# Chapter 5
# Diversity of Pollinator Moths

Atsushi Kawakita and Makoto Kato

**Keywords** Active Pollination • *Epicephala* • Gracillariidae • *Flueggea* • *Glochidion* • Ornixolinae • *Phyllanthus* • Phylogeny • Proboscis • Seed parasite

## 5.1 Diversity and Classification of Gracillariidae

Gracillariidae, the family to which *Epicephala* belongs, is a large group of miniature moths with roughly 100 recognized genera and 2000 recognized species (De Prins and De Prins 2016). They have a global distribution and are found almost everywhere there are plants, except for extremely harsh environments (e.g., the arctic). Gracillariidae is one of several lepidopteran families that consist almost entirely of leaf-mining species, although the leaf-mining habit itself is known to occur in about 30 moth families (Powell et al. 1999). In most gracillariid species, early-instar larvae have remarkably flat head capsules without chewing mandibles, and feed exclusively on cell sap within the nongreen, epidermal layer of the leaf (sap feeder; Fig. 5.1). Later-instar larvae then feed on the palisade layer and finally the spongy layer of the leaf with functional mandibles, and excrete granular frass (tissue feeder; Fig. 5.1). The larvae of the genus *Phyllocnistis* are exceptional in that they spend all their instars as sap feeders in the leaf epidermal layer. Gracillariid moths are thus unique among insects in that they undergo hypermetamorphosis, a process by which some larval instars become functionally and morphologically distinct from other instars.

Sap feeding is an apomorphy among Gracillariidae that clearly distinguishes them behaviorally from other leaf-mining Lepidoptera. The extent to which their evolutionary success has been facilitated by sap feeding is a fascinating question

---

A. Kawakita (✉)
Center for Ecological Research, Kyoto University, 2-509-3 Hirano, Otsu, Shiga 520-2113, Japan
e-mail: kawakita@ecology.kyoto-u.ac.jp

M. Kato
Graduate School of Human and Environmental Studies, Kyoto University, Yoshida-Nihonmatsu-cho, Sakyo, Kyoto 606-8501, Japan
e-mail: kato@zoo.zool.kyoto-u.ac.jp

M. Kato, A. Kawakita (eds.), *Obligate Pollination Mutualism*, Ecological Research Monographs, DOI 10.1007/978-4-431-56532-1_5

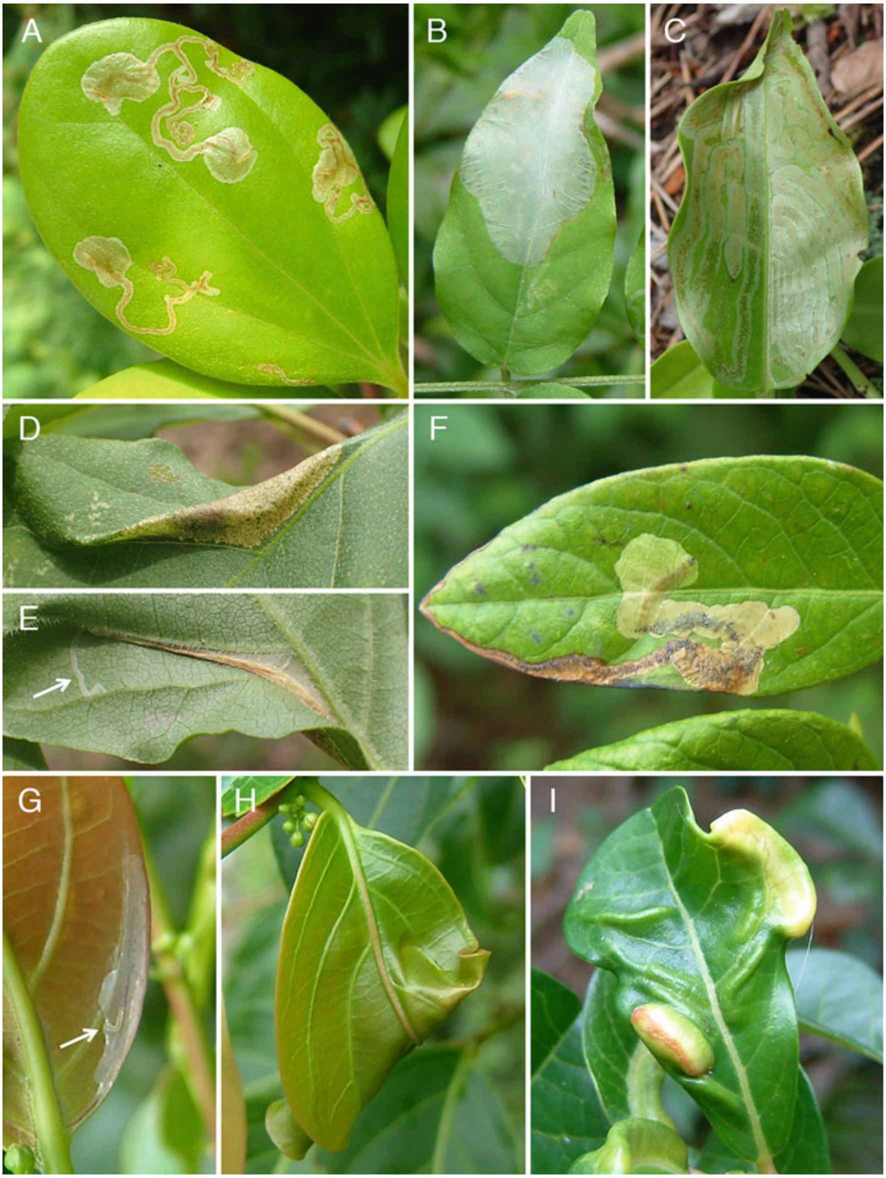

**Fig. 5.1** Various feeding habits of gracillariid moths. (**a**) Early-instar *Gibbovalva quadrifasciata* (Acrocercopinae) larvae mining *Cinnamomum doederleinii*. Sap-feeding larvae mine the epidermal layer of the leaf and produce linear mines, and later tissue-feeding larvae consume the leaf tissue within the expanded blotch mine. (**b**) Late-instar *Psydrocercops wisteriae* (Acrocercopinae) larva-mining a leaflet of *Wisteria floribunda*. (**c**) Mine of *Phyllocnistis* sp. (Phyllocnistinae) on *Ilex pedunculosa*. Pyllocnistinae larvae are sap-feeders throughout the larval stage and only use the epidermal layer of the host leaf. (**d, e**) Tentiform mine of *Phyllonorycter lyoniae* (Lithocolletinae) viewed from adaxial (**d**) and abaxial (**e**) sides of the leaf. *Phyllonorycter* larvae line the inner walls of mines with silk on the abaxial side, causing the mine eventually to become tentiform as the silk dries and wrinkles. Note that the initial instars are sap-feeding (*arrow*). (**f**) *Diphtheroptila*

that merits further study. Sap-feeding instars are secondarily lost in *Spinivalva* (leaf miner of *Passiflora*; Brito et al. 2013) and in several non-leaf-mining genera, including *Epicephala*.

The seed-feeding habit of *Epicephala* is unique within Gracillariidae. Nevertheless, deviations from the typical leaf-mining habit occur in a number of groups. *Caloptilia* species, which are common herbivores of *Glochidion* plants (Chap. 7), are sap-feeders as early instars, but later-instar larvae construct leaf rolls in which they live for the rest of the larval period. Larvae inside leaf rolls feed externally on the inner portions of folded leaves (Fig. 5.1). Galling has evolved in a number of gracillariid genera independently, for example, in *Caloptilia* (Kumata 1966; Fig. 5.1), *Borboryctis* (Kumata et al. 1988), and *Parectopa* (Wise 1962). Some species mine plant parts other than the leaf, such as stems or branches (*Marmara*, *Dendrorycter*, *Spulerina*) or fruit peel (*Marmara*, *Spulerina*). Others are plant-borers that use the seed (*Epicephala*, *Conopomorpha*), bud (*Stomphastis*, *Conopobathra*, *Chileoptilia*; Vargas and Landry 2005), or stem gall induced by rust fungi (*Polysoma*; Bashford 2002). Presumably, many more feeding types await discovery. Although the ultimate factor facilitating transitions from leaf mining to alternative feeding habits remains unknown, escape from parasitoid attack is probably a major reason for its evolution, as the structures they construct (leaf roll), induce (gall), or utilize (seed, bud, or fungal gall) are often protective against oviposition by parasitoids.

Gracillariidae species generally have narrow diets, and each specializes upon one or a few species in a single plant genus or, less commonly, in multiple related genera. Exceptions include *Acrocercops transecta*, which feeds on Juglandaceae and Ericaceae (Ohshima 2008); *Calybites phasianipennella*, which uses Polygonaceae and Myrsinaceae (Kumata 1982); and several stem- or fruit-feeding species with exceptionally wide host ranges spanning multiple families (*Marmara*, *Conopomorpha*). Hosts of Gracillariidae are found in 93 diverse angiosperm families, although only three gracillariid species are known to feed on monocots: *Acrocercops maranthaceae* (Maranthaceae; Busck 1934), *Marmara smilacisella* (Smilacaceae; Braun 1909), and *Gibbovalva tricuneatella* (Typhaceae; Sugisima et al. 2005). *Parectopa leucocyma*, the only known gymnosperm leaf-miner, feeds on *Agathis australis* in New Zealand (Wise 1962), and several undescribed gracillariid species are found on *Podocarpus* and *Gnetum* in Japan and Southeast Asia, respectively. No gracillariids are known to attack ferns or bryophytes. For reasons presently unknown, Gracillariidae predominantly use woody hosts, a pattern also observed in certain other groups of leaf-miners (e.g., Nepticulidae,

◄

**Fig. 5.1** (continued) scriptulata (Ornixolinae) larva mining *Glochidion acuminatum*. Many species of Ornixolinae expand the mine as they feed on the leaf tissue. (**g**, **h**) *Caloptilia ryukyuensis* (Gracillariinae) on *Glochidion zeylanicum*. Most *Caloptilia* species are sap-feeders as early instars (**g**), whereas late-instar larvae roll the leaf apex and feed on the leaf externally within the rolled leaf (**h**). (**i**) Galls of *Caloptilia cecidophora* (Gracillariinae) on *Glochidion obovatum*. This species is exceptional among Gracillariidae for its gall-inducing habit

**Table 5.1** Subfamilies of Gracillariidae. Number of genera and approximate number of described species are given for each subfamily.

| Subfamily<br>Genus group | Genera | Species | Representative genera |
|---|---|---|---|
| Acrocercopinae | 28 | 380 | *Acrocercops*, *Dialectica*, *Spulerina*, *Eucosmophora* |
| Gracillariinae | 25 | 700 | *Caloptilia*, *Gracillaria*, *Aristaea*, *Calybites* |
| Lithocolletinae | 11 | 550 | *Phyllonorycter, Cameraria* |
| Marmarinae | 2 | 30 | *Marmara, Dendrorycter* |
| Oecophyllembiinae | 6 | 20 | *Metriochroa, Eumetriochroa* |
| Ornixolinae | 28 | 300 | *Epicephala*, *Conopomorpha*, *Diphtheroptila*, *Philodoria* |
| Parornichinae | 4 | 90 | *Parornix*, *Callisto* |
| Phyllocnistinae | 1 | 100 | *Phyllocnistis* |

Information is based on Kawahara et al. (2017)

Heliozelidae). The scarcity of monocot hosts may thus reflect the rarity of woody monocots rather than a true lack of preference.

Gracillariidae species have traditionally been classified into four subfamilies: Gracillariinae, Lithocolletinae, Phyllocnistinae, and Oecophyllembiinae (Kumata et al. 1988a,b; Kumata 1998; note that the circumscription of subfamilies varies among authors). Among these families, Gracillariinae contains by far the largest number of species (>1380), and it has been further divided by Kumata et al. (1988a, b) into four genus groups: *Parornix*-, *Acrocercops*-, *Gracillaria*-, and *Parectopa*-groups. Kawahara et al. (2017) recently provided a robust phylogenetic framework for the family based on the sequences of 22 genes, and proposed a new classification consisting of eight subfamilies: Acrocercopinae, Gracillariinae, Lithocolletinae, Marmarinae, Oecophyllembiinae, Ornixolinae, Parornichinae, and Phyllocnistinae (Table 5.1).

*Epicephala* belongs to the Ornixolinae subfamily (corresponding to the *Parectopa* group of Kumata et al. 1988a,b), which, unlike other subfamilies that have high species diversity in temperate zones, is concentrated in the tropics. Therefore, the numbers of genera and species are likely to increase dramatically with further taxonomic studies. Ornixolinae is also unique among Gracillariidae in that it contains a disproportionately high number of non-leaf-mining species. In fact, the plant borers listed above (*Epicephala*, *Conopomorpha*, *Stomphastis*, *Conopobathra*, *Chileoptilia*, and *Polysoma*) all belong to Ornixolinae. Thus, there may have been an evolutionary precursor within the Ornixolinae lineage that enabled repeated transitions from leaf-mining to boring feeding habits, giving rise to *Epicephala*.

## 5.2 Phylogeny of *Epicephala*

The genus *Epicephala* was described by Meyrick (1881) based on specimens collected in Sydney, Australia. Meyrick, the founder of modern Microlepidoptera systematics, made the greatest contribution to *Epicephala* taxonomy, eventually describing more than 20 *Epicephala* species from various parts of the Old World. However, several of the species described by Meyrick (e.g., *Stomphastis chalybacma*) were transferred to other genera after detailed examination of genital morphology by later authors. A few more species currently placed in *Epicephala* will likely be subject to such taxonomic rearrangements. Concurrently with Meyrick, Turner greatly advanced the knowledge of *Epicephala* fauna in Australia, naming 11 currently accepted species. He was the first to note that *Epicephala* are seed-feeders; he observed *Epicephala* adults emerging from the capsules of *Glochidion ferdinandii* in Brisbane, Australia (Turner 1913). He named the moth *E. frugicola*, but later synonymized the species with *E. colymbetella*, the type species of the genus. Later, Vári (1961) described six *Epicephala* species from South Africa and provided the first detailed illustrations of the genitalia for *Epicephala* (as well as for many other genera of Gracillariidae). The study of *Epicephala* slowed down during the late twentieth century, during which time only three species were described (from Russia, Nigeria, and the Marquesas Islands).

The taxonomy of *Epicephala* has attracted renewed interest since the discovery of obligate pollination mutualism (Kato et al. 2003). Li and colleagues, working on *Epicephala* found in China, described 20 species associated with *Glochidion*, *Breynia*, and *Phyllanthus*, and provided detailed descriptions of adult behavior and morphology for some of them. Kawakita and Kato (2016) reviewed the Japanese fauna of *Epicephala* and described seven species. As of April 2016, the genus contains 64 species (Table 5.2; Fig. 5.2). However, ecological, molecular, and biogeographical data suggest that the genus contains several hundred species (Kawakita 2010). For example, some clades of *Epicephala* are confined to Madagascar or New Caledonia, where none of the described *Epicephala* species occurs (Kawakita and Kato 2009). These regions are known for hotspots of *Phyllanthus* diversity, and thus potentially have large numbers of undescribed *Epicephala* species. There is also a high level of *Phyllanthus* diversity in the New World, where *Epicephala* has not been previously recorded; furthermore, as detailed in the following section, *Epicephala* is also prevalent in the Neotropics. Accelerating the taxonomy of *Epicephala* at a global scale is therefore critical for facilitating the ecological and evolutionary study of this model group.

Figure 5.3 shows the most recent analysis of the phylogenetic relationships among *Epicephala* (Kawakita and Kato 2016). Seven clades can be recognized, each of which is generally associated with a particular clade of Phyllantheae. An exception is Clade 2, which consists of species associated with herbaceous *Phyllanthus* belonging to various *Phyllanthus* subgenera. *Conopomorpha flueggella*, which is a nonpollinating seed-feeder of *Flueggea suffruticosa*, is

**Table 5.2** The 66 *Epicephala* species named as of April 2016, with known host associations

| Species | Distribution | Known Host | References | Notes |
|---|---|---|---|---|
| *E. acinacephora* | Australia | – | Turner (1947) | |
| *E. acrobaphes* | Australia | – | Turner (1900) | |
| *E. acrocarpa* | Samoa Islands | – | Meyrick (1927b) | |
| *E. albifrons* | India, Moluccas, Sri Lanka, Thailand | *Phyllanthus* sp. | Stainton (1859) and Robinson et al. (1994) | The larvae feed on the seeds |
| *E. albistriatella* | Australia | – | Turner (1894) | |
| *E. ancistropis* | Java | *Callicarpa candicans* | Meyrick (1935b) and Robinson et al. (2001) | Doubtful placement in *Epicephala* |
| *E. ancylopa* | India, China | *Glochidion* cf. *khasicum* | Meyrick (1918b) and Li and Zhang (2016) | The larvae feed on the seeds |
| *E. angustisaccula* | China | *Glochidion wrightii*, *G. sphaerogynum* | Li et al. (2015a) | The larvae feed on the seeds |
| *E. anthophilia* | Japan | *Glochidion acuminatum* | Kawakita and Kato (2016) | The larvae feed on the seeds; active pollinator |
| *E. assamica* | China | *Glochidion assamicum* | Li and Zhang (2016) | The larvae feed on the seeds |
| *E. australis* | Australia | – | Turner (1896) | |
| *E. bathrobaphes* | Australia | – | Turner (1947) | |
| *E. bipollenella* | China, Japan | *Glochidion zeylanicum* | Zhang et al. (2012a) and Kawakita and Kato (2016) | The larvae feed on the seeds; active pollinator |
| *E. bromias* | India | – | Meyrick (1910a) | |
| *E. calasiris* | India | – | Meyrick (1908a) | |
| *E. camurella* | China | *Glochidion wrightii*, *G. sphaerogynum* | Li et al. (2015a) | The larvae feed on the seeds |
| *E. colymbetella* | Australia | *Glochidion ferdinandii* | Meyrick (1881) and Turner (1913, 1940) | Type species of the genus; the larvae feed on the seeds; the adult insect emerges inside the capsule where it remains until liberated by the dehiscence. |

(continued)

**Table 5.2** (continued)

| Species | Distribution | Known Host | References | Notes |
|---|---|---|---|---|
| *E. corruptrix* | Japan | *Glochidion rubrum*,<br>*G. obovatum* | Kawakita and Kato (2016) | The larvae induces galls on the female flower; active pollinator |
| *E. daltonii* | China | *Glochidion daltonii* | Li and Zhang (2016) | The larvae feed on the seeds |
| *E. domina* | China | *Glochidion sphaerogynum* | Li et al. (2015a) | The larvae feed on the seeds |
| *E. duoplantaria* | China | *Glochidion lutescens*,<br>*G. daltonii* | Li and Zhang (2016) | The larvae feed on the seeds |
| *E. epimicta* | Australia | – | Turner (1913) | |
| *E. eriocarpa* | China | *Glochidion eriocarpum* | Zhang et al. (2012a) | The larvae feed on the seeds |
| *E. euchalina* | Myanmar | – | Meyrick (1922) | |
| *E. eugonia* | Australia | – | Turner (1913) | |
| *E. exetastis* | Sri Lanka | – | Meyrick (1908a) | |
| *E. flagellata* | Sri Lanka | – | Meyrick (1908a) | |
| *E. frenata* | Sri Lanka, Java | *Glochidion* sp. | Meyrick (1908a) and Robinson et al. (2001) | In fruits |
| *E. haplodoxa* | South Africa | – | Vári (1961) | |
| *E. homostola* | South Africa, Namibia | – | Vári (1961) | |
| *E. impolliniferens* | China | *Glochidion sphaerogynum* | Li et al. (2015a) | The larvae feed on the seeds |
| *E. jansei* | Zimbabwe | – | Vári (1961) | |
| *E. jianfenglingina* | China | *Glochidion* cf. *rubrum* | Li and Zhang (2016) | The larvae feed on the seeds |
| *E. laeviclada* | China | *Phyllanthus microcarpus* | Li and Yang (2015) | The larvae feed on the seeds |
| *E. lanceolaria* | China | *Glochidion lanceolarium* | Zhang et al. (2012a) | The larvae feed on the seeds |
| *E. lanceolatella* | Japan | *Glochidion lanceolatum* | Kawakita and Kato (2016) | The larvae feed on the seeds; active pollinator |
| *E. lativalvaris* | China | *Breynia fruticosa*,<br>*B. rostrata* | Zhang et al. (2012a, b) | The larvae feed on the seeds; active pollinator |
| *E. lomatographa* | Australia | – | Turner (1913) | |
| *E. microcarpa* | China | *Phyllanthus microcarpus* | Li and Yang (2015) | The larvae feed on the seeds |

(continued)

**Table 5.2** (continued)

| Species | Distribution | Known Host | References | Notes |
|---|---|---|---|---|
| *E. mirivalvata* | | *Breynia fruticosa*, *B. rostrata* | Zhang et al. (2012a, b) | The larvae feed on the seeds; active pollinator |
| *E. nephelodes* | Australia | – | Turner (1913) | |
| *E. nudilingua* | Japan | *Phyllanthus ussuriensis* | Kawakita and Kato (2016) | The larvae feed on the seeds |
| *E. orientale* | India, Malaysia | *Bauhinia* spp. | Stainton (1856a) and Fletcher (1921) | Clearly belongs to *Conopobathra*; the larvae feed on the flower buds |
| *E. obovatella* | Japan | *Glochidion obovatum*, *G. rubrum* | Kawakita and Kato (2016) | The larvae feed on the seeds; active pollinator |
| *E. parasitica* | Japan | *Phyllanthus lepidocarpus* | Kawakita and Kato (2016) | The larvae feed on the seeds; pollination behavior absent |
| *E. pelopepla* | South Africa | – | Vári (1961) | |
| *E. periplecta* | New Guinea | – | Diakonoff (1955) | |
| *E. perplexa* | Japan | *Glochidion lanceolatum* | Kawakita and Kato (2016) | The larvae feed on the seeds; active pollinator |
| *E. pyrrhogastra* | South Africa | – | Meyrick (1908b) | |
| *E. relictella* | Russia, China, Korea | *Flueggea suffruticosa* | Kuznetzov (1979), Kawahara et al. (2010) and Hu et al. (2011) | The larvae feed on the seeds |
| *E. scythropis* | Myanmar | *Phyllanthus* sp. | Meyrick (1930) and Fletcher (1933) | The adults were bred from galls on *Phyllanthus* |
| *E. sphenitis* | India | *Breynia rhamnoides*; *B. vitis-idaea* | Meyrick (1931d) and Robinson et al. (2001) | The larvae feed on the seeds |
| *E. spinula* | Marquesas Islands | – | Clarke (1986) | |
| *E. spumosa* | Australia | – | Turner (1947) | |
| *E. squamella* | Vietnam | – | Kuznetzov and Baryshnikova (2001) | Doubtful placement in *Epicephala* |
| *E. stauropa* | India | – | Meyrick (1908a) | |

(continued)

**Table 5.2** (continued)

| Species | Distribution | Known Host | References | Notes |
|---|---|---|---|---|
| *E. strepsiploca* | India | – | Meyrick (1918b) | |
| *E. subtilis* | India | – | Meyrick (1922) | |
| *E. suttoni* | Nigeria | – | Bland (1980) | Treated as a species of *Ectropina* in De Prins and De Prins (2005) but clearly an *Epicephala* |
| *E. tephrostola* | South Africa | – | Vári (1961) | |
| *E. tertiaria* | China | *Phyllanthus microcarpus* | Li and Yang (2015) | The larvae feed on the seeds |
| *E. trigonophora* | Australia | – | Turner (1900) | |
| *E. venenata* | Taiwan | – | Meyrick (1935b) | |
| *E. vermiformis* | Java | *Cajanus cajan* | Meyrick (1936) | Doubtful placement in *Epicephala* |
| *E. vitisidaea* | China, Japan | – | Zhang et al. (2012a) and Kawakita and Kato (2016) | The larvae feed on the seeds; active pollinator |
| *E. zalosticha* | Australia | – | Turner (1940) | |

depicted as a sister to *Epicephala* in Fig. 5.3. However, the phylogenetic position of *C. flueggella* is contentious because the species is sometimes placed as a sister to Clade 7 (Fig. 5.3). *Conopomorpha flueggella* clearly lacks pollination behavior, and lays eggs in buds or young fruits as well as flowers (Fig. 5.4). It also lacks an ovipositor, placing its eggs on the surface of the ovule (Fig. 5.4). Interestingly, ovipositing females sometimes take nectar with their proboscises prior to oviposition (Fig. 5.4) in a manner similar to *Epicephala*'s use of its proboscis to pollinate. The nectaring behavior of *C. flueggella* may have been the evolutionary precursor for active pollination; however, this question requires a robust understanding of the phylogenetic placement of *C. flueggella* with respect to *Epicephala*. At present, it is equally probable that the behavior of *C. flueggella* represents a secondary loss of pollination behavior. A hatched *C. flueggella* larva usually consumes all six seeds contained in each fruit of *Flueggea suffruticosa* (Fig. 5.4).

*Conopomorpha flueggella* was described by Hu et al. (2011), who placed it in the genus *Conopomorpha* based mainly on wing morphology. However, the true *Conopomorpha*, which include species that attack seeds of tropical fruit trees such as lychee and longan (Sapindaceae) and cacao (Malvaceae), are distantly related to *Conopomorpha flueggella* or *Epicephala* within Ornixolinae. Therefore, *C. flueggella* can likely be more accurately placed in a separate genus.

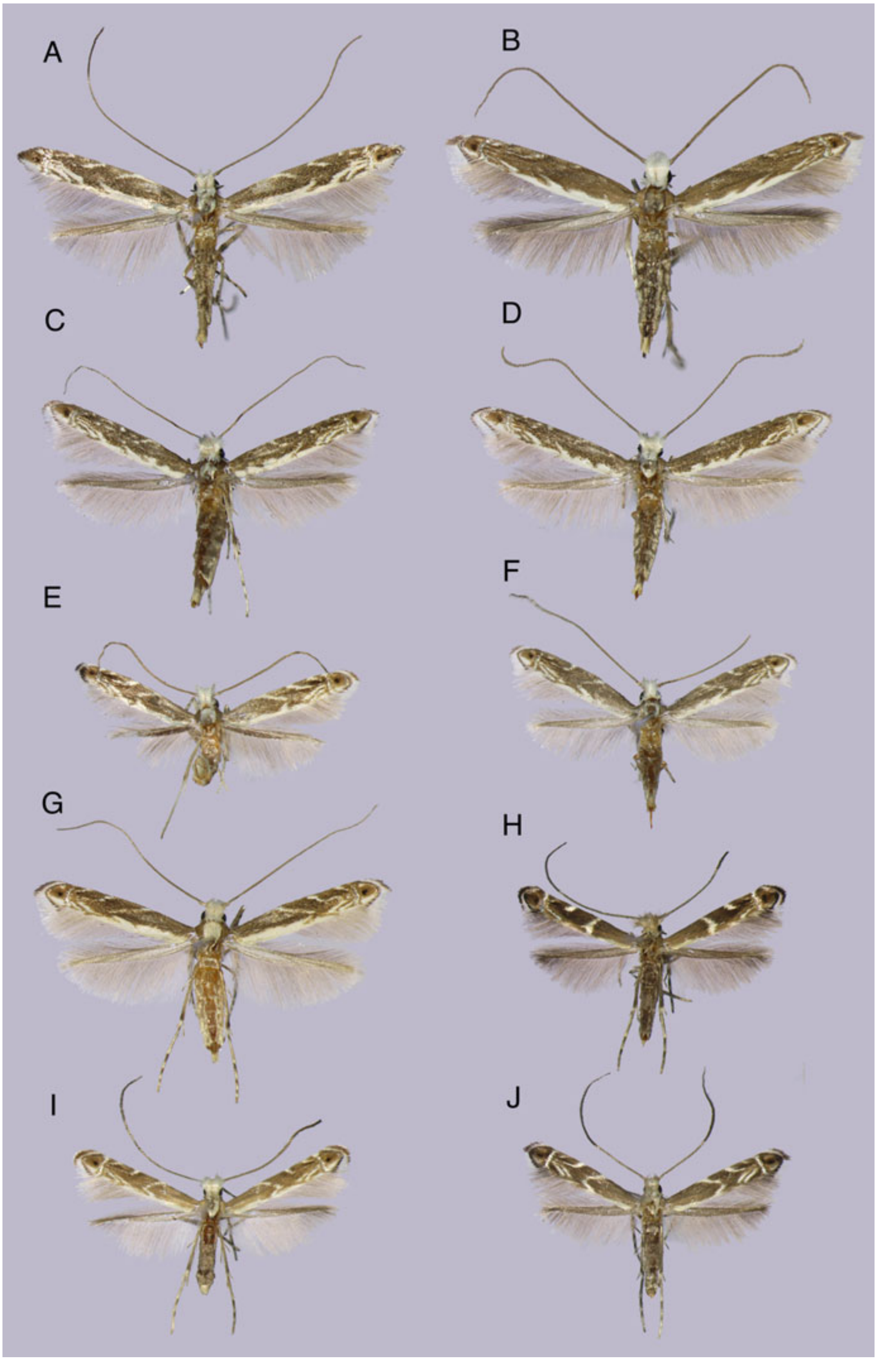

**Fig. 5.2** Representative specimens of the nine *Epicephala* species in Japan. Variation in wing pattern among species is low, and is of minor importance in morphological identification. Variation in size largely reflects host seed size. The wing pattern of *E. parasitica* is sexually

The basal-most *Epicephala* are the clade of moths associated with the *Phyllanthus* section *Gomphidium* in New Caledonia (Fig. 5.3). They clearly exhibit active pollination behavior (Chap. 3), but lack the sensilla on the proboscis and ovipositor that characterize the derived members of *Epicephala*. As such, they retain the morphology of the earliest *Epicephala* to acquire active pollination behavior. They lay eggs on the surface of the pistils (Fig. 5.5), and a single hatched larva consumes all the seeds contained in each fruit. However, moth mortality is very high, probably owing to desiccation during the egg stage. This high mortality is necessary for a fraction of the seeds to remain intact despite destructive seed-feeding by the larvae (Chap. 3). Because there is a large morphological disparity between moths of this clade and those of the derived *Epicephala*, especially with respect to the female genitalia, the former should probably be placed in a separate genus. However, determining whether it is congeneric with *C. flueggella* requires a closer examination of morphology and a better resolution of the basal phylogenetic relationships.

The remaining moths can safely be placed in *Epicephala*, and share the female ovipositor as a morphological synapomorphy (Clades 1–6; Figs. 5.3, 5.5). Ovipositors are not known in any other Gracillariidae genus; thus, aside from pollination behavior, they represent the trait that best characterizes *Epicephala*. The development of the ovipositor is most likely an adaptation for laying eggs internally in floral tissue, thereby avoiding egg desiccation (Fig. 5.5).

Interestingly, there is considerable variation in egg placement behavior among *Epicephala* species. Most *Epicephala* species associated with *Glochidion* lay eggs either through the apical pit of the stylar column into the stylar tissue or laterally through the ovary wall on the surface of the ovule (Fig. 5.6). This difference in oviposition mode is reflected in morphology; the ovipositors of laterally ovipositing species are distinctly more angular than those of apically ovipositing species (Kawakita and Kato 2016; Fig. 5.6).

Certain species associated with *Phyllanthus* and *Glochidion* lay eggs in the pedicels of female flowers (Chap. 10). In such species, the hatched larvae initially bore through the pedicel to enter the ovary. *Epicephala vitisidaea* and *E. mirivalvata* lay eggs in the narrow space between the sepals and ovary of their host *Breynia* plants, having thus reverted to external oviposition (Kawakita and Kato 2004; Zhang et al. 2012a), although both species have retained functional

**Fig. 5.2** (continued) dimorphic, so specimens of both sexes are shown for this species. (**a**) *E. anthophilia* (Amami Island, Kagoshima, ♀, host: *Glochidion acuminatum*). (**b**) *E. bipollenella* (Henoko, Okinawa, ♀, host: *G. zeylanicum*). (**c**) *E. lanceolatella* (Cape Hedo, Okinawa, ♀, host: *G. lanceolatum*). (**d**) *E. perplexa* (Cape Hedo, Okinawa, ♀, host: *G. lanceolatum*). (**e**) *E. obovatella* (Tomogashima, Wakayama, ♂, host: *G. obovatum* and *G. rubrum*). (**f**) *E. corruptrix* (Takae, Okinawa, ♀, host: *G. obovatum* and *G. rubrum*). (**g**) *E. vitisidaea* (Yona, Okinawa, ♀, host: *Breynia vitis-idaea*). (**h**) *E. parasitica* (Yonaguni Island, Okinawa, ♀, host: *Phyllanthus lepidocarpus*). (**i**) *E. parasitica* (Hateruma Island, Okinawa, ♂, host: *P. lepidocarpus*). (**j**) *E. nudilingua* (Watarase-yusuichi, Tochigi, ♀, host: *P. ussuriensis*). Scale bar: 5 mm

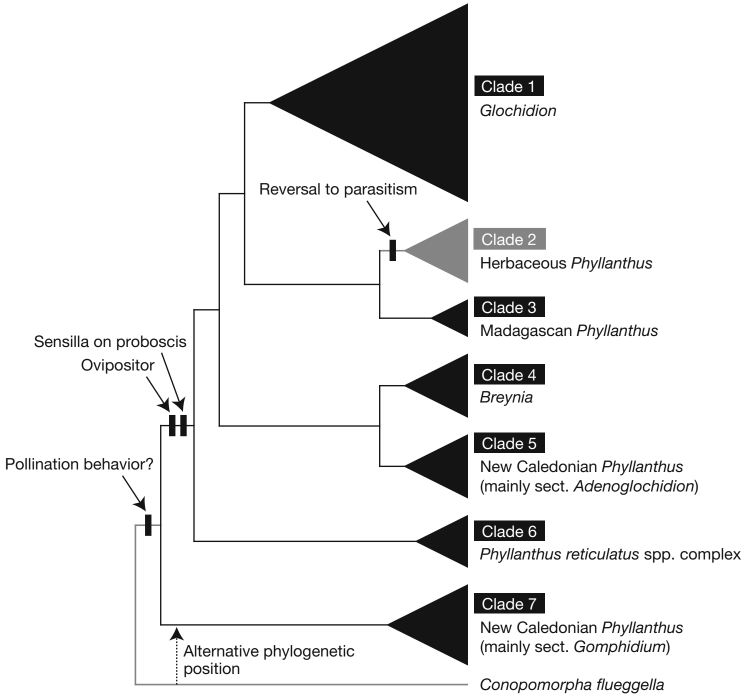

**Fig. 5.3** Phylogeny of *Epicephala*. The tree is based on a molecular phylogenetic analysis by Kawakita and Kato (2009). Host plant associations are provided below clade numbers. The phylogenetic position of *Conopomorpha flueggella* is inconsistent among analyses; an alternative placement as a sister to Clade 7 is also indicated. Clade triangle size is proportional to the estimated number of species in each clade. Major evolutionary events are listed on branches. Lineages indicated in *grey* do not possess the pollination behavior

ovipositors. Because Phyllantheae plants are known to abort selectively those flowers with heavy egg loads and abortion is likely based on the extent of mechanical damage to flowers (Chap. 9), external oviposition in these species may have evolved to circumvent the abortion response in their host plants.

However, the adaptive significance of other oviposition modes is less clear. In cases where two or more *Epicephala* species co-occur on the same Phyllantheae host, the different species exhibit different oviposition behaviors (Kawakita et al. 2015; Kawakita and Kato 2016). This may indicate that different oviposition strategies are necessary for stable coexistence on shared hosts.

Another distinguishing characteristic of the core *Epicephala* clade is the numerous sensilla on the proboscises of females (Fig. 5.7). Sensilla are absent in males, and a sensilla-bearing proboscis is not known in any other genera of Gracillariidae. Thus, we can infer that the function of the sensilla is linked to active pollination. Because most pollen is held on the basal area of the proboscis of pollen-carrying females where the density of sensilla is highest, females are able to carry more pollen than would be possible in the absence of such a structure (Fig. 5.7). Support

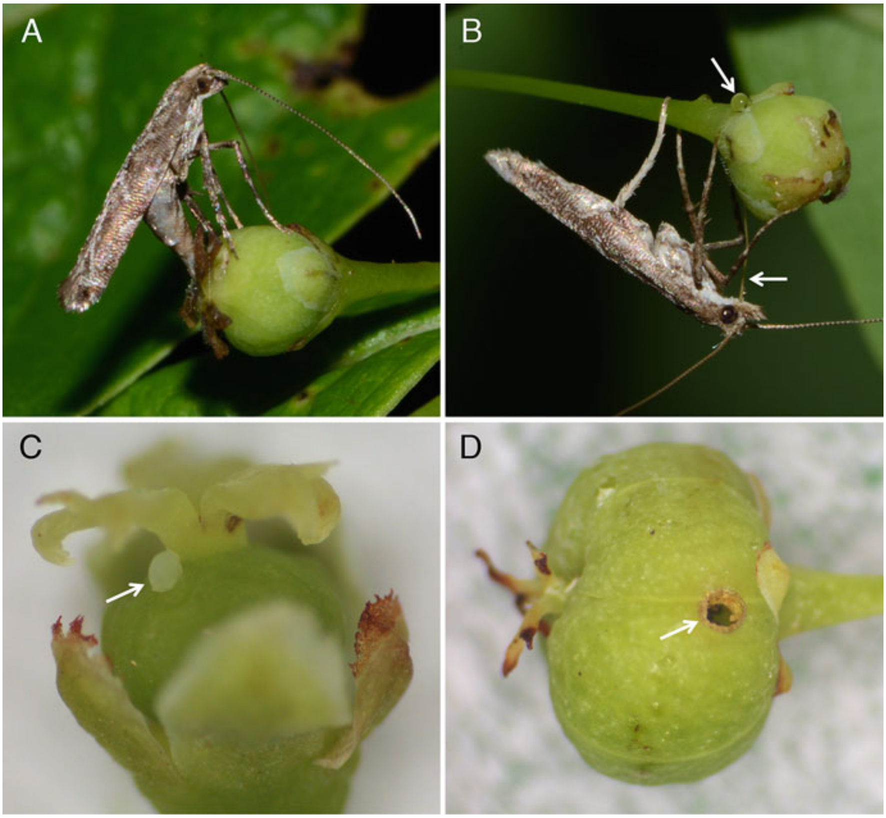

**Fig. 5.4** A nonpollinating, seed-parasitic moth, *Conopomorpha flueggella*. (**a**) Female moth depositing an egg underneath the horizontally spread styles. (**b**) Female moth taking nectar from nectary at the base of the ovary. Note that the moth proboscis lacks sensilla and does not bear pollen (*arrow*). A droplet of nectar collected at the base of the ovary and protruded through the tepals can be seen (*arrow*). (**c**) Moth egg laid externally on the surface of the ovary. (**d**) Mature fruit with exit hole excavated by moth larva. Seeds are usually entirely destroyed in such fruits

for the interpretation that the sensilla assist in pollination comes from the absence of sensilla in species that have secondarily lost the pollination behavior. For example, of the six major lineages of the core *Epicephala* clade, the herbaceous *Phyllanthus* clade consists of species that lay eggs in young fruits of herbaceous *Phyllanthus* and thus do not pollinate flowers. Accordingly, the sensilla on their proboscises are lost entirely or are rudimentary (Fig. 5.7). The *Phyllanthus reticulatus* clade represents another *Epicephala* lineage that lost the sensilla. Of the six species associated with plants of the *Phyllanthus reticulatus* species complex in Taiwan, three induce gall formation on female flowers, although two of them retain pollination behavior. Sensilla are completely lost in the three gall-inducing species, probably because selection to transfer pollen is relaxed or absent owing to their ability to induce galls (Chap. 11).

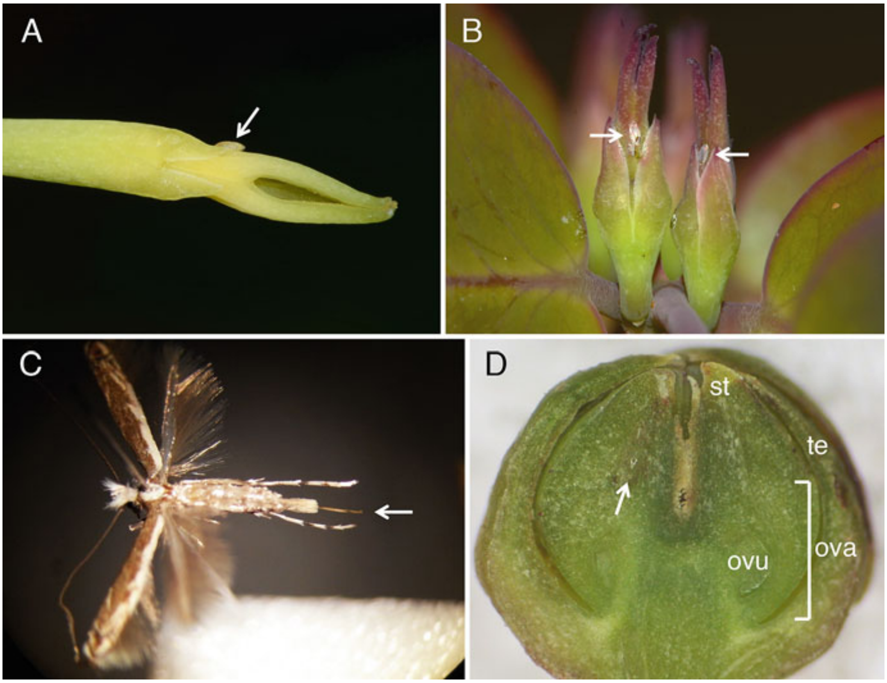

**Fig. 5.5** Difference in the placement of eggs by *Epicephala* moths with and without ovipositors. Roughly half of the species associated with New Caledonian *Phyllanthus* (Clade 7 in Fig. 5.3) do not possess ovipositors and oviposit externally (**a, b**) whereas species of derived clades have well-developed ovipositors and lay eggs internally in floral tissue (**c, d**). (**a**) Female flower of *Phyllanthus kouaouaensis* with an *Epicephala* moth egg laid externally on style surface (*arrow*). (**b**) Female flowers of *P*. cf. *koniamboensis* with *Epicephala* eggs. (**c**) Female *E. eriocarpa* with extended ovipositor (*arrow*). (**d**) Vertical section of *Glochidion zeylanicum* female flower containing an *Epicephala* moth egg (*arrow*). *Ova*, ovary; *ovu*, ovule; *st*, style; *te*, tepal

As mentioned in the previous section, gall-inducers have arisen repeatedly in the course of gracillariid evolution, but the evolution of galling may be particularly common in *Epicephala*. In the Ryukyu Archipelago of southern Japan, *E. corruptrix* is associated with two *Glochidion* species (*G. obovatum* and *G. rubrum*). On both hosts, *E. corruptrix* exhibits pollination behavior, but the resulting fruits do not develop normally. Instead, the locule in which the larva develops grows irregularly and eventually becomes a gall (Fig. 5.8). Populations of *G. obovatum* and *G. rubrum* associated with *E. corruptrix* suffer very low seed production. This contrasts sharply with populations elsewhere in their ranges, where the plants are pollinated by non-gall-inducing *E. obovatella*, and produce large numbers of normal fruits and seeds. *Epicephala corruptrix* is distantly related to the gall-inducers of the *Phyllanthus reticulatus* species complex, so the galling habit has independently evolved at least twice in *Epicephala*. Similar gall-like development is found in *P. humbertii* in Madagascar and *P. cuscutiflorus* in Australia. It is therefore interesting to consider how many other lineages of

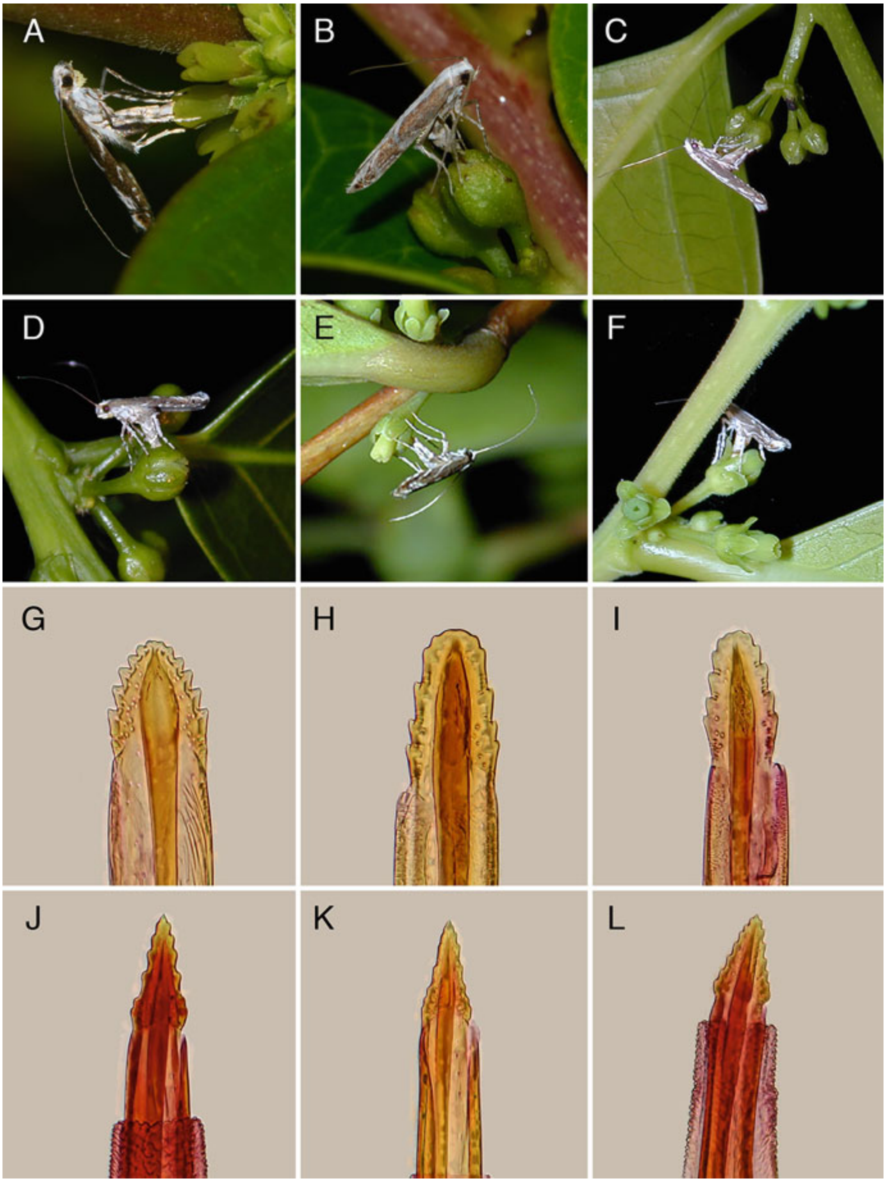

**Fig. 5.6** Oviposition behavior (**a–f**) and ovipositor morphology (**g–l**) of six *Epicephala* species associated with *Glochidion* in Japan. (**a**) *E. anthophilia* ovipositing through stylar pit of *G. acuminatum* flower. (**b**) *E. bipollenella* ovipositing through stylar pit of *G. zeylanicum* flower. (**c**) *E. lanceolatella* ovipositing through stylar pit of *G. lanceolatum* flower. (**d**) *E. perplexa* ovipositing through lateral ovary wall of *G. lanceolatum* flower. The ovipositor of this species penetrates both the tepal and the ovary wall. (**e**) *E. obovatella* ovipositing through lateral ovary wall of *G. obovatum* flower. The ovipositor of this species is inserted directly into the ovary and does not penetrate the tepal. (**f**) *E. corruptrix* ovipositing through ovary wall of *G. rubrum* flower. Similar to that of *E. obovatella*, the ovipositor of this species also does not penetrate the tepal. (**g**) *E. anthophilia*. (**h**) *E. bipollenella*. (**i**) *E. lanceolatella*. (**j**) *E. perplexa*. (**k**) *E. obovatella*. (**l**) *E. corruptrix*. Note that the ovipositors of species that oviposit through lateral ovary walls are distinctly angular

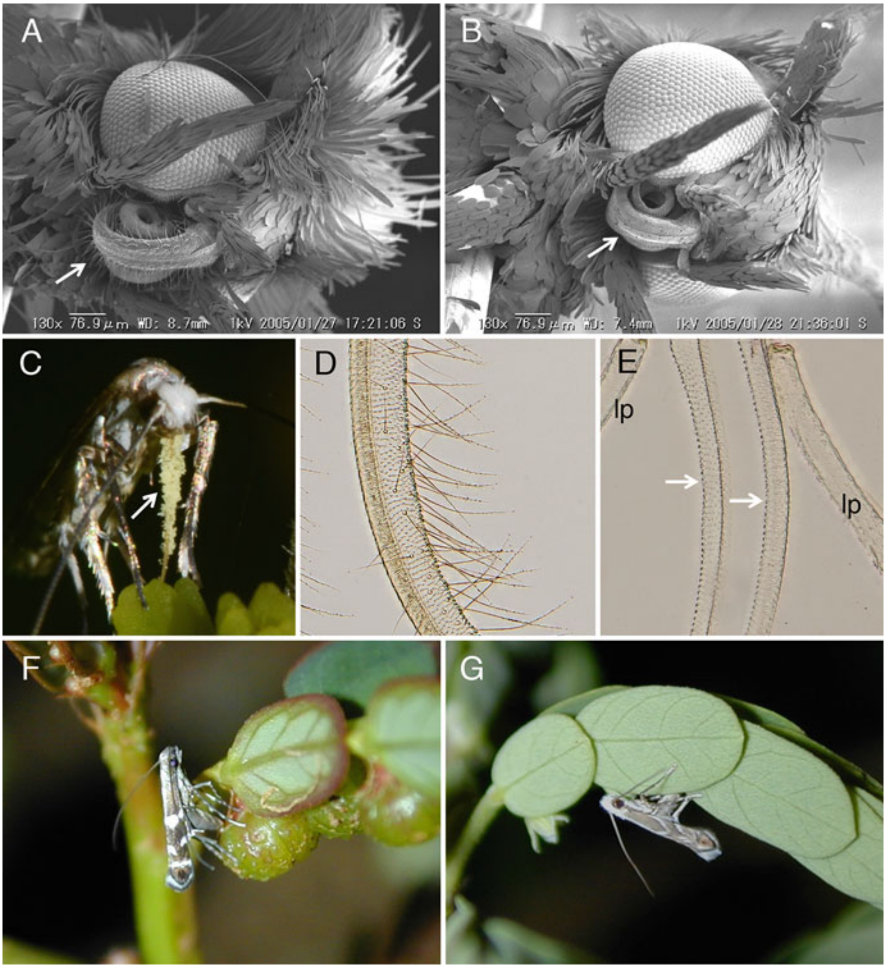

**Fig. 5.7** Proboscis sensilla. (**a, b**) Scanning electron micrographs of female (**a**) and male (**b**) proboscises of actively pollinating *Epicephala* species (*Epicephala* sp. associated with *Phyllanthus reticulatus*). Note that numerous sensilla are present on the female proboscis, whereas they are absent on the male proboscis. (**c**) *E. anthophilia* female actively depositing pollen on *G. acuminatum* stigma in Amami Island, Japan. Sensilla on moth proboscises allow more pollen to be held on the proboscis. (**d**) Proboscis of female *E. bipollenella* bearing sensilla. (**e**) Proboscis of female *E. nudilingua*, a nonpollinating species, lacking sensilla. (**f**) *E. parasitica*, a nonpollinating species, ovipositing in a young fruit of *P. lepidocarpus* in Ishigaki Island, Japan. (**g**) Another nonpollinating species (*E.* sp.) ovipositing in a very young fruit of *P. amarus* through leaves folded at night in Thakhek, Laos. Scale bar: 0.1 mm

*Epicephala* evolved the gall-inducing ability. The adaptive significance of galling is still unclear, but is probably linked to escape from parasitoid attack (Chap. 11).

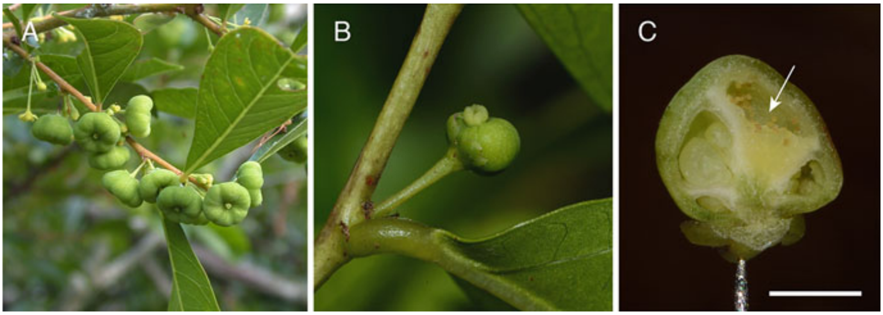

**Fig. 5.8** Fruits and galls produced by *Epicephala* species on *Glochidion obovatum*. (**a**) Fruit produced after pollination by *E. obovatella*. (**b**) Gall induced on female flower by *E. corruptrix*. (**c**) Cross-section of the gall induced by *E. corruptrix*. *Arrow* indicates the galled locule with feeding traces of *Epicephala* larva. Note that the irregularly developed ovules of the galled locule have merged and become indistinguishable from septa. Scale bar: 2 mm

## 5.3 Global Diversity of *Epicephala*

The genus *Epicephala* has thus far been described only in the Old World tropics, but they are also prevalent in the Neotropics. Observation of *Phyllanthus* in Cuba and Jamaica indicate that several species in the subgenus *Xylophylla* are associated with seed-feeding *Epicephala*. Some of the these *Phyllanthus* species (e.g., *P. chamaecrystoides*, *P. myrtilloides*) bear female flowers with fused styles characteristic of *Epicephala*-pollinated plants in the Old World, whereas others possess bifid and spread styles indicative of pollination by nonspecialized insects (e.g., *P. angustifolius*, *P. nutans*). Whether obligate pollination mutualism occurs in *Xylophylla* is still unclear, but the outcome of association between *Xylophylla* and *Epicephala* appears to be highly variable among species. There are roughly 60 species of *Xylophylla*, with the highest concentration of species being in the Caribbean islands, although the subgenus occurs as far south as Andean Peru.

The widespread occurrence of *Epicephala* in the New World is further demonstrated by the presence of *Epicephala* larvae and pupae on herbarium specimens (Fig. 5.9). Because plant specimens bearing fruits may sometimes contain *Epicephala* larvae at the time of collection, the larvae are occasionally found on *Phyllanthus* specimens, especially those in which the capsules dehisced while being dried. The larvae of most *Epicephala* species have a characteristic red color with narrow white bands, and cannot be mistaken for those of other Lepidoptera. Mature larvae inside fruits may also spin cocoons on the edges of leaves before they are completely dried. While spinning cocoons, the larvae of *Epicephala* excrete bubblelike balls from the anus, grab them with the mandibles, and attach them to the surface of the cocoon through a hole punched from inside the cocoon (Fig. 5.9). Although the adaptive role of ball production is unknown, such a habit is known only in *Epicephala* and several related genera of the Ornixolinae. Therefore, the

**Fig. 5.9** Larva and cocoon of *Epicephala* moth. (**a**) Larva of *Epicephala* sp. associated with *P. reticulatus* in Taiwan. The number and position of white bands varies among species. (**b**) Cocoon of *E. bipollenella* whose surface is decorated with bubbles. The moth has emerged from the cocoon, and the exuvia can be seen. (**c**) Herbarium specimen of *Phyllanthus mocinianus* at the herbarium of the University of California, Davis. The herbarium sheet is attached with an envelope (*arrow*) containing detached plant parts (mostly dehisced capsules and seeds), among which *Epicephala* larvae are sometimes found. (**d**) Dried *Epicephala* larva found inside the envelope. (**e**) An *Epicephala* cocoon found on a detached leaf in the envelope

presence of such cocoons on herbarium specimens provides reliable evidence of an association with *Epicephala*.

*Epicephala* larvae and cocoons are often found on specimens of *Phyllanthus* section *Nothoclema* (subgenus *Conami*; Fig. 5.9; Table 5.3). The group contains 10 species distributed from Mexico to Argentina, and is often a prominent

**Table 5.3** List of *Flueggea* and *Phyllanthus* species associated with *Epicephala*

| Genus | Subgenus | Section | Species | Geographic Location | Evidence | *Epicephala* Species |
|---|---|---|---|---|---|---|
| *Flueggea* | | | *F. suffruticosa* | Temperate east Asia | f, l | *E. relictella*, *Conopomorpha flueggella* |
| | | | ***F. elliptica*** | **Ecuador** | **h** | |
| *Phyllanthus* | *Kirganelia* | *Anisonema* | *P. reticulatus* | China, Taiwan, Laos | f | |
| | | | *P. microcarpus* | China, Taiwan, Laos, Malaysia | f, l | *E. microcarpa*,<br>*E. laeviclada*,<br>*E. tertieria* |
| | | | ***P. ciccoides*** | **Australia** | **f** | |
| | | | ***P. duplanchei*** | **New Caledonia** | **f** | |
| | | | ***P. casticum*** | **Madagascar** | **h** | |
| | | | ***P. pervilleanus*** | **Madagascar** | **h** | |
| | *Swatziani* | | *P. amarus* | Laos | f | |
| | | | ***P. abnormis*** | **USA** | **h** | |
| | ***Afroswartziani*** | | ***P. lokohensis*** | **Madagascar** | **f** | |
| | | | ***P. madagascariensis*** | **Madagascar** | **f** | |
| | *Gomphidium* | *Gomphidium* | *P. bourgeoisii* | New Caledonia | f | |
| | | | *P. buxoides* | New Caledonia | f | |
| | | | ***P. cuscutiflorus*** | **Australia** | **f** | |
| | | | *P. koumacensis* | New Caledonia | f | |
| | | | *P. mangenotii* | New Caledonia | f | |
| | | | *P. pancherianus* | New Caledonia | f | |
| | | | *P. poumensis* | New Caledonia | f | |
| | | | *P. tenuipedicellatus* | New Caledonia | f | |
| | | | *P. tiebaghiensis* | New Caledonia | f | |
| | | | *P. caudatus* | New Caledonia | f | |
| | | | *P. chamaecerasus* | New Caledonia | f | |
| | | | *P. koghiensis* | New Caledonia | f | |

(continued)

**Table 5.3** (continued)

| Genus | Subgenus | Section | Species | Geographic Location | Evidence | *Epicephala* Species |
|---|---|---|---|---|---|---|
| | | | *P. koniamboensis* | New Caledonia | f | |
| | | | *P. pilifer* | New Caledonia | f | |
| | | *Andenoglochidion* | *P. aeneus* | New Caledonia | f | |
| | | | *P. gneissicus* | New Caledonia | f | |
| | | | *P. guillauminii* | New Caledonia | f | |
| | | | *P. vulcani* | New Caledonia | f | |
| | *Gomphidium* sensu Ralimanana and Hoffmann (2011) | | *P. marojejiensis* | Madagascar | f | |
| | | | *P. humbertii* | Madagascar | f | |
| | ***Conami*** | ***Nothoclema*** | ***P. graveolens*** | **Peru** | **h** | |
| | | | ***P. mcvaughii*** | **Mexico** | **h** | |
| | | | ***P. mocinianus*** | **Mexico** | **h** | |
| | ***Xylophylla*** | ***Elutanthos*** | ***P. botryanthus*** | **Venezuela** | **h** | |
| | | | ***P. huallagensis*** | **Peru** | **h** | |
| | | | ***P. nutans*** | **Jamaica** | **f** | |
| | | ***Orbicularia*** | ***P. chamaecristoides*** | **Cuba** | **f** | |
| | | | ***P. myrtilloides*** | **Cuba** | **f** | |
| | | ***Xylophylla*** | ***P. angustifolius*** | **Jamaica** | **f** | |
| | *Emblica* | | *P. lepidocarpus* | Japan | f | *E. parasitica* |
| | | | ***P.* sp.** | **Laos** | **f** | |
| | Unplaced | *Macraea* | *P. ussuriensis* | Japan | f | *E. nudilinguae* |
| | | | ***P. chrysanthus*** | **New Caledonia** | **f** | |
| | **Unplaced** | ***Microglochidion*** | ***P. chimantae*** | **Venezuela** | **h** | |
| | | | ***P. duidae*** | **Venezuela** | **h** | |
| | | | ***P. obfalcatus*** | **Venezuela** | **h** | |
| | **Unplaced** | ***Paraphyllanthus*** | ***P. liebmannianus*** | **USA** | **h** | |

Data in bold indicate those newly reported here component of the local flora. The female flowers of the *Nothoclema* species have spread styles and do not appear to be specialized to *Epicephala*; thus, it would be interesting to clarify the pollination system of *Nothoclema* plants to understand whether obligate pollination mutualism occurs in the New World. One surprising finding was an association between *Epicephala* and *Flueggea elliptica* (Table 5.3), a plant that is only known from a small coastal area in southernmost Ecuador. The Old World *Flueggea suffruticosa* is host to *Conopomorpha flueggella* and *Epicephala relictella*, but other *Flueggea* species, such as the widespread and abundant *F. virosa*, have never been found hosting *Epicephala*. The *Flueggea*–*Epicephala* association in the New World is probably phylogenetically independent from that found in Asia, but determining where the Ecuadorian species belong within the *Epicephala* phylogeny is critical for the global understanding of *Epicephala* diversity and evolution.

Finally, one exciting possibility is the occurrence of obligate pollination mutualism on the tepuis of the Guiana Highlands. The tepuis are table-top mountains of granite arenite sandstone rising abruptly from the Amazonian rainforest that are host to a spectacular array of endemic plants and animals. The majority of the tepuis occur in Venezuela and Western Guyana, but some are also found in Colombia, Suriname, French Guiana, and in northernmost Brazil.

The *Phyllanthus* species found in the Guiana Highlands are grouped into a well-defined section, *Microglochidion* (Webster and Carpenter 2002, 2008). Many species possess elongated, nonbifid, and fused styles, which strikingly resemble those of *Glochidion* or New Caledonian *Phyllanthus*. Although neither larvae nor cocoons of *Epicephala* were found on herbarium specimens, the seeds of several *Microglochidion* species have holes that are typical of those made by *Epicephala* larvae, suggesting that the association with *Epicephala* is widespread among the ~10 species of Guiana Highland *Phyllanthus* (Table 5.3). It is exciting to think of the possibility that obligate pollination mutualism, which probably originated in the Old World, has reached some of the most exotic biota on earth and produced an impressive number of endemic species.