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SCIENTIFIC NOTE

A Dazzling Number of Beetles (Coleoptera) in a Hibernating Nest of Red Wood Ants, *Formica rufa* Linnaeus (Hymenoptera: Formicidae)

T. Parmentier
Terrestrial Ecology Unit, Department of Biology, Ghent University
K. L. Ledeganckstraat 35, B-9000 Gent, BELGIUM
Laboratory of Socioecology and Socioevolution, KU Leuven
Naamsestraat 59, B-3000 Leuven, BELGIUM
Research Unit of Environmental and Evolutionary Biology
Namur Institute of Complex Systems and Institute of Life, Earth, and the Environment
University of Namur, Rue de Bruxelles 61, 5000 Namur, BELGIUM
Thomas.Parmentier@ugent.be

AND

R. Claus
Terrestrial Ecology Unit, Department of Biology, Ghent University
K. L. Ledeganckstraat 35, B-9000 Gent, BELGIUM

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A diverse group of beetles permanently lives in the nests of ants (Kistner 1982; Hölldobler and Wilson 1990). It has long fascinated naturalists how these so-called myrmecophiles are able to bypass ant aggression and make a living in the nest (Wasmann 1894). Gradually, we are gaining more insight into the variety of their strategies and the interactions with their hosts (Parker 2016; Parmentier *et al.* 2016, 2018a, b; von Beeren *et al.* 2018). Their life history makes them good models to test hypotheses on topics such as co-evolution, the evolution of host specialization, and the spatial dynamics of symbionts (Ivens *et al.* 2016). Unfortunately, myrmecophilous beetles are typically rare and are seldom collected in large numbers. Therefore, many studies on these organisms fail to collect enough data points to make general predictions. Here, however, we report an unexpected finding of a bestiary of 1,935 beetles belonging to 11 species in a fragment of a single ant nest.

As part of an ecological study on myrmecophiles associated with red wood ants (*Formica rufa* group), we collected 18 L of nest material (both organic thatch and soil) from a nest of *Formica rufa* Linnaeus, 1761 in Boeschepe, France on 15 February 2019. The nest was constructed around a hollow tree and not active at the time of sampling. We only sampled a fragment (50 cm below the surface and adjacent to the tree) of the total nest (~20% of the total volume). We were not able to take material out from the hollow tree, in which the main part of the hibernating colony likely resided.

We searched for myrmecophilous beetles by spreading out small volumes of nest material in a large tray in the laboratory. The beetles were collected by using an aspirator and housed in plastic containers with a moist plaster bottom. The beetles were identified using the keys in Freude *et al.* (1964, 1974), grouped by species, and counted.

We found 1,935 beetles belonging to 11 species (Fig. 1, Table 1, Video S1 in Supplementary Material). The nest fragment that we sampled also contained 602 ants. All the collected beetle species are obligate myrmecophiles and known to consume brood and pilfer ant-collected prey to varying degrees (Parmentier *et al.* 2016). They were found in the adult stage, except for the leaf beetle *Clytra quadripunctata* Linnaeus, 1758 (Chrysomelidae). This beetle has an alternating life cycle with the adults living near wood ant nests, whereas the larvae develop inside the nest over a period of two years (Donisthorpe 1902). The rather large larvae carry a case in which they can hide (Fig. 1). Six species belonged to Staphylinidae.

The important role of the organic nest mounds of red wood ants as hubs for a diverse community of myrmecophiles is well recognized (Parmentier *et al.* 2014). However, this is the first time that such high numbers of beetles have been reported from a single red wood ant nest. We found a density of 107.5 beetles per liter of nest material. *Amidobia talpa*

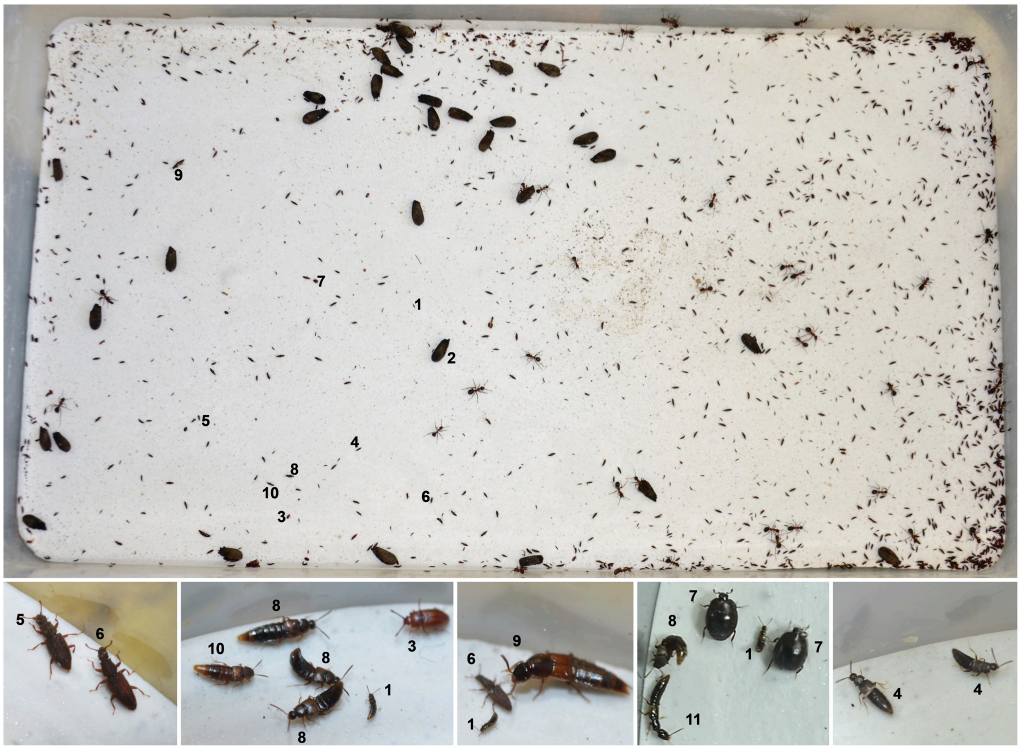

Fig. 1. Overview of the beetle community in a fragment of a nest of red wood ants, *Formica rufa*. Top figure displays all collected beetles with a handful of workers. Figures in the lower panel show the different beetle species in detail: 1) *Amidobia talpa*; 2) *Clytra quadripunctata*; 3) *Spavius glaber*; 4) *Lyprocorrhe anceps*; 5) *Monotoma angusticollis*; 6) *Monotoma conicicollis;* 7) *Myrmetes paykulli*; 8) *Nototheca flavipes*; 9) *Quedius brevis*; 10) *Thiasophila angulata*; 11) *Leptacinus formicetorum*.

(Heer, 1841) (Staphylinidae) was the most abundant beetle, reaching a density of 40.1 beetles per liter of nest material. Previously reported densities of beetles in red wood ant nests are hitherto modest, and include: a mean number of 18 beetle individuals per nest using pitfalls (Päivinen *et al.* 2004); a maximum number of four conspecific beetles per liter of nest material (Robinson and Robinson 2013); a mean number of 7.5 beetles per liter of nest material (Härkönen and Sorvari 2014); a maximum number of 18 conspecific beetles and a mean number of 9.3 beetles per liter of nest material (Parmentier *et al.* 2015). Most reports recorded only few specimens per species in nests of other ant species as well (*e.g.*, Donisthorpe 1927; Akre and Rettenmeyer 1966; Akino 2002; Lapeva-Gjonova and Lieff 2012; Lenoir *et al.* 2012, 2013; von Beeren *et al.* 2010, 2018). Exceptionally, some authors found myrmecophilous beetle species in large numbers: 300 specimens of *Ecitomorpha arachnoides* Wasmann and *Ecitophya simulans* (Wasmann) in a colony of *Eciton burchellii* (Westwood) (Akre and Rettenmeyer 1966); over 60 specimens of *Lomechusoides strumosus* (Fabricius) in nests of *Formica sanguinea* Latreille (Donisthorpe 1927); over 100 specimens of *Amphotis marginata* (Fabricius) in a nest of *Lasius fuliginosus* (Latreille) (Donisthorpe 1927); and 135 larvae of *C. quadripunctata* in a colony of *Formica polyctena* Foerster (Parmentier 2019). Nevertheless, our observation of large abundances of multiple beetle species in a fragment of a single ant nest is clearly unprecedented.

The beetles in the ant nest feed, mate, and may disperse to other nests in spring and summer (Zagaja *et al.* 2017; Parmentier 2019). During the sampling of several hibernating red wood ant mounds in previous years, we found that all the beetles hibernate near or among a cluster of thousands of workers and queens (unpublished data). Our fragment only contained 602 workers and no queens. A couple of days later on a sunny day, thousands of workers were sunning on the nest. This observation indicates that we only sampled a minor fraction of the ant colony. The main cluster of the colony, including the queens, was possibly hidden in the tree

Table 1. Overview of the collected myrmecophilous beetles from a nest of red wood ants, *Formica rufa*, and their abundance (*n*).

Species	*n*
Staphylinidae	
Amidobia talpa (Heer, 1841)	722
Thiasophila angulata (Erichson, 1837)	380
Lyprocorrhe anceps (Erichson, 1837)	205
Notothecta flavipes (Gravenhorst, 1806)	92
Quedius brevis Erichson, 1840	11
Leptacinus formicetorum Märkel, 1841	1
Monotomidae	
Monotoma conicicollis (Gyllenhal, 1827)	226
Monotoma angusticollis (Gyllenhal, 1827)	82
Cryptophagidae	
Spavius glaber (Gyllenhal, 1808)	155
Histeridae	
Myrmetes paykulli Kanaar, 1979	31
Chrysomelidae	
Clytra quadripunctata (Linnaeus, 1758)	30

or deeper in the soil. It is likely that many more beetles were hibernating in this part of the nest and in other parts around the nest as well. The winter nest is small, loose, and not maintained. The nest greatly expands in summer, huge amounts of organic material is collected, and nest chambers are constructed in the organic mound and earth nest. It is likely that a similar number of beetles are present in the summer nests, but the individuals would then be distributed over a much larger nest volume, explaining the modest densities of beetles reported before.

Although the social organization of *F. rufa* is typically monodomous and monogynous, the sampled mound was part of a polydomous red wood ant colony spread over *ca.* 30 nests in an area of 0.11 km^2. Each nest ("mound") contains a large number of queens (unpublished data). A similar organization was reported in neighboring *F. rufa* populations (Dekoninck *et al.* 2010). Ants showed no aggression towards ants of other mounds in the study site, and there was a continuous exchange of brood and workers among the mounds. This robust and stable organization may facilitate the distribution and high abundances of myrmecophilous beetles.

Overall, our study suggests that myrmecophilous beetles can be extremely abundant. It also hints that collecting large numbers of these organisms might be easier during hibernation when they clump together with their host.

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