

Phylogeny and Genetic Variation of *Reticulitermes* (Isoptera: Rhinotermitidae) from the Eastern Mediterranean and Middle East

by

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ABSTRACT

Recent genetic evaluations of *R. lucifugus* from France and Italy with *R. lucifugus* from Turkey suggests that these geographic groups likely constitute separate species. A more extensive review of eastern Mediterranean termites is needed to clarify their respective classifications. DNA sequencing analysis of the mitochondrial 16S rRNA region was used to examine genetic variation in the termite genus *Reticulitermes* Holmgren from eastern Europe and the Middle East. Sequencing of a 437 bp region from 39 individuals and from 3 sequences obtained from GenBank revealed 47 polymorphic sites within the genus. Tajima-Nei distances from species ranged from 0.0 to 4.9%, and parsimony and maximum likelihood analysis revealed clades within the genus which follow biogeographic patterns of dispersal that may support recent glacial refugia theories. European *Reticulitermes* were delimited from Turkish *R. lucifugus* with samples from Greece, Israel, and Iraq. *Reticulitermes lucifugus* from Turkey share a topology with *R. chypeatus* from Israel and *R. sp.* from Iran, which were distinct relative to other *Reticulitermes* from Eastern Europe. There was some genetic support for combining *R. balkanensis* to this group. However, this study suggests that *Reticulitermes* from this region likely constitute two genetically isolated, but sympatric populations which have not been resolved with morphological examinations, but are clearly different from the *R. lucifugus* complex from Europe.

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SPECIES OF THE GENERA

Reticulitermes Holmgren (Isoptera: Rhinotermitidae) are the major termite pests infesting wooden structures in Europe and the near east. However, reports of damage associated with these termites from the eastern Mediterranean and Middle East are less common. The Mediterranean termite *Reticulitermes lucifugus* (Rossi) was first described by Weber (1954) as occurring in the Zubair-desert region (Southern Iraq), and it has been hypothesized by Weidner (1972) that *R. lucifugus* may in fact belong to *R. clypeatus* Lash. *Reticulitermes lucifugus* has been the only documented species of the genus *Reticulitermes* to occur in Turkey (Bodenheimer 1958, Weidner 1972, Lodos 1982, Karaat & Göven 1983, Austin *et al.* 2002). Although *R. lucifugus* occasionally contributes to structural damage, it is considered a minor crop pest of various fruits, nuts, grains and vegetables throughout the eastern Mediterranean (Austin *et al.* 2002, Bodenheimer 1958, Lodos 1982, Sekendiz 1982, Iren & Ahmed 1973).

Weidner (1959) was unable to find *Reticulitermes* from Iran during collecting endeavors there, but suggested that species from neighboring Iraq, which were known to be similar to *R. clypeatus* may present a limit to their eastern distribution. Because morphological comparisons from disjunct populations throughout the Middle East exhibit overlap (Lash 1952, Weidner 1959, 1960), and information on more current distributions remain unknown. We can only speculate on the sympatry of these groups based on a small number of samples with sufficient quality for comparison by molecular analyses (Austin *et al.* 2002).

Although not supported from the results presented herein, Marini & Mantovani (2002) describe a new *Reticulitermes* species-group from northeastern Italy and demonstrate that they constitute a unique group (Figs. 1 and 2). Uva *et al.* (2004) further demonstrated that this group was genetically similar to a new "urban" phenotype identified from disjunct locations in France, now called *R. urbis* Uva. This is also supported by more recent genetic evaluations of Luchetti *et al.* (2004) which demonstrate the similarity of conspecific groups from the eastern Mediterranean region. It remains unclear whether these groups

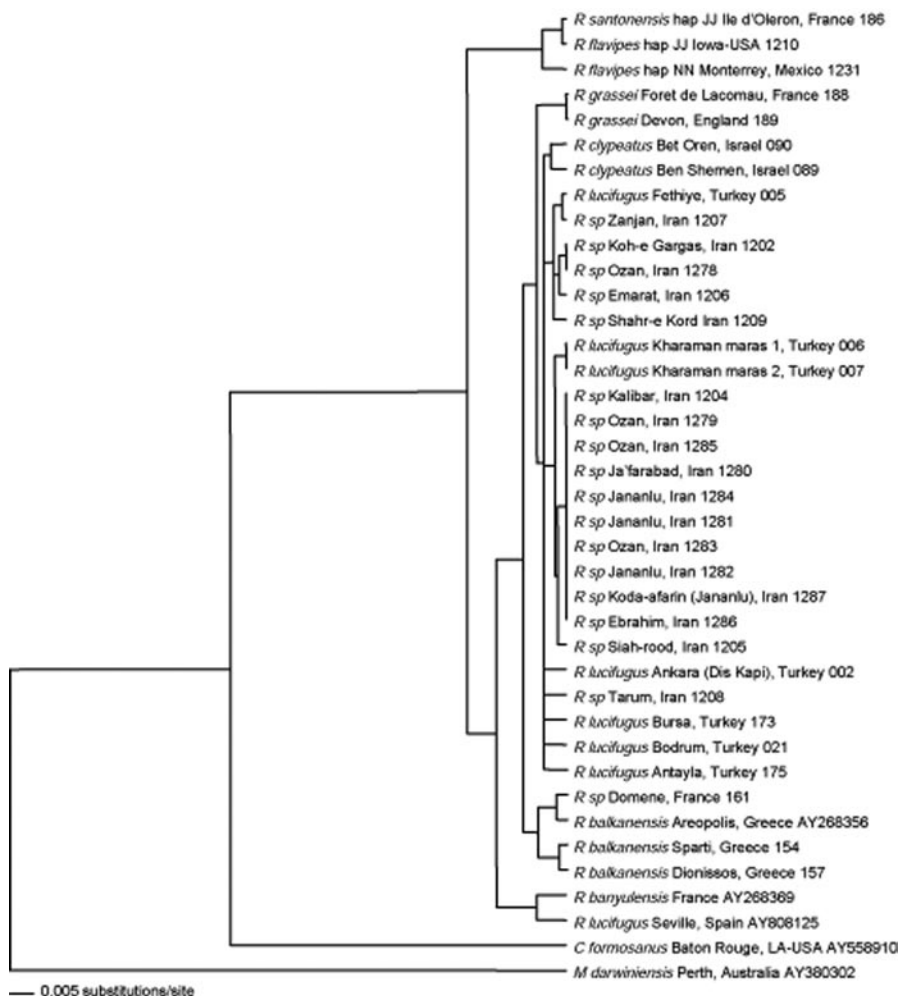


Fig. 1. Topology obtained by maximum-likelihood analysis based on the HKY85 model ($-\ln L = 1436.10583$).

occur through multiple anthropogenic introductions, or through geological events that influenced their current locations. Low genetic divergence of conspecific populations of *R. balkanensis*, *R. lucifugus* from Turkey, and *R. clypeatus* from Israel, coupled with their phylogenetic topologies which use multiple genetic markers, suggest that these species likely constitute a single group, distinct from other *Reticulitermes* in Europe. Additional genetic studies which focus on these groups, inclusive to this hypothesis, need to be evalu-

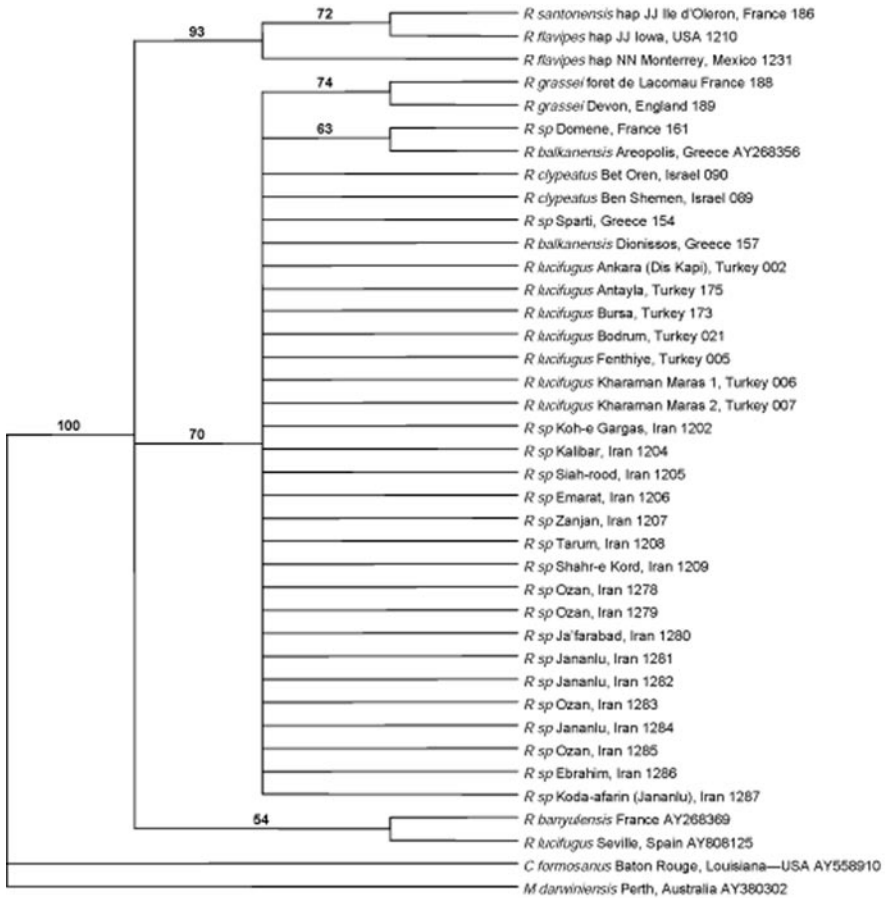


Fig. 2. 16S rRNA single most parsimonious tree during a heuristic search using PAUP*. Bootstrap values for 1000 replicates are listed above the branches supported at $\geq 50\%$.

ated. Recent collections of *Reticulitermes* from Iran have been obtained, and we include these taxa with other *Reticulitermes* from this geographic region to evaluate what their relationships may be. The western migration of these termites through glacial refugia, some 14,000 years ago, has been proposed (Clément *et al.* 2001, Uva *et al.* 2004), and further support the discontinuity of *Reticulitermes* belonging to the *R. lucifugus* “complex” from the eastern Mediterranean and Near east (Luchetti *et al.* 2004, 2005) and are affirmed by their respective rates of molecular divergence which suggest a fourth glacial refugia that may account for these differences (JWA, unpublished).

MATERIALS AND METHODS

Termites were collected from various locations in North America, Europe, and the Middle East, and preserved in 100% ethanol (Table 1). Morphological identification of *R. lucifugus* was performed in Austin *et al.* (2002): Identification of other *Reticulitermes* collected in this study was applied by the keys of Weidner (1959, 1960, 1972), Krishna & Weesner (1969), Clément (1978, 1979), Lash (1952), Scheffrahn & Su (1994), and Donovan *et al.* (2000). Identities of some specimens were verified with mtDNA 16S sequences (Szalanski *et al.* 2003). Three additional taxa (Table 1) were included as outgroup taxa to corroborate relationships within the genus for phylogenetic analysis. Voucher specimens, preserved in 100% ethanol, are maintained at the Arthropod Museum, Department of Entomology, University of Arkansas, Fayetteville, AR.

From alcohol-preserved specimens dried on filter paper, the DNA was extracted according to Liu & Beckenbach (1992) and Jenkins *et al.* (1999) from individual worker termites with the Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, MN). Extracted DNA was resuspended in 50 μ l of Tris:EDTA and stored at -20°C . Polymerase chain reaction (PCR) was conducted with the primers LR-J-13007 (5'-TTACGCTGTTATCCCTAA-3') (Kambhampati & Smith 1995) and LR-N-13398 (5'-CGCCTGTTTATCAAAAACAT-3') (Simon *et al.* 1994). These PCR primers amplify an approximately 428 bp region of the mtDNA 16S rRNA gene. The PCR reactions were conducted with 1 μ l of the extracted DNA (Szalanski *et al.* 2000), having a profile consisting of 35 cycles of 94°C for 45 s, 46°C for 45 s and 72°C for 60 s. Amplified DNA from individual termites was purified and concentrated with minicolumns according to the manufacturer's instructions (Wizard PCRpreps, Promega). Samples were sent to University of Arkansas Medical Sciences DNA Sequencing Core Facility (Little Rock, AR) for direct sequencing in both directions using an ABI Prism 377 DNA sequencer (Foster City, CA). **GenBank accession numbers were _____ to _____ for termites** subjected to DNA sequencing in this study. Consensus sequences for each sample were obtained using BioEdit 5.09 (Hall 1999). The position of variable nucleotide sites

Table 1. Collection data for *Reticulitermes* and outgroup taxa.

Species	Location	Accession No.	Country	Sample code(s)	N
<i>R. lucifugus</i>	Antalya	xxx	Turkey	175	1
	Ankara (Diş kapa)	xxx	Turkey	002	1
	Bodrum	xxx	Turkey	021	1
	Bursa	xxx	Turkey	173	1
	Fethiye	xxx	Turkey	182	1
	Kharman Maraş 1	xxx	Turkey	006	1
	Kharman Maraş 2	xxx	Turkey	007	1
	Seville	AY808125	Spain	---	
<i>R. chypeatus</i>	Ben Shemen	xxx	Israel	089	1
	Bet Oren	xxx	Israel	090	1
<i>R. balkanensis</i>	Aeropolis	AY268356	Greece	---	1
	Dionissos	xxx	Greece	157	1
	Sparti	xxx	Greece	154	1
<i>R. banyulensis</i>		AY268369	France	---	1
<i>R. flavipes</i> (= <i>R. santonensis</i>)	Iowa- haplotype JJ	xxx	U.S.A.	1210	1
	Ile d'Oleron	xxx	France	186	1
	Nuevo Leon-Monterrey	xxx	Mexico	1231	1
<i>R. grassei</i>	haplotype NN fofet de Lacomau	xxx	France	188	1
	Devon		England		
<i>R. sp.</i>	Ozan	xxx	Iran	1279, 1283, 1285	3
	Koh-e gargas	xxx	Iran	1202	1
	Emarat	xxx	Iran	1206	1
	Zanjan	xxx	Iran	1207	1
	Tarum	xxx	Iran	1208	1
	Shar-e Kord	xxx	Iran	1209	1
	Koda afarin	xxx	Iran	1287	1
	Ebrahim	xxx	Iran	1286	1
	Jananlu	xxx	Iran	1281, 1282,	3
	Ja'farabad	xxx	Iran	1284 1280	1
	Kalibar	xxx	Iran	1204	1
	Siah rood	xxx	Iran	1205	1
	<i>Coptotermes formosanus</i>	Louisiana—Baton Rouge	AY558910	U.S.A.	outgroup
<i>Mastotermes darwiniensis</i>	Perth	AY380302	Australia	outgroup	1

among the DNA sequences was obtained using MacClade v4 (Sinauer Associates, Sunderland, MA).

The distance matrix option of PAUP* 4.0b8 (Swofford 2001) was used to calculate genetic distances according to the Kimura 2-parameter model (Kimura 1980) of sequence evolution (Table 2). Mitochondrial 16S rRNA sequences from *Mastotermes darwiniensis* Froggatt (GenBank AY380302) and the Formosan termite, *Coptotermes formosanus* Shiraki (GenBank AY558910) were added to the *Reticulitermes* DNA sequences as outgroup taxa. Maximum likelihood and unweighted parsimony analysis on the align-

ments were conducted using PAUP* 4.0b8 (Swofford 2001). Gaps were treated as missing data. The reliability of trees was tested with a bootstrap test (Felsenstein 1985). Parsimony bootstrap analysis included 1000 resamplings using a heuristic search from PAUP*. For maximum likelihood analysis, the default likelihood parameter settings were used (HKY85 6-parameter model of nucleotide substitution, empirical base frequencies, and transition/transversion ratio set to 2:1). These parameters were used to carry out a heuristic search using PAUP*, using either the single most parsimonious tree as the starting tree, or step-wise addition.

RESULTS

DNA sequencing of the mtDNA 16S rRNA amplicon revealed that it averaged 432 bp in size. The average base frequencies were A = 0.41, C = 0.22, G = 0.13, and T = 0.23. The mtDNA 16S rRNA *Reticulitermes* sequences were aligned with CLUSTAL W software (Thompson *et al.* 1994) using *Coptotermes formosanus*, and *Mastotermes darwiniensis*, as outgroup taxa. Of these characters, 68 (21%) were variable and 47 (15%) were phylogenetically informative.

This dataset had only one most parsimonious tree (Fig. 1), (length = 206, CI = 0.0665, RI = 0.537), as documented using the Heuristic search algorithm of PAUP*. Bootstrap analysis of the aligned *Reticulitermes* species and the outgroup taxa resulted in a consensus tree with distinct branches (Fig. 1). These distinct clades included: *Reticulitermes lucifugus* from Spain and *R. banyulensis* Clément from France, *R. flavipes* with *R. santonensis* DeFeytaud, and *R. grassei* Clément. Both *R. flavipes* and *R. santonensis* were distinct, both in the parsimony and likelihood analyses. This relationship has been repeatedly proposed with various molecular markers (Jenkins *et al.* 2001, Marini & Manatoni 2002, Austin *et al.* 2002, Ye *et al.* 2004) and demonstrates their synonymy (Austin *et al.* 2005). Other groups distinguished include *R. balkanensis* Clément from Greece forming a distinct group with an unknown *Reticulitermes* from Domene, France (proposed as *R. urbis* by Bagnères *et al.* 2003), and a distinct clade for Israeli, Iranian, and Turkish samples. Maximum parsimony analysis suggests that Turkish, Israeli, and Iranian samples likely constitute a single *Reticulitermes* species, while maximum likelihood suggests that Balkan *Reticulitermes* are most probably distinct.

Table 2 continued. Mean morphological measurements of *Reticulitermes* soldiers from Iran, Iraq, Israel, Turkey, and Europe.

Species and cited source	location	Head length with mandibles (M) (mm)	Head length to base of mandibles (L) (mm)	Max head width (W) (mm)	Length of labrum (mm)	Width of labrum (mm)	Length of mandible (mm)	Length of post-mentum (mm)	Width of post-mentum (mm) Max.	Length of pronotum (mm)	Width of pronotum (mm)	W/L	M/L
<i>R. lucifugus</i> Sile	Turkey	2.67	1.64	1.11	--	--	--	1.22	.45	.59	.94	.68	1.63
Weidner (1960)		n=1	n=1	n=1	--	--	--	n=1	n=1	n=1	n=1	n=1	n=1
<i>R. lucifugus</i> Istanbul-Bebek	Turkey	2.51	1.56	1.07	--	--	--	1.24	.45	.54	.82	.69	1.61
Weidner (1960)		2.45-2.66 n=5	1.49-1.63 n=5	1.04-1.09 n=5	--	--	--	1.14-1.30 n=5	.44-.45 n=5	.52-.55 n=5	.79-.85 n=5	--	--
<i>R. lucifugus</i> Location*	Turkey	2.75	1.76	1.20	--	--	--	1.35	.50	.56	.94	.68	1.56
Weidner (1972)		2.38-3.12 n=67	1.49-2.03 n=67	1.03-1.37 n=67	--	--	--	1.12-1.58 n=67	.44-.56 n=67	.49-.62 n=67	.72-1.16 n=67	--	--
<i>R. lucifugus</i> Athens	Greece	2.45	1.58	1.06	--	--	--	1.12	.46	.53	.85	.67	1.55
Weidner (1960)		2.38-2.52 n=3	1.51-1.61 n=3	1.03-1.08 n=3	--	--	--	1.12 n=3	.44-.47 n=3	.52-.53 n=3	.83-.86 n=3	--	--
<i>R. santonensis</i> La Rochelle Lash	France	2.97	1.93	1.29	.34	.34	1.20	1.13	.50	.50	.95	.67	1.54
(1952)		2.87-3.02 n=8	1.73-2.08 n=8	1.28-1.30 n=8	.27-.39 n=8	.33-.37 n=8	1.17-1.23 n=8	1.04-1.17 n=8	.49-.50 n=8	.50 n=8	.94-1.00 n=8	--	--

*Soldiers taken from Ankara—Middle East Technical University Campus in 2000

*Soldiers were combined and examined from Istanbul, Bursa, Muğla, Köyceğiz, Konya, Ankara, Sinan-Bücke, Erzincan, Nallischerit, Tunceli, Siirt, Diyarbakır, Hatay, and Cyprus (see Weidner 1972).

Tajima-Nei (1984) pairwise distances ranged from 0.0 to 4.9% among *Reticulitermes* species (Table 2). *Reticulitermes* from Iran exhibited the least intraspecific variation ranging from 0.0 to 0.9%, while *Reticulitermes* from Greece and Israel ranged from 0.2% to 2.1%. Intraspecific variation of Turkish *Reticulitermes* was 0.2 to 1.6%.

Based on the maximum parsimony analysis, we find strong bootstrap support for combining *R. lucifugus* populations from Turkey with some samples described as *R. balkanensis* from Greece, and *Reticulitermes* sp. from Iran (Fig. 1). There was low bootstrap (54%) support for the *R. banyulensis* from France and *R. lucifugus* from Spain clade, and their relationships are more clearly observed in the maximum likelihood tree (Fig. 2).

Regardless of whether the starting tree was the most parsimonious tree or was obtained via step-wise addition, the maximum likelihood search found only one tree (Fig. 1). The maximum likelihood tree was consistent with the observed clades presented in the maximum parsimony tree. The consensus tree from the maximum likelihood analysis (-ln L = 1436.10583) was generally in consensus with the maximum parsimony analysis, with strong support for the genetic relationships of *Reticulitermes* from Iran to their congeners from Israel and Turkey.

DISCUSSION

The results of this analysis suggest that the relationships among *Reticulitermes* from the eastern Mediterranean and the Middle East likely constitute one large eastern complex (Fig. 3), extending from Iran west to Israel, through Turkey to Greece (Fig. 3). This complex appears to live in sympatry with other *Reticulitermes* species (e.g., *R. balkanensis*), whose historical identities may be suspect, particularly *R. urbis*. Significant morphological overlap of groups which occupy this region contribute to confusion about the phyletic relationships of these populations (Table 2). Weidner (1960, 1970) has demonstrated clinal variations in an east to west direction throughout the Mediterranean for *R. lucifugus*. Likewise, evaluation of *Reticulitermes* from North America has demonstrated considerable morphological variation which has only been clarified through molecular approaches (Szalanski *et al.* 2003, Austin *et al.* 2005b). In most applications of cuticular hydrocarbons, clear segregation of populations has yet to be achieved with the same efficiency of molecular approaches (Szalanski *et al.* 2003, Foster *et al.* 2004). For example, the original identity of *R. lucifugus* from Turkey was performed by evaluation of cuticular hydrocarbons (Austin *et al.* 2002), even though genetic evaluation suggested their identification as *R. lucifugus* was questionable. Kaib *et al.* (2002) suggests that great care should be given when applying cuticular hydrocarbons to taxo-



Fig 3. Distribution of *Reticulitermes* species from the Eastern Europe and the Middle East. Closed circles represent samples evaluated in the current study. Open circles represent samples reported in known literature or from personal collections from the region.

nomie groups like termites because substances acquired from the environment may affect the cuticular hydrocarbon composition of all members of a colony; for taxonomic identification, cuticular hydrocarbons should remain fixed, but diet and extrinsic factors can alter hydrocarbon phenotypes, leading to false or incorrect identification. For this reason, identification through molecular approaches would seem more resolute. Because samples from Greece and Israel are few in number, it is difficult to ascertain the extent of the variation which occurs within these areas. More substantial collections from the Balkans would be helpful to clarify the relationships of these groups. Sequence data obtained through GenBank for *R. lucifugus* in Italy and *R. balkanensis* in Greece appear to constitute different species from *Reticulitermes* from the Middle East. This fact has been observed by other researchers with multiple genetic markers (Austin *et al.* 2002, Uva *et al.* 2004, Luchetti *et al.* 2004). In the present study, maximum likelihood analysis grouped three *R. balkanensis* samples with one *R. sp.* from Domene, France (also recently described by Bagnères *et al.* (2003) as *R. urbis*).

Table 3. Tajima-Nei pairwise distances among *Reticulitermes* from France, Greece, Turkey, Israel, and Iran.

Sample (UARK collec- tion code)		1	2	3	4	5	6	7	8	9	10	11	12	13
1	<i>R. sp</i> Domene, France (161)	-												
2	<i>R. clypeatus</i> Bet Oren, Israel (090)	0.016	-											
3	<i>R. clypeatus</i> Ben Shemen, Israel (089)	0.021	0.009	-										
4	<i>R. balkanensis</i> Sparti, Greece (154)	0.009	0.011	0.016	-									
5	<i>R. balkanensis</i> Aeopolis, Greece	0.004	0.016	0.021	0.009	-								
6	<i>R. lucifugus</i> Dionissos, Greece (086)	0.011	0.009	0.014	0.002	0.011	-							
7	<i>R. lucifugus</i> Ankara (Diş kapı) Turkey (002)	0.016	0.009	0.014	0.011	0.016	0.009	-						
8	<i>R. lucifugus</i> Antalya Turkey (175)	0.023	0.011	0.016	0.018	0.023	0.016	0.011	-					
9	<i>R. lucifugus</i> Bursa, Turkey (173)	0.014	0.007	0.011	0.014	0.018	0.011	0.007	0.009	-				
10	<i>R. lucifugus</i> Bodrum, Turkey (021)	0.018	0.007	0.011	0.014	0.018	0.011	0.007	0.009	0.004	-			
11	<i>R. lucifugus</i> Fethiye, Turkey (005) Kharaman	0.016	0.011	0.016	0.014	0.016	0.011	0.007	0.014	0.009	0.009	-		
12	<i>R. lucifugus</i> Maras 1, Turkey (006) Kharaman	0.021	0.009	0.014	0.016	0.021	0.014	0.009	0.011	0.007	0.007	0.011	-	
13	<i>R. sp</i> Maras 2, Turkey (007) Koh-e Gargas, Iran	0.021	0.009	0.014	0.016	0.021	0.014	0.009	0.011	0.007	0.007	0.011	0	-
14	<i>R. sp</i> (1202)	0.018	0.009	0.014	0.016	0.018	0.014	0.009	0.011	0.007	0.007	0.007	0.009	0.009
15	<i>R. sp</i> Kalibar, Iran (1204)	0.021	0.009	0.014	0.016	0.021	0.014	0.009	0.011	0.007	0.007	0.011	0.004	0.004
16	<i>R. sp</i> Siah-rood, Iran (1205)	0.021	0.009	0.014	0.016	0.021	0.014	0.009	0.011	0.007	0.007	0.011	0.004	0.004
17	<i>R. sp</i> Emarat, Iran (1206)	0.023	0.014	0.018	0.021	0.018	0.018	0.014	0.016	0.011	0.011	0.011	0.014	0.014
18	<i>R. sp</i> Zanjan Iran (1207)	0.018	0.009	0.014	0.016	0.018	0.014	0.009	0.011	0.007	0.007	0.002	0.009	0.009
19	<i>R. sp</i> Tarum, Iran (1208) Shahr-e Kord, Iran	0.021	0.009	0.014	0.016	0.021	0.014	0.009	0.011	0.007	0.007	0.011	0.009	0.009
20	<i>R. sp</i> (1209)	0.016	0.007	0.011	0.014	0.016	0.011	0.007	0.009	0.004	0.004	0.004	0.007	0.007
21	<i>R. sp</i> Ozan, Iran (1278)	0.018	0.009	0.014	0.016	0.018	0.014	0.009	0.011	0.007	0.007	0.007	0.009	0.009
22	<i>R. sp</i> Ozan, Iran (1279)	0.021	0.009	0.014	0.016	0.021	0.014	0.009	0.011	0.007	0.007	0.011	0.004	0.004
23	<i>R. sp</i> Ja'farabad, Iran (1280)	0.021	0.009	0.014	0.016	0.021	0.014	0.009	0.011	0.007	0.007	0.011	0.004	0.004
24	<i>R. sp</i> Jananlu, Iran (1281)	0.021	0.009	0.014	0.016	0.021	0.014	0.009	0.011	0.007	0.007	0.011	0.004	0.004
25	<i>R. sp</i> Jananlu, Iran (1282)	0.021	0.009	0.014	0.016	0.021	0.014	0.009	0.011	0.007	0.007	0.011	0.004	0.004
26	<i>R. sp</i> Ozan, Iran (1284)	0.021	0.009	0.014	0.016	0.021	0.014	0.009	0.011	0.007	0.007	0.011	0.004	0.004
27	<i>R. sp</i> Jananlu, Iran (1285)	0.021	0.009	0.014	0.016	0.021	0.014	0.009	0.011	0.007	0.007	0.011	0.004	0.004
28	<i>R. sp</i> Ozan, Iran (1283)	0.021	0.009	0.014	0.016	0.021	0.014	0.009	0.011	0.007	0.007	0.011	0.004	0.004
29	<i>R. sp</i> Ebrahim, Iran (1286)	0.021	0.009	0.014	0.016	0.021	0.014	0.009	0.011	0.007	0.007	0.011	0.004	0.004
30	<i>R. sp</i> Koda afarin, Iran (1287)	0.021	0.009	0.014	0.016	0.021	0.014	0.009	0.011	0.007	0.007	0.011	0.004	0.004

more eastern group represented in this study (Fig. 3) and by what we would conventionally know as *R. lucifugus* from the whole of Italy.

In Iran, it would appear that there is a single distinct group of *Reticulitermes* which extend their range south towards the Persian Gulf and west towards Iraq. Most of the Iranian samples in the present study come from the Tabriz region (Northwestern Caspian Sea area) at the western edge of the Elburz mountains (Fig. 3). Some populations were more clearly revealed through maximum likelihood than with parsimony analysis. For example, two populations of *R. lucifugus* from Turkey (Kharaman Maraş 1 and 2) appear more distinct morphologically (JWA, unpublished) from other Turkish *Reticulitermes* (Figs. 1 and 2). This may be attributed to the remote location from where these samples were taken, isolated on the eastern edge of the Mediterranean faunistic zone and the southeastern Taurus mountains in Turkey.

The distribution of *Reticulitermes* in eastern Turkey remains unknown. Weidner (1972) has documented a few populations from eastern Turkey, and more recent anecdotal information from local villages suggests that *Reticulitermes* are present (JWA, unpublished). There have been reports of *Reticulitermes* attacking some crops in Turkey, like eggplant from Urfa (Karaat & Göven 1983) or citrus in Mersin (Gerini 1971). Additional populations from the east (Diyarbakır, Siirt, Batman, Kharaman Maraş), all from the southeast- inner-Anatolian region, have only scantily been reported (Bodenheimer 1958, Iren & Ahmed 1973, Lodos 1982, Karaat & Göven 1983).

Previous surveys of termites from this region by Weber (1954) and Weidner (1958, 1959) in Iran and Iraq, Weidner (1972) in Turkey, Lash (1952) in Israel, and more recently Austin *et al.* (2002) provide needed information about the abundance of *Reticulitermes* from this region. Lists of economically important termite species from neighboring Syria (Hariri 1971, 1972ab) and Egypt (El-Sherif & Kaschef 1974, Krajewski 1996) have been compiled, but there is no mention of *Reticulitermes*. The southern distribution of this genus is likely more restricted due to the contrasting bioclimatic zones of this region, most notably the availability of water and changing steppe vegetation (Atalay 1994). However, experience with other members of this genus have demonstrated exotic establishments in numerous locations around the world when artificial habitat resources are met (e.g., *R. grassei* introduced to England) and human assisted introductions can not be ruled out.

Morphological comparisons of *Reticulitermes* from Europe and the Middle East (Weidner 1960) clearly show the morphological variability of this genus from this region. Our measurements (Table 2) of soldiers from Iran overlap with previous estimates from Lash (1952) and Weidner (1959, 1960, 1972). This incongruity between morphological features of *Reticulitermes* species from this region casts doubt on their validity as discrete species. This viewpoint was supported by our maximum parsimony analysis which places three described species together—*R. clypeatus*, *R. balkanensis*, and *R. lucifugus* Turkey. However, some groups such as *Reticulitermes* from the Peloponnesian region of southern Greece appear more varied than historical records from the region, a fact more recently supported by descriptions of new species thought to occupy this area (Clément *et al.* 2001, Uva *et al.* 2004, Luchetti *et al.* 2004). Clearly, further sampling from this region would greatly enhance our understanding of events which have fostered the observed differences reflected in the present study. Unfortunately, given the political unrest and safety concerns for western researchers collecting in this region, it may be many years before more information can be garnered.

Recent theory of *Reticulitermes* speciation in Europe has been attributed to colonization routes following glacial patterns of biotic radiation after the last ice age (Clément *et al.* 2001, Uva *et al.* 2004). A 4th glacial radiation (refugium) theory proposed by Luchetti *et al.* (2004a) is strongly supported with a molecular clock (JWA, unpublished), suggesting that sympatric populations of *Reticulitermes* from Israel, Iran, Turkey, and Greece, all point towards a geological event some 13-14,000 years ago as originally proposed by Clément *et al.* (2001) and Uva *et al.* (2004), thus providing the best working explanation for the observed genetic relationships presented herein. This observation was recently supported by Luchetti *et al.* (2005) who recognized the existence of two refugia in the eastern Mediterranean area, i.e., the southern Balkans-Greece and the Near East. Some of the more apparent differences between supposed species may be an artifact of more selective environments that have fostered isolated micro-allopatric speciation within some groups. Additional data is needed to more clearly support this hypothesis, and increased sampling from the Balkans would be an important next step.

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