Cavities in bromeliad stolons used as nest sites by Euglossa cordata (Hymenoptera, Euglossini)

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Abstract
Herein, we describe nests of the orchid bee Euglossa cordata that were constructed in cavities of Aechmea distichantha (Bromeliaceae) stolons. We present data about nest and cell size, number of adults and brood, and analyses of larval provisions. The presence of E. cordata carcasses embedded in the resin of nest partitions indicates that these nests were used by multiple generations. Based on larval provisioning, E. cordata is polylectic and relies heavily on a few plant species.

Keywords
island, larval provision, natural cavity, nesting biology, orchid bees

Introduction
Orchid bees (Apidae: Euglossini) are important pollinators in the Neotropical region, as they pollinate hundreds of plant species from many families (Ramírez et al. 2002), including orchids. Previous research indicates a large degree of variation in Euglossini nesting substrates, including soil, termite nests, bamboo canes, orchid roots, and man-made cavities (Bodkin 1918, Sakagami et al. 1967, Garófalo et al. 1993, Gonzales and Gaiani 1990, respectively). Among the five different genera and ~200 species of orchid
bees (Roubik and Hanson 2004), some species build freestanding nests with a resin involucre (Eberhard 1988, Wcislo et al. 2012), while other species search for empty cavities (Garófalo 1985, Gonzales and Gaiani 1990). Dressler (1982) hypothesized that species nesting inside cavities may develop sociality, whereas species building aerial nests would be solitary. Although aerial nest with multiple adult females have been described for *Euglossa*, the study did not clarify the degree of sociality (Eberhard 1988).

Besides sociality, the nesting biology of many orchid bee species is still unknown because females are rarely observed in nature and only males are known for some species (Roubik and Hanson 2004). Human-made cavities in bamboo canes or wooden boxes have increased the knowledge of the nesting biology and social organization of orchid bees (Zucchi et al. 1969, Augusto and Garófalo 2009, Boff et al. 2015, 2017, Andrade et al. 2016). However, such methods are limited to cavity nesting species, with little information regarding natural nesting cavities (Wcislo et al. 2012), even for common species such as *Euglossa cordata* Linnaeus. *Euglossa cordata* is an orchid bee species that is widely distributed in South America (Nemésio and Silveira 2007) and abundant in Southeastern Brazil (Cordeiro et al. 2010, Aguiar and Gaglianone 2012).

This species is primitively eusocial, with a single dominant female who has full reproductive skew towards subordinates (Freiria et al. 2017). Studies about population genetics of *Euglossa cordata* indicate large population sizes and that environmental discontinuity does not play a strong role on genetic diversity in this species (Cerântola et al. 2010, Rocha-Filho et al. 2013, Boff et al. 2014). Herein we report females of *E. cordata* using cavities in stolons of the bromeliad *Aechmea distichantha* Lem. as nest sites. We also describe the pollen included in the larval provisions of this bee. This is the first study to describe orchid bees using bromeliads as nesting sites.

**Material and methods**

Natural nests were found on the Ilha da Vitória (45°01′W, 23°45′S), a small island (221 ha) in the state of São Paulo, Brazil ca. 40 Km away from the mainland (Figure 1) in November 2009. Ilha da Vitória is covered with plants commonly found in the Atlantic Forest, but has less floral diversity than the neighboring islands: Ilhabela, and Ilha de Búzios (Ângelo 1989). Ilha da Vitória has only rocky shores, where plant species that are adapted to this extreme habitat grow.

One such species is the bromeliad *Aechmea distichantha* which grows over the rocks at the edge of the island. These bromeliads are fixed to the rocks by very thin, root-like structures that arise from the plants’ stolons. Typically, stolons are solid structures that emerge from plant rhizophora and grow parallel to the rocky substrate. Close to their termini they turn upward, grow vertically, and lead to the next generation bromeliad (pup). An *Aechmea* stolon presents three layers: a coat (consisting of the lignified epidermis and/or a stratified layer of cork), a cortex, and vascular cylinder (Proença and Sajo 2008). All together the stolon forms a bridge linking two individual plants (Suppl. material 1: Figure S1a–b).
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Figure 1. Location of study site. **a** The data was sampled on Ilha da Vitória, archipelago of Ilhabela, in São Paulo state. **b** The habitat of the bromeliad on the rocks of the board, arrow highlights stolon of *A. distichantha*.

We sampled a total of seven stolons, either from the foliage or on the bare rock. Five of them were old (dry) stolons and two were living stolons. All stolons were carefully removed and brought to the Bee Laboratory at the University of São Paulo. In the lab,
all the old stolons were dissected and found to contain cavities. In 2018, we dissected living stolons, which linked two living plants. Living stolons presented no cavities, but instead were filled with aqueous white tissues (Suppl. material 1: Figure S1c–d).

We found nests of *Euglossa cordata* in the dry stolons of *Aechmea distichantha* (Suppl. material 1: Figure S2a–b), with three nests in two different stolons. These stolons were similar to a “clean gallery”, with little or no plant tissue left inside. After opening stolons and exposing the cavities, adult and immature individuals were fixed in ethanol. Adult bees found with undamaged wings were considered newly emerged, while bees with damaged wing ends were classified as not newly emergent. Nest architecture was described, brood cells were measured with a digital vernier caliper, and larval provisions were analyzed using brood feces (n_cell = 10) and fresh pollen content (n_cell = 1). In the laboratory, we analyzed pollen samples from nests 1 and 3 following acetolysis. We were unable to determine pollen from nest 2 since open cells were empty and the closed cell was undisturbed. Microscope slides were mounted and pollen grains were identified by a specialist (CFP Luz). We estimated pollen type frequencies using 300 pollen grains per slide (n = 2 slides per cell) (see Boff et al. 2011). We used the package bipartite in R to visualize network-level analyses (Dormann et al. 2009).

Vouchers of bee specimens were deposited in the entomological collection “Paulo Nogueira-Neto” in the Ecology Department at the University of São Paulo. Pollen slides were deposited in the Palynology Collection at the Institute of Botany of São Paulo state.

**Results**

We found a total of 25 cells in three nests. Nests 2 and 3 were found inside the same stolon, but the nest cavities were not connected to each other. In nests 1 and 2 we found ten brood cells each. In nest 3 we found five cells. In the closed cells of all nests we observed a cell collar. The brood cells (n = 13) were 9.04 mm (±0.98) wide and 12.3 mm (±2.62) long. In nest 1 we found two living adults (one male and one female) classified as recently emerged brood. We also found the carcass of another female. In the laboratory two other individuals (a female and a male) emerged. Additionally, we found fresh pollen inside an open cell (Figure 2).

A mixture of resin and carcasses acted as a barrier between the two nests in connected stolons. In nest 2 we found one dead female with damaged wings, a dead male inside a brood cell, and a fragmented bee carcass whose body parts were embedded in resin and unable to sex. In nest 3 we found two open and three closed brood cells. In the lab, two bees (one male, one female) emerged from the closed cells and one non-sexed dead larva was extracted from their cell (Table 1). In nests 1 and 2 the cells were aligned inside the cavities, with each cavity length about 13 cm (see Figure 2). In nest 3, cells were built in a single cluster inside a smaller cavity with 5.5 cm length (Figure 3). For all nests resin was only used in the brood cells and around the nest entrance.
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Figure 2. Details of two nests found inside stolons of bromeliads. In nest 1 only a part of the cell is shown. On the left side of nest 1 it is possible to see an open brood cell with fresh pollen in caramel color.

Table 1. Snapshot of intranidal data of Euglossa cordata. Details of three nests found inside the stolons of A. distichantha. No = Non-observed.

<table>
<thead>
<tr>
<th></th>
<th>Nest 1</th>
<th>Nest 2</th>
<th>Nest 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resident female alive</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Males alive</td>
<td>1</td>
<td>1</td>
<td>No</td>
</tr>
<tr>
<td>Brood cell with fresh provision</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Closed cells</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Cell under construction</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Old open cells</td>
<td>6</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Emerged individuals</td>
<td>2 ♀/ 2 ♂</td>
<td>1 ♂</td>
<td>1 ♀/ 1 ♂</td>
</tr>
<tr>
<td>Dead bee(s) or carcass(es)</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Pollen types (n)/cells (n)</td>
<td>16/7</td>
<td>No</td>
<td>9/4</td>
</tr>
</tbody>
</table>

We found several pollen types associated with the diet of immature Euglossa cordata. We determined 21 pollen types, corresponding to species from seven families (Figure 4). The frequency of pollen types varied from two to nine in one single brood cell. Four plant species (Centrosema sp., Dalechampia sp2, Myrcia sp. and Solanum sp1)
Figure 3. Stolon of *A. distichantha* with two connected nests of *Euglossa cordata*. Nest 2 and 3 were separated by resin and bee carcasses (arrow between nest 2 and nest 3; see details in Suppl. material 1: Fig. S1c). The entrance of nest 3 is indicated (arrow) and nest 3 is in detail after dissection (with scale, 2 cm).

were found in both nests. In five brood cells, we found that at least 80% of all pollen grains came from a single plant species. Species with the highest pollen frequencies were *Myrcia*, *Solanum*, *Baccharis*, *Genipa americana*, and *Eucalyptus* (Figure 4).

Discussion

*Euglossa cordata* females build nests in empty cavities. Even though their nests have been documented in bamboo canes or observation boxes, this species is not restricted to such substrates. Herein, we report that *E. cordata* uses naturally lignified cavities, specifically stolons of the bromeliad *Aechmea distichantha*, as nesting sites. The cavities inside the stolons seem to be an efficient site to build nests. We found carcasses of old *Euglossa* females in all nests, which may indicate that nests were reused by multiple generations.

We observed that internal cavities in the stolon may be due to drying of moist internal tissues after plant death. However, we found one stolon that was occupied by an unidentified species of termite. In this stolon, we presume that the termites were foraging in the internal tissue, since we did not find termite eggs, or a queen or king in the cavity (characteristics of a termite nest) (personal communication T Carrijo 2018). Therefore, although cavities occupied by the orchid bees may have been caused by
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**Figure 4.** Network-level analysis of larval provision of *Euglossa cordata*. Each orange rectangle represents the pollen type found in the brood cells. The green rectangles represent individual brood cell. The first number inside each green rectangle corresponds to the nest identity and the second number to the brood cell itself. The connection between rectangles (blue) shows the pollen types used as food for immature *E. cordata*. The width of each blue link corresponds to the frequency of pollen grains inside each brood cell. The content from brood cell N1.7, correspond to open cell in the nest 1 (see Fig. 2).

Drying of internal tissues after plant death, *E. cordata* may benefit from prior foraging/cleaning activity of termites in the tissues of the stolon. Furthermore, we found nests inside empty galleries, where rare roots pieces remained (see the bottom of nest 2, Figure 2). Females of *Euglossa cordata* built their cells in two different ways inside the cavities. In long and narrow cavities, brood cells were linear, while in wider and shorter cavities, cells were arranged in a cluster. Besides that, nest size seemed to be dependent on the dimensions of the stolon. Zillikens et al. (2001) reported that *Augochlora esox* (Halictidae: Augochlorini) nested in the rosettes of *Aechmea* plants, and that rosette size limited the nest architecture of the sweat bees. Similar findings have
been documented for other bee species nesting in human made cavities (Alonso et al. 2011, Seidelmann et al. 2016). We assigned *E. cordata* as a heteromorphic bee species because nest size and structure is dependent on the cavity (see Michener 2007, p.25). This behavioral plasticity of *E. cordata* may indicate that there is no single nest architecture or substrate for this species. Instead, females of *E. cordata* seem to optimize the size of a cavity when building their nests, and search for cavities in different substrates.

The nesting biology of *Euglossa*, including *E. cordata* (Garófalo 1985, Gonzales and Gaiani 1990), is documented for circa 20% of the species (see Wcislo et al 2012). Interestingly, this group of neotropical bees seems to either build nests in cavities or build aerial nests. Cavity users, as their name suggests, use cavities to build nests, as we observed herein. Other *Euglossa* species build aerial nests made of plant resources, such as resin that anchors the steam or leaf of a plant (see video in Wcislo et al. 2012). Such remarkable differences in nesting behavior within a genus may extend to other bee genera, such as *Centris*, who either nest in empty cavities or excavate nests underground (Vinson et al. 2010, Sabino et al. 2017, respectively).

**Larval provision**

Studies suggest that *Euglossa* species are polyletic species (Ramírez et al. 2002, Cortopassi-Laurino et al. 2009, Villanueva-Gutierrez et al. 2013, Silva et al. 2016), which means that the pollen diet of the brood consists of multiple plant families. However, it seems as though *Euglossa* bees collect higher amounts of pollen grains from a few species of plants in the same family. In the current study 10 out of 11 brood cells had one single plant species with pollen frequency higher than 70 %. From the same ten brood cells, 80 % had pollen from either Myrtaceae (n = 6) or Solanaceae (n = 2). Similar findings have been reported for *E. annectans* Dressler and *E. townsendi* Cockerell (eg. Cortopassi-Laurino et al. 2009, Silva et al. 2016, respectively).

Directly comparing studies about *Euglossa* diet is difficult because these studies are not uniform, with study areas distributed across the Neotropical region in different habitats and vegetation. Furthermore, studies are based on many different *Euglossa* species with different nest numbers (and brood cells) [ie. Arriaga and Hernandez (1998), (n\textsubscript{nest} = 57); Cortopassi-Laurino et al. (2009), (n\textsubscript{nest} = 12); Villanueva-Gutierrez et al. (2013) (n\textsubscript{nest} = 27); Silva et al. (2016) (n\textsubscript{nest} = 6)]. However, the most frequently reported pollen grains are from Melastomataceae, Myrtaceae, and Solanaceae species. We analyzed the pollen content of 11 cells and found the same amount of pollen types that were reported for *Euglossa townsendi* (Silva et al. 2016) (50+ brood cells were analyzed).

Besides nesting sites, *Aechmea distichantha* may also offer food resources to *Euglossa* species. Pollen grains of several species of *Aechmea* have been reported in brood cells of *E. annectans* (Cortopassi-Laurino et al. 2009), but were not found in the samples we analyzed. Asteraceae pollen in the bees’ diet seems to be novel for *Euglossa* species. We found *Baccharis* (Asteraceae) pollen in three different brood cells, and in one cell it ac-
counted for more than 95 % of all pollen grains. This may indicate that either broods differed in their pollen need or that brood cells were filled in different seasons, when flower availability changed.

**Conclusion**

Given the importance of orchid bees as pollinators of Neotropical plants, aspects related to their maintenance, such as nesting sites, deserve priority in conservation, especially on islands where nesting habitats are less common than in other continuous environments. Previous studies on Ilha da Vitória showed low diversity of orchid bees on this island (Cordeiro et al. 2010) and, at the molecular level, there is lower genetic diversity than compared to bigger islands and the mainland (Boff et al. 2014). Due to the fact that stolons of *Aechmea distichantha* are suitable cavities for nests of *Euglossa cordata*, this plant may be an important resource for sustaining the population of orchid bees on this small island. Thus, *E. cordata* on the island may regularly use stolons for multiple generations. Lastly, they are polylectic but rely heavily on a few species of plants.

**Acknowledgment**

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**References**


**Supplementary material 1**

**Figure S1. Stolon of living bromeliads**
Authors: Samuel Boff, Isabel Alves-dos-Santos
Data type: species image
Explanation note: The internal moist tissues of stolons from living plants.
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Link: https://doi.org/10.3897/jhr.62.22834.suppl1

**Supplementary material 2**

**Figure S2. Details of Euglossa cordata nests**
Authors: Samuel Boff, Isabel Alves-dos-Santos
Data type: species image
Explanation note: Nests were found inside stolons of bromeliads (Aechmea distichantha) on Ilha da Vitória, in Brazil.
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Link: https://doi.org/10.3897/jhr.62.22834.suppl2