Coevolutionary Diversification of Leafflower Moths (Lepidoptera: Gracillariidae: *Epicephala*) and Leafflower Trees (Phyllanthaceae: *Phyllanthus* sensu lato [*Glochidion*]) in Southeastern Polynesia

By

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Abstract

Coevolution between phylogenetically distant, yet ecologically intimate taxa is widely invoked as a major process generating and organizing biodiversity on earth. Yet for many putatively coevolving clades we lack knowledge both of their evolutionary history of diversification, and the manner in which they organize themselves into patterns of interaction. This is especially true for mutualistic associations, despite the fact that mutualisms have served as models for much coevolutionary research. In this dissertation, I examine the codiversification of an obligate, reciprocally specialized pollination mutualism between leafflower moths (Lepidoptera: Gracillariidae: *Epicephala*) and leafflower trees (Phyllanthaceae: *Phyllanthus* sensu lato [*Glochidion*]) on the oceanic islands of southeastern Polynesia.

Leafflower moths are the sole known pollinators of five clades of leafflowers (in the genus *Phyllanthus* s. l., including the genera *Glochidion* and *Breynia*), and thus this interaction is considered to be obligate. Female moths actively transfer pollen from male flowers to female flowers, using a haired proboscis to transfer pollen into the recessed stigmatic surface at the end of the fused stylar column. The moths then oviposit into the flowers' ovaries, and the larva which hatches consumes a subset, but not all, of the developing fruit's seed set. This interaction is known as a pollinating seed predation mutualism, because the same insect responsible for pollinating the flowers also feeds on the seeds of the same plant as a larva. Furthermore, as best as is known, this mutualism is characterized by high reciprocal species-specificity, with each leafflower species associated with only one or two moth species, and vice versa. In these salient characteristics, the leafflower/leafflower moth mutualism is thus analogous to the classically known mutualisms between fig trees and fig wasps, and yucca plants and yucca moths.

In my dissertation I examined the best-studied leafflower clade, the genus *Glochidion*, with 300 described species distributed from Pakistan to Japan, south to Australia, and east to the Pitcairn Islands. Because of their high species diversity and presumably complex biogeographic history, I focused on the co-radiation of 24 *Glochidion* species and their *Epicephala* moths on the oceanic islands of southeastern Polynesia (Cook Islands, French Polynesia, and the Pitcairn Group). All these islands are formed by volcanoes as the Pacific plate moves over a series of stationary hotspots in the earth's mantle, and the majority are of Plio-Pleistocene age (>5 Ma). All the *Glochidion* species are endemic to this region, and nearly all are described as endemic to single archipelagos; they constitute one of the largest endemic plant radiations in this biodiversity hotspot. These species differ primarily in the morphology of female flowers. Most islands have 1—3 species, but large islands have 4—7, with multiple species occurring sympatrically. All these factors suggest this diversification has occurred over very short evolutionary timescales.

In Chapter 1, I demonstrate that the mutualism between *Glochidion* and *Epicephala*, previously described on continents, is present in southeastern Polynesia. *Glochidion* had previously been reported from nearly all high islands in this region, but *Epicephala* from only two (Nuku Hiva and Fatu Hiva, Marquesas). Because of the difference in generation time between *Glochidion* and *Epicephala*, it is unlikely that they could colonize a new island in a single dispersal event.

With my co-authors, I observed and photographed *Epicephala* pollinating *Glochidion* flowers on two islands in the Society Archipelago (Tahiti and Raiatea), discovered pollen on museum specimens of *Epicephala* from several other Pacific islands, and reared *Epicephala* from the fruit of 19 species of *Glochidion* on 17 islands where it had not previously been reported. These results indicate that this mutualism has been able to repeatedly dissemble and reassemble on remote islands. This conclusion is of interest because it is in contrast to a long-standing island biogeography paradigm that organisms with specialized biotic interactions should be unable to colonize remote islands, and also because of concern over the resiliency of specialized mutualisms to global change.

In Chapter 2, I demonstrate as a result of a three-year rearing inventory on 21 islands that not only *Epicephala*, but several other lineages of insects specialized on the leafflower family (Phyllanthaceae) have colonized southeastern Polynesia. These include the leafmining moth *Diphtheroptila* (Gracillariidae) and seed-feeding moth *Tritopterna* (Tortricidae), and potentially also the leafrolling moths *Caloptilia* (Gracillariidae) and *Dudua* (Tortricidae). This indicates that niche conservatism in host plant use may play an important role in the assembly of phytophagous insect communities even on remote islands. I discuss this result in the context of the literature on adaptive radiation (onto new host plants) and niche conservatism (on the same host plant) in phytophagous insect faunas on oceanic islands generally.

In Chapter 3, I use molecular phylogenetic methods to reconstruct the evolutionary history of southeastern Polynesian *Glochidion* and their *Epicephala* within the geographic context of the entire Asia-Pacific region. *Glochidion* trees have colonized southeastern Polynesia twice, with one colonization spreading throughout the southern Cook, Society, Austral, Marquesas, and Tuamotu archipelagos, and a separate colonization of Mangareva (Gambier Islands). Epicephala moths have colonized southeastern Polynesia at least twice. An older colonization has spread throughout the southern Cook, Society, Marquesas, Tuamotu archipelagos and Rapa (Australs), and a younger colonization has spread through the southern Cooks, northern Australs, and Societies. Not only has this obligate mutualism diversified in a manner that has not resulted in phylogenetic congruence, but one pollinator lineage (the younger) has rapidly shifted onto a large number of novel host species in *Glochidion* over a wide geographic area. These results indicate a role both for geographic isolation and host-shifts in the diversification of this mutualism. More importantly, these results indicate that despite all the constraints that might be expected to prevent multiple colonizations by *Epicephala* (geographic isolation, reciprocal specialization between *Glochidion* and *Epicephala* species, and competitive exclusion between older and younger *Epicephala* clades), *Epicephala* have been able to establish multiple times on these remote islands and spread rapidly onto new hosts once they arrive. Consequently, patterns of association are likely to be similarly dynamic in these between *Glochidion* and *Epicephala* on continents. Implications of these findings for understanding how pollinating seed-predation mutualisms diversify are discussed.

In Chapter 4, I use molecular phylogenetic and network analysis methods to ask whether the patterns of interaction between *Glochidion* and *Epicephala* species are reciprocally specialized and modular to the same degree as in continental regions. I focus on the three youngest Society Islands with *Glochidion* present (Huahine, Moorea, and Tahiti), which collectively have 10

species of *Glochidion*. I find that unlike on continents, *Glochidion-Epicephala* networks on Tahiti and Huahine lack modularity. These findings may provide a view onto the early stages of diversification in pollinating seed-predation mutualisms, and suggest that coevolving clades may pass through different patterns of interaction as they diversify.

Taken together, these results indicate that these free-living mutualists have colonized remote oceanic islands repeatedly, but not congruently. These repeated colonizations have led to a dynamic coevolutionary history over only a few million years, with evidence for host-shifts, diversification on different archipelagos, and patterns of interaction which are less modular than are previously known from this and similar systems on continents.

Being limited to one planet and a small number of continents and archipelagos is the curse of evolutionary biology.

E. O. Wilson, *The Diversity of Life* (1992)

Read what the worms write on the madrone leaf, and walk sideways.

Ursula K. Le Guin, *Always Coming Home* (1985)

I dedicate this dissertation to the many peoples of the South Pacific, who let me play in their beautiful gardens over the past few years.

And to Erica Newman, for being there.

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INTRODUCTION

Mutualisms are pervasive in nature, but both theory and data suggest that they are intrinsically unstable because of conflicts between partners (Sachs and Simms 2006). Additionally, reciprocal specialization between mutualists that are not vertically transmitted might pose constraints on their ability to successfully colonize new environments. As early as 1878, Wallace (1878) hypothesized that the lack of suitable pollinators is a major constraint on the colonization of remote islands by flowering plants (Carlquist 1974). Island angiosperms are predominantly pollinated by wind or generalist insects and depend on generalist vertebrates for fruit dispersal (Kaiser-Bunbury et al. 2010). Difficulties of synchronous co-establishment are thought to also restrict island colonization by plants or animals that require specialized fungal symbionts (Florence 1997; Nobre et al. 2010). Alternatively, specialized mutualisms may be lost when plants colonize islands in the absence of mutualists (Janzen 1973; Armbruster and Baldwin 1998).

Obligate, pollinating seed-predation mutualisms, such as between figs and fig wasps (Cook and Rasplus 2003) and yuccas and yucca moths (Pellmyr 2003) would appear to be especially ill-suited to island colonization. In these mutualisms, specialized insects pollinate the host plants' flowers, but feed as larvae on a subset of the host's seeds; these interactions have high reciprocal species-specificity, but are not vertically transmitted (Cook and Rasplus 2003, Pellmyr 2003). These mutualisms are either absent, or not species-rich, on oceanic islands. Native yuccas are absent from islands off North America, including the West Indies (Pellmyr 2003). Native figs and fig wasps are widely distributed on Pacific islands, but are species-poor on most oceanic islands and absent from Hawaii (Corner 1963; Wiebes 1994). The islands of southeastern Polynesia (Cook Islands, French Polynesia and Pitcairn Islands), the region in the Pacific farthest from continents (≥6000 km from Australia, ≥4800 km from Mexico and ≥3000 km from Fiji), are considered to have only one native, non-endemic fig species (*Ficus prolixa*) (Corner 1963; Florence 1997; Staddon et al. 2010), which is apparently pollinated by a single wasp species throughout its range (Wiebes 1994; Staddon et al. 2010).

A similar mutualism between *Glochidion* trees (Phyllanthaceae) and *Epicephala* moths (Lepidoptera: Gracillariidae) is widely distributed throughout tropical Asia and Australasia (Kato et al. 2003). *Glochidion* are monoecious, with minute, unisexual flowers that do not produce nectar (Florence 1997). Female flowers have a reduced perianth, fused styles and concealed stigmas (Kato et al. 2003); this suite of traits has evolved convergently in four other *Epicephala*-pollinated Phyllanthaceae lineages (Kawakita and Kato 2009). Two Japanese *Glochidion* species

have been shown to be self-compatible (A. Kawakita 2011, personal communication). *Epicephala* are the sole-known pollinators of *Glochidion* (Kato et al. 2003; Kawakita and Kato 2006); each *Glochidion* species appears to be associated with only one or two *Epicephala* species and vice versa (Kawakita and Kato 2006). Adult *Epicephala* pollinate their host in a highly stereotyped behaviour in which they transfer pollen to the host's concealed stigmas with the proboscis and then oviposit into the ovaries; larvae consume a subset (typically 30%) of the host's seeds (Kato et al. 2003). Female *Epicephala* bear pollen on proboscis hairs; these hairs are absent in males (and females of one non-pollinating *Epicephala* lineage known from another genus of Phyllanthaceae) (Kawakita and Kato 2009).

Glochidion are also found throughout the oceanic islands of Micronesia and Polynesia, except Hawaii (Govaerts et al. 2000). The 25 described species in southeastern Polynesia constitute one of the largest endemic plant radiations in this biodiversity hotspot (Florence et al. 1995; Florence 1997; Meyer 2004; McCormack 2007). Twenty of these 25 species are considered single-archipelago endemics; most islands have one to three species, but the larger Society Islands have four to seven species (Florence 1997; McCormack 2007). These *Glochidion* retain the reduced flowers with fused styles and concealed stigmas seen in continental taxa (Florence 1997); style fusion is a strong predictor of *Epicephala* pollination in Phyllanthaceae (Kawakita and Kato 2009). Within Polynesia and Micronesia, however, *Epicephala* have only been reported from Samoa (Meyrick 1927) and the Marquesas Islands (Clarke 1986), suggesting that this mutualism may have been lost as *Glochidion* colonized the remote Pacific (Kawakita et al. 2004). It is unlikely that *Glochidion* and *Epicephala* could establish on a new island through a single dispersal event, since if a fruit containing an *Epicephala* larva were to land on a new island, the moth would eclose several years before a germinated *Glochidion* would be mature enough to produce flowers. Here, we report on the first survey of the presence of *Epicephala* moths on *Glochidion* trees in southeastern Polynesia, and assess whether on these islands *Epicephala* show evidence of pollinating *Glochidion* flowers.

MATERIAL AND METHODS

(a) Rearing
Glochidion fruit were collected from 22 species on 18 islands in the Cook Islands and the Society, Austral, Marquesas and Tuamotu archipelagos in French Polynesia (see Appendix 2) and kept in plastic bags to rear larvae. Fruit were also dissected to search for *Epicephala* larvae. Adult *Epicephala* were recognized by the two-lobed valvae of the male genitalia, sclerotized ovipositor and forewing pattern. Female *Epicephala* were examined for pollen-bearing hairs on the proboscis (Kawakita and Kato 2006).

(b) Flower observations
We observed flowers of [1] *Glochidion grayanum* at the Belvédère, Taravao Plateau, Tahiti (Society Islands, French Polynesia; 560 m elevation, 178°46.70' S, 149°815.50' W) on the nights of 21 November 2007, 27–28 July 2008 and 7 March 2009; and [2] *Glochidion temehaniense* on Te Mehani Rahi Plateau on Raiatea (Society Islands; 400 m elevation, 168°46.00' S, 151°827.90' W) on the nights of 30–31 July 2006 and 15–16 July 2008, for a total

of 15 h between 18.00 and 1.00 h. All flower visitors were observed and photographed while on flowers, captured, preserved dry in glassine envelopes and examined for pollen. *Epicephala* specimens were photographed using a Microptics XLT digital imaging system (Ashland, USA), and automontaged using COMBINEZM (Hadley 2006).

(c) Examination of museum specimens
We additionally examined four female *Epicephala* specimens from the Smithsonian Institution for pollen. These specimens were collected by J. F. Gates Clarke on Fatu Hiva and Nuku Hiva (Marquesas; two specimens of *Epicephala spinula*) in 1968 (Clarke 1986) and on Pohnpei (Micronesia; two specimens of an undescribed species) in 1953.

RESULTS

We reared *Epicephala* adults from larvae in fruits of 17 species of *Glochidion* from 14 islands in four archipelagos in French Polynesia and obtained *Epicephala* larvae from the fruits of four additional species of *Glochidion* from five islands in three archipelagos in the Cook Islands and French Polynesia (figure 1h,i and Appendix 2). Three species of *Glochidion* examined yielded no *Epicephala* (Appendix 2); in these cases, 0–1 fruiting trees and less than five fruits were found. This is, to our knowledge, the first published report of *Epicephala* from the Society, Austral, Tuamotu or southern Cook archipelagos. Based on the valvae of male genitalia, male adult specimens represent five distinct morphospecies endemic to southeastern Polynesia (*E. spinula* and four undescribed species), each of which appears geographically restricted to one to three archipelagos. All female *Epicephala* had proboscis hairs similar to those of pollinating *Epicephala* in Asia, and unlike non-pollinating *Epicephala* (Kawakita and Kato 2009). We also reared *Tritopterna* (Lepidoptera: Tortricidae); these are non-pollinating seed parasites of *Glochidion* in Asia (Kawakita et al. 2010).

We photographed and captured *Epicephala* visiting flowers of *G. grayanum* on Tahiti and *G. temehaniense* on Raiatea (Society Islands). *Epicephala* were observed visiting male flowers (figure 1a), pollinating female flowers with their proboscides (figure 1b) and subsequently ovipositing into the flowers' ovaries (figure 1c,d). Photographs show the insertion of the proboscis into both male and female flowers (figure 1a,b). Captured *Epicephala* bore pollen on their proboscides (figure 1e). Only three other insects were observed perched on the cylindrical flowers: one bug (Hemiptera: Miridae) and two midges (Diptera: Nematocera). None of these were observed contacting the vicinity of the recessed stigma and none bore pollen when examined. We also found that museum specimens of *Epicephala* from the Marquesas and Micronesia bore pollen (figure 1f,g).

DISCUSSION

We find that both *Glochidion* trees and *Epicephala* moths have colonized the remote Pacific, and that on at least several islands, *Epicephala* pollinate *Glochidion* as in continental Asia and Australasia (Kato et al. 2003). Conservation of traits involved with mutualism (proboscis hairs;

minute flowers with fused styles and concealed stigmas) between Asia and Polynesia suggests this interaction may be mutualistic throughout southeastern Polynesia. Although many insects are found on *Glochidion* foliage and some occasionally perch on flowers, our results do not suggest that they transport pollen or actively pollinate in the manner of *Epicephala*.

Successful colonization of remote islands by specialized mutualists that probably do not disperse together may appear paradoxical, and in contrast to the island biogeography paradigm that taxa with specialized biotic interactions should not be able to establish on islands (Carlquist 1974). It is unclear what mechanism has permitted this interaction to reassemble on these islands, but several hypotheses are possible. Reassembly may be permitted by long plant generation time, high insect dispersal rates or facultative species-specificity between *Glochidion* and *Epicephala* lineages that have no recent coevolutionary history. Although all evidence to date suggests that this mutualism is obligate (Kato et al. 2003; Kawakita and Kato 2006), and we have no evidence of alternative pollinators, vegetative propagation or selfing, we cannot rule out the possibility that any of these processes may operate at a low rate that permits the persistence of a small *Glochidion* population on an island until later colonization by *Epicephala*.

Glochidion and *Epicephala* in southeastern Polynesia appear to constitute a remarkable example of the colonization of some of the youngest and remotest landmasses on the planet by specialized mutualists that probably do not disperse together. This finding is of interest given concerns over the disruption of pollination systems, the increasing disturbance of island ecosystems, the rarity of many island plants and the response of mutualisms to global change.
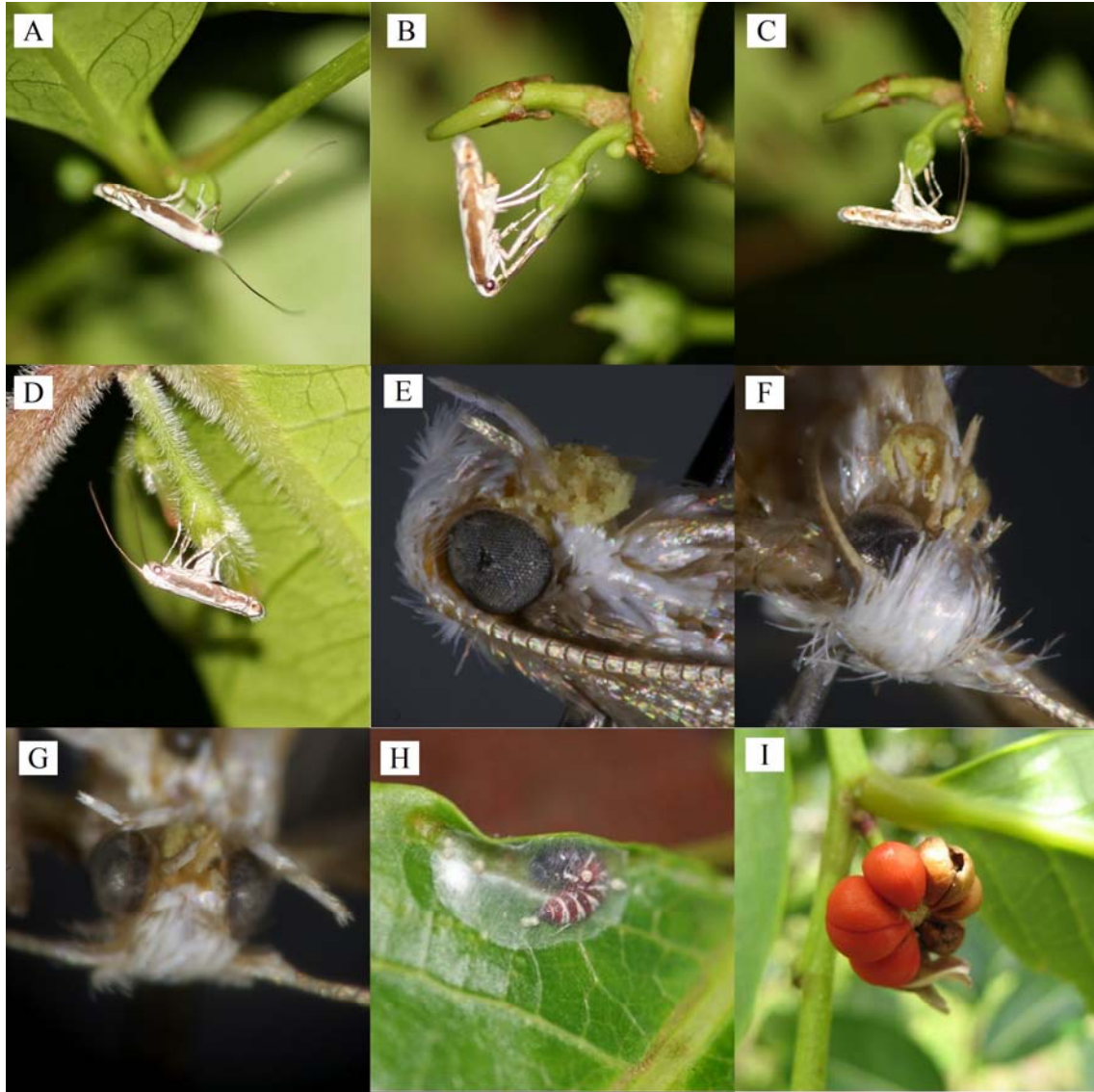
Figure 1: *Glochidion–Epicephala* interaction on Pacific islands. (a) *Epicephala* moth visiting male *G. grayanum* flower, Tahiti. (b) *Epicephala* inserting proboscis into female *G. grayanum* flower, Tahiti. (c) *Epicephala* inserting ovipositor into female *G. grayanum* flower, Tahiti. (d) *Epicephala* inserting ovipositor into female *G. temehaniense* flower, Raiatea. (e) Pollen on the proboscis of an *Epicephala* captured after being observed pollinating and ovipositing into flowers of *G. temehaniense*, Raiatea. (f) Pollen on the proboscis of a female *E. spinula* collected in 1968 on Nuku Hiva, Marquesas Islands. (g) Pollen on the proboscis of a female *Epicephala* collected in 1953 on Pohnpei, Micronesia. (h) *Epicephala* larva emerged from *G. grayanum* fruit and spinning a cocoon, Tahiti. (i) Dehisced fruit of *G. manono*, showing intact seeds (red, left) and seeds consumed by *Epicephala* larva (right, brown), Moorea.

Chapter 2: Phytophagous insect community assembly through niche conservatism on oceanic islands

Chapter 2: Phytophagous insect community assembly through niche conservatism on oceanic islands

INTRODUCTION

The roles of adaptive radiation and niche conservatism in community assembly are a major area of inquiry in community ecology and biogeography (Ackerly 2004; Melville et al. 2006; Donoghue 2008; Crisp et al. 2009; Moen et al. 2009). Nowhere are these questions more clearly envisioned than on islands. The islands of the world are known for a number of spectacular adaptive radiations, but a quick consideration of the limited number of such spectacular examples (Darwin 1859; Perkins 1913; Lack 1947; Carlquist 1974; Schluter 2000; Carlquist et al. 2003; Gillespie 2004; Pratt 2005; Grant and Grant 2008; Losos 2009) will make it clear that adaptive radiations are not inevitable on islands (Losos 2010), and that niche conservatism is likely also widespread in island taxa. Here, adaptive radiation is defined as the evolution of ecological differences among members of a rapidly multiplying lineage (sensu Schluter 2000); it encompasses the evolution of ecological differences between sister lineages, including what is referred to as *in situ* evolution (e.g., Moen et al. 2009). Niche conservatism is defined as conservation of important ecological traits among members of a diversifying lineage; it includes non-adaptive radiations (Rundell and Price 2009). Whether island biotas are assembled through adaptive radiation or by niche conservatism over evolutionary timescales ultimately has great implications for the study of community assembly, as well as island community structure and function.

Research on community assembly on oceanic islands has primarily focused on single lineages that undergo adaptive radiations to form an entire guild or community of species, such as passerine birds, lizards, spiders, and snails (Lack 1947; Chiba 2004; Gillespie 2004; Harmon et al. 2007, 2008; Grant and Grant 2008). In contrast, few studies have explicitly compared adaptive radiation and niche conservatism in phytophagous (plant-feeding) insect lineages on oceanic islands (but see Roderick and Percy 2008; Percy 2011). This neglect is surprising given that insects are a major proportion of both island and continental faunas, and that "niches", "ecomorphs", and "communities" can be defined with much greater precision in specialized phytophagous insects than in many other radiations. Plants on remote islands can provide colonizing insects numerous opportunities to radiate adaptively by shifting to novel hosts and speciating, but adaptive radiation is not the insects' inevitable evolutionary fate. The phytophagous insect community on a given host plant taxon on an island or archipelago can consist of any combination of the following: (a) generalist species that feed on other plant taxa on the same island; (b) specialized species whose closest relatives elsewhere feed on the same plant taxon (niche conservatism); (c) specialized species whose closest relatives feed on some other plant taxon (adaptive radiation); (d) empty niches (plant resources not used by any insects).

The colonization of remote islands by specialized plant-feeding insects—or any other ecologically specialized organism—poses a paradox. Specialists that disperse independently to remote islands might not be able to establish if their host or prey is absent (Holt 2009). It is unlikely that a woody host plant and insects specialized to feed on it could colonize a new island

in a single dispersal event, since any insects would reach reproductive maturity several years before a germinated host plant was mature enough to serve as a host. Synchronous colonization could, however, be permitted by insect dormancy or asexual plant propagules that reproduce vegetatively but also carry insects. Consistent with the hypothesis that it is difficult for specialized insects to colonize islands, it has long been noted that island plant taxa often lack their coevolved mutualistic and parasitic insects from the mainland (Janzen 1973, 1975; Armbruster and Baldwin 1998; Terborgh 2009), and some ecologically dominant insular phytophagous insects are host plant generalists (e.g., *Rhyncogonus* weevils in southeastern Polynesia; Claridge 2006).

However, a number of putative examples of adaptive radiation by insects onto different host plant taxa on oceanic islands are known (Gagné 1968, 1997; Gressitt 1978; Zimmerman 1978; Paulay 1985; Asquith 1995; Percy 2003; Jordal and Hewitt 2004; Jordal et al. 2004; Magnacca et al. 2008; Goodman 2010; Bennett and O'Grady 2011; Polhemus 2011). In contrast, few examples exist of specialized host associations being conserved among oceanic archipelagos or between oceanic archipelagos and continents (Garin et al. 1999; Wiebes 1994; Staddon et al. 2010). There is some degree of overlap between these two processes, since it has been hypothesized that the progenitors of some adaptive radiations were only able to establish on islands because of the presence of hosts closely related to the ancestral hosts (Asquith 1995; Gagné 1997; Percy 2003), and some of these adaptive radiations could also be considered niche conservatism since they did not leave their ancestral host family (Percy 2003; Jordal and Hewitt 2004). Furthermore, adaptive radiations may contain subclades which show niche conservatism. For the purposes of this paper, we will be considering only either end of this continuum: cases of niche conservatism in which insect lineages remain associated with the same host plant family, and cases of adaptive radiation in which insect lineages radiate onto multiple host plant families.

One system ideally suited for examining niche conservatism in phytophagous insects on oceanic islands is the radiation of endemic species of trees in the genus *Glochidion* J. R. Forst. & G. Forst. (Phyllanthaceae; Euphorbiaceae s. l.) in southeastern Polynesia (Figure 1a). The ~300 described species of *Glochidion* are distributed from tropical Asia and Australasia across the south Pacific as far east as the Pitcairn Islands (Govaerts et al. 2000). In both Asia and the Pacific islands, *Glochidion* spp. are found in a wide range of wet to mesic habitats, and many show an affinity for open areas with a history of disturbance (Butaud et al. 2008; Kawakita 2010). The community of phytophagous insects on *Glochidion* in Asia and Australia is well-known (De Prins and De Prins 2005; Horak 2006; Kawakita et al. 2010; D. Hembry, unpublished, A. Kawakita, personal communication, 2010), and includes many internally-feeding taxa which are likely to have constrained host preferences. It is already known that the sole pollinator of *Glochidion* (Lepidoptera: Gracillariidae: *Epicephala* Meyrick) in Asia has successfully co-colonized southeastern Polynesia with its host (Hembry et al. 2012 [Chapter 1, this volume]). The islands of southeastern Polynesia are some of the world's most isolated from continents (≥6,000 km from Australia and ≥3,000 km from Fiji; Fig 1a) and are all formed by midplate volcanoes, meaning that they are likely to pose numerous ecological opportunities for phytophagous insect colonizers.

The goal of this study is to determine the roles of adaptive radiation (host shifts), niche conservatism (conserved host associations), or generalist host associations in the assembly of the insect community on *Glochidion* trees on remote Pacific islands. To accomplish this, we ask what comprises the fauna of internally-feeding insects associated with *Glochidion* in southeastern Polynesia and whether the close relatives of these taxa feed on *Glochidion*, Phyllanthaceae, or other hosts in Asia and Australasia. Finally, we address putative mechanisms that may be responsible for these biogeographic patterns.

MATERIALS AND METHODS

Field collection and rearing
Internally-feeding insect larvae were collected from 202 trees representing 23 spp. of *Glochidion* on 20 islands in the southern Cook, Austral, Society, Tuamotu-Gambier, and Marquesas archipelagos in the Cook Islands and French Polynesia (Figure 1b) over a period of three years from 2007 to 2009. Insect larvae were searched for visually and reared from fruits, leaf mines, leaf rolls, tied leaves, the surface of leaves, and pupae found on *Glochidion* spp. Rearing was done in plastic bags or in plastic rearing containers in the field. To assess whether leaf-mining gracillariids had host ranges broader than *Glochidion*, we reared all other leaf-mining insect larvae found on other host plants during this fieldwork in southeastern Polynesia. We also examined the three native, extant *Phyllanthus* L. sensu stricto in this region (*P. societatis* on 'Ātiu in the Cooks, *P. pinaiensis* on Moorea in the Societies, and *P. pacificus* in the Marquesas) for leaf-feeding and seed-feeding insects. The only other potentially native Phyllanthaceae in southeastern Polynesia is *Bischofia javanica*, of doubtful indigenity and extreme rarity (McCormack 2007; Butaud et al. 2008); we did not examine it.

Most adult insects that eclosed, and some larvae, were preserved in ethanol for future molecular work. Rearing from larvae provides stronger records of host-plant use than some other methods (e.g., sweeping). Most Cook Islands larvae were killed immediately in ethanol because it was prohibitive to obtain an import permit into French Polynesia, where the lead author (DHH) was based. Rearing records from the Cook Islands thus do not accurately reflect abundances in the field.

Voucher specimens of *Glochidion* (same as Hembry et al. 2012 [Chapter 1, this volume]) have been deposited in the Herbarium Pacificum (BISH), Bishop Museum, Honolulu, USA (Cook Islands specimens), and the University Herbarium (UC), University of California, Berkeley, USA (French Polynesian specimens). Voucher specimens of moths will be deposited in the Bishop Museum.

Literature and museum search for host records
To determine whether host associations from southeastern Polynesia represented examples of niche conservatism, we concurrently conducted a literature search for other host records worldwide for each of the most abundant insect genera collected in the field (which were all moths). For additional information on host associations of Gracillariidae, we examined host records of specimens at the Hokkaido University Museum (Sapporo, Japan), based on the extensive field rearing surveys by Tosio Kumata in tropical Asia. Specifically, we aimed to

determine whether each of the genera under consideration is known only from Phyllanthaceae or Euphorbiaceae s. l. on continents and on Pacific islands; such cases would represent examples of niche conservatism.

Although niche conservatism is usually assessed through the analysis of traits on a phylogeny, the taxonomy-based approach we used here is valid for assessing niche conservatism in this system because extensive information on the relevant ecological trait (host plant) was available from the literature and museum collections for each of these genera.

Taxonomic status of Pacific island Glochidion

Numerous analyses support the monophyly of *Glochidion* sensu lato (s. l.) within a paraphyletic *Phyllanthus* sensu stricto (s. s.) (Kathriarachchi et al. 2006; Kawakita and Kato 2009; Luo et al. 2011). Accordingly, Hoffman et al. (2006) proposed that the genus *Glochidion* be placed in synonymy within a monophyletic *Phyllanthus* sensu lato, but refrained from formally transferring all *Glochidion* species to *Phyllanthus* because of the large number of species and the numerous synonymous epithets requiring resolution. For the Flora of the Marquesas project, Wagner and Lorence (2011) transferred all described Polynesian *Glochidion* to *Phyllanthus* and erected new species epithets where necessary to prevent homonymy. Because we expect all species of *Glochidion* s. l. to be eventually given valid names in *Phyllanthus* (Chakrabarty and Balakrishnan 2009), here we use the Wagner and Lorence combinations (if available) in *Phyllanthus* to refer to individual species of *Glochidion* s. l. from southeastern Polynesia. However, we use the name *Glochidion* to refer to *Glochidion* s. l. (the clade *Glochidion*, including the Polynesian species), *Glochidion* s. s. to refer to continental *Glochidion*, and *Phyllanthus* s. s. to refer to species not in *Glochidion* s. l. (e.g., the traditional circumscription of *Phyllanthus* ignoring Wagner and Lorence). There are four native species of *Phyllanthus* s. s. in southeastern Polynesia (Florence 1997; McCormack 2007). For synonyms, see Wagner and Lorence (2011).

RESULTS

Field surveys

A total of 509 insect individuals were reared from internally-feeding larvae and pupae on *Glochidion* on 20 islands (Table 1; Appendices 3, 4). This number is much lower than the total number of insects collected, due to mortality during the rearing process. Based on our experience learning to recognize different larval taxa and their feeding damage during this process, we believe that the sample of insects reared is an accurate reflection of the fauna of insects collected as larvae. Ninety percent of these insect specimens fall into five taxa that are also known to attack *Glochidion* in Asia and Australasia (Table 1; Appendix 5). These taxa are *Epicephala* (Lepidoptera: Gracillariidae; 50% of the total), leaf-mining *Diphtheroptila* Vári moths (Gracillariidae; 16%), leaf-mining *Caloptilia* Hübner moths (Gracillariidae; 1%), seed-feeding *Tritopterna* Meyrick moths (Tortricidae; 18%), and leaf-rolling *Dudua* Walker moths (Tortricidae; 4%).

To the best of our knowledge, these are the first published records of *Diphtheroptila* from the Society, Austral, and Tuamotu archipelagos; of *Caloptilia* from the Societies; of *Tritopterna* from the northern Australs; and of *Dudua* from the northern Australs and Tuamotus.

The remaining 10% of insect specimens reared represent several rare taxa. The two most distinctive were an unidentified moth (Lepidoptera: Gelechioidea) whose leaf-scraping larvae made frass and silk tubes along the upper surface of *Glochidion* leaves, and leaf-mining flies (Diptera: Agromyzidae). Frass-tube gelechioids and their distinctive feeding damage were restricted to the Windward Society Islands (Tahiti and Moorea); similar frass-and-silk tubes were never observed on other native or introduced plants (D. Hembry, unpublished notes). *Glochidion*-mining agromyzids and their mines, which are distinctive, were restricted to the Leeward Societies and Rapa, although they were only successfully reared on Rapa. However, agromyzids were observed and reared from a number of other native and introduced plant genera on many islands (D. Hembry, unpublished data) and will not be considered further.

A striking feature of the rearing survey was the small number of hymenopterans, including parasitoids, which eclosed (10 individuals), despite the hundreds of lepidopteran larvae reared. Those we did find were from leafrolls (one each from Maupiti and Moorea, Societies), frass-tube gelechioids (two from Tahiti), or leafmines (three from Ua Pou, Marquesas). Three braconids were reared from unrecorded host larvae on Fatu Hiva (Marquesas). In contrast, seed-feeding or *Epicephala*-parasitic braconids are common in *Glochidion* fruit in Asia, Fiji, and American Samoa (D. Hembry, unpublished data; A. Kawakita, personal communication, 2010).

No leaf mining larvae or empty mines were found on *Phyllanthus wilderi* on Mangareva (Gambier Islands), in contrast to all other archipelagos. Although we examined three species of native *Phyllanthus* s. s. for insect larvae in seeds and leaves, the only insects found were *Diphtheroptila* mining the leaves of *P. pinaiensis* on Moorea (Societies). Very few other leaf-mining Lepidoptera were found on other native plants, consistent with earlier surveys which suggested a depauperate fauna (Clarke 1971, 1986). Taxa found were *Macarostola* (Gracillariidae) on *Metrosideros* (Myrtaceae) in the Societies, northern Australs, and Rapa (previously reported by Clarke 1971); unidentified larvae not successfully reared from *Pipturus*, *Boehmeria*, and *Cypholophus* (Urticaceae) in the Societies and northern Australs; and unidentified microlepidoptera on *Myrsine* (Myrsinaceae) in the Societies.

Literature and museum surveys for host records
Host record information is provided in Appendix 5.

DISCUSSION

Conserved host associations and niche conservatism between Asia and Polynesia
This study finds that the numerically dominant insect taxa that feed internally on *Glochidion* in southeastern Polynesia are taxa which also feed on the same host plants in the west Pacific and Asia. The three most abundant of these taxa (*Epicephala*, *Diphtheroptila*, *Tritopterna*; 84% of the insects reared) are only known from Phyllanthaceae or Euphorbiaceae s. l. in Asia and Australasia (Meyrick 1880; Meyrick 1907; Vári 1961; Meyrick 1969; Robinson et al. 1994; Kato

et al. 2003; De Prins and De Prins 2005; Horak 2006; Kawakita and Kato 2009; Kawakita et al. 2010; Hu et al. 2011; T. Kumata, personal communication, 2010) and so their presence on *Glochidion* in southeastern Polynesia represents examples of niche conservatism. This result is robust to phylogenetic analysis, because each of these insect genera is specialized on this host plant family. If *Glochidion*-feeding *Caloptilia* (Kumata 1966, 1982; Clarke 1986; Kawakita et al. 2010; Robinson et al. 2010) and *Glochidion*-feeding *Dudua* (Bradley 1953; Esaki et al. 1971; Clarke 1976, 1986; Horak 2006) are each monophyletic across the Asia-Pacific region (Appendix 5), they may also represent examples of niche conservatism. The association of this set of taxa with the same host plants in both the Asian and Australasian (sub)tropics and the central Pacific indicates a substantial role for niche conservatism in the assembly of this phytophagous insect community. More importantly, they indicate that specialization on particular host plants does not prevent insects from successfully colonizing islands, provided their hosts are present.

Whereas these results demonstrate the role of niche conservatism in the assembly of this insect community, they do not preclude the possibility that some of these lineages have adaptively radiated onto other host plant families since colonizing this region. (This possibility is however unlikely in the case of *Diphtheroptila* and *Caloptilia*, given that leaf-miner larvae were searched for and reared on all other woody plant taxa.) These results also do not bear on the possibility that these insects have evolved narrower or broader host breadth within *Glochidion* or Phyllanthaceae than their continental relatives (Kawakita et al. 2010), or on the process of lineage diversification within each taxon across southeastern Polynesia.

Such niche conservatism between continents and oceanic islands across ≥6000 km of ocean has rarely been demonstrated (Wiebes 1994; Hembry et al. 2012 [Chapter 1, this volume]). However, despite the great distance between Asia/Australasia and southeastern Polynesia, because of the high density of islands in this region, inter-island distances are always ≤1000 km. Specialized phytophagous insects might be able to colonize this area in a stepwise fashion by using islands of the western and central Pacific archipelagos as stepping stones to colonize southeastern Polynesia. Consequently, the effective isolation of southeastern Polynesia to colonists may be much less than its distances from the nearest continents suggests. Most of western and central Pacific islands have *Glochidion* on them, with the exception of atolls (Smith 1981; Govaerts et al. 2000). As a result, herbivorous insects specialized on *Glochidion* may have been able to colonize the islands of the south Pacific in a stepping-stone fashion, and establish successfully on each new island as long as their host was already present there.

It is extremely unlikely that human-mediated dispersal has played a role in the assembly of this community. *Glochidion* are not considered to have been among the plants transported by Polynesians between archipelagos (Whistler 2009), and there is no evidence in the ethnobotanical literature or language dictionaries from the Cooks or French Polynesia that *Glochidion* were planted or facilitated by Polynesians (Chabouis and Chabouis 1954, Pétard 1986, Buse 1995; Académie Tahitienne 1999; Whistler 2009). If *Glochidion* were transported by Polynesians, it would be expected that some species would be shared between the Polynesian homeland (Samoa and Tonga; Kirch 2000) and southeastern Polynesia, or between archipelagos within southeastern Polynesia. However, all southeastern Polynesian *Glochidion* are endemic, with one exception (*P. concolor* in Fiji and Rarotonga; Wagner and Lorence 2011) that is likely

to be two distinct taxa based on molecular data (Chapter 3, this volume). Additionally, with one exception which is likely overlumped (*P. florencei*; D. Hembry, unpublished) all species are single-archipelago endemics (Florence et al. 1995; Florence 1997; Wagner and Lorence 2011). There is no widespread, low-elevation species of *Glochidion* throughout southeastern Polynesia, and today they rarely grow near sea level where the majority of people live.

Pacific diversity gradient
We also find that the insect fauna feeding on *Glochidion* in southeastern Polynesia is a subset of the taxa known to feed on *Glochidion* in Asia and the west Pacific (Figure 3). Taxa absent from southeastern Polynesia include leaf-mining bucculatricid moths (Japan; A. Kawakita, personal communication, 2010); unidentified stem-boring moths (in Japan and Malaysia; A. Kawakita, D. Hembry, unpublished data); another seed-feeding moth taxon (Pyralidae: *Cryptoblabes*; Kawakita et al. 2010); leaf-rolling weevils (Attelabidae in Malaysia; D. Hembry, unpublished data); braconid wasps (Hymenoptera), some species of which parasitize *Epicephala* larvae and some of which gall *Glochidion* fruit (A. Kawakita, D. Hembry, unpublished data; Japan, Malaysia, Fiji, American Samoa); and *Aristotelia galeotis* (Gelechiidae) (Robinson et al. 1994). The apparent absence of many of these taxa in southeastern Polynesia is consistent with the Pacific diversity gradient, in which species or lineage diversity of numerous terrestrial and nearshore marine taxa decreases, and taxonomic disharmony increases, from west to east across the Pacific Basin (reviewed in Steadman 2006; however, this pattern is in some cases confounded by island area).

The absence of certain taxa (predicted by theory; Holt 2009) may have strong effects on the densities of other community members. For instance, almost no parasitoids were obtained despite the huge number of lepidopteran larvae reared. Absence of parasitoids may be responsible for the high densities of *Diphtheroptila* seen on many islands in southeastern Polynesia. Likewise, the absence of braconids (with the possible exception of Fatu Hiva in the Marquesas), which either compete with or parasitize *Epicephala* larvae in Asia (R. Goto, personal communication, 2011), may have a strong effect on the stability and fitness outcome of the mutualism between *Glochidion* and *Epicephala* in Polynesia. These effects may have important consequences that lead to differences in community structure and function between islands and continents.

Adaptive radiation and niche conservatism in phytophagous insects on islands
This study finds that niche conservatism plays an important role in assembling this phytophagous insect community on remote oceanic islands thousands of kilometers from continents. Is this a common pattern for phytophagous insects on islands? Besides this study, a few examples are known of apparent niche conservatism between oceanic archipelagos, or between archipelagos and continents. These include multiple lineages of leaf beetles in the Canary Islands (Garin et al. 1999) which conserved their ancestral host associations but failed to diversify *in situ*; the pollinating seed-predatory fig wasp *Platyscapa innumerabilis* (Hymenoptera: Agaonidae) which apparently uses the same fig host (*Ficus prolixa*) in the Cooks, Marquesas, and Guam (Fullaway 1913; Grandi 1939; Wiebes 1994; Staddon et al. 2010); and leafmining *Macarostola* moths (Gracillariidae) on *Metrosideros* in the southern Cooks, Australs, and Societies (Clarke 1971; McCormack 2007; this study). In contrast, numerous putative adaptive radiations onto different

host plant families have been reported, primarily from the Hawaiian insect fauna. These include Hawaiian *Drosophila* (Magnacca et al. 2008), Hawaiian *Plagithmysus* beetles (Cerambycidae; Gressitt 1978), Hawaiian *Nesophrosyne* leafhoppers (Cicadellidae; Bennett and O'Grady 2011), Hawaiian leafmining *Philodoria* moths (Gracillariidae; Zimmerman 1978), multiple clades of Hawaiian plant bugs (Miridae: *Nesiomiris*, *Cyrtopeltis*, *Sarona*, *Orthotylus*; Gagné 1968, 1997; Asquith 1995; Polhemus 2011), *Nesosydne* planthoppers in Hawaii and the eastern Pacific (Delphacidae; Goodman 2010), *Miocalles* weevils on Rapa, Austral Islands (Curculionidae; Paulay 1985), and *Liparthrum* bark beetles in the Canary Islands and Madeira (Curculionidae; Jordal et al. 2004).

Taken at face value, these examples suggest that that niche conservatism may be uncommon in phytophagous insects on islands; in other words, it might be more likely that niches on host plants will be filled by adaptive radiation in situ than by specialist insects colonizing from elsewhere. What, then, determines whether phytophagous insects on oceanic islands continue to attack their ancestral hosts or evolve to attack new hosts? Broadly, there are two categories of explanations: factors intrinsic to the organisms themselves (evolutionary constraints or evolvability), and factors extrinsic to the organisms (ecological opportunity) (Schluter 2000, Losos 2010). Intrinsic factors would include aspects of the biology of host-plant specialization that make host-shifts difficult to evolve. It is well-recognized that host-plant specialization is a complex trait, and many barriers exist to host-shifts (Schoonhoven et al. 1998, Matsubayashi et al. 2010), although the extent to which host-shifts are evolutionarily constrained may vary across insect groups. Extrinstic factors in this case would include island area and age, the presence of suitable host plants, and the absence of competitors (presumably correlated with isolation).

It is worth noting that most of the putative examples of adaptive radiation in host use are seen in Hawaii (Gressitt 1978; Zimmerman 1978; Gagné 1968, 1997; Asquith 1995; Magnacca et al. 2008; Bennett and O'Grady 2011). In principle, the colonizing insect fauna should be similar across all these midplate Pacific archipelagos, and thus the intrinsic evolutionary constraints for the fauna as a whole on each archipelago should be similar. In contrast, southeastern Polynesia differs from Hawaii in two extrinsic factors that might predispose its biota towards less adaptive radiation: first, it is less isolated (because of the presence of many intermediate islands), and second, its islands are much smaller (Tahiti, by far the largest at 1000 km^2, would be the fifth largest of the Hawaiian Islands). Consequently, assuming the observed patterns are not an artifact of investigator bias (see below), it is possible that in this plant-insect community in southeastern Polynesia, dispersal occurs sufficiently frequently and ecological opportunities are sufficiently restricted, that dispersal plays a more important role than adaptive radiation in assembling its biota relative to Hawaii. It is worth noting that radiations of many other taxa (including passerine birds, woody Asteraceae, lobeliads, and *Tetragnatha* spiders) have far less ecological diversity in southeastern Polynesia than in Hawaii (Carlquist 1974; Gillespie 2003a, 2003b, 2004; Steadman 2006; Fleischer et al. 2008; Givnish et al. 2009; Lerner et al. 2011; Price and Wagner 2011). This hypothesis would imply that adaptive radiation onto new host plants is difficult, and overcome only when islands are sufficiently large and/or isolated to reduce the importance of dispersal and increase that of *in situ* evolution, as in Hawaii.

However, these apparent patterns of many adaptive radiations in certain archipelagos and a few examples of niche conservatism in others may also result in part from investigator bias. Hawaii and the Canaries have been well-studied because of their proximity to the United States and Europe, and extremely isolated islands such as Rapa have long attracted a great deal of interest from entomologists (Clarke 1971; Paulay 1985). It is likely that there are unreported and unrecognized adaptive radiations of phytophagous insects in southeastern Polynesia. Ultimately, our understanding of adaptive radiation and niche conservatism in these systems is hampered by our poor understanding of phytophagous insect diversity and their host associations among archipelagos and between archipelagos and continents. Comparisons of the same taxa across multiple archipelagos, as well as standardized surveys of insects in numerous functional groups associated with the same host plant taxon between archipelagos and continents would be particularly revealing. These kinds of studies would be illuminating not only in our understanding of the evolution of host breadth and host shifts in phytophagous insects, but in our understanding of the roles of niche conservatism and adaptive radiation in assembling biological communities in general.

Conclusions
This study finds that specialization to particular hosts does not necessarily prevent phytophagous insects from colonizing remote oceanic islands, and that consequently, niche conservatism can play a strong role in the assembly of these phytophagous insect communities. These findings indicate that even remote oceanic island communities can represent less species-rich examples of their tropical continental equivalents, and have implications for efforts to understand why certain organisms remain phylogenetically conservative in their ecology, whereas others undergo adaptive radiation.

Figure 1. a. Map of Pacific Basin with southeastern Polynesia indicated. b. Map of southeastern Polynesia, with archipelagos and islands mentioned in text indicated.
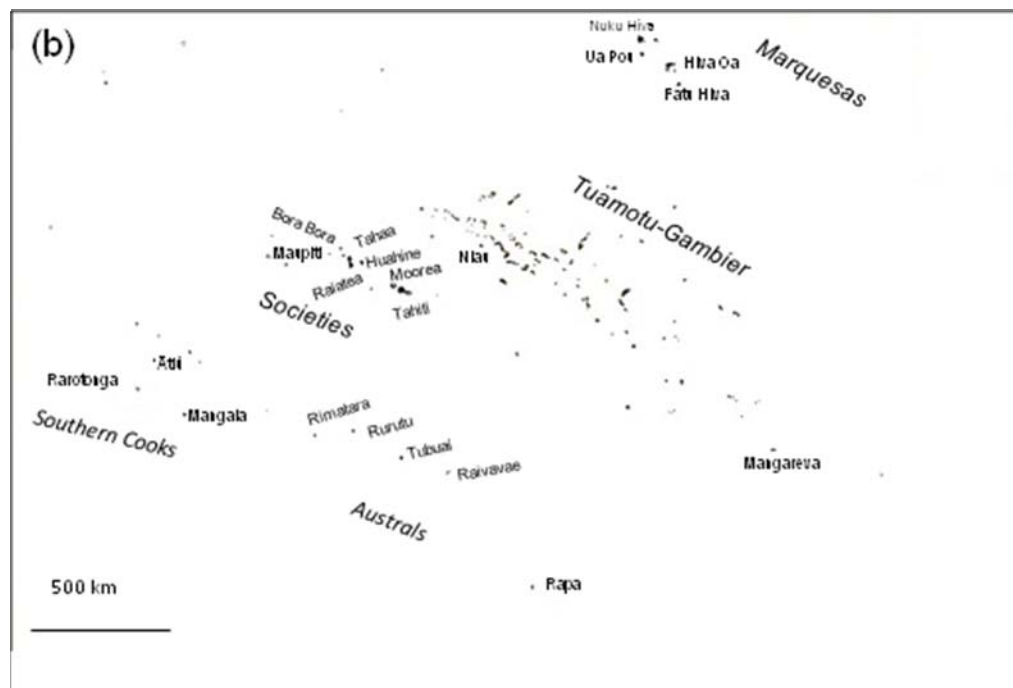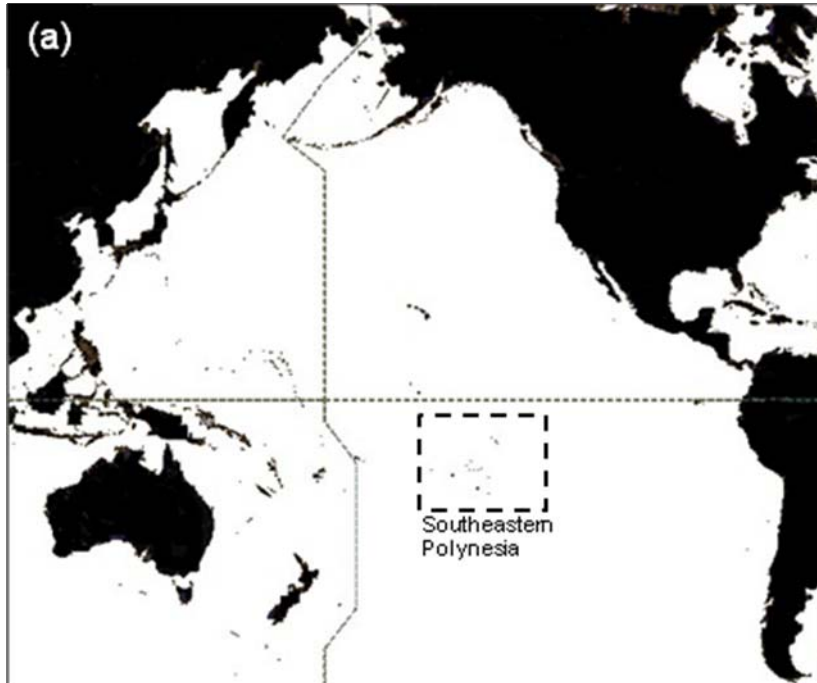
Table 1: Results of the rearing survey compared to information from continental faunas. Number of specimens of each insect taxon reared from *Glochidion* s. l. in each archipelago. Numbers do not include larvae or pupae that died before adult eclosion. Archipelagos are subdivided to reflect geology and geography: the Australs are divided into the Northern Australs and Rapa, and the Societies into the Leeward and Windward Islands. Niau is in the Tuamotus. Percent total refers only to southeastern Polynesian samples from this survey, out of the total of 509 insect specimens reared (Appendix 3).

*Previously reported on *Glochidion* s. l. (Clarke, 1986; McCormack, 2007; Hembry *et al*. 2012 [Chapter 1, this volume]) but not reared in this study.

†Agromyzid mines present but adults never successfully reared.

	Regions										Total	%
Taxa	Japan	Southeast Asia	Australia	Southern Cooks	Northern Australs	Rapa	Societies (Leeward)	Societies (Windward)	Niau	Marquesas		
Epicephala (Lepidoptera)	P	P	P	P*	3	22	97	104	3	26	255	50
Diphtheroptila (Lepidoptera)	P	P	P	1	12	0	24	43	2	0	82	16
Caloptilia (Lepidoptera)	P	0	P	1	0	1	1	2	0	1	6	1
Tritopterna (Lepidoptera)	P	P	P	1	10	13	20	26	0	22	92	18
Dudua (Lepidoptera)	P	0	P	1	8	0	9	2	2	P*	22	4
Frass-tube gelechioids (Lepidoptera)	0	0	0	0	0	0	0	8	0	0	8	2
Bucculatricidae (Lepidoptera)	P	0	0	0	0	0	0	0	0	0	0	0
Peragrarchis (Lepidoptera)	P	0	0	P*	0	0	0	0	0	0	0	0
Cryptoblabes (Lepidoptera)	P	0	0	0	0	0	0	0	0	0	0	0
Stem-boring moths (Lepidoptera)	P	P	0	0	0	0	0	0	0	0	0	0
Aristotelia (Lepidoptera)	0	P	0	0	0	0	0	0	0	0	0	0
Agromyzidae (Diptera)	P	0	0	0	0	2	P†	0	0	0	2	0
Attelabidae (Coleoptera)	0	P	0	0	0	0	0	0	0	0	0	0
Braconidae (Hymenoptera)	P	P	0	0	0	0	0	0	0	0	0	0

Chapter 3: Uncoupled colonizations of southeastern Polynesia reveal dynamics of diversification in a coevolving, obligate pollination mutualism

Chapter 3: Uncoupled colonizations of southeastern Polynesia reveal dynamics of diversification in a coevolving, obligate pollination mutualism

INTRODUCTION

Coevolution, or reciprocal selection between two or more taxa, is widely invoked as a major force promoting the continuing diversification of life on earth (Ehrlich and Raven 1964; Vermeij 1994; Schluter 2000; Thompson 2005; Futuyma and Agrawal 2009; Yoder and Nuismer 2010). Over the past two decades, the advent of geographic mosaic theory of coevolution (Thompson 1994, 2005) has led to great advances in understanding coevolutionary diversification among populations, and the mechanisms by which it occurs (Benkman et al. 2001; Thompson and Cunningham 2002; Brodie et al. 2002; Toju and Sota 2006). However, coevolutionary diversification at the level of speciation or clade diversification remains less well understood, with studies conflicting on the importance of coevolution (Parchman and Benkman 2002; Smith and Benkman 2007; Godsoe et al. 2008, 2009; Kay and Sargent 2009; Yoder and Nuismer 2010; Althoff et al. 2012), and suggesting a range of mechanisms (Ehrlich and Raven 1964; Vermeij 1987; Thompson 1989; Schluter 2000; Machado et al. 2005; Godsoe et al. 2008; Smith et al. 2008; Rabosky and Glor 2010; Ricklefs 2010; Althoff et al. 2012). As in other areas of biology, phylogenetic information has great potential to illuminate our understanding of coevolutionary diversification, particularly at the scale of coevolving clades where important coevolutionary dynamics are likely taking place. However, despite great interest in both coevolution and phylogenetics over the past two decades, very few detailed phylogenetic studies of putatively coevolving clades are available (Thompson 2005, 2011; but see Ramírez et al. 2011; Althoff et al. 2012).

For a long time, efforts to incorporate phylogenetic information into coevolutionary biology have focused heavily on cophylogenetic analyses, in which the phylogenies of reciprocally specialized hosts and associates are compared to determine if the topologies are significantly similar (Page 2003; Kawakita et al. 2004; Quek et al. 2004). The field of cophylogenetics often uses the term "coevolution" to refer to long-term association that results in phylogenetic congruence; here, we instead refer to such processes as "cospeciation" and use "coevolution" to refer to "reciprocal natural selection among taxa", the definition most widely used over the past three decades (Janzen 1980; Thompson 1994, 2005). Absolute congruence is rare in non-endosymbiotic mutualisms, but many host-associate interactions show more phylogenetic congruence than would be expected by chance (Page 2003; Kawakita et al. 2004). Incongruence can be explained by a number of factors, including the process of phylogenetic inference itself, gene introgression, host-shifts, associate duplication (speciation) on the same host, lineage sorting (analogous to the same process in population genetics), and the failure of either the host or associate to speciate when the other does. Distinguishing amongst these mechanisms has proven difficult (Page 2003), and is often contingent on taxon sampling. Furthermore, non-coevolutionary processes, such as shared responses to the same vicariant event, can result in phylogenetic congruence, and some host-associate interactions may not be coevolutionary.

Whereas congruent phylogenetic patterns between host and associate alone neither proves the role of coevolution in diversification (Thompson 2005) nor demonstrates how speciation occurs (Losos and Glor 2003), such congruence leads to predictions for the diversification of two coevolving clades, and can narrow the range of potential mechanisms operating in their diversification. For instance, the potential role and mechanisms of coevolution in speciation are very different if hosts and associates cospeciate frequently, than if associates track changes in their hosts after hosts have already diversified, if associates shift hosts frequently, or if phylogenies show no congruence. It has been argued that phylogenetic congruence should not be expected in cases where antagonistic coevolution is promoting diversification (Thompson 1989) and the same may also be true for mutualistic coevolution. Phylogenies can indicate a role for geographic isolation (Thompson and Rich 2011; Althoff et al. 2012), asynchronous diversification by hosts and associates (Ramírez et al. 2011; Althoff et al. 2012), or widespread host-sharing by pollinators that may facilitate or constrain speciation (Machado et al. 2005).

Some of the major study systems in the field of coevolutionary diversification have been pollinating seed-predation mutualisms. These interactions, such as the fig/fig wasp (Herre et al. 2008), yucca/yucca moth (Pellmyr 2003), leafflower/leafflower moth (Phyllantheae/*Epicephala*; Kawakita 2010), globeflower/globeflower fly (Pellmyr 1992), and saxifrage/*Greya* moth (Thompson 2011) mutualisms, have only arisen a few times in the entire coevolutionary history of insects and angiosperms (Pellmyr 1997). The focus of study on these associations is primarily because in these systems pollinators often control gene flow in their hosts (Thompson 1994), reciprocal coadaptations indicate that coevolutionary selection occurs (Pellmyr 2003; Herre et al. 2008; Kawakita 2010), and high species diversity and reciprocal specialization are sometimes evident (Pellmyr 2003; Herre et al. 2008; Kawakita 2010). In these mutualisms, specialized insects pollinate flowers of their hosts, but oviposit in the inflorescences such that their larvae can feed on a subset of the hosts' developing seeds. Furthermore, these interactions are usually marked by high reciprocal species specificity, in which most plant species are pollinated by one or a few insect species locally, and vice versa. Cophylogenetic analyses of these systems suggest that phylogenies of plants and pollinators are significantly more congruent than would be expected by chance, at least at some phylogenetic scales (Kawakita et al. 2004; Herre et al. 2008; Althoff et al. 2012), but exact congruence is limited to some cases of sister-species pairs (Smith et al. 2008). This lack of exact congruence has been attributed to host-shifts (Kawakita et al. 2004), lineage sorting (Kawakita et al. 2004), and the coexistence of multiple pollinators on the same host (Kawakita and Kato 2006; Herre et al. 2008). However, only a few studies have appropriate taxon sampling from demonstrably recent monophyletic groups to allow for distinguishing amongst these processes, or for obtaining insights into the mechanisms by which diversification occurs. The most taxonomically complete study, on yuccas and yucca moths (Althoff et al. 2012) suggested more cospeciation than expected at random, but also a large number of host-shifts and cases where the yucca host speciated but its pollinator failed to do so. Studies that explicitly address incipient speciation in these systems tend to suggest that geographic isolation is important for diversification, even if coevolution also has a role (Yokoyama 2003; Godsoe et al. 2008; Smith et al. 2008; Althoff et al. 2012). Also, it is uncertain to what extent these different systems diversify similarly, given the important biological differences among them. For this reason, among others, parallel studies of different mutualisms are needed.

Cophylogenetic understanding of the recently discovered mutualism between leafflower plants (Phyllanthaceae: Phyllantheae) and leafflower moths (Lepidoptera: Gracillariidae: *Epicephala*) (Kato et al. 2003; Kawakita 2010) is potentially of great value for comparison to patterns observed in the yucca/moth and fig/wasp systems. In this research, we focus on the best-studied clade of leafflower plants, the genus *Glochidion* sensu lato (Kato et al. 2003; Kawakita and Kato 2006; Okamoto et al. 2007; Goto et al. 2010). As with yuccas, figs, and clades of *Epicephala*-pollinated leafflowers in general, *Glochidion* is highly diverse, with ~300 species described from tropical Asia, Australasia, and the Pacific islands (Govaerts et al. 2000). The biological intimacy of the mutualism (Kawakita 2010), co-adapted traits of the associates (Okamoto et al. 2007; Kawakita 2010), dependency of the two clades on each other for their life cycles (Kato et al. 2003; Kawakita 2010; Hembry et al. 2012 [Chapter 1, this volume]), and convergent evolution of floral morphology of *Glochidion* with other *Epicephala*-pollinated Phyllantheae lineages (Kawakita and Kato 2009) all indicate that *Glochidion* and *Epicephala* have coevolved, but do not indicate how they diversified. Previous analyses have found that the phylogenies of *Glochidion* and its clade of *Epicephala* pollinators are more congruent than would be expected by chance, but are not exactly congruent (Kawakita et al. 2004) and, in at least one case, two *Epicephala* species coexist sympatrically on the same host (Kawakita and Kato 2006), suggesting either host shifts or sorting during their diversification.

In this paper, we examine the co-radiation of 24 described species of *Glochidion* and their *Epicephala* pollinators in southeastern Polynesia (Cook Islands, French Polynesia, and Pitcairn Islands; Figure 1a) (Florence et al. 1995; Florence 1997; McCormack 2007; Hembry et al. 2012 [Chapter 1, this volume]), which is likely to represent a recent, *in situ* radiation resulting from a limited number of colonization events. The high islands of southeastern Polynesia comprise a series of archipelagos (the southern Cook, Society, Austral, Tuamotu-Gambier, Marquesas, and Pitcairn islands; Figure 1b), all of which were formed by volcanoes as the Pacific plate moved over a series of stationary hotspots (Clouard and Bonneville 2001). With the exception of the Miocene-age northern Austral and Gambier islands, these islands were either formed or subaerially uplifted during the Pliocene and Pleistocene (past 5 million years; see references in Clouard and Bonneville 2005). All *Glochidion* species in this region are endemic to single archipelagos (with two exceptions (Wagner and Lorence 2011)), show great diversity in floral morphology (Florence 1997), and constitute one of the largest endemic plant radiations in this biodiversity hotspot (Meyer 2004). Most islands in this region have 1—3 described *Glochidion* species, but the larger islands have 4—7 species (Florence et al. 1995; Florence 1997; McCormack 2007), where multiple species often occur sympatrically (Meyer and Butaud 2003; Meyer and Taputuarai 2006; Meyer 2009; Jacq and Butaud 2010). These factors suggest that this great diversification has occurred on very recent evolutionary timescales.

Glochidion and *Epicephala* in southeastern Polynesia represent a specialized, tropical insect-plant mutualism that has co-radiated across oceanic islands and thus provides an opportunity to examine patterns of diversification between coevolving clades in fine detail. Here we ask (1) how many times *Glochidion* and *Epicephala* have each colonized southeastern Polynesia, (2) whether *Glochidion* and *Epicephala* show phylogenetic congruence in this region, and (3)

whether detailed taxon sampling shows evidence for cospeciation, sorting (duplication followed by extinction), or host shifts in the codiversification of these mutualists.

MATERIALS AND METHODS

a. Sampling
We collected specimens of 22 (out of 24) species of *Glochidion* from 21 islands in southeastern Polynesia (Cook Islands and French Polynesia) for a total of 35 OTUs (one sample per species per island) and added to these 60 additional *Glochidion* taxa from American Samoa, Wallis and Futuna, Fiji, New Caledonia, Australia, and Asia (Table 1). From the same *Glochidion* trees in southeastern Polynesia, we collected 32 *Epicephala* specimens (one per host plant species per island) and acquired an additional 46 *Epicephala* specimens from *Glochidion* in American Samoa, Fiji, New Caledonia, Australia, and Asia (Table 1). Both sets of specimens were augmented with *Glochidion* and *Epicephala* data from 18 taxa each from a previous study (Kawakita et al. 2004).

b. Molecular phylogenetic methods
For *Glochidion*, we sequenced 1500 bp of nuclear ribosomal internal transcribed spacer (ITS) and external transcribed spacer (ETS) regions, as well as 700 bp of chloroplast maturase K (matK). For *Epicephala*, we sequenced 500 bp of cytochrome oxidase I (COI) and 1500 bp of nuclear arginine kinase (ArgK) and elongation factor 1-α (EF1- α). Primers, polymerase chain reactions, and Sanger sequencing followed previously published protocols for these genes (White et al. 1990; Downie and Katz-Downie 1996; Kawakita et al. 2004; Kawakita and Kato 2009). We discarded matK because the percentage of variable sites was too low throughout *Glochidion*.

c. Phylogenetic analysis
DNA sequence data was edited in Sequencher (Gene Codes Corporation, Ann Arbor, MI, USA), aligned using MUSCLE (Edgar 2004), and trimmed using Mesquite 2.74 (Maddison and Maddison 2011). Alignments were checked by eye. Alignment of the ITS and ETS regions of *Glochidion* required several gaps of 1—10 base pairs between the two non-*Glochidion* outgroups and the remainder of the dataset; these gaps were left in for phylogenetic analyses. The three regions sequenced for *Epicephala* are protein-coding and contained no gaps. We conducted phylogenetic analysis of both datasets using Bayesian inference as implemented in MrBayes 3.1 (Ronquist and Huelsenbeck 2003), with initial model selection using jModelTest (Guindon and Gascuel 2003; Posada 2008). Bayesian analyses were run for 20 million generations, with convergence determined by average standard deviation of split frequencies, and 25% of generations discarded as burn-in. The outgroup for the *Glochidion* analyses was *Breynia distica* (Phyllanthaceae) and for *Epicephala* was the undescribed *Epicephala* species from *Phyllanthus amarus*, following results from a previous study (Kawakita and Kato 2009).

d. Divergence time estimation
We estimated ages of the southeastern Polynesian clades of both taxa in BEAST v. 1.7 (Drummond et al. 2012). Available time-calibration points for the *Glochidion* and *Epicephala* phylogenies were limited. For *Glochidion*, we conservatively set the age of the divergence between *Glochidion* and its sister taxon (*Phyllanthus roseus*) (Kawakita and Kato 2009) to the

minimum age of two *Glochidion* fossils from the middle Miocene of India (11.6 Ma) (Prasad 1994; Antal and Prasad 1996), and constrained the age of the three *Glochidion* endemic to Rapa (*P. longfieldiae*, *P. rapaense*, and *P.* sp.) to less than the age of Rapa (4.8 Ma) (Clouard and Bonneville 2005). For *Epicephala*, we used a lognormal relaxed clock (Drummond et al. 2006) and set the age of the divergence between the *Glochidion*-associated clade and its sister taxon to a secondary calibration (10 Ma) based on a previous molecular dating study (Kawakita and Kato 2009) and also constrained the age of the Marquesas clade to younger than the age of the Marquesas archipelago (5.5 Ma, the age of the oldest island, Eiao; Clouard and Bonneville 2005). The only seamounts that may have derived from the Marquesas hotspot date to 100-145 Ma and would have been underwater long before the formation of Eiao (Clouard and Bonneville 2001). The *Epicephala* dataset was pruned to achieve species-level sampling prior to BEAST analyses. Because of the limited number of available calibrations and the assumptions that go into their use, we consider the results to be for illustrative purposes only and do not consider comparisons between divergence times between *Glochidion* and *Epicephala* to be informative.

e. Biogeography

We used Bayes factor comparisons implemented in MrBayes 3.2 (Ronquist and Huelsenbeck 2003) to statistically test for the observed polyphyly of southeastern Polynesian *Glochidion*, and southeastern Polynesian *Epicephala*. This method constrains the topologies sampled from the posterior distribution of an analysis to contain a particular grouping (i.e., the monophyly of southeastern Polynesian taxa) or to not have that grouping.

f. Glochidion *taxonomy*

Glochidion has been estimated to be monophyletic within a paraphyletic *Phyllanthus* sensu lato (Kawakita and Kato 2009). Accordingly, many *Glochidion* species (such as the Pacific island ones; Wagner and Lorence 2011) have recently been treated in *Phyllanthus*, and we use these names where they exist, with the expectation that all taxa of *Glochidion* will soon be formally transferred to *Phyllanthus*. See Chapter 2 for further discussion.

g. Epicephala *taxonomy*

Taxonomy of *Epicephala* species is primarily based on the morphology of male genitalia (Vári 1961; Zhang et al. 2012). We dissected genitalia of the male southeastern Polynesian *Epicephala* (all of which were endemic (Hembry et al. 2012 [Chapter 1, this volume]) and, with one exception (Clarke 1986), undescribed) to determine if morphology was congruent with the patterns revealed by molecular phylogenetic analysis.

RESULTS

a. Phylogeny of Glochidion

All phylogenetic analyses resolved that southeastern Polynesian members of *Glochidion* fall into two clades (Figure 2), both of which are deeply nested within an Asian-Australasian grade of taxa. All taxa sampled from the Cook, Society, Austral, Marquesas, and Tuamotu archipelagos fall into a single clade (Clade A), along with one taxon from Samoa (*Phyllanthus cuspidatus*). Resolution within Clade A is poor, so it is not possible to draw further conclusions about within-southeastern Polynesian biogeography, although a clade was found uniting the three taxa from

Rapa. The remaining Southeastern Polynesian species, *P. wilderi* from Mangareva, is more closely related to taxa from Samoa, Wallis, and Fiji (Clade B). This grouping may seem unusual biogeographically since Samoa and Mangareva are nearly 4000 km apart, but is supported by two specimens of *P. wilderi* from two different sites on Mangareva.

b. Phylogeny of Epicephala

All analyses resolved that *Epicephala* from southeastern Polynesia fall into two clades (Figure 3). The first, Clade Y, is distributed across multiple archipelagos (Cooks, Societies, Rapa, Tuamotus, and Marquesas) and has diversified into a number of subclades, each of which appears to have a geographic distribution that is restricted to a single island or archipelago. The second, Clade Z, is distributed across the Cooks, Societies, and northern Australs. Clade Y appears to contain a clade of *Epicephala* from Samoa and Fiji, but this pattern is seen only in the COI dataset and the combined dataset, and is not supported by any of the individual nuclear-gene datasets (not shown).

c. Phylogenetic congruence

At the broadest possible scale, southeastern Polynesian *Glochidion* and *Epicephala* lack phylogenetic congruence and thus show no evidence either for cospeciation or phylogenetic tracking. The southeastern Polynesian members of *Glochidion* (excluding those from Mangareva) fall into a single clade, whereas the *Epicephala* associated with them fall into two distantly related clades. This polyphyly of southeastern Polynesian *Epicephala* is statistically supported both by the number of significantly supported nodes separating the two *Epicephala* clades in the Bayesian phylogenetic analysis, as well as by Bayes factor comparisons (see below). Because of this fundamental non-congruence, it is not possible to use any of the available methods for assessing cophylogeny.

d. Divergence time estimation

Two independent BEAST runs of 100,000,000 generations for divergence time estimation in *Glochidion* converged on similar distributions. The root age recovered (11.6 Ma, 95% intervals between 9.7-13.6 Ma) is the same as the prior minimum age calibration provided. The mean age estimate of Clade A converged to 9.5-9.8 Ma (intervals 5.5-13.3 Ma) and that of Clade B to 3.8-4.0 Ma (1.6-6.8 Ma). The mean age estimate age of the Rapa clade, under a prior constraint of less than 4.8 Ma, converged to a younger date (2.2 Ma; intervals 0.3-3.9 Ma). These Clade A dates are interesting because hardly any of the presently subaerial high islands in southeastern Polynesia existed by 9.5 Ma, and very few by 5.5 Ma. Those present by 9.5 Ma would have been Tubuai (and possibly Rurutu) in the Australs, and potentially Moruroa and Fangataufa in the Tuamotus (Hereheretue-Mangareva-Pitcairn alignment), which are now atolls (Clouard and Bonneville 2005). However, given the tendency of these analyses to return the root age as the previously specified calibration, and the very low amount of sequence variation across Asia-Pacific *Glochidion* in this dataset, these results should be interpreted with caution.

A single BEAST run of 100,000,000 generations with the *Epicephala* dataset yielded a root age of 9.7 Ma (95% intervals 7.7-11.7 Ma), congruent with the specified prior secondary calibration of 10 Ma. The age of clade Y appeared to be 2.6 Ma (1.4-4.1 Ma), and that of Clade Z to be 0.34 Ma (0.065-0.71 Ma). The Marquesas clade, constrained to be less than 5.5 Ma, moved towards a

mean estimate of 0.48 Ma (0.16-0.88 Ma). Although literal interpretation of these results is unwarranted, the tenfold difference in mean divergence time estimates for the roots of Clade Y and Z does suggest that Clade Z colonized southeastern Polynesia more recently than Clade Y. All of the distributions of divergence time estimates for southeastern Polynesian *Epicephala* are younger than the majority of extant high island chains (Societies, Marquesas, Australs, and Gambier; Clouard and Bonneville 2005), although they are also much younger than the divergence time estimates for *Glochidion* Clade A. Since these analyses are very sensitive to the root calibration provided, better calibration points, additional runs, and more informative datasets will be needed to refine divergence time estimates in these groups and to draw defensible conclusions about the absolute timing of their history of association.

e. Statistical tests of biogeographic patterns
Bayes factor comparisons support the polyphyly of southeastern Polynesian *Glochidion* and the polyphyly of southeastern Polynesian *Epicephala* observed in the unconstrained Bayesian consensus trees (see Tables 2 and 3).

f. Genital morphology
The groupings of *Epicephala* obtained via molecular phylogenetic analysis are also supported by male genital morphology. In particular, Clade Z is a single morphospecies at least within the Societies and northern Australs, and each of the geographically restricted subclades within Clade Y are morphologically distinct.

DISCUSSION AND CONCLUSIONS

(a) Multiple colonizations of southeastern Polynesia
This study finds that *Glochidion* and *Epicephala* have each colonized southeastern Polynesia multiple times independently (Figures 2 and 3). *Glochidion* trees in most of the region (Cook, Austral, Society, Tuamotu, and Marquesas archipelagos) appear to result from a single colonization, whereas *Glochidion* from the Gambier Archipelago appear to result from a separate colonization. Likewise, *Epicephala* moths in southeastern Polynesia fall into two clades, one of which is distributed throughout the entire region (Cooks, Australs, Societies, Tuamotus, and Marquesas) and another of which is distributed across the western part of this region (Cooks, Australs, and Societies). *Glochidion* and *Epicephala* are thus additional examples added to the growing list of organisms that have repeatedly colonized southeastern Polynesia (crab spiders (Garb and Gillespie 2006), reed-warblers (Cibois et al. 2011); *Cyrtandra* shrubs (Clark et al. 2009), *Melicope* trees (Harbaugh et al. 2009)). Individual islands in the Society archipelago (Raiatea, Huahine, Moorea, and Tahiti) also are shown here to have been colonized multiple times by *Epicephala*, an apparently rarer occurrence in the southeastern Polynesian biota (but see Clark et al. 2009). Independent colonizations of the same island by distantly related *Epicephala* lineages is also seen in this study on Tutuila (American Samoa) and Viti Levu (Fiji), suggesting that this may be a general pattern in the diversification of *Epicephala* in the Pacific. Both the grouping of *P. cuspidatus* from Samoa with the southeastern Polynesian *Glochidion*, and the nesting of several Fiji and Samoa *Epicephala* within Clade Y may offer evidence of "back-colonizations" of the west Pacific from southeastern Polynesia.

Phyllanthus wilderi from the Gambier Archipelago appears to be more closely related to several taxa from Samoa, Wallis, and Fiji, ≥4000 km distant, than to the remainder of southeastern Polynesian *Glochidion*. Although this is an unusual biogeographic association, it should be noted that the Gambier Islands are old (≥5.7 Ma; Guillou et al. 1994) and distant from the nearest high islands to the west (1000 km from Rapa, 1500 km from the Marquesas); other, analogous long-distance sister relationships are known in Pacific plants (e.g. Australia and the Juan Fernández Islands; Harbaugh and Baldwin 2007); and most of the diversity of western Polynesian *Glochidion* is missing from this analysis. Unfortunately, like the rest of the Mangarevan flora, *P. wilderi* is extremely rare due to prehistoric deforestation and invasive plants (Conte and Kirch 2004; Butaud 2009), and *Epicephala* were not collected from this species; however, photographs of mature *P. wilderi* fruit with exit holes suggest that these moths persist on large trees on the cliffs of Mangareva (J.-F. Butaud, personal communication, 2012). Biogeographic affinities of the Gambier biota in general are poorly known (but see Cibois et al. 2011).

With the sampling and resolution shown here, four inferences can be made about the biogeographic history of *Glochidion* and *Epicephala* within southeastern Polynesia. Although *Glochidion* Clade A is essentially a single polytomy, a common pattern in island plant radiations (Gemmill et al. 2002; Knope et al. 2012), the three *Glochidion* species from the old, isolated island of Rapa form a clade with strong statistical support (1.0; Figure 2). *Epicephala* Clade Y has differentiated into a set of morphologically and phylogenetically distinguishable subclades, each of which appears geographically restricted to one island or a group of adjacent islands. The grouping of a moth from Niau with specimens from the Leeward Society Islands (Raiatea and Huahine) may be due to colonization of Niau from the Societies following its secondary uplift due to lithospheric flexure from Tahiti (Bonvallot et al. 1994). In contrast, Clade Z shows no statistically supported substructure across three archipelagos. The presence of geographic structure in *Epicephala* Clade Y is consistent with a role for geographic isolation in the diversification of this clade, consistent with findings from similar systems (Smith et al. 2008; Thompson and Rich 2011; Althoff et al. 2012).

Although it may be tempting to ascribe unusual biogeographic patterns in Polynesia to human-mediated dispersal, we consider that possibility extremely unlikely on multiple grounds. *Glochidion* is not one of the plant taxa considered to have been transported by Polynesians between islands (Whistler 2009), and there is no evidence of their use or cultivation from southeastern Polynesian language dictionaries or ethnobotanical literature (Whistler 1985; Pétard 1986, Buse 1995, Académie Tahitienne 1999; Whistler 2009). If *Glochidion* or *Epicephala* were transported by Polynesians, we would expect to see *Glochidion* species shared between the Polynesian homeland in western Polynesia (Samoa and Tonga; Kirch 2000) and southeastern Polynesia, and between archipelagos in southeastern Polynesia, neither of which is observed. If these taxa were transported by humans in the modern era, we should expect groupings between archipelagos that reflect modern political barriers to commerce, but this is not the case in Clade Z, which is shared between the Cooks and adjacent parts of French Polynesia. It is unlikely that insects with such specialized host associations could be carried about by indigenous peoples through any medium other than host plant material, without leaving evidence that host plants were carried around. *P. wilderi* and its relatives in Samoa, Wallis, and Fiji are closely related but

they differ in floral morphology, and it is unlikely that such trait divergence unrelated to any putative human use could evolve in 1000 years (the time since human colonization of Mangareva; Conte & Kirch 2004) due to human-mediated selection.

(b) Phylogenetic incongruence
Not only have *Glochidion* and *Epicephala* colonized southeastern Polynesia repeatedly, they have done so incongruently: two clades of *Epicephala* are associated with a single clade of *Glochidion*. Consequently, *Glochidion* trees and *Epicephala* moths in this region have neither diversified according to a process of cospeciation, nor according to a process in which *Epicephala* moths track the phylogeny of their hosts after the hosts have already diversified. Such dramatic phylogenetic incongruence at fine scales has not previously been demonstrated in obligate pollination mutualisms. These findings are however not necessarily in contradiction with the only previous study comparing phylogenies of these taxa (Kawakita et al. 2004), since the earlier study compared only 18 species of distantly related *Glochidion* with single *Epicephala* specimens from each, and found that whereas there was evidence for more phylogenetic congruence than would be expected by chance, the phylogenies were far from identical. Thus it is possible that at a fine scale, closely related species of *Glochidion* and their *Epicephala* diversify according to a process that results in phylogenetic incongruence, but that still produces some detectable phylogenetic congruence at larger phylogenetic scales. Similar patterns are apparent in fig wasps (Machado et al. 2005; Herre et al. 2008) as well as yuccas and yucca moths (Althoff et al. 2012). These results underscore that we should not necessarily expect coevolving clades undergoing diversification to show phylogenetic congruence, even in highly specialized mutualisms (Thompson 2005; but see Weiblen and Bush 2002).

c. Dynamics of diversification in coevolving clades: rapid host shifts
Phylogenetic incongruence between mutualists can be explained by several processes: limitations of the process of phylogenetic inference itself, duplication followed by differential extinction (lineage sorting), host-shifts, and failure-to-diverge events (Page 2003). Each of these processes except the first has different implications for the process of coevolutionary diversification, but distinguishing amongst them has proven difficult. By examining recently diversified clades with extensive sampling, this study finds evidence for the latter two processes (host shifts and failure-to-diverge events) operating in the coevolutionary diversification of *Epicephala* and *Glochidion*. These results also indicate, more importantly, that a pollinator lineage can shift onto many species of hosts over a wide geographic area in a short period of time. Host-shifts have previously been postulated as a mechanism explaining phylogenetic incongruence between *Glochidion* and *Epicephala* (Kawakita et al. 2004; Kawakita and Kato 2006). It is likely that similar long-distance host shifts occur in this system on continents as well, but are harder to detect than in this case because of the difficulty of comprehensively sampling from a wide geographic area.

The evolutionary consequences of host-shifts in specialized pollination mutualisms are not clear. Multiple pollinator species may stably coexist on one host (Thompson et al. 2010). Alternatively, competition between two pollinators may result in extinction or extirpation of one species, or divergence in the plant may be accompanied by coevolution with different pollinators. Local coexistence of two distantly related *Epicephala* species on one *Glochidion* species has been

reported from the Ryukyu Islands (Kawakita and Kato 2006).   Because host-shifts may potentially be a mechanism of diversification mediated by coevolution, a better understanding of their frequency, ecology, and selective consequences in this and similar systems may reveal a great deal about how coevolving clades diversify.

Figure 1. a. Map of Pacific Basin with southeastern Polynesia indicated. b. Map of southeastern Polynesia, with archipelagos and islands mentioned in text indicated.

Figure 2. Bayesian consensus tree for *Glochidion* globally. Outgroup *Breynia distica* pruned. Node labels represent posterior probabilities.

Phyllanthus roseus Laos
G.spB_Laos138
G.spB_Vietnam153
1 G.spD_Vietnam155
G.spH_Laos144
G.spD_Laos140
G.eriocarpumCN147 China
G.spE_Laos141
0.66 G.eriocarpumVN152 Vietnam
G.spA_Lambir127
0.84 G.spC_Vietnam154
0.st G.spF_Laos142
G.spF_Lambir132
G.spA_Vietnam151
G.acuminatumVN156 Vietnam
1 G.acuminatum54 Japan
0.72 G.spC_Laos139
0.st G.sp_Assam136
1 G.assamicum150 China
G.spA_Laos137
G.pindai78 New Caledonia
0.72 G.pungens103 Australia
Dhh10047p Fiji
0.81 1 0.s9 Dhh10091p Fiji
Dhh10032p Fiji
Dhh10058p Fiji
Dhh10059p Fiji
0.78 0.99Dhh10054p Fiji
0.69 0.56 G.cordatum71 Fiji
1 G.collinum69 Fiji
dhh10018p Fiji
G.littorale207 Malaysia
0.57 G.benthamianum Australia
G.harveyanum Australia
0.94 *seemanii* Fiji
1 *concolor* Fiji
ramiflorum Wallis
dhh10061p Fiji
0.55 *wilderi* Mangareva 1
wilderi Mangareva 2
0.65 *samoanus* Ofu (American Samoa)
samoanus Ta'ū (American Samoa)
samoanus Tutuila (American Samoa)

Clade B:
Fiji, Samoa, Wallis, SEP (Mangareva)

0.61 *marchionicus* Nuku Hiva
temehaniensis Huahine
florencei Rimatara
manono Tahiti
1 *"fosbergii"* Rapa
longfieldiae Rapa
rapaense Rapa
0.98 *grayanus* Tahiti
marchionicus 07151 Nuku Hiva
orohenense Tahiti
taitensis Tahiti
florencei Huahine
temehaniensis Tahaa
st-johnii Bora Bora
florencei Raiatea
sp. Mangaia
manono Moorea
marchionicus Hiva Oa
0.85 *raiateensis* Raiatea
emarginatus Raiatea
brothersonii Raiatea
temehaniensis Raiatea
tuamotuensis Niau
raivavense Raivavae
papenooense Tahiti
0.75 *marchionicus* Fatu Hiva
marchionicus Ua Pou
cuspidatus Tutuila (American Samoa)
florencei Maupiti
hivaoaense Hiva Oa
0.9 sp. 'Atiu
nadeaudii Moorea
huahineense Huahine
raivavense Tubuai
concolor aff. Rarotonga
raivavense Rurutu
st-johnii Moorea

Clade A:
Southeastern Polynesia
+ 1 Samoan species

G.rubrum14 Japan
0.st G.obovatum27 Japan
G.spI_Laos145
1 G.velutinum112 Myanmar
0.81 G.acuminatum_like Lambir
1 G.spJ_Lambir208
G.spD_Lambir130
1 G.spE_Lambir131
1 G.lanceisepalum66 Lambir
G.spI_Lambir135
1 G.ferdinandii102 Australia
0.7 0.55 G.spC_Lambir129
G.spB_Lambir204
0.97 G.zeylanicum1 Japan
G.caledonicum77 New Caledonia
1 G.lanceolatum10 Japan
G.zeylanicum148 Japan
0.87 1 G.hirsutum149 China
0.99 G.spG_Laos143
G.spG_Lambir205
G.phillipicum15 Taiwan

0.02

33

Figure 3. Bayesian consensus tree for the *Epicephala* clade associated with *Glochidion* globally. Outgroups (*Epicephala* from *Phyllanthus amarus* and *P. marojejiensis*) pruned. Node labels represent posterior probabilities. Tip labels refer to host plant species and locality.
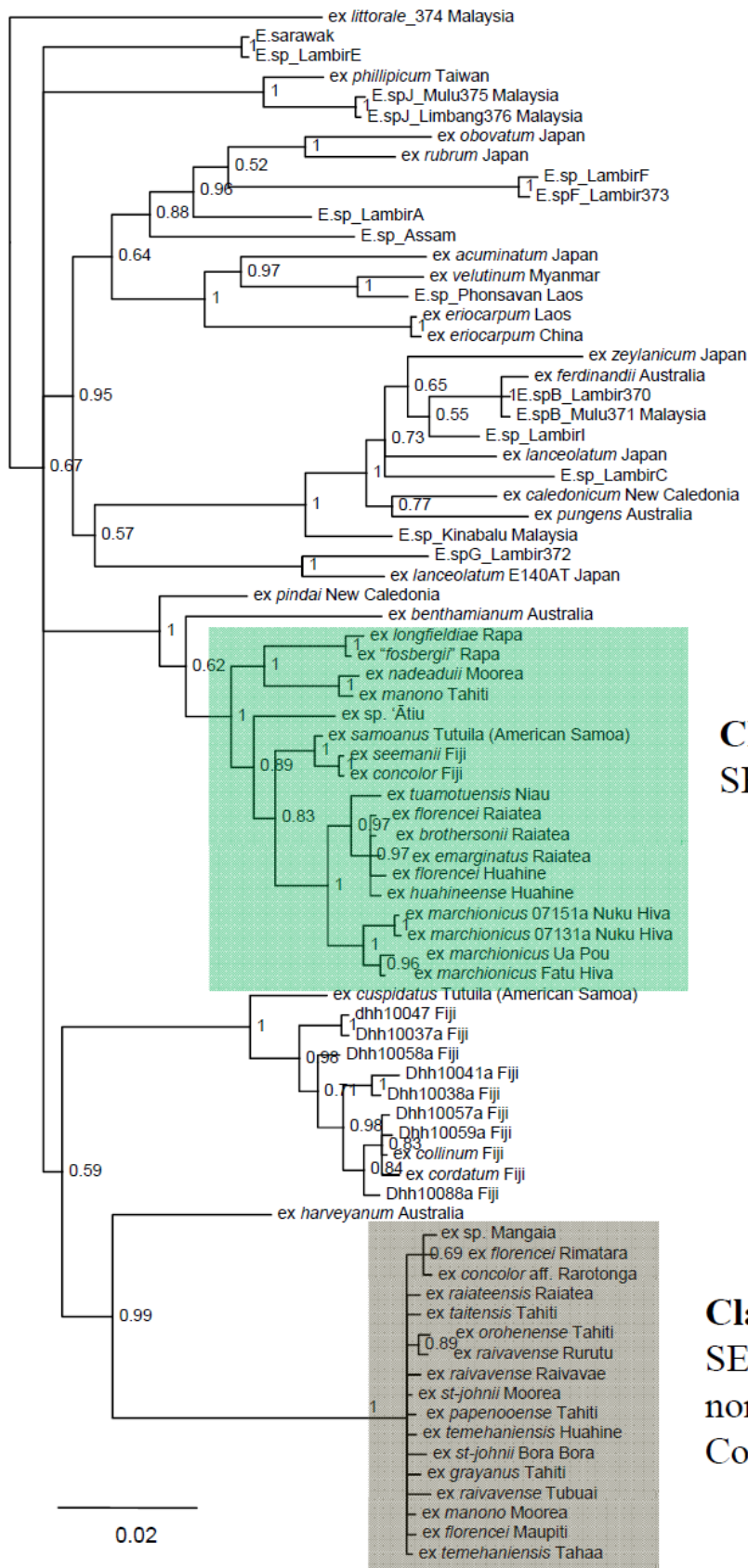
Clade Y:
SEP + Fiji, Samoa

Clade Z:
SEP (Societies, northern Australs, Cooks)

0.02

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Table 1. Specimen data table. Specimens of *Glochidion* and *Epicephala* (and outgroups) sampled for phylogenetic analysis in this study.

Abbreviations: FP, French Polynesia; NP, National Park

Collector abbreviations: AK, Atsushi Kawakita (Kyoto University); DHH, David Hembry (University of California, Berkeley); JYM, Jean-Yves Meyer (Délégation à la Recherche); MK, Makoto Kato (Kyoto University); NEG, Neil Gurr (American Samoa Community College); SL, Shixiao Luo (South China Botanical Garden).

*Two species of *Epicephala* are known to be associated with *G. lanceolatum* in the Yaeyama Islands (Japan; Kawakita and Kato 2006); both are included.

Plant name	Synonym	Collection locality	Number	Plant	Moth	Collector
P. concolor aff.	*G. concolor* aff.	Cook Islands: Rarotonga	08-308	Y	Y	DHH
P. sp.	*G.* sp.	Cook Islands: ʻĀtiu	08-329	Y	Y	DHH
P. sp.	*G.* sp.	Cook Islands: Mangaia	08-318	Y	Y	DHH
P. raivavense	*G. raivavense*	FP: Austral Islands: Raivavae	08-179	Y	Y	DHH
P. raivavense	*G. raivavense*	FP: Austral Islands: Rurutu	08-161	Y	Y	DHH
P. raivavense	*G. raivavense*	FP: Austral Islands: Tubuai	08-194	Y	Y	DHH
P. longfieldiae	*G. longfieldiae*	FP: Austral Islands: Rapa	08-255	Y	Y	DHH
P. rapaense	*G. rapaense*	FP: Austral Islands: Rapa	08-235	Y	N	DHH
P. sp. nov. ("*fosbergii*")	*G.* sp. nov ("*fosbergii*")	FP: Austral Islands: Rapa	08-285	Y	Y	DHH
P. florencei	*G. societatis*	FP: Austral Islands: Rimatara	08-143	Y	Y	DHH
P. florencei	*G. societatis*	FP: Society Islands: Huahine	08-473	Y	Y	DHH
P. florencei	*G. societatis*	FP: Society Islands: Maupiti	07-450	Y	Y	DHH
P. florencei	*G. societatis*	FP: Society Islands: Raiatea	08-445	Y	Y	DHH
P. st-johnii	*G. myrtifolium*	FP: Society Islands: Bora Bora	07-483	Y	Y	DHH
P. st-johnii	*G. myrtifolium*	FP: Society Islands: Moorea	07-383, 07-442	Y	Y	DHH
P. temehaniensis	*G. temehaniense*	FP: Society Islands: Huahine	08-030	Y	Y	DHH
P. temehaniensis	*G. temehaniense*	FP: Society Islands: Raiatea	07-307	Y	N	DHH
P. temehaniensis	*G. temehaniense*	FP: Society Islands: Tahaa	08-443	Y	Y	DHH
P. raiateensis	*G. moorei*	FP: Society Islands: Raiatea	07-310	Y	Y	DHH
P. emarginatus	*G. emarginatum*	FP: Society Islands: Raiatea	08-456	Y	Y	DHH
P. brothersonii	*G. brothersonii*	FP: Society Islands: Raiatea	09-151	Y	Y	DHH
P. huahineense	*G. huahineense*	FP: Society Islands: Huahine	08-478	Y	Y	DHH
P. nadeaudii	*G. nadeaudii*	FP: Society Islands: Moorea	07-353	Y	Y	DHH
P. manono	*G. manono*	FP: Society Islands: Moorea	07-335	Y	Y	DHH
P. manono	*G. manono*	FP: Society Islands: Tahiti	07-429	Y	Y	DHH
P. grayanus	*G. grayanum*	FP: Society Islands: Tahiti	07-434	Y	Y	DHH
P. orohenense	*G. orohenense*	FP: Society Islands: Tahiti	08-485	Y	Y	DHH

P. papenooense	*G. papenooense*	FP: Society Islands: Tahiti	PAPE 07	Y	Y	DHH
P. taitensis	*G. taitense*	FP: Society Islands: Tahiti	07-411	Y	Y	DHH
P. marchionicus	*G. marchionicum*	FP: Marquesas Islands: Fatu Hiva	07-194	Y	Y	DHH
P. marchionicus	*G. marchionicum*	FP: Marquesas Islands: Hiva Oa	07-188	Y	N	DHH
P. marchionicus	*G. marchionicum*	FP: Marquesas Islands: Nuku Hiva	07-131	Y	Y	DHH
P. marchionicus	*G. tooviianum*	FP: Marquesas Islands: Nuku Hiva	07-151	Y	Y	DHH
P. marchionicus	*G. marchionicum*	FP: Marquesas Islands: Ua Pou	07-169	Y	Y	DHH
P. hivaoaense	*G. hivaoaense*	FP: Marquesas Islands: Hiva Oa	07-211	Y	N	JYM
P. tuamotuensis	*G. tuamotuense*	FP: Tuamotu Islands: Niau	08-404	Y	Y	DHH
P. wilderi	*G. wilderi*	FP: Gambier Islands: Mangareva	08-391	Y	N	DHH
P. wilderi	*G. wilderi*	FP: Gambier Islands: Mangareva	08-394	Y	N	DHH
P. cuspidatus	*G. cuspidatum*	American Samoa: Tutuila	NEG-1a	Y	Y	NEG
P. samoanus	*G. ramiflorum*	American Samoa: Tutuila	NEG-5a	Y	Y	NEG
P. samoanus	*G. ramiflorum*	American Samoa: Ofu	09-087	Y	N	DHH
P. samoanus	*G. ramiflorum*	American Samoa: Ta'ū	09-084	Y	N	DHH
G. ramiflorum		Wallis and Futuna: Wallis ('Uvea)	JYM-427	Y	N	JYM
P. sp.	*G.* sp.	Fiji: Viti Levu: Monasavu	10-061	Y	N	DHH
P. sp.	*G.* sp.	Fiji: Viti Levu: Mt. Tomanivi	10-047	Y	Y	DHH
P. sp.	*G.* sp.	Fiji: Viti Levu: Mt. Batilamu (Koroyanitu NP)	10-091	Y	N	DHH
P. sp.	*G.* sp.	Fiji: Viti Levu: Namosi-Naitasiri border	10-032	Y	N	DHH
P. cordatus	*G. cordatum*	Fiji: Viti Levu: between Nadarivatu and Lewa	10-058	Y	Y	DHH
P. sp.	*G.* sp.	Fiji: Viti Levu: Monasavu	10-059	Y	Y	DHH
P. sp.	*G.* sp.	Fiji: Viti Levu: between Nadrau and Nadarivatu	10-054	Y	N	DHH
P. sp.	*G.* sp.	Fiji: Viti Levu: Colo-i-Suva	10-018	Y	N	DHH
P. sp.	*G.* sp.	Fiji: Viti Levu: Wainimakutu	10-037	N	Y	DHH
P. sp.	*G.* sp.	Fiji: Viti Levu: Wainimakutu	10-041	N	Y	DHH
P. sp.	*G.* sp.	Fiji: Viti Levu: Wainimakutu	10-038	N	Y	DHH
P. cordatus	*G. cordatum*	Fiji: Viti Levu: between Nadarivatu and Lewa	10-057	N	Y	DHH
P. sp.	*G.* sp.	Fiji: Viti Levu: Monasavu	10-088	N	Y	DHH
G. sp. A Lambir		Malaysia: Sarawak: Lambir Hills NP	Lambir 127	Y	Y	AK & DHH
G. sp. B Lambir		Malaysia: Sarawak: Lambir Hills NP	Lambir 204	Y	Y	AK & DHH
G. sp. B Lambir		Malaysia: Sarawak: town of Mulu	371	N	Y	AK & DHH
G. sp. C Lambir		Malaysia: Sarawak: Lambir Hills NP	Lambir 129	Y	Y	AK & DHH
G. sp. D Lambir		Malaysia: Sarawak: Lambir Hills NP	Lambir 130	Y	N	AK & DHH
G. sp. E Lambir		Malaysia: Sarawak: Lambir Hills NP	Lambir 131	Y	Y	AK & DHH
G. sp. F Lambir		Malaysia: Sarawak: Lambir Hills NP	Lambir 132	Y	Y	AK & DHH

G. sp. F Lambir		Malaysia: Sarawak: Lambir Hills NP	373	N	Y	AK & DHH
G. sp. G Lambir		Malaysia: Sarawak: Lambir Hills NP	Lambir 205	Y	Y	AK & DHH
G. littorale (sp. H)		Malaysia: Sarawak: Miri	Lambir 207	Y	Y	AK & DHH
G. sp. I Lambir		Malaysia: Sarawak: Kuching	Lambir 135	Y	Y	AK
G. sp. J Sarawak		Malaysia: Sarawak: Limbang	Lambir 208	Y	Y	AK & DHH
G. sp. J Sarawak		Malaysia: Sarawak: town of Mulu	375	N	Y	AK & DHH
G. sp. *acuminatum*-like		Malaysia: Sarawak: Lambir Hills NP		Y	N	AK & DHH
G. sp. Kinabalu		Malaysia: Sabah: Mt. Kinabalu		N	Y	AK
G. sp. Assam		India: Assam	136	Y	Y	MK
G. sp. A Laos		Laos	Laos 137	Y	N	AK
G. sp. B Laos		Laos	Laos 138	Y	N	AK
G. sp. C Laos		Laos	Laos 139	Y	N	AK
G. sp. D Laos		Laos	Laos 140	Y	N	AK
G. sp. E Laos		Laos	Laos 141	Y	N	AK
G. sp. F Laos		Laos	Laos 142	Y	N	AK
G. sp. G Laos		Laos	Laos 143	Y	N	AK
G. sp. H Laos		Laos	Laos 145	Y	N	AK
G. sp. I Laos		Laos	Laos 144	Y	N	AK
G. eriocarpum		Laos		N	Y	AK
G. sp		Laos: Phonsavan		N	Y	AK
G. eriocarpum		China	147	Y	Y	SL & AK
G. zeylanicum		China	148	Y	N	SL & AK
G. hirsutum		China	149	Y	N	SL & AK
G. assamicum		China	150	Y	N	SL & AK
G. sp. A Vietnam		Vietnam	VN 151	Y	N	AK
G. eriocarpum		Vietnam	VN 152	Y	N	AK
G. sp. B Vietnam		Vietnam	VN 153	Y	N	AK
G. sp. C Vietnam		Vietnam	VN 154	Y	N	AK
G. sp. D Vietnam		Vietnam	VN 155	Y	N	AK
G. acuminatum		Vietnam	VN 156	Y	N	AK
G. obovatum		Japan: Kyushu: Miyazaki Prefecture	27	Y	Y	Kawakita et al. (2004)
G. rubrum		Japan: Ryukyu Islands: Ishigaki	14	Y	Y	Kawakita et al. (2004)
G. acuminatum		Japan: Ryukyu Islands: Amami	54	Y	Y	Kawakita et al. (2004)
G. seemannii		Fiji: Viti Levu: Navai	70	Y	Y	Kawakita et al. (2004)
G. concolor		Fiji: Viti Levu: Namosi	72	Y	Y	Kawakita et al. (2004)
G. pindai		New Caledonia: Pindai	78	Y	Y	Kawakita et al. (2004)

G. collinum		Fiji: Viti Levu: Mt. Tomanivi	69	Y	Y	Kawakita et al. (2004)
G. cordatum		Fiji: Viti Levu: Namosi	71	Y	Y	Kawakita et al. (2004)
G. zeylanicum		Japan: Ryukyu Islands: Okinawa	1	Y	Y	Kawakita et al. (2004)
G. lanceolatum		Japan: Ryukyu Islands: Ishigaki	10	Y	Y*	Kawakita et al. (2004)
G. lanceolatum		Japan: Ryukyu Islands: Yaeyama Group	E140AT	N	Y*	Kawakita & Kato (2006)
G. caledonicum		New Caledonia: Hienghéne	77	Y	Y	Kawakita et al. (2004)
G. phillipicum		Taiwan: Nanjin	15	Y	Y	Kawakita et al. (2004)
G. lanceisepalum		Malaysia: Sarawak: Lambir	66	Y	Y	Kawakita et al. (2004)
G. harveyanum		Australia: Queensland: Mt. Windsor	101	Y	Y	Kawakita et al. (2004)
G. ferdinandii		Australia: Queensland: Mt. Lewis	102	Y	Y	Kawakita et al. (2004)
G. pungens		Australia: Queensland: Mt. Lewis	103	Y	Y	Kawakita et al. (2004)
G. benthamianum		Australia: Queensland: Mt. Molly	111	Y	Y	Kawakita et al. (2004)
G. velutinum		Myanmar: Mt. Popa	112	Y	Y	Kawakita et al. (2004)
Breynia distica		New Caledonia: Pindai	81	Y	N	Kawakita et al. (2004)
Phyllanthus roseus		Laos	143	Y	N	AK
P. amarus		Japan: Ryukyu Islands: Ishigaki		N	Y	Kawakita & Kato (2009)
P. marojejiensis		Madagascar: Mt. Marojeji		N	Y	Kawakita & Kato (2009)

Table 2: Results from constrained Bayesian analyses to estimate support for monophyly or polyphyly of southeastern Polynesian (SEP) *Glochidion* using Bayes factors. Unconstrained analyses recovered polyphyly of southeastern Polynesian taxa. The larger value (i.e., the "less negative" value) is considered to be the better supported model, with a log difference above 5 units considered to be strong evidence in support of the better model (Kass and Raftery 1995).

Constraint	Total harmonic mean (across two runs)
Positive (SEP monophyly)	-5840.99
Negative (SEP polyphyly)	**-5775.95**

Table 3: Results from constrained Bayesian analyses to estimate support for monophyly or polyphyly of southeastern Polynesian (SEP) *Epicephala* using Bayes factors. Unconstrained analyses previously recovered polyphyly of southeastern Polynesian taxa (clades Y and Z as separate clades). Bayes factor comparison analyses constrained monophyly of clades Y and Z (including Fijian and Samoan members of Clade Y), or constrained to not permit monophyly of taxa in clades Y and Z. The larger value (i.e., the "less negative" value) is considered to be the better supported model, with a log difference above 5 units considered to be strong evidence in support of the better model (Kass and Raftery 1995).

Constraint	Total harmonic mean (across two runs)
Positive (SEP monophyly)	-8814.89
Negative (SEP polyphyly)	**-8736.17**

Chapter 4: Phylogeography and network structure of *Glochidion* trees and *Epicephala* moths on three Society Islands

Chapter 4: Phylogeography and network structure of *Glochidion* trees and *Epicephala* moths on three Society Islands

INTRODUCTION

Biologists have been fascinated by specialization for over one hundred years (Darwin 1862; Simpson 1953), but the realized patterns of interactions among species in communities has recently received renewed attention in ecology and coevolutionary biology (Thompson 1994; Bascompte et al. 2003; Guimarães et al. 2007, 2011). In particular, understanding is poor of what factors promote specialization in mutualistic interactions, despite the fact that specialization has great implications for the function and stability of mutualistic interactions, as well as diversification within them. Current coevolutionary theory suggests a number of hypotheses for the evolution of specialization in mutualistic interactions. One hypothesis states that specialization is not necessarily selected for in mutualisms, but is rather a constraint conserved from non-mutualistic ancestors (Thompson 1994); another holds that mutualistic selection itself promotes specialization (Kawakita et al. 2010); yet another states that reciprocal specialization should be promoted by symbiotic interactions, in which individuals of one partner species spend significant portions of their life cycle inside the tissues of or in close proximity to individuals of the other partner (Guimarães et al. 2007). However, the importance of interactions in driving such reciprocal patterns has been questioned by other studies suggesting an important role for extrinsic factors such as climate (Mueller et al. 2011), and certainly geography and the evolutionary history of lineages themselves may also play a role in determining their realized patterns of interactions.

The advent of molecular methods has also permitted a deeper understanding of the diversity of partner lineages involved in symbiotic interactions (Mueller et al. 1998; Knowlton and Rohwer 2003; Bäckhed et al. 2005), even in non-microbial symbioses (Molbo et al. 2003, Machado et al. 2005, Kawakita and Kato 2006). The use of molecular approaches offers promise not just to examine patterns of cryptic specialization or generalization in mutualistic interactions, but to examine these patterns in an explicitly phylogenetic context. Such approaches are especially promising in that they can allow the examination of patterns of species interactions at early stages of diversification, and in conjunction with ecological studies, provide insights into the role of coevolutionary selection between mutualists in their diversification. Despite this, few molecular studies have examined recently diversified mutualists (but see Smith et al. 2008; Azuma et al. 2010). In part, this is due to the difficulties of sampling appropriately from mutualistic interactions with great cryptic diversity and poorly understood patterns of interaction. It is also a result of the fact that what may be an early stage of diversification for one partner taxon is not necessarily an early stage of diversification for the other.

The pollinating seed-predation mutualism between leafflower plants (Phyllanthaceae; *Phyllanthus* sensu lato) and leafflower moths (Lepidoptera: Gracillariidae: *Epicephala*) (Kawakita 2010) offers an ideal system for examining patterns of interaction specificity at early stages of diversification. Leafflower moths are the sole known pollinators of several clades of

leafflowers (in the genus *Phyllanthus* sensu stricto, as well as the genera *Glochidion* s. l. and *Breynia*); they actively pollinate the flowers of their host, and then oviposit into the ovaries such that the larvae feed on a subset of the developing seeds (Kato et al. 2003; Kawakita and Kato 2004a, 2004b, 2009). Those leafflower lineages that are pollinated by leafflower moths have no other known pollinators, and so the interaction is considered to be obligate and reciprocally specialized. Because the leafflower moth spends its larval stage inside the tissues of its host, the interaction is partially symbiotic. In this respect, the leafflower/leafflower moth mutualism is ecologically analogous to the obligate mutualisms between figs and fig wasps (Herre et al. 2008), yuccas and yucca moths (Pellmyr 2003), senita cacti and senita moths (Fleming and Holland 1998), and globeflowers and globeflower flies (Pellmyr 1992), as well as the non-obligate pollinating seed-predation mutualism between certain populations of greya moths and saxifrages (Thompson 2011).

The best-studied of the several clades of *Epicephala*-pollinated leafflowers is the tropical leafflower tree genus *Glochidion* (Kato et al. 2003; Kawakita and Kato 2006; Okamoto et al. 2007; this volume), with three hundred described species distributed across (sub)tropical Asia, Australasia, and the south Pacific islands (Govaerts et al. 2000), all of which are nested phylogenetically in *Phyllanthus* and best treated there, pending completion of formal name changes (see Wagner and Lorence 2011). In both Asia and Polynesia, *Epicephala* are the sole known pollinators of *Glochidion* (Kato et al. 2003, Hembry et al. 2012 [Chapter 1, this volume]). Patterns of interaction and host-specificity have been studied once, in the six species of *Glochidion* native to Japan and Taiwan; this study found that at any given site, patterns of specificity are both highly modular and reciprocally specialized (Kawakita and Kato 2006). At any given site, each *Epicephala* species is associated with a single *Glochidion* species, although a *Glochidion* species may have either one or two *Epicephala* pollinators at a site. A single *Epicephala* species may associate with more than one *Glochidion* species across its range, but never sympatrically. These findings are thus roughly consistent with findings from similar systems in which reciprocal specialization is high, but usually not absolute (Herre et al. 2008, Pellmyr 2003). However, these six *Glochidion* species and their *Epicephala* pollinators are distantly related to each other, so it is unclear if these patterns are representative of this system at early stages of diversification.

The endemic co-radiation of *Glochidion* trees and *Epicephala* moths on the oceanic islands of southeastern Polynesia (Cook Islands, French Polynesia, and the Pitcairn Group; Hembry et al. 2012 [Chapter 1, this volume]) offer an opportunity to examine patterns of interaction and host-specificity at early stages of diversification in this mutualism. The islands of southeastern Polynesia are formed by volcanoes produced as the Pacific plate moves over a series of stationary hotspots in the earth's mantle; the majority of these islands are less than five million years old. The twenty-plus endemic species of *Glochidion* found in these archipelagos represent a very recent radiation (Chapter 3, this volume); most islands have 1-3 species of *Glochidion*, but the larger ones have 4-7 species, with multiple species occurring sympatrically (Meyer and Butaud 2003; Meyer 2009; D. Hembry, unpublished data). The *Epicephala* moths associated with these *Glochidion* fall into two clades, each resulting from a separate colonization. Both clades of *Epicephala* are found on the largest of the Society Islands (French Polynesia). A preliminary phylogenetic analysis suggests that the older of these two clades has diversified into

a number of locally endemic species (each of which is endemic to a single archipelago and is associated with at least 1-4 host species), whereas the younger clade is a widespread morphospecies associated with at least ten species of *Glochidion* across ten islands. It is unclear, however, whether both clades of *Epicephala* coexist on the same host plant species, or individual in nature.

In this paper, we examine patterns of species interactions between *Glochidion* and *Epicephala* on three of the Society Islands (Tahiti, Moorea, and Huahine) as a model for studying the early stages of diversification in coevolving mutualisms. Each of these islands has 3-5 species of *Glochidion*, has both clades of *Epicephala*, and is less than 3 Ma. First, we ask whether *Epicephala* moths show signs of differentiation by geography or by host plant taxon. Second, we ask what is the pattern of species-specificity between *Glochidion* trees and *Epicephala* moths on each of these islands. Third, we ask how these patterns differ from each other, and from the patterns seen previously in this and other ecologically similar obligate pollination mutualisms.

MATERIALS AND METHODS

The islands
Tahiti, Moorea, and Huahine are the three youngest of the Society Islands with *Glochidion*, located in the central south Pacific (Figure 1). These islands are aligned from the northwest (Huahine) to the southeast (Tahiti) in an age progression reflecting the motion of the Pacific Plate over a stationary hotspot in the earth's mantle. K-Ar dated basalt samples from each of these islands suggest that Huahine was volcanically active from 2.5-2.6 Ma, Moorea from 1.5-1.7 Ma, and Tahiti (which consists of the three volcanoes Tahiti Nui, Taravao, and Taiarapu) from 0.38-1.37 Ma (Demougeot 2007). Island age is positively correlated with degree of erosion, and inversely correlated with elevation, such that Tahiti is the tallest at 2241 m (Mt. Orohena), with Moorea at 1207 m (Mt. Tohiea) and Huahine at 670 m (Mt. Turi). The currently active volcano in the Societies chain is the tiny island of Mehetia 110 km to the southeast of Tahiti, which lacks *Glochidion* (Meyer et al. 2009). *Glochidion* is also absent from the tiny high island of Maiao between Moorea and Huahine (Meyer 2007). Although Huahine, Moorea, and Tahiti have never been connected to one another, Moorea and Tahiti are relatively close (17 km at their closest point) and are cartographically grouped together as the Windward Islands (Îles-du-Vent). Moorea and Huahine are farther apart (150 km), and Huahine is cartographically grouped together with the nearby islands to its northwest as the Leeward Islands (Îles-sous-le-Vent). Each of these islands is similar in size (Tahiti 1045 km^2; Moorea, 134 km^2; Huahine 75 km^2) to those continental islands (e.g., Okinawa, 1200 km^2; Amami Oshima 712 km^2; Ishigaki, 139 km^2) examined in the previous study of *Epicephala* host specificity (Kawakita and Kato 2006).

The plants
In this study we examined ten species of *Glochidion* trees native to the islands of Tahiti, Moorea, and Huahine (Table 1), all of which have new names in *Phyllanthus* (Wagner and Lorence 2011). Five of these species are found on Tahiti, three on Moorea, and three on Huahine. Only one species is found on more than one of the islands examined (*P. manono* on both Moorea and Tahiti), while another three (*P. florencei*, *P. temehaniensis*, and *P. st-johnii*) are also found on other Society Islands (Florence 1997). The species were last revised by Florence (1997), prior to

transfer to *Phyllanthus* by Wagner and Lorence (2011). With the exception of two coexisting species pairs that are distinguished solely by the presence/absence of pubescence (*P. orohenense* and *P. taitensis* on Tahiti; *P. florencei* and *P. huahineense* on Huahine; Florence 1997) and another species pair which morphologically intergrades among populations (*P. manono* and *P. st-johnii* on Moorea; D. Hembry, unpublished data), these species are all morphologically distinct and can primarily be distinguished based on pistillate floral morphology. They are found from sea level to ~1500 m elevation on the three islands, on basaltic substrate, in a variety of mesic and wet forest and degraded vegetation types (Florence 1997; Butaud et al. 2008). *Phyllanthus huahineense* and *P. florencei* are additionally found on (although not restricted to) calcareous substrate on offshore islets or cays (*motu* in Tahitian) on Huahine (Florence 1997; Hembry, unpublished data). Multiple species are found growing sympatrically or along elevational contact zones at many locations on these islands.

The insects

All *Epicephala* species in the Society Islands are undescribed, but they have been reported from all ten *Glochidion* species considered here (Hembry et al. 2012 [Chapter 1, this volume]). Previous phylogenetic analysis (Chapter 3, this volume) suggests that at least three clades of *Epicephala* are present on Tahiti, Moorea, and Huahine: one clade on *Phyllanthus manono* on Tahiti and *P. nadeaudii* on Moorea; one clade on *P. huahineense* and *P. florencei* on Huahine; and one clade on the remaining taxa (*P. temehaniensis* on Huahine, *P. st-johnii* and *P. manono* on Moorea, and *P. taitensis*, *P. papenooense*, *P. orohenense*, and *P. grayanus* on Tahiti). *Epicephala* moths were sampled by collecting fruits from *Glochidion* species in the field and rearing larvae from them in either plastic bags or plastic rearing containers. Adult larvae (and in a few cases, larvae) were preserved in 96% ethanol for later DNA extraction. Strictly speaking, this procedure generates data on trophic interactions rather than pollination interactions, but based on the natural history of this interaction (Kawakita 2010) the former is expected to serve as an informative proxy for the latter. Numbers of *Epicephala* specimens sampled from each species of tree on each island are shown in Table 1. Collection information on the specimens collected is shown in Table 2. In 15 cases, multiple moths were collected from the same tree individuals (Table 2). Voucher specimens of the individual *Glochidion* hosts were collected for all of the trees sampled for *Epicephala* larvae in this study.

Molecular methods

Total genomic DNA was extracted from the thoraxes of ethanol-preserved adult *Epicephala*, and in a few cases, from the posterior ends of ethanol-preserved larvae as well using DNEasy kits (Qiagen Corp., Hilden, Germany). We amplified DNA from three loci using polymerase chain reaction: mitochondrial cytochrome oxidase I using HCO and LCO primers (Folmer et al. 1994), nuclear arginine kinase using ArgK-F4 and ArgK-R4 (Kawakita et al. 2004), and nuclear elongation factor-1α using ef1af2 and ef1ar2 (Kawakita et al. 2004). Previously published PCR protocols (Kawakita et al. 2004) were used for the two nuclear loci but with the annealing temperatures modified to 48° C for ArgK and 55° C for EF-1α. Products were purified using ExoSAP (Affymetrix, Foster City, CA) and Sanger sequenced, with raw sequences edited in Sequencher (Gene Codes Corporation, Ann Arbor, MI). Sequences were aligned using MUSCLE (Edgar 2004) and analyzed using Bayesian phylogenetic analysis in MrBayes 3.1 (Ronquist and Huelsenbeck 2003), with a model partitioned by codon position in each gene, for a

total of nine partitions. The model GTR+G was used for each partition. The undescribed *Epicephala* from *Glochidion obovatum* in Japan was used as the outgroup based on a previous analysis (Chapter 3, this volume). The analysis was run for 20 million generations, with convergence assessed using Tracer 1.5 (Rambaut and Drummond 2009). The first 25% of sampled trees were discarded as burn-in.

Network methods
To summarize patterns of host-specificity, network diagrams were drawn for each of the three islands. We used species designations for *Glochidion* following the revision by Florence (1997) and the nomenclator of Wagner and Lorence (2011). Each of the three minimally monophyletic clades of *Epicephala* recovered in the phylogenetic analysis was treated as a taxon. Although recognition of taxa based on monophyletic groups recovered through molecular phylogenetic analysis alone has been criticized in insects, we consider it to be warranted in this case because the fact that the same approach was used in the previous study of host-specificity in *Glochidion* and *Epicephala* and thus makes our results directly comparable (Kawakita and Kato 2006). Lines were drawn between *Epicephala* taxa and *Glochidion* species, with line thickness representing number of *Epicephala* individuals reared from each host plant taxon (i.e., interaction events). Available methods such as NODF (Almeida-Neto et al. 2008) for the quantitative examination of network properties (e.g., nestedness and modularity) do not work on small networks with 2-5 numbers of taxa in each trophic level, as found here (Paulo Guimarães, personal communication, 2011), so analysis of these networks was done qualitatively pending development of appropriate quantitative methods.

RESULTS

The phylogenetic hypothesis recovered for *Epicephala* from Huahine, Moorea, and Tahiti is shown in Figure 2. *Epicephala* fall into three clades, corresponding to the three previously reported clades from these islands (Chapter 3, this volume), and no previously unknown lineage of *Epicephala* was recovered. One clade is restricted to Tahiti and Moorea (Y1), another is restricted to Huahine (Y2), and the third is found across all three islands (Z). The clade Y2 found only on Huahine in this study is presumably found also on the neighboring Leeward Islands of Raiatea and Tahaa, based on the overlap in sampling with Chapter 3 (this volume). None of these three clades show any internal structure corresponding to either geography or host-plant taxon.

Network representation permits ease of visualizing the patterns of association between *Glochidion* species and these three *Epicephala* taxa across the three islands (Figure 3). Four of the *Glochidion* species (*Phyllanthus huahineense*, *P. florencei*, *P. grayanus*, and *P. taitensis*) are visited by two *Epicephala* moth species on the same island, with the remaining seven visited only by a single *Epicephala* species on any given island. *Phyllanthus manono* is visited by *Epicephala* clade Z on Moorea but clade Y1 on Tahiti, in an example of a geographic mosaic (Thompson 2005). However, each *Epicephala* taxon on each island is associated with more than one *Glochidion* species (with one exception, namely clade Y1 on *P. nadeaudii* on Moorea). As a consequence, whereas the network on Moorea is modular (is broken into two disconnected

subnetworks) as is generally expected for obligate pollinating seed predation mutualisms, the networks on Huahine and Tahiti lack modularity.

This lack of modularity on Tahiti and Huahine is upheld even if each of three morphologically similar species pairs of *Glochidion* (*P. orohenense* and *P. taitensis* on Tahiti; *P. florencei* and *P. huahineense* on Huahine; *P. manono* and *P. st-johnii* on Moorea) are treated as a single taxon in the network analysis. However, collapsing these species pairs increases by two the number of *Epicephala* taxa that locally visit a single *Glochidion* host species (Y2 on Huahine and Z on Moorea).

Finally, out of 15 individual *Glochidion* trees from which two moths were sampled, these results find two cases in which more than one *Epicephala* taxon visits the same individual tree: one example each for clades Y2 and Z visiting *P. florencei* and *P. huahineense* on Huahine.

DISCUSSION

a) Limited differentiation by geography but none by host-plant
Using molecular phylogenetic methods, this study finds no genetic differentiation of *Epicephala* on different host plants, and limited differentiation by geography (namely, clades Y1 and Y2 on the Windward and Leeward Society Islands, respectively). Phylogenetic differentiation on different host plants is widely known in phytophagous insects (Singer and Stireman 2005; Ohshima and Yoshizawa 2006; Althoff 2008; Matsubayashi et al. 2010) and, additionally, coevolution between host plant and mutualistic pollinator might be expected to promote pollinator differentiation by change in host plant, as has been argued for *Epicephala* (Kawakita et al. 2010).

This study does, however, find evidence of geographic differentiation in the *spinula* clade of *Epicephala* between the Windward and Leeward Society Islands, as suggested by a previous study (Chapter 3, this volume). This clade is older than Clade Z and has previously been found to show geographic differentiation by island or archipelago. Evidence for divergence between the Windward and Leeward islands (the greatest interisland distance in the Society Islands) has been previously reported in *Misumenops* crab spiders (Garb and Gillespie 2009). However, in contrast to these patterns in *Epicephala*, phylogeographic studies of a number of other Society Island arthropods/insects have shown diversification not only among islands but also within them (Claridge 2006, Lee et al. 2009). Such patterns have also been demonstrated in a number of other arthropod radiations on other volcanic Pacific islands, particularly Hawaii (Hormiga et al. 2003; Jordan et al. 2003; Garb and Gillespie 2006; Lapoint et al. 2011; Bennett and O'Grady in review).

Geographic differentiation has also previously been demonstrated in pollinating seed-predatory insects (see Smith et al. 2008, for instance). Since these three islands are so young (< 2.6 Ma), it is possible that not enough time has passed to permit further differentiation by host plant or geography to occur, especially within Clade Z. It is also possible, of course, that gene flow is restricted between *Epicephala* populations on different host species, or between islands, or that selection is acting differentially on particular loci in different *Epicephala* populations, in a

manner that is invisible to conventional molecular phylogenetic analysis. Such patterns might provide insight into speciation in these systems.

b) Network patterns between Glochidion and Epicephala

This study finds that network patterns between *Glochidion* and *Epicephala* appear to be modular on one island (Moorea) but are not modular on the other two (Huahine and Tahiti). The Huahine and Tahiti networks are in contrast with those reported from the Ryukyu Islands, which are modular (Figure 4). On many of the Ryukyu Islands, such as Okinawa and Amami, patterns of interaction have absolute modularity (made up only of one-to-one relationships) (Kawakita and Kato 2006).

These networks also provide information about the degree of dietary specialization by individual *Epicephala* taxa. They reveal that locally a single *Epicephala* taxon may interact with 1-4 species of *Glochidion*, and that a single *Glochidion* species may interact with 1-2 *Epicephala* taxa. It is difficult to statistically compare the symmetry of specialization in the absence of a quantitative method for analyzing small networks, but this finding is in contrast to that from Japan and Taiwan, where every *Epicephala* species thus far examined is locally restricted to a single host *Glochidion* species. However, the reverse (two species of *Epicephala* on the same host) has been previously observed on the adjacent islands of Yonaguni, Iriomote, and Ishigaki (the Yaeyama group), although not elsewhere in the Ryukyu Islands.

Out of 15 *Glochidion* trees from which more than one *Epicephala* was sampled, only two trees (one each of *P. florencei* and *P. huahineense*, both from Huahine) were found to have more than one *Epicephala* taxon present. This indicates that in at least some cases, an individual tree may interact with more than one pollinator taxon.

Why modularity and local dietary specialization of *Epicephala* differs between the Ryukyus and the Societies is not clear. It may be that the Society networks provide a window on the early stages of diversification in pollinating seed-predation mutualisms, and that, given enough time, these patterns of interaction will evolve into more modular networks through a process of lineage sorting, interspecific competition within each trophic level, and speciation. Consistent with this hypothesis, the phylogenetic distance among taxa in the Ryukyu *Glochidion* flora is far greater than that in the southeastern Polynesian *Glochidion* flora. However, the divergences between the *Epicephala* species found within any given island in both the Societies and in the Ryukyus spans the deepest nodes in the tree. Additionally, the fact that the Moorea network appears modular while the neighboring (and phylogenetically similar) Huahine and Tahiti communities do not suggests that closely related sets of lineages can assemble themselves into different realized patterns of interaction.

An alternative hypothesis is that climate may also act as a filter on patterns of specialization (Mueller et al. 2011), constraining the *Glochidion* or *Epicephala* lineages that can exist in the Ryukyus' subtropical climate, with the effect of making host-specificity more modular. It is not clear if the patterns observed in either the Ryukyus or in the Society Islands are representative of those between these taxa in tropical continental regions of Asia, New Guinea, and Australia, where regional diversity of *Glochidion* is highest (Govaerts et al. 2000). For example, at least

nine *Glochidion* species are found in the immediate vicinity of the research station at Lambir Hills National Park, Sarawak, Malaysia (Borneo; Chapter 3, this volume). Ultimately, distinguishing amongst these hypotheses will require information about the network structure of additional *Glochidion-Epicephala* communities not taxonomically autocorrelated with those considered here.

c) Network patterns in other specialized pollination systems
Pollination mutualisms are generally known to be nested (asymmetrically specialized) and only in some cases to be modular (Bascompte et al. 2003; Olesen et al. 2007). In principle, pollinating seed-predation networks have been assumed to be atypical in that they are more modular and lack nestedness (are more reciprocally specialized), but this assumption has not been examined using network theory. Whereas "low host specificity" has been reported with increasing frequency in the fig-fig wasp mutualism (Molbo et al. 2003, Machado et al. 2005, Cornille et al. 2012; see review in Herre et al. 2008), and host-specificity in yucca moths is known not to be absolute in all cases (Althoff et al. 2012), these findings have usually been expressed narratively and not with the aid of network diagrams, so it is difficult to make direct comparisons of modularity. It is possible for networks to have high modularity but "low host specificity" (i.e., for patterns of interaction to be less strict than one-to-one), so network approaches have great potential for the study of specialization in these mutualisms.

Orchid-orchid bee networks, classically thought to represent a highly specialized, obligate pollination mutualism, have been shown to be nested and not modular, but this mutualism has also recently been shown to not be obligate (Ramírez et al. 2011), and orchid bees are not seed predators of their orchid hosts. If high modularity is indeed a characteristic of pollinating seed-predation networks, this makes these mutualisms analogous not to other pollination networks, but to more symbiotic interactions such as ant-myrmecophyte networks (Guimarães et al. 2007) and potentially to anemone-anemone fish networks (Ricciardi et al. 2010), in which one trophic level spends much of its life cycle inside the tissues of the other.

d) Reproductive consequences for host plants
The apparent sharing of the same *Epicephala* pollinator across multiple, morphologically distinct but co-occurring species of *Glochidion* raises questions about the level of gene flow between *Glochidion* species. Morphologically intermediate, apparent hybrid trees are occasionally observed in the field (e.g., at an elevational contact zone between *P. temehaniensis* and *P. florencei* on Mt. Pohue Rahi, Huahine Iti; Hembry, unpublished data). Available phylogenetic markers do not distinguish amongst Society Islands *Glochidion* species (Chapter 3, this volume), but it may be possible to assess degree of gene flow among species with advances in sequencing technology. Such reproductive isolation or lack thereof may have great consequences for coevolution between *Glochidion* and *Epicephala*, as well as the process by which they codiversify.

CONCLUSIONS

By examining a recent, oceanic island co-radiation of mutualistic insects and plants, this study finds unexpected patterns of interaction that may provide a window into early stages of

diversification in coevolving, specialized mutualisms. We find lower host-specificity and greater modularity in the *Glochidion-Epicephala* interaction than previously reported from continents. Limited evidence is seen for geography driving divergence in the pollinating *Epicephala* moths at a broad scale, but no evidence is seen for host plant driving pollinator divergence. Different patterns on each island suggest that closely related lineages can assemble into very different realized patterns of interaction over short evolutionary timescales; and differences in modularity and specialization between continental and island networks in this system suggest that coevolving clades may pass through different patterns of interaction as they diversify.

Figure 1. Map of the Society Islands (French Polynesia), with Huahine, Moorea, and Tahiti indicated.

Huahine

Moorea

Tahiti

Figure 2. Bayesian consensus tree of *Epicephala* from Tahiti, Moorea, and Huahine (Society Islands).

nadeaudii Mo'orea 09123b
nadeaudii Mo'orea 09128a
manono Tahiti 07429a
nadeaudii Mo'orea 09112a
nadeaudii Mo'orea 09121e
nadeaudii Mo'orea 09114k
nadeaudii Mo'orea 09129a
grayanum Tahiti 11057a
manono Tahiti 09101a
nadeaudii Mo'orea 09110b
manono Tahiti 11071b
nadeaudii Mo'orea 09147a
nadeaudii Mo'orea 09109a
nadeaudii Mo'orea 09114d
manono Tahiti 09100a
taitense Tahiti 07376a
manono Tahiti 11072a
nadeaudii Mo'orea 09121a
nadeaudii Mo'orea 09123a
nadeaudii Mo'orea 07413a
nadeaudii Mo'orea 09107a
nadeaudii Mo'orea 09112b
manono Tahiti 11090a
nadeaudii Mo'orea 07329a
nadeaudii Mo'orea 09124a

Clade Y1:
Mo'orea and Tahiti

1

huahineense Huahine 11109a
huahineense Huahine 11109b
societatis Huahine 11122a
societatis Huahine 11126b
societatis Huahine 11107a
societatis Huahine 11124a
societatis Huahine 11111b
societatis Huahine 11108a
societatis Huahine 08472a
huahineense Huahine 08474a
societatis Huahine 08473b
societatis Huahine 11111a
societatis Huahine 11106a
societatis Huahine 11105a
societatis Huahine 08473a
huahineense Huahine 08478a
societatis Huahine 11106b
societatis Huahine 11110a
societatis Huahine 11110b

Clade Y2:
Huahine

1

1

manono Mo'orea 07438a
temehaniense Huahine 11132a
societatis Huahine 11123b
temehaniense Huahine 11133a
?temehaniense Huahine 11139a
manono Mo'orea 07438b
manono Mo'orea 11140a
huahineense Huahine 11125a
temehaniense Huahine 08030b
orohenense Tahiti 07356a
temehaniense Huahine 11120a
grayanum Tahiti 11053a
grayanum Tahiti 11083b
taitense Tahiti 11073a
orohenense Tahiti 08485a
manono Mo'orea 09143a
societatis Huahine 11126a
societatis Huahine 11123a
huahineense Huahine 08478b
myrtifolium Mo'orea 07442b
manono Mo'orea 07440a
taitense Tahiti 07404a
manono Mo'orea 07391a
temehaniense Huahine 11138a
?taitense Tahiti 11068a
myrtifolium Mo'orea 07442a
taitense Tahiti 07419a
taitense Tahiti 11093b
?orohenense Tahiti 07372a
?grayanum Tahiti 07420a
taitense Tahiti 07219a
grayanum Tahiti 08anaoriia
taitense Tahiti 11082a
grayanum Tahiti 11056a
temehaniense Huahine 08030a
taitense Tahiti 07411b
manono Mo'orea 07335b
grayanum Tahiti 07434a
papenooense Tahiti 08pape07a
huahineense Huahine 11113a
manono Mo'orea 08432a
manono Mo'orea 09142a
temehaniense Huahine 11137a
temehaniense Huahine 11129a
grayanum Tahiti 11059a

Clade Z:
Huahine,
Mo'orea, and
Tahiti

1

obovatum Japan

54

Figure 3. Network diagrams representing patterns of interaction between *Glochidion* species and *Epicephala* clades on three Society Islands. Line thickness represents number of *Epicephala* moths sampled. (i.e., interaction events).

Figure 4. Network diagrams representing patterns of interaction between *Glochidion* species and *Epicephala* clades on two islands in the Ryukyu Archipelago, Japan. Network diagrams derived by summarizing phylogeographic data from Kawakita and Kato (2006). Line thickness represents number of *Epicephala* moths sampled. (i.e., interaction events).

Amami (Japan)

Glochidion — *Epicephala*

acuminatum ———— sp. 2

lanceolatum ———— sp. 5

obovatum ———— sp. 4

zeylanicum ———— sp. 6

Ishigaki (Japan)

Glochidion — *Epicephala*

lanceolatum ———— sp. 5

rubrum ———— sp. 3

zeylanicum ———— sp. 4

———— sp. 6

Table 1. *Glochidion* species present on the islands of Tahiti, Moorea, and Huahine (elevational data from Florence 1997).

Name	Synonym	Distribution	Elevational range (m a.s.l.)
P. florencei	*G. societatis*	Huahine[1]	0-225
P. huahineense	*G. huahineense*	Huahine	1-4
P. temehaniensis	*G. temehaniense*	Huahine[2]	0-600
P. nadeaudii	*G. nadeaudii*	Moorea	420-800
P. st-johnii	*G. myrtifolium*	Moorea[3]	30-680
P. manono	*G. manono*	Moorea, Tahiti	30-1000
P. grayanus	*G. grayanum*	Tahiti	60-1040
P. orohenense	*G. orohenense*	Tahiti	900-1750
P. papenooense	*G. papenooense*	Tahiti	ca. 500
P. taitensis	*G. taitense*	Tahiti[4]	50-1500

1. Also present on Raiatea, Tahaa, Maupiti, and Rimatara
2. Also present on Raiatea and Tahaa
3. Also present on Raiatea, Tahaa, and Bora Bora
4. Collected once on Moorea; never found on that island in this study.

Table 2. Collection data for *Epicephala* moth specimens examined in Chapter 4.

Specimen ID	Island	Host species	Locality
DHH-08-030A	Huahine	*temehaniensis*	Mt. Pohue Rahi
DHH-08-030B	Huahine	*temehaniensis*	Mt. Pohue Rahi
DHH-08-472A	Huahine	*florencei*	Fare
DHH-08-473A	Huahine	*florencei*	Fare
DHH-08-473B	Huahine	*florencei*	Fare
DHH-08-474A	Huahine	*huahineense*	Fare
DHH-08-478A	Huahine	*huahineense*	Fare
DHH-08-478B	Huahine	*huahineense*	Fare
DHH-08-478C	Huahine	*huahineense*	Fare
DHH-11-105A	Huahine	*florencei*	Motu de Maeva, yard of Pension Fare Maeva
DHH-11-106A	Huahine	*florencei*	Motu de Maeva, road to airport
DHH-11-106B	Huahine	*florencei*	Motu de Maeva, road to airport
DHH-11-107A	Huahine	*florencei*	Motu de Maeva, road to airport
DHH-11-108A	Huahine	*florencei*	Motu de Maeva, road to airport
DHH-11-109A	Huahine	*huahineense*	Motu de Maeva, road to airport
DHH-11-109B	Huahine	*huahineense*	Motu de Maeva, road to airport
DHH-11-110A	Huahine	*florencei*	Motu de Maeva, road to airport
DHH-11-110B	Huahine	*florencei*	Motu de Maeva, road to airport
DHH-11-111A	Huahine	*florencei*	Motu de Maeva
DHH-11-111B	Huahine	*florencei*	Motu de Maeva, road to airport
DHH-11-113A	Huahine	*huahineense*	Motu de Maeva, east end
DHH-11-120A	Huahine	*temehaniensis*	Mt. Turi
DHH-11-122A	Huahine	*florencei*	Mt. Mou'a Tapu
DHH-11-123A	Huahine	*florencei*	Mt. Mou'a Tapu
DHH-11-123B	Huahine	*florencei*	Mt. Mou'a Tapu
DHH-11-124A	Huahine	*florencei*	Mt. Mou'a Tapu
DHH-11-125A	Huahine	*huahineense*	Mt. Mou'a Tapu
DHH-11-126A	Huahine	*florencei*	Mt. Mou'a Tapu
DHH-11-126B	Huahine	*florencei*	Mt. Mou'a Tapu
DHH-11-129A	Huahine	*temehaniensis*	Mt. Pohue Rahi
DHH-11-132A	Huahine	*temehaniensis*	Mt. Pohue Rahi
DHH-11-133A	Huahine	*temehaniensis*	Mt. Pohue Rahi
DHH-11-137A	Huahine	*temehaniensis*	Mt. Pohue Rahi
DHH-11-138A	Huahine	*temehaniensis*	Mt. Pohue Rahi
DHH-11-139A	Huahine	putative *temehaniensis* x *florencei*	Mt. Pohue Rahi
DHH-07-329	Moorea	*nadeaudii*	Mt. Rotui

DHH-07-335B	Moorea	*manono*	'Opunohu Valley: Sommet des Trois Pinus
DHH-07-383A	Moorea	*st-johnii*	Vaianae
DHH-07-391A	Moorea	*manono*	Mt. Fairurani
DHH-07-438A	Moorea	*manono*	near Marae Teti'iroa
DHH-07-438B	Moorea	*manono*	near Marae Teti'iroa
DHH-07-440A	Moorea	*manono*	Mou'aroa
DHH-07-442A	Moorea	*st-johnii*	Vaianae
DHH-07-442B	Moorea	*st-johnii*	Vaianae
DHH-08-432A	Moorea	*manono*	Col des Trois Cocotiers
DHH-09-107A	Moorea	*nadeaudii*	Mt. Rotui
DHH-09-109A	Moorea	*nadeaudii*	Mt. Rotui
DHH-09-110B	Moorea	*nadeaudii*	Mt. Rotui
DHH-09-112A	Moorea	*nadeaudii*	Mt. Rotui
DHH-09-112B	Moorea	*nadeaudii*	Mt. Rotui
DHH-09-114D	Moorea	*nadeaudii*	Mt. Rotui
DHH-09-114K	Moorea	*nadeaudii*	Mt. Rotui
DHH-09-121A	Moorea	*nadeaudii*	Mt. Mou'aputa
DHH-09-121E	Moorea	*nadeaudii*	Mt. Mou'aputa
DHH-09-123A	Moorea	*nadeaudii*	Mt. Fairurani
DHH-09-123B	Moorea	*nadeaudii*	Mt. Fairurani
DHH-09-124A	Moorea	*nadeaudii*	Mt. Fairurani
DHH-09-128A	Moorea	*nadeaudii*	Mt. Fairurani
DHH-09-129A	Moorea	*nadeaudii*	Mt. Fairurani
DHH-09-142A	Moorea	*manono*	'Opunohu Valley: Sommet des Trois Pinus
DHH-09-143A	Moorea	*manono*	'Opunohu Valley: Sommet des Trois Pinus
DHH-09-147A	Moorea	*nadeaudii*	Pitons de Vaianae
DHH-11-140A	Moorea	*manono*	Opunohu Valley, near Belvédère
DHH-07-219A	Tahiti	*taitensis*	Mt. Marau
DHH-07-356A	Tahiti	*orohenense*	Mt. Aora'i: Fare Mato
DHH-07-372A	Tahiti	*?orohenense*	Mt. Aora'i
DHH-07-376A	Tahiti	*taitensis*	Mt. Aora'i
DHH-07-404A	Tahiti	*taitensis*	Mt. Marau road, stand à tirs
DHH-07-411B	Tahiti	*taitensis*	Mt. Marau
DHH-07-413A	Tahiti	*manono*	Papeno'o Valley
DHH-07-419A	Tahiti	*taitensis*	Papeno'o Valley
DHH-07-420A	Tahiti	*?grayanus*	Papeno'o Valley
DHH-07-429A	Tahiti	*manono*	Papeno'o Valley
DHH-07-434A	Tahiti	*grayanus*	Belvédère de Taravao

DHH-08-485A	Tahiti	*orohenense*	Mt. Marau
DHH-08-ANAORIIA	Tahiti	*grayanus*	Papeno'o Valley: Anaori'i Plateau
DHH-08-PAPE07A	Tahiti	*papenooense*	Papeno'o Valley: Anaori'i Plateau
DHH-09-100A	Tahiti	*manono*	Taravao Plateau: Toahotu
DHH-09-101A	Tahiti	*manono*	Taravao Plateau: Toahotu
DHH-11-053A	Tahiti	*grayanus*	Hitia'a lavatubes
DHH-11-056A	Tahiti	*grayanus*	Hitia'a lavatubes
DHH-11-057A	Tahiti	*grayanus*	Hitia'a lavatubes
DHH-11-059A	Tahiti	*grayanus*	Hitia'a lavatubes
DHH-11-068A	Tahiti	*?taitensis*	Mt. Aora'i
DHH-11-071B	Tahiti	*manono*	Mt. Aora'i
DHH-11-072A	Tahiti	*manono* (pubescent)	Mt. Aora'i
DHH-11-073A	Tahiti	*taitensis*	Pic Vert
DHH-11-082A	Tahiti	*taitensis*	Belvédère de Taravao
DHH-11-083B	Tahiti	*grayanus*	Belvédère de Taravao
DHH-11-090A	Tahiti	*manono* (pubescent)	col du Tahara'a
DHH-11-093B	Tahiti	*taitensis*	col du Tahara'a

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Appendix 1: Résumé en français

La coévolution entre les espèces qui sont phylogénétiquement éloignées mais écologiquement liées est souvent considérée comme jouant un rôle central dans l'origine et le maintien de la biodiversité sur Terre. Cependant, chez beaucoup de clades considérés comme étant « en voie de coévolution », nous ne connaissons pas leur histoire évolutive de diversification ni la manière dont elles s'organisent en réseaux d'interactions. Ce manque est particulièrement remarquable au sein des associations mutualistes, malgré le fait que les mutualismes sont des systèmes modèles pour de nombreuses recherches en coévolution. Dans ma thèse de doctorat, j'étudie la codiversification d'un mutualisme obligatoire et spécialisé de façon réciproque entre les papillons de nuit du genre *Epicephala* (Lepidoptera : Gracillariidae) et les arbres du genre *Glochidion* (Phyllanthaceae : *Phyllanthus* sensu lato) sur les îles océaniques de la Polynésie orientale.

Les papillons *Epicephala* sont les uniques pollinisateurs connus de cinq clades de *Phyllanthus* sensu lato (y compris les genres *Glochidion* et *Breynia*) ; cette interaction mutualiste est donc considérée comme étant obligatoire. Les papillons femelles transfèrent de manière active le pollen des fleurs mâles aux fleurs femelles en utilisant une trompe poilue, via laquelle le pollen est déposé sur la surface stigmatique concave située au bout de la colonne stylaire. Les papillons pondent ensuite un œuf à l'intérieur des ovaires des fleurs. Leurs larves vont se nourrir d'une part infime des graines qui vont se développer. Le papillon est donc à la fois un pollinisateur et un prédateur des graines de son hôte. D'ailleurs, ce mutualisme est remarquable de par le très haut niveau de spécialisation réciproque qui le caractérise; chaque espèce de plante s'associe uniquement avec une ou deux espèces de papillon et vice-versa. Toutes ces caractéristiques importantes indiquent que le mutualisme entre *Phyllanthus* et ses papillons est similaire à ceux connus entre les figuiers et leurs micro-guêpes pollinisatrices ou entre le genre *Yucca* (Agavaceae) et ses papillons pollinisateurs.

Dans cette thèse j'ai étudié le genre *Glochidion* (*mahame* ou *mānono* en tahitien; *tevai* ou *hi'itevai* en marquisien; *kaema* en rapa; *motoi* en mangarévien; *ma'ame* ou *makai* en māori des îles Cook), qui est le clade de *Phyllanthus* sensu lato le mieux connu. Il existe 300 espèces décrites de *Glochidion*, de l'Asie tropicale du Pakistan au Japon au nord, en Australie au sud et jusqu'aux îles Pitcairn à l'est. A cause de la grande diversité d'espèces au sein de ce genre, je me suis concentré sur la co-radiation de 24 espèces de *Glochidion* avec leurs papillons *Epicephala* sur les îles océaniques de Polynésie orientale (îles Cook, Polynésie française, et îles Pitcairn). Toutes ces îles sont d'origine volcanique et ont été créées quand la plaque du Pacifique s'est déplacée au-dessus d'une série de points chauds du manteau terrestre. La plupart d'entre elles datent du Pliocène ou du Pléistocène (moins de 5 millions d'années). Toutes les espèces de *Glochidion* rencontrées dans cette région en sont endémiques ; la majorité des espèces est endémique d'un seul archipel. *Glochidion* est donc l'une des plus grandes radiations de plantes endémiques dans ce « *hotspot* » de biodiversité. Ces espèces diffèrent les uns des autres par la morphologie des fleurs femelles. Sur la plupart des îles, on rencontre de une à trois espèces de *Glochidion* mais on peut en rencontrer de quatre à sept sur les îles les plus grandes. Plusieurs

espèces se retrouvent en sympatrie. Tous ces facteurs suggèrent que cette diversification a eu lieu très rapidement.

Dans le chapitre 1, je démontre que le mutualisme entre *Glochidion* et *Epicephala*, déjà connu des régions continentales, se retrouve également en Polynésie orientale. *Glochidion* avait été déjà répertorié dans presque toutes les îles hautes de cette région, mais *Epicephala* n'avait été répertorié que dans deux d'entre elles (Nuku Hiva et Fatu Hiva, îles Marquises). A cause des différences de temps de génération entre *Glochidion* et *Epicephala*, il était peu probable que les deux espèces puissent arriver sur une nouvelle île durant le même événement de dispersion. Avec mes co-auteurs, j'ai découvert et photographié les *Epicephala* en train de polliniser les fleurs de *Glochidion* sur deux îles de l'archipel de la Société (Tahiti et Raiatea), découvert du pollen sur d'autres échantillons d'*Epicephala* récoltés dans d'autres îles du Pacifique et élevé des *Epicephala* à partir des fruits de 19 espèces de *Glochidion* sur 17 îles où ils n'étaient pas connus auparavant. Ces résultats indiquent notamment que ce mutualisme a été capable de se dissocier et de se réassembler plusieurs fois sur certaines îles isolées. Cette conclusion est en contradiction avec un paradigme en biogéographie des îles qui veut que les espèces qui dépendent d'interactions biotiques spécialisées ne devraient pas pouvoir s'établir sur les îles isolées. Ces résultats sont aussi intéressants pour les études portant sur la réponse des mutualismes spécialisés aux changements globaux.

Dans le chapitre 2, je démontre que non seulement *Epicephala*, mais aussi plusieurs autres lignées d'insectes parasites (antagonistes) spécialistes des Phyllanthacées ont colonisé la Polynésie orientale. Ces insectes incluent le papillon de la « mineuse des feuilles » *Diphtheroptila* (Gracillariidae), le papillon « prédateur de graines » *Tritopterna* (Tortricidae), et peut-être aussi les papillons *Caloptilia* (Gracillariidae) et *Dudua* (Tortricidae), dont les larves tordent les feuilles dont elles se nourrissent. Les résultats de cet inventaire indiquent que le conservatisme de niche au niveau de la plante-hôte peut jouer un rôle important dans l'assemblage des communautés d'insectes phytophages, même sur les îles très éloignées. Ces résultats sont discutés dans le contexte de la littérature sur la radiation adaptive (aux nouvelles plantes-hôtes) et la conservation de niche (sur la même plante-hôte) chez les faunes d'insectes phytophages des îles océaniques en général.

Dans le chapitre 3, j'utilise les méthodes de phylogénie moléculaire pour reconstruire l'histoire évolutive de *Glochidion* et de leurs *Epicephala* en Polynésie orientale, en l'incluant dans le contexte biogéographique de toute la région Asie-Pacifique. Les *Glochidion* ont colonisé la Polynésie orientale deux fois : une colonisation s'est établie et répandue à travers les îles Cook (sud), les îles de la Société, les îles Australes, les Marquises, et Tuamotu; une deuxième s'est établie uniquement sur Mangareva (îles Gambier). Les papillons *Epicephala* ont colonisé la région au moins deux fois. Une première colonisation s'est répandue à travers les îles Cook (sud), les îles de la Société, les îles Marquises, Tuamotu, et sur Rapa (îles Australes), pendant qu'une deuxième colonisation plus récente s'établissait aux îles Cook, sur les îles Australes du Nord, et sur les îles de la Société. Ces résultats indiquent non seulement que le mutualisme *Glochidion-Epicephala* s'est diversifié d'une manière qui ne permet pas la congruence phylogénétique entre ces deux lignées, mais aussi qu'une des lignées de pollinisateurs (la plus récente) s'est répandue rapidement sur une dizaine de nouvelles plantes-hôtes à travers une grande région géographique. La phylogénie d'*Epicephala* révèle donc un rôle de l'isolement

géographique et du changement d'hôtes (*host-shifts*) dans la diversification de cette association mutualiste. De manière particulièrement intéressante, ces résultats démontrent que malgré toutes les contraintes envisageables pouvant empêcher plusieurs colonisations indépendantes par *Epicephala* (isolement géographique, spécialisation réciproque entre les espèces de *Glochidion* et *Epicephala* et exclusion compétitive entre les deux clades d'*Epicephala*), ces papillons ont pu s'établir de façon répétée sur ces îles éloignées et se répandre rapidement sur de nouveaux hôtes une fois arrivés. Par conséquent, la dynamique des réseaux d'interaction entre *Glochidion* et *Epicephala* dans les régions continentales doit être similaire à celle observée en Polynésie orientale. Les implications de ces résultats sont aussi discutées dans le cadre de la diversification de mutualismes plantes-polinisateurs où les pollinisateurs sont également prédateurs des graines.

Dans le chapitre 4, j'utilise les méthodes de phylogénie moléculaire et d'analyse de réseaux pour tester si la topologie des réseaux d'interactions entre les espèces de *Glochidion* et d'*Epicephala* est spécialisée de manière réciproque et modulaire telle qu'elle peut l'être dans les régions continentales. Je me suis concentré sur les trois îles de la Société les plus récentes où se retrouve le genre *Glochidion* (Huahine, Moorea, Tahiti). Ces trois îles ont en tout dix espèces de cette plante. Mes résultats montrent que les réseaux d'interactions *Glochidion-Epicephala* sur deux de ces îles (Huahine et Tahiti) manquent de modularité, ce qui les distingue des réseaux connus entre ces deux genres en Asie. Ces résultats pourraient être caractéristiques des premières étapes de diversification chez ces mutualismes et suggèrent que les clades « en voie de coévolution » peuvent changer de topologie d'interaction au cours de leur diversification.

Pris tous ensemble, ces résultats indiquent que ces mutualistes à cycle de vie partiellement symbiotique ont colonisé les îles océaniques éloignées de manière répétée, mais pas de manière congruente. Ces colonisations multiples ont engendré une histoire coévolutive dynamique pendant plusieurs millions d'années, dont en témoignent les changements de plante-hôte, la diversification allopatrique sur les différents archipels et les réseaux d'interaction moins modulaires dans ces archipels que dans ce qui est déjà connu de ces mutualismes et de mutualismes similaires sur les continents.

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Traduction: Auteur
Rédaction: Juliane Casquet

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Appendix 2: Rearing and specimen data for Chapter 1

List of Eastern Polynesian *Glochidion* species examined for *Epicephala*, with specimen data and details of *Epicephala* reared from *Glochidion* fruits. Southern Cook archipelago is part of the Cook Islands; all other archipelagos are part of French Polynesia. Coordinates are not available for certain taxa for the following reasons: 1) Data have been withheld ("withheld") for 3 species of *Glochidion* (*G. hivaoaense, G. huahineense,* and *G. papenooense*) that are protected as "*Classe A*" species in French Polynesia (Arrêté n° 306 CM, 20 February 2008) as well as for a fourth species (*G. wilderi*) that is critically endangered but not protected by French Polynesian law. The withholding of these data was requested of us by the Délégation à la Recherche, one of two French Polynesian government agencies responsible for issuing research permits for this study. 2) Coordinate data are missing for several specimens ("N/A") due to an accident in which a GPS unit was destroyed in the field by heavy rain.
Epicephala specimens share the same collection number (and specimen number) as the individual trees from which they were collected.

Abbreviations and institutional codes: BISH: Herbarium Pacificum, Bishop Museum, Honolulu, Hawaiʻi, USA; UC: University Herbaria, University of California, Berkeley, California, USA; Essig: Essig Museum of Entomology, University of California, Berkeley, California, USA; Bishop: Bishop Museum, Honolulu, Hawaiʻi, USA

Glochidion species	Archipelago	Island	Site	Elevation (m)	UTM UPS coordinates	Date collection	Date emergence	*Glochidion* voucher institution	*Epicephala* voucher institution	Collectors, number
brothersonii	Society	Raiatea	Mt. Oropiro	53	05 K 0670845 8132465	24-Nov-09	preserved as larva	UC	Bishop	D. Hembry, E. Brotherson, and A. Yang 09-151
concolor aff.	Southern Cook	Rarotonga	Near start of Te Ko'u track, Avarua	171	04 K 0419676 7651855	7-May-08	preserved as larva	BISH	Bishop	D. Hembry and G. McCormack 08-308
emarginatum	Society	Raiatea	Te Mehani Rahi Plateau	711	05 K 0665206 8144297	17-Jul-08	4-Aug-08	UC	Essig	D. Hembry 08-456
grayanum	Society	Tahiti	Tahiti Iti: Taravao Plateau	796	N/A	21-Nov-07	9-Dec-07	UC	Essig	D. Hembry and E. Spotswood 07-434
hivaoaense	Marquesas	Hiva Oa	Mt. Temetiu	999	withheld	20-Aug-07	no *Epicephala* larvae found	UC	N/A	D. Hembry leg. J.-Y. Meyer 07-211
huahineense	Society	Huahine	Motu Oavarei	sea level	withheld	19-Jul-08	4-Aug-08	UC	Essig	D. Hembry 08-478
longfieldiae	Austral	Rapa	Morogouta	177	06 J 0761863 6942383	30-Mar-08	18-Apr-08	UC	Essig	D. Hembry and C. Ewing 08-255
manono	Society	Moorea	Sommet des Trois Pinus, Opunohu Valley	N/A	N/A	1-Oct-07	22-Oct-07	UC	Essig	D. Hembry 07-335
manono	Society	Tahiti	Tahiti Nui: Papenoo Valley	428	N/A	20-Nov-07	10-Dec-07	UC	Essig	D. Hembry and E. Spotswood 07-429
marchionicum	Marquesas	Nuku Hiva	Hatiheu	93	07 L 0599756 9025148	4-Aug-07	19-Aug-07	UC	Essig	D. Hembry, E. Claridge, S. McEvey, and P. Oboyski 07-131
marchionicum	Marquesas	Ua Pou	Road between Haakuti and Hakamaii	279	07 L 0707879 8724244	10-Aug-07	28-Aug-07	UC	Essig	D. Hembry 07-169
marchionicum	Marquesas	Fatu Hiva	Ridge between Omoa and Hanavave	c. 650	N/A	16-Aug-07	31-Aug-07	UC	Essig	D. Hembry, E. Claridge, and C. Ewing 07-194
moorei	Society	Raiatea	Te Mehani Rahi Plateau	c. 400	N/A	12-Sep-07	30-Sep-07	UC	Essig	D. Hembry, E. Pellé, D. Polhemus, and J. Polhemus 07-310
myrtifolium	Society	Bora Bora	Mt. Pahia	N/A	N/A	31-Dec-07	14-Jan-08	UC	Essig	D. Hembry and C. Hetherington 07-483
myrtifolium	Society	Moorea	Vaianae Valley	401	N/A	24-Oct-07	17-Nov-07	UC	Essig	D. Hembry and L. Long 07-383

nadeaudii	Society	Moorea	Mt. Fairurani	738	06 K 0204118 8062922	28-Oct-09	12-Nov-09	UC	Essig	D. Hembry and A. Yang 09-123
orohenense	Society	Tahiti	Tahiti Nui: Mt. Aorai	1423	06 K 0231325 8051069	23-Jul-08	10-Aug-08	UC	Essig	D. Hembry and M. McElroy 08-485
papenooense	Society	Tahiti	Tahiti Nui: Papenoo Valley	N/A	withheld	28-Jul-08	10-Aug-08	UC	Essig	D. Hembry and R. Taputuarai PAPE-07
raivavense	Austral	Rurutu	Mt. Manureva massif	N/A	N/A	28-Feb-08	11-Mar-08	UC	Essig	D. Hembry 08-161
raivavense	Austral	Raivavae	Mt. Taraia, south slope	168	06 K 0431616 7358872	5-Mar-08	17-Mar-08	UC	Essig	D. Hembry and J.-Y. Meyer 08-179
rapaense	Austral	Rapa	Southeast slope Mt. Vaitu, Vallée Ruapai	127	06 J 0763379 6945268	26-Mar-08	no *Epicephala* larvae found	UC	N/A	D. Hembry and C. Ewing 08-235
societatis	Austral	Rimatara	Centre of island	N/A	N/A	24-Feb-08	preserved as larva	UC	Bishop	D. Hembry 08-143
societatis	Society	Huahine	Motu Oavarei	sea level	05 K 0769906 8152776	19-Jul-08	4-Aug-08	UC	Essig	D. Hembry 08-473
societatis	Society	Maupiti	Mt. Hotuparaoa	N/A	05 K 0579756 8180736	11-Dec-07	28-Dec-08	UC	Essig	D. Hembry 07-450
societatis	Society	Raiatea	Mt. Tapioi	258	05 K 0665006 8149295	13-Jul-08	30-Jul-08	UC	Essig	D. Hembry, S. Faraire, and D. Faraire 08-445
taitense	Society	Tahiti	Tahiti Nui: Mt. Marau	1423	N/A	18-Nov-07	3-Dec-07	UC	Essig	D. Hembry 07-411
taitense	Southern Cook	ʻĀtiu	Tarapaku Landing	15	04 K 0595580 7788891	15-May-08	preserved as larva	BISH	Bishop	D. Hembry and K. Henry 08-329
taitense	Southern Cook	Mangaia	North of Oneroa, west side of island	5	04 K 0609241 7578003	13-May-08	preserved as larva	BISH	Bishop	D. Hembry and A. Tuara 08-318
temehaniense	Society	Huahine	Huahine Iti: Mt. Puhaerei	397	N/A	4-Jan-08	20-Jan-08	UC	Essig	D. Hembry and C. Hetherington 08-030
temehaniense	Society	Raiatea	Te Mehani Rahi Plateau	c. 400	N/A	12-Sep-07	3-Oct-07	UC	Essig	D. Hembry, E. Pellé, D. Polhemus, and J. Polhemus 07-307
temehaniense	Society	Tahaa	Col Vaiautea	61 m	05 K 0659600 8160473	10-Jul-08	30-Jul-08	UC	Essig	D. Hembry 08-443
tooviianum	Marquesas	Nuku Hiva	Toovii Plateau	847	07 L 0592334 9020572	6-Aug-07	2-Sep-07	UC	Essig	D. Hembry 07-151
tuamotuense	Tuamotu	Niau	North side of atoll	8	06 K 0565640 8215608	14-Jun-08	30-Jun-08	UC	Essig	D. Hembry 08-404
wilderi	Gambier	Mangareva	Forest at base of cliffs of Mt. Duff	110	withheld	6-Jun-08	no fruits	UC	N/A	D. Hembry and R. Taputuarai 08-391

Appendix 3: Insect taxa reared from *Glochidion* species on each island.

Number of specimens of each insect taxon reared from each species of *Glochidion* on each island. Numbers do not include larvae or pupae that died before adult eclosion. *Glochidion* species are grouped by archipelago, and then by island. Archipelagos are subdivided to reflect geology and geography: the Austral Islands are divided into the Northern Australs and Rapa, and the Society Islands are divided into the Leeward Islands and Windward Islands. Niau is in the Tuamotu Islands.

"Gelechioid" refers specifically to the unidentified gelechioids that produce frass and silk larval tubes. "Other" includes Hymenoptera (see Results) as well as some Lepidoptera: two *Imma* (Lepidoptera: Immidae) from the Societies (Moorea and Raiatea), six unidentified gelechioids from the Australs (Rurutu) and Societies (Maupiti, Tahaa), as well as a few other singletons belonging to unidentified moth taxa.

Archipelago	Island	Host species	Number of trees	Epicephala	Diphtheroptila	Caloptilia	Tritopterna	Dudua	gelechioid	Agromyzidae	Other
Southern Cook	Rarotonga	*P. concolor*	1	0	0	1	0	0	0	0	0
	Mangaia	*G.* sp.	2	0	1	0	0	2	0	0	0
	ʻĀtiu	*G.* sp.	2	0	0	0	1	1	0	0	0
Austral (Northern)	Rimatara	*P. florencei*	2	0	1	0	0	1	0	0	0
	Rurutu	*P. raivavense*	9	3	3	0	10	7	0	0	3
	Tubuai	*P. raivavense*	0	0	0	0	0	0	0	0	0
	Raivavae	*P. raivavense*	4	0	8	0	0	0	0	0	0
Austral (Rapa)	Rapa	*G.* sp.	3	5	0	0	5	0	0	0	0
	Rapa	*P. longfieldiae*	10	14	0	1	4	0	0	2	4
	Rapa	*P. rapaense*	2	0	0	0	1	0	0	did not rear	0
	Rapa	*G.* sp. (hybrid?)	1	3	0	0	3	0	0	0	0
Society (Leeward)	Maupiti	*P. florencei*	8	2	14	0	7	3	0	did not rear	5
	Bora Bora	*P. st-johnii*	4	8	1	0	0	3	0	0	1
	Tahaa	*P. florencei*	1	0	0	0	0	0	0	0	2
	Tahaa	*P. temehaniensis*	3	4	1	0	1	0	0	0	0
	Raiatea	*P. florencei*	2	3	0	0	0	0	0	0	0
	Raiatea	*P. st-johnii*	8	13	5	0	0	0	0	did not rear	0
	Raiatea	*P. temehaniensis*	2	6	0	0	0	0	0	0	0
	Raiatea	*P. raiateensis*	2	8	0	0	1	0	0	0	0
	Raiatea	*P. emarginatus*	2	16	0	0	0	0	0	0	0
	Raiatea	*P.* sp. (not identified)	5	4	1	0	0	0	0	0	1
	Huahine	*P. florencei*	8	11	0	0	7	0	0	0	0
	Huahine	*P. huahineense*	7	17	1	1	4	3	0	0	0
	Huahine	*P. temehaniensis*	4	5	1	0	0	0	0	0	0

Society (Windward)	Moorea	*P. st-johnii*	**2**	7	0	0	1	0	0	0	0
	Moorea	*P. nadeaudii*	**21**	38	9	0	15	1	4	0	2
	Moorea	*P. manono*	**24**	21	20	2	9	1	2	0	4
	Moorea	*P.* sp. (not identified)	**6**	1	8	0	0	0	0	0	0
	Tahiti	*P. manono*	**7**	12	3	0	1	0	0	0	0
	Tahiti	*P. grayanus*	**3**	6	0	0	0	0	0	0	2
	Tahiti	*P. papenooense*	**1**	0	1	0	0	0	0	0	0
	Tahiti	*P. taitensis*	**7**	16	1	0	0	0	2	0	0
	Tahiti	*P. orohenense*	**5**	3	1	0	0	0	0	0	2
Tuamotu	Niau	*P. tuamotuensis*	**3**	3	2	0	0	2	0	0	0
Marquesas	Nuku Hiva	*P. marchionicus*	**10**	5	0	1	3	0	0	0	7
	Ua Pou	*P. marchionicus*	**6**	2	0	0	8	0	0	0	3
	Hiva Oa	*P. marchionicus*	**0**	0	0	0	0	0	0	0	0
	Hiva Oa	*P. hivaoaense*	**0**	0	0	0	0	0	0	0	0
	Fatu Hiva	*P. marchionicus*	**5**	19	0	0	11	0	0	0	4
Gambier	Mangareva	*P. wilderi*	**10**	0	0	0	0	0	0	0	0
Totals			**202**	**255**	**82**	**6**	**92**	**24**	**8**	**2**	**40**

Appendix 4: Images of larvae and adults of insects associated with *Glochidion* trees in southeastern Polynesia.

a. *Epicephala* pollinating *Phyllanthus grayanus*, Belvédère, Taravao Plateau, Tahiti, Society Islands

b. *Diphtheroptila* larva mining leaf of *P. tuamotuensis*, Niau, Tuamotu Islands

c. *Diphtheroptila* adult, reared from *P.* sp., Mangaia, southern Cook Islands

d. *Diphtheroptila* larva mining leaf of *P. pinaiensis*, rim of Opunohu Valley near Mouaroa, Moorea, Society Islands

e. *Caloptilia* larva mining leaf of *P. marchionicus*, Mt. Tekao, Nuku Hiva, Marquesas Islands. At later stages in larval development the edge of the leaf is rolled to form a leaf edge roll.

f. *Caloptilia* adult reared from *P. concolor* aff., Rarotonga, southern Cook Islands.

g. *Tritopterna* adult reared from *P.* sp., ʻĀtiu, southern Cook Islands

h. *Dudua* larval leaf roll, *P. manono*, Maatea Valley, Moorea. Leaves rolled by *Dudua* larvae at the ends of *Glochidion* shoots turn brown and are distinctive.

i. *Dudua* adult reared from *P.* sp., Mangaia

j. Frass tube on leaf of *P. orohenense*, Mt. Marau, Tahiti. Larva hides in tube and scrapes upper surface of *Glochidion* leaf to feed.

k. Adult gelechioid reared from frass tube on leaves of *P. nadeaudii*, Mt. Rotui, Moorea.

l. Agromyzid mines containing pupa in leaves of *P. florencei*, Mt. Tapioi, Raiatea, Society Islands.

Appendix 5: Literature and museum surveys for host records

Epicephala **(Gracillariidae: Gracillariinae)**

Epicephala are seed-feeders and often pollinators of *Phyllanthus* s. s., *Glochidion* s. l., *Breynia*, and *Flueggea* (all Phyllanthaceae), throughout the old World tropics (Robinson et al. 1994, Kato et al. 2003; De Prins and De Prins 2005; Kawakita and Kato 2009; Hu et al. 2011; Hembry et al. 2012 [Chapter 1, this volume]). They have been reported from *Glochidion* s. l. in the southern Cook, Austral, Society, Tuamotu, and Marquesas archipelagos (Clarke 1986; Hembry et al. 2012 [Chapter 1, this volume]).

Phyllanthaceae-associated *Epicephala* are strongly supported as monophyletic (Kawakita and Kato 2009). Although a few continental *Epicephala* species are described from non-Phyllanthaceae hosts (De Prins and De Prins 2005), these are morphologically distinct from the Phyllanthaceae-associated taxa and should be removed from the genus *Epicephala* in order to make the latter monophyletic (T. Kumata, personal communication, 2010).

Diphtheroptila **(Gracillariidae: Gracillariinae)**

Three described and five undescribed species of *Diphtheroptila* are known from southern Africa, Australia, Japan, and Taiwan (De Prins and De Prins 2005; Kawakita et al. 2010). *Diphtheroptila* appears to have also been collected but identified as *Stomphastis* or *Conopomorpha* from Rarotonga, Cook Islands (McCormack 2007). All *Diphtheroptila* host records are from Phyllanthaceae, in which they mine the leaves. *D. ochridorsellum* is recorded from *G. ferdinandi* in New South Wales, Australia (Meyrick 1880; Meyrick 1907), and four undescribed *Diphtheroptila* species are reported from six species of *Glochidion* s. s. in Japan and Taiwan (Kawakita et al. 2010). *D. brideliae* and *D. oxyloga* from South Africa and another undescribed species from Japan are also reported from *Bridelia* (Phyllanthaceae) (Vári 1961; Kawakita et al. 2010).

Diphtheroptila specimens (all undescribed species) in the Hokkaido University Museum were reared from various genera of Phyllanthaceae (*Glochidion* s. l., *Breynia, Bridelia, Aporusa* or *Cleistanthus*) and Euphorbiaceae s. s. (*Macaranga, Mallotus*) in Japan, Taiwan, Nepal, west Malaysia, and Borneo. *Diphtheroptila* as currently circumscribed should probably be split into two genera, one feeding on Phyllanthaceae and one feeding on Euphorbiaceae, based on morphological and larval mine characters (T. Kumata, personal communication, 2010).

Caloptilia **(Gracillariidae: Gracillariinae)**

Caloptilia is an enormous genus of over 300 species, found worldwide on 36 families of host plants (De Prins and De Prins 2005), and is likely to be paraphyletic (Kawakita et al. 2010; Kawahara et al. 2011). Within southeastern Polynesia, two species of *Caloptilia* (*C. deltanthes, C. insidia*) have been reported from the Marquesas (Clarke 1986), and a third species, *C. hilariopis* from Rapa (Austral Islands) and Rarotonga (Cook Islands) (Clarke 1971; McCormack 2007).

At least seven species of *Caloptilia* are previously reported feeding on *Glochidion* s. l., either as leaf miners, rollers, or gallers. *Caloptilia ryukyuensis*, a leaf-roller, is recorded as feeding on *Glochidion lanceolatum* and *G. zeylanicum* in Japan (Kumata 1982; Kawakita et al. 2010). *Caloptilia cecidophora* forms galls in the leaves of *G. obovatum, G. rubrum, and G. acuminatum* in Japan and Taiwan (Kumata 1966, 1982; Kawakita et al. 2010). Two undescribed species of leaf-rolling *Caloptilia* are reported from two species of *Glochidion* in Taiwan (Kawakita et al. 2010). In southeastern Polynesia, *C. deltanthes* has been recorded on *G. "ramiflorum"* (presumably *Phyllanthus marchionicus*) in the Marquesas (Clarke 1986). Additionally, two species (*C. lepidella, C. xanthopharella*) have been reported from *Glochidion* in Australia, and an unidentified species from *G. zeylanicum* in Hong Kong (Robinson et al. 2010). None of these five described *Caloptilia* species have been reported from hosts other than *Glochidion* s. l. (De Prins and De Prins 2005; Robinson et al. 2010).

It is not clear if *Glochidion*-feeding *Caloptilia* are monophyletic. Kumata (1982) placed leaf-rolling *C. ryukyuensis* and galling *C. cecidophora* in separate subgenera based on differing larval characters, while noting that their adult morphology was extremely similar. A few species are also known from other Phyllanthaceae (*Bridelia, Flueggea*) in the Paleotropics (De Prins and De Prins 2005; Bai et al. 2009).

Tritopterna (Tortricidae: Olethreutinae)

Six described species of *Tritopterna* are known, from Sri Lanka, Indonesia, Australia, Micronesia, Samoa, the southern Cook Islands, Rapa, and the Marquesas (Horak 2006); the genus has also been reported from Japan (Kawakita et al. 2010). The monophyly of the genus is supported by a number of unique morphological apomorphies (Horak 2006). Within Eastern Polynesia, *T. eocnephaea* has been described from Rapa and the Marquesas (Clarke 1971, 1986), and an unidentified species reported from the southern Cooks (Horak 2006).

Host records are available for two described species of *Tritopterna*. *T. anachastopa* has been reared from fruit of *Glochidion* s. s. and flowers of *Mallotus* (Euphorbiaceae) in Java, Indonesia (Meyrick 1969); and *T. capyra* has been reared from the fruit of *G. ferdinandi* in New South Wales, Australia (Horak 2006). In addition, an unidentified species of *Tritopterna* has been reared from the fruit of five species of Japanese *Glochidion* s. s. (Kawakita et al. 2010). Several undescribed specimens of *Tritopterna* reared from *Phyllanthus* s. s. in the Philippines are held in the Hokkaido University Museum. It is worth noting that *Mallotus* is absent from southeastern Polynesia (Florence 1997; McCormack 2007).

Dudua (Tortricidae: Olethreutinae)

Thirty-one species of *Dudua* are described, from Madagascar, Indian Ocean islands, South and Southeast Asia, China, Taiwan, Japan, New Guinea, Australia, New Caledonia, Vanuatu, Fiji, Tonga, Niue, and southeastern Polynesia (Horak 2006). Within southeastern Polynesia, the widely distributed *D. aprobola* is known from Rarotonga (southern Cooks), the Australs, and Societies (Clarke 1971; Horak 2006; McCormack 2007), *D. eumenica* from Nuku Hiva, Hiva Oa,

and Fatu Hiva in the Marquesas (Clarke 1986), and an undescribed species from Rarotonga (McCormack 2007).

Host-plant records are available for 8 *Dudua* species; of these, one species (*D. aprobola*) is widely distributed in the Paleotropics and feeds on leaves and flowers of at least 11 plant families (Diakonoff 1973; Horak 2006) and another, *D. cellifera*, is known from Myrtaceae in Southeast Asia and Micronesia (Clarke 1976); the remaining six species are known only from *Glochidion* s. l. These include *D. ptarmicopa* (Meyrick) from *G.* sp. in Japan (Esaki et al. 1971), *D. phyllanthana* and *D. siderea* from *G. ferdinandi* in Australia (Horak 2006), *D. anisoptera* from *G.* sp. on Guam (Clarke 1976), *D. anaprobola* from *G.* sp. in Fiji (Bradley 1953), and *D. eumenica* from *G.* "*ramiflorum*" (presumably *Phyllanthus marchionicus*) in the Marquesas (Clarke 1986).

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