Research article

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First record of fragrance collection by euglossine bees (Apidae: Euglossini) in *Ganoderma* (Polyporales, Ganodermataceae)

Primer registro de la recolección de fragancias por abejas euglosinas (Apidae: Euglossini) en *Ganoderma* (Polyporales, Ganodermataceae)

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Abstract:

Background and Aims: Orchid bees and their relationships have been studied since the 1960's. Data show that orchids are not the only source of fragrance for euglossine bees. Although mushrooms have been reported as a source of fragrance for orchid bees, there is no information about this between *Ganoderma* cf. *applanatum* and Euglossini bees. The aim of this work was to identify the bee tribe of Euglossini species that collect fragrances on the fruit body of *Ganoderma* cf. *applanatum*.

Methods: Five samplings were carried out using an entomological aerial net directly on the fruit body of *Ganoderma* cf. *applanatum* during the dry season in April and May 2022 between 11:00 and 13:00 hours. Samplings were carried out in an agroforestry coffee system in the municipality of Jilotepec, Veracruz, Mexico. *Ganoderma* cf. *applanatum* was at the end of its spore releasing phase during this period.

Key results: In the fruit body of *Ganoderma* cf. *applanatum*, particularly in the hymenium, male orchid bees were recorded collecting fragrances, of three genera and six euglossine species. *Euglossa* was the genus with the highest visit frequency of the hymenium of *Ganoderma* cf. *applanatum*.

Conclusions: This report contributes to the documentation of the collection of fragrance by male euglossines on a mushroom (a non-floral scent source) and this activity is recorded for the first time in Mexico.

Key words: *Euglossa dilemma*, *Euglossa viridissima*, insects, mushroom, orchid bees.

Resumen:

Antecedentes y Objetivos: Las abejas de las orquídeas y sus relaciones han sido estudiadas desde la década de 1960. Existen datos que muestran evidencia de que las orquídeas no son la única fuente de fragancias para las abejas euglosinas. A pesar de que los hongos han sido reportados como fuente de fragancias empleadas por las abejas Euglossini, no existe información sobre esto entre *Ganoderma* cf. *applanatum* y Euglossini. El objetivo de este trabajo fue identificar las especies de abejas de la tribu Euglossini que recolectaban fragancias del cuerpo fructífero de *Ganoderma* cf. *applanatum*.

Métodos: Cinco muestreos de abejas euglosinas recolectando fragancias en un hongo fueron realizados usando una red entomológica directamente sobre el cuerpo fructífero de *Ganoderma* cf. *applanatum*, durante la temporada de sequía, de abril a mayo de 2022, entre las 11:00 y 13:00 horas. Los muestreos fueron realizados en un sistema agroforestal de café en el municipio Jilotepec, Veracruz, México. Durante este tiempo *Ganoderma* cf. *applanatum* se encontraba al final de la etapa de esporulación.

Resultados clave: En el cuerpo fructífero de *Ganoderma* cf. *applanatum*, particularmente en el himenio, fueron registrados machos de abejas euglosinas, correspondientes a tres géneros y seis especies, recolectando fragancias. *Euglossa* fue el género que tuvo la mayor frecuencia de visitas al himenio de *Ganoderma* cf. *applanatum*.

Conclusiones: Este reporte contribuye a la documentación de la recolecta de fragancias por abejas euglosinas en un hongo (una fuente no floral de esencias) y esta actividad se reporta por primera vez para México.

Palabras clave: abejas de las orquídeas, *Euglossa dilemma*, *Euglossa viridissima*, hongo, insectos.

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Introduction

The bee tribe Euglossini of the Apidae family consist of five genera and 245 species widely distributed in the Neotropical region (Moure and Melo, 2023). The euglossines are popularly known and studied for the reproductive habits of males, which involve the collection of fragrant oils from orchids and other resources such as sap, ripe and rotting fruits, feces, corpses, leaves, stems, mushrooms, and even on other male bees' dead bodies, prior to courtship with females (Roubik and Hanson, 2004; Michener, 2008; Lozano Rodríguez et al., 2022; Karremans et al., 2023; Moure and Melo, 2023; Henske et al., 2024). Recently, it has been reported that orchid bees collect herbicides that mimic natural fragrances (Pemberton and Kindt, 2024). Information about the interaction between these bees and plants is widely recognized. Orchid bees are considered as ecological indicators, as they are easy to sample, their taxonomy is well-known, and information about the influence of disturbances that affect them is available (Gonçalves and Faria, 2021).

Emissions of volatile organic compounds (VOCs) play important ecological and physiological roles for many organisms (Müller et al., 2013). Fungal VOCs are relatively understudied compared to VOCs of bacterial, plant, or synthetic origin (Hung et al., 2015). Approximately 250 VOCs have been identified from fungi (Chiron and Michelot, 2005; Korpi et al., 2009; Ortíz-Castro et al., 2009). Monoterpenes like α-Pinene, camphene, β-Phellandrene, limonene and linalool have been detected (Breheret et al., 1997). Another compound found in fungi is 1,8-Cineole (Morath et al., 2012), which is widely reported as one of the main VOC recollected by Euglossini bees (Liu et al., 2024). The most common VOC in mushrooms is 1-Octen-3-ol (octanol) (Venkateshwarlu et al., 1999); this is responsible for the particular mushroom aroma (Fahlbusch et al., 2003; Combet et al., 2006). VOCs in fungi play the role of insect attractants of (Morath et al., 2012), such as flies and mosquitoes, which disseminate spores and play a role in the fungal reproduction process (Chiron and Michelot, 2005). Insects (Diptera and Coleoptera) have been reported as spore dispersers in *Ganoderma* P. Karst. (Mayra et al., 2024; Syarif et al., 2024) and as potential vectors for *Ganoderma* basal stem rot disease in oil palm plantations (Syarif et al., 2024). Interactions of orchid bees and mushrooms are poorly explored, even though there are several reports (Roubik and Hanson, 2004; Henske et al., 2024). The objective of this work is to describe the interaction between Euglossini bees and *Ganoderma* and identify the involved euglossine species.

Material and Methods

Male euglossine bees were recorded in the fruiting body of a specimen of *Ganoderma*, growing 30 cm from the ground on a dead trunk of *Bursera simaruba* (L.) Sarg., within a shaded coffee plantation located in the municipality of Jilotepec, Veracruz, Mexico (Fig. 1). The individual of *Ganoderma* was identified using the key of Cappello-García et al. (2023), it was photographed and the images were shown to Dr. Gerardo Mata (Mycologist from INECOL), who identified the specimen as *Ganoderm* cf. *applanatum* (Pers.) Pat. He suggested including cf., taking into account that it would be necessary to perform *in vitro* culture of the hyphae to confirm that it was this species, which was not within the scope of work. This group of bees was monitored between the months of April and May 2022, during the dry season. *Ganoderma* cf. *applanatum* was at the end of its spore releasing phase during this period. Five samplings were carried out using an entomological aerial net, between 11:00 and 13:00 hours.

The collected bees were identified with the keys by Roubik and Hanson (2004) and Ayala and Engel (2008). The material was deposited in the entomological collection of the Instituto de Ecología, A.C. in Xalapa, Veracruz, Mexico (IEXA). Additionally, the behavior of the bees was recorded by photographs and on video using a Nikon D3100 digital SLR camera (Nikon, Tokyo, Japan). The individual of *Ganoderma* cf. *applanatum* was not collected because it was at the end of the spore releasing and the individual died a few dayls later.

Results

In total, 70 individuals from three genera and six species of Euglossini were collected (Table 1). Two specimens of *Lestrimelitta nitkib* Ayala, 1999 (Apidae: Meliponini) were

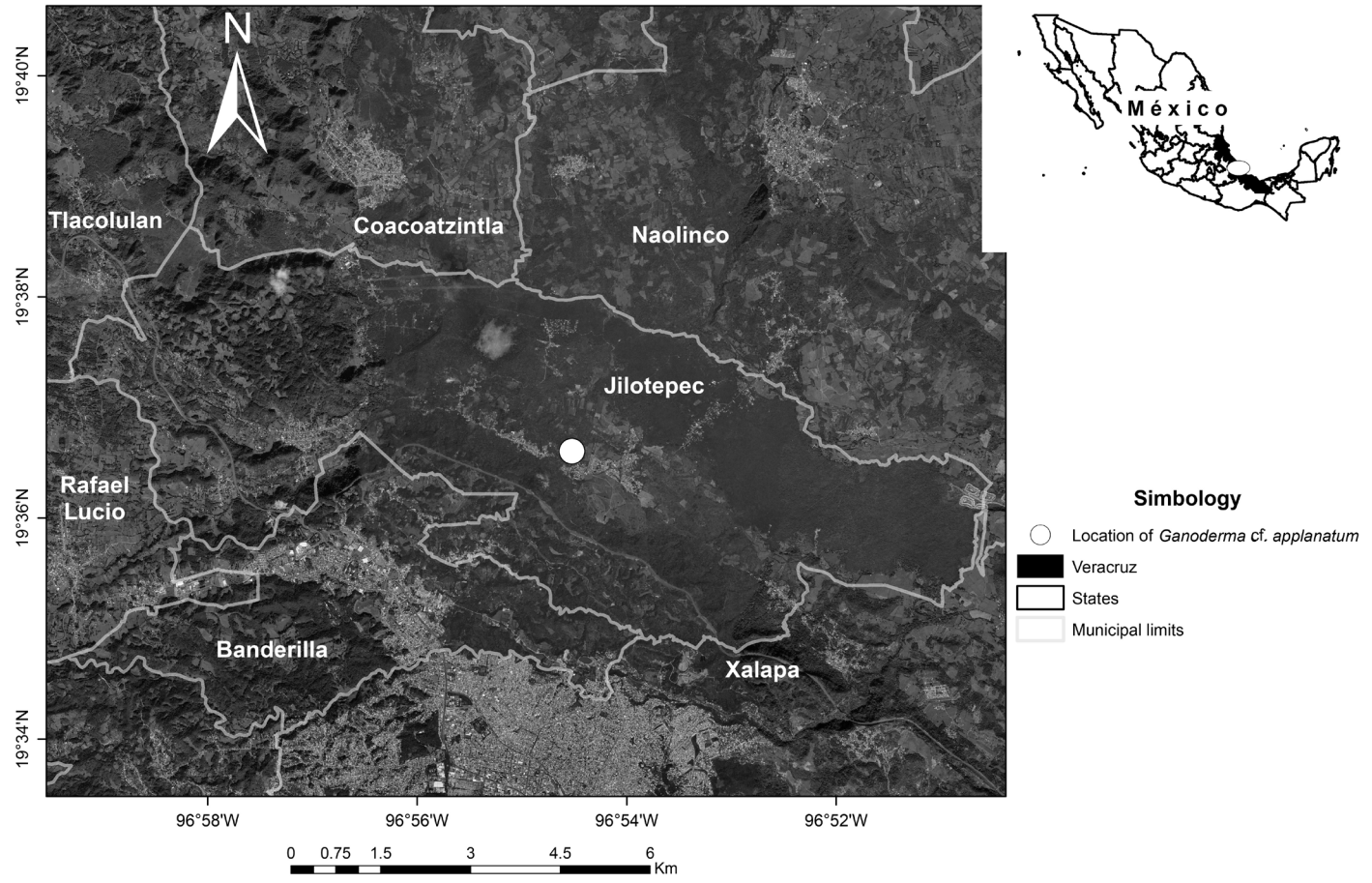

Figure 1: Location of *Ganoderma* cf. *applanatum* (Pers.) Pat. in an agroforestry coffee system in municipio Jilotepec, Veracruz, Mexico, where Euglossini bees were collecting fragrances.

also collected on *Ganoderma* cf. *applanatum*, although this species was not part of the study group. All species of euglossine bees had the typical fragrance collection behavior described by Roubik and Hanson (2004) directly on the hymenium of the fruiting body of *Ganoderma* cf. *applanatum* (Fig. 2; **supplementary material**). Morever, during collection of fragrances, we were able to see spores become attached to the bee bodies (Fig. 3). *Euglossa dilemma* Bembé and Eltz 2011, and *Euglossa viridissima* Friese 1899, represent 94% of all Euglossini bees identified. Males of both species are highly attracted to the hymenium of *Ganoderma* cf. *applanatum* but there is no information about the specific VOCs that attract them.

Discussion

There is evidence that many of euglossine male bees have generalist habits, collecting fragrances from different aromatic sources (Ramírez et al., 2002). The six species reported have been seen collecting fragrances from more than three genera of orchids and have been observed to visit different types of aromatic attractants, specifically the two species of *Eulaema* Lepeletier, 1841 (Williams and Whitten, 1983; Pearson and Dressler, 1985; Ramírez et al., 2002; Roubik and Hanson, 2004). In the case of other non-floral aromatic sources, *Euglossa variabilis* Friese, 1899 has been observed collecting fragrances on decaying logs, probably within small formations of mycelium or mosses (Williams

Table 1: Male (♂) euglossine bee species identified collecting fragrances in *Ganoderma* cf. *applanatum* (Pers.) Pat.

Genera	Species	Material studied	Individuals
Eufriesea Cockerell, 1908	*Eufriesea surinamensis* Linnaeus, 1758	(1 ♂): MÉXICO. Veracruz, municipio Jilotepec, La Concepción, 19°36'35.6''N, 96°54'25.5''W 1000 m a.s.l., 15.IV.2022, ex *Ganoderma* cf. *applanatum*, *G. Quintos-Andrade LACONC023* (IEXA).	1
Euglossa Latreille, 1802	*Euglossa dilemma* Bembé and Eltz, 2011	(39 ♂): MÉXICO. Veracruz, municipio Jilotepec, La Concepción, 19°36'35.6''N, 96°54'25.5''W 1000 m a.s.l., 13.IV.2022, ex *Ganoderma* cf. *applanatum*, *G. Quintos-Andrade LACONC019, LACONC020* (IEXA) (2 ♂); loc. cit., 15.IV.2022, *LACONC022 to LACONC045* (24 ♂); loc. cit., 29.IV.2022, LACONC053 to LACONC060 (8 ♂); loc. cit., 4.V.2022, *LACONC066, LACONC067* (2 ♂); loc. cit., 5.V.2022, *LACONC073 to LACONC075* (3 ♂).	39
	Euglossa variabilis Friese, 1899	(1 ♂): MÉXICO. Veracruz, municipio Jilotepec, La Concepción, 19°36'35.6''N, 96°54'25.5''W 1000 m a.s.l., 29.IV.2022, ex *Ganoderma* cf. *applanatum*, *G. Quintos-Andrade LACONC061* (IEXA).	1
	Euglossa viridissima Friese, 1899	(27 ♂): MÉXICO. Veracruz, municipio Jilotepec, La Concepción, 19°36'35.6''N, 96°54'25.5''W 1000 m a.s.l., 15.IV.2022, ex *Ganoderma* cf. *applanatum*, *G. Quintos-Andrade LACONC046 to LACONC050* (IEXA) (5 ♂); loc. cit., 29.IV.2022, *LACONC062 to LACONC065* (4 ♂); loc. cit., 4.V.2022, *LACONC068 to LACONC072* (5 ♂); loc. cit., 5.V.2022, *LACONC076 to LACONC086* (11 ♂); loc. cit., 13.V.2022, *LACONC087, LACONC088* (2 ♂).	27
Eulaema Lepeletier, 1841	*Eulaema polychroma* Mocsáry, 1899	(1 ♂): MÉXICO. Veracruz, municipio Jilotepec, La Concepción, 19°36'35.6''N, 96°54'25.5''W 1000 m a.s.l., 15.IV.2022, ex *Ganoderma* cf. *applanatum*, *G. Quintos-Andrade LACONC053* (IEXA).	1
	Eulaema cingulata Fabricius, 1804	(1 ♂): MÉXICO. Veracruz, municipio Jilotepec, La Concepción, 19°36'35.6''N, 96°54'25.5''W 1000 m a.s.l., 15.IV.2022, ex *Ganoderma* cf. *applanatum*, *G. Quintos-Andrade LACONC054* (IEXA).	1
		Total	70

and Whitten, 1983; Whitten et al., 1993). This topic has recently been discussed by Henske et al. (2024), who identified more than 20 different non-floral sources collected by Euglossini bees, but no fungal source was reported.

Whitten et al. (1993) recorded *Euglossa purpurea* Friese, 1899 and *Eulaema cingulata* Fabricius, 1804 visiting fungi in organic litter, in Costa Rica and Panama. Cappellari and Harter-Marques (2010) reported males of *Eufriesea violacea* Blanchard, 1840 collecting fragrances in the fruiting body of the mushroom *Hypholoma* aff. *ericaceum* P.D. Orton in southern Brazil. However, to date, this work is the first report of this interaction in Mexico and the first evidence that these six species collect fragrances from *Ganoderma*.

There is no information about which VOCs are related to the attraction between *Ganoderma* and Euglossini. It has been reported that mushroom sporulation coincides with an increase of 1-Octen-3-ol and 3-Octanone levels,

Figure 2: Male of *Euglossa* Latreille, 1802 collecting fragrances on the hymenium of *Ganoderma* cf. *applanatum* (Pers.) Pat.

Figure 3: Spores of *Ganoderma* cf. *applanatum* (Pers.) Pat. covering the hairs and wings of *Euglossa* Latreille, 1802 during the collection of fragrances.

supposedly to recruit insects to disseminate the fungal spores (Fäldt et al., 1999). In addition, the major aroma compounds identified in *Ganoderma lucidum* (Curtis) P. Karst. 1881, are 1-Octen-3-ol (Alcohol) and 3-Methylbutanal (Aldehyde) (Taşkin et al., 2013). 1-Octen-3-ol and 3-Methylbutanal can be found in Orchidaceae (Knudsen et al., 2006), which is one of the sources of VOC collected by Euglossini bees (Roubik and Hanson, 2004). Hence, these findings suggest that bees (Euglossini) may be attracted to *Ganoderma* to collect 1-Octen-3-ol as a reward for spore dispersal, such as flies and beetles in other *Ganoderma* species (Mayra et al., 2024; Syarif et al., 2024). It is unknown if in *Ganoderma* there is a relationship between the phenological stage and the production of fragrances, as occurs in other cases like the one reported by Fäldt et al. (1999) and whether this increase of fragrances attracts male euglossine bees. Although spore development trials were not performed on the collected individuals, this correlation among compounds can suggest a potential behavior of the bees as spore dispersers of *Ganoderma*. Future research should focus on VOCs of *Ganoderma applanatum* in order to identify the mayor compounds that could attract male Euglossini bees, and evaluate their role in spore dispersal by counting the spores transported by each individual, and whether they are deposited in some wounds of trees from they collect resins, as in some species of the Burseraceae family.

This report constitutes a contribution to the documentation of fragrance collection by male euglossines on a *Ganoderma* cf. *applanatum*, a non-floral scent source. Also, this is the first report of this activity wich could constitute a mutualism although further studies are needed. It is unknown if 1-Octen-3-ol is the main VOC that *Euglossa* Latreille, 1802 bees collect from *Ganoderma* cf. *applanatum* and responsible of this interaction. Finally, *Euglossa* bees can act like spore dispersal of *Ganoderma* cf. *applanatum*.

Author contributions

Conceptualization: MLR; Investigation: MLR, GQR; Writing writing – original draft: MLR, GQR; Writing – review & editing: MLR, GQR; Validation: MLR, GQR.

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Data Availability Statement

The dataset that supports the results of this study was published in SciELO Data and can be accessed at DOI: https://doi.org/10.48331/SCIELODATA.5RTP35

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Supplementary material: *Euglossa* Latreille, 1802 bees collecting fragrances on *Ganoderma* cf. *applanatum* (Pers.) Pat. Movie by Miguel Ángel Lozano-Rodríguez