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SHORT NOTE

Occurrence of Gregarines (Protozoa: Apicomplexa) in the Neotropical Soldierless *Ruptitermes* spp. (Isoptera, Termitidae, Apicotermitinae)

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Abstract

This is the first record of gregarine infection in the worker midgut of the Neotropical termites *Ruptitermes pitan* and *Ruptitermes reconditus*. Gregarines have already been described in the gut and hemocoel in workers of other termite species, and although these protozoa are not able to kill these insects, they may affect their fitness. In the present study, gregarines were only observed in the gut lumen or linked to the midgut wall of termite workers. Histological sections of the worker midgut of *Ruptitermes* spp. showed three phases of the gregarine life cycle with the same individual worker displayed up to 4 gamonts and 2 trophozoites.

Termites are part of the most important fauna which contributes to the enhanced productivity in the tropical ecosystem (Bourguignon et al., 2016). They are grouped into lower and higher termites based on the presence of flagellated protist symbionts in the hindgut of the former and absence in the latter. Thus, the higher termites are included in the family Termitidae while lower termites comprise the other families, which are dependent on cellulolytic flagellated protozoa (Lo & Eggleton, 2011).

Gregarines are common as parasites of invertebrates, especially arthropods (Criado-Fornelio et al., 2017). These unicellular organisms may reach a length of 10 mm (Manwell, 1961) and belong to the phylum Apicomplexa, class Conoidasida and order Eugregarinorida (Adl et al., 2019). Generally, the arthropod host becomes infected with these protozoa through ingestion of mature cysts, which liberate sporozoites that reach the hemocoel and target tissue (Schimid-Hempel, 1998). Many researches showed a certain costs for hosts which harbor gregarines, for instance, in damselfly species, the presence of gregarines alters survival and egg production, while it increases susceptibility to a fungal pathogen in the cockroach *Blattella germanica* (Arcila & Meunier, 2020; Lopes & Alves, 2005). These protozoan parasites affect the fitness of their hosts and can cause a delayed development, decreased survival and reduced reproduction (Zuk, 1987; Cordoba-Aguilar, 2003). According to Gigliolli et al. (2016), the lack of nutrients induced by parasitic gregarines cause the already mentioned effects to the host. However, besides parasitic relationships, gregarines also can develop mutualistic or neutral association with their hosts and the type of this interaction is highly species-specific (Zuk, 1987; Rueckert et al., 2019).

Termites are social cockroaches and according to Bouwma et al. (2005), the behavior of a social insect reflects a selection of parasites since society favors infection transmission among nestmates. However, termite societies have evolved mechanisms to limit or prevent disease agents within their colonies, making such observation unusual (Chouvenc et al., 2011). Previous records of termite gregarine infections were reported in the families Hodotermitidae (Uttangi & Desai, 1962), Rhinotermitidae (Uttangi & Desai, 1962; Huger & Lenz, 1976; Kalavati & Narasimhamurti, 1978; Hall & Hostettler, 1993; Costa-Leonardo et al., 2008), Archotermopsidae (Grassé, 1986) and Termitidae (Kalavati & Narasimhamurti, 1978; Kalavati & Narasimhamurti, 1980; Zhang et al., 2021). Gregarines present a life cycle with an endogenous stage within the insect body and an exogenous stage in the external environment (Fig 1). Infections by gregarines occur when termites and other insects ingest the parasite oocysts (mature cysts), which initiate the infection via the digestive tract (Logan et al., 2012). Then, oocysts release sporozoites, which develop into large stages named trophozoites that are attached to the intestinal wall. Later, when trophozoites mature and become gamonts, they are released from the gut wall and appear free in the gut lumen. The gamonts of opposite sex adhere to one another in a reproductive process called syzygy and form a gametocyst, which is a cyst that forms around gamont pairs. Gametocysts are then expelled in the environment together with the termite feces (Kolman et al., 2015).

The termite genus *Ruptitermes* is endemic in South America and includes 13 species and subspecies with several of them being litter-feeders (Acioli & Constantino, 2015; Constantino, 2022). *Ruptitermes pitan* and *Ruptitermes reconditus* (Apicotermitinae, Termitidae) are soldierless species of termites which have subterranean nests and forage in the open on the surface litter (Matews, 1977; Acioli & Constantino, 2015). The present study is the first record of gregarines in the Neotropical genus *Ruptitermes*, which is included in the poorly studied group Neotropical Apicotermitinae.

Foraging workers of *Ruptitermes reconditus* (Silvestri, 1901) and *Ruptitermes pitan* Acioli & Constantino, 2015 were collected in different areas located at the São Paulo State Campus of University (UNESP), Rio Claro, SP, Brazil (22°23′S, 47°32′W). For histology, 8 abdomens from each termite species were fixed in FAA (absolute alcohol, glacial acetic acid, 40% formaldehyde, in the proportion of 3:1:1) and dehydrated in increasing ethanol concentrations (70, 80, 90 and 95%). Next, the samples were transferred to a Leica historesin solution and stored for seven days in a refrigerator. The abdomens were embedded with historesin (Leica) plus catalyzer for polymerization and sectioned (3µm thick) using a Leica RM 2245 microtome. The sections were stained with toluidine blue/fuchsin and xylidine-Ponceau and documented using a Leica photomicroscope.

The abdomen histological sections showed the occurrence of gregarines infecting the midgut in both species of *Ruptitermes* (Fig 1). Although gregarines occupy a variety of niches inside their hosts, from extracellular cavities, as gut lumen and coelom, to epicellular and intracellular sites, these eukaryotes were not observed in the coelomic cavity or intracellularly in the gut of *Ruptitermes* spp. workers. We found 4 workers of *R. reconditus* and two of *R. pitan* infected from 8 examined specimens of each species. Gregarines have

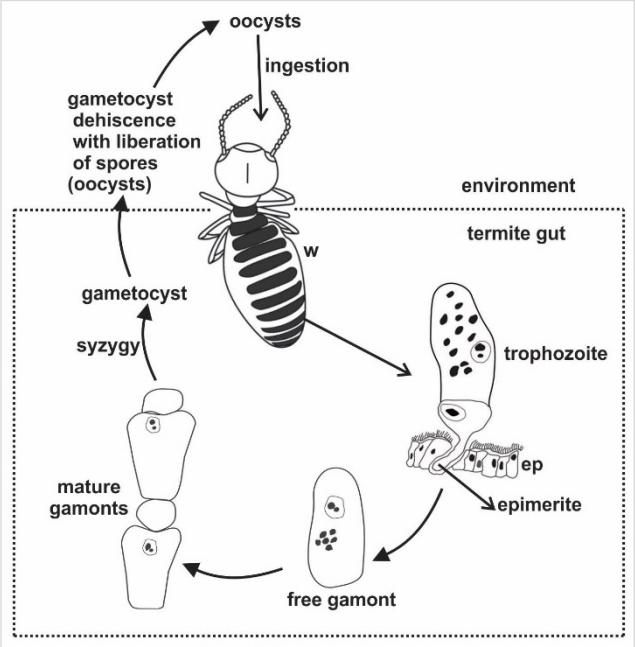

Fig 1. Schematic representation of the gregarine life cycle which infect workers of *Ruptitermes* spp. Workers (w) ingest oocysts, containing sporozoites which develop into large stages named trophozoites. The trophozoites will attach to the gut epithelial cells (ep) through a special structure known as epimerite. After, the trophozoites will detach from the epithelium and form free gamonts. These free gamonts mature and undergo syzygy, originating the gametocyst that will be released into the environment.

already been described in the gut and hemocoel in workers of several termite species (Grassé, 1982; Costa-Leonardo et al., 2008), and although these protozoa do not cause termite death, they may decrease their longevity (Villanueva, 2004). Studies with wasps of the species *Polybia occidentalis* parasitized by gregarines indicated that infected individuals decrease their foraging rates and, consequently, compromise the productivity of the colony (Bouwma et al., 2005).

Free gamonts were observed in the midgut lumen in both species of *Ruptitermes* (Figs 2a, 2b and 3) and trophozoites in the inner wall of the midgut in *R. pitan* (Figs 2a and 2d). Gamonts in syzygy were also observed in the midgut lumen in *R. pitan* (Fig 2c). Previous studies with gregarine infection also displayed some of the cycle phases of as gamonts, oocysts and trophozoites in the midgut of other termites (Kalavati & Narasimhamurti, 1978; 1980; Hall & Hostettler, 1993). Additionally, the stages of oocysts and gametocysts have already been described in the hindgut of *Zootermopsis angusticollis* and hemocoel of *Coptotermes gestroi* (Henry, 1933; Costa-Leonardo et al., 2008). Free gamonts, gamonts in syzygy and gametocysts were observed only in the worker caste of the termitid *Macrotermes barneyi*, being absent in soldiers and reproductives (Zhang et al., 2021). Although the stages of gametocyst and sporozoites were not observed in the present study, it was possible to detect up to 4 gamonts and 2 trophozoites in the same worker. The trophozoite was attached to the mesenteric epithelium through an epimerite, which is a characteristic elongated structure, observed in *R. pitan* (Figs 2d and 2e). *Ruptitermes* spp. feed on litter and *R. reconditus* workers store this food in subterranean nest galleries, a fact that should facilitate the ingestion of mature cysts and, consequently, increase the infection by gregarines. However, future studies on the *Ruptitermes* genus will be needed to clarify this gregarine infection.

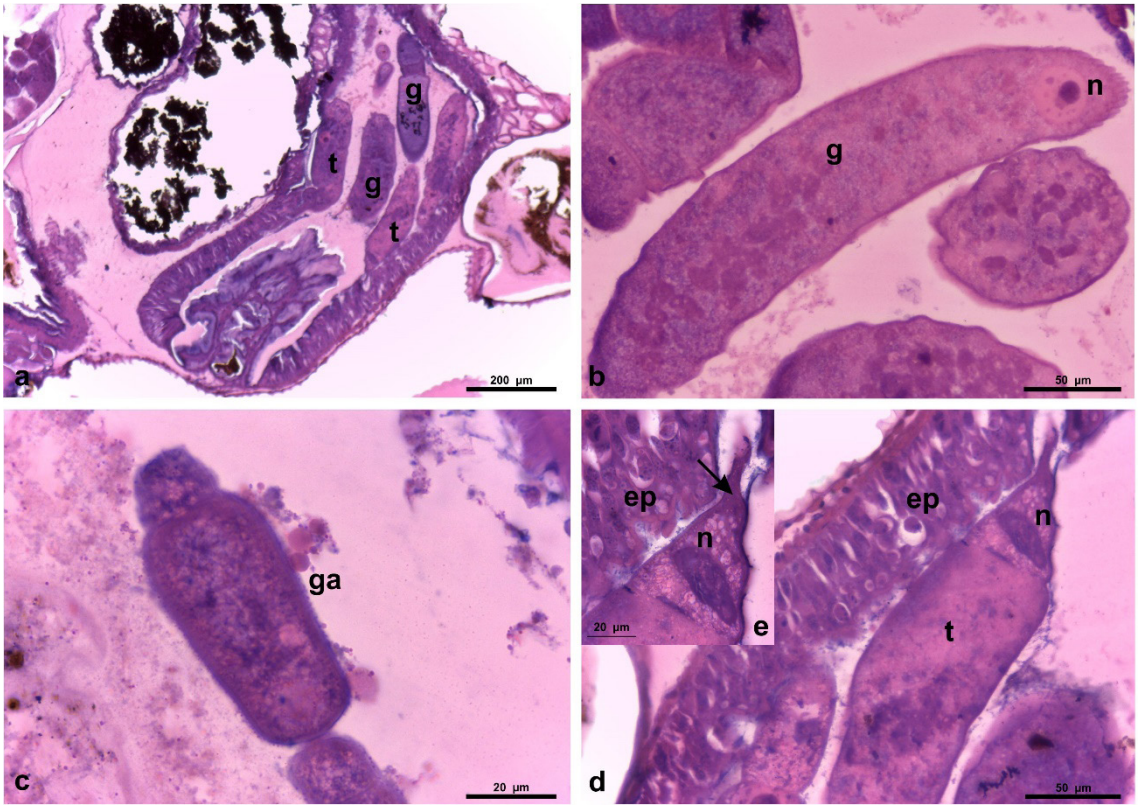

Fig 2. Histological sections of the midgut of *Ruptitermes pitan* worker stained with toluidine blue/fuchsin. **a** Free gamonts (g) in the midgut lumen and trophozoite (t) attached to the mesenteric epithelium. **b** Detail of the free gamont (g) in longitudinal and transversal section. **c** Gamonts in syzygy (ga). **d** Trophozoite connected to the epithelium (ep). **e** Detail of the epimerite (arrow) which is a elongated structure used by the trophozoite (t) for connection to the in epithelium (ep). n = nucleus

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Authors' Contributions

VJ: investigation, visualization, writing-original draft; review & editing.

AMCL: conceptualization, methodology, writing-review & editing, supervision.

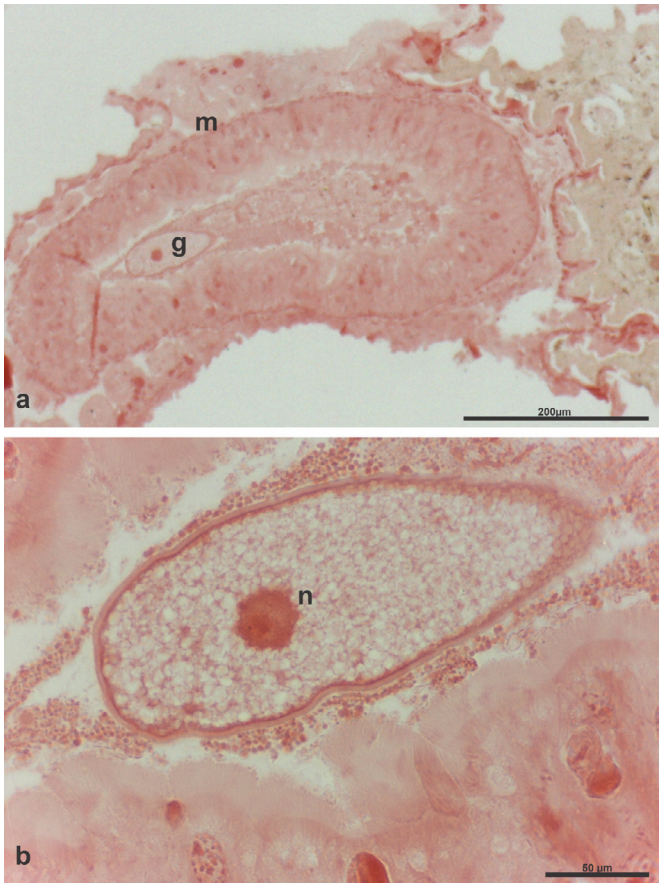

Fig 3. Histological sections of the worker midgut (m) of *Ruptitermes reconditus* stained with xylidine Ponceau. **a**, **b** Free gamont (g) in the gut lumen. n = nucleus.

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