

Description of a new termite species from Cyprus and the Aegean area: *Reticulitermes aegaeus* sp. nov.

Silvia GHESINI, Mario MARINI

Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Bologna, Italy

Abstract

Reticulitermes aegaeus sp. nov. (Rhinotermitidae) is described, based on samples of alates and soldiers collected on the island of Cyprus. The range of the species includes the island of Lošinj (Croatia), north-eastern Greece (Halkidiki and north-eastern Greece), Eastern Peloponnese, Northern Turkey, Crete, Cyprus and Amorgos (Cyclades Islands). The ascertained range of *R. aegaeus* does not overlap with the ranges of other species occurring in the eastern Mediterranean lands. From the phylogenetic point of view, *R. aegaeus* belongs to the eastern Mediterranean *Reticulitermes* clade, that also includes *R. urbis*, *R. balkanensis* and *R. clypeatus*, as well as taxa not yet described from Turkey and the island of Samos. *R. aegaeus* is genetically distinct from other eastern Mediterranean *Reticulitermes* species, while its morphological characters do not allow a reliable diagnosis without further support. DNA analysis provides such corroboration.

Key words: Isoptera, Blattodea, Termitoidae, Rhinotermitidae, subterranean termite, Cyprus.

Introduction

For more than a century, *Reticulitermes lucifugus* (Rossi), described in 1792, was believed to be the only native *Reticulitermes* species in the entire Mediterranean area. In 1952, *Reticulitermes clypeatus* Lash was described from Israel (Lash, 1952). Only in recent decades, has the taxonomy of the genus *Reticulitermes* in Europe been studied in greater detail. Clément (1978) found that what was believed to be a single species (due to morphologic similarity) was in fact a species complex, and described new species from France, the Iberian peninsula and Greece. To date, in addition to *R. lucifugus* and *R. clypeatus*, four native *Reticulitermes* species have been described for the Mediterranean area: *Reticulitermes grassei* Clément (Spain, Portugal, France), *Reticulitermes banyulensis* Clément (Spain, France), *Reticulitermes urbis* Bagnères et Clément (South East France, Adriatic and Ionian coasts of Italy, Croatia and Greece, including the Peloponnese), and *Reticulitermes balkanensis* Clément (Greece: Attica) (Clément, 1978; Plateaux and Clément, 1984; Bagnères *et al.*, 2003). Genetic analyses have shown that *Reticulitermes* populations from eastern Mediterranean lands are also different from *R. lucifugus*, even though some of them are still referred to as “*R. lucifugus* Turkey” for lack of a formal description (Austin *et al.*, 2002; Luchetti *et al.*, 2007; Velonà *et al.*, 2010). Therefore, it is now clear that the range of *R. lucifugus* is limited to Italy and southern France, with three subspecies: *R. l. lucifugus*, *R. l. corsicus*, and a third taxon, distributed only in Sicily, not yet described.

Phylogenetic studies have shown that Mediterranean *Reticulitermes* populations form two large clades, the first comprising western species (*R. banyulensis*, *R. grassei*, *R. lucifugus*), the second comprising eastern species (*R. urbis*, *R. balkanensis*, *R. clypeatus*) and taxa not yet described (Austin *et al.*, 2002; 2006; Luchetti *et al.*, 2004; 2007; Velonà *et al.*, 2010; Ghesini and Marini, 2012). Here we describe one of these last mentioned taxa, distributed in northeastern Greece (Halkidiki pen-

insula, East Macedonia and Thrace), eastern Peloponnese, Ankara, Crete, Cyprus, Amorgos (Cyclades) and Lošinj (Croatia) (figure 1), as *Reticulitermes aegaeus* sp. nov. The description is based on samples from Cyprus that include alates and soldiers. Some morphometric data on soldiers from other areas of the range are also reported (table 1). Scanning electron microscope images of alate, soldier and worker of *R. aegaeus* are available in Ghesini and Marini (2012).

Within its known range to date, *R. aegaeus* is the only *Reticulitermes* species with ascertained presence. Old reports of *R. lucifugus* in Cyprus (Hagen, 1858; Georgioui, 1977), dating back to times when *R. lucifugus* was believed to be the only *Reticulitermes* species in the Mediterranean area, are probably attributable to *R. aegaeus* (Marini *et al.*, 2015).



Figure 1. Distribution of *Reticulitermes* species in Aegean and Eastern Mediterranean lands. Question marks indicate localities where *Reticulitermes* samples were collected for which only 16S sequences are available and the phylogenetic position is not well resolved.

Table 1. Measurements of soldiers (mm): range of variation, mean \pm standard deviation, and number of individuals measured (or, in the case of antennal segments, number of antennae measured).

	Cyprus	Lošinj	Halkidiki	Thrace	Crete	All
Head length						
range	1.46 - 1.83	1.53 - 1.71	1.59 - 1.83	1.56 - 1.65	1.53 - 1.80	1.46 - 1.83
mean \pm s.d.	1.67 \pm 0.12	1.59 \pm 0.07	1.73 \pm 0.09	1.61 \pm 0.03	1.67 \pm 0.09	1.67 \pm 0.10
n	29	5	13	10	17	74
Head width						
range	1.00 - 1.15	1.02 - 1.07	1.05 - 1.23	1.02 - 1.11	1.05 - 1.20	1.00 - 1.23
mean \pm s.d.	1.07 \pm 0.05	1.04 \pm 0.02	1.13 \pm 0.06	1.07 \pm 0.03	1.11 \pm 0.05	1.09 \pm 0.05
n	29	5	13	10	17	74
Left mandible length						
range	0.88 - 1.06	0.93 - 0.99	0.90 - 1.07	0.99 - 1.04	0.93 - 1.08	0.88 - 1.08
mean \pm s.d.	0.97 \pm 0.04	0.96 \pm 0.03	0.98 \pm 0.05	1.01 \pm 0.02	1.00 \pm 0.04	0.98 \pm 0.04
n	29	5	13	8	17	72
Tibia length						
range	0.82 - 1.00	0.87 - 0.98	0.84 - 1.05	0.84 - 0.96	0.87 - 1.05	0.82 - 1.05
mean \pm s.d.	0.92 \pm 0.04	0.94 \pm 0.04	0.94 \pm 0.06	0.90 \pm 0.03	0.94 \pm 0.06	0.93 \pm 0.05
n	29	5	13	10	16	73
Antennal segments						
range	13 - 17	15	16 - 17	15 - 16	16	13 - 17
mean \pm s.d.	15.03 \pm 0.91	15.00 \pm 0.00	16.29 \pm 0.49	15.25 \pm 0.50	16.00 \pm 0.00	15.26 \pm 0.92
n	38	1	7	4	3	53

Materials and methods

All the samples used for morphological observations were collected by one of the authors (M.M.) during several collecting trips carried out in the years 2002-2009.

Measurements were obtained using a stereomicroscope with an ocular micrometer. Measurements were taken following the criteria in Ghesini and Marini (2012). Nine alates (from Cyprus), and 74 soldiers were measured, of which 29 were from Cyprus (8 colonies) and 45 from other areas of *R. aegeus* range (13 colonies) (table 1).

COII and 16S sequences of east-Mediterranean *Reticulitermes* taxa, obtained in previous studies (Austin *et al.*, 2002; Luchetti *et al.*, 2004; 2007; Velonà *et al.*, 2010; Ghesini and Marini, 2012), were drawn from GenBank. Sequences of the American *R. flavipes* and of the west-European *R. lucifugus* were used as outgroups (GenBank A.N. GU070788, GU070789, AF291723, AF292021). Models of nucleotide substitution were evaluated with JModeltest 2.1.2 (Darriba *et al.*, 2012), using the corrected Akaike Information Criterion. The maximum likelihood tree was obtained with PhyML 3.1 (Guindon and Gascuel, 2003). Bootstrap values were calculated based on 200 replicates. The Bayesian phylogenetic tree was obtained with MrBayes 3.2.2 (Ronquist *et al.*, 2012), and convergence was reached after one million generations.

Results and discussion

The results of the morphological analysis of samples from Cyprus are shown in the description of the new species.

Additional data on measurements of soldiers, grouped for geographic areas (corresponding to different sub-

clades, see figure 2), are shown in table 1. For most of the variables, the ranges of variation largely overlap among geographic regions.

The phylogenetic tree in figure 2, summarizing the results of previous phylogenetic studies (Austin *et al.*, 2002; Luchetti *et al.*, 2004; 2007; Velonà *et al.*, 2010; Ghesini and Marini, 2012), aims to illustrate the collocation of *R. aegeus* in the eastern Mediterranean *Reticulitermes* clade, as well as the relationships among *R. aegeus* subclades (figure 2). The subdivision of *R. aegeus* in subclades corresponds fairly well with the geographic origin of the samples.

The areas where the presence of *R. aegeus* is ascertained (figure 1) do not overlap with the ranges of other *Reticulitermes* species. *R. balkanensis* and *R. urbis* are also found on the western coasts of the Aegean Sea, but based on available data, they appear to be vicariant species.

The occurrence of *R. aegeus* in Lošinj (Croatia), far from the Aegean area, can be considered either the result of human-mediated dispersal or an insularised population of a taxon with a larger ancient range (Ghesini and Marini, 2012). The presence of *R. aegeus* in the city of Ankara, a small area separated from the core range of the species, may have different explanations. The phylogenetic affinity of the population from Ankara with those from northeastern Greece (figure 2) suggests the possibility that the range of *R. aegeus* might extend to the southern coasts of the Black Sea, an area where *Reticulitermes* populations have not yet been studied. However, it is unlikely that the range extends to the area surrounding Ankara, which is characterized by an altitude of about 900 m a.s.l. and subject to a continental climate, with cold winters - conditions unfavourable for termites. Therefore, the presence of *R. aegeus* in Ankara could be explained by human-mediated dispersal or as a biogeographic relict.

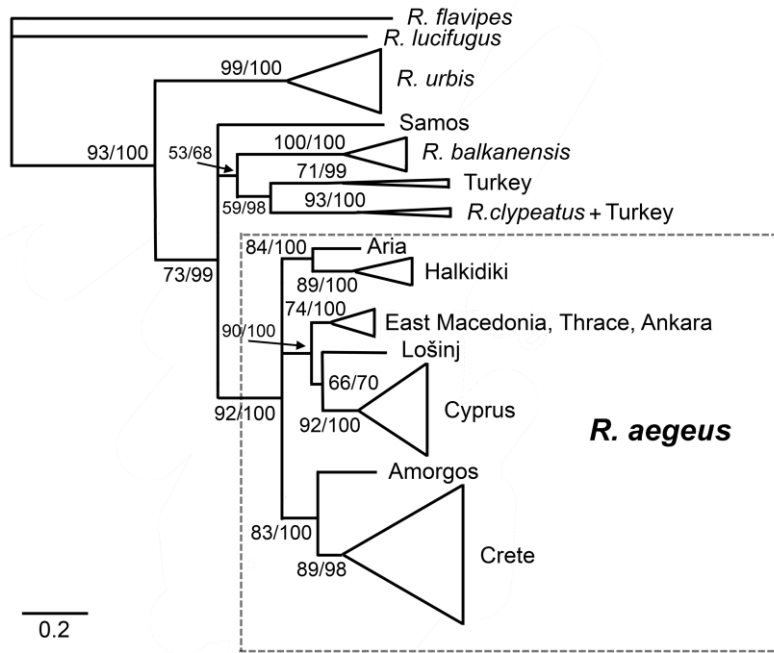


Figure 2. Maximum likelihood and Bayesian phylogenetic tree (topologies are coincident) based on COII and 16S sequences of east-Mediterranean *Reticulitermes* taxa. Numbers at nodes are support values (bootstrap/posterior probability). The scale bar indicates genetic distance.

Reticulitermes aegeus sp. nov.

Holotype: male alate from Kakopetria. Paratypes (8 male alates and 29 soldiers in total): samples Kakopetria, Vasileia, Alagadi, Rizokarpaso, Trikomo, Sotira, Pentakomo, and Kidasi. All these series are in the M. Marini collection, Department BiGeA, University of Bologna, Italy. Data on sampling localities are shown in table 2.

Alate (holotype measurements in parentheses)

Body dark brown, almost black, densely covered with hairs. Clypeus and postclypeus light yellowish-brown. Tarsi and distal portion of tibiae light whitish-brown. Wing membrane smoky grey.

Protruding postclypeus. Eye with 94-102 ommatidia. Maximum eye diameter: 0.21-0.24 mm. Minimum eye diameter: 0.16-0.20 mm. Ocellus separated from the marginal line of ommatidia of the compound eye by a distance approximately equal to the ocellus diameter. Antennae 17- or 18-segmented (18), sometimes with a different number of segments in the two antennae of the

same individual. Head length: 0.97-1.06 mm (1.02 mm). Head width: 0.99-1.04 mm (1.00 mm).

Pronotum length: 0.44-0.50 mm (0.47 mm). Pronotum width: 0.79-0.87 mm (0.87 mm). Forewing length from basal suture to wing tip: 7.27-8.11 mm. Hindwing length from basal suture to wing tip: 6.98-7.62 mm. Tibia length in the third right leg: 0.94-1.09 mm (1.06 mm). Arolia absent.

Soldier

Head ochre, postclypeus lighter. Mandibles ochre at the base, darkening toward the apex. Thorax and abdomen whitish.

Antennal segments 13-17. Antennal asymmetry, with different number of articles in the two antennae of the same individual, quite frequent. Third antennal segment shortest and smallest. Head and mandible measurements: see table 1. Postmentum length: 0.91-1.15 mm. Maximum postmentum width: 0.43-0.47 mm. Minimum postmentum width 0.15-0.19 mm.

Arolia absent.

Table 2. Sampling localities of holotype and paratypes: geographic coordinates, elevation (m a.s.l.), and collection date. Localities are listed in clockwise order, starting from the north-western end of the island.

Sampling localities	Coordinates	Elevation (m a.s.l.)	Collection date
Kakopetria	35°03'N 32°55'E	400	25 II 2009
Vasileia	35°21'N 33°07'E	40	28 XI 2009
Alagadi	35°20'N 33°29'E	10	29 XI 2009
Rizokarpaso	35°36'N 34°21'E	15	30 XI 2009
Trikomo	35°17'N 33°51'E	40	30 XI 2009
Sotira	35°02'N 33°57'E	60	01 III 2009
Pentakomo	34°44'N 33°14'E	54	25 II 2009
Kidasi	34°48'N 32°42'E	250	28 II 2009

Ecology

Samples were collected in diverse environments, from semi-arid to shaded forests, from 0 to 400 m a.s.l. Most colonies were found in natural environments.

Host plants: colonies were found in *Pinus brutia* Tenore, *P. pinea* L., *Cupressus sempervirens* L., *Tamarix* sp., *Salix* sp., and *Dittrichia viscosa* (L.) Greuter. *Morus* sp. and *Acacia saligna* (Labillardiere) H. L. Wendland (cited as *A. cyanophylla*) are also cited by Georgioui (1977). However, it is very likely that many other plants could be attacked, as is usual for European *Reticulitermes* species.

Diagnosis

Due to the high morphological similarity of East-Mediterranean *Reticulitermes* species, and to the high intra-specific variability of morphometric characters, genetic analysis is highly recommended for a correct identification. *R. aegeus* sequences available in GenBank have been obtained in previous studies (Austin *et al.*, 2002; Luchetti *et al.*, 2004; 2007; Velonà *et al.*, 2010; Ghesini and Marini, 2012).

Distribution

NORTHEASTERN GREECE: Jeroussi, Komotini, Messimvria, Nea Fokea, Nea Marmaras, Paliouri, Sarti, Stagira, Xanthi; Amorgos island (Cyclades); EASTERN PELOPONNESE: Aria; NORTHERN TURKEY: Ankara; CRETE: Agia Triada, Agios Pavlos, Aradaina, Elafonisi, Hame-toulo, Hohlakies, Gerani, Georgioupoli, Ierapetra, Kalami, Kalo Horio, Kamilari, Kastelli, Kato Zakros, Kokkino Pirgos, Malia, Panormo, Perivolakia, Sfinari, Sisi, Sitia, Vai; CYPRUS: Alagadi, Argaka, Asgata, Rizokarpaso, Episkopi, Trikomo, Kakopetria, Kapouti, Davlos, Vasileia, Kidasi, Pentakomo, Petra Tou Romiou, Sotira; and CROATIA: Lošinj.

Etymology

The name refers to the currently known distribution range of the species, having its core presence in lands surrounding the Aegean Sea.

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Authors' addresses: Mario MARINI (corresponding author, mario.marini@unibo.it), Silvia GHESENI (silvia.ghesini5@unibo.it), Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Alma Mater Studiorum Università di Bologna, via Selmi 3, I-40126 Bologna, Italy.

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