



First record of *Cephalotes pusillus* Latreille, 1802 (Hymenoptera: Formicidae) nesting in a *Sceliphron* sp. (Hymenoptera: Sphecidae) nest

Rogério Henrique Custódio^{1✉}, Maria Julia Ricci da Costa Alvarenga², Andressa Inacio Bernal², Gabriel de Castro Jacques³ & Marcos Magalhães de Souza²

1. Universidade de São Paulo, Ribeirão Preto, SP, Brazil. 2. Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais, Inconfidentes, MG, Brazil. 3. Instituto Federal de Educação, Ciência e Tecnologia de Minas Gerais, Bambuí, MG, Brazil.

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Abstract. Solitary wasp nests (Sphecidae) are often occupied by other arthropods when abandoned. However, occupation by ants such as *Cephalotes* Latreille, 1802 is still uncertain. Species of *Cephalotes* are arboreal and occupy pre-existing cavities, commonly tree hollows. In this respect, the objective of this study is to report the first record of the ant *Cephalotes pusillus* (Klug, 1824) (Hymenoptera: Formicidae) nesting in a Sphecidae nest in areas cultivated with coffee, *Coffea arabica* L. The nest was collected on July 5, 2024, in a coffee plantation area in the municipality of Inconfidentes, Minas Gerais, Brazil. This area is associated with a fragment of Atlantic Forest. In the nest of *Sceliphron* sp. (Hymenoptera: Sphecidae), 64 pupae, 94 adults and four queens of the ant were counted. The successful occupation by the ant may have occurred due to different factors, such as the size and resistance of the nest, the size of the entrance and the multiple cells that are formed inside the nest, which allowed the use of this nest as an incubation site for the ant's larvae. This is the first record of *C. pusillus* nesting in a Sphecidae nest, but this species is a generalist in terms of the cavities it uses, requiring further observations to confirm the frequency of this behavior.

Keywords: Atlantic Forest; Ecology; Interaction; Reuse; Wasp.

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✉ Corresponding author:

Rogério Henrique Custódio
henriquero235@gmail.com



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Solitary wasps of the Sphecidae family are cosmopolitan (Bohart *et al.* 1976), their nests are made of mud, which serve as incubation sites for their larvae. In the natural environments, nests are built on shaded and protected substrates, such as rock ledges, protected places in trees or hollow logs (Naumann 1983). Active nests, as well as abandoned cavities, can be utilized by other arthropods (Naumann 1983; Yuan *et al.* 2022), serving as a valuable resource for insects that nest in pre-existing cavities (Fateryga & Kovblyuk 2014). In relation to ants, it is known that they attack and/or occupy abandoned and active nests of wasps with eusocial behavior (Souza *et al.* 2022), as well as preying on species of the Sphecidae family (Cane & Miyamoto 1979; Grajales & Wcislo 1998), but it is uncertain whether there is occupation of sphecid nests.

Ants of the genus *Cephalotes* sp. have a wide distribution in Brazil (Fernandes *et al.* 2024), and nest mainly in tree cavities (Moretti & Ribeiro 2006). However, more information is needed to understand whether these ants use other cavities for nesting.

Understanding the ethology of species helps to define strategies for the conservation of biota, particularly important in the Atlantic Forest region, which is suffering a rapid reduction in insect biodiversity (Lima *et al.* 2020). In this respect, the aim here is to report the first nesting record of the ant *Cephalotes pusillus* (Klug, 1824) (Hymenoptera: Formicidae) in a Sphecidae nest, in areas of coffee cultivation associated with a fragment of Atlantic Forest in southern Minas Gerais.

MATERIAL AND METHODS

The record occurred occasionally, on July 5, 2024, in an area of coffee plantations (*Coffea arabica* Linnaeus) associated with a fragment of semi-deciduous forest, in the Atlantic Forest domain. This area is located at the Federal Institute of Education, Science and Technology of Southern Minas Gerais (-22.309083, -46.329472), in the municipality of Inconfidentes, Southern Minas Gerais, Brazil. After spotting the Sphecidae nest and checking for the presence of ants inside, photographs were taken (with a Nikon coolpix p600). The nest was then collected by hand and placed in a container with 70% alcohol. The biological material was then taken to the Zoology Laboratory for sorting, where it was deposited in the CBVS collection (number 12.221-2024 to 12.318-2024). The nest was then opened and the number of ants, castes and stages of development were quantified. The ant was identified by Prof. Dr. Ricardo Ribeiro de Castro Solar from the Federal University of Minas Gerais (UFMG) and the wasp, identified by the shape of the nest, by Prof. Dr. David Barros Muniz from the Federal University of Maranhão (UFMA). The laboratory images were taken by Prof. Dr. Diogo Vilela (IFSULDEMINAS) using a Digilab DI-106T stereomicroscope and assembled using Inkscape software.

RESULTS AND DISCUSSION

A nest of the solitary wasp *Sceliphron* sp. (Hymenoptera: Sphecidae) was recorded on a *C. arabica* plant, approximately 1.80 cm above the ground. The nest was occupied by the ant *C. pusillus* (Figure 1), which was reusing the structure for its nest, that apparently had the entrance to the colony blocked by ants (Figure 2). There were 64 pupae, 94 adults and four queens in the occupied nest.



Figure 1. Workers of *Cephalotes pusillus* and *Sceliphron* sp. (Sphecidae) nest with the ants occupation.

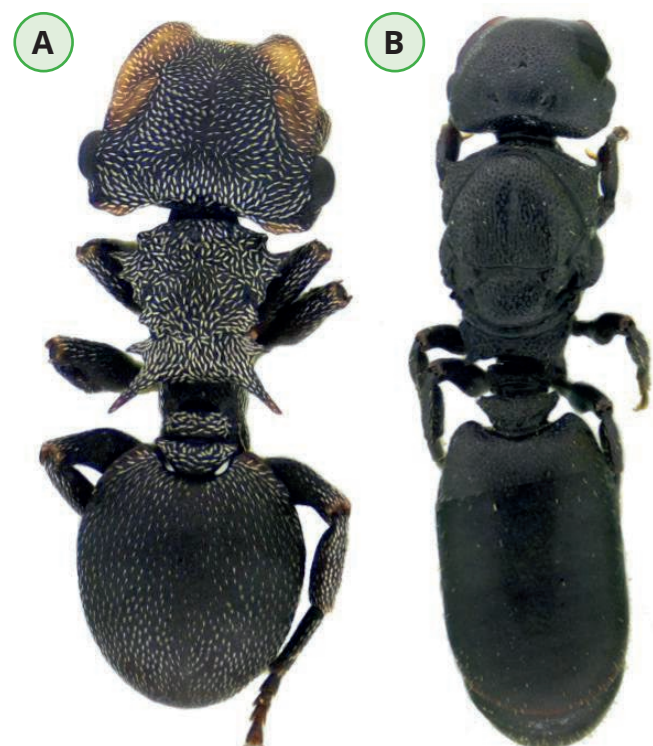


Figure 2. A. - worker of *Cephalotes pusillus* B - Queen of *Cephalotes pusillus*.

The successful occupation by the ants may have occurred as a result of different factors: first, the size of the nest entrance, as ants of the genus *Cephalotes* Latreille, 1802 (Hymenoptera: Formicidae) select cavities according to the ideal fit between the cavity entrance area and the soldiers' head area, to defend their nests against predators and other ants (Andrade & Urbani 1999; Powell 2008), as suggested in Figure 2; second, the multiple cells inside the wasp nest (Joothi *et al.* 2021; Messaitfa *et al.* 2023), used for incubating the ant larvae; third, due to the resistance of the nests of Sphecidae sp. (Park *et al.* 2022), which adds protection to the ants, since the usurpation of cavities by other ants is a constant threat to those that

live in tree cavities (Powell 2009; Powell *et al.* 2017); finally, the size of the Sphecidae nest was sufficient to support the colony of *C. pusillus*, a small ant, where the worker is 3 mm and the queen is 1 cm, that forms small colonies of less than 200 individuals (Del-Claro *et al.* 2002).

The genus *Sceliphron* Klug, 1801 (Hymenoptera: Sphecidae) is composed of 35 species (Pulawski 2003), two of which occur in Brazil: *Sceliphron asiaticum* (L., 1758) and *Sceliphron fistularium* (Dahlbom, 1843). Both species have nests with multiple mud cells (Freeman 1982), which form excellent shelters for different insect species, which can be tenants, parasites or scavengers (Yuan *et al.* 2022). Among the tenants, other families of Hymenoptera stand out, such as Crabronidae, Vespidae, Colletidae and Megachilidae (Fateryga & Kovblyuk 2014; Yuan *et al.* 2022). However, there is no record of nests of *Sceliphron* occupied by ants, although nests of other genera of Sphecidae have been reported attacked by ants (Cane & Miyamoto 1979; Rosenheim 1987).

Cephalotes pusillus has an arboreal habit (Del-Claro & Oliveira 1999), feeds on nectar and pollen, and commonly nests in cavities, such as in the dead trunks of different plant species (Del-Claro *et al.* 2002; Powell *et al.* 2014). This could explain its ability to locate the wasp's nest in a plant 1.8m above the ground. This ant uses chemical/odor clues, such as the feces of drilling beetles, to locate a nesting cavity (Del-Claro *et al.* 2021), and this may have happened with the Sphecidae nest. Although there is no record of *C. pusillus* nesting in a wasp's nest, it has shown itself to be a generalist in species of trees, as well as nesting in artificial wood hollows (Camarota *et al.* 2020), which suggests that this species has a high degree of plasticity when it comes to using cavities.

Sceliphron asiaticum and *S. fistularium* occur in all five regions of Brazil (Rosa & Muniz 2024), as does *C. pusillus*, which is present in all Brazilian biomes (Oliveira *et al.* 2021; Fernandes *et al.* 2024). Therefore, the use of nests of these species by this ant may be frequent, but the occupation cannot be dismissed as something casual, so further observations are recommended.

This is the first documented record of *C. pusillus* nesting in a Sphecidae nest. However, since this ant species is known to be a generalist when it comes to nesting sites, further research is needed to determine how common this behavior is and to gain a deeper understanding of its ecological implications.

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AUTHORS CONTRIBUTION

RHC: Investigation, methodology, Writing – original draft; Writing – review & editing; MJRCA: Writing – original draft and investigation; AIB: Writing – original draft; GCJ: Writing – original draft and investigation; MMS: Investigation, methodology, Writing – original draft and Writing – review & editing

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CONFLICT OF INTEREST STATEMENT

The authors state that there is no conflict of interest.

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