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Scientific Note

Record of parasitoidism in nests of the primitively social wasp *Zethus miniatus* Saussure, 1858 (Hymenoptera, Vespidae)

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Abstract. This note presents two new records of parasitoidism in the nest of the subsocial wasp species *Zethus* (*Zethoides*) *miniatus* Saussure, 1858. The nests were collected in two different localities in the Neotropical Savanna - "cerrado" (Minas Gerais State, Brazil). Two species from different insect orders were recorded parasitizing each of the nests. The parasitoid wasp *Brachymeria paraguayensis* (Brèthes, 1916) (Hymenoptera: Chalcididae); and the bee fly *Anthrax oedipus* Fabricius, 1805 (Diptera: Bombyliidae). Our note reinforces the importance of new studies describing the biology of vespids, especially in relation to natural enemies and ecological interactions.

Keywords: nesting; parasitoid, subsocial behavior, wasps, Zethinae.

Hymenoptera, an order of insects that comprises wasps, bees and ants, presents a variety of species of ecological, economic and evolutionary interest (Brock et al. 2021; Blaimer et al. 2023; Nastasi et al. 2023). It is in this order that we find the greatest diversity of described parasitoids (Nastasi et al. 2023). In addition to a range of taxa that exhibit behaviors related to sociality: from the construction of nests by just one female to colonies formed by hundreds of individuals, in which there is division of reproductive labor, overlapping generations and sterile castes (Hunt & Toth 2017; Sumner 2022).

Among the Hymenoptera, the Vespidae family stands out in the context of sociability since there are species described as solitary, others with social behaviors considered "intermediate" and truly social species (eusocial *sensu* Wilson) (Hunt & Toth 2017). It is interesting to note that sociality, with the sharing of nests between females, evolved independently in several lineages of Vespidae (Bank et al. 2017; Piekarski et al. 2018). An interesting record of this independent origin of social behaviors occurs in the genus *Zethus* Fabricius, 1804 (Vespidae: Zethinae) *sensu* (Piekarski et al. 2018).

This genus has the largest number of species in Vespidae, with 100 of them described from Brazil (Lopes et al. 2021; Lopes 2022; Hermes & Somavilla 2023). In recent studies, the genus has gained prominence due to its phylogenetic position: sometimes included in a clade with other solitary vespids (Eumeninae *sensu* Hermes et al. 2014), sometimes as a sister-group of vespids characterized as eusocial (Bank et al. 2017; Piekarski et al. 2018). New species of *Zethus* have been described and internal relationships within the genus have been elucidated (Lopes et al. 2021), however, as with many groups of Hymenoptera, aspects of the bioecology of the genus still need to be investigated.

Although recent studies related to the study of nesting and colony cycles of *Zethus* species have been published, other aspects related to the bioecology of the group still need to be complemented with new studies. Among them, the probable natural enemies that interact with species of the genus. This becomes even more relevant when we consider those species in which social behaviors are recognized, since such behaviors can be adaptive in defense against predators and parasitoids. Therefore, the objective of this work is to report the first cases of parasitoidism in the nest of the primitively social wasp *Zethus* (*Zethoides*) *miniatus* Saussure, 1858 (Hymenoptera: Vespidae).

Two nests were recorded and collected in the following locations: (A) in the city of Bambuí, Minas Gerais, Brazil, on the campus of the Federal Institute of Education, Science and Technology of Minas Gerais (20°1'56.280" S, 46°0'34.200" W), on November 21, 2021, during the hot and rainy season; (B) on a rural property, called "Rancho Areia", in the municipality of Papagaios, also in Minas Gerais (19°29'26.524" S, 44°45'46.811" W) on August 5, 2023, during the cold and dry season. Both locations are in the central-western region of the state and are approximately 215 km away. The institute's campus and the rural property are located in areas characterized as Cerrado biome phytophysiognomies, but with green areas affected by human presence; with greater human impact on the institute's campus.

Nest "A" was observed on a copper wire in one of the institute's buildings (Fig. 1A), while nest "B" was built on a rope support of an ornament present in the main residence of the rural property (Fig. 1B). It is interesting to note that in both cases the records were made associated with human constructions, with photographs being taken *in situ* before collections. Nest "A" was collected without the presence of adults and placed in a plastic container covered by a fabric structure, ensuring ventilation. Nest "B" was collected using a similar method, but with the presence of seven adult individuals: five that remained inside the cells; one flying over the nest; and one walking on it.

Before storage, the number of open and closed cells as well as the presence of adults inside the cells were recorded. The containers with the nests were kept in B.O.D-type incubators for approximately 40 days, at 25°C and 70% relative humidity (Jacques et al. 2022). During this period, the nest was monitored, recording the emergence of vespids as well as possible parasitoids from the sealed cells.

The insects that emerged were fixed in 70% alcohol and identified to the family level using the identification keys available in Nastasi et al. (2023). The identification of the vespids that emerged was carried out under a stereoscopic microscope, using the identification key of Carpenter & Garcete-Barrett (2002) for the generic level and the works of Lopes et al. (2021), Bohart & Stange (1965) and Lopes & Noll (2024) for the subgeneric, species group and specific levels, respectively. Additionally, the collected nest was compared with the descriptions

by Ducke (1914). The nest's parasitoids were sent for identification to specialists. The identification of the flies was carried out, from the insect's exuviae, using the work of Lamas & Couri (1991). The identification of the chalcid wasp genus was based on the keys provided by Bouček (1992) and Andrade & Tavares (2009). The identity of the chalcid species was reached by analyzing the type specimen deposited at the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (Buenos Aires, Argentina). The vespids and flies are deposited at the Federal University of Lavras (UFLA) and the chalcid wasps are in the entomological collection of the Federal University of Espírito Santo (UFES).

Figure 1. Nests recorded in the present study and collected in the following locations: (A) Bambuí, Minas Gerais, Brazil; (B) Papagaios, Minas Gerais, Brazil.

The nests were identified as belonging to the species *Z. (Z.) miniatus* (Fig. 2); being characterized by the aggregation of exposed barrel-shaped cells, with the presence of more than one individual per nest (Ducke 1914). In both nests, there was macerated green plant material covering sealed cells and other parts of the nest surface; especially in the nest "A". Nest "A" was 19.8 cm long, with 31 open cells, seven of which contained larvae, and 29 closed cells. Nest "B" was 4.3 cm long, with 17 open cells and 10 closed cells.

Figure 2. *Zethus (Zethoides) miniatus* collected in Bambuí, MG, Brazil.

Over the observation period, 34 individuals of *Z.* (*Z.*) *miniatus* emerged from nest "A", 13 males and 21 females. Nest "B" was collected containing seven adult individuals (five females and two males), and over the observation period three more individuals emerged from the nest: two males and one female. During the observation period, a larva was monitored in an open cell of nest "B", which did not pupate and emerge as an adult.

Ten parasitoids of the species *Brachymeria paraguayensis* (Brèthes, 1916) (Hymenoptera: Chalcididae) (Fig. 3A) emerged from nest "A", fifteen days after the collection, while all *Z.* (*Z.*) *miniatus* specimens had already emerged. From nest "B", two flies of the species *Anthrax oedipus* Fabricius, 1805 (Diptera: Bombyliidae) emerged (Fig. 3B). The first fly emerged from the nest 30 days after the collection; and the second one, two days after the first. In the nest "B", only one specimen of *Z.* (*Z.*) *miniatus* emerged after the emergence of the parasitoids.

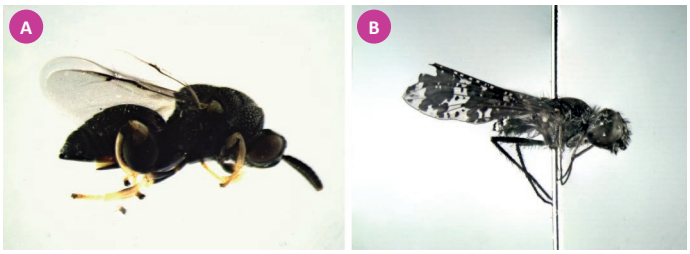

Figure 3. (A) *Brachymeria paraguayensis* (Brèthes, 1916) (Hymenoptera: Chalcididae) emerged from nest "A" collected in Bambuí, Minas Gerais, Brazil; (B) *Anthrax oedipus* Fabricius, 1805 (Diptera: Bombylidae) emerged from nest "B" collected in Papagaios, Minas Gerais, Brazil

Zethus (*Z.*) *miniatus* have primitive social behavior, in which females cooperate in building a nest, which is constructed from pieces of chewed leaves glued together with saliva. However, cooperation in brood care is not present, with each wasp caring only for its own offspring (Ducke 1914, as *lobulatus*).

The main natural enemies of solitary wasps are birds, ants (Ecitoninae), bats and parasitoids (West-Eberhard et al. 1995). The latter develop by feeding on immature stages of other arthropods causing the death of the host (Doutt 1973; Godfray 1994). However, there are few records of parasitoids of these vespids in South America (Auko et al. 2014) and for the genus *Zethus*, there is only one record for Ichneumonidae, in Hong Kong (Barthélémy & Broad 2012).

Brachymeria Westwood, 1829 is one of the most common and widely distributed genera in Chalcididae, parasitizing a number of important insect pest species (Gauld & Bolton 1988). In Brazil, there are records of parasitoidism in flies from the Sarcophagidae and Calliphoridae families (Marchiori et al. 2003), and in several Lepidoptera, such as *Thyrinteina leucocerae* (Rindge, 1961) (Lepidoptera: Geometridae) (Zaché et al. 2012) and *Historis odius* (Fabricius, 1775) (Nymphalidae) (Gil-Santana & Tavares 2005). In relation to Hymenoptera, some genera and species have already been reported being parasitized, such as: *Trypoxylon* Latreille, 1796 (Crabronidae) (Peruquetti 2001); and *Leipomeles spilogastra* (Cameron, 1912) (Vespidae) (Somavilla et al. 2015). However, this is the first record of this genus parasitizing Eumeninae/Zethinae.

Anthrax oedipus is a highly generalist fly, and females do not invade their hosts' nests and their larvae indirectly attack brood cells (Gerling & Hermann 1976). This Diptera has been frequently associated with nests of several Hymenoptera taxa, such as Apidae (Garófalo et al. 1998; Lamas & Couri 1999; Augusto & Garófalo 2004; Freiria et al. 2017; Gazola & Garofalo 2009), Megachilidae, Sphecidae (Lamas & Couri 1999) and Crabronidae (Rocha-Filho et al. 2019). For Eumeninae, there is a record of parasitism in *Pachodynerius brevithorax* (Saussure, 1852) (Lamas & Couri 1999), but there is no record in Zethinae.

Despite recent efforts to understand aspects of the biology of non-eusocial wasps from the Neotropics, there are still several gaps to be filled. As is the case for most groups of insects, information about the biology and ecological interactions of these vespids is scarce. Furthermore, knowledge of the natural enemies of Zethinae, especially of a primitively social species, can aid in the interpretation of their nesting behavior, and provide clues about how social behaviors evolved in Vespidae.

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Authors' Contributions

GCJ: Conceptualization; Methodology; Writing - Review & Editing. WDF: Methodology; Writing - Review & Editing. LCSA: Investigation; Writing - Original Draft. VLS: Investigation; Writing - Original Draft. LCPS: Resources; Writing - Review & Editing.

Conflict of Interest Statement

The authors declare no conflict of interest.

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