FLORAL VISITORS OF THREE SPECIES OF *CORALLORHIZA* IN MONTE TLÁLOC, TEXCOCO, ESTADO DE MÉXICO, MEXICO

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ABSTRACT. *Corallorhiza* is a genus of mycoheterotrophic orchids that includes 12 species, most of them restricted to North and Central America. In Mexico, there are seven taxa, distributed throughout the country, except for seven of the 32 Mexican states; in Estado de México all of them are present. The Orchidaceae is known for its diversity of pollination syndromes; however, for the more than 200 species of mycoheterotrophic orchids, little has been studied about their floral visitors and pollinators, and for the Mexican species of *Corallorhiza* nothing is known to date. Our goal was to document photographically and identify all the floral visitors of three sympatric species of the genus in Monte Tláloc, municipality of Texcoco, Estado de México. We observed individuals of Araneae, Coleoptera, Diptera, Hemiptera, and Hymenoptera visiting flowers of *Corallorhiza macrantha*, *C. macrantha × C. maculata*, *C. maculata*, and *C. striata*. Only *Ocyptamus coerules* and *Platycheirus* sp. (Diptera: Syrphidae) were found carrying pollinia on their thorax. These are the first records of potential pollinators for the genus *Corallorhiza* in Mexico.

RESUMEN. *Corallorhiza* es un género de orquídeas micoheterótrofas que comprende 12 especies, la mayoría de ellas restringidas a Norte América y Centroamérica. En México habitan siete de ellas que se distribuyen en todo el país, excepto en siete de los 32 estados; en el Estado de México están todas presentes. Orchidaceae es conocida por su diversidad en síndromes de polinización; sin embargo, para las más de 200 especies de orquídeas micoheterótrofas poco se ha estudiado sobre sus visitantes florales y polinizadores, y para el caso de las especies de *Corallorhiza* mexicanas nada se conoce. Nuestro objetivo fue documentar fotográficamente e identificar a todos los visitantes florales de tres especies simpatrías del género en el Monte Tláloc, municipio de Texcoco, Estado de México. Observamos individuos de Araneae, Coleoptera, Diptera, Hemiptera e Hymenoptera en las flores de *Corallorhiza macrantha*, *C. macrantha × C. maculata*, *C. maculata* y *C. striata*. Únicamente *Ocyptamus coerules* y *Platycheirus* sp. (Diptera: Syrphidae) fueron encontrados transportando polinias en el tórax. Éstos son los primeros registros de polinizadores potenciales para el género *Corallorhiza* en México.

KEYWORDS / PALABRAS CLAVE: flower visitors, hoverflies, mycoheterotrophic plants, plants micoheterótrofas, orchideas terrestres, sífridos, Syrphidae, terrestrial orchids, visitantes florales.

Introduction. The genus *Corallorhiza* Gagnebin (gr. κοράλλι, coral; ρίζα, root, referring to rhizomes that resemble coral structures) belongs to the subtribe Calypsoinae of the subfamily Epipendraceae (Chase et al. 2015). It is a genus of terrestrial orchids comprising 12 species (Freudenstein & Barret 2014, Pridgeon et al. 2005) restricted to North and Central America, except for *C. trifida* Châtel. (Freudenstein 1997, 1999, Magrath & Freudenstein 2002), which is circumboreal, and the recently described *C. sinensis* G.W.Hu & Q.F.Wang (Yang et al. 2021), an endemic taxon of China. Seven species are present in Mexico (Soto-Arenas et al. 2007), three of them with four infraspecies (Solano Gómez et al. 2020); all of them present in the Estado de México (Martínez de la Cruz et al. 2018, Szeszko 2011); five are Mexican endemics and *C. bul-
bosa A.Rich. & Galeotti is restricted to Megaméxico 2 sensu Rzedowski (1991) (Espejo Serna 2012). *Corallorhiza* lack leaves and are mycoheterotrophic (Shefferson et al. 2010).

It is well known Orchidaceae has a very diverse array of pollination syndromes, the most common involves bees (melonophilia) and flies (myophilia) (Ackerman et al. 2023, Nidup et al. 2023, van der Cingel 2001); it is estimated that 15–30% of the whole family is pollinated by flies (Ackerman et al. 2023, van der Pijl & Dodson 1966). Nevertheless, for most orchid species pollinators are still unknown, especially for the more than 200 species of mycoheterotrophic orchids (Mercek et al. 2013), the only available studies are from Asia (Suetsugu 2013, Sugiura 1996, 2016, Zhou et al. 2012), Europe (Claessens & Kleyneen 2014, 2018), and Oceania (Lehnebach et al. 2005). Pollinators for just three species of *Corallorhiza* have been identified (Claessens & Kleyneen 2018, Freudenstein 1997, Kipping 1971).

Dressler (1981) suggested that Syrphidae flies pollinate *Corallorhiza* species, although the taxonomic identity of these visitors is known only for *C. trifida* (Kipping 1971); additionally, there are reports of self-pollination in some members of the genus. There exist few published data on floral visitors and pollinators for *C. maculata* (Raf.) Raf. var. *mexicana* (Lindl.) Freudenst., *C. striata* Lindl. var. *striata*, *C. odontorhiza* (Willd.) Poir. var. *odontorhiza*, *C. odontorhiza* var. *pringlei* (Greenm.) Freudenst., *C. bentleyi* Freudenst., and *C. trifida* (Argue 2012, Claessens & Kleyneen 2018, Freudenstein 1997).

In Mexico, the genus *Corallorhiza* has been little studied, even on the reproductive biology of its species, so as a first approach to this subject, the purpose of this study was to document photographically and identify at the best possible taxonomic rank all the arthropods that rest or perch on *Corallorhiza* flowers for three species present in Monte Tláloc, Estado de México.

**Material and methods.** The study area is located on the west slopes of Monte Tláloc, municipality of Texcoco, Estado de México, it is part of the Trans-Mexican Volcanic Belt and the Sierra Nevada (Priority Terrestrial Region) (Arriga et al. 2000) (Fig. 1). The three *Corallorhiza* species habitat are coniferous forests of *Abies religiosa* (Kunth) Schltdl. & Cham., known locally as bosque de oyamel (Fig. 2). This vegetation type is present in ravines and lower slopes of mountains, between 3100 and 3500 m of elevation, with steep slopes greater than 40% (Sánchez-González & López-Mata 2003). The climate is humid-temperate, with an annual precipitation of 900 to 1000 mm, and an average annual temperature of 10 to 12°C (Ortiz Solorio & Cuanalo de la Cerda 1977). The type of soil is dark, deep, rich in organic matter, with medium texture (crumbs or loam), and pH values from 5.5 to 7.1 (Sánchez-González & López-Mata 2003).

A bibliographic revision of all literature regarding the genus *Corallorhiza* was undertaken (Freudenstein 1999, Lukasiewicz 1999). In addition, various digital repositories and databases were consulted to find the most complete background information on *Corallorhiza*’s floral visitors. Moreover, to compare and identify the studied species *in situ*, herbarium specimens of the genus were consulted and studied at the herbaria: CHAPA, MEXU, and UAMIZ (herbarium acronyms according to Index Herbariorum, Thiers continuously updated). The vouchers of this work are housed at UAMIZ. In the study area, *Corallorhiza* species are sympatric and in co-flowering, the plants generally are grouped in patches of up to eight individuals per species. To document the flower visitors, random walks were made between patches, when a visitor was detected, the observer approached with camera in hand to record the event. Observations were made from 8:00 am to 5:00 pm. during May 2018, 2019, and 2023. The photographs were taken with three digital cameras (Nikon model D800, Canon models Rebel T3 and SX50 HS), equipped with a macrophotography lens (Tokina atx-i 100mm, f/2.8 AF) and flashes with light diffusers. The species names for the insects were determined using identification guides (Triplehorn & Johnson 2005, Vockeroth & Thompson 1987).

To evaluate whether the species studied offer nectar as a reward, extractions were carried out on twelve flowers (three flowers per species, including the hybrid) using microcapillary tubes of 1 µl; for each sample the sugar concentration (°Brix) was recorded using a field refractometer (Mod. HRT32, range: 0–32° Brix, precision: 0.2%; A. Krüss Optronic, Germany). Extractions were carried out between 10 and 11 am.
Results

Literature and herbaria review.— Two species of *Corallorhiza* were previously reported from the studied area: *C. macrantha* Schltr. and *C. striata* var. involuta (Greenm.) Freudenst. (Sánchez-González et al. 2006), and we observed two more taxa: *C. maculata* var. mexicana (Lindl.) Freudenst. [I. N. Gomez-Escamilla & B. E. Tellez-Baños 222 (UAMIZ 85400)], and the hybrid *C. macrantha × C. maculata* [I. N. Gomez-Escamilla & B. E. Tellez-Baños 220 (UAMIZ 85398, UAMIZ 85397)] (Fig. 3), all growing sympatrically in the ravines *Abies* forest in Monte Tláloc.

All species of *Corallorhiza* grow on a moss substrate with abundant litter; their flowering period begins in late April ending in early June, while the fruiting season from July to December. The populations of *C. macrantha* were registered and collected for the first time in 1976, according with the specimens data [E. García Moya s. n. (CHAPA), Stephen D. Koch 76104 (CHAPA, MEXU)] and 45 years later, populations are still present in the area. The first specimen of *C. striata* var. involuta was collected in 1978 [José García P. 636 (MEXU), 637 (CHAPA, MEXU)].

Floral visitors.— Individuals belonging to five orders, eight families, four genera, and two species of insects were observed (Table 1, Fig. 4, 5, 6). The hybrid *C. macrantha × C. maculata* was visited by seven different insects, *C. maculata* var. mexicana by five, *C. macrantha* by three and *C. striata* var. involuta only by one. The total duration of observations for all species was 54 hours.

Nectar extractions.— For *Corallorhiza macrantha* a nectar volume of 0.8 μl, with a sugar concentration of 15°Brix was recorded while for *C. maculata* and the hybrid a volume of 0.6 and 0.4 μl respectively were obtained, which were insufficient to measure their sugar concentration. For *C. striata* no nectar was obtained.

Potential pollinators.— Two species of syrphids were recorded transporting and depositing pollinia: *Ocyptamus coeruleus* (Williston 1891) on flowers of *Corallorhiza macrantha, C. maculata* and *C. macrantha × C. maculata*, and *Platycheirus* (Lepeletier & Serville 1828) on flowers of *C. macrantha* and *C. macrantha × C. maculata* (Table 1, Fig. 6). In addition, these insects made the highest number of visits (46) to the flowers (Table 1); most of them were recorded between 11:00 and 14:00 hrs.

The insects usually visit more than one flower of the same inflorescence and more than one individual in a floral patch. They fly in front of the flower for 2–8 seconds before landing on the labellum apex which is tilted downwards due to the weight of the insect, once it moves towards the base of the labellum in search of nectar guided by the purplish spots and lines, the labellum returns to its original position, pushing the syrphids against the column. With this mechanism, the thorax of the insect is positioned below the viscidium so that when the syrphid finishes drinking the nectar and move back to leave the flower, it makes contact with the viscidium and the pollinarium adheres, on some occasions with the anther, to the dorsal thorax (scutum) of the syrphid. Subsequently, when the insect visits another receptive flower, the pollinia carried on its thorax touch the stigmatic surface and adhere to it along with the insect’s body, so to free itself, the syrphid must struggle by holding and pushing the labellum with its legs, taking to 20 seconds to do so (Fig. 7).
Figure 2. Corallorhiza species growing sympatrically in the Abies forest in Monte Tláloc A. C. macrantha × C. maculata, B. C. maculata. C. C. striata. Photographs by B. Téllez-Baños.
**Figure 3.** Flowers of **A.** *Corallorhiza macrantha*. **B.** *C. macrantha × C. maculata*. **C.** *C. maculata*. **D.** *C. striata*. Photographs by B. Téllez-Baños. Scale bars are indicated.
**Discussion.** Suggested pollinators for the Calypsoinae subtribe are bumblebees, hover flies, empididae flies, mosquitoes, and bees (Valencia-Nieto *et al.* 2018). However, in this tribe the only genera with mycoheterotrophic members are *Cremastra* Lindl., *Corallorhiza* and *Yoania* Maxim. The information about their floral visitors and pollinators is very scarce (Claessens & Kleynen 2018, Freudenstein 1997, Kipping 1971, Sugiura 1996).

Bumblebees, unlike other insects, require a surface area to allow them to roost before starting to suck nectar from a flower, it has been reported that they pollinate flowers with large petals and lips (Blionis & Vokou 2001, Ortega-Olivencia *et al.* 2012). We found *Bombus huntii* (Greene 1860), as the floral visitor of *Corallorhiza macrantha × C. maculata*, a hybrid that has a large lip enough to support the landing of this insect, however the insect cannot fully access the flower due to its stout body, so by staying away from reproductive structures it is unlikely to remove or deposit a pollinia, limiting its pollinating role.

The syrphids were the only insects that made legitimate visits to the flowers of *Corallorhiza* (in terms of transporting and depositing pollinias), and they also made the highest number of visits. Therefore, we agree with Dressler (1981), that syrphids are the most likely pollinators of these orchids, in addition they may also be responsible for their hybridization. The evidence presented here, strongly suggest that *Corallorhiza* might be a group with a myophylic pollination syn-

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**Table 1. Comparative data of the species of Corallorhiza and their floral visitors in the study area.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Order</th>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
<th>Number of visits</th>
<th>Carried pollinia</th>
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<td></td>
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<tr>
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<td>Bombus</td>
<td>huntii</td>
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<td>No</td>
</tr>
<tr>
<td></td>
<td>Diptera</td>
<td>Syrphidae</td>
<td>Ocyptamus</td>
<td>coerules</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Platycheirus</td>
<td>sp.</td>
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</tr>
<tr>
<td></td>
<td>Tachinidae</td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>No</td>
</tr>
<tr>
<td>Corallorhiza maculata var. mexicana</td>
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<td>Theridiidae</td>
<td>Theridion</td>
<td>sp.</td>
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<td>Cicadellida</td>
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<td>Ocyptamus</td>
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<tr>
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<td>Tachinidae</td>
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<td></td>
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<td>Ocyptamus</td>
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<tr>
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<td></td>
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<td>Platycheirus</td>
<td>sp.</td>
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<tr>
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<td></td>
<td></td>
<td>5</td>
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</tr>
<tr>
<td>Corallorhiza striata var. involuta</td>
<td>Coleoptera</td>
<td>Cantharidae</td>
<td></td>
<td></td>
<td>1</td>
<td>No</td>
</tr>
</tbody>
</table>

Previous works have documented the visit of a syrphid species in *C. trifida* (Claessens & Kleynen 2018), and for *C. maculata* var. *maculata*, the pollinators were identified as members of the genus *Empis* L. (Linnaeus 1758) (Diptera) (Kipping 1971). In other genera of mycoheterotrophic orchids such as *Epipogium aphyllum* Sw. (Jakubska-Busse et al. 2014) and *Cremastra appendiculata* (D. Don) Makino var. *variabilis* (Blume) I.D.Lund (Sugiura 1996) syrphids are also floral visitors.

The Hymenopteran *Pimpla pedalis* Cresson (1865) has been identified as a pollinator (Freudenstein 1997) of *C. striata* var. *striata*, while Freudenstein (1999) suggest the existence of autogamy for *Corallorhiza striata* var. *involuta*, hence we think that it is necessary to make more detailed observations in the populations of the orchid species to detect the presence of its pollinators. We would like to highlight the importance of the use of photography in this type of studies (Suetsugu & Hayamizu 2014, Suetsugu et al. 2017), since it allows the registration and taxonomic identification of the floral visitors, as well as the observation of the pollinia on their bodies.

*Corallorhiza* includes a group of orchids very vulnerable to environmental alterations to its habitat. The species require soils rich in organic matter and places with high humidity; therefore, it is important to preserve sites with the right conditions for the species thrive, including its dependency on its ectomycorrhizal fungi (Lee Taylor & Bruns 1999, Barrett et al. 2020). Currently, *C. macrantha* is cataloged as subject to special protection (Pr) in accordance with the NOM-059-SEMARNAT-2010 (SEMARNAT 2019). It is a species is an orchid that cannot be cultivated, therefore *in situ* conservation is the only viable strategy to preserving it safely (Soto-Arenas & Solano-Gómez 2007).

Future studies should include more pollination observations and experimental manipulation of breeding systems, especially since 71% of mycoheterotrophic orchids are likely autogamous (Ackerman et al. 2023);
Figure 5. A. *Platycheirus* sp. visiting *Corallorhiza macrantha × C. maculata* B. *Ocyptamus coerules* (Syrphidae, Diptera) visiting and transporting pollinia from *C. macrantha* C. *Platycheirus* sp. visiting *C. macrantha × C. maculata* D. *Ocyptamus coerules* (Syrphidae, Diptera) visiting and transporting pollinia from *C. maculata* E. Tachinidae (Diptera), visiting *C. macrantha × C. maculata*. F. Theridion (Araneae: Theridiidae) visiting *C. maculata*. Photographs by B. Téllez-Baños.

Figure 6. A. *Platycheirus* sp. visiting *Corallorhiza macrantha* A1. Pollinia of *C. macrantha* A2. Anther of *C. macrantha* B. *Platycheirus* sp. visiting *C. macrantha × C. maculata* C. *Ocyptamus coerules* visiting *C. maculata* C1. Pollinia of *C. maculata* C2. Pollinia with anther of *C. maculata*. Photographs by B. Téllez-Baños. Scale bars are indicated.
furthermore, of ca. 200 mycoheterotrophic species, only for 38 (17.67%) have studies been carried out regarding floral biology and/or floral visitors. It is important therefore to carry out studies in other mycoheterotrophic orchids to better understand the evolution of pollination strategies in this group of orchids that grow in temperate, temperate-tropical, and temperate subtropical areas.

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LITERATURE CITED


Thiers, B. M. (continuously updated).

Cremastra appendiculata


