Flower biology of four epiphytic Malesian Gesneriads

Wiguna Rahman

Cibodas Botanic Gardens, Indonesian Institute of Sciences (LIPI),
Jl. Kebun Raya Cibodas, Sindanglaya, Cianjur 43253, Indonesia
wiguna.rahman@gmail.com

ABSTRACT. Floral traits, flowering events, nectar production and reproductive success of *Aeschynanthus horsfieldii* R.Br., *A. pulcher* (Blume) G.Don, *A. longiflorus* A.DC. and *Agalmyla parasitica* C.B.Clarke were observed for sympatric populations in the Cibodas area, Mount Gede-Pangrango, West Java. All traits were significantly different among the species, but were associated with a bird pollination syndrome. Many flowers of *Aeschynanthus longiflorus* and *Agalmya parasitica* failed to develop mature stigmas. *Agalmyla parasitica* flowers take a longer time to attract pollinators and receive pollen than the others and the filaments begin to bend earlier than the others. *Aeschynanthus pulcher* produces more nectar than the other species at the female phase, but the concentration was lower than in *Aeschynanthus horsfieldii* and *Agalmyla parasitica*. These seem to be correlated with the reproductive success of the respective species, with flowers of *Aeschynanthus longiflorus* and *Agalmyla parasitica* setting fewer fruit than the other two species. Flower traits and pollination shift are discussed in light of evidence that *Aeschynanthus horsfieldii* also attracts bumble bees (*Bombus rucifes*).

Keywords. *Aeschynanthus*, *Agalmyla*, Gesneriaceae, flower biology, Malesia, pollination

Introduction

Among 28 genera of Gesneriaceae that occur in Malesia, only *Aeschynanthus* Jack and *Agalmyla* (Blume) G.Don have epiphytic representatives. *Aeschynanthus* comprises approximately 160 species, while *Agalmyla* has about a hundred. The distribution areas of these two genera overlap: *Agalmyla* (Hilliard & Burtt 2002) seems to be restricted to Malesia and its distribution nested within that of *Aeschynanthus* (Mendum et al. 2001), a genus that is also significant outside of the region (e.g., Middleton 2007, 2009).

With a lack of direct observation of pollinators for every plant species, pollination syndromes are usually inferred. However, Ollerton et al. (2009) and Merxem et al. (2009) have recently cautioned that presumed pollination syndromes do not always successfully predict the actual pollinators. Based on flower characters, *Aeschynanthus* and *Agalmyla* have the characteristic association with bird pollination. There is evidence that flowers of *Aeschynanthus longiflorus*, *A. pulcher* and three other species of *Aeschynanthus* are usually visited by both *Arachnothera* spiderhunters and sunbirds (Leeuwen 1937, McClure 1966). *Agalmyla* flowers have also been noted as bird-visited (Hilliard & Burtt 2002). The nectar content of *Aeschynanthus* flowers have the strength associated with bird pollination (Freeman et al. 1991).

This paper compares the phenotypic traits of flowers of four plant species from the presumedly bird-pollinated genera *Aeschynanthus* and *Agalmyla*, in view of evidence for bee-pollination in one of the species, *Aeschynanthus horsfieldii*.

Material and methods

The epiphytic Gesneriaceae species studied were *Aeschynanthus horsfieldii* R.Br. (section *Microtrichium*), *Aeschynathus longiflorus* A.DC. (uncertain sectional affiliation), *Aeschynanthus pulcher* (Blume) G.Don (section *Aeschynanthus*), and *Agalmyla parasitica* C.B.Clarke (section *Agalmyla*). All are widely distributed in West Malesia. The observations were carried out at Cibodas, on the northern slope of Mount Gede-Pangrango, West Java, from November 2009 to March 2010.

Flowers of these species were randomly tagged before they opened. For each tagged flower, the day of flower opening was recorded and the flower was harvested following 0, 1, 3, 5, 7, 10, and 13 days after opening. Some flowers, however, dropped before harvesting. The sample size for each harvesting time was 5–10 flowers. For each harvest time, the length of the calyx, corolla, flower tube, stamen, gynoecium; the width of the flower mouth; and the diameter of the stigma were measured using calipers (to ±0.05 mm). Planar projection and en-face areas were measured followed Dafni (1992).

The flowering event was observed using other flowers, with a sample size of 27–50 flowers per species. These flowers were observed every day from January to February, 2010. During this period and for each tagged flower, the day of flower opening, convexing of the stigma, curvature and wilting of the filament, and the corolla dropping, was recorded.

To observed nectar production, some sample flowers were bagged before their opening, using flipped plastic. Nectar was extracted from detached flowers using 50 μl micropipettes, at the staminate, sexual overlap and pistillate phases for each species. At each flower phase, nectar was extracted at four different times, 0700–0800 hrs, 1000–1100 hrs, 1300–1400 hrs, and 1600–1700 hrs. Sugar concentration in the nectar was measured for each flower using a portable sugar refractometer (Kenko Refractometer, 0–80 % Brix).

A whole shoot bearing flowers was monitored through making a "flower map" when it was difficult to individually tag every flower for observation. Flowers setting fruits were counted and the percentage fruitset of the total flower number observed was computed.

Results

Flower traits

Flower traits of the four study species are presented in Table 1. All flowers of observed taxa are red (e.g., Fig. 1). The calyces are free, divided to the base, except

Table 1. Flower traits of the four study species. Values are expressed as mean ± SD (with n, sample size, in brackets); those marked by the same superscripts in each row are not significantly different.

Flower trait	*Aeschynanthus horsfieldii*	*Aeschynanthus longiflorus*	*Aeschynanthus pulcher*	*Agalmyla parasitica*	Statistical analysis	
					F	*P*
Flower colour	red	red	red	red		
Calyx shape	free, divided to base	free, divided to base	cup-shaped	free, divided to base		
Flower attachment	pendent	erect	erect	erect		
Filament curvature	curled down	curled down	curled down	curled back		
Corolla length (mm)	27.99±1.28[a] (20)	81.58±2.54[d] (30)	63.95±3.11[c] (65)	43.78±2.04[b] (20)	1989.793	*<0.001*
Corolla tube length (mm)	22.03±0.99[a] (20)	74.84±2.42[d] (30)	49.49±2.71[c] (65)	36.33±2.70[b] (20)	2156.605	*<0.001*
Flower mouth width (mm)	7.98±0.62[a] (20)	14.63±1.42[c] (30)	16.29±1.28[d] (65)	12.48±0.92[b] (20)	266.055	*<0.001*
Filament length (mm)	26.88±1.37[a] (20)	95.11±3.63[c] (30)	63.11±9.28[b] (65)	64.45±2.73[b] (20)	284.820	<0.001
En-face area (cm^2)	0.90±0.19[a] (20)	2.17±0.48[b] (30)	4.42±0.66[c] (65)	1.049±0.28[a] (20)	394.289	<0.001
Profile planar area (cm^2)	1.71±0.21[a] (20)	6.89±0.66[d] (30)	5.45±0.63[c] (65)	3.85±0.48[b] (20)	425.266	<0.001
Ratio of profile planar to enface area	1.96±0.35[a] (20)	3.31±0.83[b] (30)	4.42±0.66[d] (65)	3.94±1.28[c] (20)	51.109	<0.001

for *Aeschynanthus pulcher*, which has a cup-shaped calyx. Flowers of *A. horsfieldii* are pendulous, while in the three others they are erect. The filaments of the three *Aeschynanthus* species are curled downwards, whereas those of *Agalmyla parasitica* are curled back. The longest corolla length, corolla tube length, and filament length are found in *A. longiflorus*. The widest flower mouth, en-face area, and ratio of profile planar to en-face ratio are found in *A. pulcher*.

Due to protandrous development, gynoecium length in all four species increased gradually after flower opening (Fig. 2). However, as much as 30.22 % (n = 225) of *Agalmyla parasitica* and 80.89% (n = 178) of *Aeschynanthus longiflorus* flowers failed to developed mature stigmas.

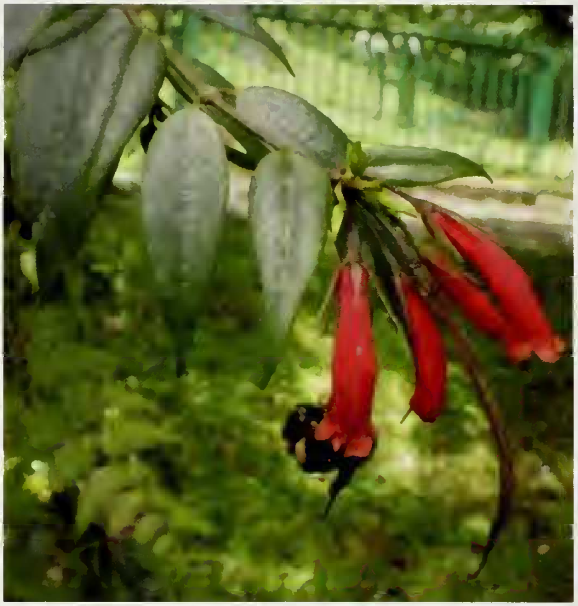

Fig. 1. Bumble bee (*Bombus rufipes* Lep.) visiting and pollinating flowers of *Aeschynanthus horsfieldii* R.Br.

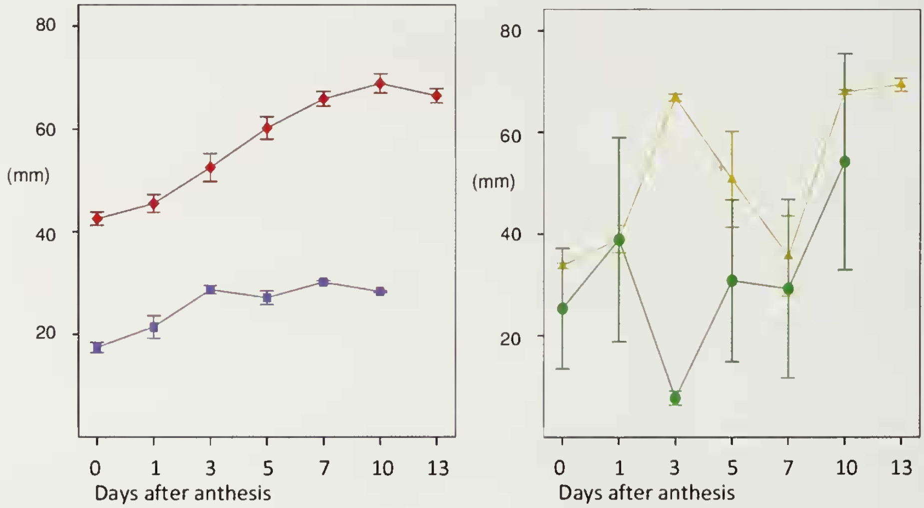

Fig. 2. Mean gynoecium length (±1 SE), in mm, of four species of Gesneriaceae after anthesis (flower opening): *Aeschynanthus pulcher* (◆), *A. horsfieldii* (■), *A. longiflorus* (●) and *Agalmyla parasitica* (▲). Note the horizontal axes are not to scale.

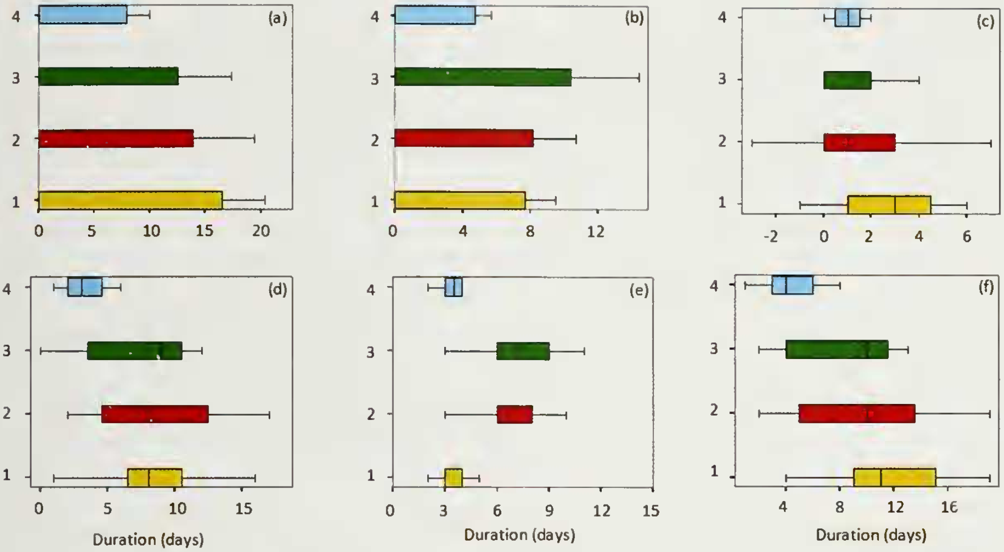

Fig. 3. Boxplots of the duration (in days) of (a) flower longevity, (b) male phase, (c) sexual overlap phase, (d) female phase, (e) filament curvature, and (f) stigma receptivity in *Aeschynanthus horsfieldii* (■ Bar 4 in each plot), *A. longiflorus* (■ Bar 3 in each plot), *A. pulcher* (■ Bar 2 in each plot) and *Agalmyla parasitica* (■ Bar 1 in each plot).

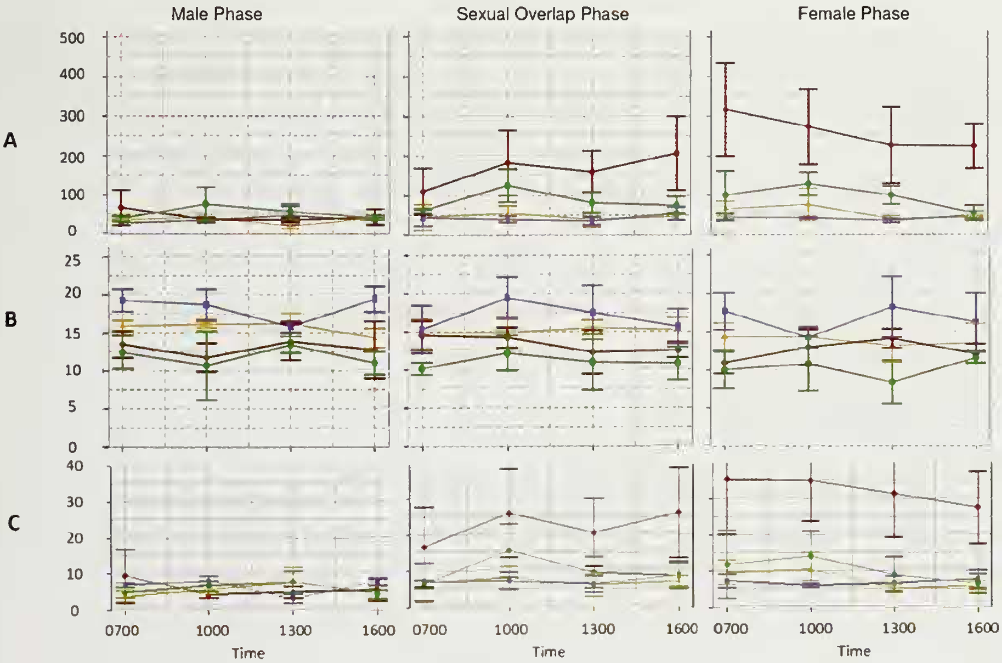

Fig. 4. Nectar volume (µl) (**A**), sugar concentration (% sucrose w/w) (**B**) and sugar amount (mg) (**C**) in *Aeschynanthus pulcher* (–), *A. horsfieldii* (–), *A. longiflorus* (–) and *Agalmyla parasitica* (–).

Flowering event

Characteristics of the flowering event (flower longevity, duration of the male and female phases, timing of filament curvature, and duration of stigma receptivity) are significantly different across the species, except the sexual overlap phase (i.e., when stamen functionality and stigma receptivity show an overlap), which showed weak differences (Fig. 3). The longest flower longevity was observed in *Agalmyla parasitica* ($F_{(3.145)}$=18.506, p<0.001). The longest male phase was found in *Aeschynanthus longiflorus* ($F_{(3.89)}$= 13.410, p<0.001), and the shortest female phase in *A. horsfieldii* ($F_{(3.86)}$=7.314, p<0.001). The duration of the overlap phase is only weakly different among the species ($F_{(3.83)}$=3.09; p<0.05). The sexual overlap phase is negative when the stigma becomes receptive after filaments have wilted, or when flowers do not display such overlap. The filaments of *A. longiflorus* were more slow to curve than in the three other species ($F_{(3.115)}$=16.955; p<0.001). The shortest stigma receptivity was observed for *A. horsfieldii* ($F_{(3.109)}$=15.667, p<0.001).

Flower nectar production

Nectaries of the four species are located at the flower base. In the *Aeschynanthus pulcher* flower, the corolla has a swollen (slightly bulbous) base which is not found in the other three species. The pattern of nectar production showed in Fig. 4. Nectar volume, and nectar sugar amount and concentration, vary over the time of day. There were also significant differences in nectar volume between the species and between flower phases, except in *A. horsfieldii* (Table 2).

The mean nectar sugar concentration was significantly different between species, but not between the flower phase in each species, except *Agalmyla parasitica* (Table 2). The highest nectar sugar concentration was found in *Aeschynanthus horsfieldii.*

The mean nectar sugar amount was significantly different between species in the overlap and female phases, but not in the male phase, when it was relatively minimal (Table 2). The mean nectar sugar amount was also significantly different between flower phases in *Aeschynanthus pulcher* and *A. longiflorus*, but not in *A. horsfieldii* and *Agalmyla parasitica*. The highest nectar sugar amount was found in *Aeschynanthus pulcher* flowers.

Reproductive success

More flowers of *Aeschynanthus pulcher* and *A. horsfieldii* successfully set fruit than in *A. longiflorus* and *Agalmyla parasitica* (Table 3). This unequal fruitset between the species could possibly indicate the presence of competition between species for pollinator services.

Discussion

According to conventional interpretation of pollination syndromes, bird pollination flowers have tubular shapes, are frequently red and odourless, and produce copious

Table 2. Nectar volume (µl), nectar sugar concentration (% sucrose w/w) and amount of sugar in nectar (mg) of *Aeschynanthus pulcher*, *A. horsfieldii*, *A. longiflorus*, and *Agalmyla parasitica*, during the male, sexual overlap and female phases of flowering. Values are means with standard deviations (SD) and sample size (n, in brackets) indicated.

Flowering Phase	Volume (µl)				Statistic	
	Aeschynanthus pulcher	*Aeschynanthus horsfieldii*	*Aeschynanthus longiflorus*	*Agalmyla parasitica*	F	*P*
Male (µl)	45.87±26.35 (20)	34.77±9.71 (20)	53.19±26.72 (20)	34.75±15.43 (20)	3.753	0.014
Overlap (µl)	164 .95±76.69 (20)	43.09±15.51 (20)	85.74±34.82 (20)	46.37±20.89 (20)	33.08	<0.001
Female (µl)	259.88±95.17 (20)	42.23±8.31 (20)	95.06±43.59 (20)	56.11±26.32 (20)	68.578	<0.001
Statistic	F=44.13; p<0.001	F=3.108; p=0.052	F=7.574; p<0.01	F=5.014; p<0.05		

Flowering Phase	Nectar sugar concentration (%)				Statistic	
	Aeschynanthus pulcher	*Aeschynanthus horsfieldii*	*Aeschynanthus longiflorus*	*Agalmyla parasitica*	*F*	*p*
Male (%)	12.96±2.48 (20)	18.28±2.06 (20)	11.87±2.7 (20)	15.61±1.22 (20)	34.24	<0.001
Overlap (%)	13.41±2.02 (20)	16.96±3.18 (20)	11.05±2.37 (20)	15.11±1.59 (20)	22.57	<0.001
Female (%)	12.49±1.91 (20)	16.57±3.25 (20)	10.14±2.68 (20)	13.79±1.93 (20)	22.80	<0.001
Statistic	F=0.9; p=0.412	F=1.936 ; p=0.154	F=2.22; p=0.118	F=6.85; p=0.002		

Flowering Phase	Nectar sugar amount (mg)				Statistic	
	Aeschynanthus pulcher	*Aeschynanthus horsfieldii*	*Aeschynanthus longiflorus*	*Agalmyla parasitica*	F	*P*
Male (mg)	6.14±4.29 (20)	6.52±2.21 (20)	5.97±2.17 (20)	5.48±2.66 (20)	0.41	0.742
Overlap (mg)	22.49±11.23 (20)	7.35±3.42 (20)	10.06±5.58 (20)	7.06±3.35 (20)	23.58	<0.001
Female (mg)	32.27±11.69 (20)	6.91±1.85 (20)	10.31±6.55 (20)	7.75±3.58 (20)	59.37	<0.001
Statistic	F=37.15; p<0.001	F=0.519; p=0.598	F=4.53; p=0.015	F=2.59; p=0.083		

Table 3. Percentage fruitset of *Aeschynanthus pulcher*, *A. horsfieldii*, *A. longiflorus* and *Agalmyla parasitica*.

Species	No. of flowers	No. of fruits set	% fruitset
Aeschynanthus pulcher	247	82	33.19
Aeschynanthus horsfieldii	117	40	34.18
Aeschynanthus longiflorus	178	23	12.92
Agalmyla parasitica	225	50	22.22

amounts of nectar, with the stigma and anthers conspicuously exserted from the floral tube. Although the flowers of all four species of Gesneriaceae observed have this classic bird pollination syndrome, the flowers of *Aeschynanthus horsfieldii* were frequently visited by bumble bees (*Bombus rupifes* Lep.) and could be said to show a shift in floral presentation.

Shifts in flower traits in *A. horsfieldii* may include the shallower flower tube length, narrower side advertisement, shorter flower longevity and higher nectar sugar concentration. With a shallower flower tube length, the nectar is more easily foraged by bees. The reduced flower tube length also decreases the potential effectiveness of side advertisement (profile planar area). According to Dafni (1994), there are little differences in the degree of side advertisement between bird flowers and large bee flowers. Primack (1985) and Stratton (1989) have also shown that bee flowers have shorter longevity than bird flowers. In *Sinningiae* (Gesneriaceae), bee flowers also have higher nectar sugar concentration than bird flowers (Perret et al. 2001).

All four species observed have red flowers, although only *A. horsfieldii* appeared to attract bumble bees. We do not know how the red colour in *A. horsfieldii* flowers is compatible with bee vision, and suggest that perhaps flowers of *A. horsfiledii* could have UV reflectance properties. Flowers which have red colour with UV reflectance can attract bees (Chitka & Waser 1997).

In terms of reproductive success, the existence of co-flowering species with similar syndromes should increase inter-specific competition and reduce pollination success (Sargent & Ackerly 2008, Chitka & Shürkens 2001). We suggest that each of the four related species could have developed a different strategy to attract pollinators. *Aeschynathus pulcher* has developed flowers with large side advertisement and which produce high nectar volume and nectar sugar amounts. *Aeschynathus horsfieldii* has flowers able to attract more than one pollinator class (birds and bumble bees) through narrow side advertisement, high nectar sugar concentration and probably both nectar and pollen as rewards.

Aeschynanthus longiflorus and *Agalmyla parasitica* could have a "flower dimorphism syndrome", sometimes apparently showing andromonoecy, when some plants only present flowers with undeveloped gynoecia (i.e., with functionally male flowers), while others present the usual hermaphrodite condition. From a population perspective, producing more male flowers is a strategy to increase pollen transfer when there are limitations in plant resources and pollinator visitation.

References

Chitka, L. & Schürkens, S. (2001) Successful invasion of a floral market. *Nature* 411: 653.

Chitka, L. & Waser, N.M. (1997) Why red flowers are not invisible to bees. *Israel J. Plant Sci.* 45 (2–3): 169–183.

Dafni, A. (1992) *Pollination Ecology: A Practical Approach*. Oxford.

Dafni, A. (1994) Note on side advertisement in flowers. *Function. Ecol.* 8: 136–138.

Freeman, C.E., Worthington, R.D. & Jackson, M.S. (1991) Floral nectar sugar compositions of some South and Southeast Asian species. *Biotropica* 23: 568–574.

Hilliard, O.M. & Burtt, B.L. (2002) The genus *Agalmyla* (Gesneriaceae-Cyrtandroideae). *Edinburgh J. Bot.* 59(1): 1–210.

Leeuwen, W.M.D. van (1937) Observation about the biology of tropical flowers. *Ann. Jard. Bot. Buitenzorg* 48: 27–68.

McClure, H.E. (1966) Flowering, fruiting, and animals in the canopy of a tropical rain forest. *Malayan Forester* 29: 182–203.

Mendum, M., Lassnig, P., Weber, A. & Christie, F. (2001) Testa and seed appendage morphology in *Aeschynanthus* (Gesneriaceae): phytogeographical pattern and taxonomic implications. *Bot. J. Linn. Soc.* 135: 195–213.

Merxem, D.G. de, Borremans, B., Jäger, M.L. de, Johnson, T., Jooste, M., Ros, P., Zenni, R.D., Ellis, A.G. & Anderson, B. (2009) The importance of flower visitors not predicted by floral syndrome. *S. African J. Bot.* 75 (4): 660–667.

Middleton, D.J. (2007) A revision of *Aeschynanthus* (Gesneriaceae) in Thailand. *Edinburgh J. Bot.* 64(3): 363–429.

Middleton, D.J. (2009) A revision of *Aeschynanthus* (Gesneriaceae) in Cambodia, Laos and Vietnam. *Edinburgh J. Bot.* 66(3): 391–446.

Ollerton, J., Alarcón, R., Waser, N.M., Price, M.V., Watts, S., Cranmer, L., Hingston, A., Peter, C.I. & Rotenberry, J. (2009) A global test of the pollination syndrome hypothesis. *Ann. Bot.* 103(9): 1471–1480.

Perret, M., Chautems, A., Spichiger, R., Peixoto, M. & Savolaine, V. (2001) Nectar sugar composition in relation to pollination syndromes in *Sinningieae* (Gesneriaceae). *Ann. Bot.* 87: 267–273.

Primack, R.B. (1985) Longevity of individual flowers. *Ann. Rev. Ecol. Syst.* 16: 15–27.

Sargent, R. & Ackerly D.D. (2008) Plant-pollinator interactions and community assembly. *Trends Ecol. Evol.* 23: 123–130.

Stratton, D.A. (1989) Longevity of individual flowers in Costa Rican cloud forest: ecological correlates and phylogenetic constraints. *Biotropica* 21(4): 308–318.