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EATING OUT OR DINING IN: INSECT-PLANT INTERACTIONS AMONG SEVERAL SPECIES OF *PIPER* IN THE RIO ABAJO FOREST PRESERVE, PUERTO RICO

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ABSTRACT

Pollination activity was investigated for *Piper glabrescens, Piper hispidum, Piper jacquemontianum*, and *Piper umbellatum*, common species in the Rio Abajo Forest Preserve in Puerto Rico. During observation periods, the main floral visitors were syrphid and drosophilid flies, which suggests they play a prominent role as potential pollen vectors for these four species. In addition, two ant species (*Linepithema iniquum* and *Wasmannia auropunctata*) were observed to nest in the stems of three *Piper* species (*Piper abajoense, Piper aduncum,* and *Piper umbellatum*). In the case of *Piper umbellatum*, this interaction is the first documentation of an ant-plant association in *Piper* section *Pothomorphe*.

Key words: Piperaceae, Pollination, Syrphidae, Ant-plant interaction, Formicidae

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INTRODUCTION

Multiple studies have addressed insect-plant interactions involving Neotropical pipers (genus *Piper* L., family Piperaceae), including herbivory (e.g., Marquis 1990, 1991; Dyer et al. 2003, 2004, 2010; Letourneau 2004), pollination (e.g., Semple 1974; Fleming 1985; de Figueiredo and Sazima 2000, 2004; Kikuchi et al. 2007; Vargas-Rojas and Vieira 2017), and ant habitation (e.g., Risch et al. 1977; Letourneau 1998; Fischer et al. 2002; Tepe et al. 2004, 2007a, 2007b, 2009, 2014).

With respect to pollination biology, available research suggests that insects, wind, and selfing play a role for Neotropical *Piper*. For example, Semple (1974) found that several bee species of the genus *Trigona* (Jurine) and some beetles were pollinators of various *Piper* spp. in Costa Rica. Similarly, Fleming (1985) discovered that *Trigona* bees, along with syrphid flies, were the main pollinators in his study of five *Piper* species in Costa Rica. During his study of *P. arieianum* C. DC. in Costa Rica, Marquis (1988) observed pollinators that included 15 species of Diptera and Hymenoptera. For some pipers in southeastern Brazil, de Figueiredo and Sazima (2000) observed that hoverflies and bees were common visitors and therefore likely pollinators, but concluded that wind was also a factor. In their study of *Piper dilatatum* L.C. Rich. on Barro Colorado Island, Panama, Kikuchi et al. (2007) also determined that syrphids, halictids, and stingless bees (e.g., *Trigona* spp.) were the most likely pollinators, and that their activity, especially the bee species, leads to significant levels of geitonogamy. Various bees [e.g., *Apis mellifera* (L.) and *Melipona* (Illiger) spp.] and syrphids [especially *Ocyptamus* (Macquart) spp.] were common visitors based on a study of *P. caldense* C. DC. in the Atlantic forest of Brazil (Vargas-Rojas and Vieira 2017).

From a morphological perspective, individual flowers of *Piper* L. are small and lack the perianth parts (or nectar) that traditionally function to attract insects. Therefore, insects visit *Piper* flowers to gather pollen, undoubtedly attracted by the usually white to yellowish flowers clustered together in large numbers to produce elongate, spike-like inflorescences. With rare exception, Neotropical species of *Piper* have bisexual flowers, which may explain their ability to self-pollinate, but those species studied in detail display some degree of dichogamy, either protandry or protogyny (Martin and Gregory 1962; de Figueiredo and Sazima 2000; Kikuchi et al. 2007; Valentin-Silva et al. 2015; Vargas-Rojas and Vieira 2017), which presumably limits selfing to some extent.

Holldobler and Wilson (1990) indicate that the predominant evidence for myrmecophily is the presence of plant structures that serve as domatia, but not all ant affiliations are necessarily symbiotic due to the opportunistic nature of ants. A total of 11 *Piper* species from Central and South America are known to harbor nesting ants (Burger 1972; Risch et al. 1977; Tepe et al. 2004, 2009, 2014). Several ant species have been noted to inhabit *Piper* spp. (Risch et al. 1977; Holldobler and Wilson 1990; Tepe et al. 2004, 2014; Muñoz et al. 2012), but *Pheidole* (Westwood) appears to be the predominant genus, with *Pheidole bicornis* (Forel) the principal species (Letourneau 2004). Many of the nesting ants establish colonies in petiolar cavities, stems, or both (Burger 1972; Risch et al. 1977; Letourneau 2004; Tepe et al. 2004, 2009, 2014). In *Piper cenocladum* C. DC., *P. fimbriulatum* C. DC., and *P. sagittifolium* C. DC., *Pheidole* spp. initially established nests in the petiolar cavities and, as the colony increased in size, entered and excavated the stem at the base of the petiolar cavity (Risch et al. 1977). Obviously, *Pheidole* spp., as well as other ant species, are benefiting from nesting sites provided by various *Piper* species. Additionally, several *Piper* spp. are known to provide nutrients in the form of single-celled food (pearl) bodies (Risch et al. 1977; Fischer et al. 2002), which occur, depending on the species, either on the inner surface of the petiolar sheath (Risch et al. 1977) or on the surface of the stem cavity (Tepe et al. 2009).

The occurrence of ants in young *Piper* stems is facilitated by the general lack of woodiness, with only the outermost, peripheral ring of vascular bundles producing any noticeable secondary tissues (Tepe et al. 2007a). This means that a significant portion of the young stems consists of soft, parenchymatous tissue, including the pith region excavated for occupancy. Although there are some medullary vascular bundles in the pith, as well as a sclerenchymatous ring that separates the pith from the cortex (Tepe et al. 2007a), these features do not appear to significantly deter insect activity. In addition, myrmecophytic species of *Piper* studied anatomically have fewer raphides (needle-shaped crystals) and starch grains in the pith region (Tepe et al. 2007a), further facilitating excavation activity.

To date, all of the studies concerning Neotropical *Piper* pollination and ant interactions have involved mainland species. However, insect (and plant) species often have limited distributions, so it is important to examine possible interactions in different regions of the world. Therefore, the intent of this study was to observe insect visitors to the inflorescences of *Piper*, and to document any ants nesting in the stems of available *Piper* species to compare the Puerto Rican insect -*Piper* interactions with those of the mainland species.

Materials and Methods

Study Site

This study was conducted in the Río Abajo Forest Reserve in Puerto Rico, located south of Arecibo, in the karst region. The reserve was established in 1935 on land that had previously been deforested (Acevedo-Rodriguez and Axelrod 1999). The main ecosystems that have been re-established in the reserve are tropical and subtropical forests (Acevedo-Rodriguez and Axelrod 1999).

There are 11 known *Piper* species in this area, including *P. abajoense* Bornst, *P. aduncum* L., *P. amalago* L., *P. blattarum* Spreng., *P. glabrescens* (Miq.) C. DC., *P. hispidum* Sw., *P. jacquemontianum* Kunth, *P. marginatum* Jacq., *P. peltatum* L., *P. swartzianum* (Miq.) C. DC., and *P. umbellatum* L. As currently circumscribed, most of these species are widely distributed in the Neotropics, except for the endemic *P. abajoense* (Puerto Rico), *P. blattarum* (Puerto Rico), and *P. swartzianum* (Dominican Republic + Puerto Rico). Although these 11 species co-occur in the area, and previous collection labels indicate that many of them should be in flower simultaneously at various times throughout the year, the pollination study was limited to those species in flowering condition and present in sufficient abundance during the time of the study; these species were *P. glabrescens*, *P. hispidum*, *P. jacquemontianum*, and *P. umbellatum*.

Sampling Protocol

The first field season occurred from 5–10 January 2013, and prior to the first full field day the best methods, times, and locations for examining species of *Piper* and collecting potential pollinators were determined. A second field season occurred from 1–5 June 2013 to see if different pollinators might be active at that time. During both observation periods, the time of day for peak insect activity was determined to be from 10 a.m. to 3 p.m. Collecting was conducted during these times to ensure that representative insect visitors were sufficiently documented.

During the January collection period, *P. glabrescens* and *P. umbellatum* were in flower and abundant; a total of ca. 60 hours of collecting/observing occurred for the former species and ca. 30 hours for the latter. In June, *P. hispidum, P. jacquemontianum*, and *P. umbellatum* were studied for the same reasons; a total of ca. 30 hours of collecting/observing occurred for each species during this period.

Specimens of the representative *Piper* species involved in the pollination project were collected during both study periods to serve as herbarium vouchers for confirmation of identification. One voucher will remain in the herbarium at Southeast Missouri State University (SEMO), and any duplicates will be distributed to herbaria in the United States (e.g., MO) and Puerto Rico (e.g., UPRRP).

During both field periods, an area ca. 2×20 meters, in which *Piper* spp. were in flower, was established for each researcher (three plot areas in January, two of which contained *Piper glabrescens* only and one that contained *Piper umbellatum*; two plot areas in June, one of which contained both *Piper jacquemontianum* and *Piper hispidum*, and the other contained just *Piper umbellatum*). There were several individuals of a given species within each observation area, and each individual displayed multiple inflorescences at various stages of maturation. Representative individuals of the insects visiting *Piper* inflorescences were collected using an insect net or aspirator. Each individual was initially placed in a kill-jar, and then transferred to a labeled vial of 85% ethanol for preservation. These stored individuals served as references of the diversity of insect visitors because field identification for many taxa to the level of genus or species is difficult. Additionally, some insects were purposely not collected in order to observe their basic behavior (e.g., how they moved along a given inflorescence).

Plants were also visually inspected for the presence of ants nesting in the stems. If ants were actively moving into and out of openings in a stem, then that stem was cut one internode above and below the nest openings. The length of the collected stem was then sectioned and all sections were placed in a labeled, wide-mouth, 1000 ml Nalgene® container of 85% ethanol. To confirm proper field identification of the plant, representative flowers and/or fruits were collected and placed in the container, as well.

Lab Analysis

After both collecting events, insect specimens were taken back to the laboratory at Southeast Missouri State University (SEMO) for processing. All insects were identified to the lowest possible taxonomic level and sent to specialists for confirmation or further identification. A few representative insects were examined via light and scanning electron microscopy to detect the presence of *Piper* pollen. Given that ethanol preservation could potentially wash some pollen from the insect bodies, random vials (ca. 20) were also examined for pollen within the ethanol fluid. All remaining voucher specimens were deposited in the insect collection at SEMO, and any duplicates will be sent to the University of Puerto Rico-Rio Piedras, and the Smithsonian Natural History Museum, to be included in their collections.

RESULTS

Pollination Biology

A total of twelve different insect taxa were collected while visiting the inflorescences of at least one of the four species of *Piper* during the two collection periods. During the January survey, nine different species were observed as visitors to *P. glabrescens* and *P. umbellatum* with syrphids as the most common/diverse (TABLE 1; FIGURE 1A). *Leucopodella gracilis* (Williston), *Ocyptamus neoparvicornis* (Telford), and the drosophilid fly *Leucophenga* (Mik) sp. were especially prevalent visitors to *P. glabrescens* based on field observations and representative collecting effort, whereas the most frequent visitor to *P. umbellatum* was the weevil, *Peridinetus signatus* (Rosenschöld) (TABLE 1). Other syrphid visitors during this observation period included *Leucopodella incompta* (Austen), *Ocyptamus parvicornis* (Loew), *Ocyptamus ricus* (Curran), *Toxomerus luna* (Hull), and *Toxomerus violaceus* (Curran), all of which were noted on *P. glabrescens*, but only *O. ricus* occurred on *P. umbellatum*.

Insects collected from the inflorescences of *Piper hispidum*, *P. jacquemontianum*, and *P. umbellatum* during the June survey were primarily syrphids (TABLE 1), which coincides with results from January. Again, based on field observations and representative collecting effort, the most common species were *Leucopodella gracilis* and *Ocyptamus neoparvicornis*. Other species of syrphids were observed and collected, but generally in lower numbers compared to the January collection period. Individuals of *Peridinetus signatus* and *Leucophenga* sp. were also collected from the inflorescences of *P. hispidum* and/or *P. jacquemontianum* during this time. The honey bee, *Apis mellifera*, was also observed on the inflorescences of *P. hispidum* in June, but to a very limited extent.

On multiple occasions, pairs of the weevil, *Peridinetus signatus*, were observed in copulo on the inflorescences of *Piper jacquemontianum* and *P. umbellatum*.

During peak activity, syrphids were observed to alight on a given inflorescence, probe the flowers with their mouth parts, and move slowly up and down a given spike, but rarely for more than one minute. On occasion it was possible to follow a single individual as it traveled from one spike to another, most often on the same plant.

Light, and especially scanning electron, microscopy confirmed the presence of *Piper* pollen on the syrphid and drosophilid flies, especially around their mouth region, but occasionally among the setae on their thorax and legs (FIGURE 1B, C). Examination of storage vials indicated only a few pollen grains of *Piper* in less than 20% of the samples surveyed, indicating that ethanol preservation had minimal effect on pollen adherence.

Ant Habitation

Two ant species, *Linepithema iniquum* (Mayr) and *Wasmannia auropunctata* (Roger) were observed nesting in the stems of three *Piper* species. *Linepithema iniquum* nests were collected from *P. aduncum* and *P. umbellatum* stems, whereas *Wasmannia auropunctata* nests were collected from *P. abajoense* Bornst. stems.

A colony of *Linepithema iniquum* was collected from two live stems of *Piper umbellatum* (FIGURE 1D), and one dead stem of *P. aduncum*. The colony in *P. umbellatum* occupied a 146.6 cm section, consisting of 100.7 cm in the main (larger) stem and a 45.9 cm branch (smaller) arising from that main stem. On the main stem, six of the internodes contained adult ants and in two adjoining internodes adults, eggs, and immatures were present. Fourteen other internodes did not contain any individuals, but there was evidence of potential ant occupation

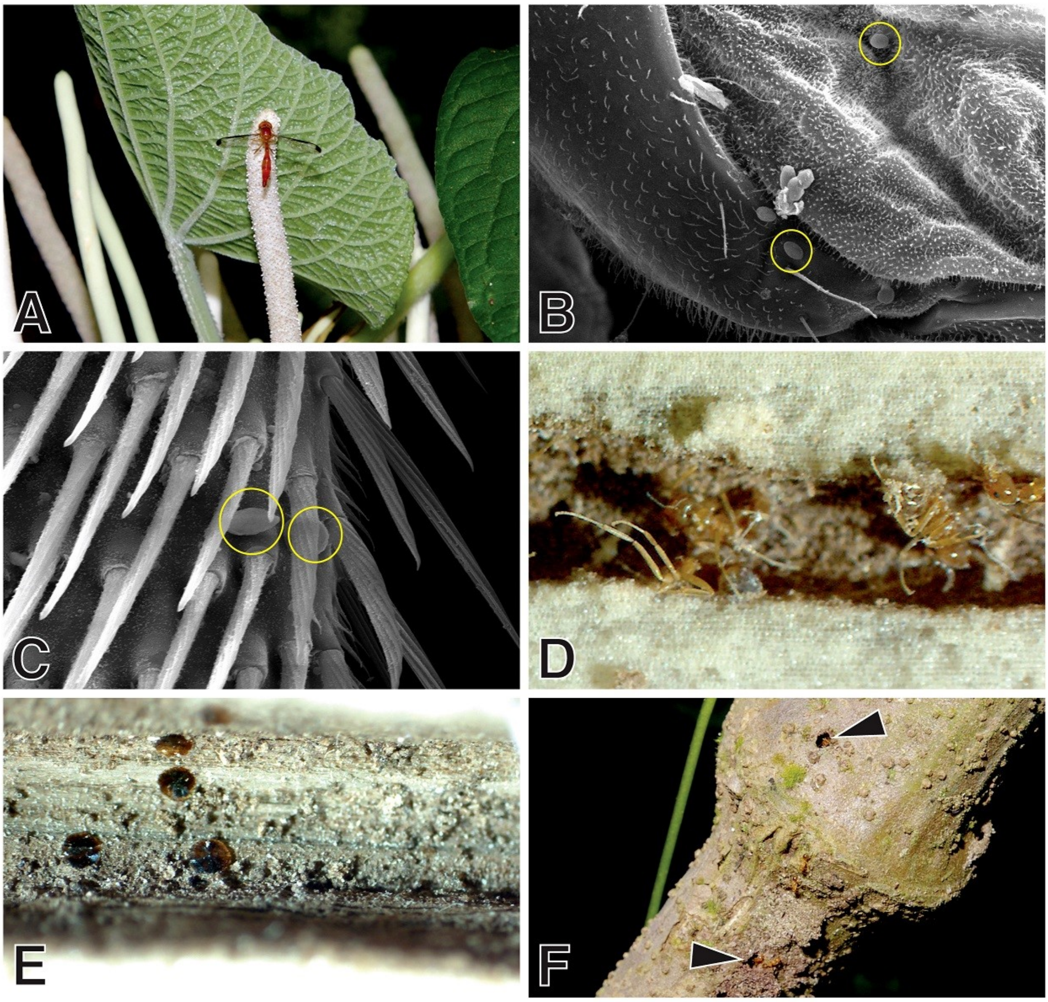

FIGURE 1. Various images of *Piper*-insect associations in Puerto Rico. **A.** Syrphid fly, *Ocyptamus parvicornis*, visiting an inflorescence of *Piper umbellatum*. **B.** Scanning electron micrograph from mouth-part region of a drosophilid fly, *Leucophenga* sp., with *Piper* pollen indicated (yellow circles). **C.** Scanning electron micrograph showing *Piper* pollen (yellow circles) among setae on leg of the syrphid fly, *Ocyptamus neoparvicornis*. **D.** Individuals of the ant, *Linepithema iniquum*, within cauline nest of *Piper umbellatum*. **E.** Close-up of resinous exudate bodies within excavated stem of *Piper umbellatum*. **F.** Entrance holes to cauline nests occupied by the ant *Linepithema iniquum*, located both above and below a node (arrowheads) in *Piper umbellatum*, with several ants visible nearby.

TABLE 1. Diversity of insect taxa collected from *Piper* spp. inflorescences at the Rio Abajo Forest Reserve in Puerto Rico (5–10 January and 1–5 June 2013). A plus sign (+) indicates individuals visited inflorescences of that species of *Piper*, a minus sign (-) indicates no visits, and an asterisk (*) indicates that a given species was not in flower during that observation period; ǂ indicates identification verified by a specialist.

Insect Taxa	*Piper* species							
	P. glabrescens		***P. hispidum***		***P. jacquemontianum***		***P. umbellatum***	
	Jan	Jun	Jan	Jun	Jan	Jun	Jan	Jun
Coleoptera								
Curculionidae								
Peridinetus signatus (Rosenschöld)	-	*	*	-	*	+	+	-
Diptera								
Drosophilidae								
Leucophenga (Mik) *sp.*	+	*	*	+	*	+	+	-
Heleomyzidae	-	*	*	-	*	+	-	-
Phoridae	-	*	*	-	*	+	-	-
Syrphidae								
Leucopodella gracilis (Williston) ǂ	+	*	*	+	*	+	-	+
Leucopodella incompta (Austen) ǂ	+	*	*	-	*	-	-	-
Ocyptamus neoparvicornis (Telford) ǂ	+	*	*	+	*	+	+	+
Ocyptamus parvicornis (Loew) ǂ	+	*	*	-	*	-	-	+
Ocyptamus ricus (Curran) ǂ	+	*	*	+	*	-	+	+
Toxomerus luna (Hull) ǂ	+	*	*	+	*	+	-	-
Toxomerus violaceus (Curran) ǂ	+	*	*	+	*	-	-	+
Hymenoptera								
Apidae								
Apis mellifera (Linnaeus)	-	*	*	+	*	-	-	-

based on excavation of the pith. In the excavated internodes, a thin pith layer lined the walls, and had a golden to light brown coloration. It is assumed that this was due to exposure to air, because undisturbed *Piper* pith tends to be solid white (E. Tepe pers. comm.). In internodes with slightly larger diameters, a double tunnel system existed, with a central partition of pith separating the adjacent tunnels. In several internodes there were small, shallow excavations directly off the main tunnel. Small, rounded, resinous exudate at the surface of the remaining pith was noted in many of the excavated internodes (FIGURE 1E), but pearl bodies were absent. In all but one internode, entrances were located at or very near the nodes (FIGURE 1F), and internodes were not interconnected as a result of excavation. The opening sizes ranged from 1.2 to 4.2 mm in diameter, with an average of 1.99 mm.

In the dead stem of *Piper aduncum*, the colony occupied a 72 cm section composed of five internodes on the main stem and a sixth internode branching off of that main stem between internodes two and three. This sixth internode contained some immatures, but no adults. It is assumed they had left the stem after being placed in the container filled with ethanol. Due to the degraded condition of the stem, excavation patterns could not be easily discerned because the pith, where present, had separated from the stem wall. Of the 12 openings, 10 were located at or near the node. The opening sizes ranged from 0.8 to 3.8 mm in diameter, with an average of 2.37 mm.

Two *Piper abajoense* individuals each contained a colony of *Wasmannia auropunctata*. One colony occurred in a single live stem and occupied a 21.7 cm section composed of three internodes. The most basal internode contained immatures, while the middle internode contained a few adults. Excavation of the internodes did not appear to have been completed by the ant colony based on the degree and irregularity of pith excavation (i.e., entirely removed or thin layer remaining). The excavated area was continuous between the three internodes. The two rounded entrances, one at the mid-point of the basal internode and the other at the middle node were 3.9 mm and 4.0 mm, respectively. No resinous exudate structures or pearl bodies were present.

The second colony of *Wasmannia* occurred in a single dead stem of *Piper abajoense* and occupied a 32.5 cm section composed of four internodes of a younger side branch from the plant's main stem. The first and second internodes contained low numbers of immatures with few adults. The excavation patterns and entrances were similar to that described above for the ant colony found in the live stems of *P. abajoense*, except that adjacent internodes were not connected via continuous tunneling. In all but one internode, entrances were located at or near the node, and the rounded openings ranged from 1.3 to 8.5 mm in diameter, with an average of 3.1 mm. No resinous exudate structures or pearl bodies were present.

DISCUSSION

Insects were using six species of *Piper* (*P. abajoense, P. aduncum, P. glabrescens*, *P. hispidum, P. jacquemontianum,* and *P. umbellatum*) in various ways. They were observed feeding on pollen within the inflorescences of *P. glabrescens*, *P. hispidum, P. jacquemontianum,* and *P. umbellatum*. Two ant species, *Linepithema iniquum* and *Wasmannia auropunctata*, were found nesting in the stems of *P. abajoense*, *P. aduncum*, or *P. umbellatum*.

Pollination Biology

The predominant insect visitors (potential pollinators) for all plants during the two collecting events were hoverflies in the family Syrphidae (TABLE 1), which agrees with the results from de Figueiredo and Sazima (2000, 2004, 2007) in their study of *Piper* and *Peperomia* species in southeastern Brazil. The syrphid fly genus *Ocyptamus*, commonly collected in this study, was also identified as a pollinator in Brazil (de Figueiredo and Sazima 2000, 2004; Vargas-Rojas and Vieira 2017) and Costa Rica (Fleming 1985). Syrphids were also frequent visitors (18%) to *Piper dilatatum* Rich. in Panama (Kikuchi et al. 2007), but identification to the genus level was not performed in that study so a more specific comparison is not possible.

The analyses conducted on Costa Rican pipers by Semple (1974) and Fleming (1985), and *Piper dilatatum* from Panama (Kikuchi et al. 2007), indicated that *Trigona* bees were the predominant pollinating group. Similarly, Vargas-Rojas and Vieira (2017) discovered that bees [*Apis mellifera* and *Melipona* spp.] were much more frequent visitors to inflorescences of *P. caldense* in Brazil than syrphid flies (84% vs. 16%). During an examination of the Puerto Rican bee fauna, Genaro and Franz (2008) did not record any *Trigona* or *Melipona* bees on the island, and these bee genera were not collected during this study. Apparently, Puerto Rico has limited hymenopteran biodiversity due to long-distance dispersal required from the mainland to establish populations on the island (Genaro and Franz 2008). The few endemic bee species present are in low abundance and exhibit restricted distributions across the island (Genaro and Franz 2008). The loss of habitat, due to agriculture, deforestation, and increasing urban sprawl, appears to be the main cause of the current decline of bee species found on the island (Genaro and Franz 2008). The low bee diversity in Puerto Rico may be why potential *Piper* pollinators collected in this study differed from those collected in Central and South America; quite simply, they have different pollinator pools.

Unlike the bees of Puerto Rico, there is diversity of syrphid fly species located on the island (Thompson 1981), which is reflected in the seven species collected (TABLE 1). The majority of these species in Puerto Rico have a Neotropical origin, with only 18% of the total species of syrphid fly being endemic to the island (Thompson 1981).

The diversity and abundance of syrphids clearly enables them to be important potential pollinators in Puerto Rico. Combined with their generalist feeding activity and stable population sizes throughout the year (Owen and Gilbert 1989; de Figueiredo and Sazima 2000), they appear to be common and efficient pollinators (Gilbert et al. 1985; Shi et al. 2009; Willmer 2011). Relying on generalist pollinators is especially important for the plants during the cold and dry seasons in tropical forests (Freitas and Sazima 2003; Dyer et al. 2004), or when bee populations have declined (Willmer 2011).

In order to facilitate collection of pollen, some species of syrphid flies have modified curly or branched hairs, modified bristles, or elongated mouth parts (Holloway 1976; Willmer 2011). These flies may also have pollen scrapers located on their legs, facilitating the transfer of pollen from hind to front legs; this occurs when the individuals are hovering in the air (Holloway 1976) and allows the pollen to be cleaned off of their bodies (Freitas and Sazima 2003). Although morphological details were not recorded for the various syrphids collected in this study, microscopy did confirm the presence of *Piper* pollen among the setae on their legs and thorax, indicating that they could be effective pollen vectors. In addition, several syrphids were observed to fly between inflorescences (occasionally on different neighboring individuals of the same *Piper* species), suggesting that pollen flow could occur. Additional studies would be required to confirm that pollen is actually being deposited between inflorescences, perhaps using fluorescent dye as was done by Kikuchi et al. (2007).

The weevil species, *Peridinetus signatus*, was also collected from *Piper jacquemontianum* and *P. umbellatum* inflorescences (TABLE 1). This weevil was seen mating on the inflorescences of *P. umbellatum*. It is certainly known that species of *Peridinetus* in the adult stage are frequent herbivores of *Piper* leaves in Costa Rica (Marquis 1991), so their occurrence on pipers in Puerto Rico is not especially surprising. Additionally, it is not uncommon for weevils to mate and oviposit on a host plant (R. Anderson pers. comm.), thus these pipers may also serve as a host for *Peridinetus signatus* larval development.

***Piper* Myrmecophytes**

Previously, known ant-plant associations involving cauline occupation in *Piper* have been limited to Central and South America, and in most cases the associated species were within the genus *Pheidole* (e.g., Risch et al. 1977; Tepe et al. 2007a, 2009, 2014). Although *Linepithema iniquum* and *Wasmannia auropunctata* have been documented to nest in *Piper cernuum* Vell. in the Atlantic forests of Brazil (Muñoz et al. 2012), this is the first documented occurrence of these species actually nesting within *Piper* stems in Puerto Rico, and the first record of such relationships beyond the mainland. In the case of *P. umbellatum*, this interaction is the first documentation of ant-plant associations in *Piper* section *Pothomorphe* (Miq.) C. DC., representing another independent evolutionary development of *Piper* plants inhabited by ants, and expands the number to four clades (see FIGURE 2).

Comparatively, the two ant species are ecologically different in several respects. *Linepithema iniquum* is considered to be arboreal and native to Puerto Rico (Wheeler 1908; Wild 2007), whereas *Wasmannia auropunctata* is described as a ground foraging species that often occupies disturbed sites (e.g., floodplains along creeks) (Hahn and Wheeler 2002; Orivel et al. 2009) and is most likely an introduced species to the island (Wetterer and Porter 2003).

Previous researchers have noted that occupation of dead stems is common for *Linepithema iniquum* (Wheeler 1908; Wild 2007, 2009; Escárraga and Guerrero 2016). However, based on excavation patterns and entrance openings, it appears that *L. iniquum* is capable of establishing a colony through their own excavation efforts within the stems of *Piper umbellatum*. The presence of resinous exudate (FIGURE 1E) is likely a wound response and not comparable to the presence of pearl bodies, as in other pipers with true cauline domatia (E. Tepe, pers. comm.).

In the *Piper aduncum* dead stems, the condition of the collected sample was too degraded to ascertain whether *Linepithema iniquum* was responsible for the excavation. If entrance opening size is indicative, then it is likely that this colony simply took advantage of a vacated stem that was previously occupied by a larger, stem-boring insect, possibly of some curculionid larva.

The excavation patterns and relatively large entrance openings suggest that *Wasmannia auropunctata* established nests within *Piper abajoense* stems that had been previously excavated by other stem-boring insects. This seems reasonable to assume given its known biology as an opportunistic species, whether in its native (Dejean et al. 2003; Wetterer and Porter 2003; Longino and Fernández 2007) or introduced range (Wetterer and Porter 2003; Orivel et al. 2009; Muñoz et al. 2012; Mikissa et al. 2013).

CONCLUSIONS

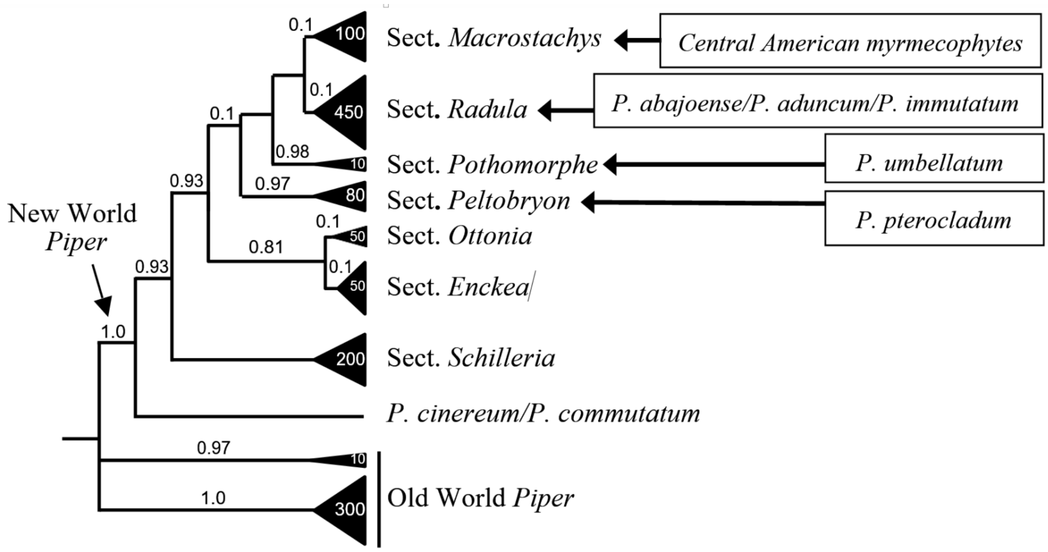

FIGURE 2. Summary phylogeny of *Piper* based on Bayesian analysis of nuclear ITS and plastid *psbJ-petA* sequence data (originally redrawn from Jaramillo et al. 2008, and subsequently modified from Tepe et al. 2009). Numbers above branches indicate Bayesian posterior probabilities, and numbers in triangles represent an estimation of species in each section. *Piper abajoense and Piper aduncum* in section *Radula* are newly documented to harbor ants, while *Piper umbellatum* represents the first documentation of an ant-plant association from section *Pothomorphe*. Each of the ant-associated species is nested within a clade of species not linked with ants, indicating a minimum of four independent evolutionary origins of ant-plant associations among New World *Piper*.

Overall, this study documents novel ecological interactions involving Puerto Rican pipers and insects. In particular, the results confirm that insects, especially syrphids (TABLE 1), likely contribute to pollination of the four *Piper* species examined, in contrast to the prevalent role of bees among the few mainland taxa studied. Regardless of previous results, to better understand the differences in natural history between island and mainland species of *Piper*, more studies need to be completed concerning possible pollen vectors, especially given the apparent difference in pollinator pools between these regions. In essence, a significant part of the sexual life history (i.e., pollination) of most pipers is unknown. Similarly, the fate of successful sexual reproduction (i.e., fruit dispersal) remains to be investigated in Caribbean pipers. Numerous studies of mainland taxa support the role of frugivorous bats as the primary dispersal agents (see Heithaus et al. 1975; Fleming et al. 1977; Fleming 1981; Fleming and Heithaus 1981; Palmeirim et al. 1989; Charles-Dominique 1991; Marinho-Filho 1991; Bizerril and Raw 1998; Thies and Kalko 2004), but comparative analyses with island taxa will first require appropriate field projects.

With respect to ants establishing cauline nests, it is not surprising that both *Linepithema iniquum* and *Wasmannia auropunctata* colonized what appear to be abandoned stems that were previously excavated by a different wood-boring insect. However, the excavation and establishment of a colony by *L. iniquum* in *Piper umbellatum* stems is significant because it involves an ant species outside of the genus *Pheidole*, the most common ant inhabitant of pipers, and represents an interaction with a species from a completely different lineage of *Piper*. Relatively few representatives (< 20 species) from four distantly related lineages of Neotropical pipers (see FIGURE 2) are known to have ants nesting within their stems and/or petioles, along with several, perhaps closely related, Old World taxa (Jaramillo and Callejas 2004; Gardner 2006). Clearly, further research efforts concerning *Piper*-ant interactions are needed to determine whether 1) these relationships exist among other lineages, 2) perhaps involve additional species within lineages already documented, and 3) might occur within other distributional regions. These studies will allow researchers to better address important questions such as why these associations exist in distantly related taxa, how these associations have evolved, and why different plant organs (stems, petioles, and leaves) are used by the ant inhabitants.

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