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# Morphological and nest notes of *Ruptitermes arboreus* (Emerson), an Amazonian soldierless termite

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**Abstract.** *Ruptitermes arboreus* (Emerson) is a litter-feeder soldierless termite that builds arboreal cartonated nests. In this paper, we describe and illustrate arboreal nests of *R. arboreus*, while also describing a fully clayish nest built upon the trunk of a fallen tree. Additionally, we provided physical and populational parameters for two nests. We also illustrate all castes except alates and record two termitophilous species occurring inside the nests.

**Keywords:** Ant-termite association; Behavior; Dehiscence; Inquilinism; Termitarium.

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*Ruptitermes* Mathews is a genus of soldierless Neotropical termites with characteristics that distinguish it from other Neotropical Apicotermitinae. While other Apicotermitinae genera feed upon the soil, *Ruptitermes* species feed on litter. Presumably, because *Ruptitermes* species are more exposed during forage, the abdomen is not as translucid as it is more sclerotized, and they have the longest tibiae/body size ratio among Apicotermitinae, which makes the tibiae visually longer when compared with other genera. They are generally more active than other Apicotermitinae because the species wander more around litter while co-familiar species into the soil (Acioli & Constantino 2015).

However, the most striking characteristic of *Ruptitermes* is their dehiscent organ, located in the anterior part of the abdomen which gives them the capability of bursting their own body, releasing a sticky secretion (Poiani *et al.* 2016). Although some other termites possess "kamikaze" workers or soldiers, including other Apicotermitinae, the dehiscent organs are an evolutionary novelty exclusive to this genus. When they burst, the liquid contains toxins, defensins, and proteolytic enzymes that may immobilize or even kill opponents such as ants (Costa-Leonardo *et al.* 2020).

Despite having such extraordinary characteristics, the biology of the *Ruptitermes* species is not well-known. Here we illustrate *Ruptitermes arboreus* (Emerson) nests and castes and report colony parameters and nest-associated fauna. *R. arboreus* distinguishes itself from other *Ruptitermes* species by having a darker body coloration and by building arboreal cartonated nests (Acioli & Constantino 2015).

## MATERIAL AND METHODS

Termite colonies were sampled in two locations in the surroundings of Manaus, Northern Brazil (92 MASL.; 3°06'07" S and 60°01'30" W), Central Amazonia. The region encompasses a dense drainage system where plateaus and valleys alternate, with altitude ranging from approximately 50 to 130 m. The terrain is rugged, alternating between clayish plateaus and sandy valleys connected through a dense drainage system. The vegetation is lowland tropical rainforest. Canopy height is around 35-40 m, with some trees reaching over 45 m, and the understory is dominated by acaulescent palms (Guillaumet 1987). The mean monthly air temperature varies from 24 to 27 °C, and the annual rainfall is 2200 mm, with a relatively dry season (less than 100 mm) from July to September (Luizão *et al.* 2004).

The nest parameters provided are from a survey carried out in 2019-2020 in 16 25 $m^2$ plots across an area of 1 ha belonging to the Universidade Federal do Amazonas (UFAM), about 38 km north of Manaus City, Amazonas State, Brazil (hereafter Fazenda UFAM, 2°38'59" S and 60°3'13" W). The plots were thoroughly inspected by two persons for *R. arboreus* nests and found nests were photographed, measured, and frozen. Later, termites were extracted using flotation (Jones *et al.* 2005), sorted according to their castes and then weighted. 1000 individuals of each caste of each colony were weighted to calculate the caste average individual weight. Finally, the found castes were photographed. In 2019, we found a third *Ruptitermes* nest on the trunk of a fallen tree during an excursion at the

Ducke Reserve (2°57'43" S and 59°55'39" W), a tropical rain forest area located northeast of the city of Manaus and 40 km distant from the Fazenda UFAM. We photographed the nest and sampled a piece of the nest. Later we identified the species as *R. arboreus* (Aciolli & Constantino 2015).

For the colony sampled at Ducke Reserve, we verified if the size of the dehiscent organs is related to termite age. For this, we calculated the index of mandible sharpness (Šobotník *et al.* 2012). This index is the ratio between the mandible height measured from the tip of the apical tooth to the end of the molar plate and its width, which is the distance between the posterior margin of the mandibular condylus and the gap between the apical and subsidiary tooth. Five *R. arboreus* workers with small dehiscent organs and five *R. arboreus* workers with large dehiscent organs were measured. As termites do not change their mandibles during mount, the weariness of mandibles can be used to estimate the relative age of the workers (Šobotník *et al.* 2012). Photographs of the mandibles were taken using a DFC 295 Leica camera attached to a M205 Leica stereomicroscope and measurements were made using FIJI software.

## RESULTS

Two *R. arboreus* nests were found during the survey in Fazenda UFAM. Nest A weighed 2.5 kg and measured 45 cm in height and 16 cm in width. Nest B weighed 6 kg and measured 55 cm in height and 25 cm in width. The nests were arboreal, cartonated, elongated, with rugous darkish surfaces usually covered with moss (Figure 1 A-B). The termites walked uncovered from the nest to the soil. Population parameters are given in Table 1. Inside both nests, there were eggs, larvae, nymphs, workers, and queen, but no alates or king were found (Figure 2).


**Figure 1:** *Ruptitermes arboreus* (Emerson) nests. A and B - Arboreal nests in normal conditions. C - nest on trunk of fallen tree, possibly rebuilt. D. Internal part of the nest.

The third nest was found at Ducke Reserve, on the trunk of a fallen tree during an excursion and was sampled opportunistically (Figure 1C). We collected a sample of the nest instead of the entire nest to preserve the colony. The nest was clayish rather than cartonated, with foraging clayish shelter tubes connecting the nest to the soil. Like other *Ruptitermes*, the workers are active compared to most termite species. The workers were fast and could climb plastic surfaces and even climb a few inches on glass. They are very aggressive, biting the tweezers and brushes used to manipulate them. Once they bite something, they do not release the grip.

**Table 1.** Populational parameters of the arboreal nests collected at Fazenda UFAM.

| | Fresh wet biomass (g) | | Average body weight (mg) | | Estimated population | |
|---|---|---|---|---|---|---|
| | Nest A | Nest B | Nest A | Nest B | Nest A | Nest B |
| Egg | 0.49 | 0.49 | 0.05 | 0.04 | 10.695 | 11.281 |
| Larvae | 9.01 | 7.97 | 0.54 | 0.37 | 16.799 | 21.784 |
| Nymph | 0.05 | 0.03 | 0.94 | 2.31 | 53 | 13 |
| Worker | 97.65 | 236.81 | 1.67 | 2.22 | 58.418 | 106.570 |
| Queen | 0.23 | - | 233 | - | 1 | - |

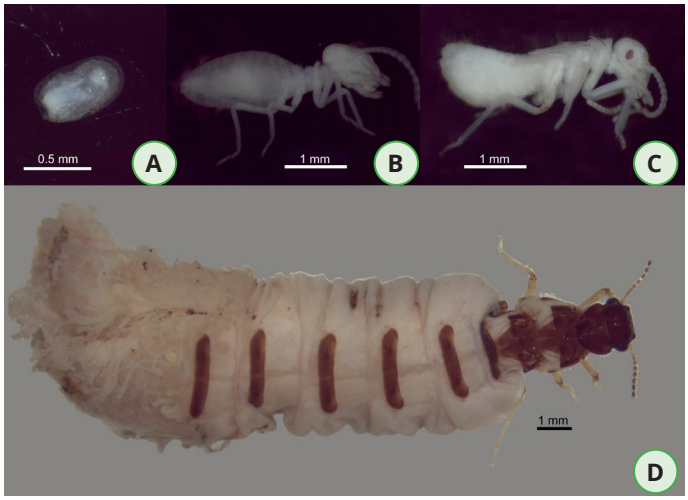


**Figure 2:** A – egg. B – larvae. C – nymph. D - Queen of *Ruptitermes arboreus* (Emerson).


**Figure 3:** Size range of the dehiscent organs of workers of *Ruptitermes arboreus* (Emerson). Red circle indicates where the dehiscent organs are located.

We found out that although there are workers with large and others with small dehiscent organs, there is a great morphological variation in the size of these organs (Figure 3). There is a gradient between small, almost non-visible dehiscent organs and very large ones, which occupy about a third of the abdominal volume. The size of the dehiscent organs was not related to mandible sharpness, as the range of the mandible sharpness index is largely overlayed between

the two groups (0.84-0.86 for individuals with small dehiscent organs and 0.83-0.86 for individuals with large dehiscent organs). For this reason, we did not find to be necessary to dissect a larger number of individuals.

In addition, there were individuals of *Camponotus* aff. *nidulans* (Smith, F.) ants in both arboreal nests (Figure 4A-B). A myriapod (Pyrgodesmidae) was found inside nest galleries (Figure 4C).

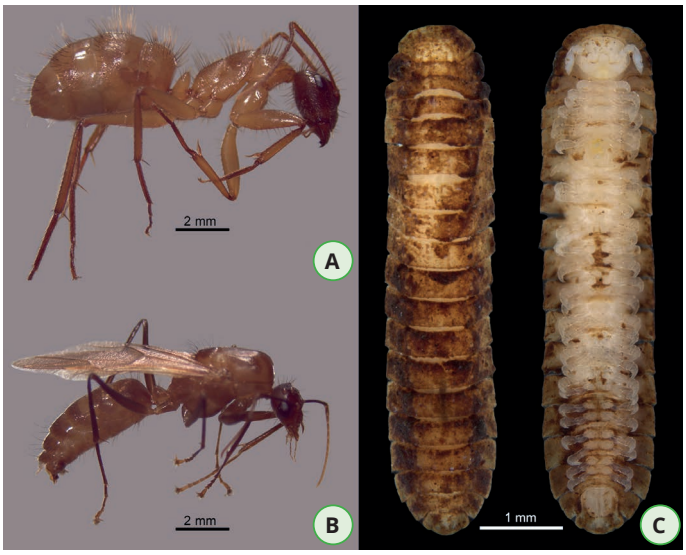


**Figure 4:** Termitophilous found inside termite nests. A and B - *Camponotus* aff. *nidulans* ant. C - Pyrgodesmidae sp.

## DISCUSSION

We have found that although *R. arboreus* colonies build cartonated nests, *R. arboreus* colonies can use clay at some parts of the nest, especially on top, presumably as a waterproof layer to protect the nest from rain. We also have also found a clayish nest at Ducke Reserve (Figure 1C-D). Some *Ruptitermes* species can habit nests made by other termite species (Acioli & Constantino 2015), and it is possible that the colony we have found colonized a nest built by another species. However, it was visible that part of the nest was recently built, which was morphologically similar to the rest of the nest. The nest was built on a horizontal surface instead of a vertical one and was exposed to rainfall. The unusual location of the nest may explain the use of clay to build the entire nest. It is possible that the nest was damaged during the fall and exposition to rainfall and needed to be reconstructed. Although some species build characteristic nests, which can be used even to identify species (Thorne 1980), others can adapt their nest morphology according to the environmental conditions (Korb & Linsenmair 1998).

As we analyzed a large number of individuals from the same colony, we saw a variation in the size of the dehiscent organs. Although they have similar body lengths, the size of the dehiscent organs ranges from inconspicuous to very large (Figure 4). Currently, there are two hypotheses about the origin of the defensive compounds in the fluid of the dehiscent organs. Either the fat body produces it in the early life stages of the termites, or it is absorbed and accumulated from food. We hypothesized that *R. arboreus* workers accumulated the defensives through life, and because of this older workers had larger dehiscent organs. A similar strategy of kamikaze workers was developed by the termite *Neocapritermes taracua* Krishna & Araujo (Šobotník *et al.* 2012) in which the development of the defensive glands is age-dependent and fully developed only in older workers (Šobotník *et al.* 2014). This strategy may benefit the colony, as young workers have more time to forage.

However, we found out that the size of dehiscent organs is not related to the sharpness of the mandibles (proxy to age). Besides the size of the dehiscent organs, there are more morphological differences between *R. arboreus* workers: individuals with larger dehiscent organs have small crops, while individuals with small dehiscent organs have larger crops (Acioli & Constantino 2015). Furthermore, Costa-Leonardo *et al.* (2021) verified that individuals varied in their secretory cycle after analyzing the proteomics of the salivary glands of two *Ruptitermes* species: some termites had small acini and less secretion, and others had larger acini and dilated secretory vesicles. This variation may be correlated with different functional roles exercised by those individuals in the colony (polyethism). This may also be the case for the different types of workers of *R. arboreus*, in which individuals with large dehiscent organs may be more apt to deter an attack on the colony. Still, polyethism in soldierless termites is not verified, and overall knowledge about the behavior of this group is incipient (Bourguignon *et al.* 2016). Two termitophilous species were found inside of the nests (Figure 4); individuals of the ant *Camponotus* aff. *nidulans* were found in the two arboreal nests and one myriapod Pyrgodesmidae was found in the clayish nest. The relation between those species and the termites is not known, as for most termite associated fauna (Pires-Silva *et al.* 2021).

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