

Chapter 10

Cospeciation and Host Shift

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10.1 Cospeciation of Intimately Interacting Partners

When two interacting lineages have been in intimate association during much or all of their diversification, as in the case of obligate pollination mutualisms or many host–parasite interactions, there is a probability that speciation in one group is paralleled by speciation in the other. This mode of diversification results in a pattern of shared evolutionary history between the two lineages, known as cospeciation. Cospeciation can be a nonadaptive process that occurs in the absence of selection. For example, repeated vicariance events followed by shared allopatric speciation can produce a pattern of parallel diversification (Roderick 1997). However, cospeciation can also be reinforced or directly result from an adaptive process. For example, in feather lice and their avian hosts, preening behavior of the host imposes selection on louse body size, which prevents lice from switching between hosts of different sizes (Clayton et al. 2003). In obligate pollination mutualisms, the pollinators are responsible for the fertilization among conspecific host flowers, and thus some adaptation in the plants to exclude nonlegitimate pollinators is likely present. In fact, there are several reciprocally selected traits that may reinforce plant–pollinator specialization, such as synchronized phenological patterns (Wiebes 1979; Patel and Hossaert-McKey 2000), species-specific olfactory signals (Hossaert-McKey et al. 1994; Song et al. 2001; Grison-Pigé et al. 2002, 2003; Okamoto et al. 2007; Svensson et al. 2010), and reciprocal adaptation between

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pollinator morphology and floral structure (Ramírez 1974; Herre 1989; van Noort and Compton 1996; Kato et al. 2003; Weiblen 2004). Thus, knowledge of the degree of cospeciation in obligate pollination mutualisms provides an essential step toward understanding the historical role of coevolution in shaping speciation and diversification in plants and pollinators.

Previous studies using the fig–fig wasp system have indicated a significant level of cospeciation at both lower and higher taxonomic levels (Herre et al. 1996; Weiblen 2000, 2001; Machado et al. 2001; Weiblen and Bush 2002). However, strict congruence of phylogenies has not been found in the fig–fig wasp association. In addition, there are several documented cases in which multiple distantly related fig wasp species associate with a single host, further indicating a lack of strict-sense cospeciation in this association (Wiebes 1979; Compton 1990; Michaloud et al. 1996; Kerdelhue et al. 1999; Lopez-Vaamonde et al. 2002; Molbo et al. 2003). Analysis of parallel cladogenesis using the yucca–yucca moth system also resulted in a similar finding; although host plant use by yucca moths is relatively conserved at higher taxonomic levels, cases of cospeciation are rare (Pellmyr and Leebens-Mack 1999; Pellmyr et al. 2007; Althoff et al. 2012).

Whereas previous studies using the fig–fig wasp and yucca–yucca moth systems provided insights into macroevolutionary patterns in these specialized interactions, the *Glochidion–Epicephala* mutualism offers a novel opportunity to corroborate and refine these earlier observations. The *Glochidion–Epicephala* system is particularly suited for such analysis, as the association is highly species-specific and diverse (Kato et al. 2003). The genus *Glochidion* comprises more than 300 species distributed in tropical Asia, Australia, and Polynesia with multiple species commonly occurring in sympatry. *Epicephala* moths associated with *Glochidion* plants are taxonomically poorly documented, but available evidence suggests that a comparable number of *Epicephala* species exist, each with a very narrow host range. This chapter provides an update to the previous analysis of cospeciation between *Glochidion* trees and *Epicephala* moths (Kawakita et al. 2004) using molecular phylogenetic analysis of 27 *Epicephala* species and 36 host *Glochidion* species (Table 10.1). Phylogenetic analyses are based on published nucleotide sequences of the internal and external transcribed spacer regions of the nuclear ribosomal DNA (ITS and ETS) for *Glochidion*, and those of the mitochondrial cytochrome oxidase subunit I gene (COI) and nuclear arginine kinase (ArgK) and elongation factor-1 α (EF-1 α) genes for *Epicephala* moths.

10.2 Phylogenetic Analysis and Cospeciation Test

A full list of species included in the analysis and their locality information is given in Table 10.1. The 36 *Glochidion* species sampled were collected from various localities within the distribution of the genus. Although there is little information concerning intrageneric taxonomy of *Glochidion* (Webster 1994; Govaerts et al. 2000), the samples cover a wide range of morphological variation found within this

Table 10.1 List of *Epicephala* and *Glochidion* species sampled for cophylogenetic analysis

Species	Host Plant	Locality	Note
<i>E. anthophilia</i>	<i>G. acuminatum</i>	Amami Island, Kagoshima, Japan	
<i>E. bipollenella</i>	<i>G. zeylanicum</i>	Okinawa Island, Okinawa, Japan	
<i>E. corruptrix</i>	<i>G. obovatum</i> , <i>G. rubrum</i> ^a	Ishigaki Island, Okinawa, Japan	
<i>E. eriocarpa</i>	<i>G. eriocarpum</i>	Lak Sao, Bolikhamsai, Laos	
<i>E. lanceolatella</i>	<i>G. lanceolatum</i>	Ishigaki Island, Okinawa, Japan	
<i>E. obovatella</i>	<i>G. obovatum</i> , <i>G. rubrum</i>	Cape Toi, Miya- zaki, Japan	
<i>E. perplexa</i>	<i>G. lanceolatum</i>	Ishigaki Island, Okinawa, Japan	
<i>E. sp. ex</i> <i>G. arborescens</i>	<i>G. arborescens</i>	Lambir, Sarawak, Malaysia	
<i>E. sp. ex</i> <i>G. assamicum</i>	<i>G. assamicum</i>	Arunachal Pradesh, India	
<i>E. sp. ex</i> <i>G. benthamianum</i>	<i>G. benthamianum</i>	Mt. Molly, Queensland, Australia	
<i>E. sp. ex</i> <i>G. caledonicum</i>	<i>G. caledonicum</i>	Hienghène, New Caledonia	
<i>E. sp. ex</i> <i>G.</i> <i>cf. zeylanicum</i>	<i>G. cf. zeylanicum</i>	Lambir, Sarawak, Malaysia	
<i>E. sp. ex</i> <i>G. collinum</i>	<i>G. collinum</i> , <i>G. cordatum</i>	Mt. Victoria, Fiji	
<i>E. sp. ex</i> <i>G. glomerulatum</i>	<i>G. glomerulatum</i>	Lambir, Sarawak, Malaysia	
<i>E. sp. ex</i> <i>G. harveyanum</i>	<i>G. harveyanum</i>	Mt. Windsor, Queensland, Australia	
<i>E. sp. ex</i> <i>G. littorale</i>	<i>G. littorale</i>	Miri, Sarawak, Malaysia	
<i>E. sp. ex</i> <i>G. lutescens</i>	<i>G. lutescens</i>	Lambir, Sarawak, Malaysia	
<i>E. sp. ex</i> <i>G. obscurum</i>	<i>G. obscurum</i>	Lambir, Sarawak, Malaysia	
<i>E. sp. ex</i> <i>G. perakense</i>	<i>G. perakense</i>	Kutching, Sara- wak, Malaysia	
<i>E. sp. ex</i> <i>G. philippicum</i>	<i>G. philippicum</i>	Nanren, Pingtung, Taiwan	
<i>E. sp. ex</i> <i>G. pungens</i>	<i>G. pungens</i>	Mt. Lewis, Queensland, Australia	

(continued)

Table 10.1 (continued)

Species	Host Plant	Locality	Note
<i>E. sp. ex G. seemanii</i>	<i>G. seemanii</i> , <i>G. concolor</i>	Navai, Fiji	
<i>E. sp. ex G. sumatranum</i>	<i>G. sumatranum</i>	Lambir, Sarawak, Malaysia	Incorrectly labeled as <i>G. ferdinandii</i> in Kawakita et al. (2004)
<i>E. sp. ex G. velutinum</i>	<i>G. velutinum</i>	Mt. Popa, Bagan, Myanmar	
<i>E. sp. ex G. sp. 1</i>	<i>G. sp. 1</i>	Pindai, New Caledonia	
<i>E. sp. ex G. sp. 2</i>	<i>G. sp. 2</i>	Lambir, Sarawak, Malaysia	Incorrectly labeled as <i>G. lanceisepalum</i> in Kawakita et al. (2004)
<i>E. sp. ex G. sp. 3</i>	<i>G. sp. 3</i>	Phonsavan, Xiangkhoang, Laos	
—	<i>G. sericeum</i>	Lambir, Sarawak, Malaysia	
—	<i>G. cf. puberum</i>	Thakhek, Khammouane, Laos	
—	<i>G. sphaerogynum</i>	Ban Phon, Xiangkhoang, Laos	
—	<i>G. cf. rubrum</i> ^a	Thakhek, Khammouane, Laos	
—	<i>G. cf. hohenackeri</i>	Lak Sao, Bolikhamsai, Laos	
—	<i>G. lanceolarium</i>	Guangzhou, Guangdong, China	
—	<i>G. daltonii</i>	Lak Sao, Bolikhamsai, Laos	

^a*G. rubrum* is used to refer to very different plants in different parts of its range. For consistency within the book, this name is used to refer to the plants called *G. rubrum* in the Japanese flora. The plants referred to as *G. rubrum* in Indochina are here labeled *G. cf. rubrum*, although a future taxonomic revision may reveal that the latter is the true *G. rubrum*

large genus. Representatives of *Breyntia* and *Sauropus* were included as outgroups, as done previously (Kawakita et al. 2004).

Epicephala moths were obtained from 29 of the 36 above-mentioned *Glochidion* species. Based on clear morphological and molecular differences, the moths were grouped into 27 species, each using usually one but rarely two host *Glochidion* species. Species names could be reliably assigned to seven of the 27 *Epicephala*

species. For outgroups, two *Epicephala* moths associated with *Breynia* and *Phyllanthus*, the close relatives of *Glochidion*, were used as in the previous analysis (Kawakita et al. 2004).

There is a large radiation of *Glochidion* and associated *Epicephala* in the French Polynesian islands (Hembry et al. 2013a), but they were not included in the present analysis because species delimitation has not been established firmly for *Epicephala*. The diversification of *Glochidion* and *Epicephala* in French Polynesia is treated in detail in Chap. 12.

Sequences used in the present analysis are all available in public databases. Alignment methods followed those described in Kawakita et al. (2004). The aligned matrices (1365 bp of ITS + ETS for *Glochidion*; 1827 bp of COI + ArgK + EF1a for *Epicephala*) were subjected to maximum likelihood (ML) phylogenetic analyses using the Treefinder software (Jobb 2011) and the substitution models chosen by the program. The robustness of the ML trees was validated by bootstrap analysis (1000 replications) using the same program. Bayesian analysis was also performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) with substitution models chosen by MrModeltest 2.3 (Nylander 2004). Trees were sampled every 100 generations, and the average standard deviations of split frequencies calculated every 1000 generations. Using the stoprule option, analyses were continued until the average standard deviations of split frequencies fell below 0.01, at which point the Bayesian chains were considered to have achieved convergence. Because the average standard deviations of split frequencies were calculated based on the last 75% of all samples, the initial 25% of sampled trees were discarded as burn-in.

To assess whether species of *Glochidion* and *Epicephala* have undergone parallel diversification, we used an event-based reconciliation analysis, as implemented in the program Jane 4.01 (Conow et al. 2010). Jane uses a dynamic programming algorithm in conjunction with a genetic algorithm to find solutions (often optimal) for reconciling two phylogenies. Costs are assigned to four types of cophylogenetic events (cospeciation, duplication, host switch, and loss; for detailed terminology, see Conow et al. (2010)); optimal solutions are reconstructions with the lowest global cost. Analyses were performed with default genetic algorithm parameters. To test whether the cost of the optimal reconstruction was lower than its null expectation, plant–pollinator associations were permuted for 1000 replicates, and optimal solutions obtained for each replicate to generate a null distribution.

In addition to the Jane analysis, the extent of cospeciation was tested using the Parafit method (Legendre et al. 2002), which, rather than tree topologies, uses matrices of patristic distances (summed branch lengths along a phylogenetic tree) or phylogenetic distances calculated directly from sequence data. Whereas Jane requires fully resolved trees and thus is sensitive to selection of different phylogenetic hypotheses, Parafit is less likely to provide different results among several optimal phylogenies. In this test, distance matrices of the two groups are transformed to principal coordinates, and the trace statistic is calculated by taking plant–pollinator associations into account. The null hypothesis that the two groups are randomly associated is tested through a permutational procedure; plant–pollinator relationships are permuted to obtain a null distribution of the test statistic

against which the observed value is tested. This method also allows one to test whether each plant–pollinator association contributes significantly to the global fit of the two phylogenies. This is done by calculating trace statistics with and without a given plant–pollinator link, and testing the difference between the two statistics by permutation. The analyses were done using the Copycat 1.14 program (Meier-Kolthoff et al. 2007).

For both Jane and Parafit analyses, the ML trees of *Glochidion* and *Epicephala* were used as input trees.

10.3 Cospeciation in the *Glochidion*–*Epicephala* Association

Figure 10.1 shows the phylogenies of *Glochidion* and *Epicephala* obtained by maximum likelihood analyses of the ITS + ETS and COI + ArgK + EF1a datasets, respectively. Although many apical branches are validated by moderate to high support values (maximum likelihood bootstrap and Bayesian posterior probability values), most higher nodes are poorly supported, highlighting the difficulty of resolving the phylogenies of *Glochidion* and associated *Epicephala*, which

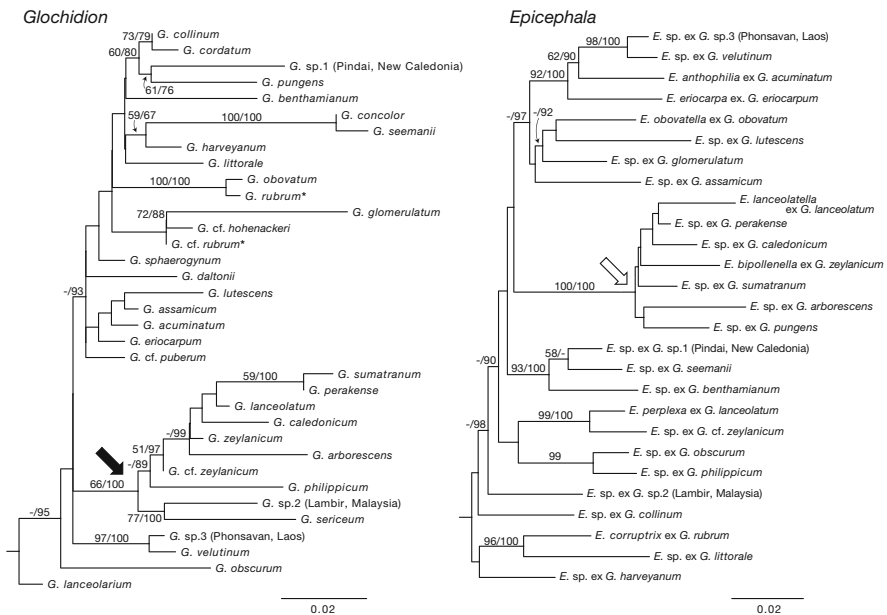


Fig. 10.1 Maximum likelihood trees for *Glochidion* and *Epicephala*. The *Glochidion* phylogeny was estimated using the combined ITS and ETS sequence data; that of *Epicephala* was based on the combined dataset of COI, ArgK, and EF-1 α sequences. Numbers above branches are bootstrap values followed by Bayesian posterior probability (See Table 10.1 for sampling details)

probably underwent rapid initial radiations (Kawakita and Kato 2009). Thus the results of the phylogenetic reconstructions, including the positions of the root, should be taken with caution. Nevertheless, these phylogenies are useful for testing the importance of cospeciation in shaping the global phylogenetic pattern in the plants and pollinators.

Figure 10.2 shows the comparison between the *Glochidion* and *Epicephala* phylogenies with information on plant–pollinator associations. Although the two phylogenies are far from identical, there is a tendency that associated plants and pollinators occupy similar positions on the phylogeny. For example, in the *Glochidion* phylogeny, there is a relatively well-supported clade of 10 species encompassing *G. sumatranum* and *G. sericeum* (indicated by the black arrow in Fig. 10.1). The *Epicephala* species associated with many of these *Glochidion* species also form a strongly supported clade (indicated by the white arrow in Fig. 10.1), suggesting that the associated plants and pollinators underwent parallel diversification. Note that the latter *Epicephala* clade contains the pollinator of *G. pungens*. Because *G. pungens* is distantly related to species of the *G. sumatranum*–*G. sericeum* clade (Fig. 10.1), this likely represents a clear case of host switch by *Epicephala*.

Statistical analyses of cospeciation corroborate visual assessment. The cost of the optimal reconstruction using Jane, under default cost settings (cospeciation = 0; duplication = 1; host switch = 2; loss = 1), was 41, but none of the optimal solutions of randomized data (1000 replicates) had lower costs (mean \pm SD, 90.5 ± 14.4), indicating that the two phylogenies are more similar than expected for a random association ($P < 0.001$). Because Jane requires fully resolved trees and thus is sensitive to phylogenetic uncertainty, the Parafit analysis, which takes tree structure into account, was performed as complementary to the Jane analysis. The Parafit analysis also found that the plant and moth phylogenies are more structurally similar than expected by chance ($P = 0.002$). Of the 31 individual plant–pollinator associations, 15 contributed significantly to the overall cophylogenetic structure (Fig. 10.2).

Studies addressing parallel diversification in plant–herbivore associations usually do not find cospeciation of the interacting lineages. Rather, host shifts are prevalent, and dramatic shifts among distantly related plant taxa are commonly observed. Therefore, the overall similarity in plant and pollinator phylogenies found in this study, together with that in the fig–fig wasp system (Herre et al. 1996; Weiblen 2000, 2001; Machado et al. 2001; Weiblen and Bush 2002), represents a special case in which plants and associated insects diversified more or less in parallel. Unfortunately, analysis of phylogenetic congruence does not identify processes that underlie the pattern of cospeciation. For example, cospeciation may arise through a number of processes including shared allopatric speciation, coevolution, and adaptation by only one group in response to the other. Thus, it is possible that plants and pollinators cospeciate as the result of shared vicariance events and that adaptive evolution may not be important in driving the overall cospeciation pattern.

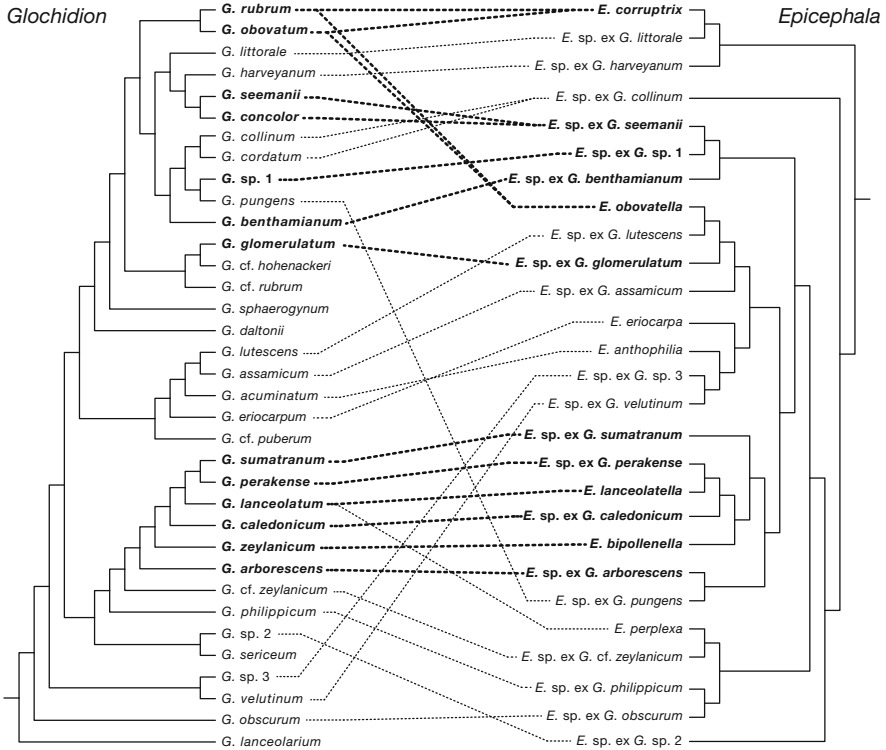


Fig. 10.2 Phylogenetic trees of *Glochidion* (left) and *Epicephala* (right) with plant–moth associations indicated using hatched lines. The *Glochidion* tree is the maximum likelihood topology inferred from the combined ITS + ETS sequences; the *Epicephala* tree is the maximum likelihood tree based on the combined COI, ArgK, and EF-1 α sequences. Plant–pollinator links with significant contribution to overall cophylogenetic structure, as inferred by the Parafit analysis, are highlighted in bold

However, several observations suggest that reciprocal selection may reinforce cospeciation in obligate pollination mutualisms. For example, Weiblen and Bush (2002) demonstrated that the degree of cospeciation between *Sycomorus* figs and *Ceratosolen* pollinators is greater than that observed between the same set of host figs and nonpollinating, gall-inducing fig wasps of the genus *Apocryptophagus*. They attributed this difference to the extent of reproductive requirements by which pollinating fig wasps are constrained, such as pollen compatibility and/or reciprocal adaptation between fig wasp morphology and narrow ostiolar entrance of the host fig (Ramírez 1974; Herre 1989; van Noort and Compton 1996). Similar constraints may also have been important in preventing host shifts by *Epicephala* moths. *Glochidion* plants produce species-specific olfactory signals that attract their own *Epicephala* species, and *Epicephala* moths have distinct preferences to the floral odor of their natal hosts (Okamoto et al. 2007). Such chemical coadaptation may lock partners into strong intimacy, making host switches difficult. In *Glochidion*,

the structure of the style exhibits great interspecific variation and is the principal species-diagnostic characteristic within the genus, which is in marked contrast with morphological uniformity of the male flowers (Airy Shaw 1978; Chakrabarty and Gangopadhyay 1995; Kato et al. 2003). Because *Epicephala* moths pollinate *Glochidion* flowers and oviposit in the styles using diverse and specific methods (Kato et al. 2003), this structural difference may further reinforce host plant specialization and may have played an important role in shaping the overall cospeciation pattern between *Glochidion* and *Epicephala*.

The relative importance of reciprocal adaptation in driving parallel diversification can potentially be assessed by the amount of pollinator and nonpollinator cospeciation with their host plants. For example, *Glochidion* plants are associated with leaf-mining *Diphtheroptila* moths that belong to the subfamily Ornixolinae together with *Epicephala* (Chap. 7). Although data on *Diphtheroptila* are only available for the Japanese fauna, these moths have distinctly broader host ranges than do *Epicephala* and consequently a lower level of phylogenetic similarity with their *Glochidion* hosts. For example, two *Diphtheroptila* species found in Japan both utilize *G. acuminatum* and *G. obovatum* (Chap. 7), which occupy distant positions in the *Glochidion* phylogeny (Fig. 10.1). These observations highlight the importance of coevolution in reinforcing plant–moth specialization and cospeciation in the *Glochidion*–*Epicephala* association.

10.4 Prevalence of Host Shift and Species-Specificity Breakdown

The intimate association between *Glochidion* and *Epicephala* is perhaps one of the most extreme cases of species-specific, plant–insect interaction known (Kato et al. 2003). It is remarkable to note that nearly all *Glochidion* species from which *Epicephala* moths were obtained had their own distinct pollinator species (Table 10.1). However, if this one-to-one rule had been maintained throughout the history of their diversification, how could the pollinators have shifted to novel hosts? If a host shift occurs successfully without violating this rule, the pollinator colonizing a new host must drive the original pollinator extinct, or the pollinator must be primarily absent on the new host. The former scenario assumes that host shift does not result in stable coexistence of two pollinator species on a single host. However, there is growing evidence showing that multiple *Epicephala* species commonly coexist on a single host. For example, *G. lanceolatum* is pollinated by *E. lanceolatella* and *E. perplexa* which can be found together on the same individual tree (Fig. 10.2; Kawakita and Kato 2006). Similarly in the fig–fig wasp system, two or more fig wasp species commonly reproduce and pollinate in a single host fig, a situation assumed to have lasted for at least a few million years (Molbo et al. 2003). Common observation of multiple pollinator yucca moth species on a single yucca host further indicates that coexistence of multiple pollinator species on a

shared host do not necessarily lead to exclusion of others by a single species (Addicott 1996; Pellmyr et al. 1996b; Addicott and Bao 1999; Pellmyr 1999). Thus the observed apparent cases of host shift by *Epicephala* moths did not result solely from the colonization/exclusion process.

The alternative scenario assuming the primary absence of a pollinator does not appear to be a plausible condition in obligate pollination mutualisms, as neither the plant nor pollinator can successfully reproduce without the other. However, this process may exert where the plant colonizes a region without its original pollinator, followed by colonization of the isolated plant population by an unassociated pollinator species. For example, independent colonization of oceanic islands (e.g., French Polynesia) by unrelated plant and pollinator could lead to the formation of novel associations involving host shift. Also, some *Glochidion* species have very wide distribution ranges (e.g., *G. acuminatum* occurs from Japan to India and *G. philippicum* from Taiwan to Australia), providing a possibility that multiple, distantly related *Epicephala* species pollinate a single *Glochidion* species allopatrically across its geographic range. In plant–herbivore interactions, there are examples in which local herbivores colonize and specialize to recently introduced host plants, supporting the plausibility of this process in the organization of novel associations.

Because the known cases of species-specificity breakdown are only found in the regions where plant–pollinator association is best studied (Japan and China), the assumed one-to-one specificity is probably routinely violated in the *Glochidion*–*Epicephala* association. It is therefore necessary to re-examine the diversity and host specificity of *Epicephala* critically on a global scale. Importantly, *Epicephala* species co-occurring on a single *Glochidion* host are not sister species in all reported cases (Fig. 10.2); thus a host shift by either moth species is minimally needed to explain the observed pattern. This situation is analogous to those in the fig–fig wasp and yucca–yucca moth associations in which multiple distantly related pollinator species are commonly observed on a single host (Addicott 1996; Pellmyr et al. 1996b; Addicott and Bao 1999; Pellmyr 1999). The observed poor concordance between plant and pollinator phylogenies is thus likely attributable to repeated host shifts and resulting breakdown of one-to-one specificity. In light of this, it is remarkable that statistical analyses commonly find significant congruence between plant and pollinator phylogenies. Examining the relative importance of cospeciation versus host shift in generating the diversity of *Epicephala* will thus be an important topic for future research.