

# Phylogeography of *Reticulitermes* Termites (Isoptera: Rhinotermitidae) in California Inferred from Mitochondrial DNA Sequences

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**ABSTRACT** Existing taxonomic studies of *Reticulitermes* spp. (Isoptera: Rhinotermitidae) from California provide information on only two described species: *Reticulitermes hesperus* Banks and *Reticulitermes tibialis* Banks. However, while conducting a genetic evaluation of the genus from North America, we find evidence of species that cannot be identified morphologically with existing information. We also update more current information about other species detected during our investigations, including the positive identification of *R. flavipes* from California. Therefore, we have conducted a molecular genetics study involving DNA sequencing of a portion of the mitochondrial DNA (mtDNA) 16S gene to determine the extent of genetic variation within *Reticulitermes* from California. We analyzed 94 samples. Twenty-five nucleotide sites were variable in *R. hesperus*, and 19 mtDNA haplotypes were observed in the 428-bp mtDNA sequence. Fourteen haplotypes (37%) occurred only once, whereas the most common haplotypes, HE4 and HE9, each occurred in 18% of the samples. Although some haplotypes were found to have a broad geographical range across the state, some were restricted to the southern region, as were all samples identified as *R. tibialis*. Twelve haplotypes of an undescribed western species, *R. n. sp.* '*R. okanaganensis*,' were found, and its distribution throughout the state is discussed. Additionally, genetic evidence of two additional undescribed *Reticulitermes* species from southern California is presented.

**KEY WORDS** *Reticulitermes*, termite, mitochondrial DNA, genetic variation

Recent studies of subterranean termites in the United States using mitochondrial DNA (mtDNA) markers (Austin et al. 2002) and cuticular hydrocarbons (Haverty and Nelson 1997, Haverty et al. 1999, Nelson et al. 2001) have indicated the existence of undescribed species of *Reticulitermes* (Isoptera: Rhinotermitidae) in California. There is a general consensus that the genus *Reticulitermes* is in desperate need of revision (Weesner 1970, Nutting 1990, Scheffrahn and Su 1994). This is an especially difficult task because of the problematic nature of this genus, namely, the lack of discrete morphological characters, which accurately identify specimens within the genus. For this reason, nonmorphological identification methods such as cuticular hydrocarbon analysis and mtDNA markers have been used.

Recently, the application of the 16S rRNA mtDNA marker has been applied to identify *Reticulitermes* populations from the south central United States

(Austin et al. 2004a,b,c) and across North America (Austin et al. 2005a). This marker has tremendous potential for molecular diagnostics of *Reticulitermes*, with increased accuracy of positive species identifications (Szalanski et al. 2003) and clarifying the identities of exotic introductions around the world (Austin et al. 2005b) and from North America (Austin et al. 2005a). Although the use of other molecular markers has been explored to resolve issues within the genus *Reticulitermes*, we have found the 16S marker to provide more consistent and reliable data. Nuclear markers such as the ribosomal internal transcribed spacer sequence (ITS) and noncoding AT-rich regions have provided inconsistent amplification in our own laboratory and have been unable to resolve phylogenetic relationships between populations and haplotypes in other studies (Foster et al. 2004, Austin et al. 2005a). We have also found amplifications of the variable cytochrome oxidase subunit two (COII) to be problematic and inefficient, perhaps because of variation in the primer region. In addition, reliable species identification and phylogenetic analysis depend on comparing sequence data across a large number of samples. A quick look at the diversity of *Reticulitermes* species represented on GenBank at the time of this study reveals that there is a wider representation of North American members of this genus using the 16S

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rRNA mtDNA marker than other nuclear markers, such as ITSs, or mitochondrial markers, such as COII.

While conducting a survey of *Reticulitermes* from North America, we found this genetic marker to provide more conservative estimates of discrete populations of *Reticulitermes* than other nonmorphological methods such as cuticular hydrocarbons (J.W.A., unpublished data). For example, Haverty et al. (1999) suggest that, because they found similar cuticular hydrocarbon phenotype patterns from disparate populations of *Reticulitermes*, they likely represent discrete taxa (26 species versus the previously described six species in North America). This number seems inflated, because we have only found genetic evidence of four undescribed species in North America applying mtDNA markers (Szalanski et al. 2006; A.L.S., unpublished data). Recently, Copren et al. (2005) found evidence for eight species of *Reticulitermes* by corroborating cuticular hydrocarbon profiles with molecular phylogenetics. This discrepancy is likely attributed to the plasticity of cuticular hydrocarbons and stresses the need for other corroborating evidence.

Chemotaxonomy largely uses insect cuticular hydrocarbons and soldier defensive excretions, identified through chromatographic means (e.g., gas chromatography), for the purpose of segregating species or populations through the frequency of various hydrocarbon compositions. The application of cuticular hydrocarbons for chemotaxonomy requires fixed patterns of hydrocarbons within taxa (Kaib et al. 1991). This approach is inherently problematic because of the plastic nature of hydrocarbon composition. Although hydrocarbon compositions are assumed to be species-specific (Kiab et al. 1991), variation between groups may be more greatly attributable to environmental differences and available food sources (Liang and Silverman 2000). Recent studies with *Coptotermes formosanus* Shiraki have shown that differences in diet can influence hydrocarbon composition and intercolonial aggression (Florane et al. 2004). Therefore, although cuticular hydrocarbons probably play a key role in nestmate recognition between colonies of termites, considerable variation of hydrocarbons across small spatial distances within an apparently single morphological species may occur and should alert taxonomist to interpret cuticular hydrocarbon patterns with care. In addition, phenotypic characters with a continuous range of values, such as hydrocarbon composition, can be difficult to delineate for taxonomic purposes. Because nucleotides are limited in variation and discretely defined, DNA sequences are less ambiguous characters from which to infer phylogenetic relationships (Olsen and Woese 1993). This being said, some studies indicate that there is a positive correlation between cuticular hydrocarbons and mtDNA data (Jenkins et al. 2000, Copren et al. 2005), whereas combinations of other nonmorphological data have been less revealing or lack spatial or geographic correlations (Fisher and Gold 2003). Finding these associations through a multidisciplinary approach incorporating morphological, biochemical,

and molecular data may be the key to revising the genus *Reticulitermes*.

Weesner (1970) and Nutting (1990) describe two species of *Reticulitermes* in California: *Reticulitermes hesperus* Banks and *Reticulitermes tibialis* Banks. Herein, we provide a genetic interpretation of the distributions of *Reticulitermes* in California. We find evidence of *R. flavipes* Kollar in two disparate locations within California, a state that has only recently had reports of *R. flavipes* (Austin et al. 2005a). Furthermore, although the likelihood of undescribed species of *Reticulitermes* in California has been previously suggested by cuticular hydrocarbon data (Haverty et al. 1999, Delphia et al. 2003), we observed genetic evidence for 12 haplotypes of an undescribed western species *R. n. sp.* '*R. okanaganensis*' (Szalanski et al. 2006) and two additional undescribed species from southern California. In addition, we provide a phylogenetic analysis of *Reticulitermes* applying the 16S mtDNA gene and discuss the geographic distribution of *Reticulitermes* species and species' haplotypes throughout the region.

## Materials and Methods

**Insect Collection.** Termites were collected from various locations in California, both from our own collecting efforts and from the 2002 National Termite Survey (Fig. 1). Samples were preserved in 100% ethanol. *R. flavipes* and *R. tibialis* were morphologically identified when alates were available using the keys of Krishna and Weesner (1969), Banks and Snyder (1920), and Hostettler et al. (1995). For the remaining samples without alates, species identification was conducted using mtDNA 16S sequences (Szalanski et al. 2003; A.L.S., unpublished data). Voucher specimens preserved in 100% ethanol are maintained at the Arthropod Museum, Department of Entomology, University of Arkansas, Fayetteville, AR.

**Polymerase Chain Reaction (PCR) and DNA Sequencing.** Alcohol-preserved specimens were allowed to dry on filter paper, and DNA was extracted according to Liu and Beckenbach (1992) on individual whole worker termites with the Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, MN). Extracted DNA was resuspended in 50  $\mu$ l of Tris:EDTA and stored at  $-20^{\circ}\text{C}$ . PCR was conducted using the primers LR-J-13007 (5'-TTACGCTGTTATCCCTAA-3') (Kambhampati and Smith 1995) and LR-N-13398 (5'-CGCCTGTTTATCAAAAACAT-3') (Simon et al. 1994). These PCR primers amplify an  $\approx 428$ -bp region of the mtDNA 16S rRNA gene. The PCR reactions were conducted with 2  $\mu$ l of the extracted DNA (Szalanski et al. 2000), having a profile consisting of 35 cycles of  $94^{\circ}\text{C}$  for 45 s,  $46^{\circ}\text{C}$  for 45 s, and  $72^{\circ}\text{C}$  for 60 s. Amplified DNA from individual termites was purified and concentrated with minicolumns (Wizard PCRpreps, Promega, Madison, WI) according to the manufacturer's instructions. Samples were sent to The University of Arkansas Medical Center DNA Sequencing Facility (Little Rock, AR) for direct sequencing in both directions. Consensus sequences for each sample were



data set for comparison along with DNA sequences from the Formosan termite, *Coptotermes formosanus* Shiraki (GenBank AY558910) and *Heterotermes aureus* (Snyder) (GenBank AY280399), which were added to act as outgroup taxa. DNA sequences were aligned using CLUSTAL W (Thompson et al. 1994). Maximum likelihood and unweighted parsimony analysis on the alignments was conducted using PAUP\* 4.0b10 (Swofford 2001). Gaps were treated as a fifth character state for the maximum parsimony analysis and as missing characters the maximum likelihood analysis. The reliability of trees was tested with a bootstrap test (Felsenstein 1985). Parsimony bootstrap analysis included 1,000 resamplings using the Branch and Bound algorithm of PAUP\*. For maximum likelihood analysis, the default likelihood parameters were used (HKY85 six-parameter model of nucleotide substitution, empirical base frequencies with the exception of the transition/transversion ratio, which was set to 2.541707). These parameters were used to carry out a heuristic search using PAUP\* by using a neighbor joining tree as the starting tree.

### Results

DNA sequencing of the 16S rRNA amplicon revealed an average size of 428 bp. The average base frequencies were A = 0.41, C = 0.23, G = 0.13, and T = 0.23. Among the 94 *Reticulitermes* mtDNA 16S DNA sequences, 21 nucleotide sites in total were variable. *R. hesperus* was found in 17 of the 25 California counties sampled (Fig. 1). Eighteen distinct haplotypes (lineages) were observed (Table 1), and genetic divergence among these haplotypes ranged from 0.23 to 1.9% (Table 2). Fourteen haplotypes occurred only once, whereas the most common haplotypes, HE4 and HE9, each accounted for 18% of the *R. hesperus* samples. Haplotype HE4 was also the haplotype found over the largest geographical area (Fig. 1). The haplotype HE7 was recovered from samples in Napa County as well as in Orange County and thus had the greatest north-south range of all *R. hesperus* in this study (Fig. 1). Twelve haplotypes of the undescribed western species *R. n. sp. 'R. okanaganensis'* were found in 17 of the counties sampled (Table 3). Three of the haplotypes occurred only once. Although haplotype O12 was the most common, occurring in 27% of the samples, it was only present in samples from the southwestern extreme of the state (Fig. 1). Haplotype O3 was also very common, occurring in 22% of the samples and covering the broadest geographic area (Fig. 1). Haplotype O1 was found in four California locations. The identification of *R. n. sp. 'R. okanaganensis'* haplotypes O1 through O13 demonstrates considerable variation in this taxon given the number of samples (43) evaluated. A single sample of a genetically distinct species, *R. n. sp. CA1*, was discovered in Arrowbear, CA, in San Bernardino County, and another genetically distinct sample, *R. n. sp. CA2*, was found in Mission Gorge, CA, in San Diego County. Two haplotypes of *R. flavipes* were obtained from Sacramento (LL) and San Diego (VV) counties. Three haplotypes

of *R. tibialis* were found in the southern region of the state, in Los Angeles (T9), Riverside (T10), and San Bernardino (T3 and T9) counties, but they were not seen in samples from other regions of the state (Fig. 1).

We conducted a phylogenetic analysis on all described *Reticulitermes* species from California to clarify the phylogenetic relationships of *R. hesperus*, *R. n. sp. 'R. okanaganensis'*, and *R. n. sp. CA1* and *CA2* within the genus. Parsimony analysis of the aligned *Reticulitermes* spp. and the outgroup taxa used 436 characters, of which 113 were variable (26%) and 76 (75%) were parsimony informative. This analysis had a single consensus tree with a length = 236 and a consistency index value of 0.614 and verified the distinct monophyly of each of the *Reticulitermes* species sampled herein to the exclusion of comparative taxa (Fig. 2). In general, there did not seem to be any correlation to geography among the *R. hesperus* haplotypes; however, haplotypes HE8, HE9, HE18, HE20, and HE21 formed a distinct clade with moderate support. Haplotypes HE13, HE14, HE19, and HE22 formed a distinct clade as did haplotypes HE1 and HE2, and HE15 and HE16 with slightly stronger support (Fig. 2). An overall look at the maximum likelihood tree shows *R. hesperus* as sister to *R. tibialis*, with the group (*R. hesperus* + *R. tibialis*) grouping more closely with *R. n. sp. 'R. okanaganensis'* than with *R. flavipes* (Fig. 3). The undescribed species *R. n. sp. CA1* and *CA2* occurred as sister to all other *Reticulitermes* species in this study (Fig. 3). However, given that only a single sample of these two undescribed species has thus far been recovered, their relationship to other *Reticulitermes* species is uncertain.

### Discussion

Phylogenetic analysis of *Reticulitermes* from California reveals discrete clades, which support the monophyletic nature of *R. hesperus* and *R. tibialis* (Figs. 2 and 3). Their monophyly is consistent with previous studies applying both COII and 16S mtDNA genes (Austin et al. 2002, 2004a,b,c). Until recently, *R. flavipes* was not thought to inhabit the western extreme of North America. During a genetic survey of *R. flavipes* in North America, Austin et al. (2005a) reported the first positive identification of this species in California. This occurrence has now been independently verified (Su et al. 2006). The identification of *R. flavipes*, the eastern subterranean termite, from Sacramento and El Cajon probably represents either extreme western distributions of the species or accidental introductions from anthropogenic sources. The latter seems more plausible given the omission of information about the species' presence in California as well as the geographic distance separating the two samples. Our experience with this group shows that it has consistently been misidentified because of prejudices on assumptions about their respective distributions (Austin et al. 2002, 2005b). Because *R. flavipes* is a primary pest of structures in the United States and around the world, assessment of the presence of this

**Table 1. Sample localities and 16S haplotype designations (Hap) for California *Reticulitermes* termite species**

Species	City/location	County	Lat/Long	Haplotype	N	
<i>R. flavipes</i>	Sacramento	Sacramento	38:28:00 N 121:19:00 W	LL	1	
	El Cajon	San Diego	32:47:41 N 116:57:42 W	VV	1	
<i>R. hesperus</i>	Novato	Marin	38:06:27 N 122:34:07 W	HE1	1	
	Riverside	Riverside	33:57:12 N 117:23:43 W	HE1	1	
	San Luis Obispo	San Luis Obispo	35:09:58 N 120:43:32 W	HE1	2	
	San Leandro	Alameda	37:42:07 N 122:09:11 W	HE2	1	
	Alabama Hills	Calaveras	38:21:18 N 120:35:27 W	HE4	1	
	Napa	Napa	38:17:50 N 122:17:04 W	HE4	2	
	San Francisco	San Francisco	37:46:30 N 122:25:06 W	HE4	1	
	San Mateo	San Mateo	37:33:47 N 122:19:28 W	HE4	1	
	Strathmore	Tulare	36:08:44 N 119:03:35 W	HE4	1	
	Visalia	Tulare	36:19:49 N 119:17:28 W	HE4	1	
	Groveland	Tuolumne	37:50:18 N 120:13:54 W	HE5	1	
	Palm Springs	Riverside	33:49:49 N 116:32:40 W	HE6	1	
	Riverside	Riverside	33:57:12 N 117:23:43 W	HE6	2	
	Westminster	Orange	33:45:33 N 118:00:21 W	HE6	1	
	Brea	Orange	33:55:00 N 117:53:57 W	HE7	1	
	Napa	Napa	38:17:50 N 122:17:04 W	HE7	1	
	Hanford	Kings	36:19:39 N 119:38:41 W	HE8	1	
	Long Beach	Los Angeles	33:46:01 N 118:11:18 W	HE9	2	
	Monarch Beach	Orange	33:47:74 N 117:69:89 W	HE9	1	
	Norco	Riverside	33:56:24 N 117:33:12 W	HE9	1	
	Santa Catalina Island	Los Angeles	33:23:09 N 118:25:47 W	HE9	2	
	Topanga Park	Los Angeles	34:06:27 N 118:37:42 W	HE9	1	
	Walnut	Los Angeles	34:01:13 N 117:51:52 W	HE9	1	
	Hanford	Kings	36:19:39 N 119:38:41 W	HE10	1	
	Stockton	San Joaquin	37:57:28 N 121:17:23 W	HE13	1	
	Stockton	San Joaquin	37:57:28 N 121:17:23 W	HE14	1	
	Porterville	Tulare	36:03:55 N 119:00:57 W	HE15	1	
	Porterville	Tulare	36:03:55 N 119:00:57 W	HE16	1	
	Los Angeles	Los Angeles	34:22:00 N 118:12:00 W	HE17	1	
	Santa Maria	Santa Barbara	34:57:11 N 120:26:05 W	HE18	1	
	Sacramento	Sacramento	38:28:00 N 121:19:00 W	HE19	1	
	Glendale	Los Angeles	34:08:33 N 118:15:15 W	HE20	1	
	Los Angeles	Los Angeles	34:22:00 N 118:12:00 W	HE21	1	
	Penn Valley	Nevada	39:11:46 N 121:11:24 W	HE22	1	
	<i>R. n. sp</i>	Chino	San Bernardino	34:00:44 N 117:41:17 W	O1	1
	<i>R. okanaganensis</i>	Grass Valley	Nevada	39:13:09 N 121:03:36 W	O1	1
		Irvine	Orange	33:40:10 N 117:49:20 W	O1	1
		Napa	Napa	38:17:50 N 122:17:04 W	O1	1
		Placerville	El Dorado	38:43:47 N 120:47:51 W	O2	1
		Auburn	Placer	38:53:48 N 121:04:33 W	O3	2
		Bakersfield	Kern	35:22:24 N 119:01:04 W	O3	1
		Davis	Yolo	38:32:42 N 121:44:22 W	O3	1
Dinuba		Tulare	36:32:36 N 119:23:10 W	O3	1	
Lafayette		Contra Costa	37:53:09 N 122:07:01 W	O3	1	
Lake Arrowhead		San Bernardino	34:15:52 N 117:11:04 W	O3	1	
Napa		Napa	38:17:50 N 122:17:04 W	O3	1	
Strathmore		Tulare	36:08:44 N 119:03:35 W	O3	1	
Walnut Creek		Contra Costa	37:54:23 N 122:03:50 W	O3	1	
Sebastopol		Sonoma	38:24:08 N 122:49:22 W	O5	1	
Walnut Creek		Contra Costa	37:54:23 N 122:03:50 W	O5	1	
Burbank		Los Angeles	34:10:51 N 118:18:29 W	O6	1	
Santa Maria		Santa Barbara	34:57:11 N 120:26:05 W	O6	1	
Mission Viejo		Orange	33:36:00 N 117:40:16 W	O7	1	
Riverside		Riverside	33:57:12 N 117:23:43 W	O7	1	
Walnut		Los Angeles	34:01:13 N 117:51:52 W	O7	2	
Pauma Valley		San Diego	33:18:12 N 116:58:50 W	O8	1	
San Marcos		San Diego	33:08:36 N 117:09:55 W	O8	1	
Davis		Yolo	38:32:42 N 121:44:22 W	O9	1	
Napa		Napa	38:17:50 N 122:17:04 W	O9	2	
Oakland		Alameda	37:48:16 N 122:16:11 W	O9	1	
Oakland		Alameda	37:48:16 N 122:16:11 W	O10	1	
Rocklin		Placer	38:47:27 N 121:14:05 W	O10	1	
Napa		Napa	38:17:50 N 122:17:04 W	O11	1	
Corona Del Mar		Orange	33:35:53 N 117:52:20 W	O12	1	
Covina		Los Angeles	34:05:24 N 117:53:22 W	O12	1	
Culver City		Los Angeles	34:01:16 N 118:23:44 W	O12	1	
Laguna Beach		Orange	33:32:32 N 117:46:56 W	O12	1	
Los Angeles		Los Angeles	34:22:00 N 118:12:00 W	O12	2	
Pomona		Los Angeles	34:03:19 N 117:45:05 W	O12	1	
San Juan Capistrano		Orange	33:30:06 N 117:39:42 W	O12	1	

(continued)

Table 1. Continued

Species	City/location	County	Lat/Long	Haplotype	N
	Upland	San Bernardino	34:05:51 N 117:38:51 W	O12	1
	W. Covina	Los Angeles	34:04:07 N 117:56:17 W	O12	1
	Walnut	Los Angeles	34:01:13 N 117:51:52 W	O12	1
	Westminster	Orange	33:45:33 N 118:00:21 W	O12	1
	Tecate	San Diego	32:36:47 N 116:41:59 W	O12	1
	Rancho Santa Margarita	Orange	33:38:56 N 117:36:17 W	O12	1
	Stockton	San Joaquin	37:57:28 N 121:17:23 W	O13	1
<i>R. tibialis</i>	-	San Bernardino	34:40:00 N 116:10:00 W	T3	1
	Diamond Bar	Los Angeles	33:58:39 N 117:50:16 W	T9	1
	San Bernardino	San Bernardino	34:40:00 N 116:10:00 W	T9	1
	Cabazon	Riverside	33:55:00 N 116:46:43 W	T10	1
<i>R. n. sp. CA1</i>	Arrowbear	San Bernardino	34:12:39 N 117:04:57 W		1
<i>R. n. sp. CA2</i>	Mission Gorge	San Diego	32:49:57 N 117:03:38 W		1
<i>Coptotermes formosanus</i>	Baton Rouge, LA			Outgroup	
<i>Heterotermes aureus</i>	Santa Rita, AZ			Outgroup	

species in California should be carefully evaluated to see whether it will compete with *R. hesperus* as a destructive pest in the future.

Although our sampling of California is far from exhaustive, some interesting distribution patterns emerge nonetheless. Although *R. hesperus* was discovered throughout the state, we recovered no samples east of the Sierra Nevada foothills (Fig. 1, inset). Based on this and other data, we suspect that its eastern distribution is restricted by the Sierra Nevada Mountain Range, by the desert regions in the south, and by the Cascades in the north (A.L.S., unpublished data). The discovery of an ecologically limited distribution of *R. hesperus* in California seems plausible given multiple studies of organisms restricted to such regions throughout the state [e.g., *Camponotus floridanus* (Buckley) carpenter ants, Gadau et al. 1996; *Ambystoma californiense* (Gray) tiger salamanders, Shaffer et al. 2004]. Whether the distribution of *R. hesperus* is limited by elevation, moisture, or vegetation clines deserves further investigation. Not surprisingly, *R. tibialis*, the arid land subterranean termite (Snyder 1954) was only recovered from samples in the southern

portion of the state in areas that border the Mojave Desert; a region classified by Jepson as the southwestern ecological zone (Fig. 1, inset) (Hickman 1993). Some haplotypes of both *R. hesperus* and *R. n. sp. 'R. okanaganensis'* seem to be limited to or excluded from this region as well. *R. hesperus* haplotype HE6 was only recovered in this region, whereas one of the most common haplotypes, HE4, was not found here at all. Two haplotypes of *R. n. sp. 'R. okanaganensis,'* O12 and O7 were found at multiple sites but only within this area. This result is particularly of note as haplotype O12 was the most common haplotype of this new species found in this study. Because this region incorporates the highly developed and sprawling urban region of Los Angeles, this distribution may be more indicative of anthropogenic sources, but it is possible that subsequent establishment has been restricted by ecological factors particular to this region. Further sampling, particularly of the northwestern mountain and southeastern desert regions, should illuminate these preliminary observations.

The identification of a genetically distinct species, *R. n. sp. 'R. okanaganensis,'* with multiple haplotypes

Table 2. Base pair differences between *R. hesperus* haplotypes from California

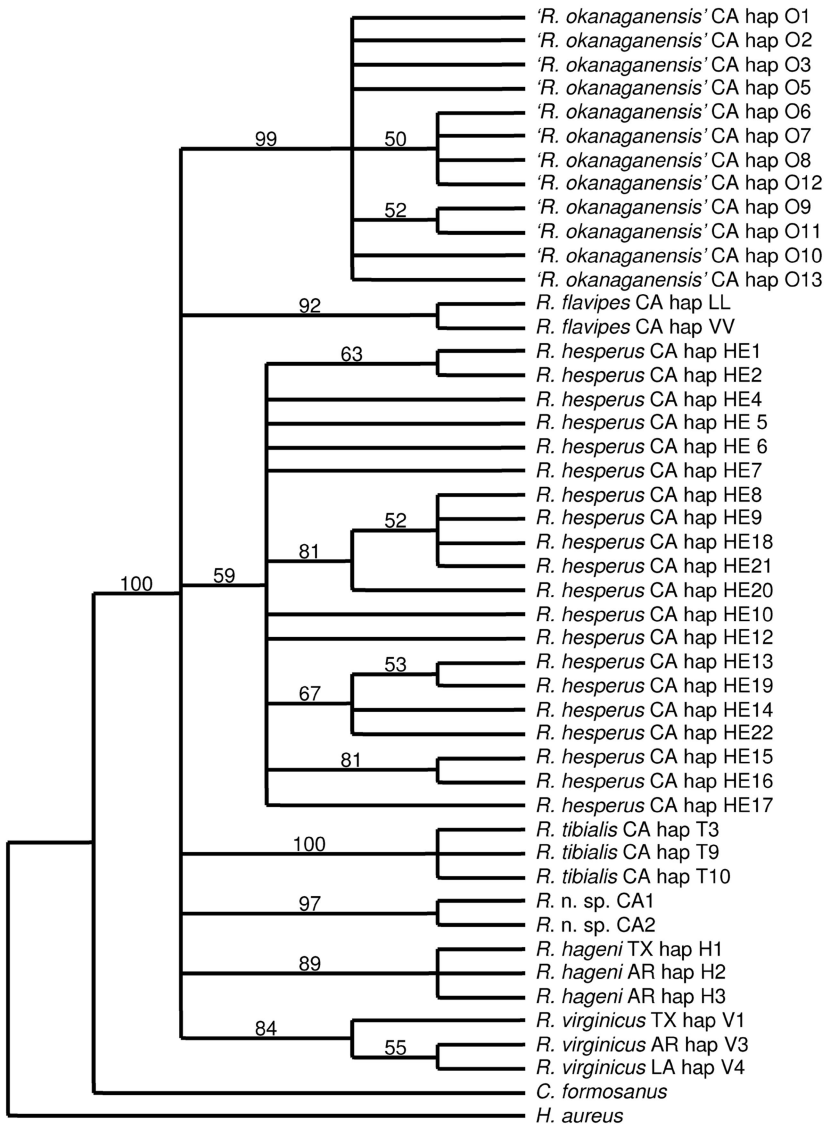
Haplotypes	55	56	80	99	127	132	140	143	154	156	159	160	167	246	247	253	260	294	325	340	362	363	366	367	373	
HE1	G	T	T	-	C	C	T	A	C	A	A	G	T	A	A	.	-	C	T	A	C	A	A	-	T	
HE2	.	.	.	-	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HE4	.	.	C	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HE5	.	.	.	A	.	.	.	G	G	.	.	.	.	.	.	.	-	T	.	.	.	.	.	.	.	
HE6	.	.	.	A	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	T	T	.	.	.	
HE7	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
HE8	.	.	.	A	.	.	C	G	.	.	G	.	.	.	.	.	.	.	.	.	T	.	.	.	.	
HE9	.	.	.	G	.	C	G	.	.	.	G	.	.	.	.	.	.	.	.	.	T	.	.	.	.	
HE10	.	.	C	A	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	
HE13	.	.	.	A	T	T	.	.	A	G	.	.	C	.	.	.	.	.	.	.	T	.	.	.	.	
HE14	.	.	.	A	T	T	.	.	G	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	C	
HE15	A	C	C	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	.	.	
HE16	A	C	C	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	
HE17	.	.	C	G	.	.	C	.	.	G	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	
HE18	.	.	.	A	.	.	C	G	.	.	G	.	.	G	.	.	.	T	.	.	T	.	.	.	.	
HE19	.	.	.	A	T	T	.	.	.	G	.	.	C	.	.	.	.	.	.	.	T	T	.	.	.	
HE20	.	.	.	G	.	.	C	G	.	.	.	.	.	.	.	.	.	.	.	A	T	.	.	.	.	
HE21	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
HE22	.	.	.	A	T	T	.	.	.	G	.	.	.	.	.	.	.	T	.	.	T	.	.	.	.	

(dot), represents identical nucleotide; - (dash), represents gap.

**Table 3. Base pair differences between *R. n. sp.* '*Reticulitermes okanaganensis*' haplotypes**

Haplotype	75	78	96	141	160	265	269	361	364	369	375
O1	C	C	C	G	A	C	C	-	T	A	T
O2	.	.	.	.	G	.	.	.	.	.	.
O3	.	.	.	.	G	.	.	.	.	.	.
O5	.	.	T	.	.	.	.	.	.	.	.
O6	.	.	.	A	.	.	.	T	.	.	.
O7	.	T	.	.	.	.	.	T	.	.	.
O8	.	.	.	.	.	.	T	T	.	.	.
O9	.	.	.	.	G	.	.	.	.	.	C
O10	.	.	.	.	G	T	.	.	.	.	.
O11	.	T	.	.	G	.	.	.	.	.	C
O12	.	.	.	.	G	.	.	T	.	.	.
O13	.	.	.	.	G	.	.	.	.	.	.

• (dot), represents identical nucleotide; - (dash), represents gap.



**Fig. 2.** Maximum parsimony cladogram of California *Reticulitermes* and related taxa. Bootstrap values for 1,000 replicates by using the Branch and Bound algorithm of PAUP\* are listed above branches supported at  $\geq 50\%$ .

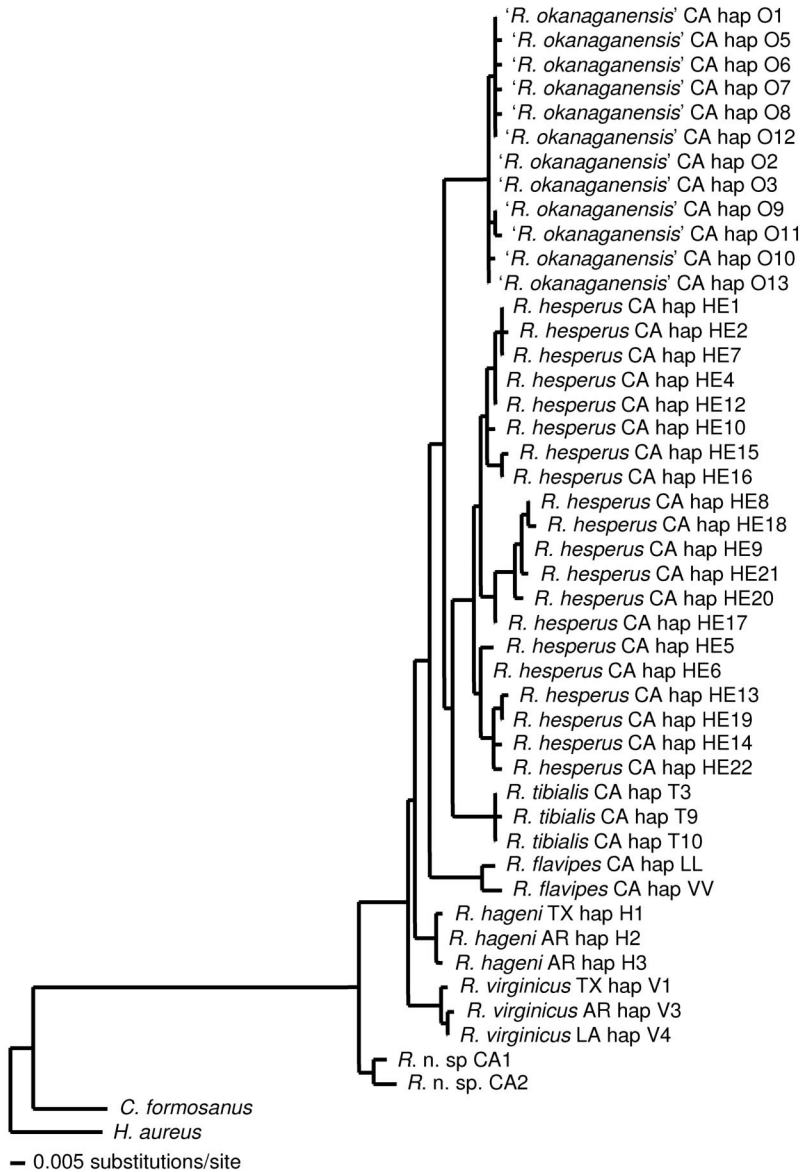


Fig. 3. Maximum likelihood cladogram of California *Reticulitermes* and related taxa.

from 17 counties across the state (Fig. 1) demonstrates that this group represents a taxonomic group that merits further investigation. The identification of new species from northern California based on cuticular hydrocarbons has been reported (Haverty and Nelson 1997, Haverty et al. 1999) and further investigated with ethological data (Getty et al. 2000a,b). This new species has become a problem in British Columbia, where there are a number of attacks to structures (Szalanski et al. 2006). Haplotype O1, which has been reported in Osoyoos, British Columbia, Canada, is distributed across the state from Nevada and Napa counties in the north to Orange and San Bernardino counties in the southern extreme (Fig. 1). Osoyoos is located in the southern interior ecoregion of Can-

ada, a region characterized as a desert climatic zone. Haplotypes O1 and O2 also have been recovered from Reno, NV, samples (Szalanski et al. 2006). We are currently investigating the distribution of termite species throughout the western United States. From this study and ongoing research, we expect that *R. n. sp.* '*R. okanaganensis*' will be found in the drier regions north of the Cascades and east of the Sierra Nevada ranges. The importation of this species either to the United States or to Canada needs to be investigated. Establishment of this species through trade is a likely scenario, whether from untreated structural timbers brought to the United States or through plant materials, but it is difficult to speculate based on our current knowledge, or lack thereof, concerning this species.

An important criterion for determining the extent of genetic variation for a species lies in the ability to sample from populations evenly distributed within the species range (Mayr and Ashlock 1991). Thus, future studies of this unknown species demand more intensive collecting data and comparative biological studies. This study represents an important first step toward this endeavor.

We have found significant genetic variation within *Reticulitermes* despite the lesser amount of intraspecific variation obtained with 16S relative to COII (Szalanski et al. 2003). In addition to the 22 haplotypes of *R. hesperus* and 13 haplotypes of *R. n. sp. 'R. okanaganensis'* reported in this article, we have found 47 haplotypes of *R. flavipes* (Austin et al. 2005a) and 31 haplotypes of *R. tibialis* (A.L.S., unpublished data). This information, combined with the ongoing expansion of our genetic database, has allowed us to review our previous COII designations for *Reticulitermes* species. In doing so, we have found that two of our California specimens have been misidentified using COII data. Previously, we had reported a *R. okanaganensis* sample from Los Angeles as *R. hesperus* (GenBank AF525329) and misidentified a *R. hesperus* sample from Catalina Island as an unidentified species, *R. n. sp.* (GenBank AF525342) (Austin et al. 2002). We have now updated this information in GenBank. These two samples were recently used as genetic type specimens to correlate hydrocarbon and molecular data to identify *Reticulitermes* species within California (Copren et al. 2005). In this study, they conclude that there are six species of *Reticulitermes* in California, *R. hesperus* and five separate, but unidentified other species. The *R. hesperus* used to identify that clade (in Copren et al. 2005, Fig. 3, designated as Clade 6: *R. hesperus*) was our aforementioned mistake, and thus that clade should be identified as *R. n. sp. 'R. okanaganensis.'* Similarly, the clade that is identified with our type specimen *R. n. sp.* (in Copren et al. 2005, Fig. 3, designated as Clade 2: *R. sp. SCB*) should be identified as *R. hesperus*. In light of this information and the high degree of variation seen in *R. hesperus*, it seems unlikely that Copren's clades 2–5 indicate four separate species. We propose that these four clades represent the genetic variation seen in *R. hesperus* and should be considered as such. Although we disagree with some of their interpretations of the data, Copren et al. (2005) have shown a strong correlation between hydrocarbon phenotype and genotype, a finding that deserves further investigation. Revising the genus *Reticulitermes* has been hampered by a lack of readily available morphological characters, misleading assumptions about distribution patterns and ecologically variable chemical phenotypes. By building a large database of discrete genetic characters, which can be used in conjunction with the above-mentioned information, we hope to soon rid the genus of synonymy and misleading assumptions about distribution in North America.

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