A DARK-NECKED DRYWOOD TERMITE (ISOPTERA: KALOTERMITIDAE) IN ITALY: DESCRIPTION OF *KALOTERMES ITALICUS* **SP. NOV.**

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Abstract

The yellow-necked drywood termite, *Kalotermes flavicollis* (F.), so called due to the yellow pronotum of its alates, is the only species in the genus *Kalotermes* known for Europe. In some Italian localities, *K. flavicollis* swarms can contain a small proportion of alates with a dark pronotum, but otherwise not different from the normally colored *K. flavicollis*. This color variation was described by G. Becker in 1955 as *Kalotermes flavicollis* var. *fuscicollis*. During collecting trips in central Italy, we found in Grosseto Marina (Tuscany) a *Kalotermes* population whose alates all have a very dark pronotum. Compared with *K. flavicollis* alates, Grosseto alates also have paler wings and smaller arolia. Grosseto soldiers have eyes smaller than those of *K. flavicollis* soldiers. *Kalotermes* sp. form Grosseto also differs morphologically from the other *Kalotermes* species known for the circum-Mediterranean lands. The population from Grosseto has mitochondrial DNA sequences (a partial sequence of the control region and a fragment including a portion of COI, tRNA-Leu and a portion of COII) quite different from *K. flavicollis* (p-distance: 5.6-7.3%). Some *Kalotermes* populations from Tuscany and Marche, morphologically classifiable as *K. flavicollis*, have sequences similar to those of Grosseto *Kalotermes* (p-distance: 0.1-1.0%). These populations are possibly hybrids between *K. flavicollis* and *Kalotermes* sp. from Grosseto. Because of its morphologic and genetic distinctive features, we describe *Kalotermes* sp. from Grosseto as *Kalotermes italicus* **sp. nov.**

Key Words: *Kalotermes flavicollis*, variant *fuscicollis*, morphology, mitochondrial DNA

Resumen

La termita de madera seca de cuello amarillo *Kalotermes flavicollis* (F.), llamada así por el pronoto amarillo en el estadio alado, es la única especie del género *Kalotermes* conocido en Europa. En algunas localidades italianas, enjambres de *K. flavicollis* pueden contener una pequeña proporción de alados con un pronoto oscuro, pero de otra manera no son diferentes de la de color normal de *K. flavicollis*. Esta variación en el color fue descrita por G. Becker en 1955 como *Kalotermes flavicollis* var. *fuscicollis*. Durante los viajes de recolección en el centro de Italia, se encuentró en Marina Grosseto (Toscana) una población de *Kalotermes* cuyos alados tienen un pronoto muy oscuro. En comparación con los alados de *K. flavicollis*, los alados de Grosseto también tienen alas más pálidas y las arolia más pequeñas. Los soldados de Grosseto tienen ojos más pequeños que los de *K. flavicollis*. La *Kalotermes* sp. forma Grosseto también se diferencia morfológicamente de las otras especies de *Kalotermes* conocidas de las tierras circum-mediterráneas. La población de Grosseto tiene secuencias de ADN mitocondrial (una secuencia parcial de la región de control y un fragmento que incluye una parte de COI, tRNA-Leu y una parte de COII) muy diferentes de *K. flavicollis* (p-distancia: 5.6-7.3%). Algunas poblaciones *Kalotermes* de la Toscana y Marche, morfológicamente clasificables como *K. flavicollis*, tienen secuencias similares a las de la *Kalotermes* de Grosseto (p-distancia: 0.1-1.0%). Estas poblaciones son, posiblemente, híbridos entre *K. flavicollis* y la especie de *Kalotermes* de Grosseto. Debido a sus características distintivas morfológicos y genéticos, se describe *Kalotermes* sp. de Grosseto como *Kalotermes italicus* **sp. nov**.

Palabras Clave: *Kalotermes flavicollis*, variante *fuscicollis*, morfología, ADN mitocondrial

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Kalotermes flavicollis (F.) is a drywood termite distributed along most of the Mediterranean coastal areas. It is the only species in the genus *Kalotermes* known to occur in Europe, and was so called (*flavus* = yellow, *collum* = neck) due to the yellowish color of the pronotum of its alates (Fa-

bricius 1793), contrasting starkly with the dark brown/black color of most of their body.

Alates with a pronotum of a darker shade of yellow, with a dark posterior margin, or even entirely dark, of the same color as the head, have been reported for some Italian localities, in the regions Veneto, Tuscany, Marche, Apulia, Campania, and Sicily (Becker 1955; Giordani Soika 1962; Springhetti 1964, 1968). Alates with a dark pronotum were found, usually in small proportions, in colonies that contained a majority of normally colored alates, and a population where all the alates had a dark pronotum has never been found (Becker 1955). Outside Italy, the occurrence of *K. flavicollis* alates with a dark pronotum has never been reported.

This color variation was described by Becker (1955) as *Kalotermes flavicollis* var. *fuscicollis*. The same author carried out experimental crosses, whose results were compatible with a monofactorial inheritance of the color of the pronotum, with an incomplete dominance of the "yellow" allele over the "dark" allele. In fact, most heterozygous alates had a pronotum of a somewhat darker shade of yellow compared with the homozygous "yellow" alates, or with a dark posterior margin. However, this difference was sometimes very slight or apparently nonexistent (Becker 1955). No morphological difference other than the color of the pronotum has been reported between the variant *fuscicollis* and the normally colored *K. flavicollis*.

From the genetic point of view (mitochondrial DNA, microsatellites, and Inter-SINE markers), *K. flavicollis* populations can be separated in 4 main groups, distributed in 4 corresponding geographical regions: i) continental France, ii) Corse and Sardinia, except the Sardinian locality Portoscuso, iii) Portoscuso, iv) central and east Mediterranean area, including peninsular Italy, Croatia, Greece, and Crete (Velonà et al. 2011) (Fig. 1). Within this latter group, genetic variability is very low (Luchetti et al. 2004; Velonà et al. 2011).

Kalotermes colonies that differ from *K. flavicollis* from the genetic point of view (mitochondrial DNA and non-LTR retrotransposon R2), but are morphologically classifiable as *K. flavicollis*, were recently found in Feniglia wildlife reserve (Grosseto province) (Ghesini et al. 2011), where colonies with typical *K. flavicollis* mtDNA haplotypes also exist (Velonà et al 2011).

In this study, we report the finding in Grosseto Marina (Tuscany) of a *Kalotermes* population whose alates all have a dark pronotum. We characterize this population from the morphological point of view, comparing it with the available morphologic data on the *Kalotermes* species known to occur in the circum-Mediterranean lands. In addition, we compare mitochondrial DNA sequences of *Kalotermes* from Grosseto with *K. flavicollis* sequences reported in literature and with sequences of samples collected in Tuscany and Marche, obtained in this study. Because Grosseto *Kalotermes* is different from *K. flavicollis*, *K. flavicollis* var. *fuscicollis*, and from the other species known to occur in the circum-Mediterranean lands, we describe it as *Kalotermes italicus* **sp. nov**.

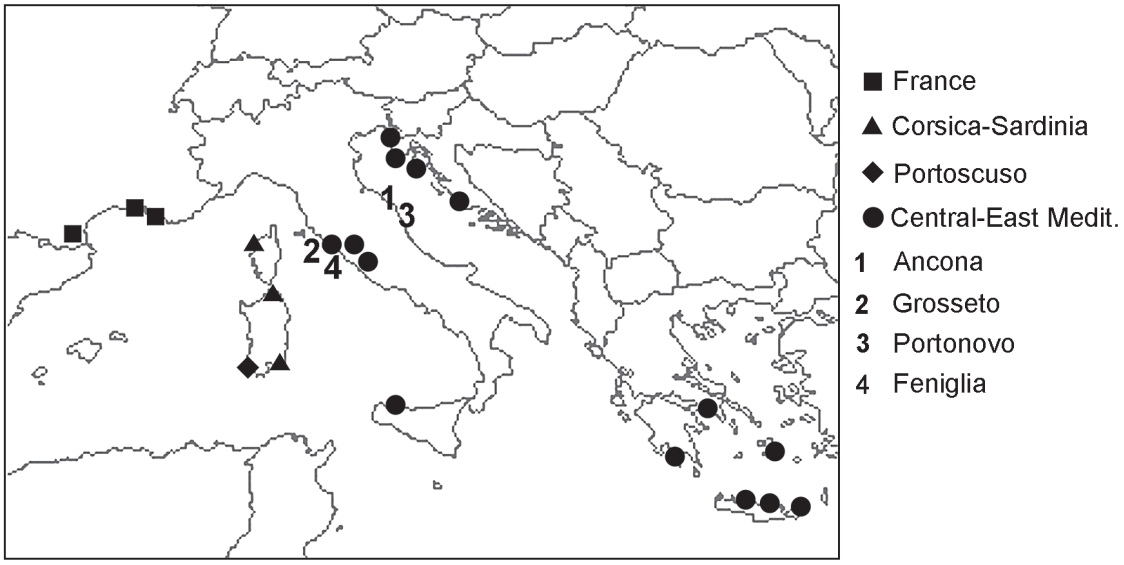

Fig. 1. Distribution of *K. flavicollis* samples analyzed in the literature, separated in the 4 main groups identified in Velonà et al. (2011), and the sampling localities considered in this study.

Materials and Methods

Termites

Three *Kalotermes* colonies with black-necked alates were collected near Grosseto (Tuscany), in Jun 2010. Colonies with yellow-necked alates, morphologically classifiable as *K. flavicollis* based on the description in Springhetti (1964), were collected in Portonovo and Ancona (Marche, 3 and 2 colonies respectively) and in Feniglia wildlife reserve (Tuscany, 2 colonies) in Jun-Jul 2010. *Kalotermes* colonies are quite small, rarely exceeding a few thousand individuals. A colony is usually confined to a single wood log, and very rarely extends over more than a few connected logs or branches. We collected samples in distant fallen tree trunks—at least 50 m from each other—so we are quite sure that we did not sample the same colony twice.

Alates were collected when they spontaneously flew from their mother colonies. Soldiers and juveniles were collected by breaking the wood they were in. The color of the pronotum and of the wings was checked in all the alates, most of which were subsequently either reintroduced in their mother colonies, kept alive in the laboratory, or used for experimental crosses that are now in progress. Samples for genetic analyses were kept in 100% ethanol. Samples for microscopy analysis were kept in 70% ethanol.

The individuals of *K. flavicollis* shown for comparison with Grosseto *Kalotermes* (Figs. 2, 3, 5, and 7) were obtained from a colony collected in Areopolis (Greece), already analyzed from the genetic point of view in previous studies (Luchetti et al. 2004; Velonà et al. 2011).

Morphology

The color of the pronotum and of the wings was checked by eye on more than 150 alates from Grosseto. The observation of other morphologic characters was carried out either with a stereomicroscope or with a scanning electron microscope (Jeol JSM-5200).

Ten alates and 7 soldiers of *Kalotermes* from Grosseto were used for SEM observations. In order to obtain pictures of *K. flavicollis* to show for comparison, 8 alates and 4 soldiers from Areopolis were also observed. Samples were dehydrated in increasing concentrations of ethanol (80%, 90%, 95%, 100%), then kept in a 1:1 solution of hexamethyldisilazane/ethanol for 2-3 h, then immersed in hexamethyldisilazane 100% and air dried. Samples were mounted on aluminium sup-

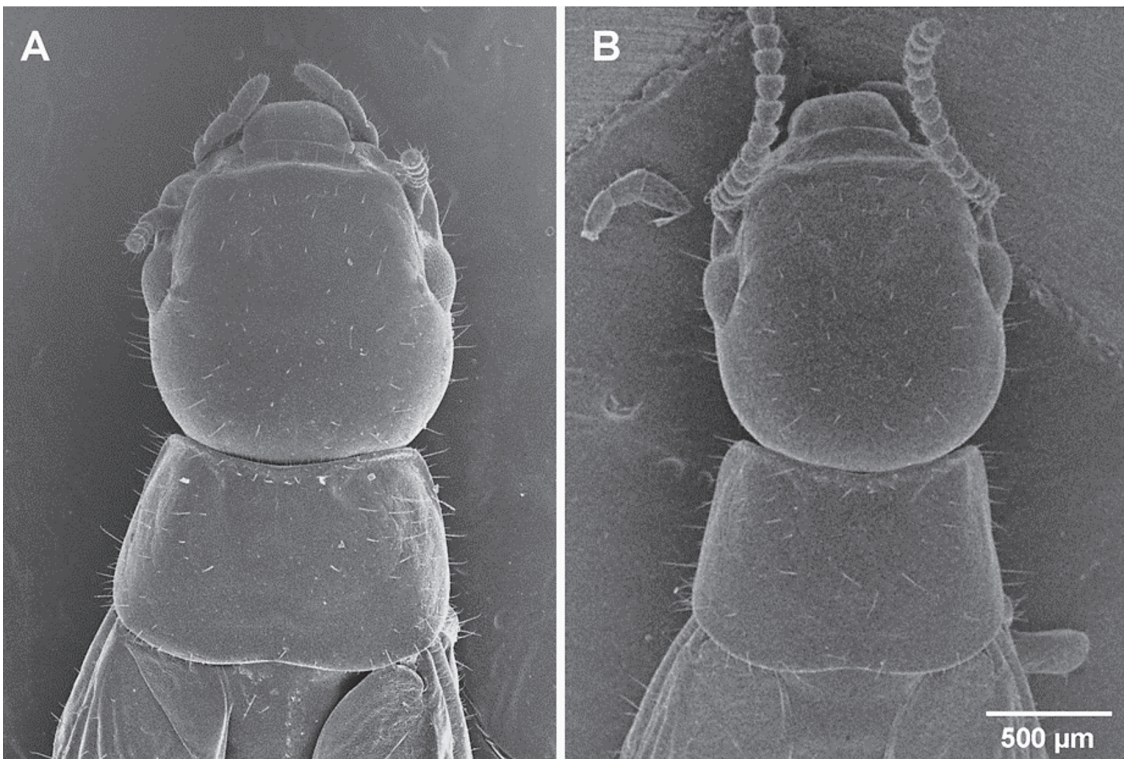

Fig. 2. Alates: head and pronotum. A) *Kalotermes* from Grosseto, B) *Kalotermes flavicollis* from Areopolis.

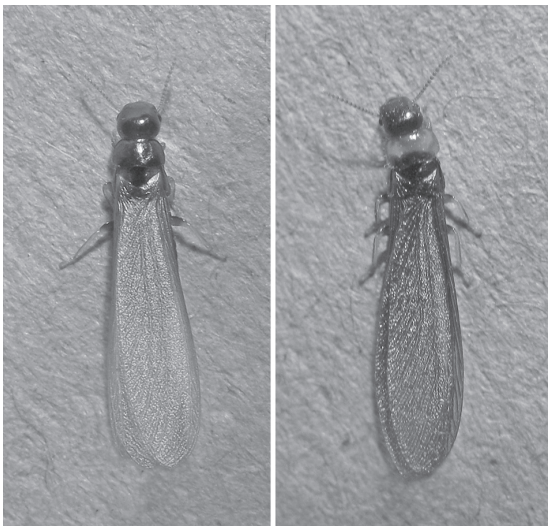

Fig. 3. Left: *Kalotermes* alate from Grosseto. Right: *K. flavicollis* alate from Areopolis.

ports and metalized with gold in a metalizer BIO-RAD SC 502 (45 s at 15 mA). The number of ommatidia forming the compound eye was counted in each of the 8 alates (an eye of each individual). Because in winged termites arolia become atrophied some days after the dispersal flight (Springhetti 1964), arolia were observed on alates that had just flown from their mother nests.

Measurements of Grosseto alates (17 females and 18 males) and soldiers (15 individuals) were taken with an ocular micrometer mounted in a stereomicroscope. For alates, the following measurements were taken: head length (from the distal margin of the clypeus to the posterior end of the head), head width, maximum eye diam, pronotum height along the median line, pronotum width, tibia length of the third right leg, forewing length (from basal suture to wing tip), maximum forewing width, hind wing length (from basal suture to wing tip), maximum hind wing width. For soldiers, the following measurements were taken: head length (from the line joining the 2 mandible insertions to the posterior end of the head), head width, pronotum height along the median line, pronotum width, tibia length in the third right leg. On the same individuals, the number of antennal segments was also counted.

Differences between measurements from female and male alates were tested with Mann-Whitney U test, performed with the software PAST 1.90 (Hammer et al. 2001).

Mitochondrial DNA

Genetic analysis was performed on 3 colonies from Grosseto, 3 colonies from Portonovo (Marche), 2 colonies from Ancona (Marche), and 2 colonies from Feniglia (Tuscany). Sampling localities are shown in Fig. 1. Two individual termites were analyzed from each colony.

Total DNA was extracted from the head of each termite using the CTAB protocol (Doyle & Doyle 1987). A 910-bp fragment (including the 3' end of the cytochrome oxidase subunit I, the tRNA-Leu, and a 5' portion of the cytochrome oxidase subunit II) and a 297-298-bp fragment of the control region were amplified in PCR with the primers C1-J-2797 (5'-CCT CGA CGT TAT TCA GAT TAC C-3')/TK-N-3785 (5'-GTT TAA GAG ACC AGT ACT TG-3') and AT-KR (5'-GTG GCT ATA CCC ACT ATA AA-3')/TM-N-193 (5'-TGG GGT ATG AAC CAG TAG C-3'), respectively. PCR was performed in a 50 µL reaction using GoTaq® Flexi DNA Polymerase kit (Promega, USA). Reaction conditions were set as follows: initial denaturation at 95 °C for 5 min; 30 cycles composed each by denaturation at 95 °C for 30 s, annealing at 48 °C for 30 s, and extension at 72 °C for 30 s; a final extension at 72 °C for 7 min. Purification and sequencing were performed by Macrogen Europe (Amsterdam, The Netherlands).

DNA sequences were aligned and analyzed with MEGA version 5 (Tamura et al. 2011). Closely related sequences were identified from GenBank using the BLAST network service (Altschul et al. 1990) at NCBI. For comparison with the sequences of *Kalotermes* sp. from Grosseto, sequences of *Kalotermes flavicollis* obtained in previous studies (Luchetti et al. 2004; Velonà et al. 2011) were drawn from GenBank and added to the alignments. Sequences of *Kalotermes* colonies from Portonovo and Ancona (Marche) and Feniglia (Tuscany) obtained in this study were also added to the alignments.

Phylogenetic trees were built based on a 665-bp fragment of the COII gene. This fragment was chosen because it was also available for many *K. flavicollis* populations (Luchetti et al. 2004; Velonà et al. 2011), as well as for some other *Kalotermes* species from Australia (Thompson et al. 2000) and Madagascar (Monaghan et al. 2009). A COII sequence from *Mastotermes darwiniensis* Froggatt (Cameron et al. 2012), was used as an outgroup. A sequence from the drywood termite *Neotermes insularis* (Walker) was also added to the alignment as a possible outgroup. Substitution models were tested using the software jModelTest 2.2.1 (Darriba et al. 2012). According to Bayesian information criterion, the best substitution model resulted Tim2 + I + G, I = 0.4490, G = 1.3740. The maximum likelihood tree was obtained with PAUP* 4.0b10 (Swofford 2003). Bootstrap values were calculated with 100 replicates. The Bayesian tree was obtained with MrBayes 3.1.2 (Huelsenbeck et al. 2001; Ronquist & Huelsenbeck 2003). Convergence was reached after 1 million generations (average standard deviation of split frequencies <

0.01). Trees were sampled every 100 generations, and the first 2,500 trees were discarded as burn-in, after graphic visualization.

Results

Morphology

Alates

The alates of *Kalotermes* from Grosseto had a number of antennal segments varying from 14 to 16, scape included. Asymmetry was very common: 27 out of 35 individuals (77.1%) were found that had a different number of segments in the 2 antennae (14-15, 15-16 or even 14-16). The number of ommatidia forming the compound eye ranged from 216 to 249, with a mean of 237.4 ± 10.43. No evident differences existed in the shape of the head between *Kalotermes* from Grosseto and *K. flavicollis* (Fig. 2).

The pronotum was a dark shade of brown, almost black (Fig. 3). When seen from above, the pronotum seemed to be variable in shape across individuals, being more or less narrowed at its anterior margin, but most of this variability was only apparent, depending on the degree to which the sides of the pronotum bent downwards.

Wings were unpigmented, and looked whitish when folded upon the body (Fig. 3). Only 2 individuals out of more than 150 were found that had wings nearly as dark as those of *K. flavicollis*. Wings of males were slightly, but significantly, longer and wider than those of females (Table 1) (Mann-Whitney test, $P < 0.05$). Wing venation was variable among individuals. In many individuals, wing venation was markedly different in the 2 wings of the same pair. As an example, in Fig. 4 are shown the 2 pairs of wings of the same individual. Arolia were present, but smaller than those of *Kalotermes flavicollis* (Fig. 5).

Measurements of alates are shown in Table 2. When measurements of female and male alates did not differ significantly, the 2 datasets were pooled.

Grosseto alates were not able to climb up the walls of the plastic containers they were kept in, such as boxes, jars and Petri dishes, whereas *K. flavicollis* alates climbed quite easily and even walked upside down under the lids (unpublished data). The flying ability of Grosseto alates seemed to be lower compared with *K. flavicollis* alates, but no tests were conducted to verify this impression.

Soldiers

The shape of the head and the shape of the mandibles of Grosseto *Kalotermes* soldiers did not show any evident differences compared with *K. flavicollis* soldiers (Fig. 6). Basal tubercles were present on the outer sides of mandibles. Most of the soldiers from Grosseto had mutilated antennae. Out of the 6 complete antennae we observed, 2 had 13, and 4 had 14 segments. Soldiers had eyes, smaller than those of *K. flavicollis* soldiers (Fig. 6). Measurements of soldiers are presented in Table 1.

Table 1 Measures (in mm) of alates and soldiers of *Kalotermes* from Grosseto.

				Alates	
	Alates	Soldiers		Females	Males
Head length			Forewing length		
Mean ± S.E.	1.3 ± 0.1	2.1 ± 0.2	Mean ± S.E.	6.9 ± 0.1	7.1 ± 0.1
Range	1.1 - 1.5	1.9 - 2.4	Range	6.5 - 7.5	6.8 - 7.9
Head width			Forewing width		
Mean ± S.E.	1.3 ± 0.0	1.5 ± 0.1	Mean ± S.E.	2.0 ± 0.0	2.1 ± 0.0
Range	1.2 - 1.4	1.4 - 1.6	Range	1.9 - 2.3	2.0 - 2.4
Mandible length			Hindwing length		
Mean ± S.E.		1.3 ± 0.1	Mean ± S.E.	7.0 ± 0.1	7.3 ± 0.1
Range		1.2 - 1.4	Range	6.6 - 7.6	7.1 - 8.1
Max. eye diameter			Hindwing width		
Mean ± S.E.	0.3 ± 0.0		Mean ± S.E.	2.1 ± 0.0	2.2 ± 0.0
Range	0.2 - 0.3		Range	1.9 - 2.2	2.0 - 2.4
Pronotum height					
Mean ± S.E.	0.8 ± 0.0	0.7 ± 0.1			
Range	0.7 - 0.8	0.6 - 0.8			
Pronotum width					
Mean ± S.E.	1.5 ± 0.1	1.4 ± 0.1			
Range	1.3 - 1.6	1.2 - 1.5			
Tibia length					
Mean ± S.E.	1.0 ± 0.0	1.1 ± 0.1			
Range	1.0 - 1.1	1.0 - 1.2			

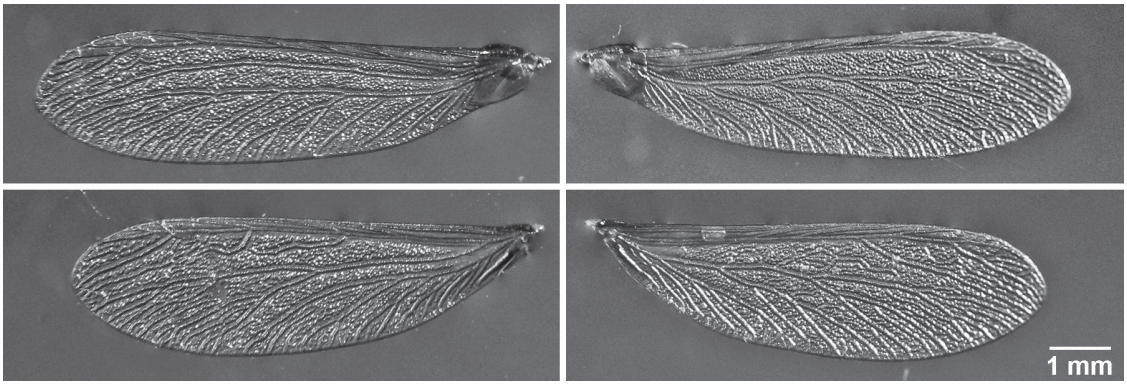

Fig. 4. The 2 pairs of wings of the same individual (a female alate from Grosseto) in dorsal view. Both pairs show asymmetries in wing-venation. Particularly evident is the absence, in the right forewing, of the median vein, that is instead normally developed in the left forewing. Right wings are also slightly shorter than left wings.

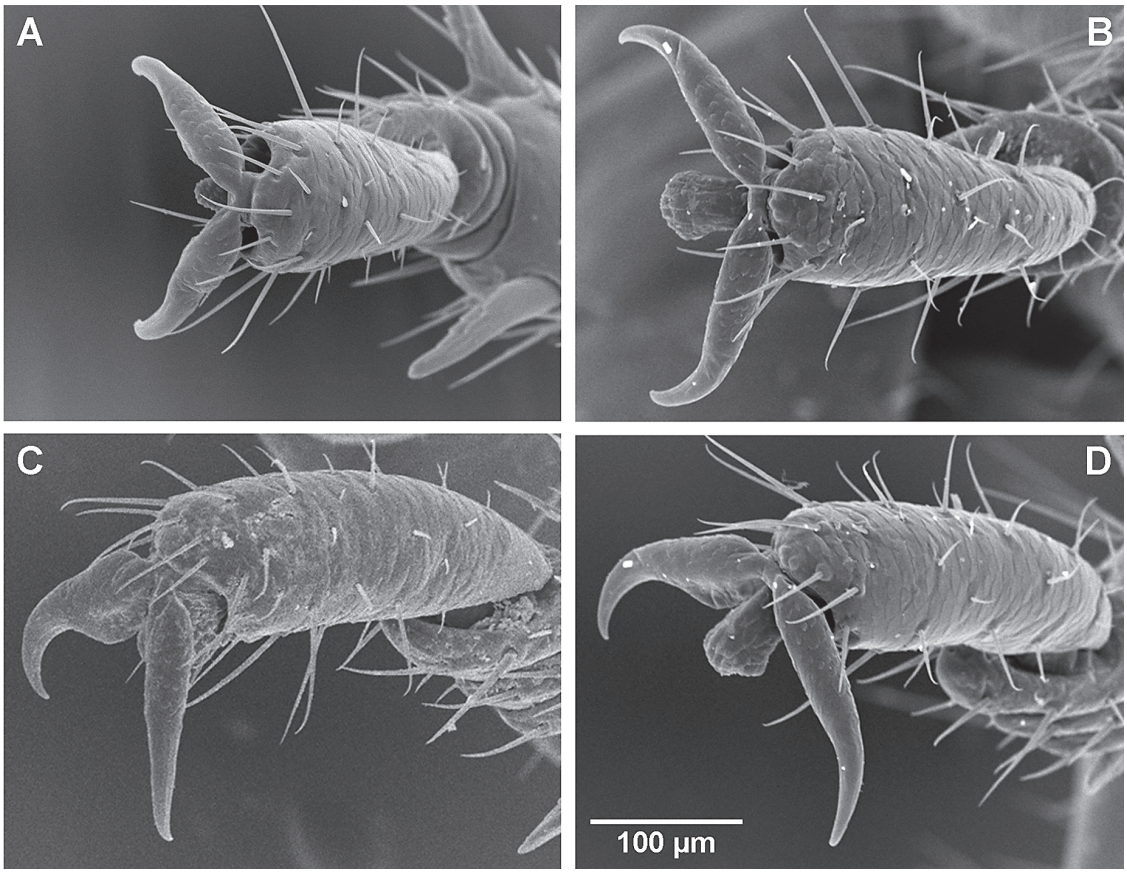

Fig. 5. Alates: tip of the left leg of the first pair. A and C: *Kalotermes* from Grosseto, B and D: *Kalotermes flavicollis* from Areopolis.

Table 2. GeneBank accession numbers of the sequences obtained in this study and species with corresponding mtDNA.

Locality	Color of the pronotum	Colony	COI/tRNA-Leu/COII	Hapl.	Control region	Hapl.	Species
Ancona	Yellow	I	JQ434269	C1	JQ434273	R1	*K. flavicollis*
		II	JQ434269	C1	JQ434273	R1	
Grosseto	Dark brown	I	JN029900	C2	JN029901	R2	*Kalotermes* sp.
		II	JN029900	C2	JN029901	R2	
		III	JN029900	C2	JN029902	R3	
Portonovo	Yellow	I	JQ434267	C3	JQ434270	R4	*Kalotermes* sp.
		II	JQ434267	C3	JQ434271	R5	
		III	JQ434267	C3	JQ434271	R5	
Feniglia	Yellow	I	JQ434268	C3	JQ434272	R4	*Kalotermes* sp.
		II	JQ434268	C3	JQ434272	R4	

Mitochondrial DNA

GenBank accession numbers of the sequences obtained in this study are shown in Table 2. Among the comparable *Kalotermes* spp. sequences available in the literature, obtained for European, African, and Australian species (Thompson et al. 2000; Luchetti et al. 2004; Monaghan et al. 2009; Velonà et al. 2011), those from Europe predictably showed the highest affinity with those obtained in this study. In particular, the 2 colonies from Ancona had typical *K. flavicollis* haplotypes, previously identified in some other Italian localities, as well as in some Greek, Croatian and Cretan localities (Luchetti et al. 2004; Velonà et al. 2011). In contrast, the colonies from Grosseto, Portonovo, and Feniglia have quite different sequences from those of *K. flavicollis* (see below). For the fragment COI/tRNA-Leu/COII, the 3 colonies from Grosseto all had the same haplotype (C2), whereas, for the control region partial sequence, 2 different haplotypes (R2 and R3), differing by a substitution and a 1-bp deletion, were found, the first one in 2 colonies and the second one in the remaining colony.

The 3 colonies from Portonovo and the 2 colonies from Feniglia shared the same COI/tRNA-Leu/COII haplotype (C3). For the control region portion, 2 haplotypes were found in Portonovo (R4 and R5), of which the first one is also found in the 2 colonies from Feniglia.

For both mtDNA fragments, the populations with the minimum p-distances from the Grosseto population were those from Tuscany and Marche (Table 3), which were morphologically classifiable as *K. flavicollis*, but genetically different (Ghesini et al. 2011). When considered together, the Grosseto population and those from Tuscany and Marche differed from *K. flavicollis* in the 5.4-7.1% of sites of the combined dataset, whereas their within-group p-distances were only 0.1-1.0%, and the within-group distances of *K. flavicollis* were 0.0-3.1%. *Kalotermes flavicollis* populations with the minimum p-distances from Grosseto population were found among those from Central and Eastern Mediterranean lands, whereas those with the maximum p-distances were those from Corsica and Sardinia.

Maximum likelihood and Bayesian phylogenetic trees were found to share the same topology (Fig. 7). Populations from Grosseto, Portonovo, and Feniglia form a well supported clade, sister to *K. flavicollis*. Inside this clade, populations from Portonovo and Feniglia form a well supported subclade. A sequence from *Neotermes insularis* was added in the alignment as a possible outgroup, but it unexpectedly turned out to be included in a clade of *Kalotermes* species from Madagascar.

Discussion

The Grosseto population is the first European *Kalotermes* population ever found whose alates all have a dark pronotum. Grosseto alates have another color peculiarity, i.e., unpigmented wings, evidently paler than those of *K. flavicollis* alates. The light color of wings was quite a striking feature, because dark wings have been indicated as a diagnostic character for the genus *Kalotermes* (Krishna 1961). The wings of Grosseto alates seemed to be less effective in flying than those of *K. flavicollis* alates. Wing venation was very variable among individuals, as usually happens in termites (Grassé 1949), and can vary sensibly between the 2 wings of the same pair. Such asymmetries are not unusual in termites, and can be very pronounced, as effectively stated by Tillyard (1931): "We have even noted an extreme case in which right and left wings from the same individual were classified into 2 distinct genera based on venational differences!".

Grosseto alates had 14- to 16-segmented antennae, and most individuals had a different number of segments in the 2 antennae. *Kalotermes flavicollis* alates usually have antennae with 17 or 18 segments, even though some authors have found

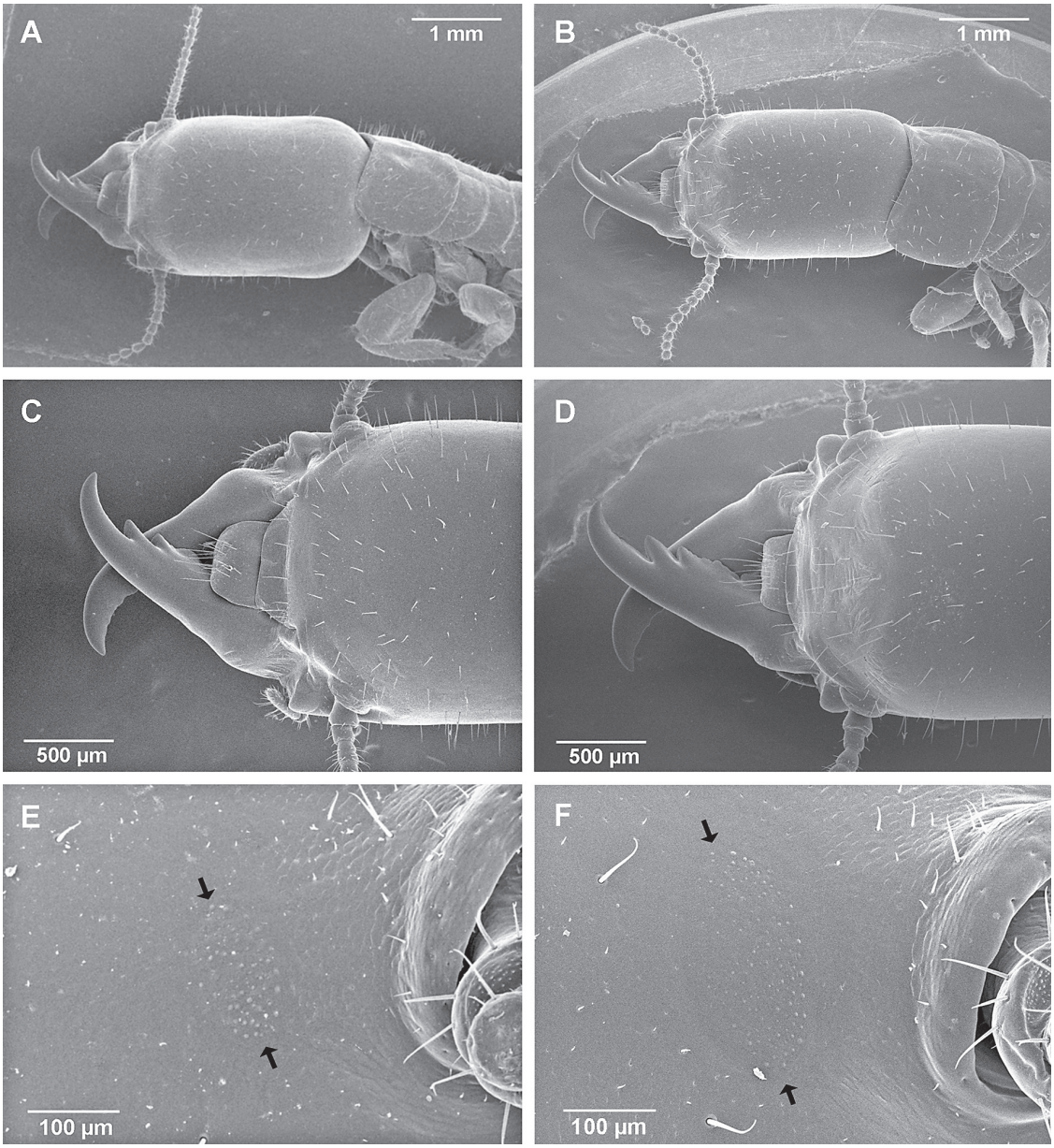

Fig. 6. Soldier head. From top to bottom: whole head, mandibles, and eye. A, C, and E: *Kalotermes* from Grosseto; B, D, and F: *K. flavicollis*. In E and F, the black arrows indicate the extremities of the eye spot, along its major axis.

a wider range of 12-20 segments (Springhetti 1964, and references therein).

Arolia are less developed in Grosseto alates than in the normally colored *K. flavicollis* alates. Because arolia are adhesive organs (Crosland et al. 2005), this afore mentioned difference probably explains the scant ability of Grosseto alates to climb up smooth surfaces, such as the walls of plastic or glass containers.

Grosseto soldiers did not show any important difference from those of *K. flavicollis* soldiers, except for the size of the eye, that in Grosseto soldiers was smaller. In *K. flavicollis*, soldier measurements can vary widely even among colonies of the same population (Springhetti 1964). The measurements of Grosseto soldiers tended to be smaller than those obtained for Italian *K. flavicollis* populations (Rossi & Springhetti 1983), even

Table 3. Per cent p-distances among the Grosseto population, populations from Tuscany and Marche, and the main European *Kalotermes* groups identified in Velonà et al. (2011)[1].

COI/rRNA^{Leu}/COII	GR	TM	CE	CS	PS	FR
GR - *Kalotermes sp.* Grosseto	0.0					
TM - *Kalotermes sp.* Tuscany, Marche	0.1 - 1.1	0.1 - 0.2				
CE - *K. flavicollis* "Central-East Medit."	5.2 - 5.5	5.7 - 6.3	0.0 - 0.6			
CS - *K. flavicollis* Corsica, Sardinia	5.9 - 6.5	6.5 - 7.3	1.2 - 2.2	0.2 - 1.1		
PS - *K. flavicollis* Portoscuso	5.4	5.9 - 6.2	0.5 - 0.9	1.4 - 1.9	0.0	
FR - *K. flavicollis* France	5.5 - 5.7	5.8 - 6.3	1.9 - 2.4	3.0 - 3.8	1.9 - 2.2	0.0 - 0.3
Control region	GR	TM	CE	CS	PS	FR
GR - *Kalotermes sp.* Grosseto	0.3					
TM - *Kalotermes sp.* Tuscany, Marche	0.3 - 1.0	0.0 - 0.3				
CE - *K. flavicollis* "Central-East Medit."	6.1 - 7.5	6.4 - 7.9	0.0 - 0.7			
CS - *K. flavicollis* Corsica, Sardinia	6.4 - 8.4	6.8 - 8.4	0.0 - 0.8	0.0 - 0.8		
PS - *K. flavicollis* Portoscuso	6.5 - 7.2	6.9 - 7.6	0.0 - 1.4	0.0 - 1.3	0.7	
FR - *K. flavicollis* France	7.2 - 7.9	6.9 - 7.6	0.7 - 1.4	0.7 - 1.3	0.7 - 1.8	0.0 - 0.4
Combined	GR	TM	CE	CS	PS	FR
GR - *Kalotermes sp.* Grosseto	0.1					
TM - *Kalotermes sp.* Tuscany, Marche	0.1 - 1.0	0.2				
CE - *K. flavicollis* "Central-East Medit."	5.4 - 5.8	5.9 - 6.4	0.0 -0.4			
CS - *K. flavicollis* Corsica, Sardinia	6.1 - 6.6	6.6 - 7.1	0.9 - 1.7	0.1 - 0.9		
PS - *K. flavicollis* Portoscuso	5.6 - 5.8	6.2 - 6.4	0.4 - 0.8	1.1 - 1.6	0.2	
FR - *K. flavicollis* France	5.9 - 6.2	6.1 - 6.5	1.6 - 2.0	2.4 - 3.1	1.7 - 2.0	0.0 - 0.3

[1]Distances computed for the COI/tRNA-Leu/COII and control region portions, considered separately and combined. Distances within groups are also shown. For each comparison, the extremes of the range of p-distances are shown.

though these data are not perfectly comparable, because Rossi & Springhetti (1983) reported only mean values, and not the ranges of variation.

Kalotermes flavicollis var. *fuscicollis* differed from the normally colored *K. flavicollis* only by its dark pronotum (Becker 1955), whereas the alates from Grosseto also had paler wings and smaller arolia. Thus, we believe that *Kalotermes* from Grosseto is not *K. flavicollis* var. *fuscicollis.*

Besides *K. flavicollis*, the other *Kalotermes* species known for the circum-Mediterranean area are *K. sinaicus* Kemner, found in Israel and Egypt, *K. monticola* (Sjöstedt), found in Algeria, and *K. maroccoensis* (Sjöstedt), found in Morocco. *Kalotermes maroccoensis* and *K. monticola* have been suspected not to be true species, but instead races of *K. flavicollis* (Harris 1970). As far as we know, no descriptions of the alates exist in the literature for these 3 taxa and no DNA sequences are available for a comparison with Grosseto sequences. Moreover, the descriptions of *K. sinaicus* and *K. maroccoensis* are based on few soldiers (5 and 1, respectively) (Kemner 1932; Sjöstedt 1925), so the interspecific variability is inevitably not well represented in the data. The description of *K. monticola* is extremely concise, and the number of soldiers examined is not stated. However, based on the available data, we think it can be reasonably excluded that Grosseto *Kalotermes* belongs to any of these species.

In particular, *K. sinaicus* soldiers are described as having a peculiarly long head, about twice as long as wide, even though a soldier was found that had a shorter head (Kemner 1932), whereas the head of Grosseto soldiers is about 1.4-1.5 times as long as wide. Unlike Grosseto soldiers, *K. sinaicus* soldiers have no basal tubercles on the outer side of mandibles and no eyes (Kemner 1932). *Kalotermes sinaicus* alates have a brown pronotum and no arolia (J. Kugler & D. Simon, personal commun.). The color of the pronotum is similar to the dark brown/black color of Grosseto alates, but the latter character is different in the 2 taxa.

The soldiers of *K. monticola* have no basal tubercles on the outer sides of the mandibles, and shorter and thicker antennae than those of *K. flavicollis* soldiers (Sjöstedt 1925), whereas the Grosseto soldiers have tubercles on the outer sides of the mandibles and their antennae are not dissimilar to those of *K. flavicollis* soldiers.

The soldier of *K. maroccoensis* has a head-width of 1.3 mm, its mandibles are as long as its head is wide, and its antennae are 15-segmented (Sjöstedt 1904), whereas Grosseto soldiers have a wider head, their head width is larger than mandible length, and their antennae are 13- or 14-segmented.

The genetic distance between Grosseto population and *Kalotermes flavicollis* populations

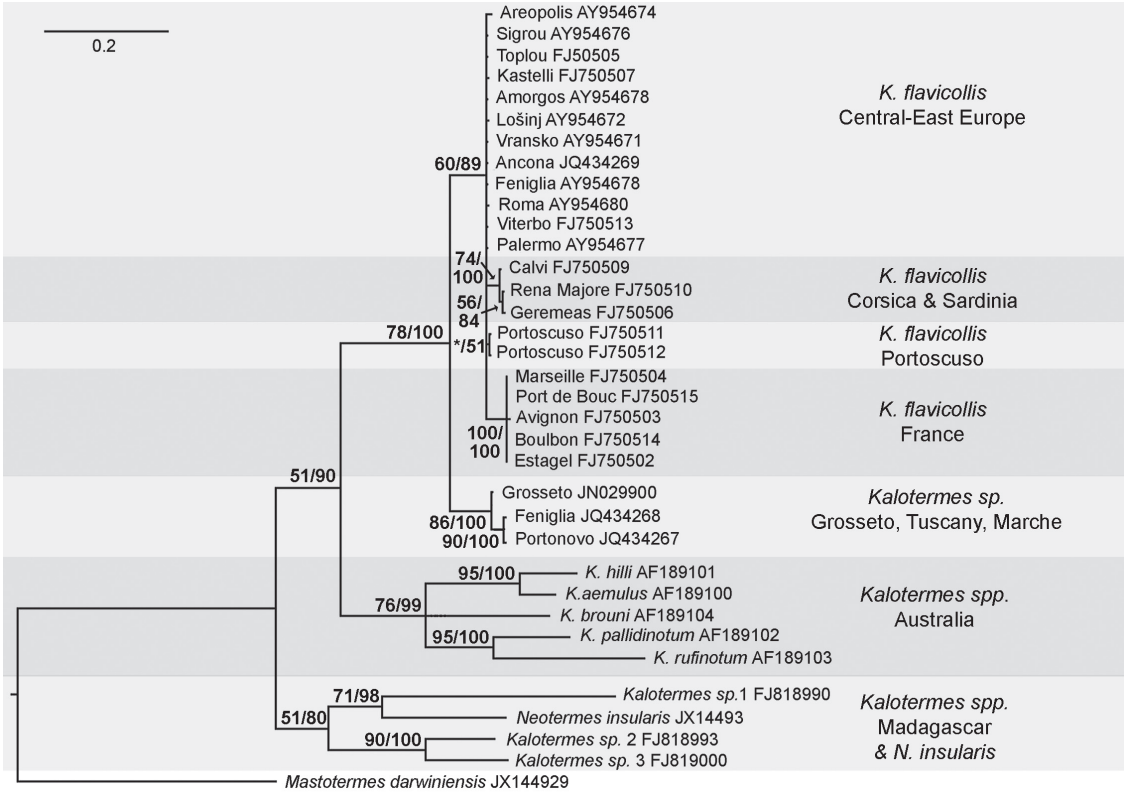

Fig. 7. Maximum Likelihood and Bayesian phylogenetic tree (topologies are coincident) based on COII gene. Numbers at nodes indicate bootstrap values and posterior probabilities. The scale bar represents the number of changes per site.

is quite high (5.2-6.5% for the COI/tRNA-Leu/COII fragment, 5.4-6.6% for the control region portion). Some *Kalotermes* colonies found in Tuscany and Marche, despite having alates with a yellow pronotum and being morphologically classifiable as *K. flavicollis*, have haplotypes similar to those found in Grosseto (p-distance: 0.1-1.0%). Together with the population from Grosseto, these populations form a well-supported clade in the COII-based phylogeny. This discordance between morphology and mtDNA could have arisen from hybridization events between *Kalotermes* sp. from Grosseto and *K. flavicollis*. Individuals of the variant *fuscicollis* are possibly hybrids homozygous for the "dark" allele, and their presence in variable (usually small) proportions in colonies with a majority of yellow-necked alates could be the result of different levels of introgression characterizing different populations. Further studies are needed to test these hypotheses.

Kalotermes italicus **sp. nov.** (Figs. 2-6)

Syntypes: sample IT-GRO1 (20 alates). Paratypes: samples IT-GRO2 (4 soldiers), IT-GRO3 (3 soldiers), IT-GRO4 (10 juveniles), IT-GRO5 (10 alates), IT-GRO6 (5 alates). All samples from the Grosseto Marina (Tuscany, Italy), 8-VI-2010, M. Cruscanti, S. Ghesini and M. Marini. All these series are in the M. Marini collection - Dipartimento di Scienze Biologiche, Geologiche e Ambientali, University of Bologna, Italy.

Imago

Head, thorax and abdominal sclerites dark brown, femura brown, antennae, clypeus, labrum and tibiae pale brown-yellowish; wings unpigmented. Antennae of 14-16 segments, often differing on either side of head. Ommatidia: 216-249. Wing venation variable among individuals, often different in the 2 wings of the same pair. Small arolium present.

Measurements: see Table 1.

Soldier

Head light brown behind, darkening towards the clypeus. Mandibles dark brown, almost black.

Basal tubercles on the outer side of mandibles. Antennae of 13-14 segments. Eye spots present.

Measurements: see Table 1.

Diagnosis

The *Kalotermes italicus* alate differs from the *K. flavicollis* alate by having a dark brown/black pronotum, unpigmented wings, and smaller arolia. It differs from *K. sinaicus* by having arolia. The *Kalotermes italicus* soldier has eyes smaller than those of the *K. flavicollis* soldier, while the *K. sinaicus* soldier has no eyes. The head of *K. italicus* is wider than that of *K. maroccoensis*, about 1.4-1.5 times as long as wide, while the *K. sinaicus* head is about twice as long as wide. *Kalotermes italicus* soldier has antennae of 13-14 segments, while *K. maroccoensis* soldier has 15-segmented antennae. The *Kalotermes italicus* soldier and the *K. flavicollis* soldier have basal tubercles on the outer side of mandibles that are absent in *K. sinaicus* and *K. monticola* soldiers.

From the mitochondrial DNA point of view, the sequences of the fragment COI/tRNA-Leu/COII and of a portion of control region of *K. italicus* (A.N. JN 029900-02) differ from those of *K. flavicollis* (p-distance 5.6-6.7%). Populations with mitochondrial DNA sequences similar to those of *K. italicus*, but morphologically classifiable as *K. flavicollis*, are found in Feniglia (Tuscany) and Portonovo (Marche), and are possibly hybrids between the 2 species.

Distribution

Until now, *K. italicus* has been found only in Grosseto Marina (southern Tuscany, Italy). The sampled area is about 200 × 100 m.

Etymology

The name refers to the currently known distribution of the species, limited to an Italian locality.

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