

A new finding of *Reticulitermes flavipes* in northern Italy

Silvia GHESINI¹, Nicola PILON², Mario MARINI¹

¹Dipartimento di Biologia Evoluzionistica Sperimentale, Università di Bologna, Italy

²Elitron, Milano, Italy

Abstract

The presence of an introduced termite species, *Reticulitermes flavipes* (Kollar), was recently reported for the first time in Italy, where it was found in Olgiate Olona (Lombardy). In this study, we report the finding of an additional *R. flavipes* infestation in Vailate (Lombardy), about 70 km from Olgiate Olona, indicating that this pest may be more widely distributed than previously known. Another sample examined in this study, collected in Rozzano (Lombardy), was found to belong to *Reticulitermes lucifugus* (Rossi), the only *Reticulitermes* species known for Italy up to 2001. Because south of Lombardy the recently described species *Reticulitermes urbis* Bagnères, Uva et Clement is also present, *Reticulitermes* distribution in Lombardy should be re-analyzed on the basis of genetic analysis.

Key words: *Reticulitermes lucifugus*, termite damage, urban environment, Lombardy.

Introduction

Reticulitermes flavipes (Kollar) (Isoptera Rhinotermitidae), commonly known as the eastern subterranean termite, is native to the eastern and central regions of North America, ranging from Ontario to Florida and from Colorado to north-eastern Mexico (Austin *et al.*, 2005a). *R. flavipes* is considered to be one of the most economically important pests of structures in the United States (McKern *et al.*, 2006).

Human-aided dispersal is responsible for its introduction to other regions of the world, including Europe. *R. flavipes* was described in 1837 from samples collected on infested plants imported from the United States to the greenhouses of the Schönbrunn Palace in Vienna-Austria (Kollar, 1837).

In the early 1900s, *R. flavipes* was found in Europe near the French Atlantic coast, and was described as a new species, *Reticulitermes santonensis* Feytaud (Feytaud, 1924). The synonymy of *R. santonensis* with *R. flavipes* was at first suggested by Jucci (1924) and later by Feytaud (1925), then supported by the analysis of cuticular hydrocarbons and defensive compounds of soldiers (Bagnères *et al.*, 1990), and lastly confirmed by mitochondrial DNA analyses (Jenkins *et al.*, 2001; Austin *et al.*, 2002; Marini and Mantovani, 2002; Uva *et al.*, 2004; Ye *et al.*, 2004; Austin *et al.*, 2005b). *R. flavipes* was most likely introduced to France from the United States during the 18th or 19th centuries (Bagnères *et al.*, 1990; Austin *et al.*, 2005b), possibly from the Mississippi River Basin area that once belonged to the French colonial Empire known as Grande Louisiana or Grande France. *R. flavipes* is now distributed in France in an area extending from the Gironde up to Paris and Normandy (Austin *et al.*, 2005b).

During the 1930s, *R. flavipes* was commonly found within wooden forepoles of channel constructions located within steam district heating of Hamburg (Germany), where it is believed to have been introduced within infested wood from the United States (Weidner, 1937; Hertel and Plarre, 2004; Austin *et al.*, 2005b).

Several records of its presence in Hamburg have appeared up until recent years (Weidner, 1978; Sellen-schlo, 1988; Hertel and Plarre, 2004).

In Italy, the presence of *R. flavipes* was first reported in 2010 in the outskirts of Olgiate Olona (Varese province, Lombardy) (Ghesini *et al.*, 2010).

New *Reticulitermes* sp. findings were brought to our attention in Rozzano (Milan province, Lombardy), a town (12.31 km², 41,000 inhabitants) adjacent to the southern outskirts of Milan, and in Vailate (Cremona province, Lombardy), a small town (9 km², 4,500 inhabitants), surrounded by cultivated lands. Rozzano and Vailate are not very far from Olgiate Olona (about 35 km and 70 km in linear distance, respectively).

We conducted this study to analyze these new samples for species identification due to the presence of native species *Reticulitermes lucifugus* (Rossi) in Lombardy. Morphologic identification is not very reliable for the genus *Reticulitermes* (Szalanski *et al.*, 2003), so we resorted to the sequencing of the mitochondrial 16S ribosomal RNA gene that shows a good resolving power for the identification of *Reticulitermes* species samples, even if they only contain a few worker termites, which is often the case in termite sampling.

In this study, we describe the infestations discovered in Rozzano and Vailate, report the results of 16S analysis, and discuss the implications of the findings.

Materials and methods

Termites were collected from a door frame in a house in Vailate (Cremona) and under corrugated cardboard in a warehouse in Rozzano (Milano). Each sample was preserved in 100% ethanol. DNA analyses were carried out on two workers per sample. Total DNA was extracted from termite heads with a CTAB protocol (Doyle and Doyle, 1987). A 519-bp portion of the mitochondrial 16S ribosomal RNA gene was amplified by PCR with the primers mtD-32=LR-J-12887 (5'-CCG GTC TGA ACT CAG ATC ACG T-3') and mtD-34=LR-N-13398

(5'-CGC CTG TTT AAC AAA AAC AT-3'). Sequencing was performed by Macrogen Inc. (Seoul, South Korea). Closely related sequences were identified from GenBank using the BLAST network service (Altschul *et al.*, 1990) at NCBI.

Results and discussion

In 2003 termites were found under corrugate cardboards in a warehouse in Valleambrosia, a suburb of Rozzano (Milan). To date, no other damage to the nearby structures has been detected.

In 2009 termites were found infesting in some houses (40 years old) in Vailate (Cremona). The first traces of termite activity were actually found four years before, but as often with these cases, the damage was not initially identified as termite activity. Termite damage was also found, but to a lesser extent, in the old town, in some houses built about a century ago. In April 2010, some swarms were observed by homeowners inside their houses. At present, at least 20 houses are still infested. In particular, damage is found on the ground floor on structures such as: window and door frames, parquet, furniture, and books.

The analysis of 16S sequences revealed that termites collected from Rozzano were *R. lucifugus*, while termites collected from Vailate were *R. flavipes*. In particular, 16S sequence of the Rozzano sample (Genbank Accession No. HQ231232) corresponds to a *R. lucifugus* haplotype found previously in several sites in peninsular Italy (haplotype 4 in Marini and Mantovani, 2002), while the 16S haplotype of the Vailate sample (GenBank Accession No. HQ231231) is identical to the *R. flavipes* haplotype present in Olgiate Olona.

The Italian *R. flavipes* haplotype is one of the four French haplotypes, that are a subset of the 47 USA haplotypes, so it seems likely, on account of the geographic proximity and of the intensity of trade between the two countries, that Italian populations were imported from France (Ghesini *et al.*, 2010).

R. flavipes findings in two different sites 70 km apart in Lombardy lead us to believe that this species could be more widespread in northern Italy and emphasizes the risk of accidental introductions of termites through human activities. *R. flavipes* is a primary pest of structures in North America (Su *et al.*, 1993, Austin, 2005a, McKern *et al.*, 2006). In non-endemic regions of the world, such as France, Hamburg (Germany) and Chile, it is also considered an economically important structural pest based on damage to buildings and trees (Lohou *et al.*, 1997; Hertel and Plarre, 2004; Su *et al.*, 2006).

Up to 2001, *Reticulitermes* findings in Italy were all referred to as *R. lucifugus*, the only *Reticulitermes* species known to be present in Italy. Further analyses based on chemical (cuticular hydrocarbons and defensive compounds of the soldiers) and genetic (mitochondrial DNA) characters revealed the occurrence of an additional species in Italy (Clément *et al.*, 2001), that was later described as *Reticulitermes urbis* Bagnères, Uva et Clément (Bagnères *et al.*, 2003). While *R. lucifugus* is generally distributed from northern Italy to the south, *R.*

urbis is typically found in eastern Italy (Marini and Mantovani, 2002; Luchetti *et al.*, 2004).

Previous reports of *R. lucifugus* populations in northern Italy, dating back to the period when *R. lucifugus* was thought to be the only Italian *Reticulitermes* species (Sbrenna and Micciarelli Sbrenna, 2008), should be re-analyzed, considering the possibility that some of those populations could actually belong to either *R. urbis* or *R. flavipes*.

Termite surveys in northern Italy should be conducted in order to determine the current distribution of each species and to identify additional *R. flavipes* populations to potentially reduce or eliminate further spread.

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Authors' addresses: Silvia GHESINI, Mario MARINI (corresponding author: mario.marini@unibo.it), Dipartimento di Biologia Evoluzionistica Sperimentale, *Alma Mater Studiorum* Università di Bologna, via Selmi 3, I-40126 Bologna, Italy; Nicola PILON, Elitron, via Capri 11/3, I-20153 Milano, Italy.

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