers and found to have large deposits of *Zaluzianskya* pollen on its proboscis (Table 3).

The day-flowering species *Z. microsiphon* was visited frequently by long-proboscid flies at the two study sites (Figs 3,4). At the Sentinel peak population, flowers were visited exclusively by the tanglewing fly *Prosoeca ganglbauri* (Nemestrinidae), while at the Mt. Gilboa site the flowers were visited by an undescribed *Prosoeca* species (Nemestrinidae). Twenty flies which were captured while visiting the flowers had dense encrustations of *Zaluzianskya* pollen adhering to the face and base of the proboscis (Table 3). Pollen is visible on the proboscides of the flies in Figures 3 and 4.

Table 2. Distribution of characters among the taxa in *Zaluzianskya* section *Nycterinia*

Z. pumila	00001 00000 00000 01010 0210
Z. microsiphon	22112 11000 00010 00011 1012
Z. spathaceae	22112 11000 00010 00000 1010
Z. natalensis	22112 11000 00010 00100 1010
Z. pulvinata	22012 12010 00011 00100 1011
Z. angustifolia	22011 02001 00011 00000 1001
Z. schmitziae	22001 12001 00011 00000 1001
Z. elongata	22001 12001 00010 00000 1001
Z. pilosa	22011 12000 01010 10000 1011
Z. pachyrrhiza	22002 12000 00010 00000 1011
Z. maritima	01001 00000 00000 00000 1001
Z. capensis	01001 00000 00000 00000 1001
Z. muirii	01001 00000 10000 00000 1012
Z. oreophila	00001 02000 00010 00000 1001
Z. chrysops	11001 00000 10000 00100 1101
Z. glareosa	12001 00000 00000 00000 1000
Z. tropicalis	22001 12100 00100 11000 1011
Z. turritella	00001 00102 10102 01100 1001
Z. katharinae	11000 00102 11100 11000 1000
Z. ovata	11000 00102 11100 11000 1101
Z. distans	11000 00101 11100 11000 1111

MORPHOLOGY AND NECTAR PROPERTIES

Marked flowers of *Z. elongata* remained tightly furled during the day and opened rapidly during a 45 min period before dusk. Other night-flowering species in this study showed the same pattern, while flowers of *Z. microsiphon*, which remain open during the day, begin closing about 2h before dusk. Flowers of the night-

Table 3. The identity, proboscis length and pollen loads of insect visitors to flowers of four *Zaluziankyia* species

Species	Site	Pollinator	N	Proboscis length (x ± SD)	Number of insects in each pollen load category		
					0	0–100	>100
Z. natalensis	Mt. Gilboa	Lepidoptera					
		Sphingidae					
		Hippotion celerio (L)	23	39.3 ± 1.4	3	9	11
		Agrius convolvuli (L)	2	110.0 ± 19.7	0	1	1
		Basiothia schenki (Möschler)	2	40.0 ± 2.8	0	1	1
		Hippotion eson (Cramer)	1	47.0 ± 0	0	0	1
		Theretra cajus (Cramer)	1	30.0 ± 0	0	1	0
		Hyles lineata livornica (Esper)	1	30.0 ± 0	0	1	0
		Deilephila nervii (L)	1	42.0 ± 0	0	1	0
		Coelonia mauritii (Butl.)	1	86.0 ± 0	0	1	0
Z. elongata	Oribi Gorge	*Basiothia charis* (Boisduval)	2	30.0 ± 1.4	0	0	2
Z. pulvinata	Mt. Gilboa	*Theretra cajus* (Cramer)	1	28	0	0	1
Z. microsiphon	Sentinel	Diptera					
		Nemestrinidae					
		Prosoeca ganglbauri (Lichtwardt)	15	22.2 ± 2.2	0	0	15
	Mt. Gilboa	*Prosoeca* sp.	5	24.2 ± 1.3	0	1	4
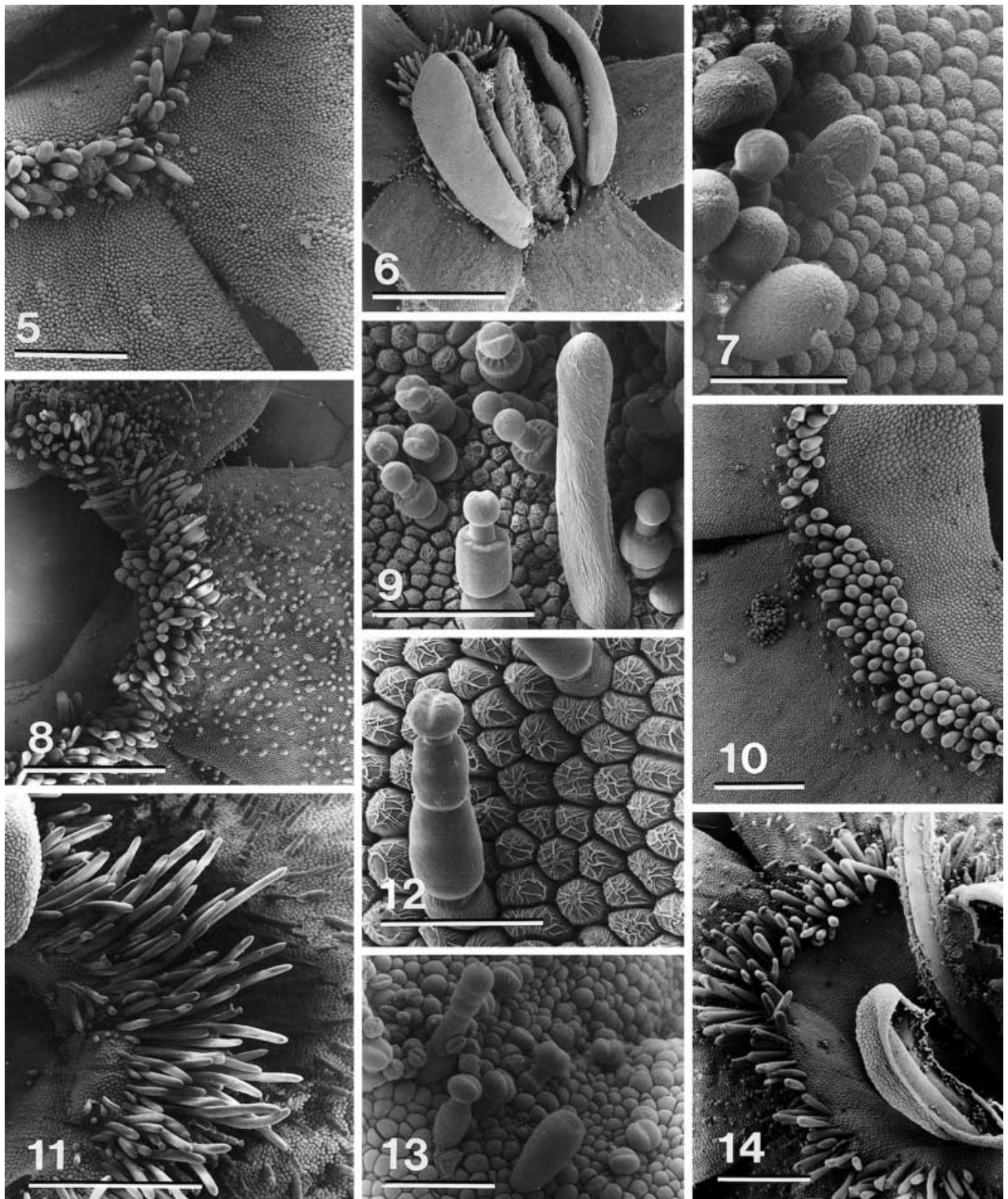
Figures 5–14. Corolla morphology in members of *Zaluziankya* section *Nycterinia*. Fig. 5. Details of the corolla of *Z. glareosa* Hilliard & Burtt. Clavate trichomes form a circlet mixed with both sessile and stalked glandular trichomes. Scale bar = 300 μm. Fig. 6 Detail of the corolla throat of *Z. elongata* showing the exerted anthers and posticous development of the clavate trichomes. Glandular trichomes are restricted to the base of the petal claws. Scale bar = 1 mm. Fig. 7. *Z. microsiphon* showing detail of the circlet vestiges from the corolla sinuses. Scale bar = 60 μm. Fig. 8. *Z. spathacea* (Benth.) Walp. showing detail of the well developed, eglandular circlet and the extensive glandular indumentum of the corolla lobes. Scale bar = 750 μm. Fig. 9. *Z. natalensis* showing detail of mixed glandular and clavate trichomes from the corolla throat. Scale bar = 100 μm. Fig. 10. *Z. ovata* (Benth.) Walp. showing detail of the sessile glandular trichomes which flank the corolla circlet. Scale bar = 300 μm. Fig. 11. *Z. natalensis* showing detail of the well developed filiform trichomes of the corolla circlet. Scale bar = 500 μm. Fig. 12. *Z. natalensis* showing detail of the glandular trichomes of the external corolla epidermis. Scale bar = 60 μm. Fig. 13. *Z. ovata* showing detail of the circlet vestiges from the posticous corolla throat. Scale bar = 100 μm. Fig. 14. *z. pachyrrhiza* Hilliard & Burtt showing detail of the glabrous corolla throat demarcated by a fringe of clavate trichomes. Adaxial corolla surfaces with stalked glandular trichomes. Scale bar = 400 μm.

Table 4. Flower opening patterns, corolla tube length and nectar properties of four *Zaluzianskya* species

| Species | Flower opening | Corolla tube length | | Nectar volume (μL) | | Nectar concentration (g/100 g) | |
		N	$\bar{x} \pm$ SD	N	$\bar{x} \pm$ SD	N	$\bar{x} \pm$ SD
Z. natalensis	Night	22	45.86 ± 2.6	11	1.05 ± 0.6	11	24.58 ± 2.9
Z. elongata	Night	21	42.56 ± 4.4	19	1.87 ± 3.3	10	23.6 ± 2.5
Z. pulvinata	Night	20	48.35 ± 4.2	20	2.6 ± 0.9	20	26.57 ± 1.3
Z. microsiphon	Day	37	36.40 ± 2.8	21	2.11 ± 0.9	9	30.22 ± 2.7

flowering species are unscented during the day, but produce a sweet clove-like scent soon after the flowers open at dusk. The mean length of the corolla tube was 45–48 mm in the hawkmoth-pollinated species and 36 mm in the fly pollinated species (Table 3).

Most of the species have a circlet of unicellular clavate hairs around the corolla throat. Within our study section (*Nycterinia*), these hairs show considerable diversity (Figs 5–14). The diurnal species, *Z. microsiphon,* displays vestiges of the circlet near the petal sinuses (Fig. 7). Glandular trichomes are confined to the proximal portions of the corolla lobes in a number of the nocturnal species and, in the extreme, are limited to a narrow zone outside the circlet of clavate trichomes (Figs 5,6,8,10,11 and 14).

The posticous filaments form a channel in which most of the nectar is located. The mean standing crop of nectar measured at the time of pollinator activity was 1–3 μL in both the hawkmoth and fly pollinated species. Nectar sugar concentration varied from 23 to 30% (Table 4).

BREEDING SYSTEMS

No fruits or seeds developed in any self-pollinated or unmanipulated flowers of *Z. natalensis* and *Z.*

microsiphon, while 100% of cross-pollinated flowers developed fruits with seeds (Table 5). This shows unequivocally that the two species possess a self-incompatibility system, and therefore depend entirely on pollinators for seed production.

CLADISTIC ANALYSIS

Parsimony analysis of the data set for *Z.* sect. *Nycterinia* yielded six trees, 65 steps long (consistency index 0.49, retention index 0.74), differing only in the positions of three basal nodes. The strict consensus tree retained all of the structure within the sublineage for which we have data on pollinators and therefore formed the basis for subsequent evolutionary interpretations. The long-proboscid fly pollinated species *Z. microsiphon* occupies a phylogenetically advanced position (Fig. 15) and is likely to have been derived from a hawkmoth pollinated ancestor, as several closely related species were observed to be pollinated by hawkmoths, and the remaining species in *Z.* sect. *Nycterinia* have traits which are consistent with hawkmoth pollination. The derived position of *Z. microsiphon* in *Z.* sect *Nycterinia,* on which this hypothesis is based, is not an artefact of the inclusion of floral characters, as a strict consensus tree based on
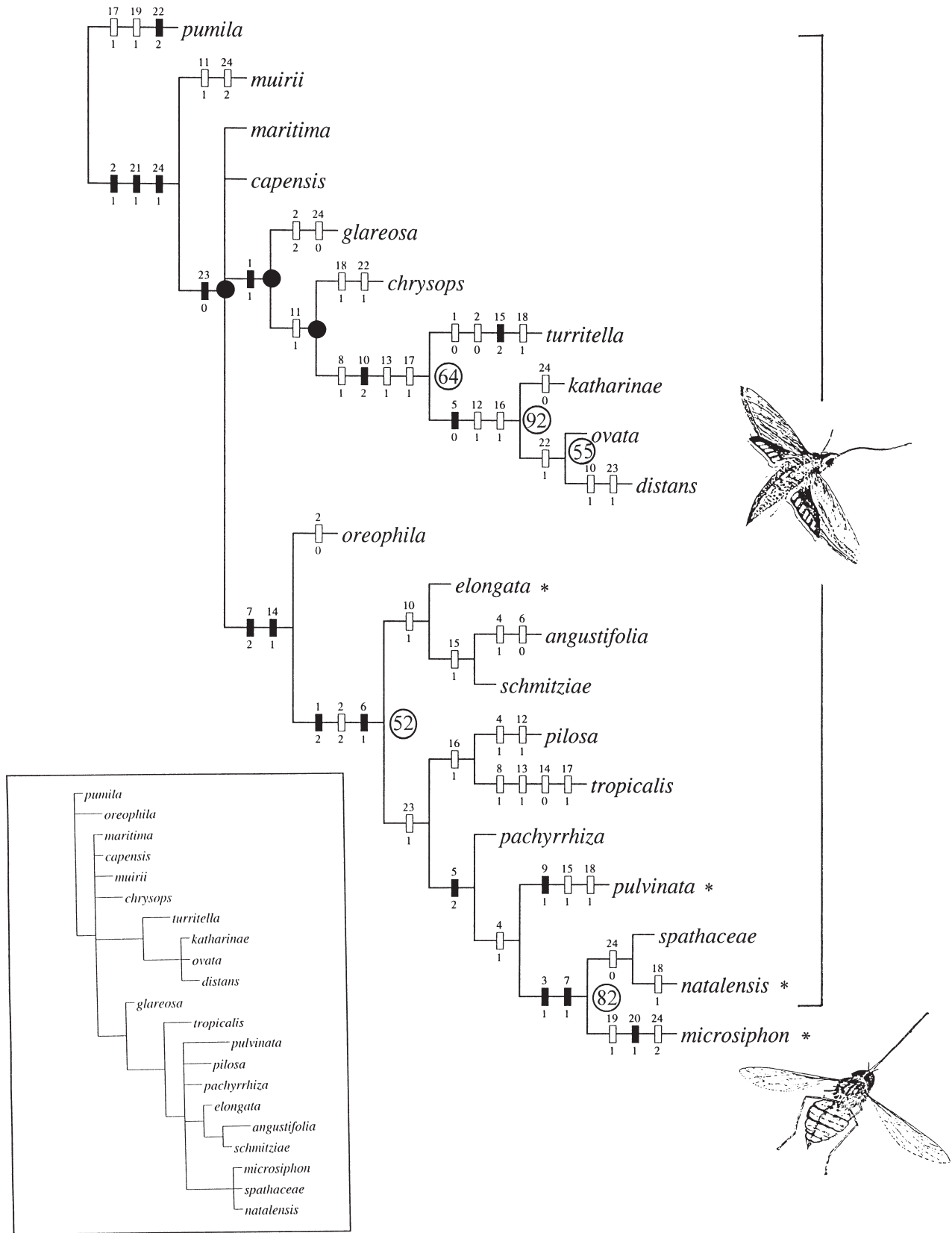
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Figure 15. One of the six most parsimonious cladograms for *Zaluzianskya* section *Nycterinia*. Nodes which collapse on the strict consensus tree are indicated by filled circles. Bootstrap values greater than 50 are indicated to the right of nodes. Long-proboscid fly pollination is hypothesized to have been derived via a single shift from pollination by hawkmoths. Taxa with pollination systems documented in this study are indicated by an asterisk. Inset: strict consensus tree based on cladistic analysis of the vegetative characters.

Table 5. Results of an experiment to determine the breeding system of two *Zaluzianskya* species. Flowers in insect-proof exclosures were either cross-pollinated (CROSS), self-pollinated (SELF)or left unmanipulated (CON) in order to test for autogamy. Selfed and unmanipulated flowers failed to set fruits or seeds, indicating that the plants are self-incompatible

	Fruit set % (n)			Seed set ($\bar{x} \pm SD$)		
Species	CROSS	SELF	CON	CROSS	SELF	CON
Z. microsiphon	100 (17)	0 (19)	0 (39)	38.1 ± 8.6	0 ± 0	0 ± 0
Z. natalensis	100 (9)	0 (8)	0 (37)	36.1 ± 23.7	0 ± 0	0 ± 0

parsimony analysis of a data set consisting only of the 14 vegetative characters (Fig. 15, inset) gave a similar topology to the consensus tree based on the total data set (Fig. 15).

DISCUSSION

Our results show that long-tubed flowers in *Zaluzianskya* section *Nycterinia* are specialized for pollination by hawkmoths and flies with correspondingly long proboscides. By restricting nectar access to these long-proboscid insects and having flowers that open for only part of the day, these plants are able to filter out more generalist visitors and thus increase the likelihood that pollen will be transferred to conspecific stigmas. This was borne out by the palynological analyses, which revealed very little pollen other than that of *Zaluzianskya* on the proboscides of the insects (Table 3, unpublished data).

Phylogenetic reconstruction indicated that the long-tubed members of *Z.* sect. *Nycterinia* are an advanced group within the genus and, that, within this clade, pollination by hawkmoths is likely to be ancestral to pollination by long-proboscid flies (Fig. 15). This conclusion was based on the direct observations of hawkmoth pollination in several species and similar traits (night opening flowers with tubes 25–60mm in length) in the remaining species of *Z.* sect. *Nycterinia*.

The use of molecular data for reconstructing the phylogeny of *Zaluziankya* would be desirable, but the generally lower reliability of the morphological data may have to be weighed against their greater practicality for complete sampling of extant taxa

within groups, which facilitates character optimization and studies of evolutionary transitions. Morphological data also provide independent hypotheses of relationships against which phylogenies based on other characters can later be assessed (cf. Prather, 1999).

The distribution of morphological characters on the phylogeny for section *Nycterinia* allowed us to identify diurnal anthesis, lack of scent, zygomorphy and vestigial hairs around the corolla mouth as autapomorphies in *Z. microsiphon* and thus likely adaptations for long-proboscid fly pollination (cf. Coddington, 1988), while long-tubes, dilute nectar, dissected corollas and white petals are plesiomorphic traits in *Z. microsiphon* that may have utility for long-proboscid fly pollination, but were originally adaptations for a different pollination system. Johnson *et al.* (1998) pointed out that homoplasy in pollination-linked floral traits is often less than expected due to this phenomenon of pre-adaptation (= exaptation *sensu* Gould & Vrba, 1982). This is consistent with Stebbin's (1970) concept of a 'line of least resistance', whereby evolutionary transitions should involve the minimal amount of floral reorganization.

Given that a shift between hawkmoth and long-proboscid fly pollination is an evolutionary line of least resistance, which has been facilitated by a common utility of traits, then such shifts should be a common feature of the evolutionary history of South African plants. There is already evidence for shifts between hawkmoth- and long-proboscid fly pollination in both the orchid genus *Disa* (Johnson *et al.*, 1998) and *Lapeirousia* (Iridaceae) (Goldblatt & Manning, 1996). Whether these shifts are statistically more

likely to occur than other shifts, such as hawkmoth to bee-pollination, will require a far larger data set for analysis.

The convergence of floral traits in unrelated plants that share the same pollinators is another strong indication for adaptation. The lack of scent in *Z. microsiphon* is a trait common to plants adapted to long-proboscid flies in other families (Goldblatt & Manning, 2000, and refs therein). There is also a striking similarity between the bifid petal lobes in many night-flowering *Zaluzianskya* species and sympatric hawkmoth-pollinated *Silene* species (Caryophyllaceae) in South Africa (Hilliard & Burtt, 1983). Dissection of the corolla in *Viola cazorlensis* Gand. (Violaceae) has been shown by experiment to increase the attractiveness of its flowers to a day flying hawkmoth (Herrera, 1993), and the repeated evolution of a fringed labellum in *Platanthera* (Orchidaceae) has been closely associated with hawkmoth pollination (Hapeman & Inouye, 1997). The resemblance between *Zaluzianskya* and *Silene* actually extends further, in that the hairs around the corolla mouth of *Zaluzianksya* closely approximate the dissected inner corona of *Silene*, and the corolla lobes in night flowering species of both genera become reflexed when fully open.

Unicellular clavate hairs often occur on the corollas of the Manuleae, including all sections within *Zaluzianskya* (Hilliard, 1994). The function of the circlet of clavate hairs around the mouth of the corolla in night-flowering *Zaluzianksya*s is at present unknown. These hairs may play a role in scent production as they are well developed in most of the night-flowering species, and reduced to a vestigial state in the unscented flowers of *Z. microsiphon*. However, in a test for the presence of osmophores, the hairs of *Z. elongata* did not preferentially stain with neutral red (unpublished data). In addition, most of the evening-scented species in subsection *Noctiflora* Hilliard do not have these hairs. In many of the nocturnal species these trichomes form a dense brush behind the posticous stamens but are reduced on the lateral and anticous sides of the throat (Fig. 6). This may prevent insects from probing for nectar from behind the posticous anthers and may facilitate pollination by increasing contact with the anthers and style.

Glandular trichomes, similar to those in *Zaluziankya*, have been recorded from other genera within the Manuleae, including *Lyperia*, *Jamesbrittenia*, *Tetraselago*, *Polycarena* and *Manulea* (Hilliard, 1994). Their function is at present uncertain. Vogel (1954) suggested that a glandular vestiture on the inner corolla surfaces is characteristic of plants adapted to hawkmoths. However, within section *Nycterinia*, glandular hairs are absent from a number of the nocturnal species but occur in the diurnal *Z. microsiphon*. It is possible that the function of the glandular trichomes on the corolla is unrelated to pollination biology and instead part of the plants' defence against flower-feeding herbivores.

Our finding of self-incompatibility in two perennial *Zaluzianskya* species (Table 5) is apparently the first for the Manuleae and contradicts Hilliard's (1994) suggestion that self-compatibility occurs throughout *Zaluzianskya*. The stigma in *Zaluzianskya* elongates after the anthers have dehisced and invariably becomes coated with self-pollen, thus self-incompatibility would be an important mechanism for promoting outcrossing. Some annual *Zaluzianskya* species belonging to other sections are apparently autogamous, as they have been reported to set seed freely in greenhouses (Hilliard, 1994).

The day-flowering *Z. microsiphon* is sympatric and co-blooming with the closely related night-flowering species *Z. natalensis* at the Mt. Gilboa site. Occasional hybrids have been found at this site (S. D. Johnson, unpublished results) and we have once seen a long-proboscid fly that had previously been visiting *Z. microsiphon*, probing flowers of *Z. natalensis* as they opened shortly before dusk. This confirms that long-proboscid flies and hawkmoths visit structurally similar flowers, and further studies are focusing on the role of traits such as floral anthesis and the timing of scent production as prezygotic isolating mechanisms in sympatric *Zaluzianskya* species.

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