Chapter 3
Biology of the Obligate Pollination Mutualism

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Keywords Active pollination behavior • *Breynia* • *Epicephala* • *Glochidion* • *Gomphidium* • *Phyllanthus* • Species specificity

3.1 Phyllanthaceae with Greenish Inconspicuous Flowers

Most zoophilous plants are adapted to attract reliable pollination agents by providing floral rewards such as nectar and pollen and by advertising their flowers with nongreen color and nonleafy odor, and facilitate pollination by protruding styles and stamens from flowers. The great diversity of angiosperm flowers is believed to be a product of coevolution and codiversification between plants and their pollinators.

However, there are some zoophilous plants whose floral characters do not correspond to these floral characteristics. For example, monoecious plants in several genera of Phyllanthaceae have small, greenish, inconspicuous flowers whose styles and stamens are fused and lodged at basal parts of flowers. Phyllanthaceae (leafflower family) is a diverse, globally distributed plant family, whereas their diversity peaks at tropical regions. Most Phyllanthaceae plants are herbaceous or shrubby plants, including some arboreal genera such as *Glochidion* and *Margaritaria*.

Glochidion, a monoecious tree genus of Phyllanthaceae has minute apetalous female flowers with highly specialized styles (Airy Shaw 1978; Chakrabarty and Gangopadhyay 1995). The genus consists of >300 species ranging from tropical Asia to Australia and Polynesia (Govaerts et al. 2000). Although its pollination system is unknown, *Glochidion* trees usually bear many fruits, most of which are infested by small moth larvae. The exclusively high proportion of the fruits infested

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by seed-parasitic insects reminds us of the situation where a fraction of seeds in a fruit of figs and yuccas is infested by fig wasps and yucca moths.

The moths associated with *Glochidion* fruits are members of Gracillariidae, most species of which are leaf miners of various angiosperms. The leaf-mining gracillariid larvae are dorsoventrally flattened, endophytic tissue feeders, and pupate in flat cocoons woven by spun silk. Thus far, no gracillariid moths have been known to be pollinators, although they infrequently visit flowers to probe floral nectar with their elongated proboscises. Compilation of circumstantial evidence of plant reproduction and our nocturnal observation on pollination revealed that the plants are certainly pollinated by these female moths whose larvae infest seeds of the flower. The discovery of obligate pollination mutualism in *Glochidion* gave us a strong incentive to study pollination systems of other related genera in Phyllanthaceae.

The genus *Breynia* is a close relative of the genus *Glochidion*, and comprises 35 species of monoecious shrubs, distributed in tropical and subtropical regions of Asia, Australia, and the Pacific Islands (Webster 1994; Govaerts et al. 2000). Unlike *Glochidion* with dehiscent fruits, *Breynia* has fleshy fruits. Observations of pollination of two *Breynia* species in Japan and Laos additionally clarified that this genus also adopts obligate pollination mutualism.

Phyllanthus sensu stricto is one of the largest genera of angiosperms and comprises more than 800 species including herbaceous annuals and perennials, woody shrubs, and small trees (Govaerts et al. 2000). Although at least some herbaceous annual *Phyllanthus* species are pollinated by ants (Kawakita and Kato 2009), the great diversity of *Phyllanthus* species in some regions of the world led us to consider that the diversity might be related to specific animal pollination systems. Among the approximately 15 subgenera currently recognized, *Gomphidium* is a group of small trees comprising about 150 species restricted to Australia, New Guinea, and Polynesia (Holm-Nielsen 1979). Notably, this subgenus has undergone extensive diversification in New Caledonia (115 species) and now constitutes the largest genus on the islands (Schmid 1991). Thus, we made observations of pollination of the diverse *Phyllanthus* species in New Caledonia.

In this chapter, we describe obligate pollination mutualism of the three plant genera *Glochidion*, *Breynia*, and *Phyllanthus*, by focusing on their remarkable floral morphology, pollinator behavior, seed parasitism, host specificity, and the nature of the mutualism.

3.2 Mutualism in *Glochidion*

3.2.1 Flowers of Glochidion

Glochidion flowers are dimorphic, consisting of a pedunculate male flower with unfolded sepals and connate ellipsoid stamens, with a sessile or shortly pedunculate female flower composed of folded sepals and a united columnar style. The style has a narrow pit at its lobed tip. The inner surface of the pit is the stigma. This cryptic stigma is unlikely to be pollinated by wind or by ordinary insect visitors. We therefore made field observations of the pollination of three *Glochidion* species in Japan.

Trees of *G. acuminatum* (Fig. 3.1a) have male and female flowers in separate axillary clusters on each branch (Fig. 3.1b). Male flowers are aggregated at the base, and the female flowers are at the apical part of each branch. Female flowers are generally six-ovuled. Neither male nor female flowers secrete nectar.

3.2.2 Nocturnal Flower Visitor

Observation on insect visits to *G. acuminatum* flowers was made at Amami-Oshima Island, in southwestern Japan. In the daytime, the flowers are rarely visited by insects, although various insects pass by the inflorescence. However, beginning in the evening and continuing until midnight, the flowers are visited frequently by a gracillariid moth, *Epicephala anthophilia*. Female moths visit male flowers to collect pollen by inserting their proboscis into the anthers (Fig. 3.1c). After flight migration among trees or within a tree, female moths visit female flowers. Female *Epicephala* moths netted around the *Glochidion* trees almost always possess numerous *G. acuminatum* pollen grains on their ciliated proboscides (Fig. 3.1f), whereas males are never found to carry pollen grains. The behavior of the female moths on female flowers is remarkable. Visiting a cluster of female flowers, a female uncoils its proboscis, deposits the pollen grains onto the cryptic stigma (Fig. 3.1d), and then bends its abdomen to insert its long ovipositor into the stylar pit (Fig. 3.1e). The series of pollination and oviposition behavior usually lasts up to 1 min. The female walks along the branch, tapping the flowers with its proboscis and visiting each female flower sequentially to repeat the stereotypic pollination/oviposition behavior.

3.2.3 Active Pollination

Pollen attachment and moth eggs in female flowers can be examined by dissecting the styles under a microscope. Normally, a female lays an egg in a flower just above the ovules at the base of each oviposited style (Fig. 3.1h), and an average female flower receives 1.7 eggs (Table 3.1). Oviposited flowers are consistently pollinated (Fig. 3.1g), whereas unoviposited flowers are very rarely pollinated (Fig. 3.2). The average number of pollen grains deposited on a stigma after a single moth visit is less than 10 but is enough to fertilize all six ovules in an ovary.

Active pollination and the oviposition into styles by *Epicephala* moths are also observed on *G. zeylanicum* and *G. obovatum*, which have different style structures

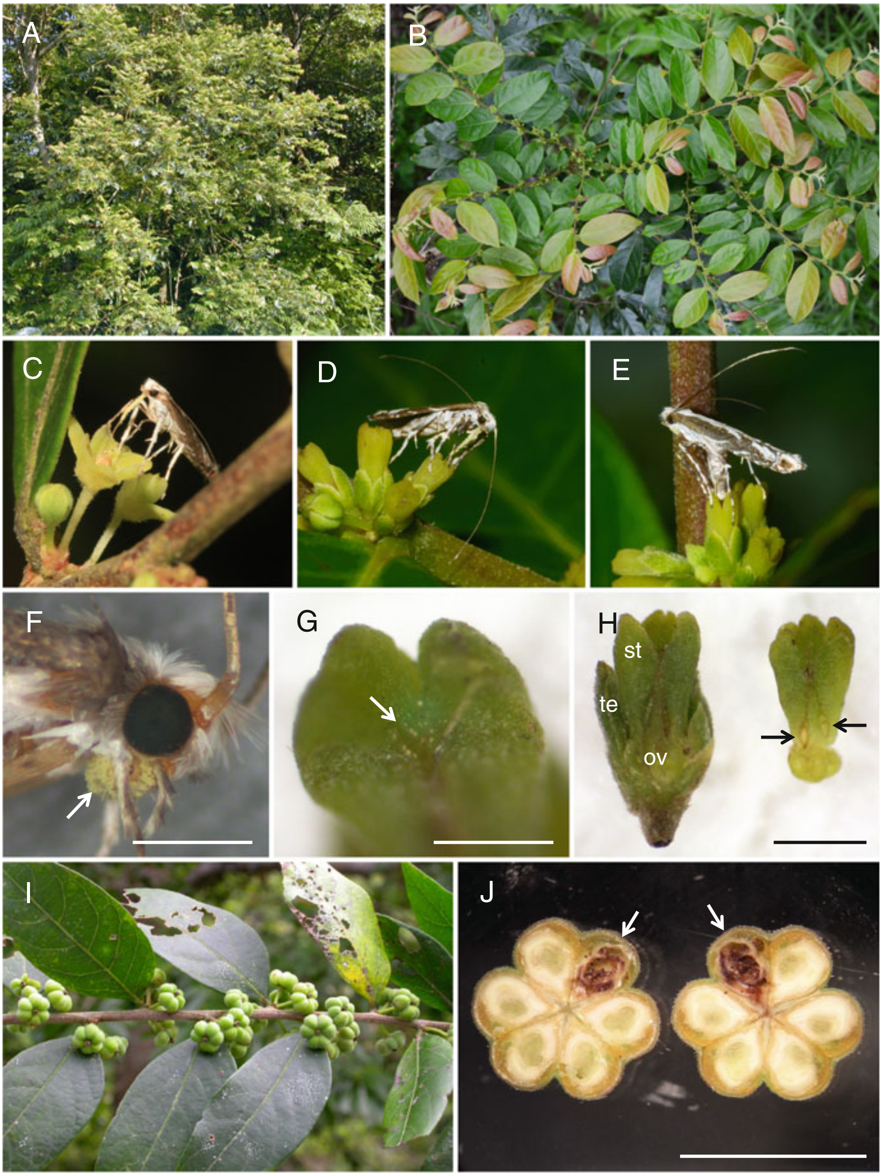


Fig. 3.1 Flowers of *Glochidion acuminatum* and its pollinator *Epicephala anthophilia*. (**a**) General appearance of the tree. (**b**) Branches bearing many male and female flowers. (**c**) A female moth collecting pollen on a male flower with its proboscis (photo courtesy of Bruce Anderson). Note that the proboscis surface is covered with pollen. (**d**) A female moth depositing pollen on stigma. (**e**) An ovipositing *Epicephala* moth. (**f**) Pollen-loaded proboscis of a female moth (*arrow*). Bar = 0.5 mm. (**g**) Apical view of a female flower, showing the pollinated cryptic stigma. Bar = 0.5 mm. (**h**) Cross-section of a female flower with two eggs (*arrows*). Abbreviations are: st, style; te, tepal; ov, ovule. Bar =1 mm. (**i**) Branch with fruits. (**j**) Cross-section of a fruit. One seed (*arrows*) has been destroyed by a moth larva. Bar =1 cm

Table 3.1 Comparison of moth pollination, oviposition, and seed infestation among three *Glochidion* species

Glochidion species	Pollen grains	Laid eggs	Ovules	Intact seeds	Infested seeds	Sterile/ Aborted seeds
acuminatum	7.8 ±4.8 (30)	1.7 ±0.8 (262)	6.1 ±0.4 (364)	3.3 ±1.7 (364)	1.8 ±1.7 (364)	0.9 ±1.2 (364)
zeylanicum	32.4 ±12.3 (51)	2.3 ±1.1 (51)	10.3 ±0.9 (104)	2.1 ±2.7 (104)	7.6 ±2.9 (104)	0.6 ±1.0 (104)
obovatum	26.7 ±11.2 (40)	1.6 ±0.6 (40)	11.9 ±0.4 (56)	3.1 ±2.6 (56)	4.8 ±2.5 (56)	1.3 ±0.6 (56)

Although the typical ovule number is different among the three species, 20–54% of seeds were intact even after multiple oviposition by *Epicephala* moths and infestation by nonpollinating seed-parasitic moths. Means ± SD are shown. Numbers in parentheses represent the number of examined fruits

(Figs. 3.2 and 3.3). *G. zeylanicum* has ovoid, budlike female flowers whose styles are almost completely enclosed by sepals (Fig. 3.2d). The flowers have small openings at the apical tip that lead to the narrow stigma pit of the fused styles. Female flowers are visited at night by *Epicephala bipollenella*, which actively pollinates the female flower (Fig. 3.2e), inserts its long ovipositor into the narrow stigma pit (Fig. 3.2f), and lays an egg. Female flowers of *G. obovatum* are columnar like those of *G. acuminatum*, but differ in having distinctly swollen ovaries (Fig. 3.2j). At night the flowers are actively pollinated by *Epicephala obovatella* (Fig. 3.2k), which inserts its abdomen between the style and calyx, and lays an egg into the locules directly through the ovary wall (Fig. 3.2l), rather than through the stigma.

3.2.4 Seed Infestation by Pollinator Moth Larva

Fertilized ovules begin to develop, and unpollinated female flowers abscise shortly thereafter. The hatched moth larva bores into the ovary and consumes a few developing seeds within a fruit (Fig. 3.1j). In *G. acuminatum*, a larva usually consumes two seeds to complete larval growth, and escapes from the fruit to pupate on the litter. The life cycles of both the plant and its pollinator moth are inseparably linked (Fig. 3.4). Seed destruction is caused mostly by *Epicephala* moths, but nonpollinating seed-parasitic moths of Pyralidae and Tortricidae also infest seeds. On average, one fruit has 6.1 ovules, of which 1.8 are infested by moth larvae, 3.3 are intact, and 0.9 are sterile or aborted. The overall outcomes are similar among the three *Glochidion* species (Table 3.1).

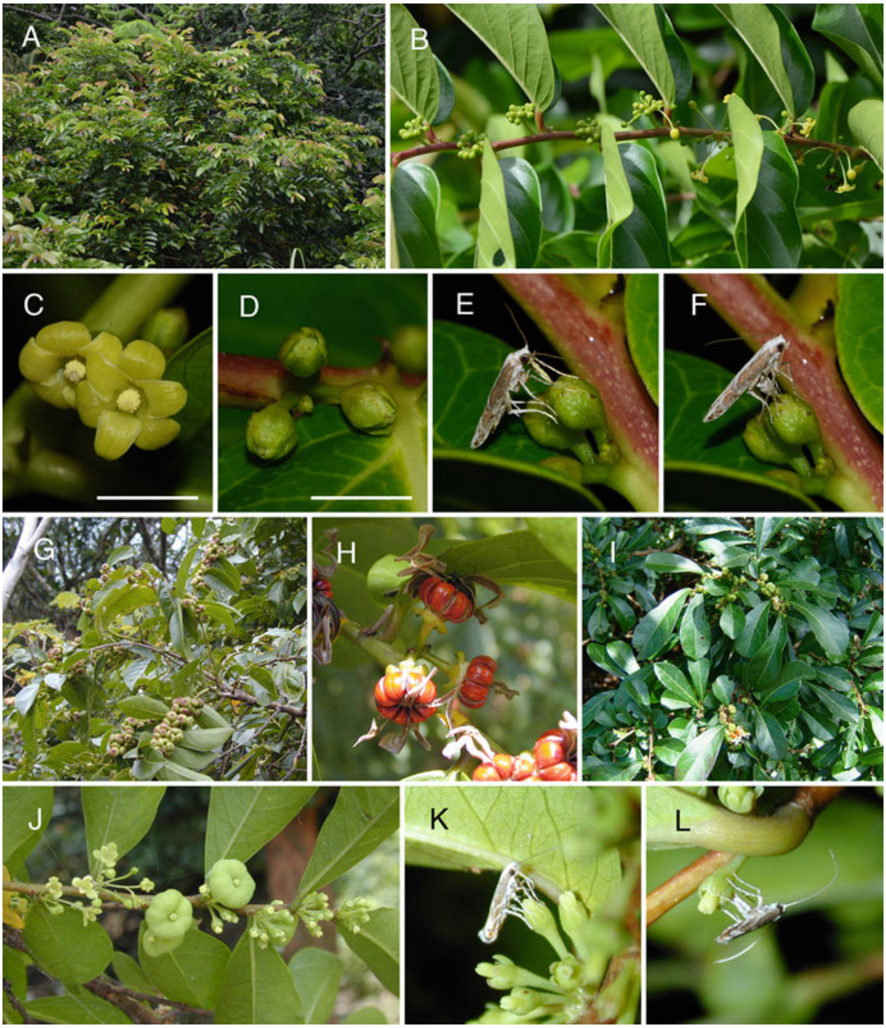


Fig. 3.2 Flowers and pollinators of *Glochidion zeylanicum* (**a**–**h**) and *G*. *obovatum* (**i**–**l**). (**a**) General appearance of *G*. *zeylanicum*. (**b**) Flowering branch with male and female flowers toward the base (*right*) and apex (*left*), respectively. (**c**) Male flowers. Bar = 5 mm. (**d**) Female flowers. Bar = 5 mm. (**e**) A female of the pollinator *Epicephala bipollenella* actively depositing pollen on the cryptic stigma. (**f**) A pollinator female ovipositing in the style. (**g**) Fruiting tree. (**h**) Dehisced fruits showing seeds with orange sarcotesta. (**i**) General appearance of *G*. *obovatum*. (**j**) Flowers and fruits. Male and female flowers are borne toward the base (*left*) and apex (*right*), respectively. (**k**) A pollinator (*Epicephala obovatella*) female actively pollinating the female flower. (**l**) A pollinator female ovipositing through the lateral ovary wall

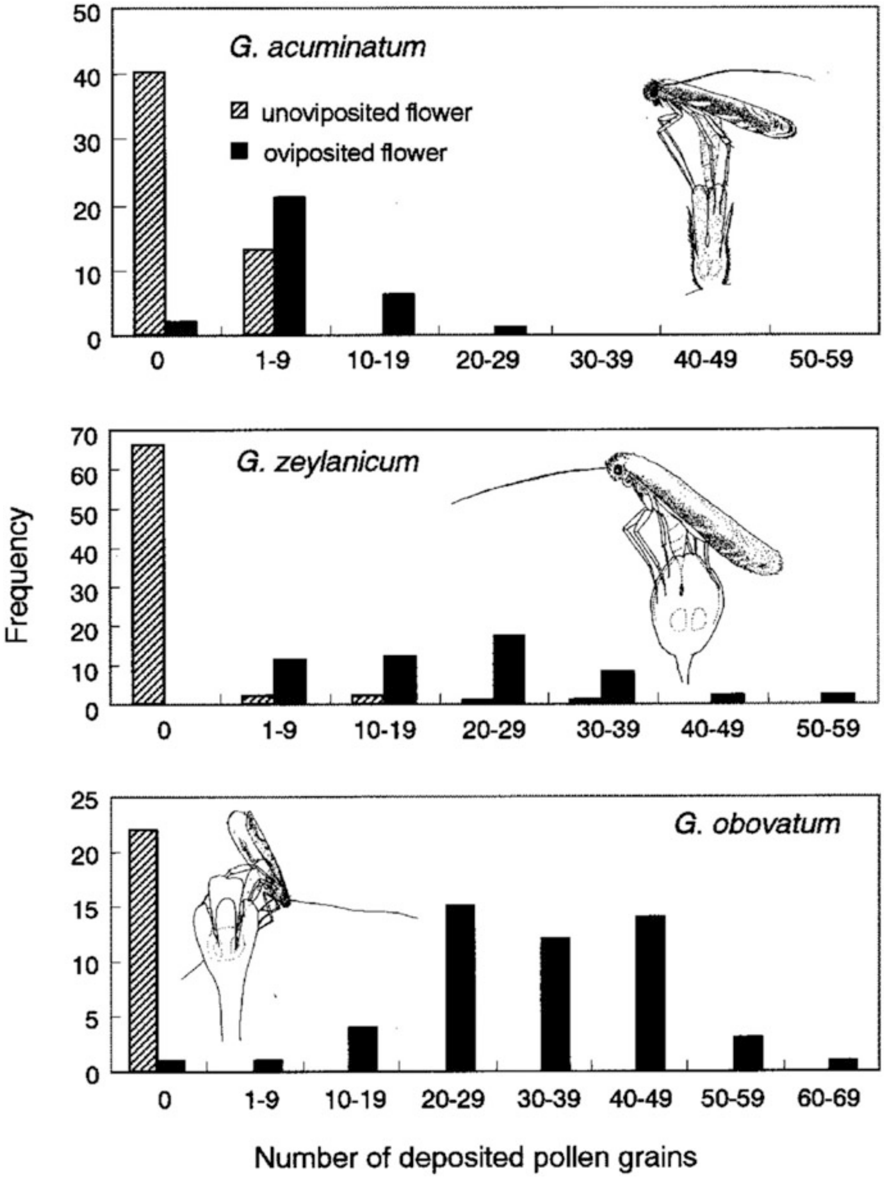


Fig. 3.3 Frequency distributions of the number of pollen grains attached to oviposited (solid) and unoviposited stigmas (shaded) of three *Glochidion* species: *G. acuminatum*, *G. zeylanicum*, and *G. obovatum*. The typical ovipositing postures and oviposited eggs of each *Epicephala* moth species are shown in each inset (Reproduced from Kato et al. (2003). Copyright (2003) National Academy of Sciences)

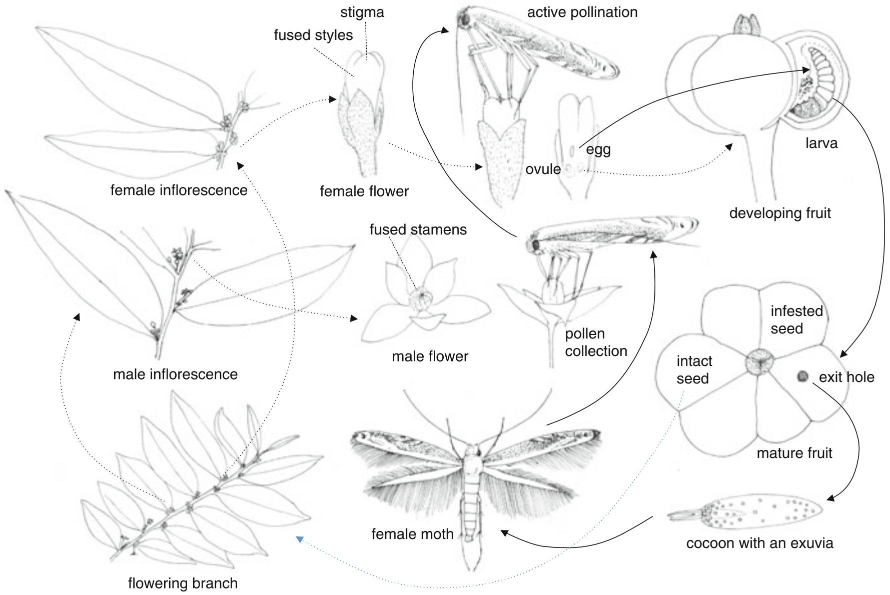


Fig. 3.4 Life cycles of *Glochidion acuminatum* (*broken arrows*) and its pollinator moth, *Epicephala anthophilia* (*solid arrows*)

3.2.5 Host Specificity of Pollinator Moth Species

Surveying other *Glochidion* species in Japan and Taiwan, we found that fruits of all six species harbored an individual (rarely two) of seed-parasitic *Epicephala* species that could be distinguished by its genitalic morphology. Host-specificity of the moths was not surprising because several *Glochidion* species often co-occur at our study sites without apparent hybridization. Host-specificity of the moths was confirmed by investigating nucleotide sequence variation in the mitochondrial cytochrome oxidase subunit I gene (COI) among sampled *Epicephala*, which clearly showed that the sequences of moths collected from different *Glochidion* hosts are highly divergent (3–9% pairwise difference), whereas the divergence between moths using the same *Glochidion* host was minimal (<0.5%).

These observations indicate that at least three *Glochidion* species are pollinated by a species-specific seed-parasitic *Epicephala* species, at the cost of infested seeds. It is notable that the female moth has an exceptionally long ovipositor to insert an egg into a style, and a ciliated proboscis to collect pollen. The moths actively pollinate flowers, similar to fig wasps and yucca moths. The seed-parasitic habit of *Epicephala* is unique in Gracillariidae, most species of which are leaf miners.

3.2.6 Widespread Mutualism in Glochidion *Plants*

Additional *Glochidion* species in New Caledonia, Fiji, Australia, Malaysia, Laos, and Myanmar all had traces of limited seed infestation by the moths. Thus, *Glochidion–Epicephala* mutualism may be widespread among the >300 known *Glochidion* species. This mutualism shares many characteristics with fig–fig wasp and yucca–yucca moth mutualisms, because the reward for the pollinator is the developing ovules or seeds. Furthermore, the sister groups of these pollinator taxa are endophytic herbivores, and the pollinators are females with elongated ovipositors. Outstanding diversification has occurred only in *Ficus* (>750 spp.) and *Glochidion*, both of which are tropical monoecious or gynodioecious woody plants that have highly specialized styles into which small pollinating insects oviposit.

In Malaysia, *Glochidion* is the largest genus (150 spp.) of Phyllanthaceae (Govaerts et al. 2000), and the principal species-diagnostic characteristic is structure of the style (Airy Shaw 1978; Chakrabarty and Gangopadhyay 1995). Because pollinating moths oviposit into styles using diverse and specific methods, the length of their ovipositor and their oviposition behavior are crucial for such specialization. Thus, the specialized structure of the *Glochidion* style and the specialized oviposition behavior of the moths may well serve as barriers against both *Glochidion* hybridization and host-shift by the moths. Plant speciation based on these traits provides a selective basis for speciation and high diversity.

3.3 Mutualism in *Breynia*

3.3.1 Flowers of Breynia vitis-idaea

Breynia vitis-idaea is a monoecious shrub that occurs in forest margins of tropical and subtropical forests in Asia (Fig. 3.5a). The species is distributed from Pakistan to the southern part of Japan, including most parts of tropical Southeast Asia (Govaerts et al. 2000). The flowers lack petals and are dimorphic, with male flowers (Fig. 3.5b) arranged toward the base and female flowers (Fig. 3.5c) at the apex of each branch. Typically, only one or two flowers are borne on axils. Male flowers have fused calyx lobes with inflexed apical ends that make the stamens unlikely to be accessible to opportunistic flower visitors (Fig. 3.5b). Female flowers are campanulate with three short styles fused at the center of the upper surface of the ovary (Fig. 3.5c). Female flowers have three locules, each containing two ovules. Fruits are produced shortly after pollination within 3–4 weeks. In the course of fruit development, pedicels become erect, and the fruit coat eventually turns red to dark purple (Fig. 3.5f). Flowering and fruiting occur throughout the year but typically peak in spring (March to May) and early fall (August to October) in southern Japan.

Fig. 3.5 Flowers and pollinators of *Breynia vitis-idaea* (**a**–**g**) and *B. fruticosa* (**h**–**j**). (**a**) General appearance. (**b**) Male flower. (**c**) Female flower. (**d**) A female of the pollinator *Epicephala vitisidaea* actively depositing pollen on the stigma. (**e**) A pollinator female laying an egg in the space between ovary and tepals. (**f**) Mature fruits. One of the fruits has an exit hole excavated by *Epicephala* larva (*arrow*). (**g**) A braconid wasp ovipositing on mature fruit. (**h**) Male flowers. (**i**) Female flowers. (**j**) Capsular fruits containing seeds with fleshy sarcotesta

3.3.2 Active Pollination

Nectar is produced at night on female flowers of *B. vitis-idaea*. Flowers of *B. vitis-idaea* are sometimes visited by ants, but the main pollinator is the moth *Epicephala vitisidaea*. Ants visit female flowers of *B. vitis-idaea* during the day and at night to forage nectar. However, these ants are not observed on male flowers, and they do not carry pollen. At night, female *Epicephala vitisidaea* moths visit female *B. vitis-idaea* flowers, depositing pollen grains with their proboscises (Fig. 3.5d) and subsequently laying an egg within the interspace between calyx lobes and ovary (Fig. 3.5e). The pollination–oviposition behavior is sometimes repeated twice on the same flower. As in *Glochidion*, all the moths that visit female flowers carry numerous pollen grains on their proboscises.

3.3.3 Obligate Pollination Mutualism

Pollinated flowers almost always have moth eggs, whereas unpollinated flowers only rarely have eggs, indicating that *Epicephala vitisidaea* moths are likely exclusive pollinators of *B. vitis-idaea*. Pollen grains are aggregated at the stigmatic part of female flowers as in *Glochidion* (Kato et al. 2003), which is unlikely to occur through passive pollination. Eggs are laid between the ovary and calyx lobes, and individual flowers receive 1–4 eggs with an average of about 1.5 eggs per flower (Kawakita and Kato 2004a).

Of the six seeds contained in a fruit, roughly half are destroyed by moth larvae, and the rest remain intact. However, fruits sometimes remain infested by the moths. These uninfested fruits contain remains of *Epicephala* moth eggs, indicating that egg/larval death of *Epicephala* moths is common in *B. vitis-idaea*. Normally, a single *Epicephala* moth larva does not consume all seeds within a fruit (Fig. 3.6a), but two moth larvae are enough to destroy all seeds of a fruit (Fig. 3.6b). Braconid wasps parasitize early instar *Epicephala* larvae by laying an egg through the fruit wall with their long ovipositors (Fig. 3.5g). When this parasitism occurs, it prevents further seed consumption by *Epicephala* larvae, resulting in greater seed survival (Fig. 3.6c).

Similar mutualism is also observed in another *Breynia* species, *B. fruticosa*, which is distributed from southern China to Indochina (Fig. 3.5h–j). Fruits of this species are dehiscent, and the seeds possess sarcotesta, contrasting with the fleshy fruit of *B. vitis-idaea*. In both *B. vitis-idaea* and *B. fruticosa*, larvae of the moths consumed the developing seeds, but in total, a fraction of the seed crop was left intact, thus resulting in a net benefit to plant reproduction.

The genus *Breynia* currently comprises 35 species distributed in tropical regions of Asia, Australia, and the Pacific Islands (Govaerts et al. 2000). Plants of this genus are characterized by the fused, obconic or turbinate calyx lobes in male flowers and minute styles that are more or less fused in female flowers (Fig. 3.5b,c,h,j; Chakrabarty and Gangopadhyay 1996). These structures likely prevent effective contact with anthers and stigmas by facultative flower visitors and suggest that the specialized *Epicephala* moth pollination is potentially widespread within the genus. Fruits of *B. disticha* in New Caledonia and *B. cernua* and *B. oblongifolia* in Australia are also infested by *Epicephala* moths (A. Kawakita and M. Kato, personal observations), which further supports the widespread occurrence of obligate pollination mutualism in the genus *Breynia*.

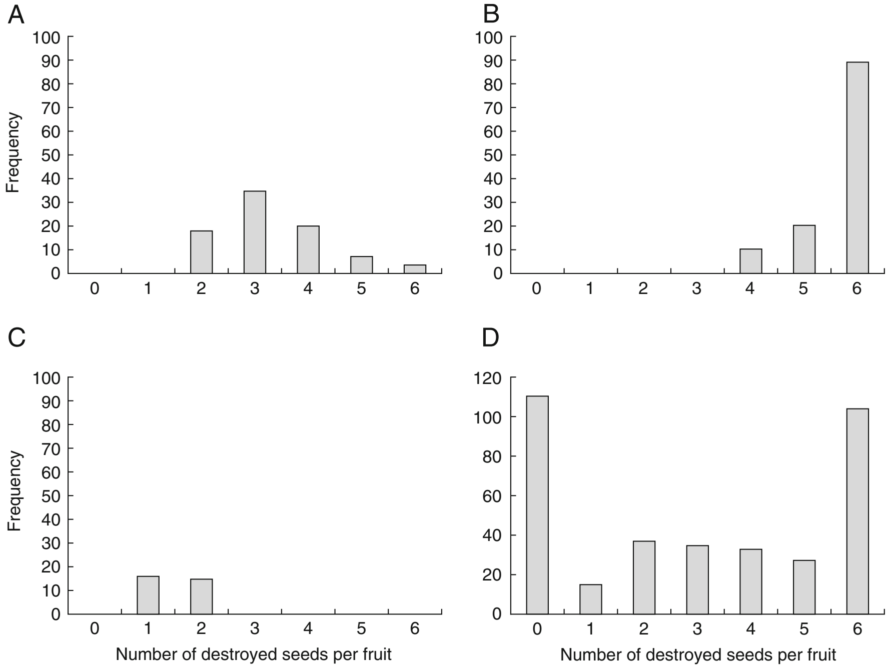


Fig. 3.6 Frequency distributions of the number of destroyed seeds per fruit in *Breynia vitis-idaea*. Fruits of *B. vitis-idaea* have six ovules. Immature larvae of *Epicephala* moths were occasionally parasitized by braconid wasps. (**a**) Fruits with one *Epicephala* larva ($N = 83$). (**b**) Fruits with more than two or more *Epicephala* larvae ($N = 119$). (**c**) Fruits with one or two parasitized moth larvae ($N = 31$). (**d**) All fruits ($N = 365$) (Modified from Kawakita and Kato 2004a)

3.4 Mutualism in New Caledonian *Phyllanthus* (*Gomphidium*)

3.4.1 Flowers of Gomphidium

In 2003, we studied the pollination biology of 25 *Phyllanthus* (all in subgenus *Gomphidium*) species at various localities in New Caledonia, and insect flower visitors were observed for two *Phyllanthus* species: *P. bourgeoisii* and *P. aeneus*. *Phyllanthus bourgeoisii* is a common rheophyte on rocky riverbanks (Fig. 3.7a), and *P. aeneus* is a shrub that is typical of serpentine scrub habitats (Fig. 3.7f). *Phyllanthus aeneus* has open male flowers, whereas those of *P. bourgeoisii* have folded calyx lobes, which make the anthers inaccessible to facultative flower visitors (Fig. 3.8). Female flowers of both species are much reduced and consist of short fused styles that are mostly covered with calyx lobes (Fig. 3.8). The flowers have three locules, each containing two ovules. The two species produce flowers and fruits throughout the year, which is also typical in *Glochidion* and *Breynia*.

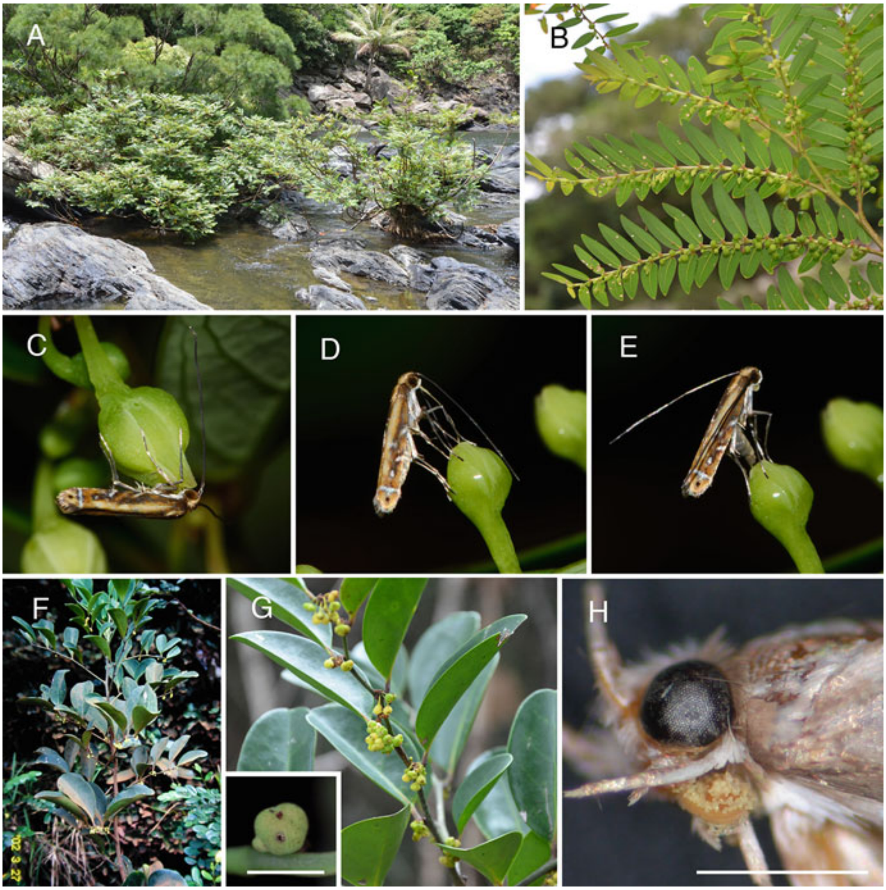


Fig. 3.7 Flowers and pollinators of *Phyllanthus bourgeoisii* (**a**–**e**) and *P. aeneus* (**f**–**h**). (**a**) Habitat of *P. bourgeoisii* on rocky riverbank. (**b**) Flowering and fruiting branches. (**c**) A pollinator female actively collecting pollen on male flower. (**d**) A pollinator female depositing pollen on the stigma, which is hidden inside the imbricate tepals. (**e**) A pollinator female ovipositing in the style. (**f**) General appearance. (**g**) Flowering branch. A fruit with the exit hole made by *Epicephala* larva is shown in the inlet. Bar = 1 cm. (**h**) Female *Epicephala* moth collected on *P. aeneus* showing its pollen-coated proboscis. Bar =1 mm

3.4.2 Active Pollination

Epicephala moths are the only visitors to the flowers of the two *Phyllanthus* species. In the evening, females of undescribed *Epicephala* species use their proboscises to collect pollen from male *Phyllanthus* flowers (Fig. 3.7c). They then deliberately deposit pollen on the stigma with their proboscises (Fig. 3.7d) and subsequently lay an egg (Fig. 3.7e). Flower-visiting females consistently carry numerous pollen grains on their proboscises (Fig. 3.7h), and their behavior on

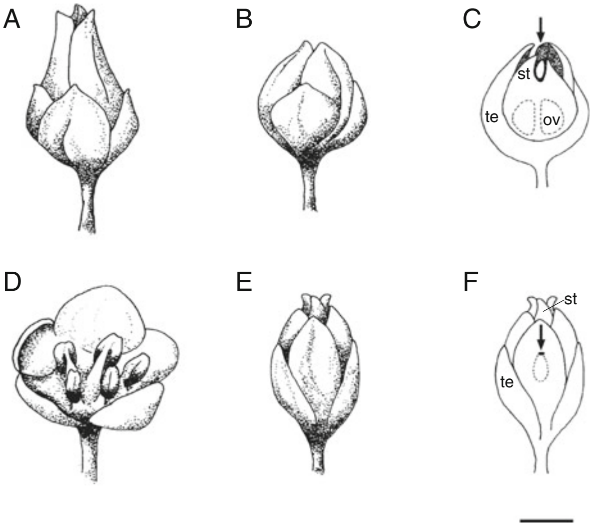


Fig. 3.8 Flowers of *Phyllanthus bourgeoisii* (**a**–**c**) and *P. aeneus* (**d**–**f**). (**a**) Male flower. (**b**) Female flower. (**c**) Longitudinal section of a female flower. The *arrow* indicates the location of an *Epicephala* moth egg. (**d**) Male flower. (**e**–**f**) Female flowers. *Epicephala* eggs are laid within the tissue of the calyx lobes (*arrow*). Abbreviations are: st, style; te, tepal; ov, ovule. Bar = 1 mm (Modified from Kawakita and Kato 2004b)

flowers is very similar to that observed in *Glochidion*- and *Breynia*-pollinating *Epicephala* moths (Kato et al. 2003; Kawakita and Kato 2004a).

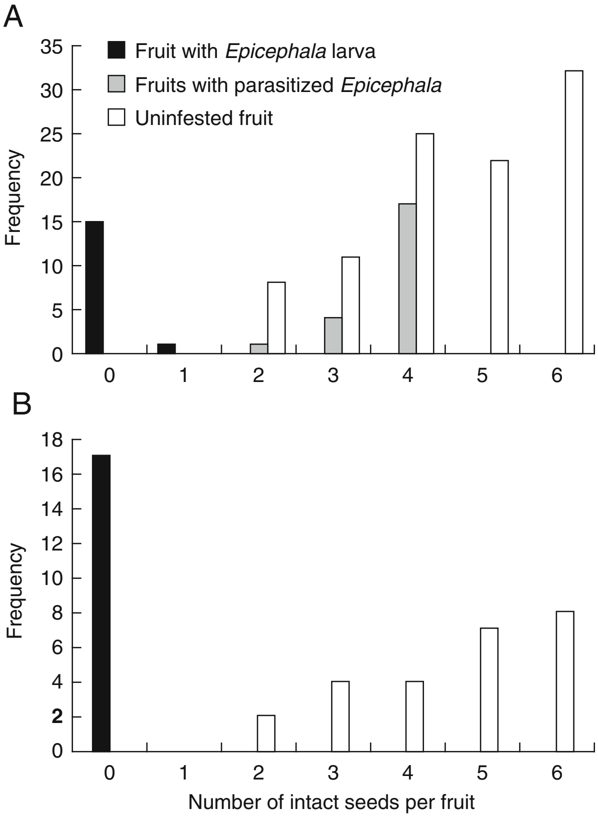
In both *Phyllanthus* species, pollen grains are deposited on the inner surface of the fused styles, which unlikely occurs through passive pollination. In *P. bourgeoisii*, moth eggs were laid into the narrow pit of the style apex (Fig. 3.8), whereas in *P. aeneus*, eggs were laid directly into the tissue of the calyx lobes (Fig. 3.8). What is very different from *Glochidion* and *Breynia* is that not all pollinated flowers contain eggs; in both species, only about 50–70% of the pollinated flowers have eggs. *Phyllanthus bourgeoisii* flowers used for oviposition invariably have one egg per flower, whereas *P. aeneus* flowers sometimes contain two and three eggs. Unpollinated flowers do not contain moth eggs.

Overall, infestation by *Epicephala* larvae occurs in less than half of the fruits produced in both species (Fig. 3.9). Each larva consumes all six ovules to complete larval growth and emerges from the fruit (Fig. 3.7g) to pupate on the host leaves or in litter. In *P. bourgeoisii*, *Epicephala* larvae are parasitized by a braconid wasp species. These parasitoids have a significant positive effect on seed set by preventing further seed consumption by the moth larvae (Fig. 3.9).

3.4.3 Seed Infestation by Pollinator Moth Larva

Seed destruction by *Epicephala* larvae occurs in most, but not all, species of *Gomphidium* in New Caledonia. When larvae enter the fruits, the seeds within these infested fruits are entirely destroyed, as observed in *P. bourgeoisii* and *P. aeneus*. Curiously, moth eggs are not found in some of the pollinated flowers; for example, moth eggs occurred in proportions ranging from 25% (*P. poumensis*)

Fig. 3.9 Frequency distribution of the number of intact seeds per fruit. (**a**) *Phyllanthus bourgeoisii*. (**b**) *P. aeneus*. *Epicephala* larvae were parasitized by braconid wasps. Fruits from which moths/wasps had already emerged were assigned to each category based on differences in the exit-hole structure. The number of intact seeds within uninfested fruits ranged from two to six due to the presence of unfertilized/aborted ovules and/or empty, sterile seeds (Modified from Kawakita and Kato 2004b)



to 95% (*P. buxoides*) of the pollinated flowers in one sampling. Eggs are laid on the external surface of the flowers, and thus oviposition by adult moths does not damage the ovary. The *Gomphidium–Epicephala* association is thus probably widespread among other members of this subgenus, but the strength of the interaction may not be as strong as in *Glochidion* and *Breynia*.

Examination of adult moths reared from various *Gomphidium* species (*P. bourgeoisii*, *P. aeneus*, *P. mangenotii*, *P. guillauminii*, *P. chamaecerasus*, *P. koniamboensis*, *P. pilifer*, *P. vulcani*, and *P. pancherianus*) suggested that high species specificity is also the rule in *Gomphidium*. In most cases, individual moths that developed from different hosts are easily distinguishable by wing pattern and relative size. The host specificity of the moths is further supported by nucleotide sequence variations of the COI gene (Fig. 3.10). Sequence differences between individuals collected from different hosts are 3–15%, whereas differences are $<1\%$ among individuals parasitizing the same host, despite regional co-occurrence of the host plants (*P. bourgeoisii* and *P. chamaecerasus* at Chutes de Ba, *P. aeneus* and *P. mangenotii* at Cap Bocage, and *P. tiebaghiensis* and *P. guillauminii* at Tiébaghi).

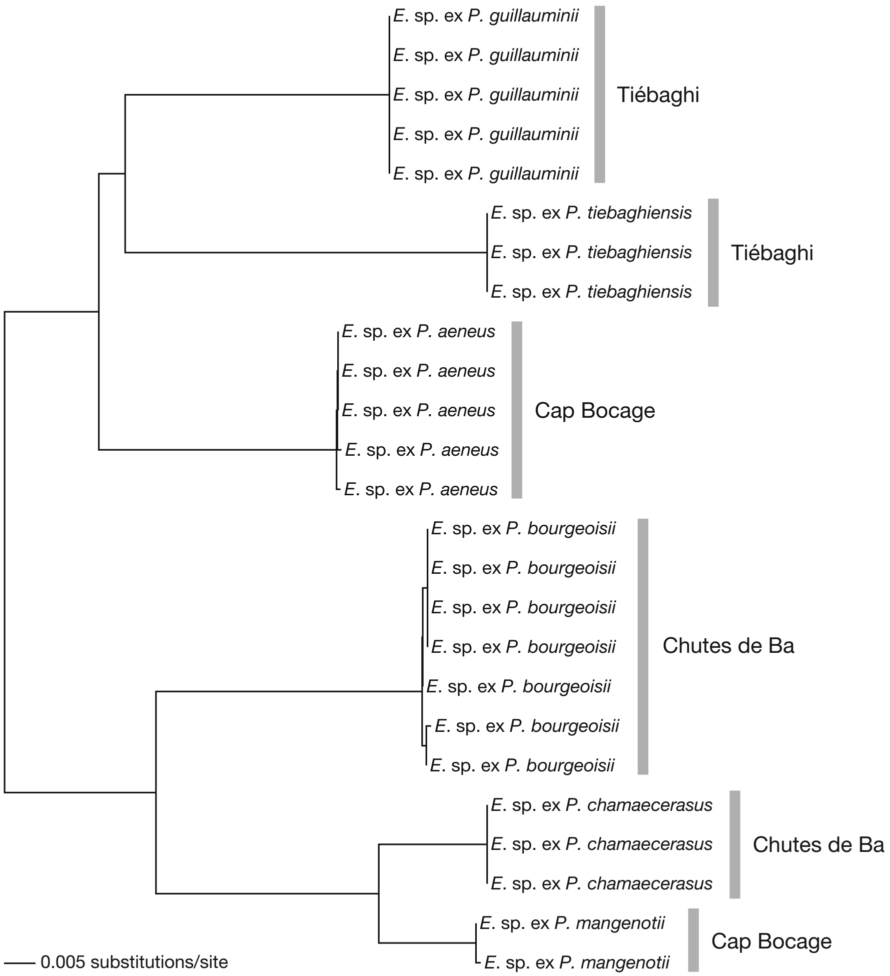


Fig. 3.10 Unrooted neighbor-joining phylogram depicting relative branch lengths within and among *Epicephala* moth individuals collected from different *Phyllanthus* host species. The tree is based on uncorrected pairwise distances within 1317 bp of the mitochondrial cytochrome oxidase subunit 1 gene (COI). All the moths used in the analysis are currently undescribed, and host affiliation of each individual moth is given in parentheses. Locality information is also provided to the right of shaded bars (Modified from Kawakita and Kato 2004b)

3.4.4 Mutualism or Parasitism?

The most critical factor underlying the *Gomphidium–Epicephala* interaction is that a fraction of the fruits is left untouched by the moths. This is most likely brought about by the absence of moth eggs in a fraction of pollinated flowers. One possible explanation for this pattern of egg distribution is that *Epicephala* eggs may be lost from some flowers, possibly by egg predation or strong desiccation. In some yucca–yucca moth interactions, high mortality of eggs and/or early instar larvae is an important process for limiting seed consumption by the moths (Addicott and Bao 1999; Csotonyi and Addicott 2001; Shapiro and Addicott 2003). However, in *P. aeneus*, moths oviposit directly into the tissue of the calyx lobes, thereby scarring the surface of the lobes. Such scars were not observed in flowers without moth eggs, which may allow exclusion of egg mortality as an explanation.

Another possibility is that the moths do not always oviposit in flowers that they pollinate. Such a behavior seems paradoxical, because the moths do not benefit from the pollinating behavior itself. However, this seemingly altruistic pollination behavior can be advantageous to the moth if the presence of uninfested fruits forces the braconid parasitoid to spend excessive time in detecting moth larvae, thus decreasing the probability of successful detection and parasitism. The last possibility is that there are as yet undiscovered copollinators in this system. The flowers of some *Gomphidium* species are reddish, unlike the characteristic greenish flowers of *Glochidion* and *Breynia* (images of flowers are shown in Chap. 4). Also, *Gomphidium* species in the section *Adenoglochidion*, which includes *P. aeneus*, possess female flowers with spread bifid styles, which are also in contrast to the reduced fused styles of *Glochidion* and *Breynia*. More detailed examination of moth pollination and of other potential visitors is clearly needed before these hypotheses can be evaluated robustly.

In some obligate pollination/seed-parasitic mutualisms, plants selectively abscise flowers that contain large numbers of eggs, thereby preventing excessive seed destruction (Pellmyr and Huth 1994; Richter and Weis 1995; Wilson and Addicott 1998; Addicott and Bao 1999; Goto et al. 2010). In light of this, it is paradoxical that *Gomphidium* trees do not abscise flowers containing moth eggs, despite the substantial cost imposed by the larvae. One explanation for the lack of selective abscission in *Gomphidium* is that the potential for such a mechanism is weak because the available resources do not limit seed set and thus need not be allocated to high-quality fruits. However, as hypothesized for some yuccas (Addicott and Bao 1999), *Gomphidium* flowers may not have proximate cues to predict whether their ovules are infested, because oviposition by *Epicephala* moths does not directly damage the ovary. Selective abscission may be more likely involved in the *Glochidion–Epicephala* mutualism, in which the ovipositor of the moth directly cuts through the ovary and/or style tissue, and the reproductive success of the plant strongly depends on the number of eggs laid per flower (Kato et al. 2003; Goto et al. 2010).

Given that *Gomphidium* plants do not possess a mechanism by which to prevent excessive exploitation by *Epicephala* moths, there is also no means by which the pollinators can retaliate against being overexploited by the plant. Once a plant acquires the ability to abscise flowers containing moth eggs selectively, it attains higher relative fitness, which would rapidly lead to pollinator extinction. It is important to note that such a pathway leading to the breakdown of the system is inherently avoided in *Glochidion* and *Breynia*, because the exclusive pollinators of the plants consistently infest the flowers that they pollinate.

Although the proximal process generating seed set in *Gomphidium* plants requires further study, there are major differences in feeding patterns between *Epicephala* moths associated with *Gomphidium* and *Glochidion*/*Breynia* fruits and different mechanisms may be responsible for the evolutionary stability of these specialized interactions.