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Article

First report of a nest of *Ceratina* (*Ceratinula*) *fioreseana* Oliveira (Hymenoptera: Apidae)

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ABSTRACT. We provide herein the first report of a nest of *Ceratina* (*Ceratinula*) *fioreseana* Oliveira, 2020, a species that was recently described for the Cerrado domain. The nest was collected in a *Passiflora edulis* Sims (Passifloraceae) plantation in Minas Gerais State, Brazil and was built in a trap nest, a dry petiole of *Cecropia pachystachya* Trécul (Urticaceae). Within the nest there was a living female and a brood cell without partition containing a pupa, whose adult female emerged 28 days after pupation. Pollen analysis revealed the dominance of *Emilia fosbergii* Nicolson (Asteraceae) pollen grains, a weed recorded a few meters from the nest. The record of an adult female within the nest with a pupa and the absence of nest partition might suggest social behavior in this bee species.

KEYWORDS. Brazilian Savanna, *Ceratinula*, pollen, trap nest.

RESUMO. Primeiro registro de um ninho de *Ceratina* (*Ceratinula*) *fioreseana* Oliveira (Hymenoptera: Apidae). É fornecido o primeiro registro de um ninho de *Ceratina* (*Ceratinula*) *fioreseana* Oliveira, 2020, uma espécie que foi recentemente descrita para o domínio do Cerrado. O ninho foi coletado em um cultivo de *Passiflora edulis* Sims (Passifloraceae) no estado de Minas Gerais, Brasil e foi construído em um ninho-armadilha, um pecíolo seco de *Cecropia pachystachya* Trécul (Urticaceae). Dentro do ninho havia uma fêmea e uma célula de cria sem partição contendo uma pupa, a qual a fêmea adulta emergiu 28 dias após pupação. A análise polínica revelou a dominância de grãos de pólen de *Emilia fosbergii* Nicolson (Asteraceae), uma espécie ruderal observada a poucos metros do ninho. O registro de uma fêmea adulta dentro do ninho juntamente com uma pupa e a ausência de partição na célula de cria podem sugerir comportamento social nesta espécie de abelha.

PALAVRAS-CHAVE. Cerrado, *Ceratinula*, ninho-armadilha, pólen.

Ceratina Latreille, 1802 (Apidae) is represented by approximately 380 species (Oliveira *et al.*, 2020; Ascher & Pickering, 2022; Moure & Melo, 2022) classified in 23 subgenera with a worldwide distribution but absent in Ireland, Iceland, New Zealand, and some small islands (Michener, 2007; Ascher & Pickering, 2022). Females nest in pithy twigs or dead branches where they excavate a burrow and construct unlined brood cells (Michener, 2007). Despite the high diversity found in the Neotropical region, more than 100 species recorded (Moure & Melo, 2022), most of the studies on the nest biology of *Ceratina* species were conducted in Asia, Australia, Europe, and North America (Sakagami & Maeta, 1987; Malaipan & Orostrirat, 1992; Maeta & Sakagami, 1995; Rehan *et al.*, 2009; Rehan & Richards, 2010; Vickruck *et al.*, 2011; Ali *et al.*, 2016; Kaliaperumal, 2019; Mikát *et al.*, 2019; Udayakumar & Shivalingaswamy, 2019; Mikát *et al.*, 2021, 2022; Oppenheimer & Rehan, 2021; Rauf *et al.*, 2022). In contrast, the information on nests of Neotropical *Ceratina* species is still scarce (Schrottky, 1914; Michener & Eickwort, 1966; Sakagami & Laroca, 1971; Gonzalez *et al.*, 2004; Rehan *et al.*, 2015).

Here, it is reported for the first time a nest of *Ceratina* (*Ceratinula*) *fioreseana* Oliveira, 2020 constructed in a trap nest in Minas Gerais State, south-eastern Brazil. This species was recently described from Goiás State from an area distant about 500 km from our study site (Oliveira *et al.*, 2020). It belongs to the subgenus *C.* (*Ceratinula*) Moure, 1941, which comprises 40 minute species of up to 6 mm distributed from southern United States to northern Argentina (Oliveira *et al.*, 2020; Ascher & Pickering, 2022; Moure & Melo, 2022). The records of nest biology or mentions of built nests of *C.* (*Ceratinula*) species were documented by Schrottky (1914) for *C. gomphrenae* Schrottky, 1909 in Paraguay, Michener & Eickwort (1966) for *C. rectangulifera* Schwarz & Michener, 1954 in Costa

Rica, Sakagami & Laroca (1971) for *C. muelleri* Friese, 1910 and *C. oxalidis* Schrottky, 1907 in southern Brazil, and Rehan *et al*. (2015) for four species from Panama.

MATERIAL AND METHODS

The nest was collected in a farm (18°34'10.1"S, 48°01'06.2"W) located north-eastern the city of Araguari, Minas Gerais State, south-eastern Brazil. The area lies at the Brazilian Savanna domain and consist of a small familiar property where 0.8 hectares were occupied by a plantation of yellow passionfruit, *Passiflora edulis* Sims (Passifloraceae), which were replaced with soybean, *Glycine max* (L.) Merr (Fabaceae), by the end of 2021. The area was visited once a month since August 2019 to sample trap-nesting solitary bees and wasps (Rocha-Filho *et al*., in prep.). The sampling station consisted of a wooden frame (30 cm height, 30 cm width, 20 cm depth) attached to a tree 1.5 m above ground and located in front of the passionfruit crop. Twenty bamboo canes, *Bambusa vulgaris* Schrad. (Poaceae), from 1.4 to 2.4 cm diameter and 15 cm length were inserted horizontally in metal screens screwed to the wooden frame and were used to attract nesting females of *Xylocopa* (*Neoxylocopa*) *frontalis* (Olivier, 1789) (Apidae). Additionally, five PVC tubes (20 cm length and 10 cm diameter) containing each 24 bamboo canes (0.5 to 2 cm diameter and 9 to 25 cm length) were attached below the wooden frame to attract nesting females of trap-nesting wasps and bees. Beside each side of the wooden frame, two dry petioles of *Cecropia pachystachya* Trécul (Urticaceae), cut straight in both ends, were attached with nails to attract nesting females of *Ceratina* species.

On November 10th, 2021, one dry petiole containing a small hole at its border was collected and taken to the laboratory for the emergence of adults. The 23.4 cm-length and 0.59 cm-diameter petiole was carefully open the next day with a cutter to check if there was a *Ceratina* nest or if it was occupied by ants such as *Pseudomyrmex gracilis* (Fabricius, 1804) (Formicidae), which can use this kind of substrate to nest (Rocha-Filho *et al*., in prep.). The emerging bees were identified with the key provided by Oliveira *et al*. (2020) and are deposited as voucher specimens at the collection of *Laboratório de Ecologia e Comportamento de Abelhas* (LECA) of *Universidade Federal de Uberlândia*.

The faeces found within the nest were collected, stored in 70% ethanol, and then transferred to absolute acetic acid for at least 24 hours to be acetolysed according to the protocol described by Erdtman (1960). The acetolysed pollen grains were glued with small glycerine gelatine cubes and placed on microscopic slides.

RESULTS

The nest contained a sinuous tunnel of 9.1 cm length (Fig. 1) and a living female, which tried to hide at the bottom of the nest, where there was a pupa surrounded by faeces. No signs of nest partitions were observed. The adult female (Fig. 2) left the nest on November 12th. Another female (Fig. 3) emerged from the nest on December 7th. Three pollen samples were used for identification and counting of all grains found in the microscopic slides. A total of 1,057 pollen grains were counted and 95.27% belonged to the weed species *Emilia fosbergii* Nicolson (Asteraceae) (Fig. 4), which had some individuals located one to three meters from the sampling station. Other pollen types, such as *P. edulis*, type *Bidens* (Asteraceae), and *Mesosphaerum suaveolens* (L.) Kuntze (Lamiaceae) accounted 1.42%, 1.42%, and 0.76%, respectively, while other four types represented less than 1.5% of the total amount counted.

Figs 1-4. 1, Dry petiole of *Cecropia pachystachya* used as nesting substrate by a female of *Ceratina fioreseana*; 2, living female found in the nest; 3, emerging female; 4, polar view of *Emilia fosbergii* pollen grain. Figs 2, 3, Scale bars: 2 mm; 4, Scale bar: 50 µm.

DISCUSSION

Most of the studied nests of *Ceratina* species were observed on natural substrates such as pithy stems or twigs (Rehan & Richards, 2010; Rehan *et al.*, 2015; Ali *et al.*, 2016; Kaliaperumal, 2019; Mikát *et al.*, 2019; Udayakumar & Shivalingaswamy, 2019; Oppenheimer & Rehan, 2021). However, Mikát *et al.* (2021) used sheaves of cut twigs attached to rods and fixed to the ground as artificial substrates for *Ceratina* (*Euceratina*) *nigrolabiata* Friese, 1896 in Czechia. Rauf *et al.* (2022) obtained nests of *Ceratina* (*Pithitis*) *smaragdula* (Fabricius, 1787) built in bamboo canes, a cardboard tube and a log of *Ziziphus mauritiana* Lam. (Rhamnaceae) in Pakistan. In Japan, Sakagami & Maeta (1987) employed glass tubes in which it was inserted the pithy core of *Kerria japonica* (L.) DC. (Rosaceae) that had already been partly excavated by a female. Dry petioles of *C. pachystachya* used herein are commonly found in different Brazilian biomes and have been demonstrated to be a useful artificial substrate for nesting *Ceratina* females, both larger species such as *Ceratina* (*Crewella*) *maculifrons* Smith, 1854 (Rocha-Filho *et al.*, 2021) and minute as *C. fioreseana*. We also encourage the use of dead branches of *Rosa chinensis* Jacq. (Rosaceae) as trap nests to attract nesting females since nests of *Ceratina* species are often recorded in such substrates and this plant species is ubiquitous in several gardens in Brazil.

Differently from what was observed in other *C.* (*Ceratinula*) spp. (Michener & Eickwort, 1966; Sakagami & Laroca, 1971; Rehan *et al.*, 2015), there was no evidence of partition in the *C. fioreseana* nest. According to Michener & Eickwort (1966), nest partitions of *C. rectangulifera* were thin but resistant, composed of bits of pith compactly cemented together. Possibly, the soft pith of *C. pachystachya* petioles may not be hard enough to build nest partitions, which could be reinforced by the fact that no partitions were detected in nests of *C. maculifrons* constructed in the same nesting substrate (L. C. Rocha-Filho, pers. comm.). The lack of partitions can be due to failure in constructing them, when the last brood cell is not sealed and the immature is guarded by the mother, as observed in *C.* (*Rhysoceratina*) *volitans* Schrottky, 1907 (Sakagami & Laroca, 1971). Another cause for the absence of partitions is later removal by the mother, which is often observed between brood cells containing old larvae or pupae, as noted in one nest of *C. oxalidis* containing older immature (Sakagami & Laroca, 1971).

The *C. fioreseana* growth lasted longer than what was reported by Michener & Eickwort (1966) for *C. rectangulifera*. The period between pupation to the emergence of adult took approximately 11 days for *C. rectangulifera* while the *C. fioreseana* female emerged 28 days since the nest collection in the field, when it was in pupal stage. This longer period of pupation was also observed in *C. volitans* in Brazil, which was estimated as 28 to 30 days (Sakagami & Laroca, 1971). The discrepancy of developmental period between the two *C.* (*Ceratinula*) species could be related to differences of climate variables between the studied areas.

The lone brood cell containing a pupa found in the *C. fioreseana* nest was also observed in one of the seven nests of *C. rectangulifera*, but the latter had no adult bee inside the nest (Michener & Eickwort, 1966). However, the authors found one nest with one cell containing a large larva and a living adult in the external part of the nest tunnel. Because of this small number of brood cells observed in the nests of *C. rectangulifera*, Michener & Eickwort (1966) conjectured that a nesting female must construct two or more nests. It could be wondered the same hypothesis for *C. fioreseana* because of the single brood cell found in the nest, but other three dry petioles were available in the sampling station and no signs of excavation were observed on them.

Only one adult female was found in the *C. fioreseana* nest. On the other hand, Sakagami & Laroca (1971) observed two females in one nest of *C. oxalidis* containing old brood cells and it was assumed that they were elder sister of the pupa and larva found within that nest. The authors also found two nests of this species without brood cells, but only two females with swollen ovaries in one of them and six individuals (three females and three males) in the other nest. *Ceratina oxalidis* has an inactive period in the year and it was presumed that both nests were on stage of hibernation (Sakagami & Laroca, 1971). Rehan *et al.* (2015) found nests with two females in three of the four *C.* (*Ceratinula*) studied species but always in a much lower proportion when compared to nests with a single female. The authors observed reproductive division of labor in these multifemale nests, in which the larger female was a reproductive forager and the smaller female a non-reproductive guard, providing thus the first evidence for social behavior of three *C.* (*Ceratinula*) species.

One important aspect to be highlighted herein is the putative social behavior of *C. fioreseana*. Despite the record of only one nest, the fact that an adult female (possibly the mother) was inside the tunnel with a pupa and the absence of nest partition indicates that this species is not solitary, as also observed in other *Ceratina* species belonging to different subgenera (Rehan *et al.*, 2009, 2015; Rehan & Richards, 2010; Oppenheimer & Rehan, 2021). Our data suggest that the living female in the nest may be long-lived and performing nest protection and maternal care, which are recurrent characteristics of several *Ceratina* species (Rehan *et al.*, 2009).

The high prevalence of *E. fosbergii* pollen grains found in the residual content of the *C. fioreseana* nest suggests that this bee species might be oligolectic, but a single sample would not be appropriate to conclude about its diet. The *C. fioreseana* specimens studied and described by Oliveira *et al.* (2020) were collected on flowers of peppermint, *Mentha piperita* L. (Lamiaceae), which could be either a pollen and/or a nectar source. Flower records of eight *C.* (*Ceratinula*) species in São Paulo State showed associations with 34 plant species belonging to 22 families, which indicates that some of these species could be polylectic given the high number of species visited (Imperatriz-Fonseca *et al*., 2011). Indeed, *C. oxalidis* females containing pollen loads were collected on several flowers belonging to various plant species of different families in southern Brazil (Sakagami & Laroca, 1971). Additionally, in five nests of *C. maculifrons* collected in an urban area, the residual content had pollen from four plant families, confirming the polylecty of this bee species (Müller & Kuhlmann, 2008; Rocha-Filho *et al*., 2021). Hence, it is plausible to assume that the use of *E. fosbergii* pollen for brood cell provisioning may be associated to the very short distance of plant individuals from the nest, likewise *P. edulis* and *M. suaveolens*, which were located one to three meters away from the sampling station, ensuring the host female short-distance flights for foraging.

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