Non-congruent colonizations and diversification in a coevolving pollination mutualism on oceanic islands

David H. Hembry[1,3,†], Atsushi Kawakita[3], Neil E. Gurr[4], Mark A. Schmaedick[4], Bruce G. Baldwin[2] and Rosemary G. Gillespie[1]

[1]Department of Environmental Science, Policy, and Management, and [2]Jepson Herbarium and Department of Integrative Biology, University of California, Berkeley, CA 94720, USA
[3]Center for Ecological Research, Kyoto University, Otsu, Shiga 520-2113, Japan
[4]Land Grant Program, American Samoa Community College, Pago Pago, AS 96799, USA

A challenge for coevolutionary theory is how different types of interaction influence the diversification of coevolving clades. Reciprocal specialization is characteristic of certain coevolving, mutualistic interactions, but whether this specialization seen in ecological time constrains changes in patterns of interaction over evolutionary time remains unclear. Here, we examine the co-radiation of *Glochidion* trees (Phyllanthaceae: *Phyllanthus* s. l.) and pollinating, seed-predatory *Epicephala* moths (Lepidoptera: Gracillariidae) on young (mostly later than 5 Ma) oceanic islands in southeastern Polynesia. *Epicephala* are the sole known pollinators of *Glochidion* trees, and show extreme reciprocal specialization in continental Asia. We find that *Glochidion* and *Epicephala* diversified across these islands through repeated, non-congruent colonizations, and that one recently colonizing *Epicephala* lineage has spread across 12 host species in three archipelagos in less than 1 Myr. These results indicate that reciprocal specialization and coadaptation do not prevent dramatic changes in associations between intimately associated taxa over short evolutionary time scales. Not only are these host associations more dynamic than previously recognized, but these changes in patterns of interaction may play an important role in the diversification of coevolving taxa.

1. Introduction

Coevolution, or reciprocal evolutionary change driven by selection between multiple taxa, is widely invoked as a force promoting the diversification of life on Earth [1–5]. Over the past two decades, the geographical mosaic theory of coevolution [4,6] has led to great advances in understanding the importance and mechanisms of coevolutionary diversification among populations [4,7–9]. However, studies conflict on coevolution's importance in diversification at higher taxonomic levels [5,10–15], and suggest a range of mechanisms [1,3,12,15–18]. Although the mechanisms and role of coevolution in promoting diversification among antagonists and competitors are relatively clear [1,3,4,10,11], evidence is mixed as to the role of coevolution in the diversification of mutualistic lineages [5,19–21]. Few studies have examined by what mechanisms coevolving mutualistic clades diversify and concurrently assemble themselves into patterns of interaction (but see [15,21]).

Some major study systems in the field of coevolution and diversification have been obligate pollinating seed–predation mutualisms (or brood pollination mutualisms) between insects and angiosperms, such as the fig/fig wasp [22], yucca/yucca moth [23], leafflower/leafflower moth (Phyllantheae/ *Epicephala*) [24] and senita cactus/senita moth [25] mutualisms. Focus on these associations is primarily because the pollinators partially control gene flow in their hosts [6], reciprocal coadaptations indicate that coevolutionary selection occurs [22–24] and high species diversity is often evident [22–24]. In these mutualisms, specialized insects pollinate flowers of their hosts, but
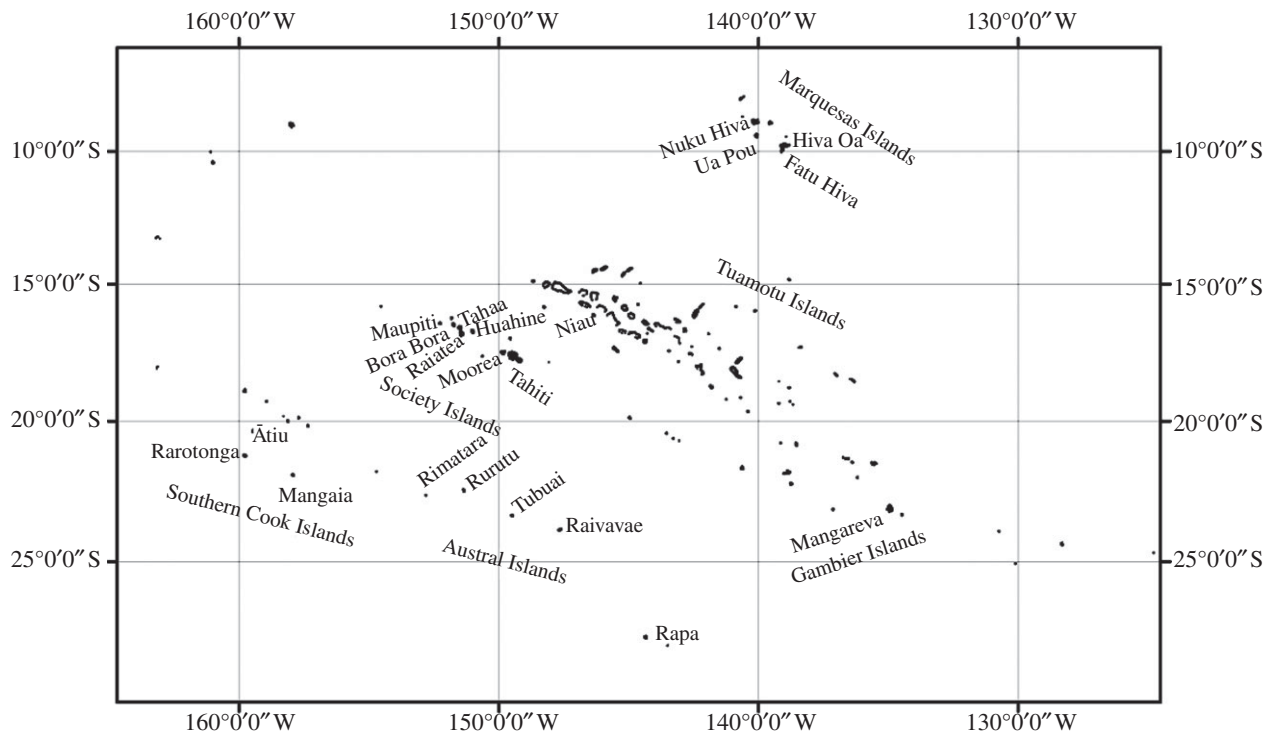
Figure 1. Southeastern Polynesia. Archipelagos and islands mentioned in the text are indicated.

oviposit in the inflorescences such that their larvae consume a subset of the hosts' seeds. Importantly, these interactions show high reciprocal specialization, in which each plant species interacts with one or a few insect species locally, and vice versa [22–24].

Despite great interest in pollinating seed–predation mutualisms, little is known about the mechanisms by which they speciate and diversify, with the exception of yuccas and yucca moths [12,13,15,17]. It is known that large-scale phylogenies of plants and their pollinators are topologically more congruent than would be expected by chance [15,24,26] (with exact congruence limited to some cases of species pairs [17]), and that extreme reciprocal specialization exists between host and pollinator at fine taxonomic scales. The common interpretation is that cospeciation or tracking of hosts by pollinators is predominant, but that, to some degree, host-shifts must occur in the diversification of pollinators. However, diversification of these systems at fine scales has hardly been examined, in part owing to the high diversity of some groups (e.g. figs, leafflowers; but see [15]). It is not known whether the factors that promote high reciprocal specialization in these mutualisms (which may include coevolutionary selection [27], ancestral specialization [6] or pollinator–pollinator competition [28]) additionally constrain diversification and changes in the patterns of interaction over evolutionary time scales. These factors might constrain taxa to cospeciate (or phylogenetically track one another) over short evolutionary time scales, with host-shifts being rare events. Alternatively, these factors might not prevent patterns of interaction among taxa from changing and reforming dynamically over short evolutionary time scales, in a manner similar to that seen in other interactions that are considered less specialized [4]. Both of the above processes would produce similar cophylogenetic and specialization patterns at large scales, but have very different implications for how diversification occurs in these coevolving mutualisms. To distinguish between these hypotheses, a system is needed that can serve as a proxy for the early

stages of diversification, and whose history of co-association can be reconstructed in fine detail to determine whether changes in the patterns of interaction are constrained over short evolutionary time scales, or are more dynamic.

Such a system exists in the co-radiation of leafflower trees (Phyllanthaceae: Phyllantheae: *Phyllanthus* s. l. [*Glochidion*]) and leafflower moths (Lepidoptera: Gracillariidae: *Epicephala*) [24,29] on oceanic islands in southeastern Polynesia. The genus *Glochidion* sensu lato (hereafter, *Glochidion*) is the best-studied clade of leafflowers [26,29–31], with approximately 300 species described from tropical Asia, Australasia and the Pacific islands [32]. *Epicephala* includes the sole known pollinators of *Glochidion*; female *Epicephala* actively pollinate *Glochidion* flowers and then oviposit into the floral ovaries, where the larvae consume a subset of the developing seeds [29]. Species specificity is very high and mediated by floral odour [30,31]. The biological intimacy of the mutualism [24], co-adapted traits of the associates [24,31], mutual dependency of the two clades for their life cycles [24,29,33] and convergent evolution of *Glochidion* floral morphology with that of other *Epicephala*-pollinated leafflowers [34] all indicate that *Glochidion* and *Epicephala* have coevolved, but do not indicate how they diversified or how their patterns of interaction have changed over time. Previous analyses have found that phylogenies of *Glochidion* and its clade of *Epicephala* on continents and west Pacific islands are significantly but not exactly congruent [26], and in one case two distantly related *Epicephala* species coexist sympatrically on one *Glochidion* host species [30].

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; electronic supplementary material, figure S1) [33,35–37], 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, Marquesas, Gambier and Pitcairn islands; figure 1), all of which were formed by

midplate volcanoes under the Pacific plate [38]. 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 (since 5 Ma [39]). *Glochidion* species in this region are endemic to single archipelagos (with two exceptions [40]), show great diversity in floral morphology [36] and are one of the largest endemic plant radiations [41]. These factors suggest that diversification of *Glochidion* and *Epicephala* in southeastern Polynesia has occurred over very recent evolutionary time scales.

Glochidion and *Epicephala* in southeastern Polynesia represent a specialized insect–plant mutualism that has co-radiated across oceanic islands. This system provides an opportunity to examine the patterns of diversification between coevolving clades in fine detail and ask whether over short evolutionary time scales these plants and insects have been constrained to co-diversify in a cophylogenetic pattern (with host-shifts being rare), or if patterns of interaction have changed dynamically over time as the clades co-diversified. Our hypothesis, based on past findings of cospeciation and reciprocal specialization [26,30,31], is that *Glochidion* and *Epicephala* have each colonized this region once, and diversified *in situ* in a manner producing phylogenetic congruence, without changes in the patterns of interaction. Alternatively, *Glochidion* and *Epicephala* may have colonized this region multiple times and non-congruently, and in their diversification they may have undergone changes in the patterns of interaction via host-shifts or other mechanisms. To test these rival hypotheses, we specifically ask (i) how many times *Glochidion* and *Epicephala* have each colonized southeastern Polynesia, (ii) whether they show phylogenetic congruence in this region, and (iii) whether detailed taxon sampling shows evidence for cospeciation, sorting (duplication followed by differential extinction) or host-shifts in their codiversification.

2. Material and methods

(a) Sampling

We collected specimens of 22 (of 24) species of *Glochidion* from 21 islands in the Cook Islands and French Polynesia for a total of 35 operational taxonomic units (one sample per species per island), plus 60 additional *Glochidion* taxa from American Samoa, Wallis, Fiji, New Caledonia, Australia and Asia (see the electronic supplementary material, table S1). From the same *Glochidion* trees in southeastern Polynesia, we collected 32 *Epicephala* specimens (one per host species per island) plus an additional 46 *Epicephala* specimens from *Glochidion* in American Samoa, Fiji, New Caledonia, Australia and Asia (see the electronic supplementary material, table S1). Both datasets included data from previous studies [26,30]. See electronic supplementary material for additional detail.

(b) Molecular phylogenetic methods

We Sanger-sequenced 1500 bp of nuclear ribosomal DNA (ITS, ETS) and 700 bp of chloroplast (*mat*K−*trn*K) DNA for *Glochidion*, and 500 bp of mitochondrial (COI) and 1500 bp of nuclear (ArgK, EF1-α) DNA for *Epicephala*, using primers and methods from previous studies [26,34,42–44] (see electronic supplementary material). GenBank accession numbers are KC912861–KC913192.

(c) Phylogenetic analysis

Sequence data were edited in SEQUENCHER (Gene Codes Corporation, Ann Arbor, MI) and aligned using MUSCLE [45]. We conducted phylogenetic analyses of both datasets using likelihood

implemented in RAxML-HPC2 [46,47] on CIPRES [48] and Bayesian inference implemented in MRBAYES v. 3.1 [49]. The outgroup was *Breynia distica* (Phyllanthaceae) for the *Glochidion* analyses, and the undescribed *Epicephala* species from *Phyllanthus amarus* for the *Epicephala* analyses, following earlier findings [34]. See electronic supplementary material for additional detail.

(d) Divergence time estimation

We estimated ages of southeastern Polynesian clades of both taxa in BEAST v. 1.7 [50], using a lognormal relaxed clock [51] on CIPRES [48]. Available time-calibration points were limited. For *Glochidion*, we used two *Glochidion* fossils from the Miocene of India [52,53] and the age of Rapa (4.8 Ma) [39]. For *Epicephala*, we used a secondary calibration from previous divergence time estimates [34] and the age of the Marquesas Archipelago [38,39]. The *Epicephala* dataset was pruned for species-level sampling. See electronic supplementary material for additional information.

(e) Biogeography

We used Bayes factor comparisons in MRBAYES v. 3.2 [49] as well as Shimodaira–Hasegawa tests [54] in the package phangorn [55] implemented in R [56] to statistically test for the observed polyphyly of southeastern Polynesian *Glochidion* and *Epicephala*, respectively. Both analyses used the results of Bayesian inferences in which southeastern Polynesian taxa were constrained to be monophyletic and to not be monophyletic (see the electronic supplementary material). Additionally, we conducted ancestral state reconstructions of biogeography on a posterior distribution of trees from the unconstrained Bayesian analyses using BAYESTRAITS [57] (see electronic supplementary material).

(f) Cophylogenetic analysis

We used the method ParaFit [58] implemented in the package ape [59] in R [56] to test the hypothesis that *Glochidion* and *Epicephala* have codiversified in a manner producing phylogenetic congruence both globally and within southeastern Polynesia. Analyses were conducted using both posterior distributions of trees from the Bayesian phylogenetic analysis and Bayesian consensus trees (see the electronic supplementary material).

(g) *Glochidion* taxonomy

Glochidion has been estimated to be monophyletic within a paraphyletic *Phyllanthus* sensu lato [34]; accordingly, many *Glochidion* species have recently been treated in *Phyllanthus* [40], and we use these names where they exist (see the electronic supplementary material, appendix).

(h) *Epicephala* taxonomy

Taxonomy of *Epicephala* species is primarily based on the morphology of male genitalia [60]. We dissected genitalia of the male southeastern Polynesian *Epicephala* (all of which were endemic [33] and, with one exception [61], undescribed) to determine whether morphology was consistent with the patterns revealed by molecular phylogenetic analysis.

3. Results

(a) Phylogeny of *Glochidion*

Bayesian 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. Maximum-likelihood analyses do not conflict
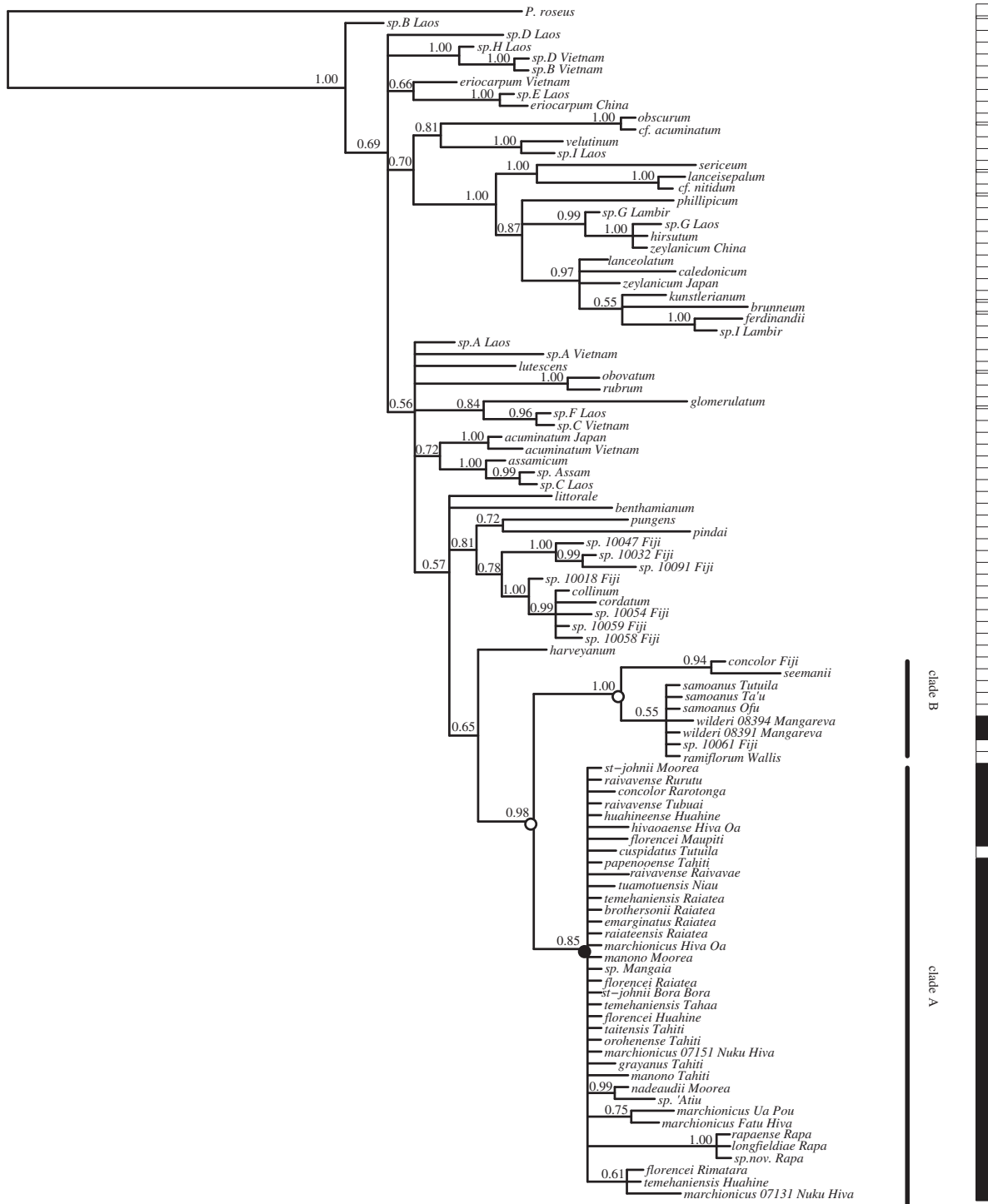
Figure 2. Bayesian consensus tree for *Glochidion* globally. Outgroup *Breynia distica* pruned. Node labels represent posterior probabilities. Tip labels refer to specific epithets of *Glochidion* species. Coloured boxes and circles represent present-day distributions and BAYESTRAITS biogeographic reconstructions (black: southeastern Polynesia; white: not southeastern Polynesia). See electronic supplementary material, table S1 for specimen information.

with the Bayesian inference tree but show less supported resolution within *Glochidion* (see the electronic supplementary material, figure S4); all further analyses use the Bayesian consensus tree. 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, although a clade was found uniting the three taxa from Rapa. The remaining southeastern Polynesian species, *Phyllanthus wilderi* from Mangareva (Gambiers), is more closely related to taxa from Samoa, Wallis and Fiji (clade B). This grouping is biogeographically unusual because

Samoa and Mangareva are nearly 4000 km apart, but is supported by two specimens of *P. wilderi* from different sites on Mangareva.

(b) Phylogeny of *Epicephala*

Both Bayesian and maximum-likelihood analyses resolved that *Epicephala* from southeastern Polynesia fall into two clades (figure 3; electronic supplementary material, figure S5). The first, clade Y, is distributed across multiple archipelagos (Cook, Society, Rapa, Tuamotu and Marquesas) and has diversified into a number of subclades, each of which appears
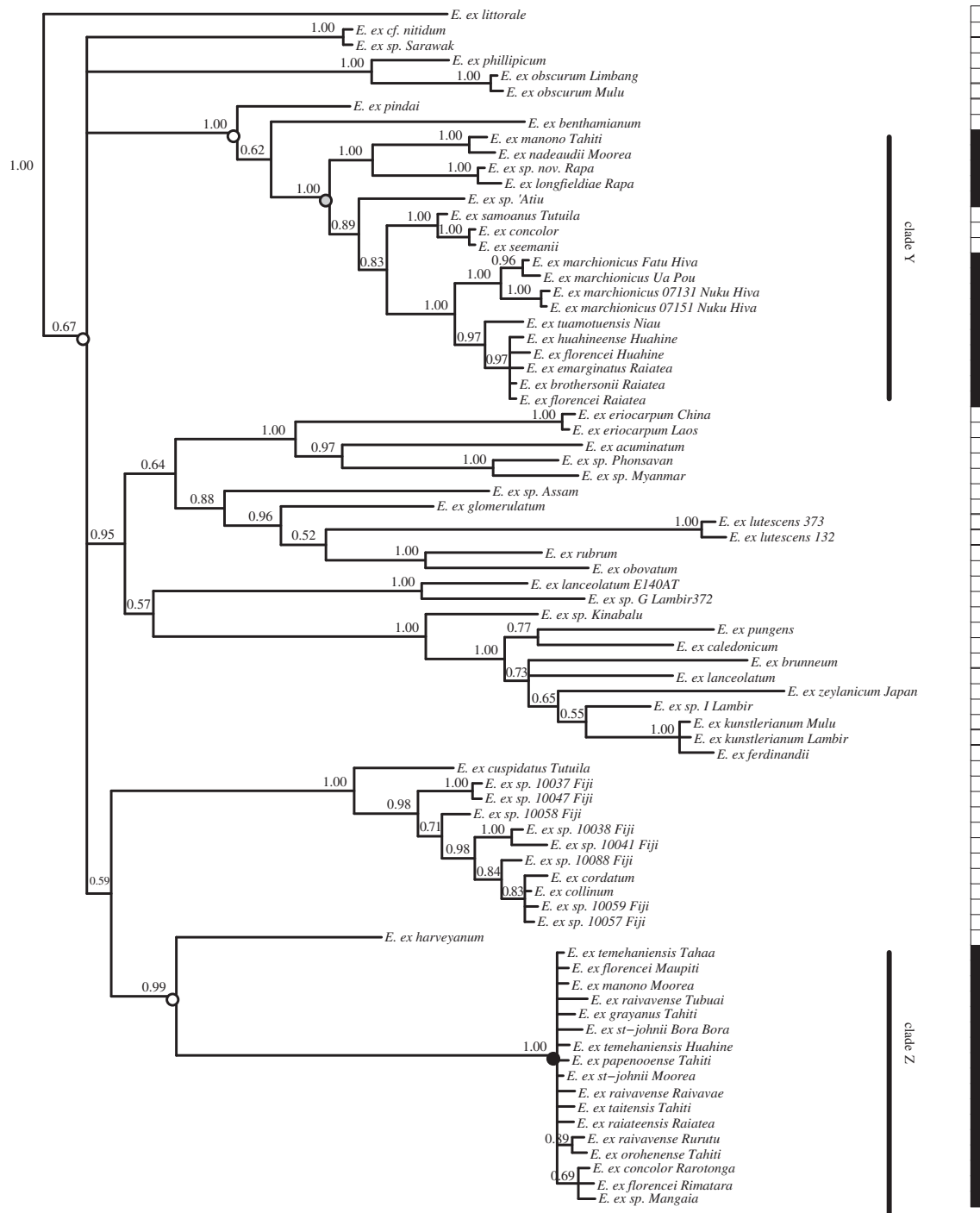
Figure 3. Bayesian consensus tree for the *Epicephala* clade associated with *Glochidion* globally. Outgroups (*Epicephala* from *Phyllanthus amarus* and *P. marojejiensis*, which are not members of the *Glochidion*-associated clade [41]) pruned. Node labels represent posterior probabilities. Tip labels refer to the specific epithets of host *Glochidion*. Coloured boxes and circles represent present-day distributions and BAYESTRAITS biogeographic reconstructions (black: southeastern Polynesia; white: not southeastern Polynesia; grey: biogeographic reconstruction equivocal). See electronic Supplementary material, table S1 for specimen information.

geographically restricted to a single island or archipelago. The second, clade Z, is distributed across the Cook, Society and northern Austral archipelagos. Clade Y appears to contain *Epicephala* from Samoa and Fiji, but this pattern of being nested within clade Y (as opposed to being sister to the southeastern Polynesian taxa) is seen only in the COI and the combined analyses, and not in the individual nuclear-gene datasets (see the electronic supplementary material, figures S6–S8).

(c) Molecular dating

The mean age of clade A was estimated at 9.5–9.8 Ma (5.5–13.3 Ma) and that of clade B at 3.8–4.0 Ma (1.6–6.8 Ma).

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; Australs), and potentially Moruroa and Fangataufa (Tuamotus; Hereheretue–Mangareva–Pitcairn alignment), which are now atolls [39]. By contrast, the age of *Epicephala* clade Y was estimated to be 3.1 Ma (1.7–4.5 Ma), and that of clade Z to be 0.41 Ma (0.08–0.81 Ma). Although these age estimates are not without error, the ten-fold difference in mean estimates for the roots of clades Y and Z does suggest that clade Z colonized southeastern Polynesia more recently than clade Y. All distributions of divergence time estimates for southeastern Polynesian *Epicephala* are younger than the majority of
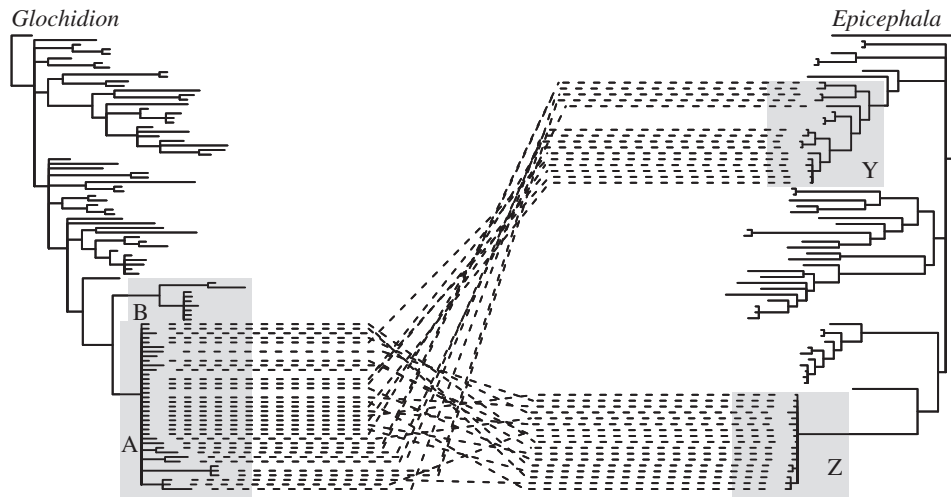
Figure 4. Cophylogenetic patterns between southeastern Polynesian *Glochidion* and *Epicephala*. Clades mentioned in text indicated. Dashed lines represent associations between southeastern Polynesian taxa; associations between other taxa omitted for clarity.

extant high island chains (Society, Marquesas, Austral and Gambier [39]) and the *Glochidion* clade A estimates. Because Bayesian divergence time estimation is very sensitive to root calibrations and the amount of sequence variation across Asia-Pacific *Glochidion* in this dataset is very low, better calibration points and larger datasets are required for rigorous inference of the absolute timing of their history of association. See electronic supplementary material for additional detail.

(d) Phylogenetic congruence

Southeastern Polynesian *Glochidion* and *Epicephala* lack phylogenetic congruence. 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 (figure 4). 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 and Shimodaira–Hasegawa tests (below).

ParaFit cospeciation analyses using trees randomly sampled from Bayesian posterior distributions support phylogenetic congruence for *Glochidion* and their *Epicephala* globally (throughout the Asia-Pacific region; *p*-values centred on $p = 0.017$, Shapiro–Wilk normality test, $W = 0.8656$, $p_W < 0.001$) and excluding southeastern Polynesian taxa (Asia, Australia and the west Pacific; centred on $p = 0.018$, $W = 0.8504$, $p_W < 0.001$), but not within southeastern Polynesia (centred on $p = 0.700$, $W = 0.9573$, $p_W < 0.001$). These results are robust to the collapse of minimally monophyletic southeastern Polynesian *Epicephala* subclades into single terminals (global analysis: centred on $p = 0.013$, $W = 0.8769$, $p_W < 0.001$; southeastern Polynesia: centred on $p = 0.58$, $W = 0.9677$, $p_W < 0.001$). ParaFit analyses using Bayesian consensus trees obtain consistent results (see the electronic supplementary material).

(e) Statistical tests of biogeographic patterns

Both Bayes factor comparisons (see the electronic supplementary material, tables S2 and S3) and Shimodaira–Hasegawa tests (*Glochidion*: $p = 0.029$; *Epicephala*: $p < 0.0001$) support the polyphyly of southeastern Polynesian *Glochidion* and southeastern Polynesian *Epicephala* observed in the unconstrained Bayesian consensus trees. Biogeographic reconstructions in

BAYESTRAITS suggest that the most recent common ancestor (MRCA) of *Glochidion* clade A was found in southeastern Polynesia, whereas the MRCA of clade B, and the common ancestor of clades A and B were found elsewhere (figure 2; electronic supplementary material, table S4), supporting the hypothesis that *Glochidion* colonized this region twice. The MRCA of *Epicephala* clade Z is strongly supported as present in southeastern Polynesia, whereas the MRCA of *Epicephala* clade Y (which contains southeastern Polynesian, Samoan and Fijian taxa) is reconstructed equivocally (figure 3; electronic supplementary material, table S5). The MRCA of clades Y and Z, as well as the MRCAs of clades Y and Z with each of their respective sister groups from the Bayesian consensus tree (clade Y with the *Epicephala* from *Glochidion benthamianum* and *Glochidion pindai*; clade Z with the *Epicephala* from *Glochidion harveyanum*) are strongly supported as found outside southeastern Polynesia. Taken together, these results suggest that *Epicephala* colonized southeastern Polynesia at least twice.

(f) Genital morphology and *Epicephala* taxonomy

The groupings of *Epicephala* obtained via molecular phylogenetic analysis are supported by male genital morphology. In particular, clade Z is a single morphospecies within the Society and northern Austral archipelagos. Each geographically restricted subclade within clade Y is morphologically distinct.

4. Discussion

(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 (Mangareva) appears 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 (Cook, Austral, Society, Tuamotu and Marquesas archipelagos) and another of which is distributed across the western part of this region (Cook, Austral and Society archipelagos). *Glochidion*

and *Epicephala* are thus additional examples of organisms that have repeatedly colonized southeastern Polynesia [62–65]. We find that four islands in the Society archipelago (Raiatea, Huahine, Moorea and Tahiti) have been colonized twice each by *Epicephala*; few repeated colonizations of the same island are known in the southeastern Polynesian biota (but see [64]). Independent colonizations of the same island by distantly related *Epicephala* lineages are 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. The grouping of *Phyllanthus cuspidatus* from Samoa with the southeastern Polynesian *Glochidion* and the nesting of several Fiji and Samoa *Epicephala* within clade Y may represent 'back-colonizations' of the west Pacific from southeastern Polynesia.

Phyllanthus wilderi from the Gambier archipelago (Mangareva) appears more closely related to several taxa from Samoa, Wallis and Fiji, at least 4000 km distant, than to the remainder of southeastern Polynesian *Glochidion*. Although this is an unusual biogeographic association, the Gambiers are old (at least 5.7 Ma [66]) and distant from the nearest high islands to the west (1000 km from Rapa, 1500 km from the Marquesas); analogous long-distance sister relationships are known in Pacific plants [67]; and most of the diversity of western Polynesian *Glochidion* is missing from this analysis. Like the rest of the Mangarevan flora, *P. wilderi* is extremely rare due to human impacts [68], and *Epicephala* were not collected from this species; however, photographs of mature *P. wilderi* fruit with exit holes and only a fraction of the seed set consumed suggest these moths persist on trees on the cliffs of Mangareva (J.-F. Butaud 2012, personal communication). Biogeographic affinities of the Gambiers biota are poorly known (but see [63]).

Three further inferences can be made about the biogeographic history of *Glochidion* and *Epicephala* within southeastern Polynesia. Although *Glochidion* clade A is a polytomy, a common pattern in island plant radiations [69], the three *Glochidion* species from the old, isolated island of Rapa form a clade with strong 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. By contrast, clade Z shows no statistically supported substructure across three archipelagos. The presence of geographical structure in *Epicephala* clade Y is consistent with a role for geographical isolation in the diversification of this clade, similar to findings from similar mutualisms [15,17,70].

These biogeographic patterns are unlikely to result from human-mediated dispersal. Evidence is lacking of the use, cultivation or transport of *Glochidion* in the southeastern Polynesian linguistic and ethnobotanical literature [71–77], and the patterns of distribution and endemism of *Glochidion* and *Epicephala* taxa are inconsistent with human patterns of migration into and within southeastern Polynesia (see the electronic supplementary material).

(b) Phylogenetic incongruence over short time scales

Reciprocal coadaptation, biogeographic isolation and competitive exclusion between pollinator lineages might be expected to constrain patterns of diversification between *Glochidion* and *Epicephala* so that they cospeciate or phylogenetically track over short evolutionary time scales. However, we find fundamental incongruence between the phylogenetic histories of

these plants and pollinators over a timespan of less than 5 Myr, indicating that despite these potential constraints, patterns of interaction are dynamic and can shift rapidly over several million years as these clades diversify. Not only did clade Z shift onto a new host when it colonized southeastern Polynesia, but it subsequently rapidly shifted onto at least 11 other host species on 12 other islands. Whatever factors promote reciprocal specialization in this mutualism in continental regions [30] and geographic isolation on different islands do not prevent rapid changes in the patterns of association between *Glochidion* and *Epicephala* as they diversify on young oceanic archipelagos. Such dramatic phylogenetic incongruence at fine scales has not previously been demonstrated in pollinating seed–predation mutualisms.

However, this and a previous study [26] find evidence of phylogenetic congruence between *Glochidion* and *Epicephala* both globally (despite the presence of southeastern Polynesian taxa) and within the Asia and west Pacific region (excluding southeastern Polynesia). Thus, it is possible that at a fine scale, closely related species of *Glochidion* and their *Epicephala* pollinators diversify in a manner that results in phylogenetic incongruence, but that still produces some detectable congruence at larger phylogenetic scales. Similar patterns are apparent in fig wasps [16,22,27,78] and yucca moths [16]. These results underscore that we should not necessarily expect coevolving clades undergoing diversification to show phylogenetic congruence, even in highly specialized mutualisms [4,79] (but see [80]).

(c) Evidence for rapid and widespread host-shifts in diversification

This study finds that rapid and widespread host-shifts may be an important factor in the diversification of *Glochidion* and *Epicephala* over short evolutionary time scales. Phylogenetic incongruence between mutualists can be explained by several processes: limitations of phylogenetic inference, duplication followed by differential extinction (lineage sorting), host-shifts and failure-to-diverge events [81]. Distinguishing among these processes has proved difficult. By examining recently diversified clades with extensive sampling, this study finds evidence for host-shifts (and probably also failure-to-diverge events) in the diversification of *Epicephala* and *Glochidion*. These results also indicate, more importantly, that a pollinator lineage (*Epicephala* clade Z) can shift onto many species of hosts (12 *Glochidion* species) over a wide geographical area (13 islands in three archipelagos) in a short period of time (approx. less than 1 Ma). Clade Z is unlikely to be a non-pollinating 'cheater' taxon because clade Z has been observed pollinating [33] (D.H.H. 2011–2012, unpublished data) and because females possess pollen-carrying proboscis hairs, unlike other *Epicephala* lineages which have lost pollination behaviour [34]. Host-shifts have previously been postulated as a mechanism explaining phylogenetic incongruence between *Glochidion* and *Epicephala* [26,30]. It is likely that similar rapid host-shifts occur in this mutualism on continents as well, but are harder to detect than in this case because of the difficulty of comprehensively sampling from a wide geographical area.

The evolutionary consequences of host-shifts in specialized pollination mutualisms are not clear [28]. Multiple pollinator species may stably coexist on one host. Alternatively, competition between two pollinators may result in displacement 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 a single leaf-flower species has been reported in Asia [30,82]. Because host-shifts may potentially be a mechanism of diversification mediated by coevolution, data on their frequency, ecology and selective consequences in this and similar systems may reveal much about how coevolving clades diversify.

(d) Comparison of leafflowers with yuccas and figs

These results provide illuminating comparisons with what is known of how diversification occurs in the yucca/yucca moth and fig/fig wasp mutualisms. Nearly complete extant taxon sampling of yuccas and yucca moths suggests the moth clade *Tegeticula* has largely tracked the phylogeny of its *Yucca* hosts millions of years after *Yucca* had already diversified, although host-shifts and failure-to-diverge events have been common [15]. As seen in *Epicephala* clade Y, speciation in both *Yucca* and *Tegeticula* has largely been allopatric. Intriguingly, it is suggested [15] that *Tegeticula* may have rapidly shifted onto many previously existing *Yucca* species, and in doing so displaced an earlier pollinator lineage (potentially the extant genus *Parategeticula*) that had previously been associated with the entire *Yucca* radiation. A similar widespread, rapid host-shift occurred when *Epicephala* clade Z colonized southeastern Polynesia, and this colonization may have involved partial displacement of one pollinator lineage by another. Few studies have examined recent diversification of figs and fig wasps (but see [83,84]), but many find multiple pollinator species per fig species [22], which may be consistent with rapid host-shifts such as those observed here in *Glochidion* and *Epicephala* [28]. Consequently, yucca/yucca moth and fig/fig wasp evolutionary patterns appear broadly consistent with those seen here in *Glochidion* and *Epicephala*, suggesting that despite trends towards reciprocal specialization and coadaptation, patterns of interaction may be highly dynamic over short evolutionary time scales in these mutualisms as well. However, neither figs nor yuccas show evidence of host-shifts as widespread and as rapid as those reported here. Cophylogenetic patterns involving the four other reciprocally associated clades of *Epicephala* moths and their host leafflowers (in *Phyllanthus* and *Breynia*) [34] would be informative in comparison with this study.

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