Pollinia Removal and Suspected Pollination of the Endangered Ghost Orchid, Dendrophylax lindenii (Orchidaceae) by Various Hawk Moths (Lepidoptera: Sphingidae): Another Mystery Dispelled

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Research

Pollinia removal and suspected pollination of the endangered ghost orchid, *Dendrophylax lindenii* (Orchidaceae) by various hawk moths (Lepidoptera: Sphingidae): another mystery dispelled

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Abstract

The ghost orchid, *Dendrophylax lindenii* (Lindl.) Bentham ex Rolfe (Orchidaceae), is a rare, leafless epiphyte restricted to forests in southernmost Florida and western Cuba. The species' appealing floral display, high public profile, and challenging cultivation contribute to its ongoing removal from the wild by unethical collectors. To effectively conserve this and other native orchids that rely on seed for reproduction, a thorough understanding of natural pollination mechanisms is essential. Digital single lens reflex camera traps were used to survey for potential pollinators visiting *D. lindenii* flowers on the Florida Panther National Wildlife Refuge during the summers of 2016 to 2018. Based on suspected *D. lindenii* pollinia affixed to photographed moths, we provide visual evidence that *D. lindenii* is pollinated by at least 2 large hawk moths (Sphingidae) in southern Florida, which include the fig sphinx moth, *Pachylia ficus* Linnaeus, and pawpaw sphinx moth, *Dolba hyloeus* Drury (both Lepidoptera: Sphingidae). Species that were documented probing *D. lindenii* flowers, but lacked pollinia, included the giant sphinx moth (*Cocytius antaeus* Drury), banded sphinx moth (*Eumorpha fasciatus* Sulzer), and streaked sphinx moth (*Protambulyx strigilis* Linnaeus) (all Lepidoptera: Sphingidae). In addition to the aforementioned species of hawk moths (sphinx moths), the seagrape spanworm moth (*Ametris nitocris* Cramer; Lepidoptera: Geometridae), palamedes swallowtail (*Papilio palamedes* Drury; Lepidoptera: Papilionidae), monk skipper (*Asbolis capucinus* Lucas; Lepidoptera: Hesperiidae), Brazilian skipper (*Calpodes ethlius* Stoll; Lepidoptera: Hesperiidae), and 3 unidentifiable geometrid moths were observed visiting *D. lindenii* flowers within the study area. During 2017 and 2018, a total of 21 different visits by Lepidoptera were recorded, and the duration of each visit was rarely longer than 1 s. Hawk moth visits were infrequent, but did show some evidence of clustering by species. Measurements of proboscis lengths of the 2 documented pollinators from museum specimens were of sufficient length (50–100 mm) to probe *D. lindenii* nectar spurs, further lending support to our field observations. Larval food sources of the 2 confirmed pollinators include plant species native to southern Florida, suggesting that these moths are natural pollinators of *D. lindenii*. Our findings, although preliminary, provide critically needed baseline information that will augment ongoing conservation efforts in southern Florida aimed at the recovery of *D. lindenii*.

Key Words: Florida Panther National Wildlife Refuge; Fakahatchee Strand; conservation

Resumen

La orquídea fantasma, *Dendrophylax lindenii* (Lindl.) Bentham ex Rolfe (Orchidaceae), es una rara epífita sin hojas restringida a los bosques en el extremo sur de Florida y el oeste de Cuba. La atractiva exhibición floral de la especie, su alto perfil público y su difícil cultivo contribuyen a su eliminación permanente de los recolectores no éticos. Para conservar de manera efectiva esta y otras orquídeas nativas que dependen de la semilla para la reproducción, es esencial comprender a fondo los mecanismos naturales de polinización. Se utilizaron trampas fotográficas digitales réflex de lente única para inspeccionar posibles polinizadores que visitan flores de *D. lindenii* en el Refugio Nacional de Vida Silvestre Florida Panther durante los veranos de 2016 para 2018. Sobre la base de la sospecha de *D. lindenii* polinios pegada a polillas fotografiadas, proporcionamos evidencia visual de que *D. lindenii* es polinizada por al menos 2 polillas halcón grandes (Sphingidae) en el sur de la Florida, que incluyen la polilla esfinge, *Pachylia ficus* Linnaeus y la papaya Esfinge polilla, *Dolba hyloeus* Drury (ambos Lepidoptera: Sphingidae). Las especies documentadas que probaron las flores de *D. lindenii*, pero carecían de polinios, incluían la polilla esfinge gigante (*Cocytius antaeus* Drury), la polilla esfinge bandada (*Eumorpha fasciatus* Sulzer), y la polilla esfinge rayada (*Protambulyx strigilis* Linnaeus) (todo Lepidoptera: Sphingidae). Además de las especies antes mencionadas de las polillas

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halcón (polillas esfinge), la polilla de la lombriz (*Ametris nitocris* Cramer; Lepidoptera: Geometridae), cola de golondrina del paladar (*Papilio palamedes* Drury; Lepidoptera: Papilionidae), patrón del monje (*Asbolis capucinus* Lucas; Lepidoptera: Hesperiidae), skipper brasileño (*Calpodes ethlius* Stoll; Lepidoptera: Hesperiidae), y se observaron 3 polillas geométridas no identificables visitando flores de *D. lindenii* dentro del área de estudio. Durante 2017 y 2018, se registraron un total de 21 visitas diferentes de Lepidoptera, y la duración de cada visita rara vez fue superior a 1 s. Las visitas de la polilla halcón fueron poco frecuentes, pero mostraron cierta evidencia de agrupamiento por especies. Las mediciones de la longitud de la probóscide de los 2 polinizadores documentados de especímenes del museo fueron de una longitud suficiente (50–100 mm) para sondear los espolones de néctar de *D. lindenii*, lo que brindó más apoyo a nuestras observaciones de campo. Las fuentes de alimento larvales de los 2 polinizadores confirmados incluyen especies de plantas nativas del sur de Florida, lo que sugiere que estas polillas son polinizadores naturales de *D. lindenii*. Nuestros hallazgos, aunque preliminares, brindan información de línea de base crítica y necesaria que aumentará los esfuerzos de conservación en curso en el sur de la Florida dirigidos a la recuperación de *D. lindenii*.

Palabras Claves: Florida Panther National Wildlife Refuge; Fakahatchee Strand; conservación

Florida is home to about 118 species and varieties of orchids, of which 106 are native (Brown 2005). The ghost orchid, *Dendrophylax lindenii* (Lindl.) Bentham ex Rolfe (Orchidaceae), is a rare leafless epiphyte restricted to dense, wet forests and hammocks in southern Florida and western Cuba (Luer 1972; Brown 2005; Mujica et al. 2018). The common name stems from its striking floral display consisting of large white, fragrant flowers that appear to hover in mid-air in its dimly lit habitat. Anthesis typically occurs during the summer mo (May–Aug) when humidity levels and insect activity are at their peak. Because of its appeal, rarity, and challenging cultivation, *D. lindenii* remains vulnerable to poaching. Other threats include periodic hurricanes (Wiegand et al. 2013), hydrological changes (Langdon 1979), and phytophagous pests (Zettler et al. 2012). The species is currently state listed as endangered in Florida (Coile & Garland 2003), where it is largely restricted to 2 counties undergoing rapid urban development (i.e., Collier and Hendry).

To effectively conserve this species, the specialized needs of *D. lindenii* must be fully understood including biotic and abiotic components alike. The 2 most critical biotic agents needed for reproduction by orchids are pollinators for fruit set, and mycorrhizal fungi for seedling recruitment (Swarts & Dixon 2009). In recent years, much has been learned about *D. lindenii*, including its specific habitat requirements (Mujica et al. 2018), artificial propagation with and without mycorrhizal fungi (Hoang et al. 2016), and greenhouse acclimatization leading to field establishment (Coopman & Kane 2019). Other than anecdotal reports (Tuttle 2007), little information exists with regards to pollination of *D. lindenii*. Sadler et al. (2011) analyzed the floral fragrance chemistry of *D. lindenii* and concluded that nocturnal moths were the likely pollinators of the ghost orchid. It has long been assumed that hawk moths (Lepidoptera: Sphingidae), in particular, serve as pollinators of *D. lindenii* based on floral characteristics that cater to these insects, i.e., white floral coloration, sweet-smelling evening fragrance, and long nectar spur (Dressler 1981). Based on the latter character, the giant sphinx moth, *Cocytius antaeus* (Drury) (Lepidoptera: Sphingidae) has been singled out as the most likely pollinator given that the proboscis length of the moth (10–15 cm) closely matches the length of a typical *D. lindenii* nectar spur (11–17 cm) (Correll 1950). However, pollination of the ghost orchid has yet to be reported in a scientific context. The primary objective of this study was to use digital single lens reflex camera traps to identify potential pollinators visiting *D. lindenii* flowers on the Florida Panther National Wildlife Refuge in Collier County, Florida.

Materials and Methods

STUDY AREA

This research was conducted on the 10,768-ha Florida Panther National Wildlife Refuge in eastern Collier County, Florida, USA (26.184124°E, 81.412493°N), which is part of the Big Cypress basin Ecoregion. The Florida Panther National Wildlife Refuge shares common boundaries with Big Cypress National Preserve to the east, Picayune Strand State Forest to the southwest, and the orchid-rich Fakahatchee Strand State Preserve State Park to the south. Over two-thirds of the Florida Panther National Wildlife Refuge is palustrine wetland, and prominent wetland features of the Florida Panther National Wildlife Refuge include the Fakahatchee Strand, Lucky Lake Strand, Stumpy Lake Strand, Mud Lake Strand, and the Okaloacoochee Slough. These cypress/mixed hardwood-dominated swamps are separated by forests of slash pine (*Pinus elliottii* Engelm.; Pinaceae), saw palmetto (*Serenoa repens* [W. Bartram] Small; Arecaceae), and cabbage palm (*Sabal palmetto* [Walter] Lodd. ex Schult. & Schult.f; Arecaceae) that grow on slightly higher ridges underlain with cap rock. Also interspersed throughout the Florida Panther National Wildlife Refuge are mixed hardwood hammocks and wet prairies. The topography of the Refuge is relatively flat, with elevations ranging from 3.7 to 4.9 m above mean sea level. As much as 90% of the Refuge is inundated to depths ranging from a few centimeters to more than 1 m of water at the height of the rainy season (i.e., typically Aug–Sep). Collectively, these habitats support a total of 27 orchid species in 17 genera (Stewart & Richardson 2008), including > 500 documented occurrences of individual *D. lindenii* (A. Herdman & E. Mujica, personal communication, and U.S. Fish and Wildlife Service internal records). All research was conducted within a unique forested wetland known as the Fakahatchee Strand, which is believed to support the highest diversity of native orchids and bromeliad species in North America (FDEP 2014).

Three digital single lens reflex camera traps were deployed within the northern portion of Fakahatchee Strand during a 3-yr period beginning in Jul 2016 and ending in Oct 2018. Camera traps were active from Jul to Oct to coincide with common *D. lindenii* flowering times at the study site (U.S. Fish and Wildlife Service internal records). The duration that time-active camera traps were focused on *D. lindenii* blooms during 2016 and 2017 amounted to 847 and 632 h, respectively. A more extensive effort to photograph insect visitors took place in 2018 from 22 Jun to 5 Oct (106 d) totaling 3,495 h. All 3 camera traps were deployed at 1 given site and ran simultaneously, with each trap focused on a different *D. lindenii* specimen in flower. The cameras were positioned to the side of each flower so that they would not obscure the flower nor physically interfere with insect activity.

CAMERA APPARATUS

The camera trap system, designed by C. Ward, consisted of Nikon (Melville, New York, USA) digital single lens reflex cameras, operating at 2 and 5 Nikon flashes, coupled with high speed Sabre light detection and ranging (LIDAR) laser triggers manufactured by Cognisys Systems, Inc. (Traverse City, Michigan, USA). Nikon flashes were preferred because of their unique ability to revert into low-energyconsumption standby mode while instantaneously activating once the camera trigger detects insect movement. Under ideal conditions, and with minimal false triggering events, these cameras and flash batteries lasted up to 1 mo during sampling, which is longer than the persistence of most *D. lindenii* blooms. The internal batteries in the Sabre triggers typically lasted < 24 h when sampling at the highest rate of every 10 msec. To keep the Sabre triggers running for longer periods of time, 35 amp-h deep cycle batteries were suspended from the camera supports to provide extra power. This configuration allowed the Sabre triggers to operate for up to 2 wk. Given that water levels were high (about 1 m deep) at the time of sampling, a platform was constructed that facilitated camera set-up at blooming height, typically 2 to 3 m above the ground or 1 to 2 m above the water surface.

The cameras and lenses were secured inside custom made waterproof boxes. Flashes were secured inside custom made waterproof tubes. One flash was connected to the camera by a wire, which allowed the camera to operate within shutter speed parameters compatible with flash synchronization. Additional flashes were fired by wireless radio triggers. No flashes or cameras were mounted to trees hosting orchids. Most equipment was mounted to the tripod or camera post. Some flashes, such as background and side lights, were attached to other trees by tree screws. Flashes were carefully aimed and adjusted so as not to overexpose the white *D. lindenii* flowers, and to provide adequate illumination for dark moths. Once activated, cameras were set to record a sequence of photos lasting between 1 to 2 s. Each sequence captured between 8 and 15 photographs at rates ranging from 5 to 8 frames per s. Lasers were aimed a few cm above and beyond the labellum of the flower, with sufficient distance so that wind and rain would not move the bloom across the beam, but close enough so that a large moth should not be able to access the *D. lindenii* flower without triggering the camera. Because of the precision of aim, flower growth or movement of a bloom often disrupted the camera trap configuration and caused false triggers. To mitigate for such occurrences, camera traps were visited weekly during the peak of *D. lindenii* blooming (typically during Jul). In cases where a single *D. lindenii* had multiple concurrent blooms, 2 Sabre triggers were used, each aimed above a different flower. There was a period of about 3 wk when 1 individual *D. lindenii* had 3 concurrent blooms, in which case only 2 blooms were sampled.

INSECT MEASUREMENTS AND IDENTIFICATION

The clarity of the color images that were obtained from the digital single lens reflex camera traps enabled identification of insects to species level. Means of proboscis lengths of 7 different hawk moth species (*Agrius cingulata* Fabricius [Lepidoptera: Sphingidae], *C. antaeus*, *D. hyloeus*, *E. fasciatus*, *Manduca rustica* Fabricius [Lepidoptera: Sphingidae], *P. ficus*, *P. strigilis*) were measured from specimens housed in the McGuire Center for Lepidoptera & Biodiversity (Florida Museum of Natural History, University of Florida, Gainesville, Florida, USA). Means were obtained from 3 male and 3 female moths of each species selected at random, and from specimens collected solely in Collier County, Florida, USA during summer mo coinciding with *D. lindenii* flowering times. To facilitate proboscis measurements, the specimens were removed from dry storage, and the coiled proboscis of each was removed and soaked in a relaxing solution of 10% KOH for 24 h. After this time, the proboscis was manually uncoiled and measured.

Results and Discussion

During 2017 and 2018, a total of 21 different visits by Lepidoptera were recorded (Table 1), and the duration of each visit was rarely longer than 1 s. Hawk moth visits were infrequent, but did show some evidence of clustering by species (Table 1). *Cocytius antaeus* was photographed only between 29 Jul and 7 Aug 2018, while the most common documented hawk moth (*P. ficus*) was photographed on 7 different occasions between 3 Jul and 26 Jul 2018 (Table 1). We were able to conclusively identify 5 different species of hawk moths (Sphingidae) visiting *D. lindenii* flowers and probing for nectar: *P. ficus* (fig sphinx; Figs. 1–3), *D. hyloeus rustica* (pawpaw sphinx; Fig. 4), *E. fasciatus* (banded sphinx; Fig. 5), *P. strigilis* (streaked sphinx; Fig. 6), and *C. antaeus* (giant sphinx; Fig. 7). Of these 5 species, *P. ficus* and *D. hyloeus* were observed with

Table 1. Confirmed Lepidoptera visits to *D. lindenii* flowers during the study.

Date	Time (EST)	Species	Notes
20 Jul 2017	12:04 AM	*Ametris nitocris*	Fig. 9
22 Jun 2018	10:13 AM	*Asbolis capucinus*	Fig. 14
2 Jul 2018	5:50 PM	*Calpodes ethlius*	Fig. 15
29 Jul 2018	8:37 PM	*Cocytius antaeus*	Fig. 7
23 Aug 2018	8:19 PM	*Dolba hyloeus*	Fig. 4, pollinia present
4 Jul 2018	2:49 AM	*Eumorpha fasciatus*	Fig. 5, top image
2 Sep 2018	9:33 PM	*Eumorpha fasciatus*	Fig. 5 bottom image
3 Jul 2018	8:55 PM	*Pachylia ficus*	Fig. 1, top two images
5 Jul 2018	5:43 AM	*Pachylia ficus*	Fig. 1, bottom image, pollinia present
8 Jul 2018	8:40 PM	*Pachylia ficus*	Fig. 2
9 Jul 2018	9:00 PM	*Pachylia ficus*	
12 Jul 2018	9:04 PM	*Pachylia ficus*	
23 Jul 2018	9:17 PM	*Pachylia ficus*	
26 Jul 2018	8:29 PM	*Pachylia ficus*	Fig. 3, pollinia present on proboscis
9 Aug 2017	10:59 AM	*Papilio palamedes*	Fig. 10
17 Aug 2018	10:07 PM	*Protambulyx strigilis*	Fig. 6
5 Oct 2018	6:43 AM	*Protambulyx strigilis*	
7 Aug 2018	12:15 AM	Likely *Agrius cingulata*	Fig. 8, note long proboscis extended towards flower
6 Aug 2018	2:02 PM	Unidentified Geometrid	Fig. 11
15 Aug 2018	6:19 AM	Unidentified Geometrid	Fig. 12
15 Aug 2018	9:25 PM	Unidentified Geometrid	Fig. 13

Fig. 1. *Pachylia ficus*, shown nectaring on *Dendrophylax lindenii* in top 2 images (3 Jul 2018, 8:55 PM Eastern Standard Time), and carrying pollinia in lower image (5 Jul 2018, 5:43 AM Eastern Standard Time). Pollinia are yellow in color, and can be observed on the moth's forehead, at base of proboscis.

pollinia affixed to the head region at the base of the proboscis (Figs. 1 & 4, respectively). The location of the pollinia on these moths coincides with the orchid's floral column, thereby signifying that these 2 moth species are likely pollinators of *D. lindenii*. In a second image (Fig. 3), pollinia appear also to be affixed to the proboscis itself in *P. ficus*. Camera traps captured a single image of a hawk moth flying towards a *D. lindenii* flower with its proboscis nearly touching a *D. lindenii* flower on 7 Aug 2018 at 12:15 AM Eastern Standard Time (Fig. 8). Because the photograph lacks sufficient evidence to conclusively identify this moth as *A. cingulata*, there are some visible characteristics which would indicate that this moth is most likely *A. cingulata* (e.g., alternating pink and black banding on abdomen). Comparisons between this photographed moth and preserved *A. cingulata* specimens at the Florida Museum of Natural History further support the likelihood that this photographed moth is *A. cingulata*. In addition to these hawk moths, other types of Lepidoptera made physical contact with the labellum of the flower including 4 geometrid moths (Figs. 9, 11–13), 1 palamedes swallowtail butterfly (*P. palamedes*; Fig. 10), 1 monk skipper (*A. capucinus*; Fig. 14), and 1 Brazilian skipper (*C. ethlius*; Fig. 15). Only 1 of the geometrid moths could be positively identified (*A. nitocris*; Fig. 9). Table 1 lists the

Fig. 2. Stages of proboscis insertion by a female *Pachylia ficus* (8 Jul 2018, 8:40 PM Eastern Standard Time). Note the close proximity of the insect's head to the column following complete insertion of the proboscis into the nectar spur (lower image).

dates and times of all Lepidoptera that were photographed visiting *D. lindenii* flowers during the study, with references to figures, if any. Although physical capture of these moths would have been preferable to photographic evidence alone, the high quality images rendered by the digital single lens reflex camera traps allowed for positive identification of most of the larger lepidopterans to the species level. For field-based pollination studies, the camera-trapping technique described herein should provide researchers with a useful tool for studying insect pollination, especially in remote areas. For orchids, which are notorious for having long-lasting flowers and few pollinator visits, this technique also may be especially useful.

This is the first documentation of apparent ghost orchid pollination in a natural setting, and supports the hypothesis that hawk moths serve this important ecological role in southern Florida. This assumption is based on photographic evidence of yellow rounded masses attached to the bases of *P. ficus* proboscises (Figs. 1 & 3), and the proboscis of *D. hyloeus* (Fig. 4). These yellow masses are similar in size, shape, and color of *D. lindenii* pollinia (Sheehan & Sheehan 1979; Fig. 16), and other orchids in the Subtribe Angraecinae (Stewart et al. 2006). Given that other orchid species inhabit the Florida Panther National Wildlife Refuge, it is conceivable that these moths carried pollinia from species other than *D. lindenii*, the leading candidate being *Epidendrum noc-*

Fig. 3. *Pachylia ficus* shown inserting its proboscis into the nectar spur opening (top). In the bottom image, pollinia are shown affixed to the proboscis itself (26 Jul 2018, 8:29 PM Eastern Standard Time).

Fig. 4. *Dolba hyloeus* photographed with *Dendrophylax lindenii* pollinia at base of proboscis (23 Aug 2018, 8:19 PM Eastern Standard Time).

turnum Jacquin (Orchidaceae). However, we are confident that these pollinia originated from *D. lindenii* because no other native epiphytic orchid species in the region could accommodate the proboscis lengths of the documented hawk moths, including *E. nocturnum*. Moreover, the pollinia of *Epidendrum* orchids typically are more elongated, and have a pronounced stalk leading down to the viscidia (Sheehan & Sheehan 1979). We also did not observe any other orchids in flower in the immediate area at the time of the study. Whether or not the flowers depicted in Figures 1, 3, and 4 were actually fertilized is not known, but several ghost orchids at the site did develop seed capsules both during and after the study. Of particular note is the ghost orchid seed capsule that can be observed in Figures 3, 5, 6, 7, and 11 to 13. This individual ghost orchid is about 22 m away from the ghost orchid depicted in Figures 1, 2, 4, 8, 14, and 15. Additionally, another known ghost orchid that is located approximately 3 m away from the ghost orchid depicted in Figures 3, 6, 7, and 11 to 13 also formed a seed capsule after the study concluded. As such, pollination did take place within the study area, and occurred in close proximity to the ghost orchids where moths carrying pollinia were photographed.

Contrary to numerous references (e.g., Hammer 2002) and public opinion, our study revealed that the giant sphinx moth (*C. antaeus*) should not be regarded as the sole pollinator of *D. lindenii*. Although no pollinia were observed affixed to *C. antaeus* within the study area, pollination by this species should not be ruled out, nor should it be assumed that *P. ficus* and *D. hyloeus* serve as the primary pollinators of *D. lindenii*. However, our evidence, along with additional evidence obtained by fellow researchers at Audubon's Corkscrew Swamp Sanctuary (M. Stone & P. Houlihan, personal communication), indicate that several hawk moths could be, or are, potential pollinators of *D. lindenii*, as opposed to a single pollinator, in this case *C. antaeus*. Pailler (2019) reported *Papilio* pollination of an epiphytic angraecoid orchid (*Neobathiea hirtula* H. Perrier; Orchidaceae) in Madagascar and the Comoros with a floral display very similar to *D. lindenii*. Thus, *Papilio* pollination of the ghost orchid should not be ruled out. Taken together, *D. lindenii* does not appear to be a "specialist orchid" in the truest sense, i.e., relying on 1 specific species for its cross pollination needs.

Of the 5 confirmed hawk moth species that were photographed during the course of this study, the most frequent was *P. ficus*, which also was a verified pollinator based on pollinia photographed (Table 1; Figs. 1–3). This was the first hawk moth to be photographed during the study (3 Jul 2018), and it was most active during Jul between 8:00 to 9:00 PM, and 5:00 to 6:00 AM Eastern Standard Time. The average proboscis length of *P. ficus* acquired from museum specimens was 53.5 mm, which is intermediate in length compared to the other 5 hawk moths described in Table 2 (i.e., 28.7–133.8 mm). Interestingly, *D. hyloeus* had the second smallest mean proboscis length of the 5 species that were measured (38.2 mm), yet was a species that was photographed with *D. lindenii* pollinia (Fig. 4). The mean *D. lindenii* nectar spur length measured on artificially propagated plants (n = 102) was 136.5 mm (H. Ray, personal communication). These artificially propagated plants were grown from *D. lindenii* seeds that were collected on the Florida Panther National Wildlife Refuge (Hoang et al. 2016). Nectar spur lengths measured on 5 natural Florida Panther National Wildlife Refuge *D. lindenii* flowers ranged from 90 to 120 mm, and averaged 100 mm (M. LaRusso & J. Lu, personal communication). Estimated lengths of the aforementioned 5 nectar spurs that actually contained nectar ranged from 31 to 62 mm, with an average of 43 mm (M. LaRusso & J. Lu, personal communication). Thus, the proboscis of *P. ficus* is of sufficient length to reach nectar within the nectar spur of a *D. lindenii* flower, especially at the onset of flowering when nectar spurs would presumably contain the highest volume of liquid. It is not known whether *D. lindenii* flowers are capable of replenishing nectar between feeding episodes, but this aspect warrants further study. If nectar spurs are not refilled frequently or sufficiently, it is conceivable that hawk moths with a longer proboscis would be needed to actually acquire nectar if it occurred only towards the bottom of the nectar spur. Under this scenario, proboscis lengths of *A. cingulata* (91–127 mm) and *C. antaeus* (104–147 mm) would fill this role. Given the extreme proboscis length of *C. antaeus*, this moth essentially may "clean up" after previous visits by other species, the tongues of which may not be long enough to drain the whole nectar load. It should be noted that hawk moths could still be attracted to a *D. lindenii* flower, and try to collect nectar even if the moth was unable to actually do so. As such, a moth with a shorter proboscis conceivably still could collect *D. lindenii* pollinia in this scenario. Once pollinia are successfully deposited onto the stigmatic surface (= pollination), floral function would be expected to "shut down" with resources being shifted into fertilization and fruit set. Indeed, ghost orchid flowers that are hand-pollinated rapidly change in appearance from white to translucent within 24 h of pollination (M. Kane, personal communication), which may reduce the visible signature of the flower as perceived by the next visitor. As such, insects would then shift their attention to flowers that remain unpollinated in the immediate vicinity.

Why the non-Sphingidae moth species appeared to be attracted to the floral display of *D. lindenii* is not known, but these insects initially may have been allured by the sweet-smelling floral scent. During 4 yr of detailed monitoring of *D. lindenii* in the Florida Panther National Wildlife Refuge, we have noted that the shape of the labellum is conducive to moisture collection from dew and rain. Thus, these non-Sphingidae moth visitors may have been attracted to pockets of water on the labellum (Fig. 10). Van der Cingel (2007) noted that chemical compounds that constitute floral scent often are located in the labellum, and that the blend of these chemicals may change as the flower matures. If true for *D. lindenii*, it is plausible that these compounds may mix with water droplets on the surface of the labellum, which might then serve as an "appetizer" of sorts for moths seeking a more substantial nectar meal.

IMPLICATIONS FOR CONSERVATION IN FLORIDA AND CUBA

Although preliminary, this study provides fundamental baseline information on the natural pollination of *D. lindenii* that now may be used to augment conservation efforts in southern Florida. For orchid conservation to be successful, Swarts and Dixon (2009) proposed 3 actions that must be met: (1) management of natural reserves with

Fig. 5. Top image: *Eumorpha fasciatus* in the act of inserting its proboscis into the nectar spur, opening just beneath the column of the flower (4 Jul 2018, 2:49 AM Eastern Standard Time). Bottom image: *Eumorpha fasciatus* flying towards *Dendrophylax lindenii* flower (2 Sep 2018, 9:33 PM Eastern Standard Time).

Fig. 6. Photo sequence of *Protambulyx strigilis* nectaring on *Dendrophylax lindenii* flower, with proboscis fully inserted into the nectar spur in the bottom image (17 Aug 2018, 10:07 PM Eastern Standard Time).

emphasis on the specialized needs of orchids (e.g., pollinators, mycorrhizal fungi); (2) establishment of ex situ seed and mycorrhiza banks for orchids under immediate threat; and (3) development of orchid restoration techniques. For *D. lindenii*, all 3 of these actions have been achieved largely due to coordinated research efforts in southern Florida and Cuba during the past decade (see Hoang et al. 2016; Mujica et al. 2018; Zettler et al. 2019; Coopman & Kane 2019). Collectively, all of these efforts have aligned in favor of *D. lindenii* with 1 important exception – virtually no information existed on the pollination biology of the ghost orchid until now. In addition to the aforementioned actions, significant hydrological restoration is needed in southern Florida to restore and enhance the hydrology of unique wetlands such as the Fakahatchee Strand. Prior to highway and canal construction, water would have flowed virtually unimpeded into the Fakahatchee Strand via the Okaloacoochee Slough and East Hinson Marsh (Sonenshein 2008; Reese 2010). Once this water entered the Fakahatchee Strand, it would have flowed southward for about 35 km before ultimately discharging into the marshes of Ten Thousand Islands. Unfortunately, the majority of the water from Okaloacoochee Slough and East Hinson Marsh never makes its way into the Fakahatchee Strand today. Instead, it is

Fig. 7. *Cocytius antaeus* flying towards *Dendrophylax lindenii* flower (29 Jul 2018, 8:37 PM Eastern Standard Time).

Fig. 10. Palamedes swallowtail butterfly (*Papilio palamedes*) visiting *Dendrophylax lindenii* flower during daylight hours (9 Aug 2017, 10:59 AM Eastern Standard Time).

Fig. 8. Suspected *Agrius cingulata* flying towards *Dendrophylax lindenii* flower (7 Aug 2018, 12:15 AM Eastern Standard Time).

Fig. 11. Unidentified Geometrid moth resting on the labellum of *Dendrophylax lindenii* flower. The head of the insect is positioned over the concave labellum's upper surface where moisture droplets from dew and rain have been known to accumulate (6 Aug 2018, 2:02 PM Eastern Standard Time).

Fig. 9. Seagrape spanworm moth (*Ametris nitocris*) on *Dendrophylax lindenii* flower (20 Jul 2017, 12:04 AM EST).

Fig. 12. Unidentified Geometrid moth resting on the lower labellum of a *Dendrophylax lindenii* flower (15 Aug 2018, 6:19 PM Eastern Standard Time).

Fig. 13. Unidentified Geometrid moth visiting *Dendrophylax lindenii* flower (15 Aug 2018, 9:25 PM Eastern Standard Time).

Fig. 14. Monk skipper (*Asbolis capucinus*) visiting *Dendrophylax lindenii* flower (22 Jun 2018, 10:13 AM Eastern Standard Time).

Fig. 15. Brazilian skipper (*Calpodes ethlius*) visiting *Dendrophylax lindenii* flower (2 Jul 2018, 5:50 PM Eastern Standard Time).

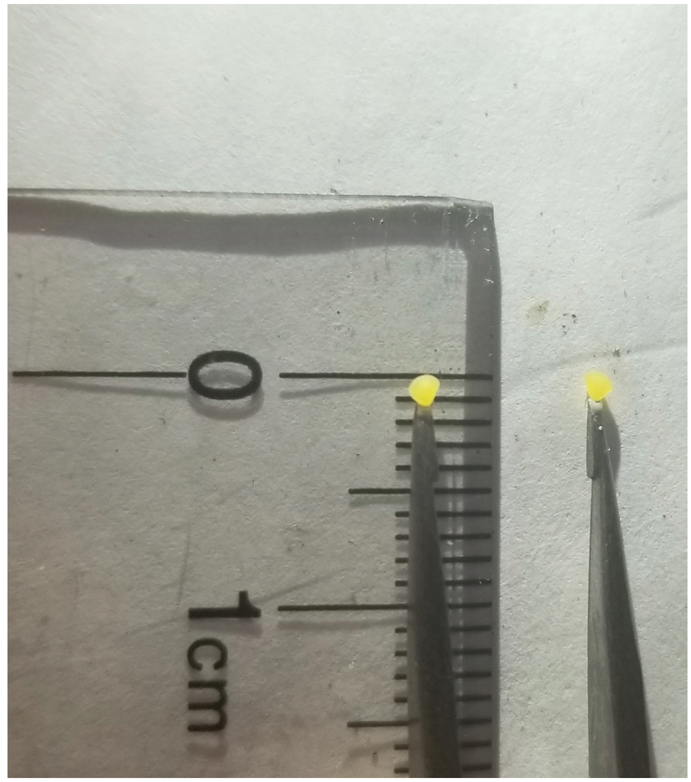

Fig. 16. Photo of *Dendrophylax lindenii* pollinia.

diverted by State Road 29 and the adjacent Barron River Canal, and is discharged into the Ten Thousand Islands at Everglades City. During the past 4 yr of intensive ghost orchid research on the Florida Panther National Wildlife Refuge, we have discovered that *D. lindenii* is found typically only within the deepest flooded portions of wetlands, which have longer hydroperiods than the surrounding areas. These deep portions are believed to provide critically needed moisture and thermal protection for *D. lindenii* and other epiphytic orchids. Hydrologic improvements to increase sheetflow into the Fakahatchee Strand would likely provide improved habitat for *D. lindenii*, its pollinators, and numerous other wetland dependent species of flora and fauna.

Clearly, before any generalizations are made about natural pollination of *D. lindenii*, more data are needed in future yr, and at different sites throughout southern Florida, to gain a more complete picture. Nevertheless, the results of our study suggest that immediate steps might be taken to safeguard these pollinators and pollinator candidates, especially the habitats and associated plants that serve as larval food sources. For the 2 documented pollinators described herein, larval food sources generally remain common in the region, e.g., *P. ficus* on *Ficus* spp., and *D. hyloeus* on *Ilex* spp. and *Asimina* spp. (Covell 1984). Assuming *C. antaeus* also pollinates *D. lindenii*, its larval food source (*Annona glabra* Forssk.; Annonaceae) also currently is common and widespread in southern Florida. As optimistic as this scenario may be, the highest densities of *D. lindenii* are confined to discrete forested wetlands within the Big Cypress Basin ecoregion, which borders agricultural land subject to frequent pesticide application and hydrologic alteration. Maintaining high water levels in the region is crucial for cold-sensitive epiphytic orchids such as *D. lindenii*, because high humidity levels beneath the forest "dome" serve as a buffer from occasional sub-freezing temperatures (Luer 1972). Unfortunately, State Road 29 and the Barron River Canal essentially have blocked a significant amount of water that would have historically flowed into orchid-rich Fakahatchee Strand. In nearby urban areas (e.g., Naples), aerial

Table 2. Proboscis lengths of 6 species of Sphingidae from peninsular Florida.

Species	Proboscis lengths (mm)						Average proboscis length (mm)
	Male 1	Male 2	Male 3	Female 1	Female 2	Female 3	
Agrius cingulata	91	111	116	109	111	127	110.8
Cocytius antaeus	104	133	135	139	145	147	133.8
Dolba hyloeus	31	37	37	40	42	42	38.2
Eumorpha fasciatus	34	38	41	44	45	46	41.3
Pachylia ficus	50	51	56	53	58	53	53.5
Protambulyx strigilis	26	28	28	26	32	32	28.7

spraying of pesticides for mosquito control also could pose a potential threat to pollinators of *D. lindenii*. Future research is needed to better understand the impacts of pesticide applications on hawk moths. As urbanization and agricultural practices are expected to intensify in the region, we urge land managers to remain diligent about maintaining suitable habitat for these moth pollinators.

From an ecological standpoint, knowing more about the pollinators of *D. lindenii* in western Cuba also warrants study given that the habitats in both countries differ (Mujica et al. 2018). Such information also would be useful in determining whether Cuban ghost orchids should be regarded as a separate subspecies in the absence of genetic comparisons. In the Guanahacabibes Peninsula, where the majority of Cuban ghost orchids are clustered, flowering occurs as early as Jun (L. Zettler, personal observation) and extends into Dec. Flowers that open earlier in the growing season have shorter nectar spurs (about 10 cm) than those that bloom later (about 15 cm; A. Camejo, personal communication), suggesting that moths with a longer proboscis (e.g., *C. antaeus*) serve as pollinators later in the yr. In Guanahacabibes National Park during a 14 d period in Nov 2018, hawk moths in 2 genera (i.e., *Manduca* and *Eumorpha*) were captured in the vicinity of 10 *D. lindenii* flowering individuals spaced 3 to 4 m apart, but neither carried pollinia (A. Camejo, personal communication). In addition, *P. ficus*, *A. nitocris*, *Eumorpha strenua* Ménétriés, and *Cocytius duponchel* Poey (all Lepidoptera: Sphingidae) were reported at the site, verified by a specialist in Havana (Alejandro Barro). Several yr prior, A. Barro observed *C. antaeus* in Guanahacabibes (A. Camejo, personal communication). The presence of *C. antaeus* in western Cuba is not surprising given that this species occurs throughout Tropical America (Covell 1984), and its larval food source (*A. glabra*) is present about 17 km from the *D. lindenii* site (A. Camejo, personal communication). Thus, the Lepidoptera fauna associated with *D. lindenii* appears to be similar to the fauna in southern Florida despite habitat differences. Given that hawk moths are known to cross-pollinate orchids separated by 2,000 to 4,000 m (Lind 1994), pollination by *C. antaeus* in Guanahacabibes should not be ruled out. Considering that few ghost orchid flowers set fruit in either country (< 12% in 2015; Mujica et al. 2018), infrequent flower visits and pollination may be typical for *D. lindenii* throughout its range, and this may be explained, in part, by limited hawk moth population numbers.

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