

**POLLEN CARRIED BY *Euglossa nigropilosa* MOURE (APIDAE: EUGLOSSINAE)
AT LA PLANADA NATURE RESERVE, NARIÑO, COLOMBIA.**

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RESUMEN

Las abejas Euglosinas son importantes polinizadores de plantas neotropicales de tierras bajas. Con el objetivo de determinar las principales fuentes de polen y néctar de *Euglossa nigropilosa* (Euglossinae), en La Reserva Natural La Planada RNLN, Nariño, Colombia, observamos su comportamiento en plantas cercanas a sus nidos durante más de 100 horas. Además, los granos de polen recolectados de las corbículas fueron recolectados de abejas arribando a los nidos y fueron identificados en el laboratorio. El polen fue preparado usando la técnica de acetólisis y caracterizado morfológicamente utilizando una serie de descriptores taxonómicos como número, posición y tipo de apertura. El polen fue identificado a nivel género incluyendo *Tibouchina*, *Miconia* y *Clidemia* (Melastomataceae), *Physalis* y *Solanum* (Solanaceae), y *Melothria* (Cucurbitaceae); este último apareció en muy baja frecuencia. La mayoría de visitas florales de *E. nigropilosa* para coleccionar polen fueron a Melastomataceae, seguidas por Solanaceae. Las fuentes de néctar de *E. nigropilosa* en el campo incluyen *Impatiens balsamina* (Balsaminaceae) y *Melothria longituba*. Este estudio muestra una fuerte preferencia de *E. nigropilosa* por fuentes de polen provenientes de floras con el síndrome de polinización por vibración. Para conservar *E. nigropilosa* en la naturaleza es importante proteger las plantas que ofrecen recursos vitales como polen, néctar y resinas para la construcción de sus nidos.

Palabras clave: Euglossini, Fuentes de Polen, Lulo, plan de Conservación, Polinización, Solanum quitoensis.

SUMMARY

Euglossinae bees are important pollinators of lowland plants in the Neotropics. With the aim of determining the main nectar and pollen sources for the Euglossinae bee *Euglossa nigropilosa*, at La Planada Nature Reserve LPNR, Nariño, Colombia, we observed the behaviour on plants near the nest for more than 100 hours. Additionally, pollen grains from corbicula were collected from bees arriving at their nests and identified in the laboratory. Pollen was prepared using acetolysis technique and characterized morphologically using a list of taxonomic descriptors with taxonomic value such as number, position and type of pollen aperture. The pollen genera identified included *Tibouchina*, *Miconia* and *Clidemia* (Melastomataceae), *Physalis* and *Solanum* (Solanaceae), and *Melothria* (Cucurbitaceae); this last one occurred at a very low frequency. The majority of *E. nigropilosa* flower visits to collect pollen were to Melastomataceae, followed by Solanaceae. The nectar plants visited by *E. nigropilosa* in the field included *Impatiens balsamina* (Balsaminaceae) and *Melothria longituba*. This study shows a strong preference by *E. nigropilosa* for pollen sources with buzz pollination syndrome. In order to preserve *E.*

nigropilosa in the field, it is important to provide plants that offer vital resources such as pollen, nectar and resin for nest construction.

Key words: Pollen sources, *Euglossini*, *Lulo*, *Pollination*, *Solanum quitoensis*, *Conservation plan*.

INTRODUCTION

One of the most important ecosystem services in agricultural economies is that of pollination. In 1997, pollination services were estimated as having a value of \$112 billions *per annum* (Costanza *et al.* 1997). However, anthropogenic activities such as habitat transformation, agricultural intensification, and agrochemical use are causing a drastic decline in pollinator populations (Steffan-Dewenter *et al.* 2005). Bees, probably the most important pollinator guild for both crop and wild plants in both tropical and temperate areas, have seen declining population sizes of recent years (Steffan-Dewenter *et al.* 2005, Gouldson & Darvill 2008). There is an urgent need for action directed to the preservation of pollinators and the development of conservation plans to protect the pollination services. The development of such plans require a detailed information of the natural history of pollinators including the nest site preferences and construction materials, social structure, as well as food sources and natural enemies.

Bees collect pollen and nectar from plant flowers to feed their broods, but the variability of pollen sources is great among bee species. While the honey bee (*Apis mellifera*) is commonly known to collect pollen from many plant species (eg. Singer 1947), in contrast, the species *Diadasis* spp (Hymenoptera: Apoidea), are much more specialized in their pollen sources (Sipes & Tepedino 2005). Bees collecting pollen from a variety of sources are referred as polylectic, those that are more specialized are oligolectic, and monolectic when they only collect pollen from a single plant species. Such variation in food preference is related to the ecological factors affecting the bee species distribution, abundance, and has important implications for conservation efforts, as specialized bees may be limited in distribution to those areas where appropriate pollen is available. In order to develop viable and effective conservation plans, it is essential to understand the pollen resources used by different bee species.

In consequence it is also important to identify and preserve the plant sources of pollen, nectar and nest material used by the focal bee species. Subsequently, botanical studies of the most important plant species, are also required, in order to understand the ecological requirements of these pollen sources.

Euglossine bees are distributed in the Neotropics, from the south of Florida to north Argentina and are classified within the subfamily Apinae. This group of bees is recognized as one of the most important pollinator guilds in the low land tropical forest of South and Central America, and is suggested as a keystone species given its pollination services (Tonhasca *et al.* 2002). The tribe Euglossini comprises five genera: *Euglossa*, *Eulaema*, *Eufriesia*, *Exaerete* and *Algae* (Roubik & Hanson 2004). *Euglossa* contains the highest number of species, including more than 200 Spp (Augusto & Garófalo 2004). Several Euglossinae bees are important pollinators of economically important plants; for example, *Eulaema nigrita* pollinates the Brazil nut (*Bertholletia excelsa* HBK) (Motta Maués 2002), and *Euglossa viridisima* and *Eulaema* sp. are reported as vanilla pollinator in Mexico (Hernandez-Hernandez 2011). Similarly, *Euglossa nigropilosa* Moure has been reported as pollinating of the “lulo” plant (*Solanum quitoense* L., also known as “naranjilla” in Ecuador) in Colombia (Otero 1996).

Nests of *Euglossa* spp. are inconspicuous and finding them in nature is not easy. Thus the acquisition of information regarding the natural history of these pollinators is slow. Through the study of accessible *E. nigropilosa* nests at La Planada Nature Reserve (LPNR), Nariño, Colombia, several natural history aspects of the bee species are now known (Otero 1996, 2001, Otero *et al.* 2008). *Euglossa nigropilosa* Moure is a communal bee inhabiting resin nests inside cavities (Otero 1996). Individual bees collect pollen and nectar for brood feeding. Pollen and nectar is stored in resin cells

before oviposition and enclosure of the cell (Otero *et al.* 2008). Previous studies on this species collected important information including observations of nests and social issues, but information on nectar and pollen sources are anecdotic (Otero 1996, Otero *et al.* 2008). Further, these few anecdotal studies were based on field observations of bee visits to plants, and it is not clear exactly how many plant species a female *E. nigropilosa* may visit in a single trip and which are their preferred pollen source.

The aim of this study was to identify the nectar and pollen sources of *E. nigropilosa* at LPNR and determine the main plant species to be included in future conservation plans for *E. nigropilosa*. Knowing the identity and importance of pollen sources is important for the development of conservation plans for those plants required by the bee species. Secondly, we aimed to determine how many plants a single bee use in a single trip, in order to further our understanding of the biology of this species.

MATERIALS AND METHODS

Sampling location and pollen collection.

The study was performed at La Planada Nature Reserve (1°15'N, 78°15'O, Municipality of Ricaurte, Nariño, Colombia; 1300-2100 m. above sea level; with annual average precipitation of 4900 mm; and average temperature of 12° to 24°C).

Field observations of bee behaviour in areas near the nests were made over a total of 103 hours of observation. A record was kept of the plant species visited by *E. nigropilosa*, and also any collection activity of floral resources – pollen or nectar. Pollen samples were collected from the pollen basket or corbicula of bees arriving to the nest (Otero 1996, Otero *et al.* 2008), with each sampling corresponding to pollen collected by the bees in a single trip.

After sampling, bees were marked for monitoring inside the nest, and liberated. Pollen samples were stored in acetic acid for preservation before transportation to the laboratory of botany at Valle University, in Melendez Campus, Cali. Pollen analysis was performed

at laboratory of pallinology and electronic microscopy at Colombian National Universidad at Palmira Campus.

To determine the nectar sources, bee visits to plants for nectar extraction was recorded over 60.6 hours of observation from July 1994 to June 1995. The sugar concentration of the nectar from the plants visited by *E. nigropilosa* was measured with an Atago hand refractometer, Model ATC-1E, Brix (0–32%) (Otero *et al.* 2008).

Material processing and data analysis.

Acetolysis (Erdtman 1952) was performed, and semi-permanent slides in glycerin-jelly seal with paraffin were prepared. Slides were observed at 40X in a Leika light microscope with metric ruler and photographic camera. Identification and pollen grain characterization was performed for 16 samples of acetolysed pollen collected from individual bee corbicula. From each slide 30 pollen grains were characterized and grouped by morphological similarity, assigning an identification code. Photographic records were stored and compared with pollen guides (Roubik & Moreno 1991) for identification at the genus level focusing on the families reported for LPNR (Mendoza-Cifuentes & Ramirez-Padilla 2000). The family level identification was carried out using size, morphology, nuclear pore complex NPC and shape. The determination at genus level was carried out using pollen morphology in equatorial and polar views. All pollen measurements were taken in micrometers (μ m). In the equatorial view we measured the equatorial (E) and polar (P) axis. With the polar index (P/E) we obtained the pollen morphology index. The size was defined as the polar axis. For all measurements we calculated the average, standard deviation, and variation coefficient. These measurements allow us to determine the pollen grains at genus level according to the palinological atlas (Roubik & Moreno 1991).

RESULTS

The main plants visited by *Eg. nigropilosa* as nectar sources were *Impatiens balsamina* L. (Balsaminaceae) with an average nectar concentration from 26.6% sucrose, and the

native vine *Melothria longituba* C. Jeffrey (Cucurbitaceae) with nectar concentration 30.2% sucrose.

Most pollen samplings (66.6%) included a single type of pollen grains. We identified ten pollen morph-types from tree plant families (Table 1). Most pollen grains were from the family Melastomataceae including six morph-types from three genera: *Miconia* (four morph-species), *Tibouchina*, and *Clidemia*; (Figure

1). Melastomataceae was followed by Solanaceae including three morph-types from two genus *Solanum* and *Physalis*; this family was dominant on only three slides. Finally, and in contrast to the observation of plant visits, a morph-type from Cucurbitaceae (*Melothria*) was observed in very low frequency (three pollen grains in a single slide). Pollen arrival showed no specific pattern with time.

Table 1. Occurrence of pollen morphs in single pollen collection trips of female *Euglossa nigropilosa* at La Planada Nature Reserve

Plant species	Nest P	Nest C	Total
Melastomataceae			
<i>Tibouchina</i> Sp1	4	4	8
<i>Miconia</i> Sp1	1		1
<i>Miconia</i> Sp2	1		1
<i>Miconia</i> Sp3		1	1
<i>Miconia</i> Sp4	1		1
<i>Clidemia</i> Sp1	2	3	5
Solanaceae			
<i>Solanum</i> Sp1	1		1
<i>Solanum</i> Sp2	1		1
<i>Physalis</i> Sp1	1		1
Cucurbitaceae			
<i>Melothria</i> Sp1	1		1
Total	13	8	21

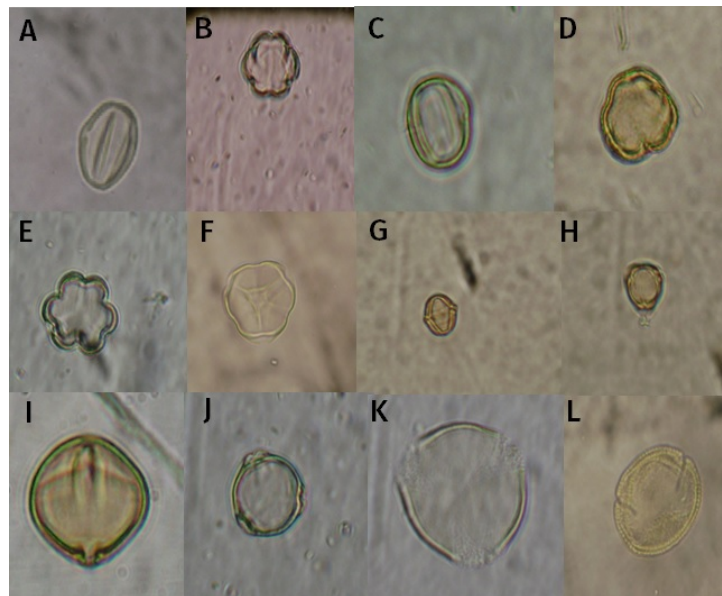


Figure 1. Diversity of pollen grains collected by *Euglossa nigropilosa* at La Planada Nature Reserve. A: *Tibouchina* Sp1 equatorial view, B: polar view. C: *Miconia* Sp.1 equatorial view, D: polar view. E: *Clidemia* Sp1

equatorial view, F: polar view. G: *Solanum* Sp1 equatorial view, H: polar view. I: *Solanum* Sp 2 equatorial view, J: polar view. K: *Melothria* Sp1. Equatorial view, L: polar view.

DISCUSSION

Bees vary in pollen preferences. According to the classification, bee species can be classified as polylectic if it use the total broad variety of pollen sources; oligolectic, if it uses a restricted number of plants as pollen source; and monolectic, if it utilizes only a single plant species as pollen source (Michener 1974). Our results showed that *E. nigropilosa* was associated with a relatively small pollen sources including mainly the plant families Melastomataceae and Solanaceae with buzz-pollination syndrome, a pollen reward pollination mechanism presenting anthers with a pore that deliver pollen after the buzz of the bee collecting the pollen (Michener 1974). In consequence, *E. nigropilosa* can be classified as oligolectic species. Previously, *Tibouchina lepidota* and *Solanum quitoense* were detected as pollen source for *E. nigropilosa* at La Planada Nature Reserve by direct observations of the female bees visiting the plants in the field (Otero 1996). Our study confirms the presence of pollen morphs in the corbicles of *E. nigropilosa*, belonging principally to two genera and enlarges the information on pollen sources for the study species. The participation of euglossine bees on buzz pollination was previously reported (Ackerman 1983, Roubik 1989). Other studies also report different pollen sources for euglossinae bees. *Euglossa annectans* and *E. anodorynchi* collect pollen from *Aechmea caudata* (Bromeliaceae) in Santa Catarina, Brasil (Kamke 2009). In Mexico, *E. atrovirens* used up to 71 floral resources during

a year, including pollen sources as: *Antirhea* sp., *Cassia* sp., *Combretum* sp., *Commelina* sp., *Cordyline terminalis*, *Dendropanax* sp., Leguminosae type 4, *Lycianthes* sp., *Saurauia* sp., *Solanum* aff. *torvum*, *Solanum* aff. *tuerckheimii* and *Tibouchina longifolia* (Ramírez-Arriaga & Martínez-Hernández 1998). Our results suggest that *Euglossa* may have considerable variation in the diversity of pollen sources used. Nevertheless, Melastomataceae and Solanaceae were used by several *Euglossa* spp.

Given that male and female Euglossinae bees are important pollinators in the Neotropics, including both wild and crop plants, and given that pollen is the main food for immature bees, the conservation of bees and their nurse plants should be a priority in Colombia and in the world. Sustainability requires plans to preserve pollen source species and ensure the pollination services.

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CITED LITERATURE

- Ackerman, J. D. 1985. Euglossine bees and their nectar hosts. In W. G. D'Arcy & M. D. Correa (Eds), The botany and natural history of Panama Pp 225-233, Missouri Botanical Garden, St. Louis, Missouri, USA.
- Augusto, S. C. & Garófalo, C. A. 2004. Nesting biology and social structure of *Euglossa* (*Euglossa*) *townsendi* Cockerell (Hymenoptera, Apidae, Euglossini). *Insectes Sociaux*, 51:400-409.
- Costanza, R., D'Arge, R., de Groot, R., Farber, S. & Grasso, M. 1997. The value of the world's ecosystem services and natural capital. *Nature*, 387:253-260.
- Erdtman, G. 1952. Pollen morphology and plant taxonomy, Angiosperms. The Chronica Botanica Co., Waltham, Mass., Printed by Almqvist Wiksell, Stockholm, Sweden.
- Goulson, D. L., G. C., & Darvill, B. 2008. Decline and Conservation of Bumble Bees. *Annual Review of Entomology*, 53:191-208.

- Hernandez-Hernandez J. 2011. Mexican vanilla production. In: Havkin-Frankel, D. and F.C. Belanger Eds. Handbook of Vanilla Science and Technology, Pp 3-25, First Eddition. Blackwell Publishing Ltd.
- Kamke, R. 2009. Polinização por abelhas em *Aechmea caudata* Lindm., uma Bromélia com características ornitófilas na ilha de Santa Catarina, Sul do Brasil. MSc Teses, Universidade Federal de Santa Catarina. Centro de Ciências Biológicas, Departamento de Botânica, Programa de Pós-graduação em Biologia Vegetal.
- Mendoza-Cifuentes, H. & Ramírez-Padilla, B. 2000. Flora de la Planada: Guía ilustrada de Familias y Géneros. Instituto Alexander von Humboldt, Fundación para la Educación superior, World Wildlife Fund, Bogotá, Colombia.
- Michener, C. D. 1974. The social behaviour of bees. The Blacknap Press, Harvard University Press, Cambridge, Massachusetts, USA.
- Motta Maués, M. 2002. Reproductive phenology and pollination of the brazil nut tree (*Bertholletia excelsa* Humb. & Bonpl. Lecythidaceae) in Eastern Amazonia. In: Kevan P. & Imperatriz Fonseca V. L. Eds. Pollinating Bees - The Conservation Link Between Agriculture and Nature, Pp 245-254, Ministry of Environment, Brasília.
- Otero, J. T. 1996. Biología de *Euglossa nigropilosa* Moure (Apidae: Euglossinae): Características de nidificación en la Reserva Natural La Planada. Boletín del Museo de Entomología de la Universidad del Valle, 4:1-19.
- Otero, J. T. 2001. *Monodontomerus argentinus* Brethes (Hymenoptera: Torymidae): a new parasitoid of *Euglossa nigropilosa* Moure (Hymenoptera: Apidae: Euglossinae). Pan-Pacific Entomologist, 77:57-60.
- Otero, J. T.; Silverstone, P.; Chacon, P.; & Giray, T. 2008. Group nesting and individual variation in behaviour and physiology in the orchid bee *Euglossa nigropilosa* Moure (Hymenoptera, Apidae). Insectes Sociaux, 55:320-328
- Ramirez-Arriaga, E. & Martines-Hernandez, E. (1998) Resources foraged by *Euglossa atrovoneta* (Apidae: Euglossinae) at Union Juarez, Chiapas, Mexico. A palynological study of larval feeding. Apidologie, 29:347-359.
- Roubik, D. W. 1989. Ecology and natural history of tropical bees. Cambridge University press, Cambridge, England.
- Roubik, D. W. & Hanson, P. E. 2004. Orchid bees of tropical America, biology and field guide. Instituto Nacional de Biodiversidad, San Jose, Costa Rica.
- Roubik, D. W. & Moreno, P. 1991. Pollen and spores of Barro Colorado Island, Monographs in Systematic Botany 36. Missouri Botanical Garden, St Lous Missouri Singer, A.D. 1947. Pollen Collection by Honeybees (*Apis mellifera*). Journal of Animal Ecology, 16:122-138.
- Sipes, S. D. & Tepedino, V. J. 2005. Pollen-host specificity and evolutionary patterns of host switching in a clade of specialist bees (Apoidea: *Diadasia*). Biological Journal of the Linnean Society, 86:487-505.
- Steffan-Dewenter, I.; Potts, S. G.; & Packer, L. 2005. Pollinator diversity and crop pollination services are at risk. Trends in Ecology and Evolution, 20:651-652.
- Tonhasca, A. J.; Blackmer, J. L.; & Albuquerque, G. S. 2002. Abundance and diversity of euglossine bees in the fragmented landscape of the Brazilian Atlantic forest. Biotropica, 34:416-422.