

Molecular characterization and phylogeny of *Kaloterme*s populations from the Levant, and description of *Kaloterme*s *phoeniciae* sp. nov.

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Abstract

The presence of the yellow-necked drywood termite, *Kaloterme*s *flavicollis* Fabr., has been reported along most of the Mediterranean coasts of Europe, Africa and Asia. While morphological and genetic data exist on European *K. flavicollis* populations from western and central Mediterranean regions, data on eastern Mediterranean *Kaloterme*s are scarce, and no genetic data exist to date. In this study, we analyzed 17 *Kaloterme*s sp. colonies from 11 localities in the Levant (Cyprus, Lebanon, and Israel), in order to characterize genetically (mitochondrial DNA: COII, 16S, and control region) these populations. We found that samples from the Levant are genetically different from *K. flavicollis*, with distance values falling in the range of interspecific distances. In the phylogeny of European *Kaloterme*s populations, samples from the Levant form a clade of their own, sister to a clade including *K. flavicollis* and *Kaloterme*s *italicus*. Inside the eastern Mediterranean clade, all the samples from Cyprus are included in a well-supported subclade, suggesting that the colonization of the island might have occurred in a single event. These findings show that the populations we examined do not belong to the species *K. flavicollis*, but to a new species peculiar to the Levant, that we describe as *Kaloterme*s *phoeniciae* sp. nov. It is possible that previous reports of *K. flavicollis* in this region can be attributed to *K. phoeniciae*.

Keywords: *Kaloterme*s *flavicollis*, Cyprus, Israel, Lebanon

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Introduction

In the Mediterranean area, drywood termites in the genus *Kaloterme*s (Isoptera, Kalotermitidae) are represented by three species: *Kaloterme*s *flavicollis* Fabr., with a wide range of distribution in the circum-Mediterranean lands, *Kaloterme*s *italicus* Ghesini & Marini, with a small range in central Italy, and *Kaloterme*s *monticola* (Sjöstedt), found on a locality in north-eastern Algeria. The Moroccan *Kaloterme*s *maroccoensis* (Sjöstedt) was recently synonymized with *K. flavicollis* (Krishna *et al.*, 2013). *Kaloterme*s *sinaicus* Kemner, distributed

in north-eastern Egypt and Israel, was recently assigned to the new genus *Longicaputerme*s, because of morphological and genetic peculiarities that differentiate it from the genus *Kaloterme*s and liken it to the genera *Comaterme*s, *Ceratokaloterme*s, and *Paraneoterme*s (Ghesini *et al.*, 2014).

While *K. flavicollis* populations from central Mediterranean regions (from France to Crete) have been analyzed with some detail (Luchetti *et al.*, 2004, 2013; Velonà *et al.*, 2011), data on eastern Mediterranean *Kaloterme*s populations are scarce. In particular, no data exist on Cypriot and Lebanese *K. flavicollis*, and for Israel only records of *K. flavicollis* presence in some localities are available (Bodenheimer, 1930; Kugler, 1988).

In this study, we conducted both genetic (mitochondrial DNA: COII, 16S and control region) and morphological analyses of *Kaloterme*s sp. from Cyprus, Lebanon, and Israel, in order to characterize these populations and to clarify their phylogenetic relationships. Because these populations form a

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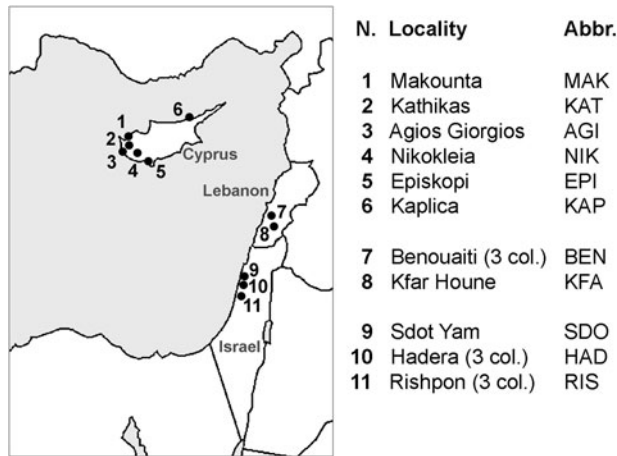


Fig. 1. Sampling localities of *Kaloterмес* sp. in Cyprus, Lebanon, and Israel. One colony was sampled in each locality, except when differently stated (3 col.).

clade sister to the clade composed of *K. flavicollis* and *K. italicus*, and because diagnostic characters exist that differentiate this taxon from west-European *Kaloterмес* species, we describe it as *Kaloterмес phoeniciae* sp. nov.

Materials and methods

Samples of *Kaloterмес* sp., morphologically compatible with *K. flavicollis*, were collected during collecting trips conducted in Cyprus, Lebanon, and Israel in the years 2009–2012. Sampling localities are shown in fig. 1. When two or more colonies were analyzed from the same locality they are identified by a capital letter (A, B, or C) added to the name of the locality. Samples from Cyprus and Lebanon contained all the castes (alates, soldiers, and pseudergates), while samples from Israel contained only pseudergates and soldiers. Samples for genetic analyses were preserved in 100% ethanol, while samples for morphological observations were preserved in 80% ethanol.

Total DNA was extracted from termite heads following the CTAB protocol (Doyle & Doyle, 1987). Two individuals for each sample were analyzed. A 662–668-bp fragment of the cytochrome oxidase subunit II gene (COII), a 515–516-bp fragment of the large mitochondrial ribosomal subunit gene (16S), and a 297–300-bp fragment including the 3' portion of the control region, tRNA-Ile, tRNA-Gln, and the 5' portion of tRNA-Met (CR), were amplified. The primers used are TL2-J-3034 (5'-AAT ATG GCA GAT TAG TGC A-3') and TK-N-3785 (5'-GTT TAA GAG ACC AGT ACT TG-3') for COII, LR-J-12887 (5'-CCG GTC TGA ACT CAG ATC ACG T-3') and LR-N-13398 (5'-CGC CTG TTT AAC AAA AAC AT-3') for 16S, and AT-KR (5'-GTG GCT ATA CCC ACT ATA AA-3') and TM-N-193 (5'-TGG GGT ATG AAC CAG TAG C-3') for CR. Polymerase chain reaction was performed in a 50 µl mixture using GoTaq® Flexi DNA Polymerase kit (Promega, USA), following the enclosed protocol. Reaction conditions were set as follows: initial denaturation at 95°C for 5 min; 30 cycles composed by denaturation at 94°C for 30 s, annealing at 48°C for 30 s, extension at 72°C for 30 s; final extension at 72°C for 7 min. Purification and sequencing were performed by Macrogen Inc. (Amsterdam, The

Netherlands). The sequences obtained in this study are deposited in GenBank under accession numbers KC914294–KC914309 and KC914316–KC914331.

The preliminary analysis and the alignment of DNA sequences were performed with MEGA version 5 (Tamura *et al.*, 2011).

Haplotype networks were obtained with Network 4.6.1.1, available at www.fluxus-engineering.com (Bandelt *et al.*, 1999), and with TCS 2.21 (Clement *et al.*, 2000), based on a COII+16S+CR alignment of the *Kaloterмес* sp. sequences obtained in this work, adding sequences of *K. flavicollis* (Viterbo) and *K. italicus* (Grosseto) as roots for the median-joining analysis.

For the construction of phylogenetic trees, COII and 16S sequences published in previous studies (Velonà *et al.*, 2011; Ghesini & Marini, 2013; Ghesini *et al.*, 2014) from *K. flavicollis* and *K. italicus* were drawn from GenBank and added to the alignment. Sequences from the Mastotermitidae *Mastoterмес darwiniensis* (GenBank A.N. JX144929) and from the Kalotermitidae *Kaloterмес browni*, *Neoterмес insularis*, *Cryptoterмес brevis*, and *Longicaputerмес sinaicus* (A.N. AF189104, KF840415, JX144933, FJ806880, FJ806145, KC914288, KC914292) were used as outgroups. CR sequences were not used for phylogenetic reconstruction because sequences from different genera cannot be reliably aligned.

In order to compare European *Kaloterмес* species with other *Kaloterмес* species for which only COII sequences are available, an alignment was built including the COII sequences used for phylogenetic reconstruction, as well as sequences of species from Australia (Thompson *et al.*, 2000) and Madagascar (Monaghan *et al.*, 2009). This alignment was also used for the construction of a Bayesian tree to be used as input for the Species Delimitation plugin (Masters *et al.*, 2010) of the software Geneious 7.1 (Biomatters), that computes some measures of taxon distinctiveness, including inter-species tree distances, Rodrigo's test, and Rosenberg's reciprocal monophyly test. Rodrigo's test estimates the probability that a clade has the observed degree of distinctiveness due to random coalescent processes; Rosenberg's test tests the hypothesis that monophyly is a chance outcome of random branching (Rosenberg, 2007; Rodrigo *et al.*, 2008; Boykin *et al.*, 2012).

Models of nucleotide substitution were tested with JModelTest 2.1.2 (Darriba *et al.*, 2012), with the corrected Akaike Information Criterion. A partition homogeneity test, to determine whether COII and 16S datasets could be included in the same dataset, was performed with PAUP* 4.0 (Swofford, 2003), and the *p*-value was determined based on 200 replicates. Maximum likelihood trees were obtained with PhyML 3.1 (Guindon & Gascuel, 2003), and bootstrap values were calculated after 300 replicates. Maximum parsimony analyses were performed with PAUP* 4.0. Bootstrap values were calculated with 1000 replicates. Bayesian trees were obtained with MrBayes 3.1.2 (Huelsenbeck *et al.*, 2001; Ronquist & Huelsenbeck, 2003). Convergence was reached after one million generations (average standard deviation of split frequencies <0.01), and 25% of the trees were discarded as burn-in.

Morphological observations and analyses were conducted as in Ghesini & Marini (2013). A total of 39 alates (17 from Lebanon and 22 from Cyprus) and 27 soldiers (4 from Lebanon, 9 from Israel, and 14 from Cyprus) were observed and measured. Mann–Whitney *U* test was conducted using the software Past (Hammer *et al.*, 2001).

Table 1. COII, 16S, an CR haplotypes of *Kaloterme s* sp. isolated in this study and corresponding GenBank accession numbers. Numbers of localities are as in fig. 1. Different colonies collected in the same locality are indicated by a capital letter (A, B, and C). Different haplotype combinations found in the same colony are indicated by a number (1, 2).

Locality	COII hapl.	16S hapl.	CR hapl.	GenBank A.N.		
				COII	16S	CR
Cyprus						
1 Makounta	C1	S1	R1	KC914294	KC914316	KC914323
2 Kathikas	C2	S1	R2	KC914295	KC914316	KC914324
3 Agios Giorgios	C3	S2	R2	KC914296	KC914317	KC914324
4 Nikokleia	C4	S3	R3	KC914297	KC914318	KC914325
5 Episkopi	C5	S3	R4	KC914298	KC914318	KC914326
6 Kaplica	C6	S4	R5	KC914299	KC914319	KC914327
Lebanon						
7 Benouaiti A	C7	S5	R6	KC914300	KC914320	KC914328
Benouaiti B	C8	S5	R7	KC914301	KC914320	KC914329
Benouaiti C1	C8	S5	R7	KC914301	KC914320	KC914329
Benouaiti C2	C9	S5	R7	KC914302	KC914320	KC914329
8 Kfar Houne	C10	S5	R6	KC914303	KC914320	KC914328
Israel						
9 Sdot Yam	C11	S5	R6	KC914304	KC914320	KC914328
10 Hadera A	C12	S5	R6	KC914305	KC914320	KC914328
Hadera B	C13	S6	R8	KC914306	KC914321	KC914330
Hadera C1	C14	S5	R6	KC914307	KC914320	KC914328
Hadera C2	C15	S5	R6	KC914308	KC914320	KC914328
11 Rishpon A	C12	S5	R6	KC914305	KC914320	KC914328
Rishpon B1	C12	S5	R6	KC914305	KC914320	KC914328
Rishpon B2	C16	S5	R6	KC914309	KC914320	KC914328
Rishpon C	C14	S7	R9	KC914307	KC914322	KC914331

Table 2. Minimum and maximum percent nucleotide p-distances between combined haplotypes (COII+16S+CR) of different colonies from Cyprus, Lebanon, and Israel and within-group distances. Standard deviations are shown in parentheses. Distances with *K. flavicollis* (Viterbo) and *K. italicus* (Grosseto) are shown for comparison.

	Cyprus	Lebanon	Israel	<i>K. flavicollis</i>
Cyprus	0.27–1.29 (0.12) (0.29)			
Lebanon	2.64–3.32 (0.45) (0.49)	0.00–0.81 (0.00) (0.21)		
Israel	2.64–3.53 (0.44) (0.50)	0.14–2.04 (0.09) (0.35)	0.00–2.04 (0.00) (0.37)	
<i>K. flavicollis</i>	8.13–8.33 (0.72) (0.75)	8.06–8.26 (0.71) (0.72)	7.99–8.26 (0.72) (0.72)	
<i>K. italicus</i>	8.03–8.58 (0.70) (0.75)	7.90–7.97 (0.69) (0.70)	7.78–8.24 (0.69) (0.69)	5.03 (0.60)

Results

In *Kaloterme s* sp. from the Levant, 16, 7, and 9 haplotypes were found, respectively, for COII, 16S, and CR sequences (table 1). The two individuals analyzed for each colony have the same haplotype, except in three colonies (Benouaiti C, Hadera C, and Rishpon B) for the gene COII. For all the three regions sequenced, the haplotypes found in Cyprus are not found in Lebanon and Israel, and vice versa. For the gene COII, Lebanese haplotypes differ from Israeli haplotypes, while for 16S and CR a haplotype (S5 and R6 respectively) is found both in Lebanon and Israel.

Based on nucleotide p-distances between combined COII +16S+CR haplotypes (table 2), samples from Lebanon show a high affinity with those from Israel, with distance levels

(0.14–2.04%) that fall within the range of distances found among Israeli samples (maximum distance 2.04%). Samples from Cyprus are more distant (2.64–3.32% with Lebanon, 2.64–3.53% with Israel).

Based on COII sequences, p-distances between *Kaloterme s* sp. from the Levant and the other European species (from $9.37 \pm 1.35\%$ to $11.03 \pm 1.38\%$ with *K. flavicollis*, from $10.88 \pm 1.24\%$ to $12.39 \pm 1.47\%$ with *K. italicus*) are comparable to or higher than several interspecific distances in the genus *Kaloterme s* (e. g., from $6.04 \pm 1.10\%$ to $8.31 \pm 1.30\%$ between *K. flavicollis* and *K. italicus*, $7.57 \pm 1.27\%$ between *Kaloterme s aemulus* and *Kaloterme s hilli*, $12.01 \pm 1.17\%$ between *K. aemulus* and *K. browni*). Based on the Bayesian COII tree, the distance between

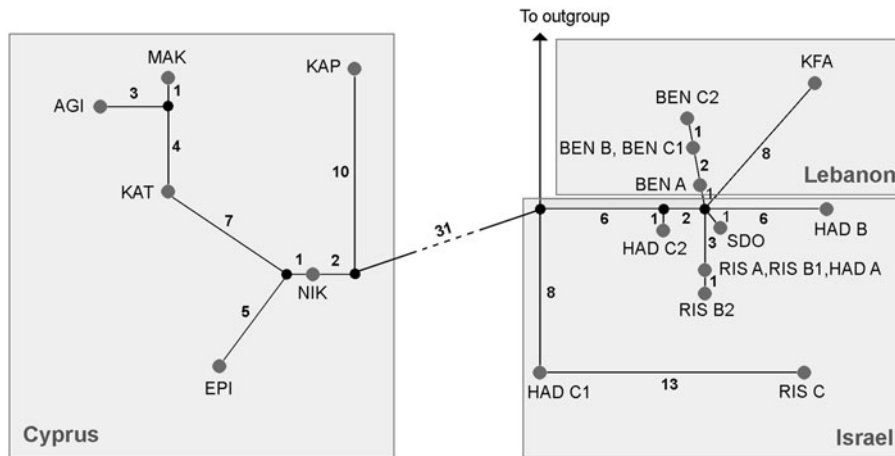


Fig. 2. Median-joining haplotype network based on the alignment COII+16S+CR. Numbers near lines represent the number of changes between haplotypes. Small black dots represent median vectors (unsampled or hypothetical haplotypes at the intersection of two or more lines). The attribution of median vectors to a geographical area is arbitrary.

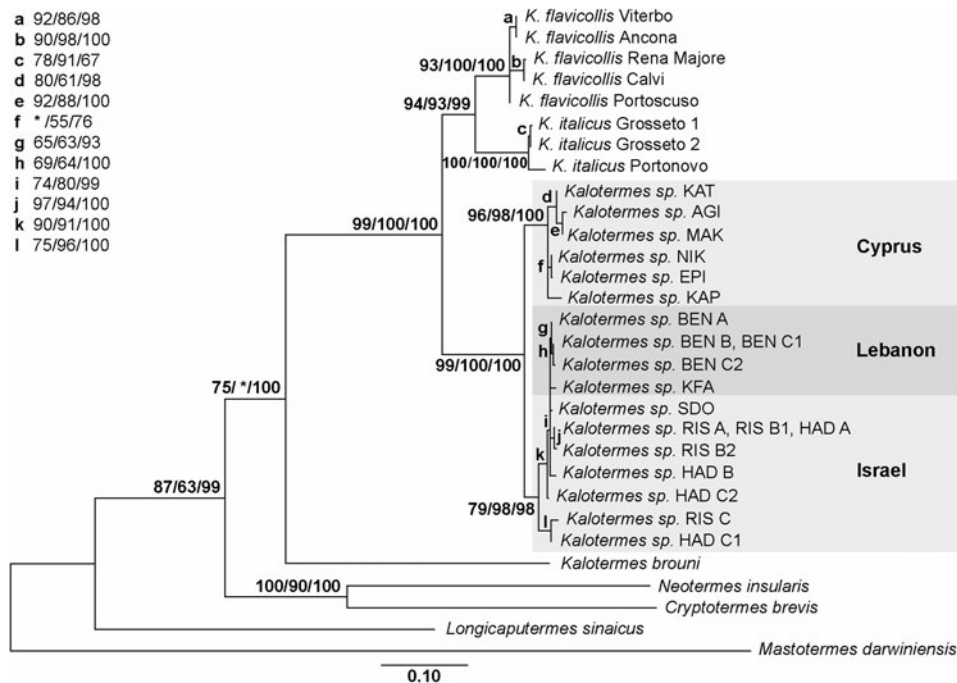


Fig. 3. Maximum parsimony, maximum likelihood, and Bayesian inference tree (topologies are coincident) based on COII+16S alignment. Numbers at nodes represent bootstrap and posterior probability values (MP/ML/BI). Support values of internal nodes are shown on the left of the figure.

Kaloterme sp. from the Levant and the group *K. flavicollis*+*K. italicus* is 24.9%. Rodrigo's and Rosenberg's tests support the distinction of the group *K. flavicollis*+*K. italicus* from *Kaloterme* sp. from the Levant ($P < 0.05$ and $P < 10^5$ respectively).

The median-joining and the parsimony haplotype networks, based on the COII+16S+CR alignment, identify two main groups, the first includes haplotypes from Cyprus and the second includes haplotypes from the mainland (Lebanon and Israel) (fig. 2). In the parsimony haplotype network, with a 95% cutoff, the two groups are disjointed. The correspondence

between the collocation of haplotypes in the network and the geographic location of sampling localities is fairly good. The Cypriot haplotype nearest to the mainland group is the one found in Nikokleia. The mainland haplotype nearest to the Cypriot group is the one found in Hadera. The sampled haplotypes nearest to *K. flavicollis* and *K. italicus* are those from the mainland group, in particular those from Hadera C.

JModeltest 2.1.2 indicates TPM3uf+I+G and TPM2uf+G as the best models of nucleotide substitution, for COII and 16S datasets respectively. The partition homogeneity test allows

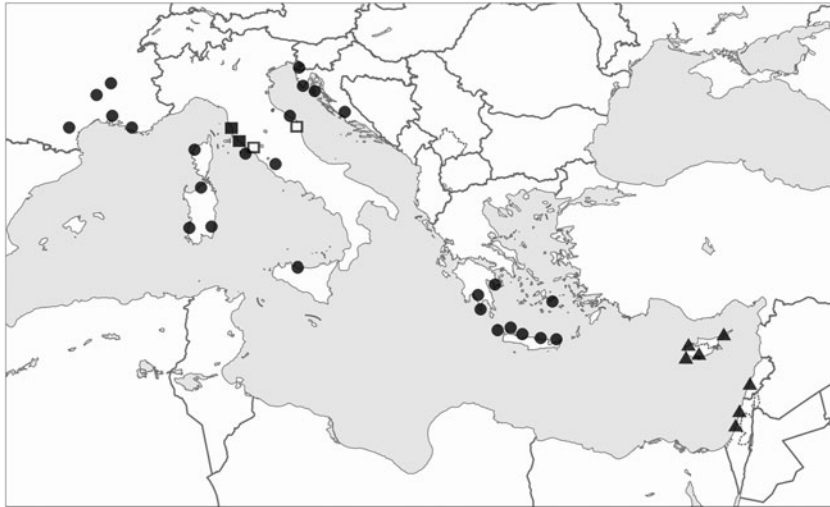


Fig. 4. Localities whose *Kalotermes* colonies have been analyzed from the genetic point of view (mitochondrial DNA and/or microsatellite analysis). Black dots: *Kalotermes flavicollis*; black squares: *K. italicus*; open squares: hybrids or probable hybrids (individuals with *K. flavicollis* phenotype and *K. italicus* mitochondrial haplotypes) between *K. flavicollis* and *K. italicus*; black triangles: *K. phoeniciae* sp. nov. (see the text for references).

to include the two genes in the same dataset ($P = 0.29$). For the combined COII+16S dataset, JModelTest 2.1.2 chooses TPM2uf+I+G as the best model of nucleotide substitution.

The topologies of maximum likelihood, maximum parsimony, and Bayesian inference trees based on the COII+16S dataset are coincident (fig. 3). In the phylogeny of central and eastern Mediterranean *Kalotermes* species, the samples of *Kalotermes* sp. from the Levant form a well supported clade, sister to the clade including *K. flavicollis* and *K. italicus*. Two main subclades are identified, one including all the samples from Cyprus, and the other including all the samples from Lebanon and Israel. The internal branching of these subclades is only partially resolved. The topology of *Kalotermes* sp. clade shows a good correspondence with the structure of the haplotype network (fig. 2).

The results of the morphological analysis are shown in the description of the new species (see below).

Discussion

In the phylogeny of Mediterranean *Kalotermes* species, samples from Cyprus, Lebanon, and Israel form a well supported clade, sister to the clade including *K. flavicollis* and *K. italicus*. Both the haplotype network and the phylogenetic analysis identify two groups, one including samples from Cyprus and the other including samples from Lebanon and Israel. The high affinity between Israeli and Lebanese samples was expected due to the geographical proximity and the absence of relevant barriers to termite dispersion between the two countries.

In the haplotype network, the haplotypes nearest to the path connecting the Cypriot group to the Israeli-Lebanese group are those found in Nikokleia and Hadera, suggesting that the colonization route between Cyprus and the mainland might have connected southern Cyprus to northern Israel. In order to rule out other possible origins of Cypriot *Kalotermes* sp., it would be useful to analyze samples of *Kalotermes* taxa from nearby lands, such as Egypt, Syria, and Turkey, for which no genetic data exist to date.

Samples from Cyprus, although genetically distinguishable from those from Lebanon and Israel, are strongly related to them. This is in contrast to what is found in the case of *Reticulitermes* sp. from Cyprus, that is genetically distant from the Israeli *Reticulitermes clypeatus*, and instead shows affinities with populations from northeastern Greece (Ghesini & Marini, 2012). Thus, the colonization of Cyprus by the two termite genera seems to have occurred through different routes.

Cyprus is an oceanic island, i.e., it was never connected with the mainland. It began emerging from the sea about 20 million years ago, and, even at times of minimum sea level, was probably separated from the surrounding lands by at least a 30–40 Km water gap (Simmons, 1999). Unlike *Reticulitermes* termites, *Kalotermes* termites can tolerate high salinity levels, such as those of sea water (Springhetti, 1959). Their 'one piece' wood nesting behavior further facilitates over-water dispersal, scarcely exploitable by *Reticulitermes* termites. The colonization of islands by wood rafting is a concrete possibility for termites (Gathorne-Hardy et al., 2000).

The monophyly of the Cypriot clade supports the hypothesis that *Kalotermes* sp. established on Cyprus following a single colonization event. This is not so surprising as it might seem, because, from a biogeographical point of view, Cyprus is one of the most isolated Mediterranean islands, with a very low rate of faunistic immigration (Corti et al., 1999). Despite the frequent and long-standing movement of people and goods between the mainland and Cyprus, we found no evidence of human-mediated dispersal of *Kalotermes* sp. between the two regions.

Until the 1970s, two termite species were believed to be widely distributed along the Mediterranean coasts of Europe and Asia: the subterranean termite *Reticulitermes lucifugus* (Rossi) and the drywood termite *Kalotermes flavicollis* (Fabr.). During the last decades, morphological, chemical, and genetic analyses of *Reticulitermes* samples collected over most of *R. lucifugus* range have shown that what was once believed to be a single species is in fact a complex of about 10 taxa, five of them already described as species (e.g., Clément, 1978; Clément et al., 2001; Velonà et al., 2010; Ghesini & Marini, 2012), so that the

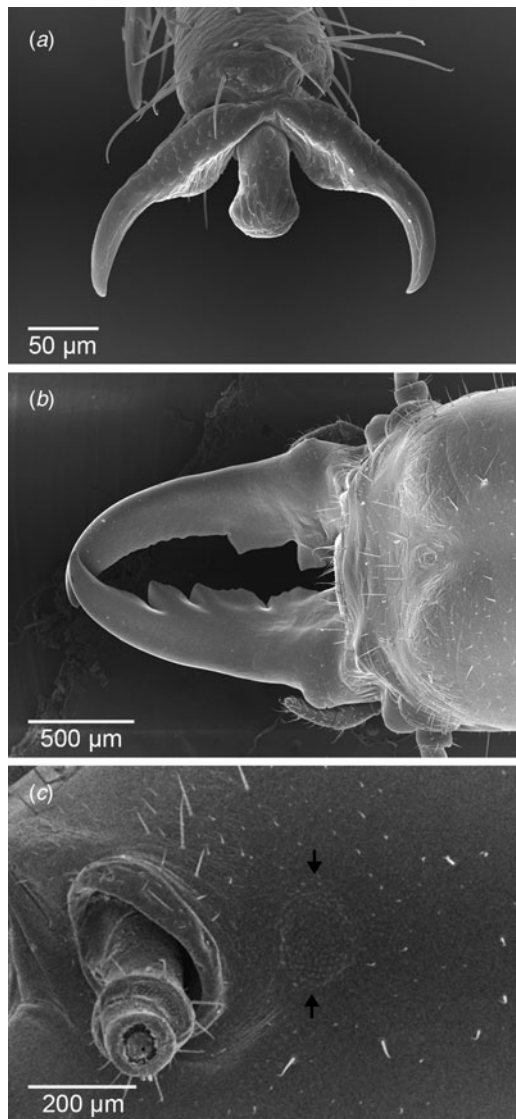


Fig. 5. Main diagnostic characters for *K. phoeniciae* sp. nov.: arolium of the alate (A), mandibles (B) and rudimentary eye (C) of the soldier. Arrows indicate the extremities of the eye, along its major axis.

range of *R. lucifugus* is actually limited to south-eastern France and Italy. Similarly, in the case of *K. flavicollis*, a more complex pattern than was previously known is now emerging from genetic and morphological analyses of long-known populations and to the acquisition of samples from new localities. The first genetic analyses, conducted on samples from Italy and the Balkan peninsula, showed a remarkable genetic homogeneity across *K. flavicollis* populations (Luchetti *et al.*, 2004). The extension of analyses to populations from southern France, Corse, and Sardinia revealed a fair degree of genetic variation (Velonà *et al.*, 2011). Then, the analysis of an Italian dark-necked *Kaloterмес* population led to the description of *K. italicus* (Ghesini & Marini, 2013). In this study, we show that eastern Mediterranean *Kaloterмес* populations from Cyprus, Lebanon, and Israel are different from *K. flavicollis*, and can be considered as a new species, that we describe below.

The distribution of Mediterranean *Kaloterмес* spp. samples whose species attribution has been verified to date through genetic analyses (Luchetti *et al.*, 2004, 2013; Velonà *et al.*, 2011; Ghesini & Marini, 2013; this study) is summarized in fig. 4. None of the samples collected in Cyprus, Lebanon and Israel – some of them collected in localities where *K. flavicollis* had been previously reported – belongs to the species *K. flavicollis*. Because *Kaloterмес* sp. from the Levant and *K. flavicollis* are morphologically similar and have the same ecology, it is likely that the previous reports of *K. flavicollis* in these regions are in fact attributable to *Kaloterмес* sp.

Kaloterмес phoeniciae sp. nov. (fig. 5)

Holotype (female alate): sample BEN A2. Paratypes (17 female alates, 21 male alates, 27 soldiers in total): samples AGI; BEN A, B, C; EPI; HAD A, B, C; KAP; KAT; KFA; MAK; NIK; RIS A, B, C; SDO. All these series are in the M. Marini collection, University of Bologna, Italy.

Alate

Body dark brown. Clypeus, antennae, tibiae, and tarsi, light brown-yellowish. Pronotum yellow. Wing membrane smoky brown.

Eye with 169–252 ommatidia (mean 204.33 ± 19.75). Ocellus separated from the eye by a distance of approximately a half ocellus diameter. Antennal segments 15–19 (Cyprus: 15–18; Lebanon: 16–19).

Table 3. Samples used for this study: collection date, collector, geographic coordinates, elevation (m a.s.l.), and castes (P: pseudergates, S: soldiers, A: alates).

Sample	Coll. date	Collector(s)	Coordinates	Elevation	Castes
MAK	27/02/2009	M. Marini	35°04'N, 32°28'E	5	A
KAT	27/02/2009	M. Marini	34°54'N, 32°22'E	340	P, S, A
AGI	26/02/2009	M. Marini	34°54'N, 32°21'E	250	P
NIK	28/02/2009	M. Marini	34°48'N, 32°42'E	250	S
EPI	26/02/2009	M. Marini	34°42'N, 32°52'E	180	S, A
KAP	29/11/2009	M. Marini	35°26'N, 33°56'E	10	P, S, A
BEN	29/12/2010	M. Marini	33°41'N, 35°31'E	300	P, S, A
KFA	29/12/2010	M. Marini	33°29'N, 35°35'E	1080	P, A
SDO	10/09/2012	M. Marini and D. Simon	32°29'N, 34°54'E	10	P
HAD	11/09/2012	M. Marini and D. Simon	32°25'N, 34°53'E	5	P, S
RIS	11/09/2012	M. Marini and D. Simon	32°12'N, 34°50'E	25	P, S

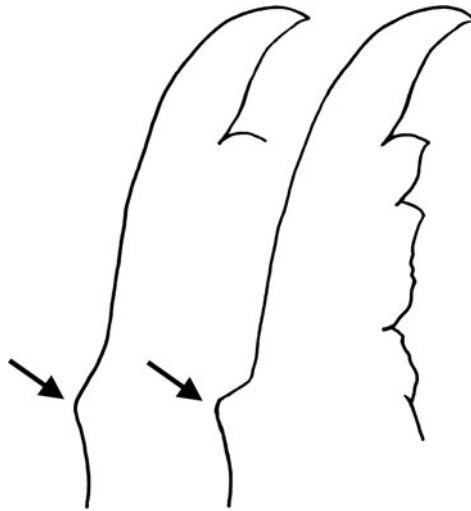


Fig. 6. Outline of the left mandible of *K. flavicollis* and *K. italicus* (left) and *K. phoeniciae* (right). Arrows indicate basal tubercles.

Arolia big (fig 5A), of about the same size in all legs (75–80 μm in length, nearly half the length of the claw). Wing venation variable among individuals and often different in the contralateral wings of the same individual. Radial sector sclerotized, with 9–11 branches in the forewing and 6–8 branches in the hindwing. Media unsclerotized, usually well developed and reaching, with its ramifications, the wing edge; sometimes short, ending before the wing edge or joining the cubitus. Cubitus unsclerotized, with a variable number of branches and sub-branches, often occupying half or more of the area of the wing.

Measurements: see table 3.

Soldier

Head light brown, darkening toward mandibles. Mandibles dark brown. Thorax and abdomen whitish.

Mandibles broad, with big basal tubercles on their outer sides (figs 5B and 6). Maximum diameter of the rudimental eye: 160–210 μm , about half the maximum external diameter of the antennal socket (fig 5C). Antennal segments 13–16 (Cyprus: 13–16; mainland: 13–15). Second and third antennal

Table 4. Measurements (mm) of alates of *K. phoeniciae* sp. nov. An asterisk (*) indicates characters with significantly different values in Cyprus and in Lebanon (Mann–Whitney test, $P < 0.01$).

	Cyprus	Lebanon	All	Holotype
Head length (*)				
Mean \pm SD	1.36 \pm 0.06	1.30 \pm 0.05	1.33 \pm 0.06	1.36
Range	1.23–1.46	1.18–1.36	1.18–1.46	
Head width (*)				
Mean \pm SD	1.24 \pm 0.04	1.16 \pm 0.04	1.21 \pm 0.06	1.23
Range	1.13–1.30	1.08–1.23	1.08–1.30	
Max. eye diameter				
Mean \pm SD	0.24–0.02	0.25–0.01	0.24 \pm 0.02	0.26
Range	0.21–0.27	0.21–0.27	0.21–0.27	
Pronotum length				
Mean \pm SD	0.82 \pm 0.05	0.79 \pm 0.05	0.80 \pm 0.05	0.92
Range	0.75–0.90	0.73–0.92	0.73–0.92	
Pronotum width (*)				
Mean \pm SD	1.39 \pm 0.08	1.32 \pm 0.07	1.36 \pm 0.08	1.29
Range	1.21–1.49	1.19–1.42	1.19–1.49	
Tibia length (*)				
Mean \pm SD	0.99 \pm 0.06	0.91 \pm 0.04	0.96 \pm 0.07	1.00
Range	0.84–1.06	0.85–1.00	0.84–1.06	

Table 5. Measurements (mm) of soldiers of *K. phoeniciae* sp. nov. Measurements on soldiers from Cyprus and from the mainland do not differ significantly, except in the case of head length (Mann–Whitney test, $P = 0.02$).

	Cyprus	Lebanon and Israel	All
Head length (*)			
Mean \pm SD	2.11 \pm 0.20	2.27 \pm 0.13	2.19 \pm 0.18
Range	1.67–2.33	2.06–2.45	1.67–2.45
Head width			
Mean \pm SD	1.38 \pm 0.09	1.38 \pm 0.06	1.38 \pm 0.08
Range	1.17–1.48	1.25–1.45	1.17–1.48
Mandible length			
Mean \pm SD	1.39 \pm 0.13	1.33 \pm 0.04	1.36 \pm 0.10
Range	1.17–1.58	1.25–1.39	1.17–1.58
Pronotum height			
Mean \pm SD	0.81 \pm 0.11	0.85 \pm 0.05	0.83 \pm 0.09
Range	0.57–0.91	0.76–0.91	0.57–0.91
Tibia length			
Mean \pm SD	1.06 \pm 0.11	1.05 \pm 0.05	1.06 \pm 0.09
Range	0.84–1.21	0.96–1.12	0.84–1.21

segment of about the same length; third shaped as a truncated cone; fourth shortest.

Measurements: see [table 4](#).

Measurements of alates and soldiers are reported for completeness ([tables 4](#) and [5](#)), but should be considered with caution, because in *Kaloterme*s spp. measurements can vary amply among colonies and even among individuals of the same colony, and the ranges of variation of measurements of different taxa often largely overlap.

Distribution

Cyprus, Lebanon, Israel

Ecology

Diverse environments, from arid or semi-arid to shaded creek banks, from 0 to 1100 m a.s.l. All colonies found in natural environment, except in the case of Rishpon (avocado orchard).

Host plants: colonies found in tree stumps, logs, or under the bark of living plants of *Ceratonia siliqua* L., *Ficus carica* L., *Juniperus* sp., *Persea americana* Mill., *Pinus pinea* L., *Platanus orientalis* L., *Salix* sp., *Tamarix* sp.

Comparison with the other Mediterranean Kalotermitidae

The pronotum of the alate of *K. flavicollis* is often of a duller shade of yellow (with dark margins, or even entirely dark, in *K. flavicollis* var. *fuscicollis*). Arolia are slightly smaller (about 60 µm in length, less than 4/10 of the length of the claw). The alate of *K. italicus* has a dark brown, almost black pronotum, unpigmented wings, and much smaller arolia (about 40–45 µm in length, less than 3/10 of the length of the claw) (Ghesini & Marini, 2013). No description of the alate of *K. monticola* exists.

Soldiers of both *K. flavicollis* and *K. italicus* mandibles with smaller basal tubercles ([fig. 6](#)). The soldier of *K. flavicollis* has bigger rudimental eyes (maximum diameter longer than 230-µm, more than half the antennal socket). Most soldiers of *K. italicus* have smaller rudimental eyes (maximum diameter shorter than 170 µm, less than half the antennal socket). The soldier of *K. monticola* lacks basal tubercles on the outer sides of mandibles and has two frontal depressions (Sjöstedt, 1925).

For the identification of the Mediterranean species in the genus *Kaloterme*s, we provide the following keys.

Identification key for alates

1. Length of arolium:
nearly half the length of the claw: *K. phoeniciae*
less than half the length of the claw: 2
2. Color of the wings:
hyaline, whitish when folded: *K. italicus*
dark: *K. flavicollis*

Identification key for soldiers

1. Outer side of mandibles:
without basal tubercles: *K. monticola*
with basal tubercles: 2
2. Size of basal tubercles:
big, as in [fig. 6](#) (right): *K. phoeniciae*
smaller, as in [fig. 6](#) (left): 3
3. Maximum diameter of the rudimental eye:
less than 170 µm: *K. italicus*
more than 230 µm: *K. flavicollis*

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