Chapter 7
Reinforced Specificity of Pollinator Moths

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Keywords *Caloptilia* • *Cuphodes* • *Diphtheroptila* • *Epicephala* • *Glochidion* • Gracillariidae • Host specificity • Phylogeny

7.1 Partner Specificity in Mutualisms

The remarkably high level of partner specificity is a hallmark feature of the leafflower–leafflower moth mutualism. Together with the fig–fig wasp and yucca–yucca moth systems, obligate pollination mutualisms provide some of the best examples of highly species-specific plant–insect associations. However, the evolutionary processes underlying these patterns are poorly understood. The high degree of specificity in pollinating seed parasites is often regarded as the fortuitous result of specialization in their ancestors because these insects are derived from endophytic herbivores that are themselves highly host-specific. This chapter focuses on the comparison of the level of host specificity in *Epicephala* to those of purely parasitic gracillariid relatives as a test of whether mutualism reinforces partner specificity. When interpreted with what is known in the fig and yucca systems, such an analysis serves as a useful approach to determine how partner specificity is shaped in coevolved mutualisms.

Parasitic lifestyles, in general, favor extreme specialization to one or few host species because they require complex adaptations to circumvent host defenses and sustain life on a single host (Ehrlich and Raven 1964; Price 1980; Thompson 1994; Strauss and Zangerl 2002). Although specific mechanisms underlying host specialization may vary among taxa, the broad general understanding is that host–parasite coevolution promotes specialization in parasitic organisms (Thompson 1994,

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2005). Strict host-specificity of parasites is often linked to high species diversity because specialization to different hosts can result in host-associated speciation (Mitter et al. 1988; Farrell 1998; Schluter 2000; Coyne and Orr 2004).

In contrast, the evolutionary processes that determine the level of specialization in mutualisms are far less understood. Although many mutualisms do not evolve to exhibit high degrees of specificity (e.g., most plant–pollinator and plant–seed disperser interactions), reciprocal partner specialization is often found in intimate mutualisms, such as those between myrmecophytic plants and their resident ants (Davidson and McKey 1993; Heil and McKey 2003; Guimarães et al. 2007), ants/termites and their cultivated fungi (Mueller et al. 1998; Aanen et al. 2002; Currie et al. 2003), or various invertebrates and their endosymbiotic microorganisms (Moran and Telang 1998; Hosokawa et al. 2006). Both ultimate and proximate causes of specialization have been proposed, including selection for elimination of less-cooperative partners (Heil et al. 2005; Poulsen and Boomsma 2005) and chemical or physical mechanisms of partner discrimination (Federle et al. 1997; Brouat et al. 2001; Edwards et al. 2006; Grangier et al. 2009). However, the general understanding of the evolutionary conditions favoring specialization in mutualisms is still very limited (Thompson 1994, 2005), and molecular approaches continue to refine our view of how mutualists are associated with one another on both local and broad geographic scales (Molbo et al. 2003; Mikheyev et al. 2006; Quek et al. 2007; Visser et al. 2009).

A currently estimated 500 species of leafflowers are each pollinated by host-specific *Epicephala* moth species (Chap. 6). Similarly, figs and yuccas have diversified into more than 700 and 40 species, respectively, and a corresponding high diversity of pollinator species has evolved, each of which is obligately mutualistic with one or few fig/yucca hosts (Weiblen 2002; Pellmyr 2003; Herre et al. 2008). This level of specificity is unusual among pollination mutualisms because, although selection may favor plants to depend on specialized visitors for effective conspecific pollination, pollinators are generally expected to maximize the range of plants they visit to optimize resource use (Pellmyr 2002; Gómez and Zamora 2006). The high specificity of pollinating seed parasites is therefore considered to be the result of their inherently parasitic lifestyle (Thompson 1994, 2005) because seed-feeding insects commonly specialize to a narrow range of host plants. Indeed, ecological and phylogenetic studies of the yucca moth family Prodoxidae have found that close relatives of the pollinators are also highly host-specific herbivores (Pellmyr and Thompson 1992; Pellmyr 1999; Pellmyr et al. 2006), suggesting that the high degree of pollinator specificity is driven by the parasitic part of the interaction and cannot be attributed to mutualistic selection (Thompson 1994, 2005).

However, observations suggest that the above view of host specificity in pollinating seed parasites may require revision. Within the yucca moth lineage, two cheater species have independently lost their pollinating behavior and oviposit in young fruits to exploit the seeds that other yucca moth species have pollinated (Pellmyr et al. 1996a, b; Pellmyr 1999). In contrast to their pollinating relatives, each of these cheater species evolved to utilize 4–6 yucca hosts (Pellmyr 1999), suggesting that host specificity in the pollinators may not be determined solely by the herbivorous habit of the moths (Pellmyr 2003). In the fig system, nonpollinating agaonid wasps that are closely related to and co-occur with pollinating fig wasps tend to be less host-specific than are the pollinators (Weiblen and Bush 2002; Marussich and Machado 2007; but see Lopez-Vaamonde et al. 2001; Jousselin et al. 2006, 2008). In addition, fig herbivores in general are dominated by insects that feed on several locally available fig hosts. Given that shared pollinators can result in hybridization among closely related, co-occurring figs (Machado et al. 2005; Wang et al. 2016), selection may favor figs to rely on specialist pollinators to achieve effective conspecific pollination. Thus, these observations indicate that pollinating seed parasites may in fact attain a higher degree of host specificity than that of their parasitic ancestors due to coevolutionary selection arising after the evolution of pollination mutualism.

Glochidion plants and associated *Epicephala* moths provide an ideal system for testing whether host specificity is greater in pollinating seed parasites than in their herbivorous ancestors because *Glochidion* is host to two other genera of Gracillariidae that are purely parasitic, namely *Diphtheroptila* and *Caloptilia* (Fig. 7.1). *Diphtheroptila*, which belongs to the subfamily Ornixolinae together with *Epicephala*, are leaf miners that utilize young *Glochidion* leaves, whereas *Caloptilia* (subfamily Gracillariinae) are leaf miners as early instar larvae and, as they develop into late instars, construct leaf rolls or induce leaf galls, depending on the species (Chap. 5). Phylogenetic analyses of Gracillariidae focused on Ornixolinae and Gracillariinae indicate that neither *Diphtheroptila* nor *Caloptilia* is the direct sister of *Epicephala* (Fig. 7.2); thus, *Epicephalae* are not derived from leaf herbivores with which they share host plants, and *Diphtheroptila* and *Caloptilia* likely each colonized *Glochidion* plants independently. Nevertheless, the shared use of *Glochidion* by the three genera allows for a rigorous test of how different life histories affect patterns of host specificity by controlling for the effect of host-plant species. Furthermore, *Glochidion* plants are attacked by three other genera of seed-parasitic moths in the families Tortricidae, Pyralidae, and Carposinidae (Fig. 7.1), whose host specificity may be determined by a common mechanism with that of *Epicephala* due to their shared larval diet.

The geographic region of focus is southwestern Japan and Taiwan, where there are five common *Glochidion* species (Fig. 7.3; two additional *Glochidion* species occur in Taiwan, but both are relatively uncommon). Of the five species, only *G. obovatum* ranges as far north as mainland Japan; the remaining four species occur in the Ryukyu Archipelago (the southern island chain of Japan) and Taiwan (and elsewhere in subtropical and tropical Asia). As many as four *Glochidion* species coexist on several of these islands. Any pair of *Glochidion* species can be found growing side by side (Fig. 7.4), although each species has more or less distinct microhabitat requirements. For example, *G. zeylanicum* is often found near streams and wetlands, and *G. obovatum* tends to occur along edges of coastal forest. Because most *Glochidion* species flower continuously from spring to autumn, there is a large overlap in the flowering period between sympatric *Glochidion* species.

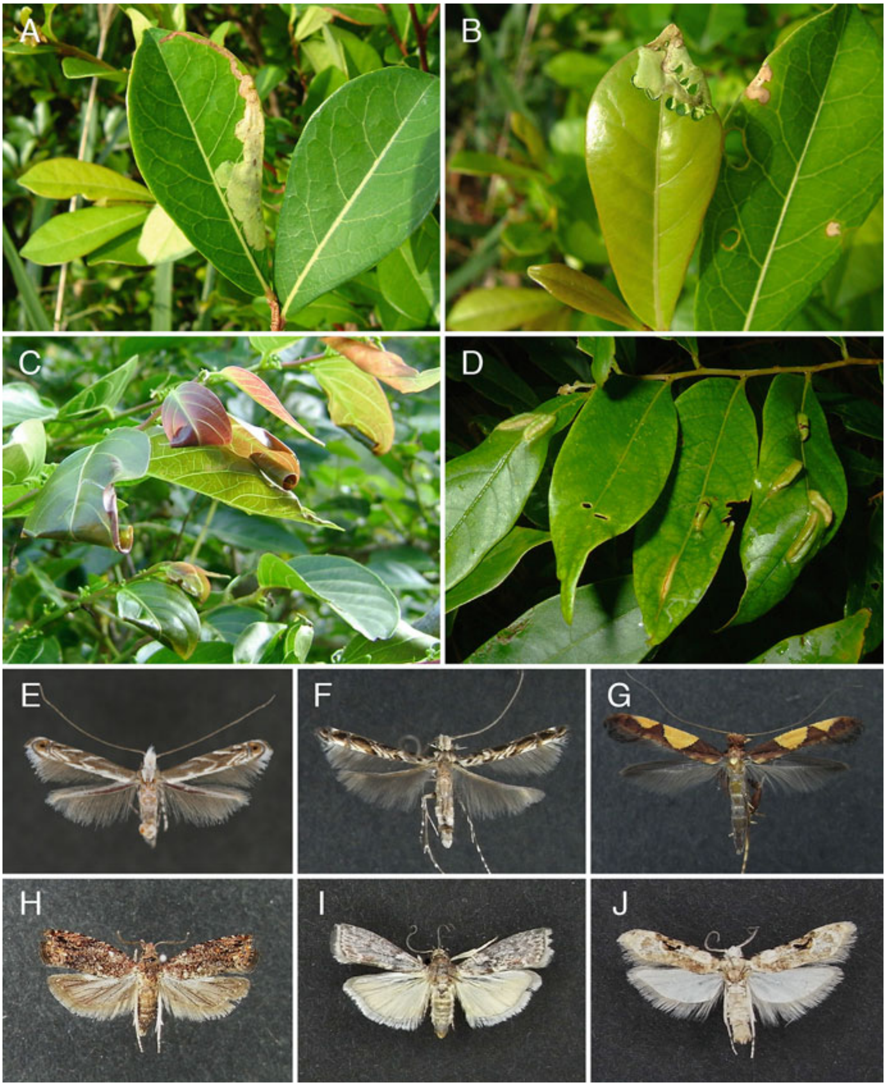

Fig. 7.1 Gracillariid moths associated with *Glochidion*. (**a**) A mature *Glochidion obovatum* leaf mined by *Diphtheroptila scriptulata* in Amami Island, Japan. (**b**) A young *G. obovatum* leaf mined by *Diphtheroptila* sp. 1 in Amami Island, Japan. Note that the leaf used by this species is distinctly younger than that used by *D. scriptulata*. Also, the larvae of this species cut the margins of the mine to defecate feces, a behavior not seen in *D. scriptulata*. (**c**) Leaf rolls produced by *Caloptilia ryukyuensis* on *G. lanceolatum* in Yonaguni Island, Japan. (**d**) Leaf galls induced by *Caloptilia cecidophora* on *G. acuminatum* in Okinawa Island, Japan. (**e**) *Epicephala obovatella*. (**f**) *Diphtheroptila scriptulata*. (**g**) *Caloptilia ryukyuensis*. (**h**) *Tritopterna* sp. (Tortricidae). (**i**) *Cryptoblabes* sp. (Pyralidae). (**j**) *Peragrarchis syncolleta* (Carposinidae)

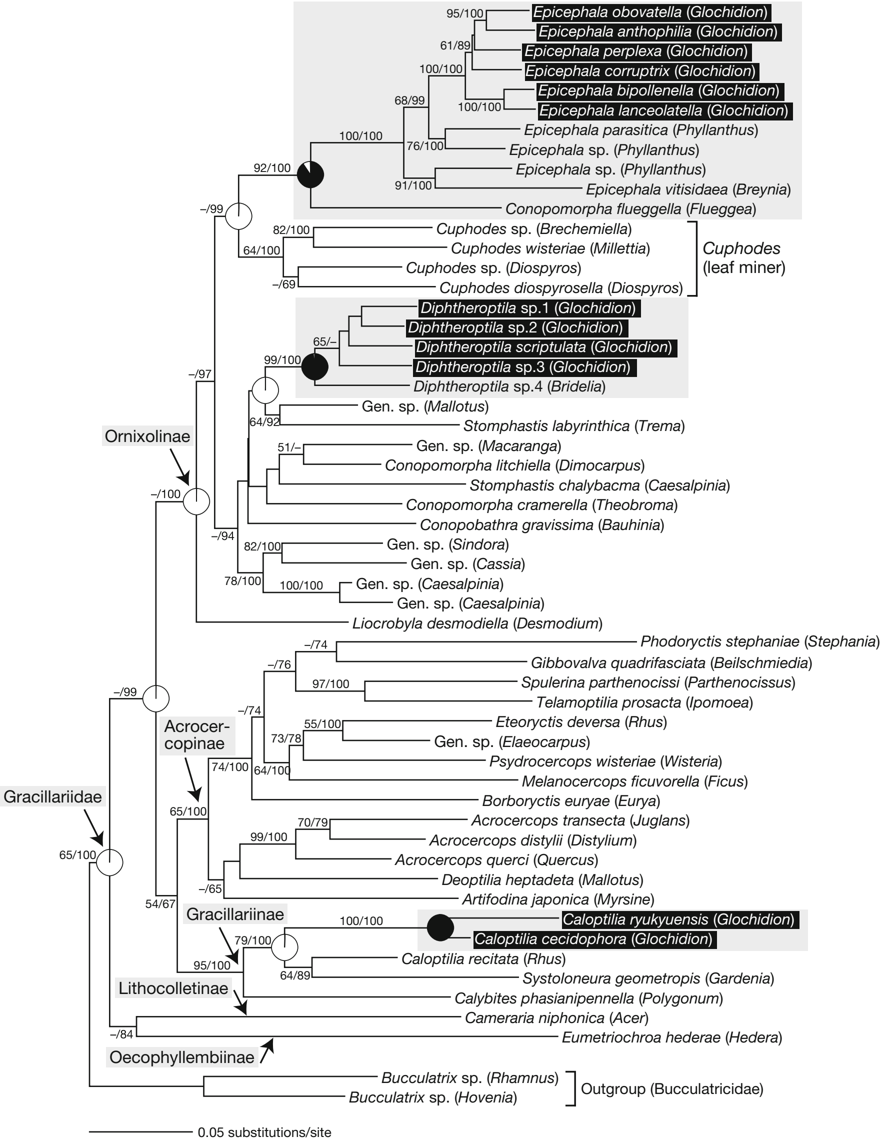

Fig. 7.2 Maximum likelihood phylogeny of Gracillariinae based on 2548 bp of the combined mitochondrial *COI* and nuclear *EF-1α*, *ArgK*, and *18S rRNA* genes. *Numbers* at nodes indicate maximum likelihood bootstrap values followed by Bayesian posterior probabilities. Clades boxed in *grey* are those feeding on Phyllanthaceae plants, and species highlighted individually in *black* are those associated with *Glochidion*. *Pie graphs* show the relative likelihoods of alternative host associations at selected ancestral nodes: *black*, Phyllanthaceae host; *white*, non-Phyllanthaceae host. Taxon names in *parentheses* indicate host plant genera

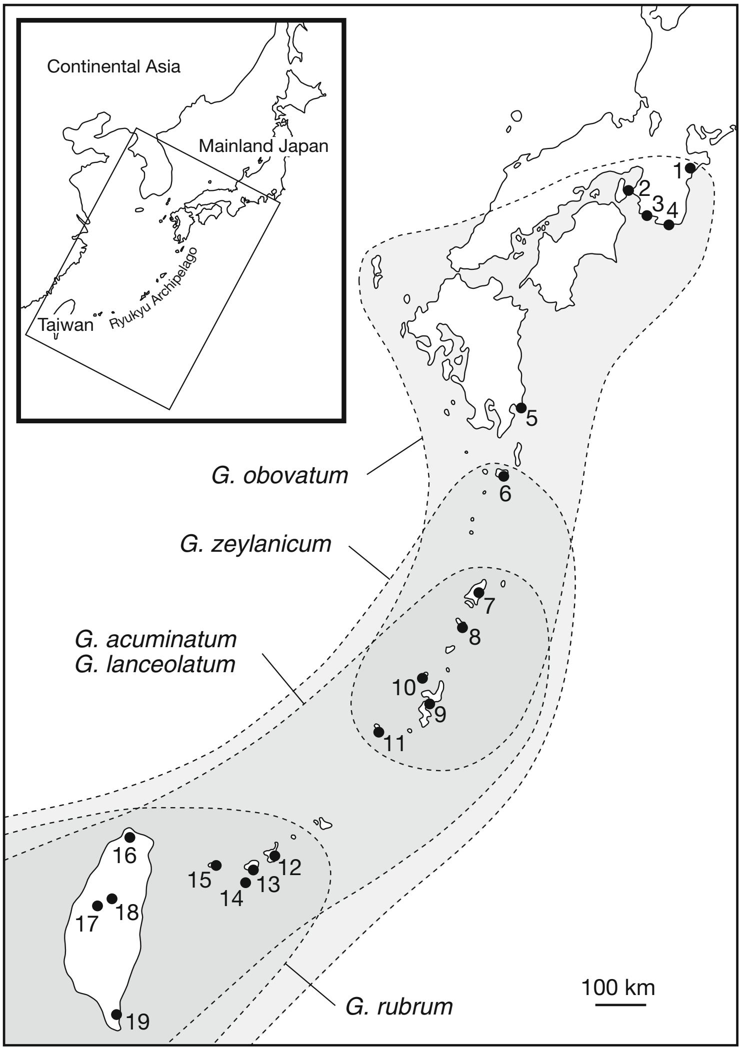

Fig. 7.3 Map of southwestern Japan and Taiwan showing moth sampling localities and distribution ranges of host *Glochidion* species. Locality numbers are: (*1*) Nantou; (*2*) Tomogashima; (*3*) Yura; (*4*) Koza; (*5*) Cape Toi; (*6*) Yaku Island; (*7*) Amami Island; (*8*) Tokuno Island; (*9*) Okinawa Island; (*10*) Iheya Island; (*11*) Kume Island; (*12*) Ishigaki Island; (*13*) Iriomote Island; (*14*) Hateruma Island; (*15*) Yonaguni Island; (*16*) Taipei; (*17*) Sun Moon Lake; (*18*) Wushe; (*19*) Nanren

Fig. 7.4 *Glochidion obovatum* (*left*) and *G. lanceolatum* (*right*) growing side by side in Amami Island, Japan

7.2 Host Specificity of *Epicephala*

Epicephala moths are generally specific to a single host species. However, an often-assumed one-to-one specificity is not perfectly met, as many Phyllantheae plants host more than one *Epicephala* species, or a single *Epicephala* species uses two or more host species either locally or in different parts of their ranges. The *Epicephala* moths associated with the five *Glochidion* species in Japan and Taiwan consist of six species that are clearly distinguishable by morphology and sequences of mitochondrial and nuclear DNA (Fig. 7.5). Of the six species, two species are specific to single host species; *E. anthophilia* is specific to *G. acuminatum*, and *E. bipollenella* uses only *G. zeylanicum*, throughout this region (Fig. 7.6). Two species, *E. lanceolatella* and *E. perplexa*, are associated with *G. lanceolatum* and are regularly found co-occurring on the same trees (Fig. 7.6). Lastly, two species, *E. obovatella* and *E. corruptrix*, share two closely related parapatric hosts (*G. obovatum* and *G. rubrum*; Fig. 7.6). Behaviorally, all six species possess the actively pollinating habit, although the larvae of *E. corruptrix* probably induce gall formation and provide little benefit to the host as compared to the other five species (Chap. 5).

Within this geographic scale, genetic variation within each *Epicephala* species is minimal (Fig. 7.5). However, individuals of *E. obovatella* in the Wushe population, which is located at 1400 m in the central mountain range of Taiwan, exhibit consistent divergence in both mitochondrial and nuclear gene sequences from

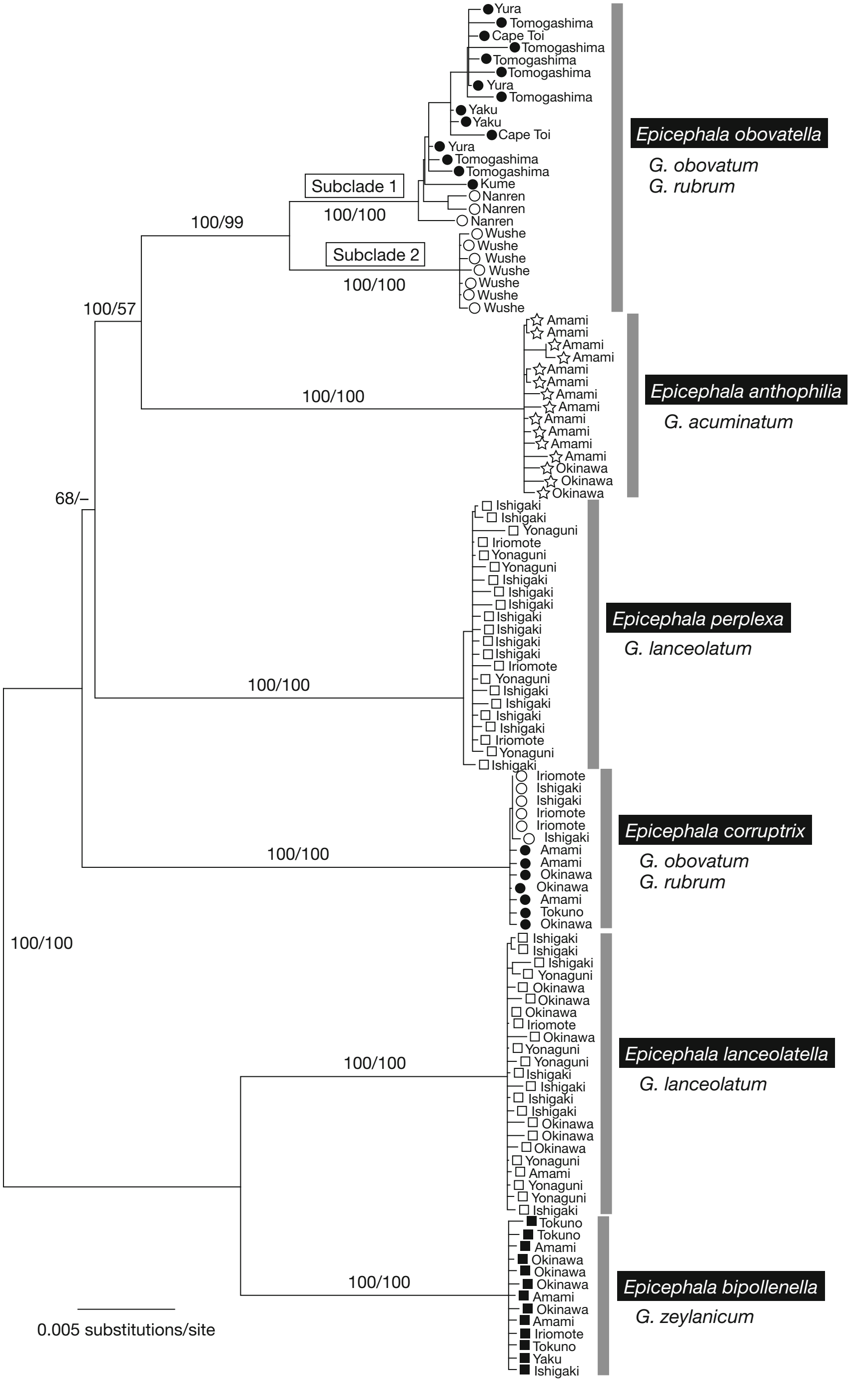

Fig. 7.5 Bayesian 50% majority rule consensus phylogram obtained by simultaneous analysis of *COI*, *ArgK* , and *EF-1α*. Terminal symbols represent host *Glochidion* species followed by locality names: *filled circle*, *G. obovatum*; *open circle*, *G. rubrum*; *filled square*, *G. lanceolatum*; *open*

individuals of other populations located at lower elevations (subclades 1 and 2 in Figs. 7.5 and 7.6), although genital morphology is uniform across all *E. obovatella* populations. Notably, *G. rubrum* trees of the Wushe population tend to have shorter pedicels and larger fruits than plants occurring elsewhere; thus, although a further quantitative study is necessary, this may represent an incipient stage of simultaneous speciation in plant and pollinator.

Phylogenetic analysis also indicates that the two *Epicephala* species co-occurring on *G. lanceolatum* (*E. laceolatella* and *E. perplexa*) are not sister taxa, so a host shift has occurred at least in one of the two species. Whether the coexistence of two pollinator species on a shared host is evolutionarily stable is unknown, because the age at which the two species started to coexist cannot be inferred from available data. Nevertheless, the prevalence of similar situations in figs, yuccas, and leafflowers (Pellmyr 1999; Molbo et al. 2003) may suggest that long-term coexistence on a shared host can occur.

In contrast, the two species associated with *G. obovatum* and *G. rubrum* (*E. obovatella* and *E. corruptrix*) were not found within the same population (Fig. 7.6). Although the number of moths sampled is limited to rule out coexistence, the pattern is in clear contrast with that observed for the two species pollinating *G. lanceolatum*. The disjunct distribution of *E. obovatella* in mainland Japan, Taiwan, and a few small islands (Fig. 7.6), coupled with a relatively high level of intraspecific sequence variation (Fig. 7.5), may indicate ancient widespread distribution of *E. obovatella* and subsequent extinction through competitive exclusion by *E. corruptrix* in most of the Ryukyu Archipelago. Because *E. corruptrix* is probably the less beneficial species (Chap. 5), it is interesting to identify the mechanism that has shaped the current distribution pattern of the two species.

Overall, the pattern of association between *Glochidion* and *Epicephala* is far more complex than a simple one-to-one relationship. However, an important pattern consistently observed throughout this region is that, at any given location, these moths are all specific to only one of multiple co-occurring *Glochidion* species. This likely prevents interspecific pollen transfer and helps maintain reproductive isolation of sympatric *Glochidion* species. For example, on Amami Island of southern Japan, four *Glochidion* species (*G. acuminatum*, *G. obovatum*, *G. zeylanicum*, and *G. lanceolatum*) co-occur and all flower simultaneously during May–June. Three of the four species other than *G. acuminatum* have a prolonged flowering season lasting from spring to autumn, so phenological isolation is virtually absent among these *Glochidion* species. Although studies are needed to quantify the strength of pollinator isolation, local host specificity of *Epicephala* is probably a necessary condition for multiple *Glochidion* species to coexist stably.

Fig. 7.5 (continued) square, *G. zeylanicum*; *open star*, *G. acuminatum*. *Numbers above branches* indicate Bayesian posterior probabilities followed by parsimony bootstrap values (shown when >50%). Species name is given to the *right* of each clade on *black background*; host plant species are given immediately *below*

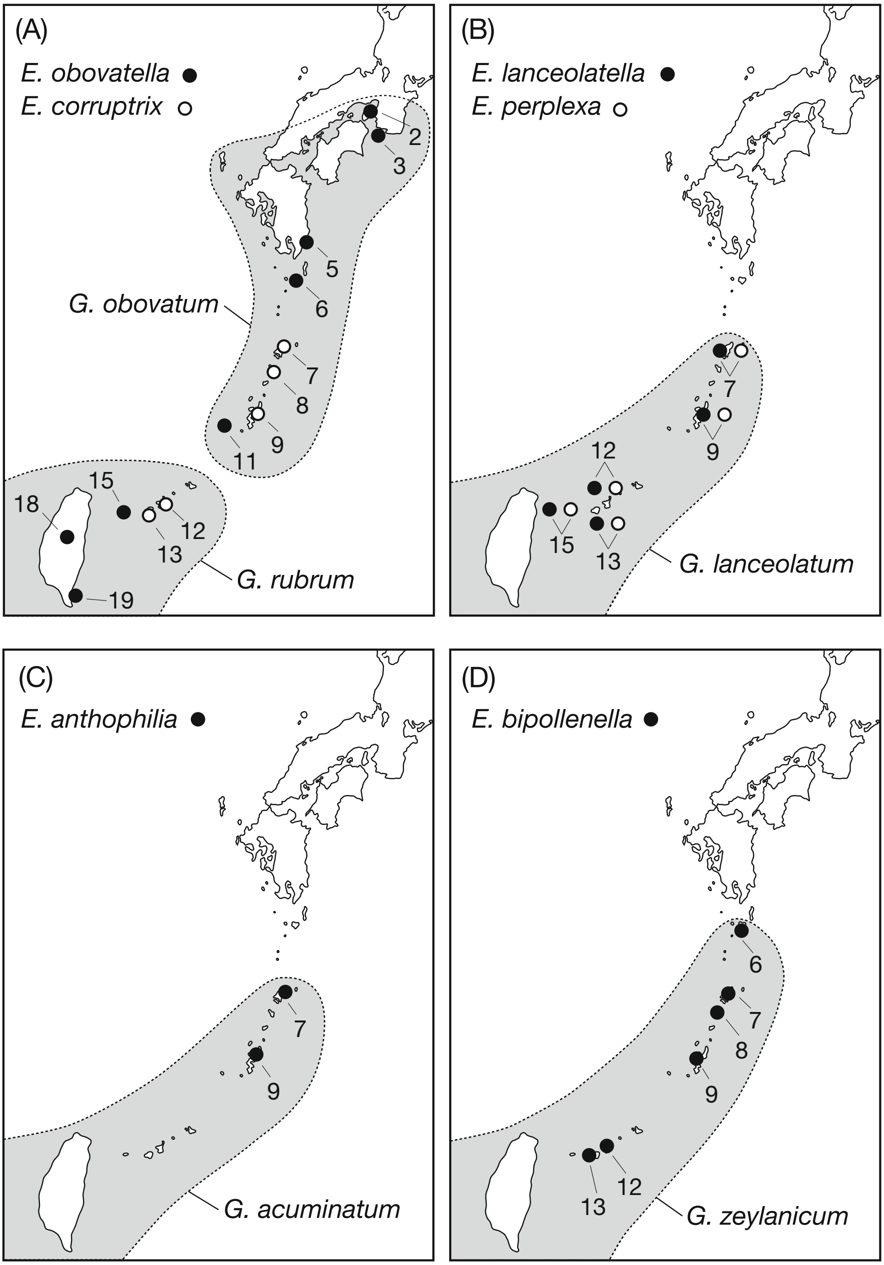

Fig. 7.6 Geographic distribution of the six *Epicephala* species in Japan along with range of their host plants. *Numbers* on the map correspond to those in Fig. 7.3. *Epicephala lanceolatella* and *E. perplexa* co-occur at each location

7.3 Host Specificity of *Diphtheroptila* and *Caloptilia*

Diphtheroptila and *Caloptilia*, sampled within precisely the same geographic range as the above *Epicephala* moths, each contained four distinct species that are widely separated genetically (Fig. 7.7). They are also distinguishable by wing pattern, male genitalia morphology, and larval feeding habit (Fig. 7.2). Notably, each *Diphtheroptila* and *Caloptilia* species commonly utilizes more than one coexisting *Glochidion* species (Fig. 7.7). Although the possibility of hidden divergence associated with *Glochidion* species cannot be ruled out, it is unlikely that all the *Diphtheroptila* and *Caloptilia* species under consideration are at incipient stages of such host-associated divergence. Moreover, the level of host-associated differentiation, if any, is considerably lower than that found in *Epicephala*, in which individuals associated with different *Glochidion* hosts in any population are morphologically distinct and divergent by at least 4% uncorrected pairwise sequence difference in the COI gene (Fig. 7.5). Therefore, available evidence suggests that *Epicephala* are more highly host-specific than are their leaf-feeding relatives that utilize the same sets of *Glochidion* hosts.

7.4 Host Specificity of Other Seed-Feeding Moths

The observed increase in the level of host specialization in *Epicephala*, however, may simply be the result of a shift to seed feeding, rather than coevolutionary selection resulting from being a pollinator. This possibility can be evaluated by comparing the level of host specificity in seed-infesting lepidopterans that share the same larval food with *Epicephala* moths. Non-gracillariid moths that emerge from *Glochidion* fruits are morphologically identified as either *Peragrarchis syncolleta* (Carposinidae) or as undescribed species of *Tritopterna* (Tortricidae) or *Cryptoblabes* (Pyralidae). However, there is only one species in each genus, as judged by the negligible divergence found in mitochondrial and nuclear gene sequences, and each species attacks 4–5 different *Glochidion* hosts. Although it is not straightforward to compare host specificity directly between moths of different families, the level of host specialization found in these groups is at the opposite extreme from the pattern expected if seed feeding is to promote higher host specificity. Therefore, there is no positive evidence that seed feeding favors a higher degree of host specialization; thus, *Epicephala* host specificity is likely determined by factors other than larval diet.

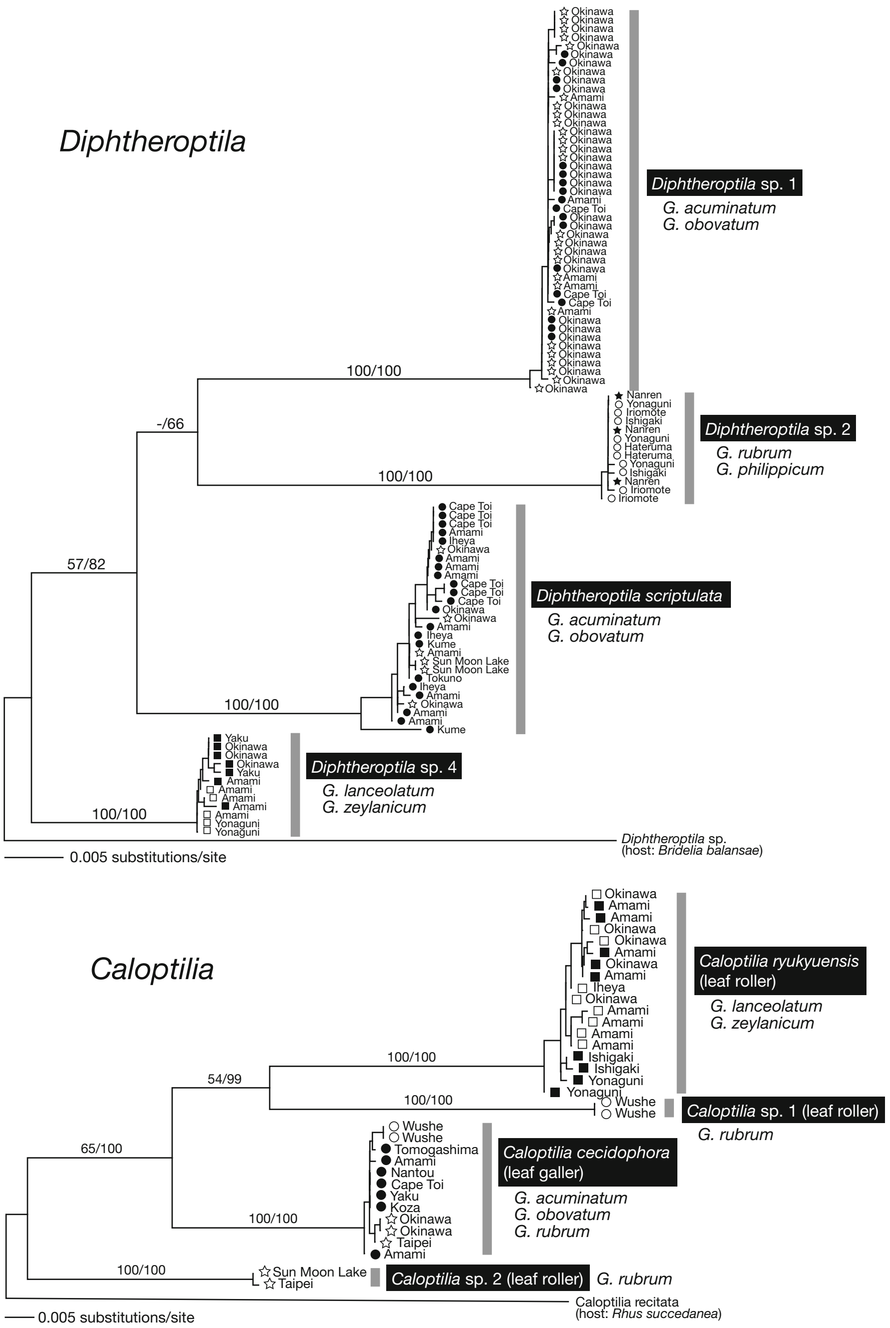

Fig. 7.7 Maximum likelihood phylogeny of *Diphtheroptila* and *Caloptilia* moths based on 1058 bp of the combined mitochondrial *COI* and *EF-1α* genes. Terminal symbols represent host *Glochidion* species followed by locality names: *filled circle*, *G. obovatum*; *open circle*, *G. rubrum*;

7.5 Host Specificity of *Cuphodes*

Another explanation for strict host specialization in *Epicephala* is that such high host specificity is a common feature among all the closest relatives of *Epicephala*, and that pollinator habit evolved against a background of high host specificity. The closest relative of *Epicephala* on the Ornixolinae phylogeny (Fig. 7.3) is *Cuphodes*, which uses species of Fabaceae, Ebenaceae, and Rhamnaceae as hosts. Although support for this relationship is low, adults of *Epicephala* and *Cuphodes* rest with their abdomens raised (Fig. 7.8), a feature not otherwise found in any genera of Ornixolinae, suggesting that *Cuphodes* is likely one of the closest relatives of *Epicephala*.

Cuphodes moths sampled from roughly the same geographic region as the above- sampled *Glochidion* feeders consisted of eight putative species (Fig. 7.8), which can be distinguished by wing pattern, male genitalia morphology, and larval mining pattern. As with *Diphtheroptila* and *Caloptilia*, single *Cuphodes* species regularly use 2–4 closely related plants (Fig. 7.8), suggesting that the closest relatives of *Epicephala* do not show the same degree of host specificity as *Epicephala*. Although the use of different host plant families in *Epicephala* and *Cuphodes* may make direct comparison difficult, available evidence suggests that *Cuphodes* species exhibit much broader host ranges than do the species of *Epicephala*. For example, *C. wisteriae* utilizes *Wisteria* and *Millettia*, which are distantly related genera within Fabaceae, having diverged at least 50 Ma (Lavin et al. 2005), whereas the age of the *Glochidion* crown group is estimated to be only <10 Ma (Chap. 6). Similarly, *Cuphodes* sp. 4 feeds on two genera (*Berchemia* and *Berchemiella*) of the Rhamnaceae family, although the antiquity of their divergence is unknown. Thus, the high degree of host specialization found in *Epicephala* is also likely not an ancestral condition predating the evolution of pollinator habit.

7.6 Reinforced Specificity of *Epicephala*

Taken together, the pattern of host specificity of *Diphtheroptila*, *Caloptilia*, *Cuphodes*, and seed-feeding non-gracillariid moths all indicate that the level of host specialization in *Epicephala* is higher than would be expected if host specificity were determined solely by the herbivorous habit of the moths. A more likely view is that pollinator habit favors higher host specificity than the ancestral parasitic lifestyle. What, then, is the ultimate cause driving the strict host specificity of *Epicephala*? As discussed in Chap. 8, there are clear differences in the chemical

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Fig. 7.7 (continued) filled square, *G. lanceolatum*; *open square*, *G. zeylanicum*; *filled star*, *G. philippicum*; *open star*, *G. acuminatum*. *Numbers above branches* indicate maximum likelihood bootstrap values followed by Bayesian posterior probabilities. Species name is given to the *right* of each clade on black background; host plant species are given immediately *below*

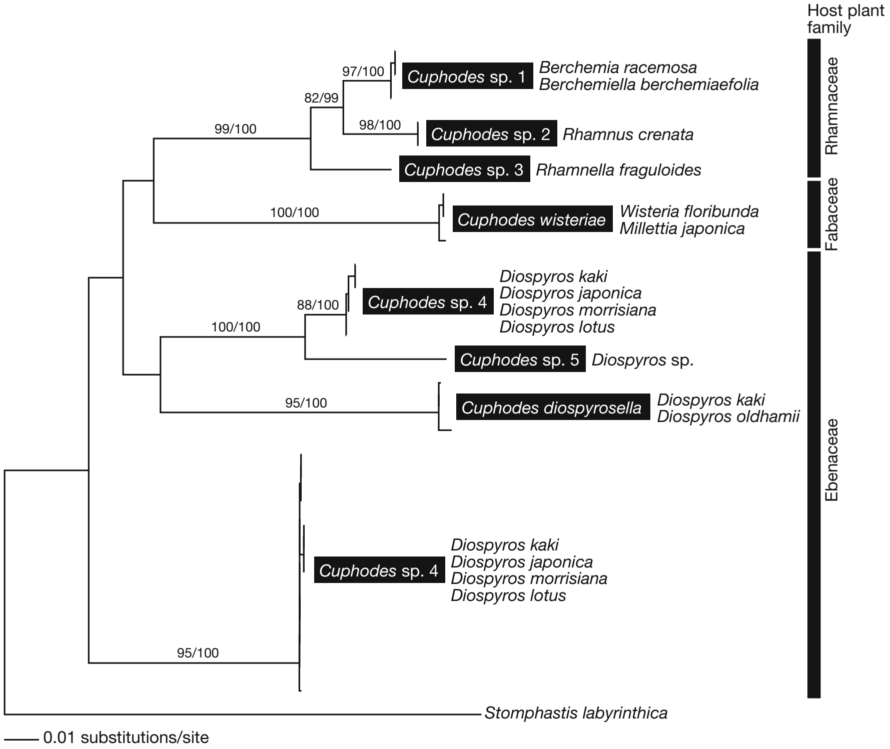

Fig. 7.8 Maximum likelihood phylogeny of *Cuphodes* based on 1601 bp of the combined mitochondrial *COI* and nuclear *EF-1α* and *ArgK* genes. Species name is given to the *right* of each clade on *black background*; host plant species are given immediately *below*. Host plant families are indicated using *bars* on the *right*. *Numbers above branches* indicate maximum likelihood bootstrap values followed by Bayesian posterior probabilities

composition of floral volatiles among coflowering *Glochidion* species. These differences are perceived by host-seeking *Epicephala* females and likely facilitate the attraction of species-specific pollinators. Thus, selection may operate on *Glochidion* plants to produce distinct floral scents and attract specific pollinators and thereby avoid hybridization. Although further experimentation is needed to determine whether interspecific crosses result in fruit production, any decrease in quantity and/or quality of hybrid fruits is likely also to facilitate specialization by *Epicephala* to species-specific floral volatiles. Thus, it is possible that the high plant–pollinator specificity in obligate pollination mutualisms is driven by a plant's interest to avoid less-advantageous hybridization.

Overall, reinforced specificity of *Epicephala* contrasts with what is known in the yucca moth lineage (Pellmyr and Thompson 1992; Thompson 1994, 2005). The closest relative of the pollinating yucca moths, *Prodoxus*, feed on inflorescence stalk, fruit, or, rarely, leaves of yucca plants and have very similar degrees of host specificity with the pollinating yucca moths (Pellmyr et al. 2006). This difference is probably due to contrasting patterns of flowering phenology between yuccas and *Glochidion*. Because both pollinating (*Tegeticula* and *Parategeticula*) and nonpollinating (*Prodoxus*) yucca moths are short lived and their life histories are strongly associated with yucca flowers (Pellmyr 1999, 2003; Pellmyr et al. 2006), the adult moths must emerge during a short period when host flowers are available. However, phenological isolation is strong between coexisting yucca species (Pellmyr 2003); thus, there is little opportunity for both pollinating and nonpollinating yucca moths to select among multiple hosts within a single population. In contrast, *Glochidion* plants produce flowers and leaves continuously from spring to autumn, and different *Glochidion* species commonly flower at the same time. Under such circumstances, both leaf-feeding and flower-infesting moths are provided with multiple available hosts, but the latter are more selective in their host choice probably due to a stricter chemical coadaptation with which they are constrained. The occurrence of multiple coflowering host species is also the case in figs, for which pollinating fig wasps are more host specific than the nonpollinators (Weiblen and Bush 2002; Marussich and Machado 2007; but see Lopez-Vaamonde et al. 2001; Jousselin et al. 2006, 2008).

Although *Epicephala* exhibits higher degrees of host specialization than do their parasitic ancestors, a more direct test of host specificity would be to include nonpollinating gracillariid seed parasites in the analysis. *Conopomorpha flueggella*, a nonpollinating seed feeder of *Flueggea* that is very closely related to *Epicephala* (Chap. 5), may be specialized to *Flueggea suffruticosa*, but a rigorous test is necessary in regions where multiple *Flueggea* species occur. Also, a derived clade of *Epicephala* has secondarily lost the pollinating habit, and presently there are three species that are each specific to a single *Phyllanthus* host (Kawakita and Kato 2009). However, closely related *Phyllanthus* hosts are rarely available within the same population, which precludes a direct comparison of host specificity with pollinating *Epicephala* in this case as well. Within the yucca moth lineage, two derived species have independently lost their pollinating behavior and oviposit in young fruits to exploit the seeds that other yucca moth species have pollinated (Pellmyr et al. 1996b; Pellmyr 1999). These cheater species evolved to utilize 4–6 yucca hosts (Pellmyr 1999, 2003), which is consistent with the view that pollinator habit promotes host specificity in pollinating seed parasites. The cheater yucca moths are likely to have a broader phenological window for successful oviposition (Pellmyr 2003); thus, selection for host specialization may have been relaxed in these derived nonpollinators.

Although further research is required to identify coevolutionary forces driving pollinator specificity, a viable hypothesis is that mutualistic selection reinforces host specificity of pollinating seed parasites in obligate pollination mutualisms. Pollinator specificity is likely to impact strongly patterns of gene flow between coexisting plant species and play an important role in facilitating reproductive isolation between diverged populations (Machado et al. 2005; Smith et al. 2008b, 2009). Thus, identifying the mechanism that shapes partner specificity is the key to understanding the role of coevolution in promoting speciation and diversification in obligate pollination mutualisms.