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First record of the subterranean termite *Reticulitermes grassei* in Switzerland

Silvia GHESINI[1], Gabi MÜLLER[2], Mario MARINI[1]
[1]*Department of Biological, Geological and Environmental Sciences (BiGeA), Università di Bologna, Italy*
[2]*Department of Public Health and Environment City of Zürich, Urban Pest Advisory Service (UPAS), Zürich, Switzerland*

Abstract

Switzerland has no native termite species. Historically, drywood termites have been introduced on six occasions, and subterranean termites were introduced on two occasions (*Reticulitermes lucifugus* and an undetermined *Reticulitermes* species). Only one of these introductions resulted in establishment; however it was subsequently eradicated. A new subterranean termite infestation was found in 2018, in the winter garden of a house on the lake of Zürich (northeastern Switzerland). Mitochondrial DNA analysis (cytochrome oxidase subunit II and 16S ribosomal RNA genes) showed that these termites belong to the Franco-Iberian species *Reticulitermes grassei*, and are genetically similar to populations from southern Spain. Termites were probably introduced with olive and palm trees that were planted in the affected property and in a neighbouring one. Damage was limited to the wooden floor and walls of the winter garden. The infestation was initially treated with diatomaceous earth followed by the insect growth regulator diflubenzuron. The infestation is nearing eradication.

Key words: *Reticulitermes grassei*, subterranean termites, termite damage, invasive species, Switzerland.

Introduction

Native European termites belong to the genera *Kalotermes* Hagen (Kalotermitidae, or drywood termites) and *Reticulitermes* Holmgren (Rhinotermitidae, or subterranean termites). The natural range of *Kalotermes* spp. is limited to areas with a Mediterranean climate: *Kalotermes flavicollis* (F.) is found along most of the circum-Mediterranean lands, while *Kalotermes italicus* Ghesini et Marini is limited to some localities in central Italy (Velonà *et al.*, 2011; Ghesini and Marini, 2013; Scicchitano *et al.*, 2018). The natural range of *Reticulitermes* spp. is more expansive, extending to areas further from the coasts. *Reticulitermes grassei* Clement is distributed in the Iberian peninsula and southwestern France, *Reticulitermes banyulensis* Clement in Spain and southern France, *Reticulitermes lucifugus* (Rossi) in Italy and southern France, *Reticulitermes urbis* Bagneres, Uva et Clement in the Adriatic side of Italy, Croatia, western Greece and southern France, *Reticulitermes balkanensis* Clement in Attica, *Reticulitermes aegeus* Ghesini et Marini in northeastern Greece, Crete and Cyprus (Clément *et al.*, 2001; Nobre *et al.*, 2006; Luchetti *et al.*, 2007; 2013; Lefebvre *et al.*, 2008; 2016; Ghesini and Marini, 2015).

Non-native termite species also have been reported in Europe, where in some cases they established populations after introduction. Among these, the most widespread and destructive, is the North American *Reticulitermes flavipes* (Kollar), that is mainly found in France, along the Atlantic coast and in some urban areas, including Paris (Baudouin *et al.*, 2018). Infestations by this species have also been reported in Austria, Germany and Italy (Weidner, 1978; Sellenschlo, 1988; Ghesini *et al.*, 2010; 2011). Several infestations by *Cryptotermes brevis* (Walker) (Kalotermitidae) are also recorded in some European countries: Great Britain, Germany, Italy, Spain and Portugal (Nunes *et al.*, 2010, and references therein). Termites of the genus *Coptotermes* Wasmann (Rhinotermitidae) have been reported twice in Italy, but they did not become established (Mancini and Priore, 2005; Ghesini *et al.*, 2011).

No native termite species have been documented in Switzerland. There have been six reports of drywood termite introduction in the database of the Urban Pest Advisory Service (UPAS) of the City of Zürich from 1998 to date. In four of these cases, termites were probably introduced via wooden objects from the tropics, while in the remaining cases the source of the introduction is not recorded. A single case of subterranean termite introduction was reported in the literature until now (Billen, 1988): *R. lucifugus* was introduced in Basel with infested potatoes imported from Naples (Italy). This introduction did not cause an infestation, as termites were readily identified and eliminated. In 2013, a *Reticulitermes* sp. infestation was found in a garden in Locarno (southern Switzerland), in the ground near a palm tree and in a wood chip shed, and was subsequently eradicated (Delano Osterwalder, personal communication). Even though no exotic termites seem to have established in Switzerland to date, the risk of subterranean termites invading the warmest areas of the country is considered as a concrete risk (Wittenberg, 2005).

In 2018, a termite infestation in northeastern Switzerland was brought to the attention of the UPAS. In this study, we describe the characteristics of this infestation, identify the species involved, and discuss the implications of these findings.

Materials and methods

Field observations were carried out from June 2018 to October 2019. Termite samples, containing individuals of the worker caste, were collected in July 2019 from two bait stations and preserved in 100% ethanol. A preliminary morphological observation showed that the samples belonged to the genus *Reticulitermes*. As the

identification of *Reticulitermes* termites based on morphological characters is not entirely reliable at the species level, the samples were identified through the analysis of mitochondrial DNA. Analyses were carried out on two workers for each sample. Total DNA was extracted from termite heads with a CTAB protocol (Doyle and Doyle, 1987). A 702-bp portion of the cytochrome oxidase subunit II gene (COII) was amplified by PCR with the primers 5'- CAG ATA AGT GCA TTG GAT TT -3' and 5'- GTT TAA GAG ACC AGT ACT TG -3'. A 494-bp portion of the 16S ribosomal RNA gene was amplified with the primers 5'- CCG GTC TGA ACT CAG ATC ACG T -3' and 5'- CGC CTG TTT AAC AAA AAC AT -3'. These genes were chosen as they are widely used for identification and phylogeny of *Reticulitermes* spp. PCR was performed using GoTaq® Flexi DNA Polymerase kit (Promega, Madison, WI, USA), following the enclosed protocol. Reaction conditions were set as follows: initial denaturation at 94 °C for 5 minutes; 30 cycles composed by denaturation at 94 °C for 30 seconds, annealing at 48 °C for 30 seconds, extension at 72°C for 30 seconds; final extension at 72 °C for 7 minutes. Purification and sequencing were performed by Macrogen Europe BV (Amsterdam, Netherlands). Closely related sequences were identified from GenBank using the BLAST network service (Altschul *et al.*, 1990; Madden *et al.*, 1996) at NCBI.

Results

Characteristics of the infestation

In April 2018, termite alates were observed dispersing from holes in the oak parquet floor of a winter garden of a house near Lake Zürich (Canton Zürich). The pest control company called by the house owner applied diatomaceous earth on two occasions into the holes in the parquet and sprayed insecticide along the windows of the winter garden.

In April 2019, alates were seen again in the winter garden, and more diatomaceous earth was applied. In June, the pest control company installed 24 bait stations (Termigard® System, Quimunsa, Spain) containing cellulose (20 ground stations and four wall stations) on the infested property, as well as three on neighbouring properties. One month later, bait stations were checked and termites were found in two of them, situated in the garden of one of the neighbouring properties, in a location with bamboo near the border of the property where termites were first detected. The bait stations on the property where the termites were initially found were not infested, possibly due to the previous applications of diatomaceous earth. In the infested stations, the cellulose bait was later substituted with cellulose impregnated with diflubenzuron 0.25%. In September, a single worker was found in one of the bait stations. In October, none of the stations contained termites.

The area where termites were found is approximately 350 m^2. Damage was overall moderate, and limited to the wood parquet and walls of the winter garden.

DNA analysis

A single COII haplotype and a single 16S haplotype were found in all the individuals analyzed (GenBank Accession No. MN727371 and MN727372), corresponding to the species *Reticulitermes grassei*. Among the more than 40 COII sequences of *R. grassei* available in GenBank, the most similar ones are those obtained by Kutnik *et al.* (2004) and Lefebvre *et al.* (2016) from samples collected in Spain. In particular, the sequence from the Swiss termites is identical to a sequence isolated from Montoro, Andalusia (JQ431002), and shows an identity percentage higher than 99.5% with sequences from nine other Andalusian localities. For the portion of 16S gene obtained in this study, only three *R. grassei* sequences are available in GenBank, all of them from samples collected in France, so this sequence is not informative as to the origin of the Swiss sample.

Discussion

R. grassei was originally described as a subspecies of *R. lucifugus* (Clément, 1977; 1978), and later considered a separate species. Its natural distribution range extends over Spain, Portugal and southwestern France (Clément, *et al.*, 2001; Nobre *et al.*, 2006; Lefebvre *et al.*, 2016).

As Switzerland is well outside the natural range of *R. grassei*, the presence of this termite in Zürich can be attributed to human-aided dispersal. Based on the genetic affinity between the samples analysed in this work and other *R. grassei* populations with available COII sequences, it seems likely that termites were introduced into Switzerland from Spain, and in particular from Andalusia. An olive tree was planted about 10 years ago near the winter garden where the termites were found, and it is speculated that this was the original source of the termites. In the neighbouring property where infested bait stations were found, there are four olive trees and two palm trees in large pots, which could also be considered as sources of the infestation.

R. grassei is considered an invasive species, as its ecology and reproductive biology enable it to establish and expand outside its native range (Evans *et al.*, 2013). Two cases of infestations outside its range, deriving from human-aided introductions, have been documented. In the 1990s, *R. grassei* was found in North Devon (UK). The infestation was the result of an accidental introduction that might have occurred more than 30 years previously (Jenkins *et al.*, 2001; Verkerk and Bravery, 2001). After the implementation of a termite control program with hexaflumuron baits, the infestation seemed to have been eradicated, but termite activity was detected again in 2009 (Verkerk and Bravery, 2010). In the 2000s, *R. grassei* was found on Faial island (Azores), to which it was possibly imported from mainland Portugal with infested soil in containerized plants or boats (Ferreira *et al.*, 2013).

Even though Switzerland is out of the natural range of European termites, its warmest areas, such as the borders of the lakes and urban areas, are at risk of being colonized by invasive termite species, and this risk could increase with global warming. As termites are introduced into new localities mainly through the importation of plants, soil, timber, and wooden objects, particular attention should be paid when importing these materials from areas where termites are present.

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Authors' addresses: Mario MARINI (corresponding author: mario.marini@unibo.it), Silvia GHESINI, Department of Biological, Geological and Environmental Sciences (BiGeA), *Alma Mater Studiorum* Università di Bologna, via Selmi 3, I-40126 Bologna, Italy; Gabi MÜLLER, Department of Public Health and Environment City of Zürich, Urban Pest Advisory Service (UPAS), Walchestrasse 31, CH-8021 Zürich, Switzerland.

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