Established populations of the indoor silverfish *Lepisma saccharinum* (Insecta: Zygentoma) in red wood ant nests

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**Keywords.** Commensalism, myrmecophile, pest species, red wood ants, thermophilous.

This paper presents the records of stable outdoor populations of the silverfish *Lepisma saccharinum* (Zygentoma: Lepismatidae) in red wood ant nests in Belgium. This hostile nest environment is a surprising niche, as this silverfish species is usually found indoors and strictly bound to humans in North-Western Europe.

Ant nests are rich micro-environments that attract a diverse array of arthropods [1]. This is partly because temperature and moisture of ant nests are often regulated, providing optimal conditions for its tenants throughout the year [2,3]. An especially rich community of ant associates can be found in the organic mound nests of red wood ants (*Formica rufa* group) [4,5]. These ants perfected thermoregulation in their mound nests [2,6]. They can keep the inner nest temperature higher than the ambient temperature from early spring to late autumn [6]. The organic mounds of red wood ants also provide a favorable humid environment buffered against drought [7].

Silverfish (Insecta: Zygentoma) are a primitive group of wingless insects of which different lineages associate with ants [5,8,9]. Some have evolved to obligate symbionts and can only be found in ant nests, whereas others engage in a facultative association and occur with and without ants [8]. Some silverfish are known to steal retrieved prey from their host ants or to beg for liquid food droplets [1,9], but commensalism is widespread as well [8]. Six silverfish species have been reported in Belgium so far: five synanthropic species that are found indoors (*Ctenolepisma longicaudatum, Ctenolepisma lineatum, Thermobia domestica, Nicoletia phytophila* and *Lepisma saccharinum*) [10,11] and one species
Atelura formicaria which lives outdoors in strict association with ants [12]. *Lepisma saccharinum* Linnaeus, 1758 is a cosmopolitan silverfish that feeds on materials rich in starch and cellulose. It is frequently found indoors in humid places, such as attics, bathrooms, and basements and can become a pest species. In warmer regions, such as in the Mediterranean area, it can also occur outdoors under stones and in leaf litter. In Spain, *L. saccharinum* has also been recorded in nests of different ant species of the genera *Lasius*, *Aphaenogaster*, *Messor*, *Tapinoma* and *Tetramorium*. An overview of the habitats of *L. saccharinum* reported in the literature is given in Table 1. Crucially, *L. saccharinum* is typically bound to urban settings in temperate Europe, and only a few individuals were reported outside buildings so far ([10], Table 1). Mendes [13] revised every citation of *L. saccharinum* known up to that date and wrote that, “with the exception of the Mediterranean subregion”, this species “seems exclusively synanthropic, except for rare cases of colonizing natural habitats.”

In the framework of a detailed monitoring campaign of arthropods associated with red wood ants (*Formica rufa* Linnaeus, 1761 and *Formica polyctena* Förster, 1850), we found over a three-year period (2018–2021) large numbers of *L. saccharinum* in and at the periphery of these ants’ nests (Fig. 1). The identification of *L. saccharinum* was verified by checking the redescription of Mendes [14]. The absence of a setal collar on the pronotum, the types of specialized antennal sensilla and the type of paramera place it within the subfamily Lepismatinae. The urotergal chaetotaxy, the type of notal trichobothrial areas (all anterior open and all posterior closed) and the hyperdeveloped paramera are diagnostic characters of the genus *Lepisma* [15]. *Lepisma saccharinum* has a characteristic combination of characters (pigmentation, structure of infralateral groups of setae of urotergites, shape of the tenth urotergite, length and number of divisions of the ovipositor, etc.) that distinguishes it from other species of the genus [15,16].

We used plastic boxes (Sunware Q-Line Box: 27 × 8.4 × 9 cm, volume: 1.3 L) with a 1 cm layer of moist plaster on the bottom as traps to collect the silverfish. The sides of these traps were too slippery for the silverfish to escape from, but ants could easily climb out of these boxes. The traps at the periphery of the nest were buried so that their top rim was level with the soil surface. We covered these traps with a plastic roof to prevent rain falling into them. The roof was positioned 2 cm above the opening of the traps by attaching plastic caps in the corners of the roof (details and pictures see [17]). Traps were left for one week and then emptied. We used the same type of traps with roofs to assess the diversity in the nests. These traps were completely buried in the organic mound of the nest. As ants gradually filled the

Figure 1 – Interaction between *Formica rufa* (red wood ants) and *Lepisma saccharinum* in the organic mound nest. The worker in the right figure tries to grab the agile silverfish.
Habitats of *Lepisma saccharinum* according to literature on Lepismatidae. Only certain identifications have been included (*`). Abbreviations: A = with ants; S = synanthropic; F = free-living (in natural habitats; N = no data. When the number of citations given in a work for a certain habitat is not mentioned, the occurrence of the species is indicated only with an X.

| Author(s) and year of publication | reference | Geographic location | Habitat |
|----------------------------------|-----------|---------------------|---------|
| **SMITH (2015)**                | [16]      | Australia           | 1       |
| **MOLERO-BALTANÁS et al. (2014)**| [21]      | Spain               | 6 20 66 |
| **LOCK (2007)**                 | [10]      | Belgium             | X 2     |
| **BLOCH (2007)**                | [22]      | Faroe Islands       | X       |
| **MENDES (2002)**               | [23]      | Portugal            | 6 7     |
| **MOLERO-BALTANÁS et al. (2000)**| [24]      | Italy (Sicily)      | 1       |
| **MENDES et al. (2000)**        | [25]      | Azores Islands      | 6 2     |
| **HAZRA et al. (1999)**         | [26]      | India               | 2 1     |
| **MOLERO-BALTANÁS et al. (1996)**| [27]      | Spain               | 29 76   |
| **MENDES (1995)**               | [28]      | Israel              | 1       |
| **MOLERO-BALTANÁS et al. (1994)**| [29]      | Spain               | 7       |
| **MENDES et al. (1992)**        | [30]      | Canary Islands      | 1       |
| **MENDES (1992)**               | [31]      | Algeria, Tunisia    | 2       |
| **MENDES (1992)**               | [31]      | Canada (British Columbia) | 1 |
| **MENDES (1989)**               | [32]      | China               | 1       |
| **MENDES (1985)**               | [33]      | Poland              | 4       |
| **MENDES (1984)**               | [34]      | Greece (Santorini Island) | 2 |
| **MENDES (1983)**               | [35]      | Cape Verde Islands  | 1       |
| **MENDES (1981)**               | [36]      | N Macedonia, Turkey | 4       |
| **MENDES (1980a)**              | [37]      | Germany, France     | 3       |
| **MENDES (1980a)**              | [37]      | Corse, Spain        | 2       |
| **MENDES (1980a)**              | [37]      | England, Algeria    | 2       |
| **MENDES (1980a)**              | [37]      | Portugal            | 3 15 32 |
| **MENDES (1980b)**              | [38]      | Malta               | 1       |
| **MENDES (1980c)**              | [39]      | Italy               | 4       |
| **WYGODZINSKY (1970)**          | [40]      | Saint Helena island | X X     |
| **WYGODZINSKY (1967)**          | [41]      | Brazil, Bolivia, Argentina | X |
| **PACLT (1966)**                | [42]      | Panama, Colombia    | 2       |
| **WYGODZINSKY (1962)**          | [43]      | Afghanistan         | 1       |
| **PACLT (1961)**                | [44]      | Austria, France     | 2       |
| **PACLT (1961)**                | [44]      | Germany             | 1 2     |
| **WYGODZINSKY (1959)**          | [45]      | St Martin Island (Lesser Antilles) | 1 |
| **WYGODZINSKY (1958)**          | [46]      | France              | 2       |
| **WYGODZINSKY (1954)**          | [47]      | Netherlands         | 10 1    |
| **UCHIDA (1954)**               | [48]      | Japan               | 1       |
| **WYGODZINSKY (1952)**          | [49]      | Cyprus              | 1       |
| **SWEETMAN (1944)**             | [50]      | United States       | X       |
| **AGRELL (1944)**               | [51]      | Sweden              | X       |
TABLE 2

Overview of the sampled nests and number of trapped *L. saccharinum* silverfish. Nest size (nest surface), coordinates and sampling period are given.

| Nest | Nest surface (m²) | Coordinates | Period | Inside nest | Periphery nest |
|------|-------------------|-------------|--------|-------------|----------------|
|      |                   |             |        | Traps (N)   | Silverfish (N) | Traps (N) | Silverfish (N) |
| Site: Poperinge | | | | | |
| A    | 0.38              | 50.885661° N, 2.698817° W | 6–13 June 2018 | 1 | 1 | 1 | 2 |
| A    |                   |             | 20–27 June 2018 | 1 | 3 | 1 | 3 |
| A    |                   |             | 14–21 July 2018 | 1 | 1 | 1 | 6 |
| A    |                   |             | 1–8 Aug. 2018 | 1 | 0 | 1 | 12 |
| A    |                   |             | 15–22 Sep. 2018 | 1 | 0 | 1 | 28 |
| A    |                   |             | 3–10 Aug. 2020 | 3 | 186 | |
| A    |                   |             | 10–17 Aug. 2021 | 3 | 68 | |
| B    | 0.05              | 50.884457° N, 2.693178° W | 3–10 Aug. 2020 | | 3 | 4 | |
| C    | 0.20              | 50.884939° N, 2.695236° W | 3–10 Aug. 2020 | | 3 | 1 | |
| D    | 0.35              | 50.885369° N, 2.697714° W | 3–10 Aug. 2020 | 3 | 3 | |
| Site: Jabbeke | | | | | |
| E    | 0.19              | 51.1750513° N, 3.136943° W | 19–26 July 2021 | 4 | 9 | |
inside traps with nest material, these boxes had to be emptied every 1–2 days. Silverfish were collected to avoid double counting and traps were put back inside the mound. Between one and four traps were placed in and/or at the periphery of a nest at the same time (details of period of sampling and number of traps used are given in Table 2).

A maximum of 186 unique individuals was collected in nest A in 2020, 68 individuals were found in this nest in 2021. Nest A is located in a deciduous forest in Poperinge, Belgium (Table 2) at a forest edge along a road. Both adults and juvenile *L. saccharinum* were recorded, hinting that the life cycle can be completed in the nests. The nearest building is 156 m away. We also recorded in 2020 some individuals in three other *F. rufa* nests in the same forest complex in Poperinge (nest B: *N* = 4, nest C: *N* = 1, nest D: *N* = 3). Apart from these records, no silverfish were collected in the 47 other *F. rufa* nests at this site. Surprisingly, we also found *L. saccharinum* in the nest of *F. polyctena* in another forest site (Jabbeke, Belgium) 44.3 km away from the Poperinge site (nest E: *N* = 9, Table 2).

In a next step, we were interested to investigate how the ants interacted with the silverfish. Some arthropods associated with red wood ants provoke little or no aggression whereas others are heavily persecuted [18]. We brought some silverfish and *F. rufa* workers from nest A to the lab to conduct behavioral assays. We prepared a circular arena with a moist plaster bottom and with the walls coated with an anti-escape layer (Fluon). Ten ant workers were put into the arena and were able to acclimatize for 30 minutes. Then we introduced a silverfish, waited for 10 s, and subsequently scored the aggression of the ants in the first twenty interactions with the silverfish. We replicated this with ten different silverfish individuals and replaced the workers for each trial.

Red wood ants responded very aggressively, 79.4% of the interactions were aggressive (biting, opening of the mandibles, chasing) on average, 8.9% of the interactions were biting (Fig. 1). The silverfish mostly tried to avoid contact with the red wood ants and ran away when detected. During the experiment, none of the tested silverfish were killed.

We recorded *L. saccharinum* in several outdoor ant nests. The very high abundances of the silverfish in nest A are especially intriguing. The presence of all stages over a three-year period indicated that a stable population has been established in the very hostile red wood ant environment. Although the red wood ants readily detected and attacked the silverfish, they may survive by hiding in the organic material, similar to some strongly attacked obligate ant associates [19,20]. The silverfish are likely attracted to the thermoregulated and humidity-controlled environment and the ample food resources. In that way, heated ant nests can serve as a stepping stone for thermophilous organisms to colonize other outdoor niches. It can be expected that *L. saccharinum* will increasingly explore outdoor habitats in temperate regions as an effect of global warming.

**Acknowledgements**

We are grateful for the sampling permission granted by the Agency of Nature and Forest (ANB) and by Karim Neirynck from Natuurpunt. This work was funded by Fonds Wetenschappelijk Onderzoek - FWO (Junior postdoctoral fellowship TP 1203020N) and the Fonds de la Recherche Scientifique - FNRS (Chargé de recherches TP 30257865).

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*Manuscript received: 17 September 2021*

*Manuscript accepted: 24 January 2022*

*Published on: 3 February 2022*

*Branch editor: Kurt Jordaens*