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SHORT NOTE

Nest camouflage in *Metapolybia cingulata* and nesting and colony defensive behavior in *Metapolybia docilis* (Vespidae: Polistinae) in the Brazilian Atlantic Forest

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Abstract
Colony defense by social wasps includes aggressive behavior and camouflage with a high diversity of adaptation of their nests to the environment, including substrate choice. The objective of this study was to present new data on nesting and colony defense by the Neotropical social wasps *Metapolybia cingulata* and *Metapolybia docilis* (Hymenoptera: Vespidae). Field observations were made on January 4th and 5th, 2022 at the State Park of Iguaçu, state of Paraná, Brazil, on *M. cingulata* and *M. docilis* nests and information on nesting of these wasps obtained in 2014 at the Pandeiros River Wildlife Refuge (REVIS), and in 2012 at the Rio Doce State Park, state of Minas Gerais, Brazil. The types of substrates used by *M. cingulata* and *M. docilis* reinforce the importance of nest camouflage as a defense strategy for their colonies. The selection of nesting sites by these species was very variable.

The genus *Metapolybia* includes 18 species of social wasps of the tribe Epiponini with distribution restricted to the Neotropics (Andena et al., 2019), 16 of which are recorded in Brazil (Cooper, 1999; Andena & Carpenter, 2011; Andena et al., 2019; Somavilla et al., 2021). Wasps of this genus build colonies by swarming and build nests on different substrates (Souza & Zanuncio, 2012), without a pedicel and classified as astelocyttarus (Richards & Richards, 1951) and, for the most part, with a protective envelope (Cooper, 1999).

Similarities between the chemical composition of social wasp nests and the plants on which they were built can provide information on the ideal physical conditions and safety and integrating the "chemical signature" of their colonies (Sguarizi-Antonio et al., 2021).

Colony defense strategies by social wasps are diverse, including repellent substances against ants (Jeanne, 1970), nest built with protective envelope or camouflage and aggressive behavior (Chavarría-Pizzarro & West-Eberhard, 2010; Barbosa et al., 2016; Milani et al., 2021).

Defense strategies of *Metapolybia* species include camouflage by homochromy and colony homotypy on tree trunks (Somavilla et al., 2012; Souza et al., 2020) and aggressive behavior in eventual disturbances, as reported for *Metapolybia cingulata* (Fabricius, 1804; Souza et al., 2020). The objective of this study was to present new data on nesting and colony defense in Neotropical social wasps *Metapolybia cingulata* (Fabricius, 1804) and *Metapolybia docilis* (Richards, 1978) (Hymenoptera: Vespidae).

M. cingulata nests were recorded in 2010, in an evergreen forest in the Rio Doce State Park (19°42' S, 42°34' W) and the others in deciduous forest in the Pandeiros River Wildlife Refuge (REVIS) (15°88' S, 45°95' W), state of Minas

Gerais in 2014. Nests of *M. docilis* were recorded in September 2014 and 2021 and in January 2022 in the State Park of Iguaçu (25°22'S, 54°2'W), state of Paraná, all in Brazil.

Behavioral aspects of one colony of *Metapolybia docilis* were observed and recorded in the State Park of Iguaçu using the *ad libitum* method (Del-Claro, 2010) for 50 minutes divided into 10-minute sessions with two at 9:30 a.m., two at 5:00 p.m. and one at 1:00 p.m. on January 08th, 2022. This colony was subjected to stress stimuli: 1) manipulating the nest; 2) seeking to catch the specimens; 3) removing part of the nest envelope to model the behavior related to the aggressiveness or docility of *Metapolybia*. A part of the colony envelope (≈ 3 cm) was removed by exposing the comb (Chavarría-Pizzarro & West-Eberhard, 2010). The height of the other colonies made these procedures impossible.

This study complied with the norms of the SISBIO (licence number: 76084-3).

Each nest of *M. cingulata* and *M. docilis* was located in different plant species and anthropic substrates (Table 1).

Table 1. Data on numbers of colonies, locality, phytophysiognomy nesting substrate, height above the ground and nest color of *Metapolybia cingulata* and *Metapolybia docilis* (Hymenoptera: Vespidae) colonies.

Data	Species	
	Metapolybia cingulata	*Metapolybia docilis*
Number of colonies	Nine	Four
Locality	State Park of Rio Doce (one nest); REVIS Rio Pandeiros (eight nests)	State Park of Iguaçu
Phytophysiognomy	Evergreen forest (one nest); Deciduous Forest (eight nests).	Evergreen forest
Nesting Substrate	*Euterpe edulis* (Arecaceae) (one nest); house construction lumber (eight nests).	Trunk of *Pliniarivularis* (Cambess.) Myrtaceae (one nest); unidentified tree species (one nest), residential walls (two nests).
Height above the ground	1.5 m (one nest); 2 to 2.4 m (eight nests).	2 m (one nest); 3.5 m (one nest); 4 and 5 m (two nests).
Nest Color	Light grey to dark gray, similar to substrate.	Light grey to dark grey, similar to substrate.

Diversity of plant species used as substrate for nesting by *M. docilis* and *M. cingulata* were similar to those reported for *Polistes actaeon* Haliday, 1836 using from tree species, as *Aspidosperma cuspa* (Khunt), to shrubs, such as *Baccharis dracunculifolia* DC., and for *Polybia platycephala* Richards, 1978 and *Protopolybia sedula* (Saussure, 1854), nesting in nine and 19 plant species, respectively (Alvarenga et al., 2010; Souza et al., 2014).

The *M. cingulata* and *M. docilis* nesting, on anthropic substrates, evidences the plasticity of nest site selection in *Metapolybia* species as reported for *Mischocyttarus*, *Polistes* and *Polybia* (Silva et al., 2019; Barbosa et al., 2020) in timber and walls (Oliveira et al., 2017; Silva et al., 2019) and *Metapolybia miltoni* Andena & Carpenter, 2011 (Silva et al., 2019), *Metapolybia fraudator* Carpenter and Andena, 2019 (Andena et al., 2019) and *Metapolybia unilineata* (Ihering, 1904) (Somavilla et al., 2012) nesting in wooden houses. Nesting in human constructions can offer greater protection against environmental factors, such as rainfall, wind and temperature extremes (Silva et al., 2022), which explains the use of these substrates near forested areas.

The color of *Metapolybia* nests, similar to the substrate, seems to be related to homochromy camouflage, since in all the records made, there is a similarity between the color of the nest and the substrate (Fig 1), whether in a natural environment, using plant substrates, such as tree trunks or palm tree stems, and in anthropic environments, nesting on walls and wooden structures of houses, as already reported and suggested for species of the genera *Mischocyttarus* and *Parachartergus* (Souza et al., 2020), *Metapolybia cingulata* (Souza et al., 2020) and *Chartergellus communis* Richards, 1978 (Silva et al., 2022), which suggests that it constitutes a defense mechanism against visual predators, such as birds.

The height of *M. cingulata* and *M. docilis* colonies on plant substrates was lower than those on anthropic substrates, the latter on roofs, and wooden or concrete structures with greater protection from the weather (Barbosa et al., 2020). This condition may be associated with the fact that nesting on anthropic substrates, below 2 m, could facilitate the destruction of nests by humans (López et al. 2012), however, this assumption needs to be better evaluated.

Individuals of *M. docilis*, after stimulation by stress, behaved as follows: upon the first stimulus (touching with the hand or the handle of the entomological net), the individuals raised their heads and the first pair of legs, moving them intensely, but without attacking (Fig 2A), characterizing bluff behavior (Chavarría-Pizzarro & West-Eberhard, 2010). This may also characterize an alarm behavior, but no contraction of the gaster or wing movement was observed, as reported for *Polistes erythrocephalus* Latreille (1813) (West-Eberhard, 1969),

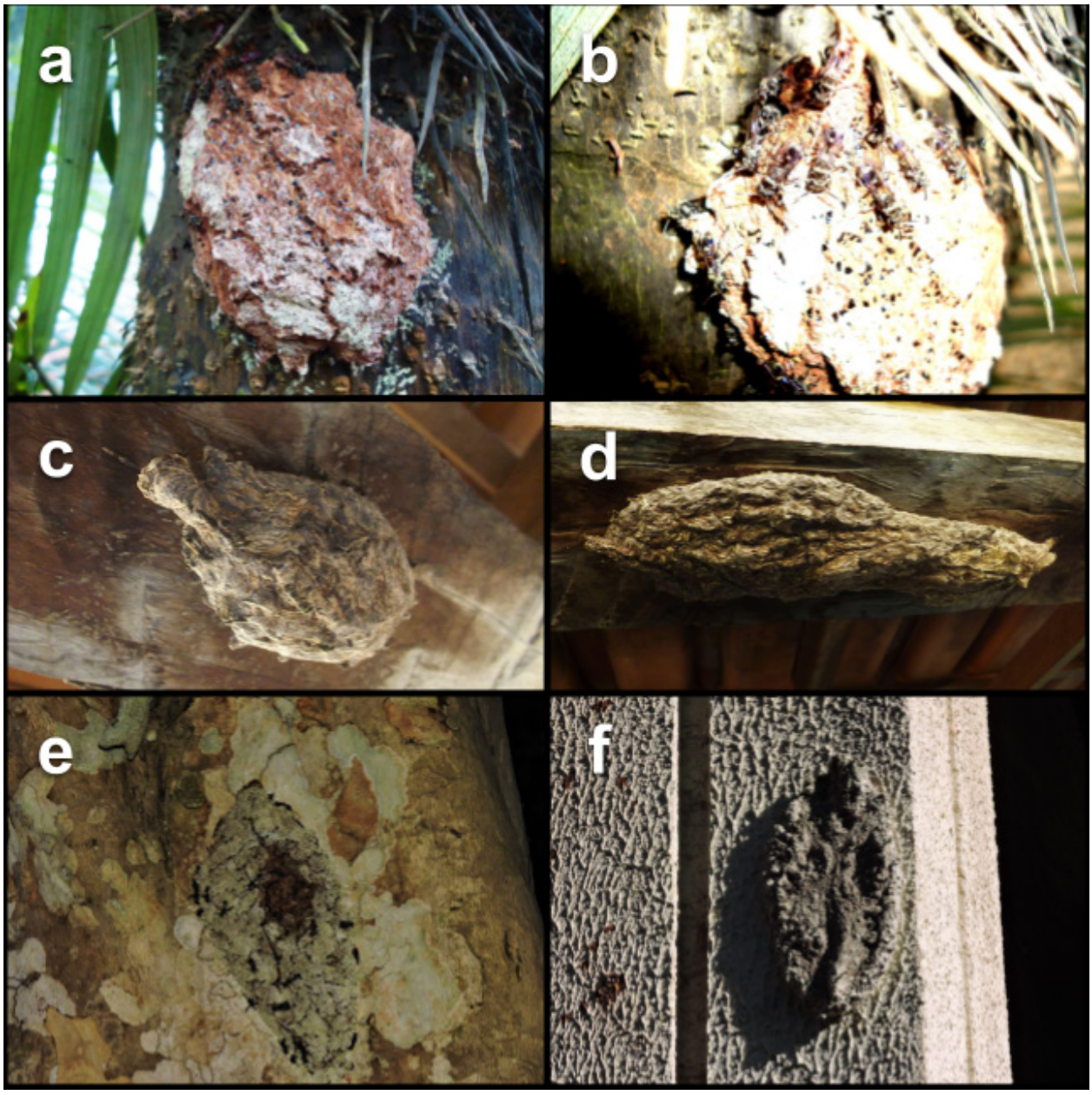

Fig 1. Nest camouflage in *Metapolybia docilis* (Hymenoptera: Vespidae) (a and b) at the State Park of Rio Doce; *Metapolybia cingulata* in the Pandeiros River Wildlife Refuge (REVIS) (c and d) and *M. docilis* in the State Park of Iguaçu (d, e and f), Brazil.

Polybia occidentalis (Olivier, 1791) (Jeanne, 1981) and *Protopolybia exigua* (Saussure, 1854) (Chadab, 1979). Alarm behavior was recorded in the morning, but not in the late afternoon, when the sun was directly shining on the colony, increasing wasp activity with foraging and colony maintenance (Giannotti et al., 1995; Santos & Presley, 2010).

Wasp individuals subjected to the second (collection of individuals) and third (attempt to remove the colony envelope) stimuli raised their heads and the first pair of legs, moving them intensely, but they flew away or remained in the surroundings without attacking (Fig 2B). The absence of attack with only bluff behavior characterizes *M. docilis* as docile, increasing the importance of nest camouflage for this species (Chavarría-Pizzarro & West-Eberhard, 2010). However, the absence of sting attempts by this wasp differs from that reported for *M. cingulata* (Souza et al., 2020), showing that the colony defense behavior by *Metapolybia* species involves camouflage and, also, some degree of aggressiveness (Forsyth, 1981), as reported for *Synoeca* wasps (Chavarría-Pizarro & West-Eberhard, 2010).

Camouflage as a defense strategy is important to select nesting substrates by *Metapolybia cingulata* and *Metapolybia docilis* and shows the plasticity in the selection of anthropic substrates or plants for nesting by these wasps.

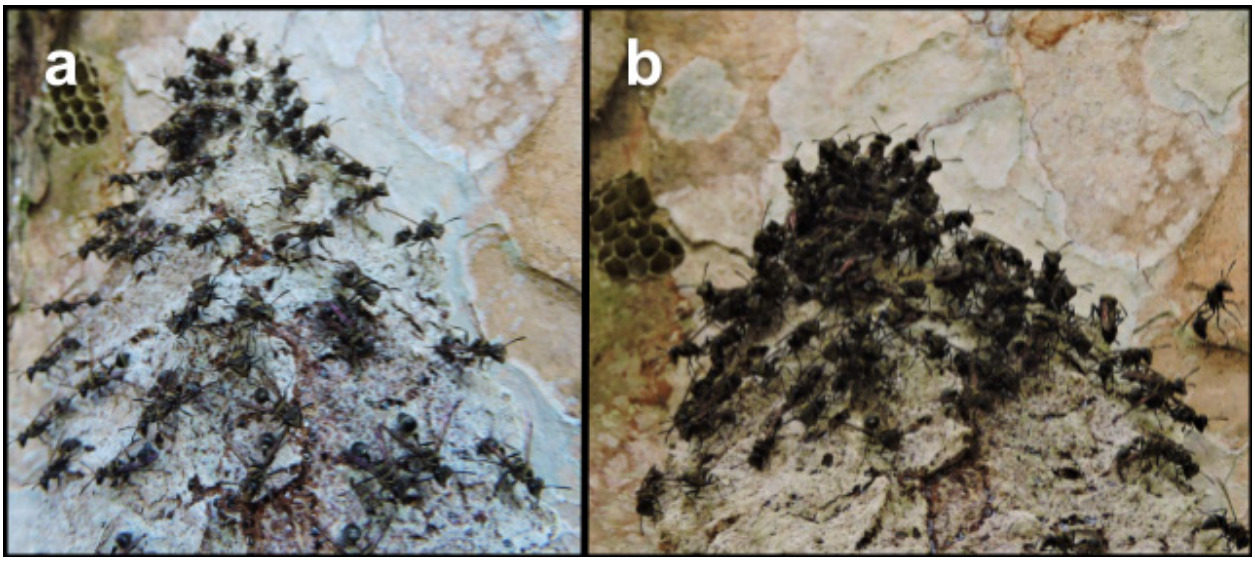

Fig 2. Bluff defense behavior of *Metapolybia docilis* (Hymenoptera: Vespidae) during the provocation of the stress stimulus (a and b).

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Authors' Contributions

MMS: contributed on conceiving and designing the analysis, collecting the data, and writing both the draft and the final paper.
NB: contributed on collecting the data, writing the draft and the final paper.
JAMA: contributed on collecting the data, formatting, writing the draft and the final paper.
GSTG: contributed on writing the draft and the final version, formatting, translating and preparing the figures.
JCZ: contributed on designing the analysis, writing the draft and the final version, translating and supervising the manuscript writing.

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