First record of an indoor pest sawtoothed grain beetle *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) from wild outdoor wood ant nest

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Abstract

Alive individual adult sawtoothed grain beetle *Oryzaephilus surinamensis* (Linnaeus, 1758) was discovered inside a nest mound of the red wood ant *Formica rufa* Linnaeus, 1758 during a survey of myrmecophilous invertebrates. The sawtoothed grain beetle is a widespread indoor pest that has not previously been found in an ant nest. It is one of the most common pests in stored grain and cereal products, but the natural life-style of the species is not known. As the site of discovery was exceptional, we verified the species identification using the DNA barcode. If the sawtoothed grain beetle can live in mounds of red wood ants, the mounds may become widespread source habitats for the future infestations of this serious stored product pest.

1. Introduction

The sawtoothed grain beetle *Oryzaephilus surinamensis* (Linnaeus, 1758) along with the merchants grain beetle *O. mercator* (Fauvel, 1889) are among the most common pests in stored grain and cereal products, but their natural life-styles are not known. They currently have worldwide distributions, but they probably have a tropical origin with closely related species in Africa (*O. parallelus* Halstead, 1980) and Middle East (*O. abeillei* (Guillebeau, 1890) (Halstead 1980). They have not been documented to live outdoors in northern Europe, but *O. surinamensis* has sometimes been caught from light traps and beneath the bark of trees in Britain (Halstead 1980, Robinson 2005).

Oryzaephilus surinamensis has wide tolerance of humidity (between 10 – 90% RH) and it is relatively cold hardy, but it fails to complete its development under the temperature of 17 °C (Halstead 1980, Robinson 2005). The optimum temperature for development is between 30 and 35 °C (Halstead 1980).

A wood ant (*Formica rufa* group) nest can maintain a constant temperature of 26–30 °C in summer and the deeper parts of the nest mound stay above the freezing point in winter (around +5°C; Rosengren et al. 1987, Sorvari & Hakkarainen 2009). Thus, in a wood ant mound, the abiotic conditions for *O. surinamensis* would be close to optimal during summer and could be one of the best outdoor environments for overwintering in the boreal zone.
Wood ant nest mounds would appear to be unlikely suitable habitats for most arthropods, because the ants are predaceous and aggressive towards nest intruders. However, wood ant nests have been proven to be hot spots for arthropod fauna (Laakso & Setälä 1998). So far at least 166 coleopteran species are found in the nests of wood ants of *Formica rufa* group (Päivinen et al. 2002). In order to investigate the diversity of the wood ant-associated

arthropods on Ruissalo Island (SW-Finland) we (SH, JS) conducted a field study where we sampled 12 nest mounds of *Formica polyctena* and 4 of *F. rufa*. One of the *F. rufa* nests contained one alive adult *O. surinamensis* individual: *Oryzaephilus surinamensis* (Linnaeus, 1758) 1 ♀. 3 VII 2009, Turku, Ruissalo, Finland (60.4154:22.1140) 9 m a.s.l. (Fig. 1.).

2. Site of discovery and sampling methods

The *F. rufa* nest was located in a nature conservation area in the Kuuva region close to the southern tip of the Ruissalo Island. The habitat was dominated by Scots pine (*Pinus sylvestris*), mixed with birch (*Betula pendula*) and Norway spruce (*Picea abies*), in that order. The nest mound was 120 cm in diameter and the height was 55 cm, which gives the nest mound a volume of 422 litres when calculated with the formula of a half ellipsoid. The nest mound situated 300 metres away from the nearest summer cottage and 530 metres from the nearest permanently inhabited house. The distance to the two nearest major grain storage was 7.7 km.

Of the nest mound material, 0.9% (3.75 l) was examined. Five sub-samples of 0.75 l, collected from the four cardinal points of the outer part of the active nest mound and one sub-sample from the top of the mound. The samples were taken about 3–5 cm beneath the outer layer of the mound. The sub-samples were pooled and sieved in the field with sieves of 2.5 mm and 1 mm. The cruder material that did not go through the 1 mm sieve was examined in the field and the fine material was brought into the laboratory and examined there. The arthropod samples were picked with forceps and put in 75% ethanol. The *O. surinamensis* individual was identified originally by comparing it with specimens in the Coleoptera collections of the Zoological Museum at the University of Turku (ZMUT), Finland. The sex was determined on the basis of the absence of spines on the basal part of the hind leg (Bousquet 1990).

3. DNA barcoding

Because the individual was found inside of a potentially hostile wood ant mound we wanted to verify the species also using the DNA barcode. Total DNA was extracted using a non-destructive method and QIAgen's DNEasy extraction kit (cat. number 69506; QIAGEN, Valencia, California, USA). The ethanol-stored sample was briefly dried at 60 °C and the whole specimen was then placed on 1.5 mL tube with extraction buffer. The sample was incubated overnight in the buffer at 55–65 °C. After the incubation, the intact sample was removed from the buffer and placed in 99.5% ethanol to stop further digestion. After this, the extraction continued according to the extraction kits' protocol. The DNA barcode region (*Cytochrome oxidase subunit I*) was amplified and sequenced using universal animal primers LCO1490: 5'- GGG TCA ACA AAT CAT AAA GAT ATT GG-3' and HCO2198: 5'- TAA ACT TCA GGG TGA CCA AAA AAT CA-3' (Folmer et al. 1994).

Polymerase chain reaction (PCR) was carried out in 11 µl reaction volumes containing 2 µl of DNA extract, 5.75 µl ddH_2O, 1.0 µl 10x buffer, 1.0 µl $MgCl_2$, 0.5 µl primerF (LCO), 0.5 µl PrimerR (HCO), 0.2 µl dNTPs, and 0.05 µl BioTaq polymerase (Bioline). Thermal cycling was performed with the following program: 95°C for 5 min, then 40 cycles of 94°C for 30 sec, 50°C for 30 sec, 72°C for 1 min 30 sec, and a final extension period of 10 min at 72°C. A blank water sample was used as a control in the PCR. The control sample was negative, indicating that there was no contamination during the PCR setup. The successful PCR product was purified and sequenced by Macrogen Inc. (South Korea). The sequence was trimmed using the software Geneious Pro 5.3.6 (Drummond et al. 2011) and then manually

confirmed by eye. The total length of the trimmed high quality sequence was 635 base pairs. The resulting sequence was uploaded to the public Finnish Arthropoda Barcoding Project (FIART) project in the Barcode of Life Data System (Ratnasingham & Hebert 2007) with process ID FIART001-11. The sequence was identified as *Oryzaephilus surinamensis* (100% match) using BOLD Identification System. The trace files and pictures of the sample are also uploaded into the FIART project.

4. Discussion

The occurrence of *O. surinamensis* in the nest mound where it was discovered and other wood ant mounds needs to be further monitored. *Formica rufa* is a member of the mound-building red wood ant species (*Formica rufa* group) distributed over Eurasia and North America (Czechowski et al. 2002, Jurgensen et al. 2005). If the sawtoothed grain beetle can live in mounds of red wood ants, the mounds may become widespread sources for future infestations of this serious stored product pest.

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Fig. 1. The *Oryzaephilus surinamensis* individual (♀) found in the red wood ant (*Formica rufa*) nest. The scale bar is 0.5 mm. Photo: E.J. Vesterinen.