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Ants Tend Ghost Orchids: Patrolling of Dendrophylax lindenii (Orchidaceae) by Crematogaster ashmeadi in Florida

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Ants tend ghost orchids: patrolling of *Dendrophylax lindenii* (Orchidaceae) by *Crematogaster ashmeadi* in Florida

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Abstract

Myriad symbioses exist between insects and orchids, especially in tropical forests where the majority of species are epiphytic. Relationships between ants and rare epiphytic orchids are underrepresented in the scientific literature. The natural history and ecological entomology of Florida's endangered and epiphytic ghost orchid, *Dendrophylax lindenii* (Lindley) Bentham ex Rolfe (Orchidaceae), remain limited. Widely recognized for long-standing hypotheses concerning the species' pollination ecology, that documentation recently overturned, other interactions between insects and ghost orchids are scarce. Here we describe the first associations between ants, *Crematogaster ashmeadi* Mayr (Hymenoptera: Formicidae), and *D. lindenii*. Ghost orchid roots provide facultative and opportunistic structures for arboreal ants to use in nesting. Furthermore, excrement from ant colonies within the root mass can increase nutrient availability in the orchid's nutrient-poor substrate; the proximity of these ants permits patrolling to defend the plant and exert control over possible extra floral nectaries that require further inquiry. This study presents novel observations that expand the known insect associations with ghost orchids, elucidating the complex ecology of one of Florida's rarest and most endangered species.

Key Words: ants; arboreal; ecology; epiphyte; Everglades; Fakahatchee

Resumen

Existen incontables números de simbiosis entre insectos y orquídeas, especialmente en los bosques tropicales donde la mayoría de las especies son epífitas. Las relaciones entre las hormigas y las orquídeas epífitas más raras están subrepresentadas en la literatura científica. La historia natural y la entomología ecológica de la orquídea fantasma epífita, *Dendrophylax lindenii* (Lindley) Bentham ex Rolfe (Orchidaceae), que esta en peligro de extinción en la Florida, siguen siendo limitadas. Ampliamente reconocida por mucho tiempo por las hipótesis sobre la ecología de la polinización de la especie, la documentación fue recientemente anulada y otras interacciones entre insectos y orquídeas fantasmas son escasas. Aquí describimos las primeras asociaciones entre las hormigas, *Crematogaster ashmeadi* Mayr (Hymenoptera: Formicidae) y *D. lindenii*. Las raíces de orquídeas fantasmas proporcionan estructuras facultativas y de oportunidad para que las hormigas arbóreas las usen en la anidación. Además, el excremento de las colonias de hormigas dentro de la masa de la raíz puede incrementar la disponibilidad de nutrientes en el sustrato pobre nutritivo de la orquídea; la proximidad de estas hormigas permite patrullar para defender la planta y ejercer control sobre posibles nectarios extra florales que requieren de una mayor investigación. Este estudio presenta observaciones novedosas que amplían las asociaciones de insectos conocidos con las orquídeas fantasmas, aclarando la compleja ecología de una de las especies más raras y en peligro de extinción de la Florida.

Palabras Clave: hormigas; ecología; epifita; los Everglades; Fakahatchee

Epiphytic plants occupy a prominent position in the research history of ant-plant mutualisms (Huxley 1980; Beattie 1985). This is likely due to the fact that epiphyte abundance and richness contribute to increased arthropod diversity in tropical tree canopies (Yanoviak et al. 2011) where arboreal ants are the dominant insect taxon (Wilson 1987). Early naturalists first noted the diversity and complexity of angiosperms that take root in arboreal ant nests and described the phenomenon of ant gardens: a flourishing of epiphytic plants associated with ant nests (Ule 1901). Benefits to the plants include the ants' waste products that provide nutrients (Huxley 1980), which enhance plant growth and production of flowers. Ant occupants regularly patrol host plants and defend them from herbivores (Jeffrey et al. 1970; Bentley 1977; Bronstein 1998), as well as aid in the harvest and dispersal of seeds. Ants benefit from this association by obtaining structural resources from epiphytes for nest construction, and often benefit from the sugary rewards from flowers or extra floral nectaries. The study of these complex interactions among ants and plants, and their role in the ecology of tropical forests, has emerged as a distinct field of inquiry. Some ant-plant associations are highly specialized, co-evolved mutualisms, but many are facultative and opportunistic associations taking advantage of available nutritional and structural resources (Fisher et al. 1990).

Orchids are among the most intriguing, diverse, and well-studied groups of epiphytes. Many of the approximately 25,000 species within the Orchidaceae produce sugary exudates through extra floral nectaries and contain cavities within living tissues suitable for nesting, both of which are attractive to ants. Relationships between ants and rare

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epiphytic orchids are underrepresented in the scientific literature because of the difficulty in documenting them: the partners are often small, inconspicuous, and interactions occur high in trees.

Florida's endangered ghost orchid, *Dendrophylax lindenii* (Lindley) Bentham ex Rolfe (Orchidaceae: Angraecinae), is one of the most widely recognized orchids in the world with only an estimated 2,000 individuals remaining in the wild. Studies of ghost orchids have investigated mycorrhizal relationships (Chomicki et al. 2014), host tree affinities (Mujica et al. 2009; Raventos et al. 2015), micropropagation (Hoang et al. 2016), and volatile compound composition (Sadler et al. 2011). Although few investigations have focused on insect associates, this orchid has generated significant publicity around the mystery of its pollination. Long standing hypotheses proposed that pollination in this species, with its long, elegant nectar spur, was carried out by a single, co-evolved mutualist (Houlihan 2018). This hypothesis was recently upended completely when the first images of hawkmoth pollinators disproved the previous hypothesis and suggested that multiple pollinator communities may be associated with *D. lindenii* (Houlihan et al. 2019). This discovery highlights how little has been documented about the ecological interactions between insects and ghost orchids, and how much more remains to be understood (Houlihan et al. 2019). Filling this gap in our understanding is an urgent need for efforts to conserve this species (Stewart & Kane 2007).

In the wild, *D. lindenii* do not always produce flowers annually; rather, clusters of nearby individuals appear to synchronize flowering years (M. O. unpublished data); this may enhance pollination success and seed pod production. Micropropagation experiments investigating the nutrient requirements of *D. lindenii* reveal that certain specific microhabitat conditions and mycorrhizal compositions generate spikes that induce flowering (Hoang et al. 2016). Although optimal environmental scenarios can be simulated in the laboratory, the availability of these nutrients does not exist in constant or regular abundance in the wild. After consecutive years of flower production, individual ghost orchids (and even entire slough clusters) often go dormant for a period of yr (P. R. H., M. O. unpublished data). This boom and bust cycle likely allows plants to reaccumulate nutrients depleted during flowering yr, ultimately improving pollination success. Here we describe a new association between *D. lindenii* and a native ant species, *Crematogaster ashmeadi* Mayr (Hymenoptera: Formicidae), from multiple observations over a 3 yr period. This is the first record of such a relationship in the *Dendrophylax* genus.

Materials and Methods

Observations were conducted in the Fakahatchee Strand Preserve State Park in Collier County, Florida. The lowest part of the Everglades and Big Cypress basins in elevation, Fakahatchee is a unique subtropical strand forest, characterized by slow-flow, seasonally flooded sloughs that are dominated by pop ash (*Fraxinus caroliniana* Mill.; Oleaceae) and pond apple (*Annona glabra* L.; Annonaceae) the primary hosts in Florida for the epiphytic *D. lindenii* (Fig. 1). The presence of royal palms and bald cypress adds to the distinctiveness of these forests, as this sympatry is not found anywhere else in the world. Fakahatchee contains the highest diversity of orchids (> 49 spp.) and bromeliads in the USA.

Fig. 1. A ghost orchid, *Dendrophylax lindenii*, flower and bud in the flooded forests of Fakahatchee State Park, Florida. One of 2 individuals observed having an association with *Crematogaster ashmeadi*. A mosquito is seen resting on the labellum of the flower.

Orchids were monitored from Jun 2014 through Jul 2017. During this time, 14 visits were made to Fakahatchee (varying in length from 1–8 d), totaling 47 d in the field and amounting to 423 h searching for and observing the orchids. A Canon XF205 HD infrared camera (Canon, Melville, New York, USA) was deployed to film flowers for 10 h overnight on 20 separate nights, totaling 200 h of camera monitoring. The number of visits includes 6 trips during the winter when *D. lindenii* was not in flower, but field conditions permitted further exploration of the flooded forest in search of new individuals to monitor later in the yr during the flowering mo. A total of 124 individuals were located in Fakahatchee. This number includes individuals observed in remote sloughs that were occasionally explored opportunistically, but may not have been revisited during the course of the study. As the predominant focus of the study was initially on pollination (Houlihan et al. 2019), a select group of 14 orchids that reliably produced flowers annually during this period were monitored most frequently. An additional 2 visits were made to Guanahacabibes National Park in western Cuba. During the first trip in Dec 2015, 12 orchids with flowers were monitored over the course of 5 d. On the following trip in early Jan 2017, more than 150 individuals were located in 3 d with only 1 in flower.

Ants on *D. lindenii* were first filmed and photographed from a distance of 2 to 3 m with a Canon 5DS SLR camera and Canon EF 100-400 mm USM ii lens (Canon, Melville, New York, USA). This permitted initial documentation without disturbing the ants and eliciting behavioral response to observer presence. A Canon EF 100 mm Macro lens was then used to record close up video on a tripod to document ant interactions with other ants and various parts of the orchid. *Crematogaster ashmeadi* was identified based on color, size, and collection locality (Mark Deyrup personal communication). Distinguishing this species from *Crematogaster pinicola* Deyrup & Cover (Hymenoptera: Formicidae) can be difficult because the 2 are morphologically indistinguishable; however, the former is often brownish rather than black when it occurs in wet or mesic areas. Voucher specimens were collected into 95% EtOH for morphological examination. Pinned voucher specimens were deposited in the Archbold Biological Station insect collection in Venus, Florida, USA.

Results

Crematogaster ashmeadi was observed on *D. lindenii* on 2 separate occasions. In Sep 2016, multiple individuals of this species were observed on 1 ghost orchid, primarily on the root mass and a spike that contained a bud. *Crematogaster ashmeadi* individuals were observed again on a different plant of *D. lindenii* in Jul 2017 that had produced a bud.

Photos, videos, and direct field observations indicated that the ants were patrolling the orchid (Figs. 2, 3). Individuals moved in and out of

Fig. 2. *Crematogaster ashmeadi* emerge and return via cavities excavated beneath roots of *Dendrophylax lindenii*. Individuals make mandible contact with the roots.

Fig. 3. An individual *Crematogaster ashmeadi* patrolling the roots of *Dendrophylax lindenii.*

a central point beneath the root mass, apparently in a cavity that appeared to have been excavated in the orchid's substrate (Fig. 2). In one case, a spider descended on a bud (Fig. 4) and began affixing a web to the sepals and labellum, possibly lured by the abundance of small prey,

such as mosquitoes (Fig. 5), also attracted to flowers. Ant activity subsequently increased on the inflorescence and individuals dismantled the web, effectively removing the spider from the flower bud and leaving it to fall into the water below (Fig. 6).

Discussion

In nutrient poor tropical forests, epiphytic plants are known to benefit from relationships that enhance the suitability of substrates and availability of nutrients (Blüthgen et al. 2001). Ants are known to have facultative associations with orchids for: (1) structural support in construction of carton nests or usage of cavities for housing (Longino 1986); (2) extrafloral nectar (Fisher & Zimmerman 1988); and (3) dispersal of seeds in ant-gardens (Kaufmann & Maschwitz 2006).

In our study, *C. ashmeadi* was observed only on developing inflorescences of *D. lindenii* with buds (Figs. 2, 3, 6), suggesting the possibility of extra floral nectaries on those structures and spikes, or a possible role of volatile compounds. Extrafloral nectaries contribute to the greatest number of ant-orchid associations (Fisher 1992) that can occur on nearly all orchid structures, and secrete nectar from glands on developing inflorescences, shoots, buds, and fruits (Marazzi et al. 2013). Furthermore, biotic and abiotic factors influence extra floral nectary production in the wild, which can strongly affect ant-plant interactions

Fig. 4. A spider of the Family Araneidae descends and begins constructing a web on a ghost orchid flower. The flowers are frequented by mosquitoes, which may be the target prey.
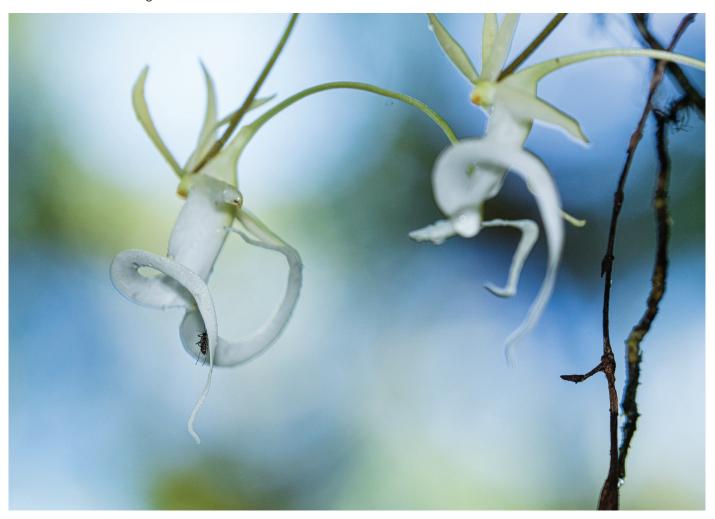
Fig. 5. A mosquito, *Aedes taeniorhynchus*, rests on a *Dendrophylax lindenii* flower. This is a common occurrence that may indicate insect attraction to volatile compounds emitted by the orchid, and also potential prey for ambush predators on its flowers.

in situ (Jones et al. 2017). In a floristic study of the Everglades of southern Florida (Koptur 1992), 9% of species (78 of 851 spp.) were reported to contain extra floral nectaries. Although *D. lindenii* was not one of the 9 orchid species identified with extra floral nectaries, that study did not state if this orchid was assessed. However, we believe that this species was not present because the primary focus of investigations were carried out in Everglades pineland and sawgrass prairie habitats rather than flooded forests characteristic of *D. lindenii* in the Big Cypress Basin. Indeed, Hoang et al. (2016) dissected numerous *D. lindenii* for anatomical studies that were germinated from southern Florida seeds and did not identify extrafloral nectaries on the stem, root, or leaf primordia (N. H. Hoang personal communication). The study of extra floral nectaries within flooded forest habitats remains limited (Koptur 1992). Based on observations of mandible prodding of roots by ants in our study (Fig. 3), root masses warrant further inspection for possible secretions. As stated earlier, it is possible also that volatiles, rather than extra floral nectaries, play a role in attracting ants to this orchid (Willmer et al. 2009).

While extra floral nectaries remain uncertain for *D. lindenii*, the root structure provides an opportunistic haven for *C. ashmeadi* (Fig. 2). The 2 instances of ant patrolling occurred in heavily flooded sloughs of the Fakahatchee Strand where tree trunks are isolated by water 1 to 2 m in depth. Ants had excavated cavities in the bark behind root structures affixed to the host tree, *A. glabra*, which has been observed

elsewhere in this tree species with epiphytic orchids and *Crematogaster* ants. In Panama, *A. glabra* has been found to host 49 ant species, with a predicted ant species richness of 56 to 71; of these, 32 nested occasionally in an epiphytic orchid, *Caularthron bilamellatum* Hooker (Orchidaceae), most commonly inhabited by *Crematogaster* spp. (Yanoviak et al. 2011).

To our knowledge, generation of volatile compounds produced by ant-gardens or carton nests have not been studied as potential supplemental attractants to pollinators. Nocturnal Lepidoptera are known to be attracted to and associate with ants (Sourakov & Houlihan 2017), and a recent study documenting orchid seed dispersal and preferential seed removal by epiphytic ant gardens in the Americas provides novel insight into a poorly understood field of orchid ecology (Morales-Linares et al. 2018). Pollination ecology of many sphingophilous flowers, remains understudied (Houlihan et al. 2019), particularly in tropical swamp forests where Lepidoptera diversity often is relatively lower than other tropical forest habitats (Houlihan et al. 2013). Pollination success and fertilization of *D. lindenii* is exceptionally low, with only a few individuals producing seed pods within Fakahatchee's population each yr. Further observations of pollinated *D. lindenii* individuals are required to assess facultative ant gardening, which could be confirmed if seed pod production is significantly higher in individuals of *D. lindenii* that associate with ants than those that do not and subsequent seed dispersal.
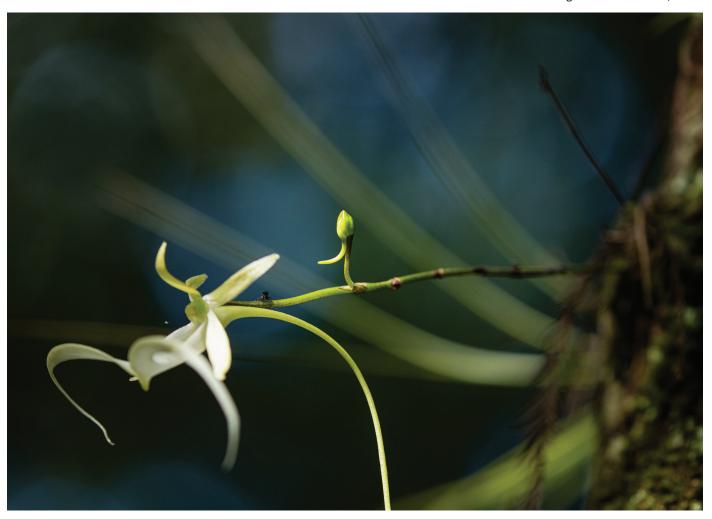
Fig. 6. Upon the arrival of the spider previously shown in Figure 3 of a ghost orchid flower, *Crematogaster* ants begin dismantling the web and ultimately remove the spider from the plant.

Epiphytic orchids are likely to have many associations with ants yet to be documented. More research in this realm is necessary across orchid species to develop a comprehensive understanding of the diversity of ant-orchid symbioses. The benefit to *D. lindenii* of having regular ant tending may follow the model of ant-gardens, in that ant waste may increase nutrient availability in the nutrient-poor substrate of this tropical epiphytic orchid. Additionally ants may defend the plant against herbivores, as well as any potential deterrence to pollination, such as spider webs.

Acknowledgments

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