Scientific Note

Natural history notes on worker size, colony size, and nest structure of *Azteca muelleri* Emery, 1893 (Hymenoptera: Formicidae) in *Cecropia glaziovii* (Rosalés: Urticaceae) from the Atlantic Forest

Victor H. Nagatani¹, Nathalia S. da Silva¹, Emília Z. Albuquerque², André L. Gaglioti³, Maria S. C. Morini¹,¹,⁴,⁵

¹Universidade Mogi das Cruzes, Núcleo de Ciências Ambientais, Programa de Pós-Graduação em Biotecnologia, Laboratório de Mirmecologia do Alto Tietê, Mogi das Cruzes, SP, Brazil. ²National Museum of Natural History, Smithsonian Institution, Washington, DC, USA. ³School of Life Science, Arizona State University, Tempe, AZ, USA. ⁴Instituto de Botânica, Núcleo de Pesquisa e Curadoria do Herbário SP, São Paulo, SP, Brazil.

Corresponding author: mscmorini@gmail.com

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Abstract. Mutualistic association between *Azteca* Forel, 1878 ants and *Cecropia* Loefl. plants are one of the most studied interactions in Neotropics, however, natural history studies of *Azteca* species still poorly investigated due to the large effort required to conduct detailed descriptive studies. Here, we describe biological aspects of *Azteca muelleri* Emery, 1893 nesting in *Cecropia glaziovii* Snethl. in a fragment of Atlantic Forest, addressing (a) colony size; (b) nest distribution on the tree; and (c) worker and queen morphometrics. We collected two *C. glaziovii* saplings and counted the characteristics of the nests and plants. We randomly selected 140 workers to measure and to determine whether intraspecific polymorphism occurs. Workers, immatures, and mealybugs were present in all hollow internodes of the plant, and a queen was found. We found isometric morphological variation in *A. muelleri*. Our study provides new data to understand the biology of *A. muelleri* nesting in *C. glaziovii*, one of the most species-rich genera of arboreal ants known for the neotropics.

Keywords: insect-plant interactions, morphometrics, mutualism, polymorphism, rain forest.
is an indicator of body size (Kaspari 1996; Kaspari & Weiser 1999). We measured ants (workers and a queen) using a Motic SMZ-168 stereomicroscope with a camera attached. Images were analyzed using the software Motic Images Plus 2.0ML.

Figure 1. Map of São Paulo state in Brazil, showing Atlantic forest fragment in Guararema municipality where Azteca muelleri ants were found nesting in Cecropia glaziovii trees.

We used a key to the queens of Cecropia-inhabiting Azteca (Longino 1991b), and then compared our specimen with Azteca specimens deposited at the MZSP collection. At the same time, high-resolution images of the workers and the queen were sent to John T. Longino to confirm the identification. We deposited voucher specimens at the University of Mogi das Cruzes (São Paulo, Brazil) and Museu Paraense Emílio Goeldi (Pará, Brazil). The identification of C. glaziovii was carried out by André Luiz Gaglioti by analyzing type specimens at the MZSP herbarium (accession numbers SP 300499 and SP 300500). We deposited voucher specimens of the Cecropia trees specimens in the SP herbarium (accession numbers SP 300499 and SP 300500).

We analyzed the number of entrance holes in relation to the regions of the tree stem through a Kruskal-Wallis test using the software BioEstat 5.0 (Ayres et al. 2007). A bivariate scatterplot was created with high-resolution images using the software RStudio (RStudio Team 2019, version 3.6.1) and the packages car, for the Wald Test, and ggplot2, for data plotting.

The spatial distribution of A. muelleri ants along the cavities in the trunk of C. glaziovii was similar to other Azteca species (see Longino 1989). In A. constructor Emery, 1896, registered in tropical rainforest in Panama, colonies are distributed according to leaf growth, and most workers and immatures, as well as the queen, are found in the upper half of the trunk (Marting et al. 2018b). Colony distribution within cavities of the trunk of Cecropia spp. is highly variable according to the Azteca species, which may be related to defense of the host plant (see Longino 1991a), resource foraging by workers, and colony size (Marting et al. 2018b).

Table 1. Characteristics of Azteca muelleri intranidal population and of nest in the host tree Cecropia glaziovii.

| Intranidal Population/Tree | Tree 1 | Tree 2 | \( \bar{x} \) | SD | SE |
|---------------------------|--------|--------|--------|-----|----|
| Ant                       | Workers | 1,552  | 3,614  | 2,583 | 1,458 |
|                          | Queen   | 1      | 0      |       |     |
|                          | Eggs    | 81     | 0      | 40.5  | 57.27 |
|                          | Immatures | 273   | 0      | 136.5 | 193.04 |
| Mealybugs                 | Number of Entrances | 15   | 12     | 13.5  | 2.12 |
|                          | Height (m) | 2.30   | 2.60   |      |     |
|                          | Diameter at Breast | 11.86 | 12.62  |      |     |
|                          | Height (cm) | 1 (DBH) |       |      |     |
| \( \bar{x} \) = mean, SD = standard deviation, and SE = standard error |

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Figure 2. A - Cecropia glaziovii tree collected in Guararema, São Paulo, Brazil. B - Hollow internode in the trunk of a C. glaziovii tree. a: mealybugs; b: immatures, and c: workers of Azteca muelleri. Red arrows indicate worker-made entrances between tree cavities.
Our results suggest that A. muelleri is monogynous, similarly to A. nigricans Forel, 1899 and A. pittieri Forel, 1899 (Longino 2007); but we emphasize that we analyzed few individuals of C. glaziovii. Other species, such as A. jelskii Emery, 1893, are polygynous (Longino 2007), and some species have secondary monogy, where multiple queens cooperate to establish a new colony, followed by a fight until only one queen remains (Mayer et al. 2018), therefore social structure is variable among species. Of note, we did not find any queens in one of the trees collected. Although unlikely, this could indicate that we missed it during field work or specimen processing in the lab, or it may have already been dead.

We counted an average of 13.5 ± 2.12 (SD) entrances along the trunk that allowed entry and exit of A. muelleri workers. We found 5.5 ± 0.71 entrances in the apical region, 4.5 ± 0.71 entrances in the middle, and 3.5 ± 2.12 entrances in the basal region. The number of entrances was not significantly different (Kruskal-Wallis = 4.2978, d.f. = 2, p = 0.1166) between the three regions of the plant. When variation occurs in the number of entrances, it is usually associated with Müllerian bodies, located at the base of leaf petioles (Vieira et al. 2010). This idea is supported by C. pachystachya trees, because the number of ant-made entrances is higher in the apical region, where most Müllerian bodies are found (Vieira et al. 2010). However, we did not find the same pattern in C. glaziovii trees. This could be related to (1) the number of plants analyzed in this study or (2) the immaturity of the colonies, which is suggested by absence of winged males and females (Longino 1991b). We selected plants of the same height because the number of entrances is positively correlated with plant growth rate (Marting et al. 2018b), but it is possible that our results represent initial colony growth. In addition, the entrances were actively open which suggests that all of them were being used by workers to search for food resources and protect the tree against herbivores (Marting et al. 2018a).

Mean head width of workers was 0.91 ± 0.15 mm, and mean Weber’s length was 1.26 ± 0.20 mm. The head width of most workers was between 0.81 and 0.92 mm, while head widths between 1.15 and 1.21 mm were less common (Fig. 3A). Our results show that A. muelleri is isometric for head width and Weber’s length (regression, $r^2 = 0.7783$, scaling coefficient (log-log slope) = 0.6806; Wald test for comparing the scaling coefficient to 1, $P = 0.7707$), suggesting no size variation among A. muelleri workers (Fig. 3B). Only one queen was found, with a head width of 1.56 mm and a Weber’s length of 3.14 mm. Workers of the same species with different sizes can indicate the influence of habitat (e.g., preserved and disturbed areas), as is the case of Gnamptogenys striatula Mayr 1884 ( Oliveira et al. 2015). That is an external factor, but there may also be intrinsic factors (genotype and development), or a combination of both (nutrition and social environment) (Wills et al. 2018). Part of the colony’s nutrition comes from hemipteran honeydew (Longino 2007), and we found mealybugs (Coccoidea) inside the hollow internodes of C. glaziovii, with workers of A. muelleri surrounding insects that occupied the inner walls of plant cavities ( Johnson et al. 2001). The relationship is so close that colonies that have many workers and immatures also have a high number of hemipterans (Marting et al. 2018b).

Thus, despite the low number of tree, our study provides new data on natural history of A. muelleri ants in C. glaziovii trees in Atlantic Forest, one of the most species-rich genera of arboreal ants known in the Neotropics. These data help fill the information gap and support future studies of these two species. Unfortunately, insects like ants and their interactions with species of plants are still neglected in environmental conservation policies due to lack of knowledge about the biology of species, and this kind of information and may be important for conservation projects (e.g., pollination; Del-Claro et al. 2019) especially those involving biomes threatened by human processes, such as the Atlantic Forest.

![Figure 3. A - Frequency distribution of head width (in mm) of 140 Azteca muelleri workers nesting in Cecropia glaziovii trees. B - Relationship between head width and Weber’s length.](image)

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**Authors’ Contributions**

V.H.N.: Substantial contribution in the concept and designer of the study; Substantial contribution to data collection; Contribution to manuscript preparation; Contribution to critical revision adding intellectual content. N.S.S.: Substantial contribution to data collection; Contribution to manuscript preparation; Contribution to critical revision adding intellectual content. E.Z.A.: Substantial contribution in the concept the study; Identified Azteca species; Contribution to critical revision adding intellectual content. A.L.G.: Substantial contribution in the concept and designer of the study; Identified Cecropia species; Contribution to critical revision adding intellectual content. M.S.C.M.: Substantial contribution in the concept and...
designer of the study; Contribution to data collection; Contribution to manuscript preparation; Contribution to critical revision adding intellectual content.

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