



Ecosistemas y recursos agropecuarios

ISSN: 2007-9028

ISSN: 2007-901X

Universidad Juárez Autónoma de Tabasco, Dirección de Investigación y Posgrado

Alanís-Méndez, José Luis; Ortiz-Santos, Luz del Carmen; Chamorro-Florescano, Ivette Alicia; Pech-Canché, Juan Manuel; Limón, Francisco  
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Ecosistemas y recursos agropecuarios, vol. 6, no. 17, 2019, May-August, pp. 361-368  
Universidad Juárez Autónoma de Tabasco, Dirección de Investigación y Posgrado

DOI: <https://doi.org/10.19136/era.a6n17.1922>

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## Pollinators and floral visitors of two orchids in a protected natural area in Tuxpan, Veracruz

### Polinizadores y visitantes florales de dos orquídeas en un área natural protegida en Tuxpan, Veracruz

José Luis Alanís-Méndez<sup>1</sup> , Luz del Carmen Ortiz-Santos<sup>1</sup> , Ivette Alicia Chamorro-Florescano<sup>1</sup> , Juan Manuel Pech Canché<sup>1</sup> , Francisco Limón<sup>1\*</sup> 

<sup>1</sup>Facultad de Ciencias Biológicas y Agropecuarias, Universidad Veracruzana. Km. 7.5 Carretera Tuxpan-Tampico, CP. 92895. Tuxpan, Veracruz, México.

\*Corresponding author: flimon@uv.mx

**Scientific note** received: July 18, 2018 accepted: February 11, 2019

**ABSTRACT.** Floral visitors, particularly pollinators, are important during the life cycle of many orchids. However, despite their biological importance, this information is still unknown in many species, particularly those in mangrove forests. The aim of this research was to study the floral visitors, as well as the pollinators, of the two orchid species *Myrmecophila grandiflora* and *Brassavola nodosa*, in a protected natural area with mangrove (RAMSAR), in Tuxpan, Veracruz, Mexico. Ants of different genera were the floral visitors of *M. grandiflora*, its pollinator is the carpenter bee, *Xylocopa nautlana*. In *B. nodosa*, were observed mosquitoes and ladybugs as floral visitors, the pollinator could not be registered, but it is possible that it is a species of moth (*Scopula* sp.). Finally, this study provides field information about floral visitors and pollinators in two orchid species in mangrove ecosystems and the evidence for pollination.

**Key words:** Epiphytes, Orchidaceae, RAMSAR, Schomburgkia, *Xylocopa*.

**RESUMEN.** Los visitantes florales, particularmente los polinizadores, son importantes durante el ciclo de vida de muchas orquídeas. Sin embargo, a pesar de su importancia biológica, esta información permanece desconocida para muchas especies, particularmente en ecosistemas de manglar. El objetivo de esta investigación, fue conocer los visitantes florales, así como polinizadores de dos especies de orquídeas *Myrmecophila grandiflora* y *Brassavola nodosa* en un área natural protegida con manglar (RAMSAR), en Tuxpan, Veracruz, México. Hormigas de diferentes géneros fueron los principales visitantes de *M. grandiflora*, su polinizador es la abeja carpintero *Xylocopa nautlana*. En *B. nodosa*, se observaron mosquitos y catarinas como visitantes florales. Este estudio provee información de campo acerca de los visitantes florales en dos especies de orquídeas en ecosistemas de manglar y la evidencia para la polinización.

**Palabras clave:** Epifitas, Orchidaceae, RAMSAR, Schomburgkia, *Xylocopa*.

## INTRODUCTION

Plants interact with numerous species from taxonomically diverse groups (Herrera 1987, Horvitz and Schemske 1990). Pollination is one of the main reasons, and it is a fundamental requirement for the reproductive success of 80 to 90% of the flowering plants and up to 97% of the orchids (Schiestl 2005, Pellegrino *et al.* 2010). In Mexico, orchids (Orchidaceae) occupy the third place in taxonomic diversity of vascular plants and it is estimated that around

40% of them are endemic (Hágsater *et al.* 1996, 2005). Their highest diversity is concentrated in three types of vegetation: tropical evergreen forest, temperate oak forests and tropical montane cloud forest (García-Cruz and Sosa 2011). On the other hand, ecosystems such as mangroves, have received less attention; these are important because of their high diversity of biological interactions and ecological importance (Basáñez 2005, Lee *et al.* 2014). Therefore, it is essential to integrate the knowledge of orchids into these ecosystems, as well as the species they

interact with, pollinators and floral visitors.

In a protected natural area of northern Veracruz (RAMSAR 2016), we studied two orchid species associated with mangrove forests: *Brassavola nodosa* and *Myrmecophila grandiflora* (Ames and Correll 1985, Carnevali *et al.* 2001, Carnevali 2009). In both cases, their pollinators are unknown. Accordingly, the objectives of the present study were (1) to document associated phorophyte species with the orchids studied in a natural protected area; (2) to identify the pollinators and the floral visitors of the orchids in the mentioned natural protected area, and (3) to determine the type of pollination.

## MATERIALS AND METHODS

### Study area

The RAMSAR 1602 is a protected natural area located in the municipality of Tuxpan, in the north of the state of Veracruz, Mexico (Figure 1). Its average altitude is 14 m, the climate is warm-subhumid with rains in winter, and the average annual temperature is 24.1 °C (INEGI 2010). It is listed among the wetlands of global conservation priority (RAMSAR 2016), and is one of the better conserved within the Biogeographical Province of the Gulf of Mexico (Basáñez 2005). This site contains the four species of mangrove that are distributed in Mexico, all of which are found under the category of special protection: *Avicennia germinans* (L.) L. (black mangrove), *Conocarpus erectus* L. (buttonwood mangrove), *Laguncularia racemosa* (L.) C. F. Gaertn (white mangrove), and *Rhizophora mangle* L. (red mangrove) (Basáñez 2005).

### Study species

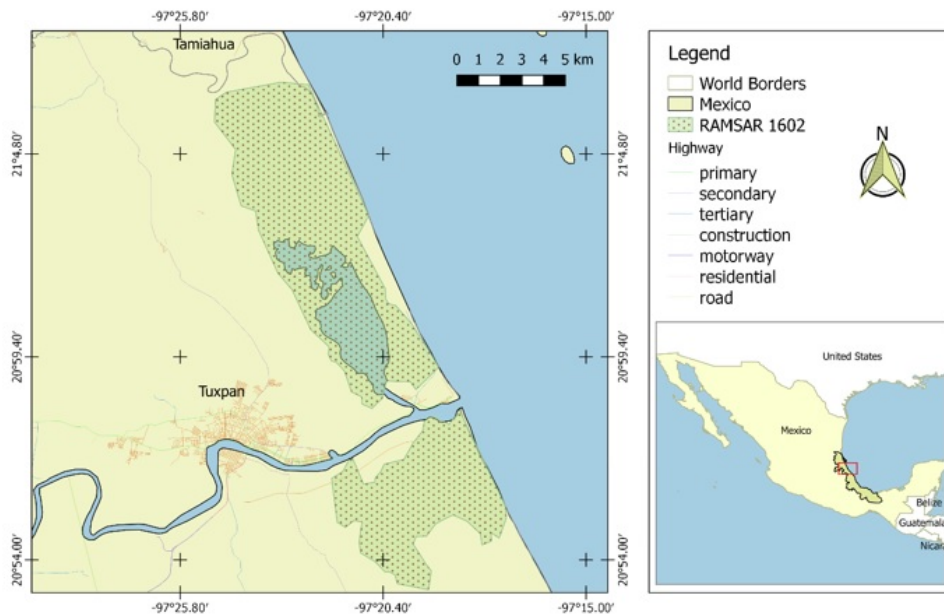
*M. grandiflora* (Lindl.) Carnevali, J.L. Tapia & I. Ramírez. it has purple-lilac or lilac-orange flowers, with quite wide and flat petals, the labellum is very open and the inflorescences are long and simple (Carnevali 2009). The production of its scent increases throughout the morning (Parra-Tabla *et al.* 2009). It is known as the ant orchid, since it is associated with ants that inhabit the interior of the pseudobulb hollows. It is endemic to Mexico, occurring in

the states of Veracruz, Chiapas and Oaxaca, and is generally epiphytic (Carnevali *et al.* 2003). Given the aforementioned characteristics (daytime production of scent, color and shape of flowers), the expected pollination syndrome corresponds to bees (Rico-Gray and Thien 1987, Faegri and Van Der Pijl 2013). *B. nodosa* (L.) Lindl. is an epiphytic species, known as the “lady of the night” because it releases a characteristic nocturnal fragrance (Jones 1975); its flowers are white, 9-15 cm in diameter (Mahr, 2004). Geographically, it is distributed throughout southeastern Mexico, Central America and the northern part of Colombia. It can be found growing on tree trunks and branches in lowland tropical forests and mangrove swamps, or on bare rocks and cliffs by the seashore (Schemske 1980, Murren and Ellison 1998, Noguera-Savelli 2010). Given the aforementioned characteristics (fragrance, nocturnal activity and white flowers), the expected pollination syndrome would correspond to moths (Schemske 1980, Faegri and Van Der Pijl 2013).

### Floral visitors and pollinators

To identify pollinators and floral visitors, we conducted trips to the RAMSAR site number 1602 during the period of March to July 2012, which corresponds to the flowering period of the studied species, attempting to cover its entire local range (Figure 1). Times for visitor observations and recordings were chosen considering the reproductive biology of *M. grandiflora* and *B. nodosa*, diurnal and nocturnal, respectively (Roebuck and Steinhart 1978, Parra-Tabla and Vargas 2004). Thus, in *M. grandiflora*, sampling was from 07:00 to 12:00 hours and in *B. nodosa*, from 21:00 to 01:00 hours. We conducted focal observations for 10 min in one individual flower (one meter away) and then moved to another flower to repeat the sampling procedure. This strategy was used in order to maximize sampling throughout the entire area. Binoculars (Mizar MR- de 10 x 50 mm) were used and images captured with a photographic camera (Canon EOS).

During observations, all visitors that arrived at the flowers of both orchid species were recorded. We considered a visitor any individual that landed



**Figure 1.** Study site, protected area RAMSAR 1602, Tuxpan, Veracruz, Mexico.

on the flower, and a pollinator when pollen was visibly attached and moved towards other flowers. Following field observations, all visitors were captured in plastic jars for examination in the laboratory under a stereoscopic microscope (Nikon, C-PS). The collected insects were deposited in the entomological collection of the Faculty of Biology and Agronomy, Tuxpan, Veracruz. Determination of species was carried out using identification guides (Delvare *et al.* 2002, Eaton and Kaufman 2007) and consulting experts.

## RESULTS AND DISCUSSION

### Host trees (phorophytes)

We recorded 54 flowers of the orchid *M. grandiflora* (30 hours of observation) and 14 flowers of *B. nodosa* (24 hours of observation), all of them growing on the stem of adult trees of *C. erectus*, *L. racemosa* and *R. mangle*. Both orchid species have been reported inhabiting coastal ecosystems, mainly mangroves (Schemske 1980, López Portillo and Ezcurra 2002). According to Murren and Ellison (1998), in coastal Belize, red, black, and white mangroves are the most

common host trees of *B. nodosa*, however, despite being present, they did not observe the establishment of *B. nodosa* in *C. erectus*. On the Yucatan peninsula, interactions with two species have been reported for *M. tibicinis*: the red mangrove (*R. mangle*) and the black mangrove (*A. germinans*) (Rico-Gray and Thien 1987, Rico-Gray 1989). It is remarkable that despite the presence of four mangrove species, we did not register any orchids in *A. germinans*. However, Dejean *et al.* (2003) reported the growth of *M. christinae* (closely related and similar in many ecological attributes to *M. grandiflora*) in *A. germinans*. It would be worth exploring such differences in detail, bearing in mind that it is also possible that these hosts exhibit generalist interactions with *Myrmecophila* orchids, as noted in recent reviews (Wagner *et al.* 2015).

### Floral visitors

The floral visitors of *M. grandiflora* were three species of ants, belonging to the genera *Crematogaster*, *Camponotus* and *Pseudomyrmex*, which were observed numerous times crossing the flowers (Table 1). The floral visitors of *B. nodosa* were mosquitoes belonging to the family Culicidae (Diptera), as well as

ladybugs (Coleoptera: Coccinellidae, Table 1). As the genus name suggests (*Myrmecophila* literally means “ant loving”), the floral visitors of *M. grandiflora* were mainly ants (Table 1). The same pattern has been observed in *M. tibicinis* and *M. christinae*, where there is an association ant-orchid in which extrafloral nectar is produced by the inflorescence and is collected by the ants. In return, they occupy the hollow pseudobulbs and defend the inflorescences and the phorophytes from herbivory (Rico-Gray 1989, Malo et al. 2001, De-jean et al. 2003).

The species *M. tibicinis* has been observed interacting with 8 to 13 species of ants on the Yucatan peninsula (Rico-Gray and Thien 1987, Thien et al. 1987). For *M. grandiflora*, in contrast, we recorded 109 individual visitors of three genera of ants; it is possible that there are several species per genera, but it was not possible to identify ants at species level. In our study, unlike *M. grandiflora*, visitors to *B. nodosa*, were scarce. This feature seems common within this genus, as suggested by the scarce data on pollinators reported by previous studies. Rech et al. (2010) only observed the bee *Plebeia remota* visiting *B. cebolleta*. In Brazil, hummingbirds (Trochilidae) were observed visiting flowers of *B. martiana* and *B. tuberculata* (Braga 1977, Van Der Cingel 2001). It is possible that by presenting a phalaenophily syndrome, the attraction of pollinators and floral visitors, is very restricted and specific to certain times of night. A possible solution to the lack of information, could contemplate observations for a longer time. Since our study only evaluated four hours at night, it is possible that interactions with other species are performed at other times that were not contemplated here. In this sense, Damon and Salas-Roblero (2007) show in Chiapas, Mexico, that several species of Lepidoptera were observed visiting flowers of *B. nodosa* during the day.

### Pollinators

In our study, the pollination of *M. grandiflora* was entomophilous, carried out by the female carpenter bee *Xylocopa nautlana* Cockerell (Figure 2A). Pollination was achieved using the thorax, on which up to eight pollinia were carried (Figure 2B, Table 1).

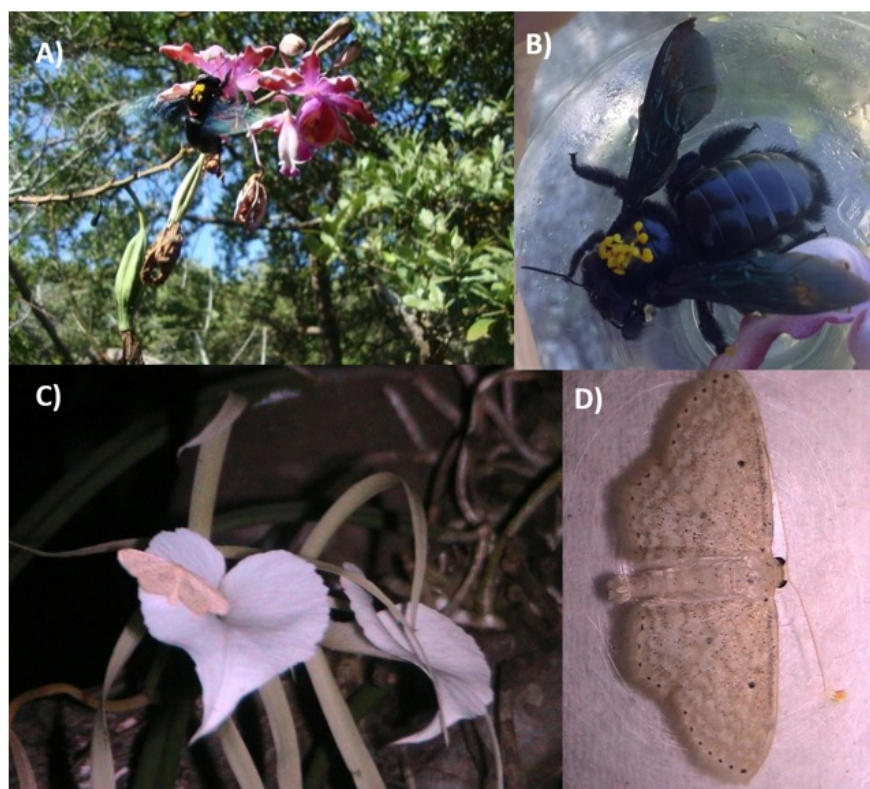
This evidence is important, not only because the pollinator of *M. grandiflora* was hitherto unknown, but also because it reinforces the information regarding pollinators of this genus, i.e., that they are pollinated by bees, which are considered to pollinate around 60% of the orchids (Pijl and Dodson 1966). For example, *M. tibicinis* and *M. christinae*, species closely related (Carnevali et al. 2003) and distributed in similar environments, are pollinated by *Eulaema polychroma* Mocsáry and another species of the genus *Xylocopa* (Rico-Gray and Thien 1987, Parra and Nates-Parra 2007).

The genus *Brassavola* is assumed to be moth-pollinated. This idea is based on some of the characteristics associated with a sphingophilous pollination syndrome (Lepidoptera: Sphingidae), the production of a nocturnal, strongly sweet but not fruity floral scents, size, morphology and the presence of a cream or white flower color (Roebuck and Steinhart 1978, Williams 1981). In our study, no pollinator was observed for *B. nodosa* during field trips; however, we observed a recurring visitor, a white moth (Geometridae) *Scopula* sp. (Figure 2 C, D, Table 1), which is a genus observed previously by other authors. Pijl and Dodson (1966) reported a sphingid moth as the potential pollinator of *B. nodosa*. Schemske (1980) refers in his study to a white moth as a pollinator; and more recently, Damon and Salas-Roblero (2007) observed a sphingid moth at any time of day. Nevertheless, these authors neither recorded any evidence of pollination. Likewise, in *B. cebolleta*, a moth of the family Notodontidae, was recorded as a potential pollinator because pollination could not be observed directly (Rech et al. 2010). Finally, it should be noted that, while the pollinator was not recorded, we did observe the occurrence of fructification, which would indicate the presence of pollinators at the site, pointing the need to explore with greater attention the pollination of this species.

In our study, we identified the pollinator of the orchid *M. grandiflora* in the area of Tuxpan, Veracruz, Mexico, which was the carpenter bee *X. nautlana*. We could not identify the pollinator of *B. nodosa*; however, it is possible that it could be a moth, considering that this insect was frequently observed and fits the polli-

**Table 1.** Floral visitors and pollinators of the two study species in Tuxpan, Veracruz, Mexico. In asterisk the pollinator.

Orchid	Floral Visitor Family: Genus species	Number of visits
	Apidae: <i>Xylocopa nautlana</i> Cockerell*	33
<i>Myrmecophila grandiflora</i> (Lindl.) Carnevali & J.L.Tapia & I.Ramírez	Formicidae: <i>Crematogaster</i>	45
	Formicidae: <i>Camponotus</i>	37
	Formicidae: <i>Pseudomyrmex</i>	27
	Salticidae	4
<i>Brassavola nodosa</i> (L.) Lindl.	Coccinellidae	3
	Culicidae	6
	Geometridae: <i>Scopula</i> *	14



**Figure 2.** A) *Xylocopa nautlana*, with polinia of *Myrmecophila grandiflora*; B) *X. nautlana*; C and D) *Scopula* sp. (Geometridae) visiting *Brassavola nodosa*.

nation syndrome of this orchid. Future studies should identify whether the ecological condition of sites under environmental protection truly favor plant reproduction. Further, they should provide quantitative data regarding the number of floral visitors and their relationship with the adaptation of the orchids that grow in mangrove ecosystems. Finally, future studies should propose strategies to record and identify the pollinator of *B. nodosa*.

## ACKNOWLEDGEMENTS

This study is part of the bachelor dissertation of Ortiz Santos LC and was supported by a Master's Thesis scholarship by CONACyT. Limón F thanks the Master of Environmental Sciences of the Universidad Veracruzana and National Council on Science and Technology (CONACyT) for a postdoctoral scholarship (2018-2019). We thank Dr. Ricardo

Ayala Barajas for the identification of the *Xylocopa* bee. Dr. Jonas Morales-Linares, for his comments on the preliminary version of this text and to the edi-

tor and the two anonymous reviewers who helped improve the quality of the manuscript.

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