New Species Assemblages Disrupt Obligatory Mutualisms Between Figs and Their Pollinators

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The reliance of each fig species on its specific pollinator wasp, and vice versa, is the archetype of both obligatory mutualism and coevolution. Pollinator sharing between host fig species is only known to occur among closely related sympatric species. On the Hawaiian island of Kauai, we gathered syconia from 23 non-native fig species, three of which contained the wasp *Pleistodontes imperialis*. Of the three fig species, one is the wasp's natural host, *Ficus rubiginosa*, and another is its sister species, *Ficus watkinsiana*, which overlaps in native ranges, although researchers have not previously documented pollinator sharing. The third fig species, *Ficus rubra*, is distant to the others both in terms of phylogenetic relationship and native range. We found viable seeds for all three fig species, whereas species without wasps did not produce seeds. To investigate similarity between these pollinator-sharing fig species, we collected morphometric data for syconia of our study fig species. We found that fig species with and without *P. imperialis* significantly differ based on the orientation of their inner ostiolar bracts. These findings suggest that pollinator sharing among these three fig species may normally be impeded by pollinator competition in the case of *F. watkinsiana*, and by geographic distance in the case of *F. rubra*. This work therefore demonstrates that coevolution depends on interactions within native species assemblages, and that mutualisms can be disrupted in new non-native communities.

Keywords: codiversification, convergent evolution, *Ficus*, fig wasps, fundamental niche, geographic mosaic theory, host breadth expansion, symbiosis

INTRODUCTION

Coevolution is the reciprocal evolution of traits between interacting species (Thompson, 2005) and is thus a major driver of biodiversity, as seen in the vast diversity of insects and flowering plants (Ehrlich and Raven, 1964; Lunau, 2004; Wiens et al., 2015). Certain insect groups that are closely associated with plants have diversification rates that reflect those of their host plants (Danforth and Ascher, 1999; Janz et al., 2006; Kawakita and Kato, 2009; Shimizu et al., 2015; Winter et al., 2017; McKenna et al., 2019). Coevolution can result in strictly obligatory symbiotic relationships, including obligatory mutualisms, which involve cospeciation demonstrated by

mirrored phylogenies (Hafner and Page, 1995; Pellmyr and Leebens-Mack, 1999; Jousselin et al., 2009; Percy, 2010).

One of the strongest cases of coevolution is between figs (Moraceae: *Ficus*) and their pollinator wasps (Chalcidoidea: Agaonidae), in which each depends upon the other to complete its life cycle (Ramírez-Benavides, 1970; Weiblen, 2002; Cruaud et al., 2012b). In particular, a specialized wasp must go through a minute labyrinthine portal known as an ostiole on a fig's closed involuted inflorescence (i.e., syconium or "fig") to deposit eggs into the ovules it can reach and pollinate those it cannot (Weiblen, 2002; Cook and Rasplus, 2003). To maintain the mutualism, the fig emits volatile organic compounds (Grison-Pigé et al., 2002; Hossaert-McKey et al., 2010; Souto-Vilarós et al., 2018) that attract a specific pollinator whose ovipositor length complements its flower styles (Herre et al., 2008). Chemical cues on the surface of the syconium (Wang et al., 2013) and the size of the wasp relative to the ostiole (Liu et al., 2013) likely also play important roles. As a result, this strong relationship is formed not by host-switching (Moe and Weiblen, 2010) but by coevolution of figs and fig wasps that largely results in cospeciation, with one obligatory pollinator species for each fig species (Anstett et al., 1997; Lopez-Vaamonde et al., 2001; Cook and Rasplus, 2003; Rønsted et al., 2005; Cruaud et al., 2012b). The interdependency of figs and their specific pollinators reinforces the obligatory mutualisms, and hybridization is hypothetically rare (Moe and Weiblen, 2012; Yang et al., 2012; Souto-Vilarós et al., 2018).

However, other studies have exposed inconsistencies in the cospeciation of figs and their pollinators (Machado et al., 2005; Cruaud et al., 2012a; Yang et al., 2015). Researchers have encountered cases of pollinator sharing, which is the sharing of a single pollinator among multiple figs (Wei et al., 2014; Wang et al., 2016), and co-pollination, whereby a single fig species has multiple pollinators (Compton et al., 2009; Wachi et al., 2016). Nonetheless, such exceptions to cospeciation involve sympatric closely related fig species complexes (e.g., Cornille et al., 2012). For the most part, fig species within these complexes maintain genetic distinction, but there are instances of hybridization (Tsai et al., 2015). No work has so far determined whether pollinator sharing or co-pollination can exist across species that are more distantly related than sympatric complexes.

Importantly, the geographic mosaic theory describes that the mechanisms supporting a mutualism evolve in response to interactions within a given species assemblage (i.e., community), where natural selection favors traits that strengthen the relationship between partners while excluding other species interactions (Thompson, 2005). In other words, coevolution is dependent on the species interactions in a given location that is more conducive to the partnership (Thompson, 2005), yet natural selection for host specificity can obviously only act on sympatric species. But, the human-aided dispersal of non-native species worldwide creates new communities (van Kleunen et al., 2015) in which obligatory mutualisms are exposed to new species interactions they did not evolve to counter. The new species assemblage could be missing a key interaction that normally encourages niche differentiation or could include new interactions with species that have evolved analogous traits (i.e., convergent evolution). These situations would thus disrupt the obligatory mutualism, because the species would no longer interact solely with its unique partner. Coyne and Orr (2004, p. 189) assert that cospeciation requires one-to-one obligatory partnerships, as opposed to codiversification whereby groups are coevolving but not necessarily by strict relationships (e.g., Winter et al., 2017; McKenna et al., 2019). By this definition, if a fig forms a new facultative pollinator relationship in a new community, the original relationship will no longer be obligatory and there would be less pressure to maintain it.

The Hawaiian island of Kauai has the potential to shed light on whether mutualisms will be compromised in a new community, having no native figs or fig wasps but ∼45 cultivated non-native fig species. Foresters purposefully introduced three fig species alongside their obligatory pollinators, and only one of these figs, *Ficus microcarpa* L.f., is documented as naturalized (Timberlake, 1924; Beardsley, 1998). A recent botanical survey found additional non-native fig species that are potentially in the early phases of naturalizing on the island (detailed herein), but without knowledge of the arrival of their natural pollinating wasps. A fig species theoretically requires the arrival of its specific pollinator to naturalize, although the restricted life cycle of fig wasps reduces the odds of accidental introductions. We therefore question whether newly establishing fig species could involve new relationships with pollinators that are already present. Given Kauai's unnatural combination of non-native figs from across the world, we furthermore suspect that new instances of pollinator sharing could manifest between distantly related figs, and thus phylogenetic relationship would be unable to explain them as it could for previously studied examples of pollinator-sharing figs. To explore whether convergent evolution can lead to novel pollinator sharing among co-occurring non-native figs, we collected syconia from nearly two dozen fig species across Kauai, examined any seeds and wasps present, and performed a morphometric analysis.

MATERIALS AND METHODS

From January–June 2017, we gathered an average of 24 syconia at various stages of development from each of 23 fig species, which included five subgenera, 10 sections, and 12 subsections (three of the sectional lineages in our study do not contain subsections). Most of Kauai's fig species have not escaped cultivation and are therefore sparsely distributed on private land, so our collections were opportunistic and often confined to the National Tropical Botanical Garden in Kalaheo. Although *Ficus microcarpa* is naturalized, we found it bearing syconia only once. Unfortunately this means the only fig species for which we were able to sample multiple trees were those that appeared to be in the early stages of naturalizing (individual tree locations in **Supplementary Table 1**). This level of sampling is nevertheless expected considering we were exploring new pollinator interactions among unestablished or newly establishing figs.

Under a stereo microscope, we dissected each syconium and collected any present fig wasps for identification and any present seeds to assess their viability to demonstrate successful pollination. We define a successful interaction as the ability for

both figs and wasps to reproduce, rather than frequency of the relationship, because the non-native species assemblage of figs on Kauai is less than a century old, hence new interactions may be in the process of emerging and not yet fully stabilized. The formation of new pollinator interactions is particularly important to understand in the context of non-native fig species that may naturalize.

Identifying Figs and Wasps

For taxonomic identification of pollinator wasps (i.e., Agaonidae) and non-pollinator wasps (e.g., Pteromalidae), we followed Ishii (1934), Wiebes (1964), Berg and Wiebes (1992), Wiebes (1994), van Noort and Rasplus (1997), Beardsley (1998), Beardsley and Rasplus (2001), Lopez-Vaamonde et al. (2002), van Noort and Rasplus (2010), and van Noort et al. (2013). To crosscheck the identity of wasps using new hosts, we sequenced the mitochondrial gene for cytochrome b (cytB) following the protocols described by Lopez-Vaamonde et al. (2001). Dichotomous keys for identifying figs included Corner (1965), Berg and Wiebes (1992), and Dixon (2003). In the case of multiple individuals of the same wild-growing fig species, we corroborated our taxonomic identification by sequencing the nuclear ribosomal internal transcribed spacer region (ITS1, 5.8S, and ITS2) according to methods outlined by Weiblen (2000) and Rønsted et al. (2008). Voucher specimens of both figs and wasps are at the Bernice Pauahi Bishop Museum in Honolulu and the National Tropical Botanical Garden in Kalaheo, Kauai (listed in **Supplementary Material**). We also deposited wasp vouchers at the University of Hawaii Insect Museum and the Hawaii Department of Agriculture, both in Honolulu. Genetic sequences are on GenBank (accession numbers in **Supplementary Table 1**).

Germination Assays

We sowed three to nine replicates of 25–50 seeds on seed germination paper (Anchor Paper, St. Paul, MN, United States) in 60-mm Petri dishes. We moistened the germination paper with a solution of 0.1% Plant Preservative Mixture^{TM} (Plant Cell Technology, Washington, DC, United States) in distilled water to inhibit fungal growth without affecting germination (Assaf Guri, personal communication). After sealing dishes with Parafilm® (Bemis, Oshkosh, WI, United States) to increase water retention, we placed them in a GR-36L germination chamber (Percival Scientific, Perry, IA, United States) with a photoperiod of 12 h light [~41 mmol/m^2/s cool white (4100 K) fluorescent light] and 12 h dark, and respective temperature regimens of 25 and 15°C. We monitored seeds until they all germinated or died, or until at least 30 days elapsed since date sown, and defined germination as radicle emergence.

Morphometric Data Collection

For each of the 23 fig species in our study, we collected morphological data regarding the syconium and the ostiole through which a wasp must navigate to reach the flowers in the interior. In other words, these characters physically restrict which wasp species could enter the syconium (**Table 1**). We included two continuous variables, mean syconium diameter and mean ostiole diameter, because size limitations could directly

impede wasps (Tsai et al., 2015). Raw data for these two variables, as well as pollinators per syconium, are on Figshare (doi: 10.6084/m9.figshare.12927287.v). We also had four categorical variables: (i) mode of reproduction (monoecy versus gynodioecy, in which different mechanisms entail different life cycles for pollinators; Suleman et al., 2015; Yang et al., 2015; Wachi et al., 2016); (ii) presence of lateral bracts on the syconium (which may not directly affect wasps but could indirectly coincide with other traits that do); (iii) external shape of the ostiole (flat, nearly flat, or convex); and (iv) the orientation of the ostiole's internal bracts (interlocking versus linearly descending into the syconium's inner cavity). The latter two traits confront a wasp with obstacles that require different skills, which may be reflected in head morphology (van Noort and Compton, 1996). To aid our characterization of ostiole morphology, we followed Corner (1962), Berg (1989), Verkerke (1989), and Berg and Wiebes (1992). Seed germination and a new generation of wasps emerging from fig ovaries demonstrate a wasp's success in both pollination and oviposition once it reaches the interior of the syconium.

Data Analysis

To assess the morphological similarity of any species that share pollinators among the 23 study figs, we first assigned numerical character states to categorical data so we could construct ordinations in R (R Core Team, 2019). A principal component analysis (PCA) of fig species allowed us to explore multidimensional trends in variation, visualized with the package rgl (Adler et al., 2018). We then used candisc (Friendly and Fox, 2017) to do a canonical discriminant analysis (CDA) to account for within- and between-group variation. A Kolmogorov-Smirnov test evaluated the normality of our morphometric data. To test the significance of morphological variables in influencing the presence of any shared pollinators, we first performed a multivariate logistic regression with Monte Carlo resampling at 1000 bootstraps using MVabund (Wang et al., 2012, 2019), which also provided a likelihood ratio test. We then conducted univariate Kruskal–Wallis analyses of variance followed by a *post hoc* Dunn's multiple comparison test using Bonferroni correction via the package dunn.test (Dinno, 2017).

RESULTS

Three of the 23 fig species in our study had saplings near mature trees, possibly indicating early stages of naturalization, which were not previously documented on Kauai (**Table 1**; detailed in **Supplementary Material**). Aside from detecting three species of pollinating wasps that landscapers intentionally introduced in the early 20th century (Timberlake, 1924; Beardsley, 1998), we also found two pollinating wasp species in the genus *Platyscapa* that were not recorded for the Hawaiian archipelago, as well as four new records of non-pollinating wasp species (**Table 1**; detailed in **Supplementary Material**), extending the known non-native ranges of these species (Beardsley, 1999; Bain et al., 2015; Wang et al., 2015). Numbers of wasps per fig species are in **Table 1** (detailed per tree in **Supplementary Table 1**).

TABLE 1 | Morphometric and seed germination data for non-native fig species on Kauai, alongside any wasps present; metrics in mean ± standard deviation; [N] = previously known naturalized species; [A] = new naturalization record for the Hawaiian archipelago; [I] = new naturalization record for Kauai Island but known on other Hawaiian islands; [P] = new potentially naturalizing species on Kauai; [S] = species outside of cultivation but without self-sustaining wild populations (see details in **Supplementary Material**).

Ficus species	Mode of reproduction	Syconia collected	Syconium diameter mean ± sd (mm)	Lateral bracts	Ostiole diameter mean ± sd (mm)	Inner ostiolar bracts	External ostiole shape	Pollinator wasp found (number)	Seeds germi-nated	Non-pollinating wasps found (number)
F. altissima Blume	monoecious	28	22.5 ± 2.8	absent	2.3 ± 0.2	Interlocking	Convex	None		*Josephiella microcarpae* Beardsley & Rasplus[N] (2)
F. benghalensis L.	monoecious	5	17.5 ± 0.9	absent	1.5 ± 0.2	Interlocking	Shallowly convex	None		None
F. benjamina L.	monoecious	31	10.0 ± 1.1	absent	1.1 ± 0.1	Interlocking	Shallowly convex	None		None
F. boninsimae Koidz.	gynodioecious	21	7.5 ± 0.7	present	1.1 ± 0.1	Interlocking	Convex	None		None
F. drupacea Thunb.	monoecious	10	20.0 ± 2.0	absent	2.4 ± 0.2	Interlocking	Convex	None		None
F. elastica Roxb. ex Hornem.	monoecious	18	11.0 ± 0.6	absent	1.0 ± 0.2	Interlocking	Convex	None		None
F. macrophylla Desf. ex Pers.[P]	monoecious	26	21.8 ± 2.0	absent	2.0 ± 0.3	Linear	Shallowly convex	*Pleistodontes froggatti* Mayr[N] (176)	Yes	None
F. microcarpa L.f.[N]	monoecious	40	8.0 ± 1.1	absent	0.5 ± 0.1	Interlocking	Convex	*Eupristina verticillata* Waterston[N] (1)	Yes	*Philotrypesis emeryi* Grandi [I] (11), *Sycoscapter* cf *gajimaru* (Ishii)[A] (20), *Walkerella microcarpae* Bouèek[I] (73)
F. nota (Blanco) Merr.	monoecious	21	27.5 ± 2.0	absent	7.5 ± 0.3	Interlocking	Convex	None		None
F. prolixa G.Forst.	monoecious	18	7.0 ± 0.4	absent	1.2 ± 0.1	Interlocking	Shallowly convex	None		None
F. pumila L.	gynodioecious	16	52.5 ± 2.5	present	10.9 ± 0.4	Interlocking	Convex	None		None
F. religiosa L.[P]	monoecious	33	12.5 ± 0.9	absent	2.5 ± 0.3	Interlocking	Convex	*Platyscapa quadraticeps* (Mayr)[A] (176)	Yes	*Otitesella digitata* Westwood[A] (10)
F. rubiginosa Desf. ex Vent.[P]	monoecious	36	14.0 ± 1.9	absent	1.3 ± 0.2	Linear	Flat	*Pleistodontes imperialis* Saunders[N] (183)	Yes	None
F. rubra Vahl	monoecious	40	17.0 ± 1.7	absent	3.5 ± 0.2	Linear	Shallowly convex	*Pleistodontes imperialis* Saunders[N] (23)	Yes	None
F. saussureana De Candolle	monoecious	30	22.5 ± 4.1	absent	2.4 ± 0.2	Linear	Flat	None		None
F. scabra G.Forst.	gynodioecious	31	17.5 ± 3.6	absent	4.3 ± 0.2	Interlocking	Convex	None		None
F. superba Miq.	monoecious	18	21.0 ± 1.6	absent	2.3 ± 0.3	Interlocking	Flat	None		None
F. sycomorus L.	monoecious	18	25.0 ± 1.5	absent	4.0 ± 0.2	Interlocking	Convex	None		None
F. tinctoria G.Forst.	gynodioecious	38	9.0 ± 0.4	present	1.5 ± 0.1	Interlocking	Flat	None		None
F. trigonata L.	monoecious	18	22.5 ± 2.3	absent	5.0 ± 0.2	Interlocking	Convex	None		None
F. vallis-choudae Delile	monoecious	18	45.0 ± 6.9	absent	3.8 ± 0.2	Interlocking	convex	None		None
F. virens Aiton	monoecious	23	9.5 ± 1.1	absent	2.8 ± 0.2	Interlocking	Convex	*Platyscapa* cf *coronata* (Grandi)[A] (105)	Yes	None
F. watkinsiana F.M. Bailey[S]	monoecious	10	32.5 ± 2.2	absent	3.3 ± 0.2	Linear	Convex	*Pleistodontes imperialis* Saunders[N] (43)	Yes	None

Overall, pollinating wasps were present in seven fig species, including the three we found to potentially be in the process of naturalizing. Although we found wide variation of germination rates, seeds from these seven species all germinated (**Table 1** and **Supplementary Table 2**), whereas the other 16 species without wasps did not develop viable seeds.

The fig wasp *Pleistodontes imperialis* Saunders (**Figure 1**) was present in three fig species: *Ficus rubiginosa* Desf. ex Vent. (the normal host for *P. imperialis*), *F. watkinsiana* F.M. Bailey, and *F. rubra* Vahl (**Figure 2A**). The first two fig species are sympatric in eastern Australia and are sister in subsection *Platypodeae* of section *Malvanthera*. The last fig species is from islands in the western Indian Ocean and is in subsection *Platyphyllae* of section *Galoglychia*. We genetically corroborated the identities of *Pleistodontes* in each host and also of both individuals of *F. rubiginosa* (GenBank numbers in **Supplementary Table 1**). Other pollinating wasps in this study associated only with their normal hosts.

The PCA of the morphometric data (**Figure 2B** and **Supplementary Table 3**) reveals that the first 4 of 6 principal component axes account for 97% of cumulative variation (eigenvalues) among the fig species. In the first PC axis (PC1), the correlation between variation and the original variables (eigenvectors) ranges from 0.264 to 0.512, with the highest input from the presence of lateral bracts. The greatest eigenvector in PC2 (0.560) is related to syconium diameter. For PC3, the highest eigenvector (0.699) corresponds with the inner ostiolar bracts.

A single canonical axis explains 100% of the variation in the CDA (**Figure 2C**), with largest eigenvector (0.983) attributed to the inner ostiolar bracts. The Kolmogorov-Smirnov test shows the morphometric data are not normally distributed ($p < 0.01$), thus we transformed our raw data into z-scores for non-parametric analysis. A multivariate logistic regression reflects the CDA in that the only variable that significantly distinguishes figs based on the presence of *P. imperialis* is the orientation of the inner ostiolar bracts (likelihood ratio = 10.69, $p = 0.004$). We further confirm this assessment with Kruskal-Wallis results ($\chi^2 = 11.88$, df = 1, $p < 0.001$) and a *post hoc* Dunn's multiple comparison test ($z = 3.45$, adjusted $p < 0.001$).

DISCUSSION

We found previously undocumented pollinator interactions between the wasp *Pleistodontes imperialis* and two fig species, *Ficus watkinsiana* and *F. rubra*, despite mechanisms that usually reinforce host-specificity (e.g., Souto-Vilarós et al., 2018). Among figs containing *P. imperialis*, the presence of both mature male and female wasps in the syconia indicates that the wasp can complete its life cycle in different hosts, and the germinated seeds confirm that the wasp is successfully pollinating ovules, thereby completing the fig's life cycle (**Figure 1**).

Two of the germination rates were notably low (**Supplementary Table 2**). First, the rate for *Ficus macrophylla* Desf. ex Pers. was merely 2.9%, even though it may be naturalizing and we found its obligatory pollinator, *Pleistodontes froggatti* Mayr, emerging from syconia. We detected two

genotypes of *P. froggatti* present on Kauai, so perhaps one can successfully reproduce but not pollinate (GenBank numbers in **Supplementary Table 1**). Further investigation is necessary to learn if Kauai has a less successful genotype of *P. froggatti*. Second, the germination rate for one *F. rubiginosa* tree was 70.2% but the other was 4.6%. We found two morphs of this species growing in the Lihue-Koloa Forest Reserve (**Supplementary Figure 4**). While genetics provided support that they are the same species, perhaps one is an unusual variety, and more research may show that it is less suitable to its obligatory pollinator, *P. imperialis*. Moreover, our germination protocols were standardized so they were not optimized per species, but regardless, all fig species containing pollinator wasps germinated, including those with new pollinator interactions.

The host breadth expansion of *P. imperialis* from *F. rubiginosa* to its sister *F. watkinsiana*, sympatric in their native range, appears to involve the absence on Kauai of *Pleistodontes nigriventris* (Girault), the normal pollinator of *F. watkinsiana* (Lopez-Vaamonde et al., 2002). Haine et al. (2006) conducted a broad-scale mitochondrial analysis across the native range of *P. imperialis* and detected no evidence of it shifting to other fig species nor of other wasps shifting to *F. rubiginosa*. Some mechanism is therefore limiting *P. imperialis* in its native range but is absent on Kauai, and the only difference is the occurrence of *P. nigriventris*, implying some form of antagonistic interaction (i.e., competition) between them in their native range. Although some researchers suggest fig wasps may not be limited by direct competition for fig ovules (Hawkins and Compton, 1992; Zhang et al., 2004), perhaps *P. nigriventris* is better adapted to *F. watkinsiana* and can ostensibly exclude *P. imperialis* in the native range. The realized niche of *P. imperialis* could thus be restricted to *F. rubiginosa* in Australia, whereas the absence of *P. nigriventris* reveals its fundamental niche to include *F. watkinsiana*.

However, we also discovered *P. imperialis* successfully using another host that is both distantly related and not overlapping in range with its normal host: *F. rubra* (*Galoglychia*: *Platyphyllae*; **Figure 2A**). Two other fig species did not contain *P. imperialis* despite being related to those that did: (i) *F. macrophylla* in section *Malvanthera* that instead contained its normal pollinator, and (ii) *F. saussureana* De Candolle in section *Galoglychia* that lacked its normal pollinator (**Table 1**). These absences suggest that neither phylogenetic relationship nor alleviated competition can fully explain which *Ficus* hosts *P. imperialis* can use on Kauai. Our results instead strongly support our hypothesis that convergent evolution is a driving factor, particularly regarding the arrangement of the inner ostiolar bracts that are tightly correlated with the presence of *P. imperialis* (**Figure 2C**). Our findings concur with work by Jousselin et al. (2003), who also found homoplasy of the ostioles across fig sections; now our analysis further implicates this trait as strongly influencing in the ability of *P. imperialis* to utilize new hosts.

This opens the investigation to other causes of the reduced specificity of *P. imperialis*, and by contrast the apparent maintained specificity of the other four pollinating wasps in this study (**Table 1** and **Supplementary Material**). We examined six morphological traits, but others may exist that further distinguish
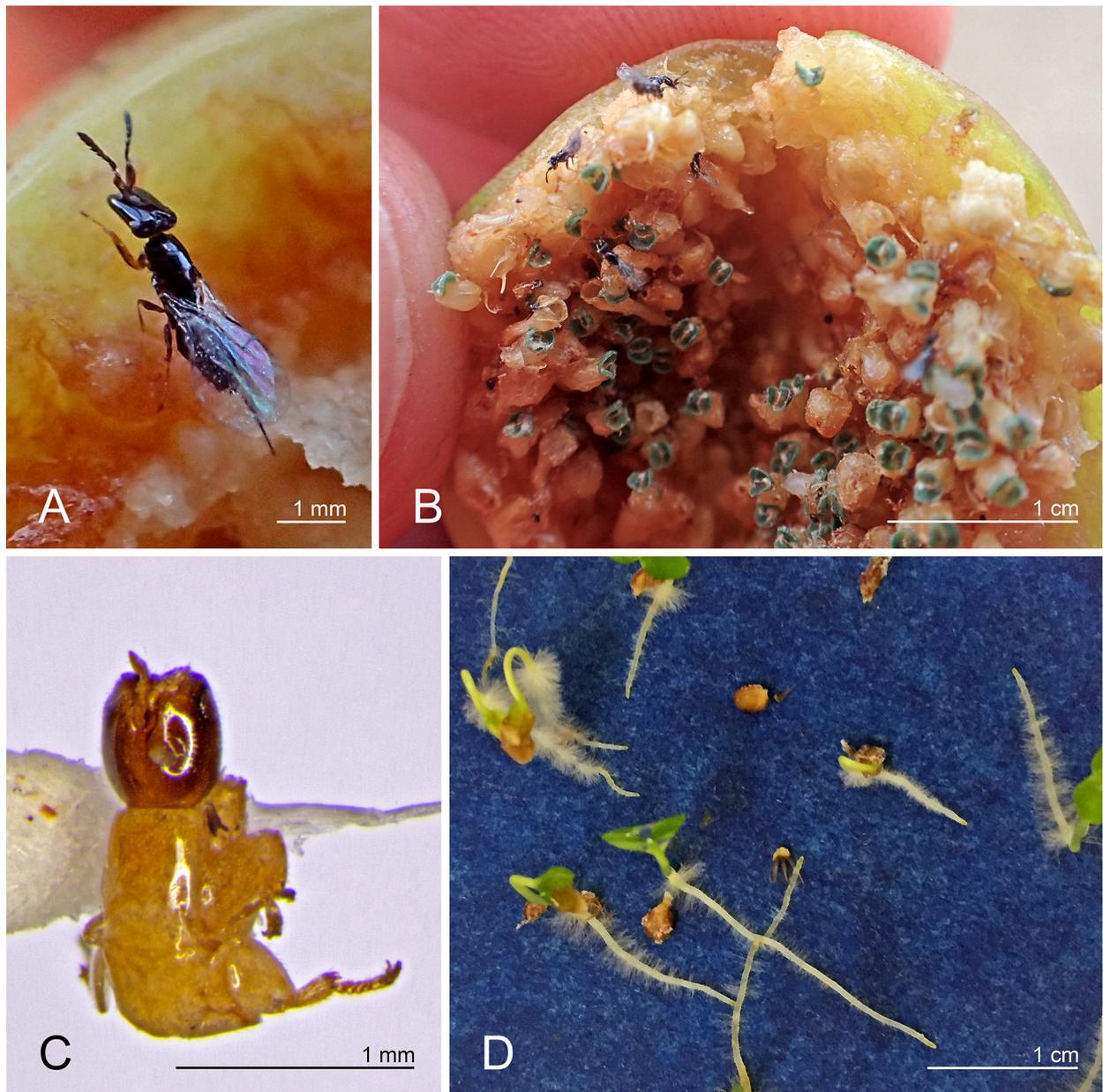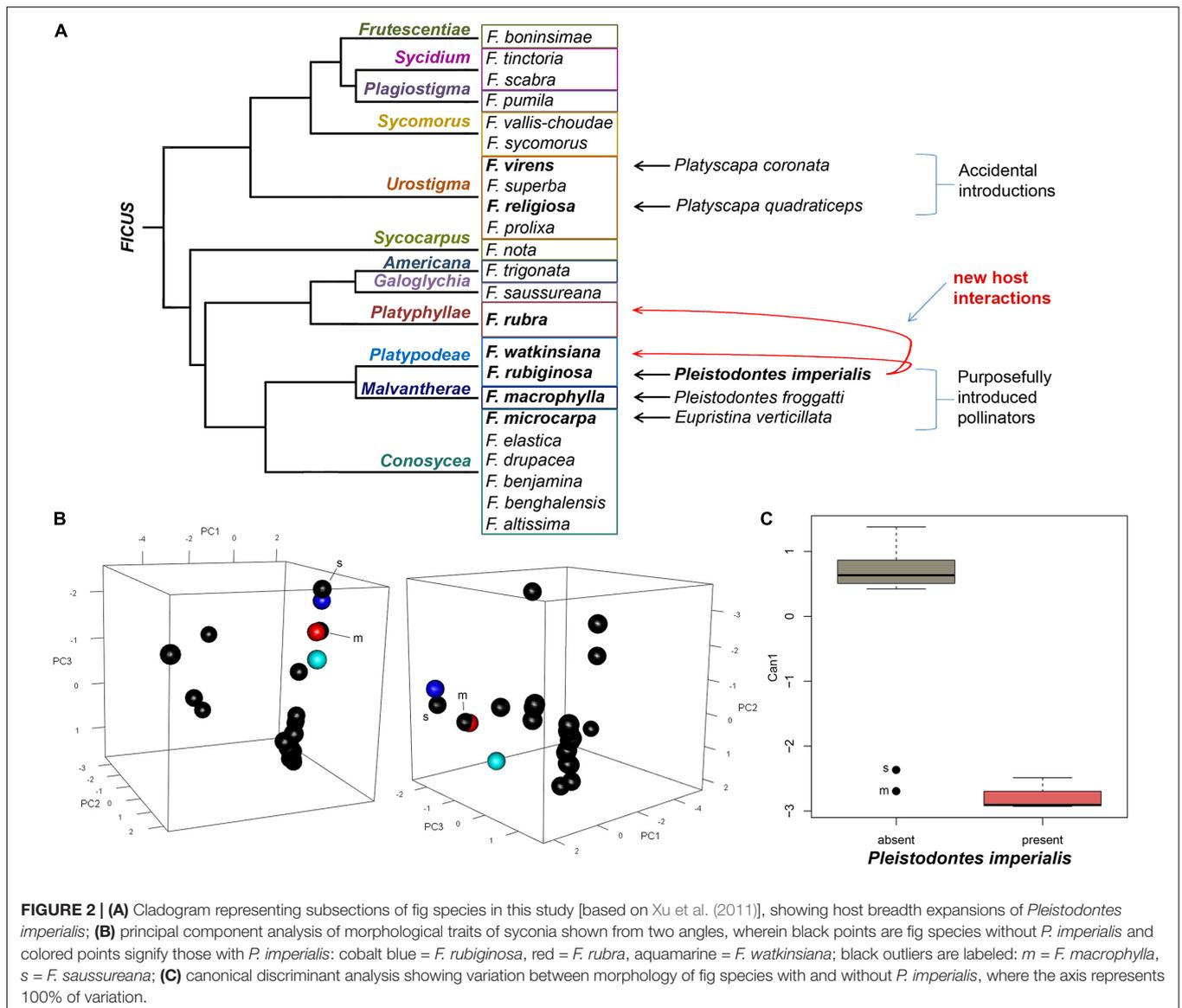
FIGURE 1 | (A) *Pleistodontes imperialis* female; **(B)** new *P. imperialis* generation emerging from *Ficus watkinsiana* syconium; **(C)** wingless *P. imperialis* male extracted from *F. watkinsiana*; **(D)** *F. watkinsiana* seeds germinating in the absence of its normal obligatory pollinator.

figs that share a pollinator, for instance synchronized phenology between pollinators and syconia development. Although we found wasp-bearing syconia on our pollinator-sharing species at numerous times during our collection period (**Supplementary Material**), additional work is needed to explore this possibility. Another trait that is almost undoubtedly involved in forming new pollinator interactions is scent. Because figs use volatile compounds to attract specific pollinators (Grison-Pigé et al., 2002; Proffit et al., 2009; Hossaert-McKey et al., 2010; Souza et al., 2015), any new pollinator would very likely need to be attracted to the same cues. Similarity of volatile compounds

alongside that of the ostiole could drive pollinator sharing, such as Cornille et al. (2012) detected between sympatric sister fig species in South Africa.

On the North Island of New Zealand, non-native figs include *F. rubiginosa* and *F. obliqua* G.Forst. (Gardner and Early, 1996), both of which are in subsection *Platypodeae* and have overlapping native ranges in Australia (Dixon, 2003; Rønsted et al., 2008), although only the former is naturalized due to the introduction of *P. imperialis*. Gardner and Early (1996) noted *P. imperialis* in many syconia of *F. obliqua*, yet despite the absence of the normal pollinator for *F. obliqua* in New Zealand, *P. imperialis*

FIGURE 2 | (A) Cladogram representing subsections of fig species in this study [based on Xu et al. (2011)], showing host breadth expansions of *Pleistodontes imperialis*; **(B)** principal component analysis of morphological traits of syconia shown from two angles, wherein black points are fig species without *P. imperialis* and colored points signify those with *P. imperialis*: cobalt blue = *F. rubiginosa*, red = *F. rubra*, aquamarine = *F. watkinsiana*; black outliers are labeled: *m* = *F. macrophylla*, *s* = *F. saussureana*; **(C)** canonical discriminant analysis showing variation between morphology of fig species with and without *P. imperialis*, where the axis represents 100% of variation.

was unable to pollinate the ovules, no seed development took place, and the authors remarked that females were "stuck in the ostiole." In this situation, *F. obliqua* probably produces similar volatile compounds to *F. rubiginosa* that are necessary to attract *P. imperialis*, but a divergent internal ostiole morphology rather than competition seems to be preventing *P. imperialis* from using *F. obliqua* as a host. This obstacle is alleviated in *F. watkinsiana* because its ostiolar morphology is homologous with that of *F. rubiginosa*, in which case competition may be a more important factor in limiting pollinators in the native range. This concept is congruent with work by Rønsted et al. (2008), which describes both *F. rubiginosa* and *F. watkinsiana* as having triradiate ostioles, whereas *F. obliqua* (in a sister series) has a bilabiate ostiole.

For the expansion of *P. imperialis*'s host breadth to include *F. rubra*, convergent evolution of the inner ostiole morphology, and most likely also the volatile compounds required to attract

the wasp (Grison-Pigé et al., 2002; Proffit et al., 2009; Cornille et al., 2012), appears to be necessary. Whether *P. imperialis* benefits from the absence of *F. rubra*'s normal pollinator is unclear. The absence of this wasp in *F. saussureana*, in spite of clustering with its other new hosts in the PCA (**Figure 2B**) and no normal pollinator, implies that a trait not measured in this study was preventing its use of *F. saussureana*. Because we found no indication that *P. imperialis* was attempting to enter the syconia of *F. saussureana*, as it did with *F. obliqua* in New Zealand (Gardner and Early, 1996), the limiting factor in this case could be volatile compounds (Proffit et al., 2009). We also did not detect *P. imperialis* attempting to enter the syconia of *F. macrophylla*, whose morphology also clusters with the pollinator-sharing figs. Whether this stems from competition with the present normal pollinator or from dissimilar volatile compounds is not clear.

Here we elaborate on Thompson's (2005) geographic mosaic theory of context-dependent coevolution by showing that an

established obligatory mutualism subjected to a new species assemblage is unstable as a result of unprecedented species interactions. *Pleistodontes imperialis* can form new relationships on Kauai because its interaction with the normal pollinator for *F. watkinsiana* is missing, which may disincentivize niche partitioning, and because it can now interact with *F. rubra*, which has an analogous ostiole shape to that of its normal host. Our findings therefore also show the relevance of the rest of the species interactions in the native community, rather than only the two in the partnership, in forming the mechanisms that maintain a relationship. A deeper exploration of new species interactions among coexisting non-native figs will likely uncover other novel instances of pollinator sharing or co-pollination. Such occurrences stipulate long-held views on coevolution by emphasizing the importance of native species assemblages in forming mutualisms, and that they can be disrupted in the new communities of the Anthropocene.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

KB conceptualized the project, surveyed Kauai for potentially naturalizing fig species, and identified fig species taxonomically. KB, JB, SW, and DW collected samples in the field. JW and GW identified *Ficus rubiginosa* with genetics. SW and DW conducted seed germination assays. JB identified wasp species taxonomically and collected, analyzed, and interpreted morphometric data, and wrote the manuscript. VT and GW verified *Pleistodontes* wasp identifications with genetics. All authors reviewed and edited the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2020.564653/full#supplementary-material

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