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Scientific Note

First nesting site record and observation of nesting behavior of the bee species *Diadasina paraensis* (Ducke, 1912) (Hymenoptera, Apidae, Emphorini)

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Abstract. It present here the first record of the nesting site of the bee species *Diadasina paraensis* (Ducke, 1912), found in the Amazon region of the Brazilian state of Pará. The nesting site comprises a dense aggregation of 198 nests constructed on flat and bare ground, in soft and humid compact sandy soil near the bank of the Paranaquara river. Each nest entrance has a typical vertical tumulus with a circular opening slightly curved in shape. Males were found patrolling the area and just one female was observed per nest, indicating the solitary behavior of the species.

Keywords: Amazon, Biology, Brazil, Ground-nesting, Solitary bee.

Bees are the most effective pollinators of nature and play an important role for the maintenance of crops plants and conservation of wild ecosystems (Michener 2007; Potts et al. 2016; Orr et al. 2021). The understanding of the diversity distribution and biological aspects of bee species is key to evolutionary, ecological, or conservation-oriented studies (Potts et al. 2016; Danforth et al. 2019; Orr et al. 2021). Details of nesting behavior and nest architecture provide meaningful insights for our understanding of adaptive traits related to the evolution and conservation of the group (Michener 2007; Danforth et al. 2019; Harmon-Threatt 2020; Antoine & Forrest 2021). Bees construct their nests excavating burrows in the soil, cavities in wood or in other substrates, for example occupying small spaces in manmade structures (Michener 2007; Danforth et el. 2019). However, they can also be found in association with social insect nests (wasps, ants, and termites) as documented for many species of stingless bees (Meliponini) (Michener 2007; Harmon-Threatt 2020; Antoine & Forrest 2021). The nests typically consist of a main burrow which gives rise to lateral burrows, each containing lined or unlined brood cells, which serve to protect the delicate immature stages and the food for the growing larvae (Michener 2007; Danforth et al. 2019).

Despite such enormous importance, the nests of most solitary bee species have not yet been described, mainly due to the difficulty of finding nests in the natural landscape, especially in the case of ground-nesting bees (Danforth et al. 2019; Harmon-Threatt 2020; Antoine & Forrest 2021; Martins et al. 2021). It present here for the first time the data and observations of a nesting site of *Diadasina paraensis* (Ducke, 1912) (Hymenoptera: Apidae), an Emphorini species restricted to Brazil which is found in the states of Amapá, Bahia, Ceará, Pará, and Rio Grande do Norte (Silveira et al. 2002; Michener 2007; Moure & Melo 2022). The records of Emphorini for tropical forests in Brazil are scarce, especially in the northern region of the Amazon rainforest. *Diadasina* Moure, 1950 is a genus of hairy and small bees (6 to 8 mm in length) restricted to South America and distributed in Argentina, Brazil, Bolivia, and Paraguay (Silveira et al. 2002; Michener 2007; Moure & Melo 2022). The biology and nesting behavior of *Diadasina* species is poorly known. At least eight species are included in the genus, however, nesting biology is only known for *Diadasina distincta* (Holmberg, 1903) and *Diadasina riparia* (Ducke, 1907) (Hymenoptera: Apidae). Biology and nesting behavior of *D. distincta* were broadly studied by Martins & Figueira (1992), Martins & Antonini (1994), Martins et al. (1999), Martins & Borges (1999), and Antonini et al. (2000) in a nesting site located in Minas Gerais (Brazil), and by Hazeldine (1997) in a nesting site located in Buenos Aires (Argentina). Nests of *D. riparia* were found in Pernambuco (Brazil) and studied by Martins et al. (2021). Females of *Diadasina* have been recorded collecting floral resources in several plant families such as Asteraceae, Malvaceae, Melastomataceae, Pontederiaceae, and Polygonaceae, but also show a high degree of specialization (oligolect) on the pollen of Onagraceae (Hazeldine 1997; Martins & Borges 1999). The genus is characterized by solitary, multivoltine, and ground-nesting species which nest in dense aggregation, in bare and flat soil, and the same natal site (or very near it) for successive generations – philopatry; each nest is a burrow ended in a single cell and each female must make several nests (Martins & Figueira 1992; Martins & Antonini 1994; Hazeldine 1997; Martins & Borges 1999; Martins et al. 1999; Antonini et al. 2000; Martins et al. 2021).

The nesting site of *D. paraensis* was found and studied on December 8, 2011, in São Judas, a community on the bank of the Paranaquara river in the Chicaia micro-region, situated in the city of Almeirim, Pará, Brazil (UTM 0284760/9821780). The area has a mosaic of rich floristic diversity, with a predominance of ombrophilous forests, estuarine floodplains, and savannas (general fields) (Renó et al. 2011). The climate is Tropical rainforest climate according to the Köppen classification. This means that it is tropically humid and hot with temperatures that do not vary significantly, and two well-defined climatic seasons: a dry period (May to September) and a rainy period (November to March) (Fisch et al. 1998). Individuals' activity and the entrance of the nests at the aggregation were ascertained by direct observation in the nesting site. The terminology used to describe the aggregation features, nest entrance, nesting site and behavior follows the same employed by Martins & Figueira (1992), Martins & Antonini (1994), Hazeldine (1997), Antonini et al. (2000), and Martins et al. (2021). Imagens in the field were taken with camera Digital Sony Cyber Shot DSC-H55.

Specimens were collected using an entomological net and stored in vials with 98% alcohol. Selected individuals were pinned and studied in the Laboratório de Entomologia de Ecossistemas, Instituto de Pesquisas Científicas e Tecnológicas do Estado do Amapá (IEPA) and in the Laboratório de Artrópodes, Universidade Federal do Amapá (UNIFAP). Bees were identified to the tribe and genus levels using the identification keys of Silveira et al. (2002) and Michener (2007), and to the species level by the specialists cited in the Acknowledgments. Voucher specimens were deposited in the Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil (MZSP) and Universidade Federal do Paraná, Curitiba, Brazil (UFPR). Images of the voucher female and male (Fig. 1) were taken with a Leica video camera MC190 HD attached to a Leica M205C stereomicroscope, and the series of images were processed in the software Helicon Focus 6.7.1 to produce confocal images.

The nesting site found comprises a large aggregation of 198 nests in an area of 15 m², counted by the number of entrances. The estimated density of nest in the aggregate was 13.2 entrances/m². Females were found excavating isolated nests on flat and bare ground, among grasses vegetation and dry leaves, and near the bank of the river (Figs. 2A-C). The substratum which the aggregation was found is characterized by a soft and humid compact sandy soil. The entrances were fully exposed to the sun. The nests were excavated with the mandibles and the soil was pushed out by the female's hind legs and abdominal movements. The mud pellets were then pushed out by her legs and deposited around the nest entrance (Figs. 2B and D). Each nest had a typical vertical tumulus with a slightly curved shape (Fig. 2E). The entrance has a circular opening of 0.5 cm diameter and a height of 2.65 cm on average (n=4) with a rugose aspect (Figs. 2D-E). Nests were not excavated and need further investigation. Males were found patrolling the area, but mating was not observed. The species appears to be solitary as multiple females sharing the same nest have not been observed. Floral resources of *Eugenia* sp., *Myrta* sp. (Myrtaceae), and one unidentified species of Asteraceae were observed near the nesting site. Also, *Astrocaryum aculeatum* G. Mey, *Mauritia flexuosa* Mart., and *Euterpe oleracea* Mart. (Arecaceae), and *Triplaris americana* L. (Polygonaceae) are also evident in the landscape where the nests are located.

The nesting behavior and nest architecture observed for *D. paraensis* were similar to other *Diadasina* species. The nesting sites of *D. riparia* and *D. distincta* are composed of a dense aggregation in compact sandy soil, with a circular nest entrance from which a short tunnel ending in one or more brood cells, and vertically constructed in relation to the surface (Martins & Figueira 1992; Martins & Antonini 1994; Martins et al. 1999; Antonini et al. 2003; Martins et al. 2021). *Diadasina riparia* can also construct their nests on the vertical surfaces of hard clay escarpments with the entrances arranged in the horizontal position and a tunnel assuming vertical orientation (Martins et al. 2021). *Diadasina paraensis* and *D. distincta* nests exhibit more elongated and curved turrets rising vertically from the nest entrance when compared to the tiny turrets observed in *D. riparia* (Martins & Antonini 1994; Martins et al. 2021). *Diadasina riparia* and *D. distincta* are multivoltine species with nesting activity in both the dry and the rainy seasons, including a diapause period as part of their developmental biology (Martins & Figueira 1992; Martins & Antonini 1994; Martins et al. 1999; Antonini et al. 2003; Martins et al. 2021), and it is plausible to

Figure 1. Voucher specimens of *Diadasina paraensis* (Ducke, 1912) from Almeirim (Pará, Brazil) deposited in the Museu de Zoologia, Universidade de São Paulo, Brazil. (A) Head of female in frontal view. (B) Body of female in lateral view. (C) Head of male in frontal view. (B) Body of male in lateral view. Scale bars (a) and (C): 0.5mm; (B) and (D): 1mm.

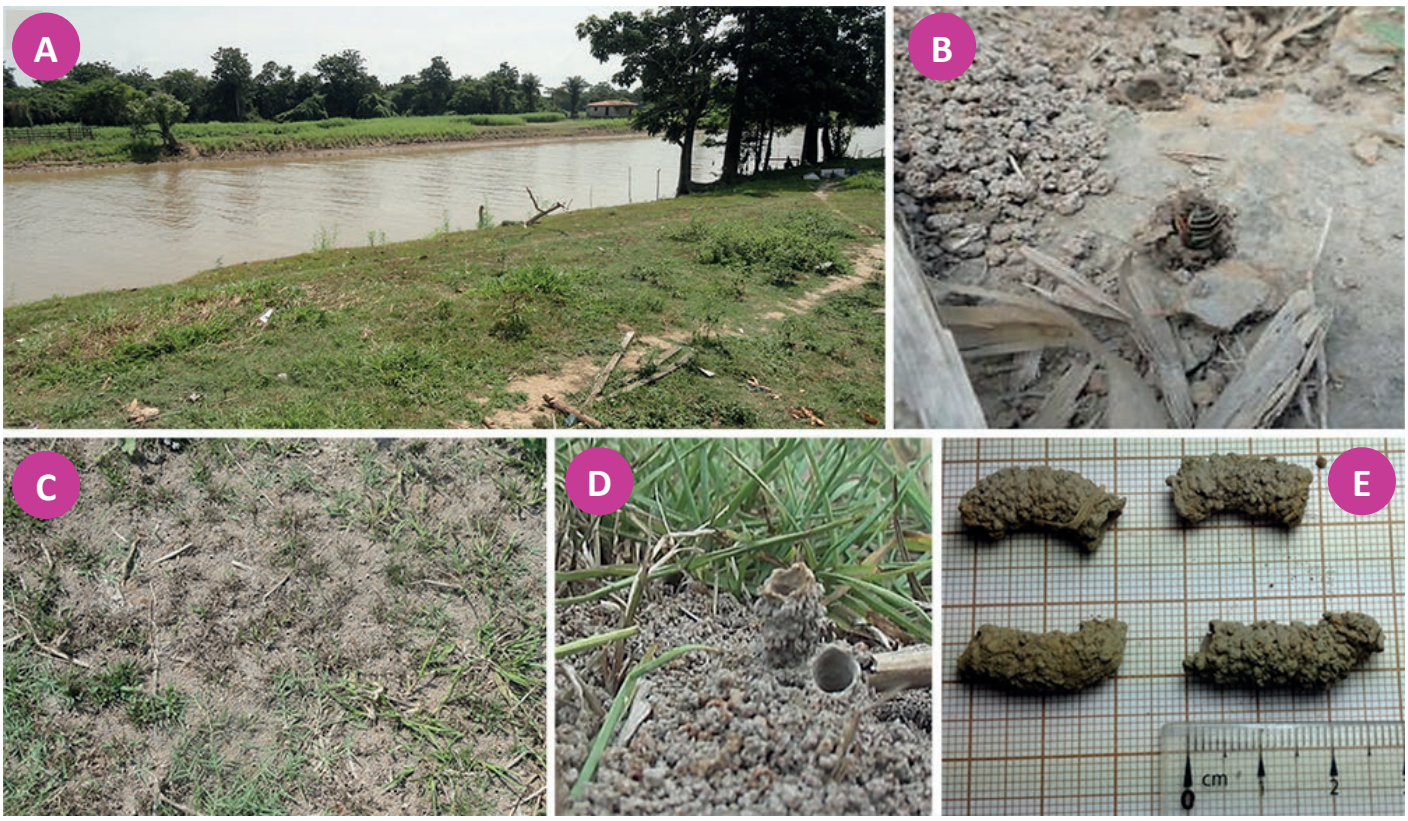

Figure 2. Nesting information of *Diadasina paraensis* (Ducke, 1912) from Almeirim, Pará, Brazil. (A) Nesting site on the bank of the Paranaquara river. (B) Female excavating a nest. (C) Ground nesting aggregation. (D) Nest entrance showing the vertical tumulus. (E) Aspect of the vertical tumulus.

infer that *D. paraensis* shares these same characteristics. A diapause period is also recorded for other Emphorini genera, and the ancestral reconstruction analysis of diapause in bees showed that *Diadasia* and *Ptilothrix* have a common ancestry in the developmental diapause (Santos et al. 2019; Martins et al. 2021).

The gregarious nesting behavior in bees is mainly associated with the availability of substrate, philopatry, and parasites. Ground-nesting behavior is a common feature among solitary bees and site selection is an important step of the nesting process, playing a key role in bee fitness (Danforth et al. 2019; Antoine & Forrest 2021; Gardein et al. 2022). The substrate directly impacts the selection of the nesting site since females of different ground-nesting bee species shows clear preferences for some specific soils which provides, for example, some favorable thermal or humidity conditions (Sabino et al. 2020; Antoine & Forrest 2021). Floral and nesting resources are the most important factors for females in relation to habitat suitability (Roulston & Goodell 2011; Harmon-Threatt 2020; Antoine & Forrest 2021; Sabino et al. 2020; Gardein et al. 2022). For bees, a local habitat quality (protected from adverse weather conditions, excessive humidity, predators, and parasites) with proximity to food plants for larval development and survival is essential to maximize the success of their brood and species persistence since solitary bees do not provide care to their offspring after provisioning and sealing the brood cells (Roulston & Goodell 2011; Harmon-Threatt 2020; Sabino et al. 2020; Antoine & Forrest 2021). In addition, the philopatry is directly related to the idea of the time a female spends searching for the ideal nesting location in relation to the time for the provision and egg laying or the construction of additional nests (Roulston & Goodell 2011; Sabino et al. 2020; Antoine & Forrest 2021). These factors are mentioned to make sense of the dense gregarious nesting sites and natal philopatric behavior observed in *D. distincta* and *D. riparia*, and future studies could show these same features to be occurring with *D. paraensis*. The natal philopatry in *Diadasina* can also be associated with oligolectic behavior since the females should prefer to nest near flowers in which they are specialized. However, the philopatry found in *D. distincta* was attributed to the specific edaphic conditions rather than proximity to the floral resource (Antonini et al. 2000).

This study gives us some insight into the nesting biology of *Diadasina paraensis* and therefore into the genus. However, it also raises many questions that need investigation and further studies are clearly necessary to understand the nesting biology of *D. paraensis,* which is unknown so far.

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Authors' Contributions

R.F.F. collected the specimens, data, and images of the nesting site. K.S.R. produced the digital images of the species. All authors wrote the manuscript, discussed the data, and contributed to its final version.

Conflict of Interest Statement

The authors have no conflicts of interest.

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