Leaf-cutting ants of the genus *Atta* Fabricius, 1805 (Hymenoptera: Formicidae) are eusocial insects found exclusively in the Neotropical region (Hölldobler & Wilson, 1990). These ants build stable and long-lasting nests that protect themselves against abiotic and biotic factors, besides growing the fungus *Leuocoprinus gongylophorus* Heim, 1957, on which they feed (Sudd, 1982; Hölldobler & Wilson, 1990; Rocha et al., 2020).

However, due to favorable conditions, like temperature and humidity, that these nests offer (Camargo et al., 2016), they also become favorable habitats for a multitude of inquilines, from invertebrates such as beetles, cockroaches, mites, crickets, dipterans and springtails (Waller & Moser, 1990; Dekoninck et al., 2007; Forti et al., 2007), to vertebrates such as frogs, lizards and snakes (Hölldobler & Wilson, 1990; Baer et al., 2009; Lima Barros et al., 2016). According to Hölldobler and Wilson (1990), these ant guests are commonly known as myrmecophiles and may be present in ant nests temporarily engaging in predation or commensalism, or may also depend on such nests for a while or throughout their life cycle. Information about their ecology, behavior, life cycles and dispersion is scarce for most of these myrmecophilous species because they are difficult to observe and can only be found when specific studies are conducted (Dekoninck et al., 2007).

Nonetheless, springtails are inquilines commonly reported and studied in nests of fungus-growing ants (Castaño-Meneses et al., 2017). Even a specific behavior known as jigging, performed by *Cyphomyrmex costatus* workers, Mann, 1922, and *Myrmicocrypta buenzlii* workers, Borgmeier, 1934, (Formicidae: Myrmicinae), has been reported as a defense strategy to keep springtails away from the fungus-garden-growing chamber (Weber, 1957, 1972; Kweskin, 2004).
In this study, we identify, quantify and report the first species of springtails of the genus *Cyphoderus* living in early colonies of *A. sexdens*. In addition, we provide information on a possible defensive behavior performed by workers when springtails are present in their fungus garden chamber.

The study design relied on sampling, six hundred colonies of *A. sexdens* (Hymenoptera: Formicidae), aged approximately four months old, were collected on February 6, 2019, in a transition area between the Atlantic Forest and the Cerrado, located in the municipality of Botucatu (22° 48' 23.5" S 48° 25’ 52.8" W), São Paulo, Brazil.

In the laboratory, a high population density of springtails was observed in four of the 600 collected colonies. These colonies were separated, and then, with the aid of an aspirator, all springtails present were removed by suction and stored in 98% alcohol. Afterwards, population density was quantified, and samples were taken for species identification. The identification was carried out by the Laboratory of Collembola Systematics and Conservation of the State University of Paraíba (UEPB). The methodology adopted for identification consisted of clearing and mounting of the specimens, following the procedures described by Jordana et al. (1997), with clearing being done using Nesbitt’s solution and a phase-contrast microscope (Zeiss Axioskop). Then, to identify the species, the classification key provided by Oliveira et al. (2017) was used. The specimens are deposited in the UEPB’s Soil Fauna Reference Collection [Coleção de Referência da Fauna de Solo] (CRFS).

*Cyphoderus innominatus* Mills, 1938 (Collembola: Paronellidae) was the species of springtails found living in association with early colonies of *A. sexdens* (Fig 1). *Cyphoderus innominatus* were found in four (0.67%) of the 600 collected colonies, with an average population density of 227 ± 212 individuals (Table 1). Most of the individuals were present in peripheral zones inside the fungus garden chamber of the colony, where some debris accumulated, but the springtails did not make direct contact with the ants (Fig 1B and video: https://youtu.be/EObSZYkZs30).

The video showed that the springtails (https://youtu.be/EObSZYkZs30) were initially present in the fungus garden chamber of the ants, apparently without causing any disturbance. Until a medium worker (ant with head width of approximately 2.2 mm), between the 38th and 46th seconds, presented a defensive behavior against the springtails. With its jaws open, it jumped three consecutive times towards some springtails. According to Kweskin (2004), a high population density of springtails ends up consuming a large amount of the mycelium grown in the fungus garden, consequently disturbing the colony (Kweskin, 2004). Some behavioral acts performed by ants against these disturbances have been reported. Among said acts, jigging is a peculiar rhythmic swinging behavior, and there are two hypotheses as to why it is performed: alerting mates about disturbances, or expelling competitors (Weber, 1957;1972). However, this behavior was not observed in our study. On the other hand, the behavioral act also performed by ants in the presence of springtails in their fungus gardens, and which was observed in our study, is jumping, when ants jump and try to capture springtails with their jaws and legs. According to Kweskin (2004), this type of behavior seems to be exclusively meant for defense against invaders, and the same behavior was also performed by *C. costatus* workers when springtails approached their fungus garden. The actual reason and the efficiency of this behavioral act against these disturbances are still not well understood.

In light of our findings, future studies should be carried out to elucidate the effect of the presence of *C. innominatus* in fungus gardens of *A. sexdens*, and whether their presence causes disturbances and induces defensive behaviors. Thus, our study provides important information about a species of myrmecophilous springtails that live in association with early colonies of fungus-growing ants, since we reported here, for the first time, the occurrence of *C. innominatus* in early colonies of *A. sexdens*. Furthermore, we provided information about a possible defensive behavior performed by *A. sexdens* workers when springtails are present in their fungus garden chamber.
Colony Number of individuals of *Cyphoderus innominatus* Mills, 1938

| Colony | Number of individuals of *Cyphoderus innominatus* Mills, 1938 |
|--------|---------------------------------------------------------------|
| 1      | 539                                                           |
| 2      | 133                                                           |
| 3      | 165                                                           |
| 4      | 70                                                            |
| Mean ± standard deviation | 227 ± 212 |

### Conflict of interest

The authors declare no conflicts of interest.

### Authors’ contributions

TMM Mota Filho, KKA Sousa, RS Camargo, N Caldato and Forti L. C. Conceptualization, investigation, data curation, Writing. JVC Oliveira and D Zeppelini carried out the identification of the species.

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