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First report of *Reticulitermes lucifugus corsicus* in the Piedmont Region of Italy

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Abstract

The presence of subterranean termites in northern Italy is not common, and generally limited to urban areas where winter microclimatic conditions are milder than in the surrounding natural environment. To date there is only one report of termite presence for Piedmont, doubtfully identified as *Reticulitermes lucifugus* (Rossi). The present paper relates on the recent finding of *Reticulitermes* spp. termites in two additional locations, where they caused damage to buildings. Termites were identified as *Reticulitermes lucifugus lucifugus* (Rossi) and *Reticulitermes lucifugus corsicus* Clement using mitochondrial DNA analysis. This is the first report of *R. lucifugus corsicus* in northern Italy. Both infestations could have originated either from human-mediated introduction or from survival of relictual populations.

Key words: *Reticulitermes lucifugus lucifugus*, subterranean termites, mitochondrial DNA, termite damage.

Introduction

Until the end of the 20th century, only two termite (Blattodea Isoptera) species were known to occur in Italy: *Reticulitermes lucifugus* (Rossi) (Isoptera Rhinotermitidae) and *Kalotermes flavicollis* (F.) (Isoptera Kalotermitidae). New diagnostic techniques, such as cuticular hydrocarbon and DNA analyses, revealed the presence of additional species and subspecies, which are difficult to differentiate morphologically. Three species have been added to the Italian fauna: *Reticulitermes urbis* Bagneres, Uva et Clement (Marini and Mantovani, 2002; Bagnères *et al.*, 2003; Luchetti *et al.*, 2004; Ghesini and Marini, 2012), *Reticulitermes flavipes* Kollar (Ghesini *et al.*, 2010; 2011a), and *Kalotermes italicus* Ghesini et Marini (Ghesini and Marini, 2013).

Thus, three *Reticulitermes* species are now known to occur in Italy: the native *R. lucifugus*, distributed through the majority of Italy; *R. urbis*, distributed mainly on the Adriatic regions and considered by different authors either native (Ghesini and Marini, 2012) or introduced from the Balkan peninsula (Luchetti *et al.*, 2007; Leniaud *et al.*, 2010); and *R. flavipes* from North America, which was recently found in Lombardy (Ghesini *et al.*, 2010; Ghesini *et al.*, 2011a). Three subspecies of *R. lucifugus* have been recognized: *Reticulitermes lucifugus lucifugus* (Rossi) in peninsular Italy; *Reticulitermes lucifugus corsicus* Clement in Sardinia, Tuscany and the Tuscan Archipelago; and a third Sicilian subspecies not yet described (Luchetti *et al.*, 2004).

Other termite species have occasionally been reported in Italy: *Cryptotermes brevis* Walker (Tremblay and Priore, 1997; Liotta and Matranga, 1999; Raineri *et al.*, 2001), *Coptotermes* sp. (Mancini and Priore, 2005) and *Coptotermes gestroi* (Wasmann) (Ghesini *et al.*, 2011b).

In Italian areas with a Mediterranean climate, *Reticulitermes* spp. termites are found both in urban areas and in natural environments, while in areas with a continental climate, such as northern Italy, they are found only in urban areas, where the warmer microclimatic conditions beneath and surrounding buildings create conditions suitable for their survival (Ghesini and Marini, 2012).

The presence of termites in urban environments in northern Italy can be explained in two possible ways: i) human-mediated introduction (e.g. with infested wooden materials, soil, or with living plants coming from infested nurseries) and ii) survival of relict populations in conservative environments.

The first hypothesis is a documented possibility, and can result in heavy infestations, as shown by the cases of *R. flavipes* in France, Austria, Germany and Italy (Ghesini *et al.*, 2010), and of *R. urbis* in Domène (Isère, France) (Leniaud *et al.*, 2009).

The second hypothesis is supported by the observation that in northern Italy termites are found almost exclusively in older urban areas, which were founded many centuries ago or even during the Roman Empire period, such as in Bagnacavallo (Ravenna), Mira (Venice), Salsomaggiore (Parma) (Jucci and Springhetti, 1957; Springhetti, 1965; Savoldelli and Lupi, 2008; Ghesini and Marini, 2012). In the climatic history of Europe, alternating climatic phases are known at various time-scale levels, and in warmer climate periods the range of distribution of termites extended to higher latitudes than today, as documented by the fossil record (Engel *et al.*, 2007; Engel and Gross, 2009). Climatic changes occurred at millennial and centennial time-scale also in more recent times. Even in the last 2000 years, European climate has experienced climatic oscillations of considerable size. A marked alpine glacier retreat peaking at 2100-1800 cal yr BP reflects warm and dry conditions (Seppä *et al.*, 2009). Another warm peak is documented in Middle Europe in AD 1200-1350 (Late Medieval Warm Optimum) and was followed by a temperature drop leading to the "Little Ice Age" (AD 1550-1850) (Glaser and Riemann, 2009). It is likely that, in colder periods, the range of distribution of termites shifted southwards, where climatic conditions were warmer, leaving relict populations in areas protected from the cold, such as in urban settlements, below and between buildings.

Even though in northern Italy termites are not common, when present they can cause heavy infestations, as in the case of *R. urbis* in Bagnacavallo (Marini and Ferrari, 1998; Ferrari *et al.*, 2011).

In Piedmont (northwestern Italy) termites are not common. A single report existed until now, for the historical centre of Asti, where an infestation doubtfully attributed to *R. lucifugus* was found nearly 20 years ago (Currado, 2000).

The present paper relates on the recent finding of *Reticulitermes* termites in two localities in the Piedmont Region: San Francesco al Campo (Turin) and Spinetta Marengo (Alessandria). The specific identification of these termites was important to define the range of *Reticulitermes* spp. in northern Italy, where *R. lucifugus*, *R. urbis*, and *R. flavipes* are all present (Ghesini *et al*., 2010; 2011a; Ghesini and Marini, 2012). The correct identification of termite species is also important for pest control management (Marini, 2011).

Materials and methods

Inspections were carried out during October and November 2017, in termite-infested buildings and their surrounding areas in San Francesco al Campo, 314 m a.s.l., and Spinetta Marengo, 97-98 m a.s.l. (figure 1). Two termite samples, containing workers and soldiers, were collected from each location, at points approximately 90 m from each other, and preserved in 100% ethanol.

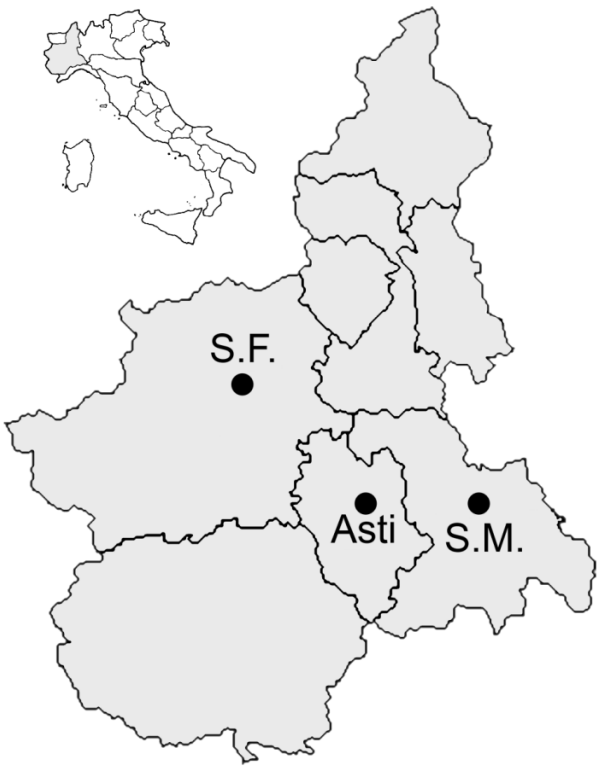

Figure 1. Localitions with documented termite presence in the Piedmont Region of Italy: Asti (Currado, 2000); S.F. = San Francesco al Campo, S.M. = Spinetta Marengo (this study).

A preliminary morphological analysis of soldiers and workers classified all termite specimens as belonging to the genus *Reticulitermes*. For the identification at the species/subspecies level, 684-bp sequences of the cytochrome oxidase subunit II (COII) gene, and 537-bp sequences of the 16S large subunit RNA mitochondrial gene, were obtained and analysed. These genes were chosen as they are widely used for termite identification and phylogenetic reconstructions, and sequences from various locations in Italy have been determined during previous studies (Luchetti *et al.*, 2004; Ghesini and Marini, 2012). DNA extraction and amplification were carried out as in Ghesini and Marini (2017) using two individual termites for each sample. Sequencing was performed by Macrogen Inc. (Madrid, Spain). Sequences obtained in this study are deposited in GenBank under accession numbers MG967459-MG967460 (COII) and MG962533-MG962534 (16S).

Closely related sequences were identified from GenBank using the BLASTN 2.8.0 service (Altschul *et al*., 1997) at NCBI (National Center for Biotechnology Information).

Results

Inspections carried out in San Francesco al Campo revealed the presence of a termite infestation in a house built in the 1970-80s. Termites were found on the ground floor in doors and door frames, as well as in holly (*Ilex aquifolium*) and forsythia (*Forsythia* sp.) stumps in the garden. Doors and door frames had to be completely renovated in 2017 due to termite damage. On the side of a neighbouring road, a decaying old stump of *Prunus* sp. was also found infested by termites.

Inspections carried out in Spinetta Marengo revealed a heavy termite infestation in a house built in the 1850s. On the ground floor, extensive damage to door frames and parquet was found (figure 2). Signs of termite activity were present in the garden inside and among roots and trunks of black locust (*Robinia pseudacacia*), horsechestnut (*Aesculus hippocastanum*) and *Prunus* sp. Termites were also found in a tree stump in a nearby park.

For both COII and 16S genes, nucleotide sequences were identical in the two individual termites from the same sample, and in the two samples from the same location. Sequences from San Francesco al Campo showed the highest correspondence with sequences from *R. lucifugus lucifugus*. In particular, the COII sequence was identical (98% coverage) to a sequence from southern Italy (Rosarno), while the 16S sequence was identical to sequences from northern Italy (Rozzano and Bologna). Sequences from Spinetta Marengo showed the highest correspondence with sequences from *R. lucifugus corsicus*. The COII sequence corresponds to the most common *R. lucifugus corsicus* haplotype (h1 in Luchetti *et al.*, 2013), which is found in central Italy and southern France, while the 16S sequence was identical to a sequence from central Italy (Parco dell'Uccellina).

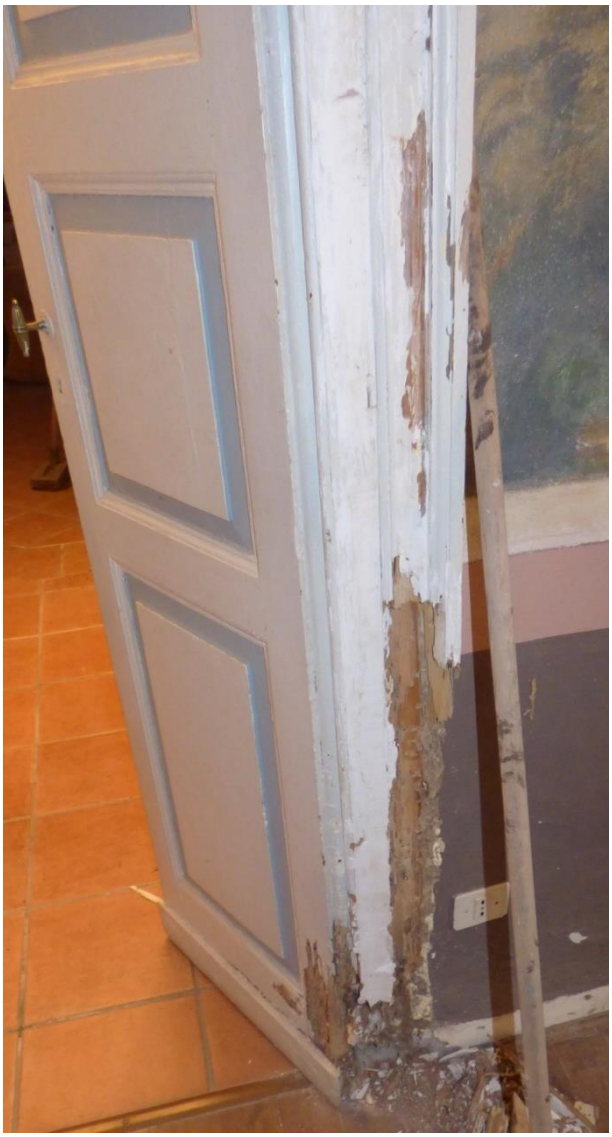

Figure 2. Termite damage in wooden door framing in Spinetta Marengo.

Discussion

There has only been one documented case of termites found in the Piedmont Region, and the termites were identified as *R. lucifugus* based only on morphological characteristics (Currado, 2000). As for many other termite reports in Italy, dating back to times when *R. lucifugus* was the only known *Reticulitermes* species in Italy (reviewed in Sbrenna and Micciarelli Sbrenna, 2008), the original identification as *R. lucifugus* cannot be considered reliable.

This paper reports termite infestations in two additional localities, in San Francesco al Campo (Turin) and Spinetta Marengo (Alessandria), due to *R. lucifugus lucifugus* and *R. lucifugus corsicus*, respectively. *Reticulitermes lucifugus corsicus* had been previously found only in Corsica, Sardinia, and on the coasts of Tuscany and Provence (Clément *et al.*, 2001; Lefebvre *et al.*, 2008), and this is the first report for northern Italy.

The termite-infested house in San Francesco al Campo was built in the 1970-80s on land previously used for agriculture, and thus subject to low winter temperatures, which are not suitable for termite survival. Therefore, it seems likely that termites were recently introduced by human activity. As non-native ornamental plants were present in the garden, it is possible that termites were introduced within soil from infested plants purchased from nurseries or brought from regions of Italy where termites are more abundant. In fact, plant nurseries are located mainly in Italian regions with mild winters.

In Spinetta Marengo, termites were found in an old house, built around 1850 in an area with a long history of settlements. In this case, it is possible that the presence of *R. lucifugus corsicus* is due to long-term survival in a favourable environment, even though the possibility of a human-mediated introduction cannot be ruled out.

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