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SHORT NOTE

Record of an Ectoparasitic Fungus on Eggs of the Neotropical termite *Nasutitermes corniger* (Blattaria, Isoptera, Termitidae)

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Abstract

Insects and fungi are abundant in many environments and often interact. However, little information exists on the fungal infestation of insect eggs. Here, we report an entomogenous fungus similar to *Hormiscioideus filamentosus* (Blackweel & Kimbrough, 1978) infesting eggs of the termite *Nasutitermes corniger* (Motschulsky, 1855). The fungus arises from egg chorion and has long and simple filaments. The small haustorial cells infiltrate the egg chorion and the fungal thalli number varies from two to ten. Future studies will elucidate the infestation levels of termite eggs by this entomogenous fungus in the Neotropical region and its effects on termite colonies.

Many interactions between insects and fungi are known, including mutualistic ones, and host-fungal interactions can range from fungi as food sources to fungi as pathogenic agents (Wilding et al., 1989). Eggs of the subterranean termites (*Reticulitermes* and *Coptotermes*) and *Nasutitermes takasagoensis* are often intermixed with brown balls ('termite ball') that mimic termite eggs and are formed from the sclerotia of a wood-decaying fungus (Matsuura & Yashiro, 2010). Termite egg mimicry by sclerotium-forming fungi has been interpreted as a facultative mutualism, since the fungus within termite nests has a competitor-free habitat and can supplement the diet of their host termite with the enzymes they provide (Ye et al., 2019; Komagata et al., 2022). Although, some ectoparasite fungi have been described in termite colonies, the mention of egg infestation is rare. According to Schmidt-Hempel (1998), the lack of information on the incidence of fungal infestations of eggs can reflect a lack of studies on this subject.

Different genera of ectoparasite fungi infesting termites have been reported from the following families: Mastotermitidae, Stolotermitidae, Kalotermitidae, Rhinotermitidae and Termitidae (Wilson et al., 2021), indicating that fungal infestations reach five families among the nine included in Isoptera (Engel et al., 2009).

A variety of microbes colonize termite colonies, including diverse pathogenic fungi (Lee & Wood, 1971; Blackwell & Rossi, 1986). Recent research showed that termite embryos have microbial protection owing to embryonic microbiomes and grooming by nestmates (Cole et al., 2020). Thus, according to this study, embryos are

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immature but not defenseless since they have extra-chorionic protection (grooming) combined with intra-chorionic defense (embryonic microbiome), which influences the fitness of the whole colony. However, some fungi breach this defense and infest the termite eggs, although their impact on egg survival has yet to be evaluated. The current scientific note describes an entomogenous fungus similar to *Hormiscioideus filamentosus* (Blackweel & Kimbrough, 1978) infesting eggs of the termite *Nasutitermes corniger* (Motschulsky, 1855). Nine genera of fungi are specialized for infesting termites, with the genus *Antennopsis* being the most common and the only one recorded as a termite egg parasite (Wilson et al., 2021). *H. filamentosus* has been described as infesting the termite *Armitermes neotenicus* (now named *Embiratermes neotenicus*) (Holmgren, 1906) from Brazil and is distinguished from other termite-infesting fungi by the combination of its basal-cell arrangement, filament arrangement, and reproduction by filament fragmentation with internal proliferation (Blackwell & Kimbrough, 1978).

In the present research, eggs were obtained from an arboreal nest of *N. corniger* collected in a small area of natural vegetation. The nest was elliptical and had 11 imaginal physogastric queens and six imaginal kings. The eggs were fixed in FAA fluid (formol, alcohol, and acetic acid; 1: 3:1), cleaned with detergent in an ultrasound apparatus, dehydrated, and critical point dried in a CPD 030 Balzers desiccator. Later, they were sputtered with gold and examined under a Philips (SEM 505) scanning electron microscope. The results show a fungus with long and simple filaments arising from the egg chorion (Figs 1 and 2). The highest number of observed filaments was ten. For comparison, Myles et al. (1998) recorded the fungus *Antennopsis gallica* (Buchli & Heim, 1951) infesting various castes of the termite *Reticulitermes flavipes* (Kollar, 1837) in Canada with several termites carrying in excess of 200 fungal thalli.

The ectoparasitic fungus was observed in the egg micropylar region (Fig 1) as well as other locations (Fig 2). Some eggs had two fungal thalli attached to a cellular mass with small haustorial cells infiltrating the egg chorion. Moreover, the basal cellular area presented a peculiar structure with a cushioned aspect. This morphological aspect is quite different from the basal cells of *Antennopsis* but very similar to the crustose mass or pulvinate structure described for the fungus *H. filamentosus.* Other characteristics of *Antennopsis*, such as the conidial heads, which are the terminal ends, are also absent. Moreover, fungi of the genus *Termitaria* and *Mattirolella* present a far more elaborate thallus.

H. filamentosus was recorded as infesting different termite castes of *E. neotenicus*, including soldiers, workers, and alate reproductives, but not eggs. Other fungi of the genus *Hormiscioideus* have been found in various insects, including beetles, ants, and flies (Blackwell & Kimbrough, 1978).

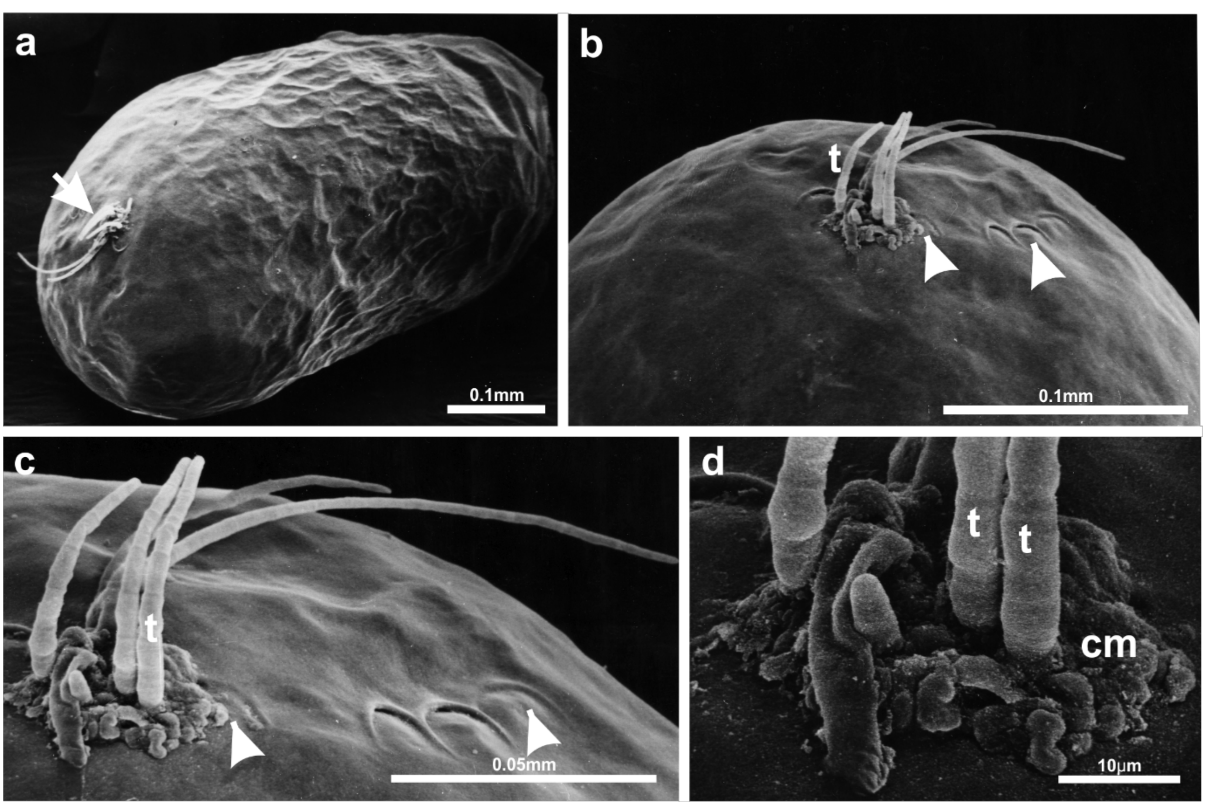

Fig 1. Egg of *Nasutitermes corniger* infested by a fungus similar to *Hormiscioideus filamentosus.* a. Thalli (arrow) of the fungus attached to the micropilar region of the egg. b. and c. Detail of the six fungal thalli arising from micropilar process (arrowheads). D. Detail of the crustose mass (cm), which is a basal feature of this fungus similar to *H. filamentosus*. t = thallus.

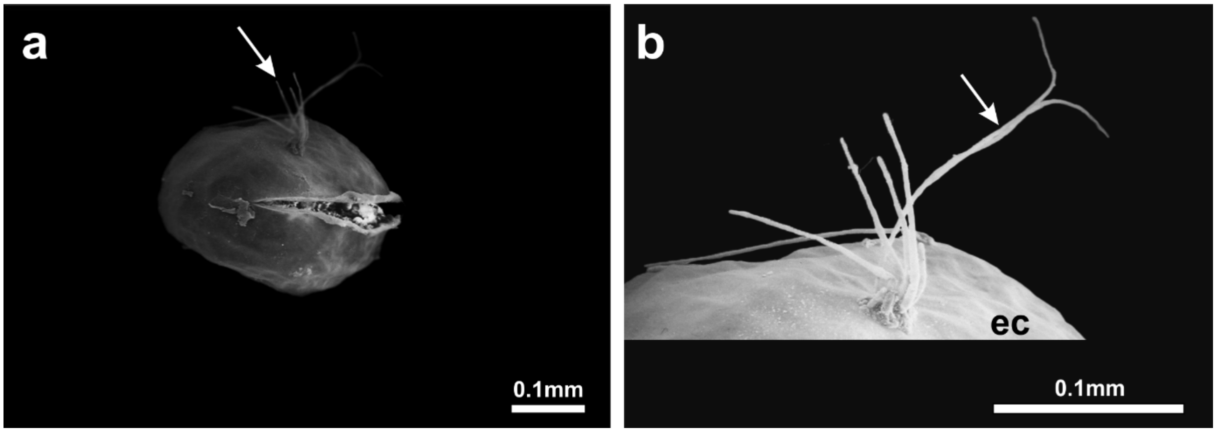

Fig 2. Other egg of *Nasutitermes corniger* infested by a fungus similar to *Hormiscioideus filamentosus.* a. Thalli (arrow) of the fungus attached outside to the micropylar region of the termite egg. b. Detail of the various fungal thalli on the egg chorion (ec), with some very long (arrow).

According to Wilson et al. (2021), most ectoparasitic fungal infestations of termites cause low-level damage to their host. Therefore, light infestations are not detrimental to the termite colony because the molting rate is fast enough to ensure the low abundance of the fungus (Myles et al., 1998). Guswenrivo et al. (2018) reported that the infestation rate and thallus number per termite can measure the strength of a fungal infestation. However, it needs to be clarified what effect a given level of infestation has on the colony. *N. corniger* and *N. ephratae* infested by *Mattirolella crustosa* died rapidly compared with non-infested individuals (Thorne & Kimbrough, 1982). Similarly, termites infested by the ectoparasitic fungus *Termitaria* sp. tended to survive less than non-infested termites (Lenz & Kimbrough, 1982). The mortality of termites infested by ectoparasitic fungi might be due to a combination of fungal toxins, physical obstruction of hemolymph circulation, nutrient depletion, and invasion of organs (Goettel & Inglis, 1997).

It is known that grooming can reduce fungi infestation because it affects fungal growth (Cole et al., 2020). However, there is a lack of modern studies showing the pathogenic relationships between fungal ectoparasites and termite hosts (Wilson et al., 2021). Moreover, factors that affect grooming activity may allow the infestation of eggs by parasitic fungi. The ectoparasite fungus *Antennopsis* is known to interfere with swarming success (infested imaginal swarming termites perish) and infests eggs. Consequently, Wilson et al. (2021) comment that it can potentially be used as a microbial control agent for termites from small confined colonies. Future studies will elucidate the infestation levels of termite eggs by entomogenous fungi in the Neotropical region and their effects on the colonies.

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Author's Contributions

AMCL: Conceptualization (equal); data curation (lead); formal analysis (equal); investigation (equal); methodology (equal); writing-original draft (lead); writing-review and editing (equal). VJ: formal analysis (equal); investigation (equal); designed the figures (equal); writing-original draft (supporting); writing-review and editing (equal).

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Declarations
Conflict of interest

The authors declare no competing interests.

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