


Scientific Note

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

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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.

Natural history studies (e.g., about ants) play a vital role in our understanding of biodiversity, evolution, and developing conservation programs (Rosumeck 2017) and they may help answer questions related to ants polymorphism; nest structure; influence of habitat on nest occupation; intranidal population and other invertebrates (other ants and non-ant arthropods) and nesting strategies (Longino 1991a). However, natural history works remain poorly explored due to the large effort required to conduct detailed descriptive studies, as in the case of ants occupying natural tree cavities (Oliveira et al. 2015a; Morais & Benson 1988).

Natural tree cavities in *Cecropia* Loefl. are colonized by *Azteca* Forel, 1878 ants by making a hole in the thinnest part of the inner wall of the trunk to nest (Longino 1991a). These natural cavities consist of small holes which open into the inside of stems or branches of the tree, and are divided by septa into a series of chambers also known as internodes (Longino 1991a; Oliveira et al. 2015a). In these mutualistic interactions, *Azteca* ants protect vegetative and reproductive *Cecropia* organs (Oliveira et al. 2015a, 2015b) while plants provide shelter and food, such as Müllerian bodies (Vieira et al. 2010). This relationship is very successful and effective due to the high level of aggressivity of *Azteca*, which responds directly to leaf herbivory damage (Marting et al. 2018a) and is one of the most studied interactions in Neotropics (Oliveira et al. 2015a, 2015b).

Azteca is a highly diverse and strictly neotropical genus of arboreal ants, and at least 21 species show a mutualistic relationship with *Cecropia* trees (Longino 1989, 1991a, 2007). In Brazil, there are twelve *Azteca* species, and nine of these were recorded in *Cecropia* (Harada & Benson 1988; Longino 1989, 2007). The biology of most species is poorly known; that includes *A. muelleri* Emery, 1893. This species occurs in the Neotropical Region and is widely distributed from southern to

northern Brazil. It is mainly distributed in the Atlantic Forest biome (Longino 2007), but there are records from other countries, such as Peru, Bolivia, and El Salvador (Guenárd et al. 2017). *Azteca muelleri* colonies form a spindle-shaped carton nest in the bole, which causes a bulging deformity in the trunk (Longino 1991a). It nests in several species of *Cecropia*, such as *C. glaziovii* Snethl, *C. angustifolia* Trécul, *C. adenopus* Mart. Ex Miq., and *C. pachystachya* Trécul (Longino 1991a; Rocha & Bergallo 1992) and their presence is beneficial for the plant, enhancing their growth and protecting against specialized herbivorous beetles *Coelomera ruficornis* Baly, 1885 (Coleoptera: Chrysomelidae) (Rocha & Bergallo 1992; Oliveira et al. 2005a).

On the literature just a few studies address *A. muelleri* biology and morphology (Longino, 1991a; Rocha & Bergallo 1992; Oliveira et al. 2015a, 2015b) and little is known about the biological characteristics of the nests of *A. muelleri* in Atlantic Forests fragments. In order to fill this gap, we describe some aspects of the natural history of *A. muelleri* related to (a) intranidal population, (b) nesting structure in *C. glaziovii* trees, and (c) worker and queen morphometrics.

We conducted fieldwork in March 2016 in at the edge of fragments of Atlantic Forest located in a rural area of the municipality of Guararema, São Paulo state, Brazil (-23.4605, -46.059), between 550 and 850 m.a.l.s. (Fig. 1). We cut down two saplings of *C. glaziovii* (between 2.30 and 2.60 m tall; growth is monopodial, i.e., from a single stem) at ground level and opened the stem at each internode. We counted the number of workers, immatures, eggs, queens, and mealybugs (intranidal population), in addition to the number of entrance holes in the trunk. We grouped all workers counted, and each received a number. We randomly selected 140 workers to measure head width and Weber's length. Head width is an important character to differentiate species (Longino 2007), while Weber's length

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.

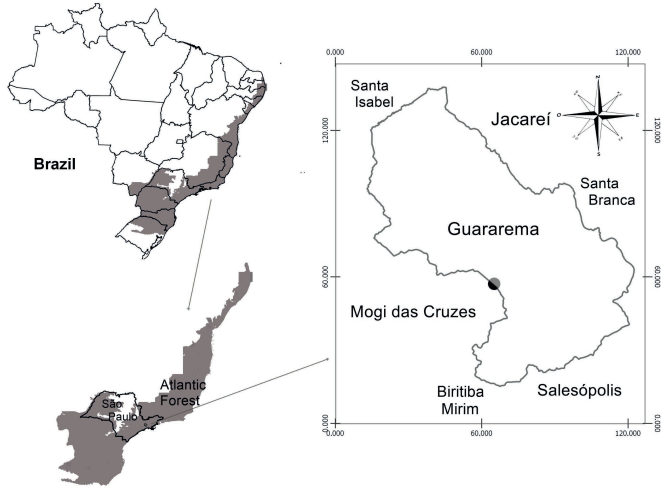


Figure 1. Map of São Paulo state in Brazil, showing Atlantic forest fragment in Guararema municipality where *Azteca muelleri* ants were found nesting in *Cecropia glaziovii* trees.

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, protologues and the collection of Herbário Maria Eneyda P.K. Fidalgo of the Instituto de Botânica de São Paulo (herbarium code: SP), Brazil. Both individuals were sterile, and the identification was confirmed using DNA sequencing (GenBank MW130832–MW130833). 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 head width data. We log-transformed the variables head width and Weber's length to analyze allometric scaling, testing if the observed scaling coefficient (log-log slope) differed from the predicted isometric scaling coefficient with a Wald test. These analyses were performed

using the software Rstudio (RStudio Team 2019, version 3.6.1) and the packages car, for the Wald Test, and ggplot2, for data plotting.

Between the two *C. glaziovii* trees, we found a total of 5,166 workers, 273 immatures, 81 eggs, and one queen of *A. muelleri*. In addition, we found mealybugs near immatures (Tab. 1). Workers, immatures, and mealybugs were found in all hollow internodes, that is, they occupied the entire trunk of the plant (Fig. 2B). The queen was found in the most apical cavity of the monopodial trunk. The colony size of *A. muelleri* found nesting in *C. glaziovii* in fragments of Atlantic rainforest was similar to the colony size of other *Azteca* species, such as *A. chartifex* Forel, 1896 and *A. alfari* Emery, 1893 also nesting in *Cecropia* in fragments of Amazon rainforest (Vasconcelos & Casimiro 1997). *Azteca*'s colony size may also depend on the species of *Cecropia* (Vasconcelos & Casimiro 1997) and size and age of the tree (Davidson & Fisher 1991), since nests are restricted by space inside trunks (Oliveira et al. 2015a).

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	20.28
	Queen	1	0			
	Eggs	81	0	40.5	57.27	6.36
	Immatures	273	0	136.5	193.04	11.68
Mealybugs		65	292	178.5	160.51	8.49
Tree	Number of Entrances	15	12	13.5	2.12	0.40
	Height (m)	2.30	2.60			
	Diameter at Breast Height (DBH) (cm)	11.86	12.62			

\bar{x} = mean, SD = standard deviation, and SE = standard error

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).

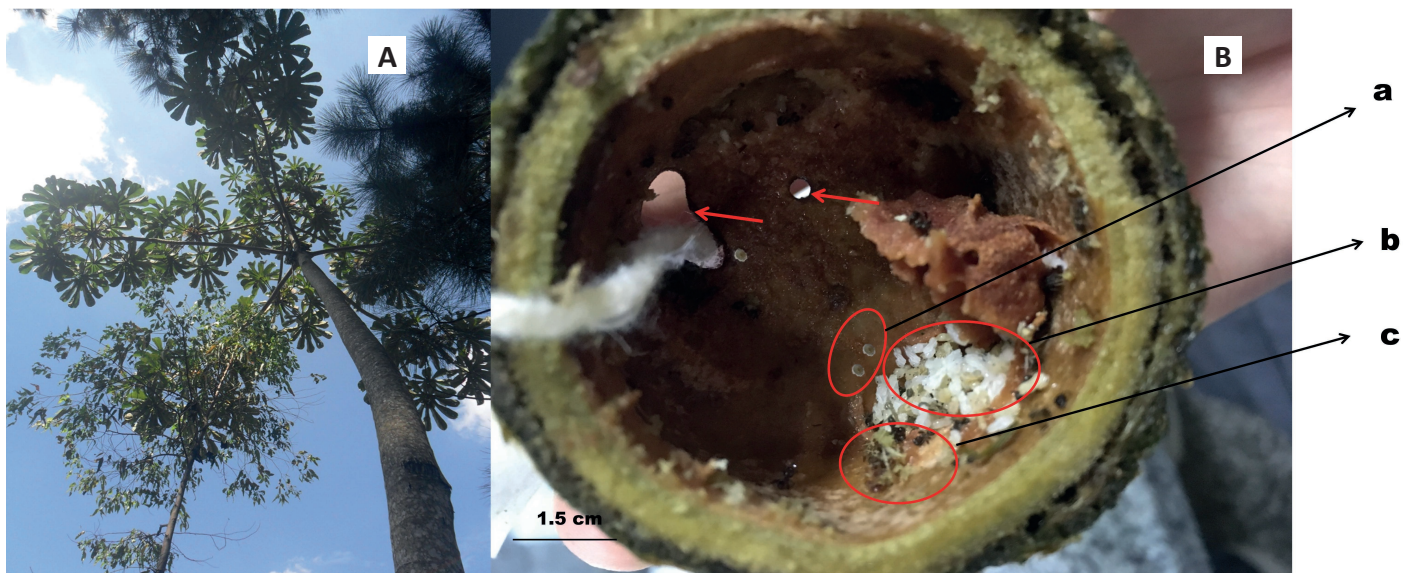


Figure 2. A - *Cecropia glaziovii* tree collected in Guararema, São Paulo, Brazil. B - Hollow internode in the trunk of a *C. glaziovii* tree. a: mealybugs; b: immatures, and c: workers of *Azteca muelleri*. Red arrows indicate worker-made entrances between tree cavities.

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 monogyny, 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.

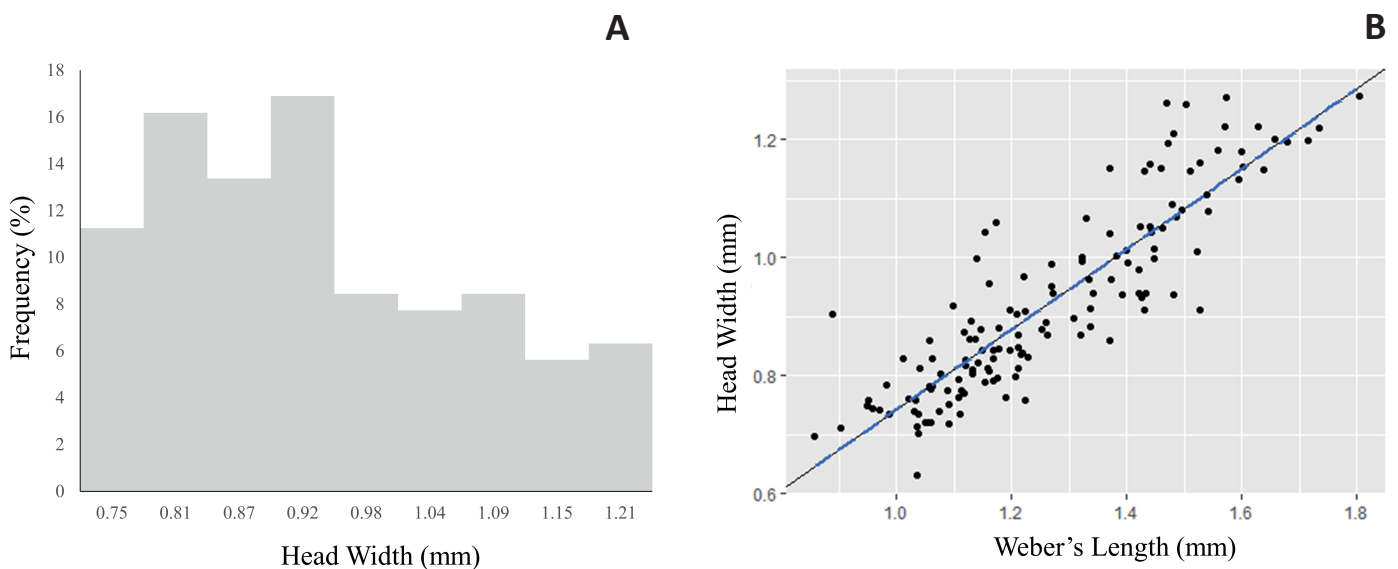


Figure 3. A - Frequency distribution of head width (in mm) of 140 *Azteca muelleri* workers nesting in *Cecropia glaziovii* trees. B - Relationship between head width and Weber's length.

Acknowledgments

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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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