J Chem Ecol (2008) 34:1452–1475
DOI 10.1007/s10886-008-9548-6

Cuticular Hydrocarbons and Soldier Defense Secretions of *Reticulitermes* in Southern California: A Critical Analysis of the Taxonomy of the Genus in North America

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Received: 1 May 2008 / Revised: 11 August 2008 / Accepted: 9 September 2008 / Published online: 22 October 2008

Abstract Cuticular hydrocarbons (CHC) and soldier defense secretions (SDS) were characterized for collections of *Reticulitermes* from six counties (Los Angeles, Orange, Riverside, San Bernardino, San Diego, and Santa Barbara) in southern California. Collection sites included the type locality for *R. hesperus*, Lake Arrowhead (formerly known as Little Bear Lake) in the San Bernardino Mountains. In southern California, there are two CHC phenotypes, SC-A and SC-B, which are easily distinguished by the presence or absence of 5-methyl pentacosane, 5-methyl heptacosane, 5,17-dimethyl pentacosane, and 5,17-dimethyl heptacosane. These phenotypes are similar, but not identical, to previously designated phenotypes of *Reticulitermes* from northern California. The SDS of termites collected from southern California were characterized; (—)-germacrene A was abundant in all but the four samples from Lake Arrowhead. Soldiers of phenotype SC-A produced >79% germacrene A. The four samples from Lake Arrowhead produced no germacrene A, but contained >78% γ-cadinene. The SDS from the Lake Arrowhead samples were more similar to those of CA-A/CA-A′ from northern California than to any of the CHC phenotypes from southern California. Soldiers of CHC phenotype SC-B produced germacrene A, with the proportion varying from 16.2 to 98.7%. The SDS of SC-B were more similar to those of SC-A than to any of the phenotypes from northern California. The CHC phenotype SC-A found in southern California likely represents *R. hesperus* and SC-B appears to be a new, as yet undescribed species. We discuss the state of current taxonomic research on *Reticulitermes*.

Keywords Isoptera · Rhinotermitidae · *Reticulitermes* · Chemotaxonomy · Cuticular hydrocarbons · Soldier defense secretions · Subterranean termites

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Introduction

Termites in the genus *Reticulitermes* (Isoptera: Rhinotermitidae) are widespread and economically, as well as ecologically, important in California (USA), where they are among the most significant pests of wooden structures. Within the past decade, there has been a great deal of interest in the genus, largely within the context of the development of new control technologies for these subterranean termites. A clear understanding of the taxonomy and distribution of the various species in North America is critical to informed work on this genus. In the field of integrated pest management, there are serious legal implications for misidentifying termites, since many pesticide labels are required to specify the target organism.

Identification of *Reticulitermes* specimens is problematic for three reasons. First, the taxonomy of North American *Reticulitermes* is outdated and in need of revision (Weesner 1970; Nutting 1990; Scheffrahn and Su 1994; Copren et al. 2005). Second, identification by using morphological keys to species level has been difficult or impossible because of inadequacies of existing keys (Haverty and Nelson 1997;

Nelson et al. 2001; Copren et al. 2005). Finally, it is difficult to distinguish *Reticulitermes* taxa solely on the basis of morphology, particularly if only workers are available.

Given this problem, numerous investigators have employed chemical characters, such as cuticular hydrocarbons (CHCs) and soldier defense secretions (SDSs), for distinguishing taxa of *Reticulitermes* (Howard et al. 1978, 1982; Parton et al. 1981; Zalkow et al. 1981; Baker et al. 1982; Clément et al. 1985, 1986, 1988; Bagnères et al. 1988, 1990, 1991; Lemaire et al. 1990; Haverty et al. 1996a, 1999b; Haverty and Nelson 1997; Clément and Bagnères 1998; Takematsu 1999; Takematsu and Yamaoka 1999; Nelson et al. 2001; Page et al. 2002; Quintana et al. 2003). Studies that correlate mitochondrial DNA sequences with CHC phenotypes of *Reticulitermes* give additional credibility to the value of CHCs as taxonomic characters (Jenkins et al. 2000; Clément et al. 2001; Copren et al. 2005).

We have successfully used CHCs and SDSs to separate taxa in studies on the foraging ecology of *Reticulitermes* in northern California (Getty et al. 1999a; Haverty et al. 1999c, 2000) and to evaluate baiting technology for control of *Reticulitermes* in structures (Getty et al. 1999b, 2000b). This chemotaxonomic information has also been critical for studies of behavior of *Reticulitermes* (Haverty et al. 1999a, 2003; Getty et al. 2000a; Delphia et al. 2003; Copren 2004; Copren et al. 2005).

Recently, we expanded our studies to locations in southern California to fill in gaps in the biogeography of *Reticulitermes* and to facilitate evaluation of bait technology. The study reported here includes more extensive collections and a complete description of the phenotypic differences first reported in Haverty and Nelson (1997), Nelson et al. (2001), and Copren et al. (2005). We also characterized SDSs for the CHC phenotypes found in southern California, and we discuss the state of current taxonomic research on *Reticulitermes*.

Methods and Materials

Insects Termites were collected from monitoring stations or naturally infested wood at locations in Los Angeles, Orange, Riverside, San Bernardino, San Diego, and Santa Barbara counties in southern California (Table 1). In San Bernardino County, we collected at the type locality of *R. hesperus*, Little Bear Lake, San Bernardino Mountains, CA (Snyder 1949). This area is now known as Lake Arrowhead (Robinson 1989). In addition, we include information from collections used in our study of the CHCs of *Reticulitermes* from northern California (Table 1) (Haverty and Nelson 1997).

Once separated from wood and debris, samples of up to 200 termite workers were placed in 20-ml scintillation vials and kept at −15°C until extraction of CHCs. Termite soldiers (1–10) were placed in *n*-pentane (ca 200–600 µl) in 4-ml vials and stored at −15°C until analysis of SDSs. Voucher specimens for each colony were placed in 70% ethanol and are maintained by the authors.

Cuticular Hydrocarbons Frozen termite workers were thawed, dried at ca 60°C, and immersed in 10 ml of *n*-hexane for 10 min. After extraction, hydrocarbons were separated from other compounds by pipetting the extract through 4 cm of activated silica gel (70–230 mesh), in Pasteur pipet mini-columns, followed by elution with 5 ml of *n*-hexane. The solvent was removed under a stream of nitrogen, and the residue was redissolved in 60 µl of *n*-hexane for gas chromatography-mass spectrometry (GC-MS) analysis.

GC-MS analyses were performed on a Hewlett-Packard (HP) 5890 gas chromatograph interfaced with an HP 5970B Mass Selective Detector. The GC-MS was equipped with an HP-1 fused silica capillary column (25 m×0.2 mm ID). Split injection (split ratio of 8:1) and a temperature program of 200–320°C at 3°C min^{-1} were used in the analysis. Electron impact (EI) mass spectra were obtained at 70 eV. Samples collected in 2001 and 2002, numbered SC-182-244 (Table 1), were analyzed under the same conditions with an Agilent 6890 GC coupled with a 5973 MSD.

n-Alkanes and methyl-branched alkanes were identified by mass spectral fragmentation patterns (Blomquist et al. 1987; Nelson 1993; Page et al. 1997). Alkenes, alkadienes, and alkatrienes were identified by mass spectra, but double bond positions were not determined (see Haverty and Nelson 1997). Equivalent chain lengths (ECL) were calculated for some of the unsaturated compounds, so as to distinguish isomers.

In the text and tables, we use shorthand nomenclature to identify individual hydrocarbons or mixtures of hydrocarbons. This shorthand uses a descriptor for the total number of carbons (CXX) in the hydrocarbon component, excluding the methyl branch(es), the location of methyl groups (X-me), and the number of double bonds following a colon (CXX:Y). Thus, normal pentacosane is *n*-C25; 5-methylpentacosane is 5-meC25; 5,17-dimethylheptacosane is 5,17-dimeC27; and pentacosatriene is C25:3. Integration of the total ion chromatogram was performed by using HP Chemstation data analysis software. GC-MS peak areas were converted to percentages of the total hydrocarbon fraction. A summary of the relative amounts of each peak is presented in table form using all samples of each phenotype.

CHC phenotypes are identified by a prefix that indicates geographic region from where specimens were collected, and a suffix indicating the distinct phenotype for that geographic region. In Haverty and Nelson (1997), northern California phenotypes were labeled with a single letter to

Table 1 Collection localities for *Reticulitermes* samples

Sample	County	Locality	Date collected	Latitude (°N)	Longitude (°W)	Elevation (m)
Phenotype SC-A						
SC-104	Santa Barbara	Goleta	7/20/2000	34.443945	119.843309	18
SC-106	Santa Barbara	Goleta	7/20/2000	34.443945	119.843309	18
SC-107	Santa Barbara	Goleta	7/20/2000	34.443945	119.843309	18
SC-108	Santa Barbara	Goleta	7/20/2000	34.443945	119.843309	18
SC-109	Santa Barbara	Goleta	7/20/2000	34.443945	119.843309	18
SC-110	Santa Barbara	Goleta	7/20/2000	34.443945	119.843309	18
SC-114	Santa Barbara	Goleta	7/20/2000	34.443945	119.843309	18
SC-184	Santa Barbara	Goleta	9/24/2001	34.443945	119.843309	18
SC-186	Santa Barbara	Goleta	10/31/2001	34.443945	119.843309	18
SC-207	Santa Barbara	Goleta	1/28/2002	34.443945	119.843309	18
SC-221	Santa Barbara	Goleta	5/22/2001	34.443945	119.843309	18
SC-222	Santa Barbara	Goleta	5/22/2001	34.443945	119.843309	18
SC-236	Santa Barbara	Goleta	3/25/2002	34.443945	119.843309	18
SC-111 (SCA2)[a]	Santa Barbara	Goleta	7/20/2000	34.450375	119.831185	24
SC-112	Santa Barbara	Goleta	7/20/2000	34.450375	119.831185	24
SC-113	Santa Barbara	Goleta	7/20/2000	34.450375	119.831185	24
SC-182	Los Angeles	Baldwin Park	9/27/2001	34.086216	117.978931	98
SC-208	Los Angeles	Baldwin Park	1/29/2002	34.086216	117.978931	98
SC-209	Los Angeles	Burbank	3/7/2002	34.174138	118.322956	169
SC-210	Los Angeles	Burbank	3/7/2002	34.174138	118.322956	169
SC-211	Los Angeles	Burbank	3/7/2002	34.174138	118.322956	169
SC-204	Los Angeles	Burbank	1/15/2002	34.168448	118.334144	171
SC-213	Los Angeles	Burbank	3/7/2002	34.168448	118.334144	171
SC-242	Los Angeles	Chatsworth	3/27/2002	34.257484	118.614576	291
SC-241	Los Angeles	Los Angeles	3/15/2002	34.091573	118.196865	200
SC-124 (SCA5)[a]	Los Angeles	Los Angeles	8/22/2000	34.093894	118.198291	222
SC-185	Orange	Irvine	10/9/2001	33.678302	117.757289	63
SC-205	Orange	Irvine	1/15/2002	33.678302	117.757289	63
SC-228	Orange	Trabuco Canyon	5/22/2001	33.663889	117.589444	317
SC-ALP2	San Diego	Alpine	4/29/1998	32.835000	116.765556	562
SC-ALP3	San Diego	Alpine	4/29/1998	32.835000	116.765556	562
Phenotype SC-B						
SC-105 (SCB2)[a]	Santa Barbara	Goleta	7/20/2000	34.443945	119.843309	18
SC-231	Santa Barbara	Goleta	3/25/2002	34.443945	119.843309	18
SC-232	Santa Barbara	Goleta	3/25/2002	34.443945	119.843309	18
SC-101	Santa Barbara	Carpinteria	7/20/2000	34.395631	119.517370	8
SC-206	Santa Barbara	Carpinteria	1/28/2002	34.395631	119.517370	8
SC-225	Santa Barbara	Carpinteria	5/22/2001	34.395631	119.517370	8
SC-239	Santa Barbara	Carpinteria	3/25/2002	34.395631	119.517370	8
SC-103	Santa Barbara	Carpinteria	7/20/2000	34.395780	119.517548	8
SC-227	Santa Barbara	Carpinteria	5/22/2001	34.395780	119.517548	8
SC-212	Los Angeles	Burbank	3/7/2002	34.174138	118.322956	169
SC-219	Los Angeles	Chatsworth	3/10/2002	34.257484	118.614576	291
SC-119	Los Angeles	El Monte	8/3/2000	34.078531	118.024463	91
SC-120	Los Angeles	El Monte	8/3/2000	34.078531	118.024463	91
SC-121	Los Angeles	El Monte	8/3/2000	34.078531	118.024463	91
SC-123 (SCB4)[a]	Los Angeles	Los Angeles	8/22/2000	34.093894	118.198291	222
SC-200	Los Angeles	Los Angeles	12/7/2001	34.093894	118.198291	222
SC-244	Los Angeles	Los Angeles	8/18/2002	34.092844	118.199101	202
SC-229	Los Angeles	Los Angeles	5/17/2001	34.089372	118.199912	194
SC-215	Los Angeles	Los Angeles	3/19/2002	34.089382	118.199431	202
SC-218	Los Angeles	Los Angeles	3/18/2002	34.087747	118.204024	176
SC-202	Los Angeles	Malibu	1/2/2002	34.104218	118.858461	549
SC-203	Los Angeles	Malibu	1/2/2002	34.070140	118.894261	351
SC-189	San Bernardino	Rialto	11/14/2001	34.092112	117.382250	361

Table 1 (continued)

Sample	County	Locality	Date collected	Latitude (°N)	Longitude (°W)	Elevation (m)
SC-190	San Bernardino	Rialto	11/14/2001	34.092112	117.382250	361
SC-196	San Bernardino	Rialto	12/4/2001	34.092112	117.382250	361
SC-197	San Bernardino	Rialto	12/4/2001	34.092112	117.382250	361
SC-198	San Bernardino	Rialto	12/4/2001	34.092112	117.382250	361
SC-SF1	San Diego	Scissors Crossing	4/29/1998	33.095350	116.475420	700
SC-SF2	San Diego	Scissors Crossing	4/29/1998	33.095350	116.475420	700
SC-TP	San Diego	Torrey Pines	4/30/1998	32.920736	117.254518	91
Phenotype SC-B'						
SC-MR2	Riverside	Motte Rimrock Reserve	4/30/1998	33.803356	117.261084	552
SC-MR3	Riverside	Motte Rimrock Reserve	4/30/1998	33.803356	117.261084	552
SC-MR4	Riverside	Motte Rimrock Reserve	4/30/1998	33.803356	117.261084	552
SC-PTF1	Riverside	UC Riverside Campus	4/30/1998	33.975478	117.331206	315
SC-PTF2	Riverside	UC Riverside Campus	4/30/1998	33.975478	117.331206	315
Phenotype CA-A/A'[b,c]						
SC-115 (LBL1)[a]	San Bernardino	Lake Arrowhead	8/2/2000	34.258360	117.168260	1587
SC-116	San Bernardino	Lake Arrowhead	8/2/2000	34.258360	117.168260	1587
SC-117 (LBL2)[a]	San Bernardino	Lake Arrowhead	8/2/2000	34.258360	117.168260	1587
SC-118	San Bernardino	Lake Arrowhead	8/2/2000	34.258360	117.168260	1587
CA-PPE	El Dorado	Placerville	8/14/1994	38.742110	120.744060	828
CA-Wc7	El Dorado	Placerville	9/16/1993	38.742110	120.744060	828
CA-Yq31	El Dorado	Placerville	9/16/1993	38.742110	120.744060	828
CA-Wk64	El Dorado	Placerville	10/24/1994	38.742110	120.744060	828
CA-Zp8	El Dorado	Placerville	6/20/1994	38.742110	120.744060	828
CA-Xi21	El Dorado	Placerville	8/14/1995	38.742110	120.744060	828
CA-L34	Marin	Larkspur	7/6/1995	37.928127	122.536179	59
CA-SAC	Sacramento	Sacramento	2/5/1995	38.582848	121.489305	7
CA-L55	Marin	Larkspur	6/15/1992	37.928127	122.536179	59
CA-St25	Marin	Novato	6/7/1995	38.108880	122.572440	7
CA-St63	Marin	Novato	4/16/1996	38.108880	122.572440	7
CA-St87 (CAAP1)[a]	Marin	Novato	6/6/1995	38.108880	122.572440	7
CA-F167	Marin	Novato	8/2/1995	38.091654	122.595103	44
CA-HAS	Monterey	Hastings Reserve	8/15/1990	36.408161	121.590645	600
CA-BM	Lassen	Blacks Mountain	8/27/1996	40.777460	121.192220	2182
CA-HC	Shasta	Hat Creek	8/29/1996	40.744640	121.488820	1117
Phenotype CA-B[b,c]						
CA-Xm14	El Dorado	Placerville	9/16/1993	38.742110	120.744060	828
CA-Wb36 (CAB5)[a]	El Dorado	Placerville	9/16/1993	38.742110	120.744060	828
CA-Yv34 (CAB1)[a]	El Dorado	Placerville	9/16/1993	38.742110	120.744060	828
CA-Zv31	El Dorado	Placerville	8/14/1995	38.742110	120.744060	828
CA-Wc10	El Dorado	Placerville	8/14/1995	38.742110	120.744060	828
Phenotype CA-C[b,c]						
CA-Zn11 (CAC2)[a]	El Dorado	Placerville	6/20/1994	38.742110	120.744060	828
CA-PV	El Dorado	Placerville	6/20/1994	38.742110	120.744060	828
CA-Wi74 (CAC1)[a]	El Dorado	Placerville	12/21/1994	38.742110	120.744060	828
CA-Yt2 (CAC3)[a]	El Dorado	Placerville	7/12/1995	38.742110	120.744060	828
Phenotype CA-D[b,c]						
CA-UCB	Alameda	UC Berkeley Campus	6/15/1994	37.872355	122.262462	76
CA-L5	Marin	Larkspur	7/6/1995	37.928127	122.536179	59
CA-St21	Marin	Novato	7/6/1995	38.108880	122.572440	7
CA-St116	Marin	Novato	7/6/1995	38.108880	122.572440	7
CA-St253	Marin	Novato	7/6/1995	38.108880	122.572440	7
CA-St314	Marin	Novato	7/6/1995	38.108880	122.572440	7

[a] Sequences of the cytochrome oxidase II region of mtDNA for these collections were reported in Copren et al. 2005.
[b] The cuticular hydrocarbon phenotypes for these samples are reported in Haverty and Nelson 1997, excluding samples SC-115–118.
[c] The soldier defense secretion phenotypes for these samples are reported in Nelson et al. 2001, excluding samples SC-115–118.

indicate the phenotype (e.g., A, A′, B, C, D). However, in subsequent publications, we added the prefix CA- to distinguish these phenotypes from those characterized from other regions, such as Arizona (AZ-) and Georgia (GA-). Here, we use the prefix SC- for southern California collections.

Soldier Defense Secretions Monoterpenes, sesquiterpenes, and diterpenes were identified and quantified by GC-MS (Nelson et al. 2001). GC-MS analysis was carried out with splitless injection, a 0.25 mm ID×30 m 5% phenyl–95% methylpolysiloxane capillary column, helium as carrier gas, and a temperature program of 35°C (held for 0.7 min) to 280°C at 6°C min^{-1}. Most compounds were identified by retention time and MS comparison with authentic material or components of well-characterized essential oils. In some cases, identification was by comparison with published GC-MS data only and is considered tentative (compound name in parentheses). Terpenoids in bold characters in the tables are novel reports for termite soldiers. Unknowns are designated by apparent molecular weight and an identifying suffix letter. The suffix designations for the unknowns correspond to the same unknowns reported in Nelson et al. (2001).

Quantities of each terpenoid are calculated as a percentage of total peak area from the total ion chromatogram without correction for response factors. Germacrene A, which gradually rearranges to β-elemene under GC conditions, appeared as a broad hump following the β-elemene peak. Its quantity was determined as the sum of the areas of the β-elemene peak plus the hump, minus the areas of the other peaks superimposed on the hump. Germacrene B behaved similarly (rearranging to γ-elemene) and was quantified by the same approach.

(+)-γ-Cadinene from pooled samples of various collections was purified by silica gel LC and preparative GC as described elsewhere (Kim et al. 1994) and positively identified by diffuse-reflectance Fourier-transform infrared spectroscopy by using ca 50 μg of the neat material on powdered KBr in a microcup. Its optical rotation (α_D) was measured in hexane at 20°C.

Enantiomeric identity of a number of the monoterpenes and sesquiterpenes was determined by enantioselective GC (EGC). Conditions were: 10% permethylated β-cyclodextrin-90% OV-1701 capillary column, 0.25 mm ID×30 m; temperature program of 60–80°C for monoterpenes, 90–140°C for sesquiterpenes, with a 1-°C min^{-1} temperature increase; splitless injection (0.7 min); flame-ionization detection; internal standards of *n*-undecane for monoterpenes, and *n*-tetradecane or *n*-hexadecane for sesquiterpenes. EGC analysis was done on one soldier collection from the CHC phenotype that was richest in the relevant terpene(s). Before analysis, oxygenated components were removed by passing the pentane extract through activated alumina. Enantiomer identification was by retention time comparison with authentic materials. In the case of (−)-germacrene A, pooled samples were purified as for γ-cadinene, although under preparative GC conditions only β-elemene was recovered. This was then analyzed by EGC and compared to authentic (−)-β-elemene. For brevity, enantiomeric designations are not specified in the text, although they are given in the tables.

Cluster Analysis of CHC Mixtures From Termites The percentage of each hydrocarbon peak was used as the response variable. The presence of co-eluting compounds precluded exact quantification of many individual hydrocarbons. Several samples of each of the five northern California phenotypes described by Haverty and Nelson (1997) were included in the cluster analysis (Table 1).

Cluster analysis was first performed with all the samples listed in Table 1. There was a definite division between samples that lacked 5-methylalkanes and 5,17-dimethylalkanes and those that contained these compounds. Thus, in order to simplify the analysis, samples were divided into two groups based on this distinction, which corresponds to the lineage designations of Page et al. (2002). The first group included collections that lacked 5-methylalkanes and 5,17-dimethylalkanes. The predominant compounds in these samples were internally branched monomethyl- and dimethylalkanes, which place this group in lineage I. Phenotypes in lineage I were CA-A/A′ and SC-A. The second group, lineage II, was composed of collections with abundant 5-methylalkanes and 5,17-dimethylalkanes in the CHCs. Phenotypes in this lineage were CA-B, CA-D, and SC-B/B′. We also included four samples designated as CA-C from Placerville, CA (Haverty and Nelson 1997); although this phenotype did not neatly fit into lineage I or II, these samples were grouped with the former because of the absence of 5-methylalkanes and 5,17-dimethylalkanes.

The standardized Euclidean distances were calculated separately for the samples in lineages I and II by using all hydrocarbon peaks (Takematsu and Yamaoka 1999; Haverty et al. 2005; Baker and Haverty 2007; Haverty and Nelson 2007) using R Statistical Language (R Development Core Team 2004). Results are displayed as dendrograms. Euclidean distances of 15.0 were used to define clusters that we infer represent discrete taxa within each of the two lineages.

Results

Cuticular Hydrocarbons As reported in Copren et al. (2005), the two CHC phenotypes found in southern California were designated SC-A and SC-B. These pheno-

types belong to lineages I and II, respectively (Page et al. 2002). Samples representing each phenotype often were found at the same sites, indicating sympatry. For example, at study sites in Goleta (Santa Barbara Co.) and Burbank (Los Angeles Co.), we collected samples of SC-A and SC-B from nearby monitoring stations on the same day (Table 1: SC-231 and SC-236; SC-209 and SC-212).

Figure 1 shows the dendrogram based on cluster analysis of Californian *Reticulitermes* samples belonging to lineage I. There were three main clusters. One, SC-A, is composed of 18 samples from southern California ranging from Goleta to Alpine in San Diego County (Fig. 1; Tables 1 and 2). A second cluster is composed of CA-A and CA-A′ samples from northern California, as well as the two *R. hesperus* topotype samples from Lake Arrowhead (SC-115 and SC-117), and one sample from Los Angeles (SC-241; Fig. 1; Tables 1 and 2). Three samples (CA-Yt2, CA-Zn11, and CA-Wi74), previously designated as phenotype CA-C (Haverty and Nelson 1997), clustered together (Fig. 1; Tables 1 and 2), and a fourth sample (CA-PV), also previously included in CA-C, fell out by itself in the cluster analysis, despite the fact that the predominant compounds present (C27:2; 11,15-dimeC27) were typical of phenotype CA-C. For this reason, this sample is considered phenotype CA-C.

Phenotype SC-A closely resembles phenotypes CA-A and CA-A′ from northern California (Table 2), with a few quantitative and qualitative differences. All three of these phenotypes were dominated by internally branched monomethylalkanes and dimethylalkanes, with the methyl branches usually on carbons 11 or 13. SC-A produced several monomethylene-interrupted dimethylalkanes, 9,11-dimeCXX, in small amounts; these were not seen in CA-A, CA-A′, nor in the *R. hesperus* topotype samples from Lake Arrowhead (Fig. 2; Table 2).

SC-A profiles contained the same pair of pentacosatrienes (C25:3, ECL 25.98 and 26.07) as CA-A′, although the relative proportions differed (Table 2). Most samples from southern California produced more of the later eluting isomer (ca 1:7; Fig. 2a), whereas CA-A′ produced approximately equal amounts of both (Fig. 2b). Topotype samples from Lake Arrowhead had more of the earlier eluting isomer (ca 4:1; Fig. 2c). CA-A lacked these two compounds (Fig. 2d).

The one sample (SC-241) from Los Angeles that clustered with the northern California phenotypes had very small amounts of 9,11-dimethylalkanes, which may have caused it to cluster as it did (Fig. 1). However, we designate this sample as SC-A based on the presence of these compounds and the proportion of pentacosatrienes.

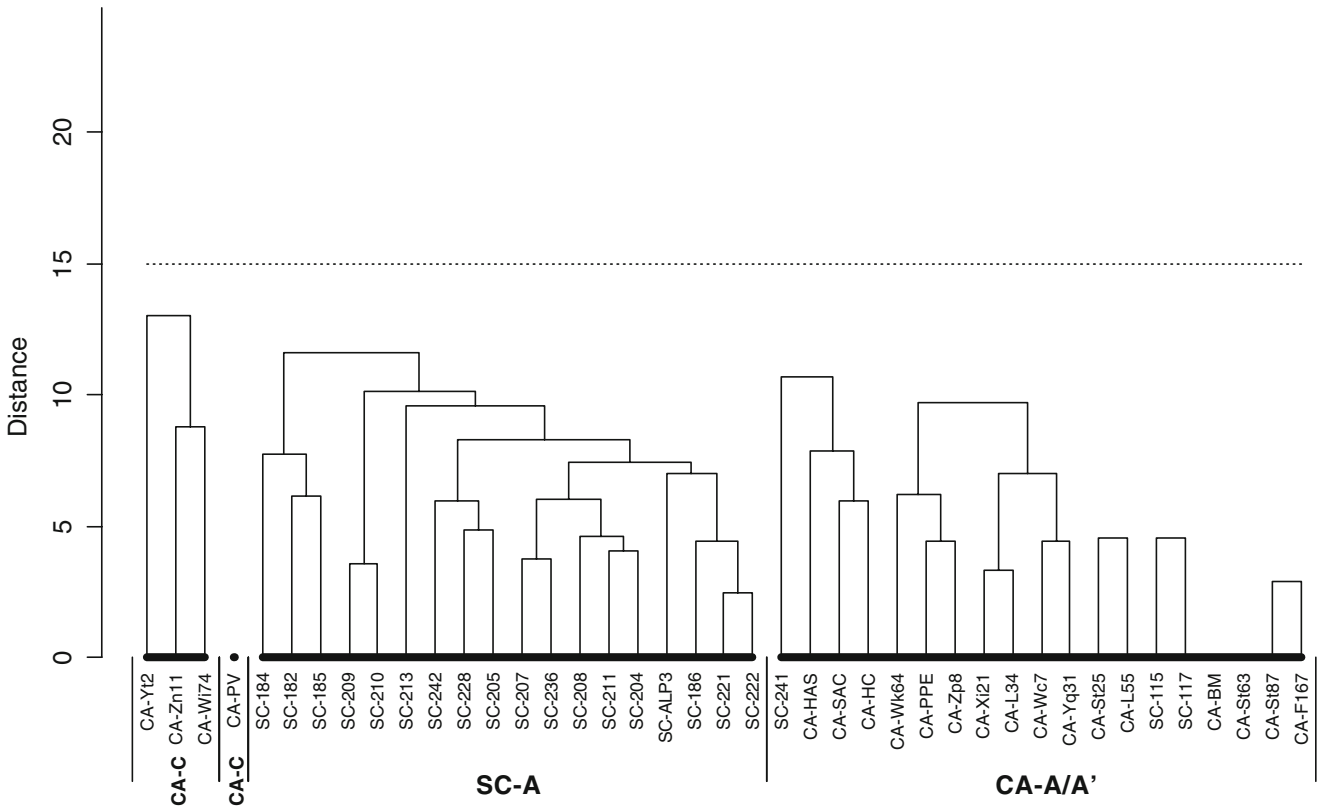

Fig. 1 Dendrograms from cluster analyses based on Euclidean distance of cuticular hydrocarbons extracted from samples of *Reticulitermes* workers from California. These samples lack 5-methylalkanes and 5,X-dimethylalkanes. See Tables 1 and 2 for data pertaining to individuals and phenotypes

Table 2 Mean percent composition (and SD) of cuticular hydrocarbons from all clusters

Hydrocarbon[a]	SC-A *N*=18	CA-A/A' *N*=16	CA-C *N*=4	SC-B *N*=23	SC-B' *N*=4	CA-B *N*=5	CA-D *N*=6
C21			0.05 (0.06)				
C22		0.08 (0.11)	0.21 (0.19)				
2-; 3-meC22		0.03 (0.06)	0.11 (0.07)				
C23:1 (ECL=22.70)		0.16 (0.27)					0.22 (0.04)
C23	2.67 (0.97)	3.85 (1.14)	1.11 (0.28)	0.86 (0.49)	0.54 (0.03)	0.23 (0.05)	3.59 (0.75)
11-; 9-meC23	0.91 (0.34)	2.79 (0.70)	0.13 (0.09)				1.66 (0.20)
9,11-dimeC23	**0.97 (0.38)**	**0.03 (0.13)**					
7-meC23				0.40 (0.38)			0.18 (0.02)
5-meC23				0.02 (0.02)			0.42 (0.10)
2-meC23	0.53 (0.17)	1.67 (0.46)	1.28 (0.19)	0.07 (0.05)	0.48 (0.27)		0.48 (0.06)
3-meC23; 9,13-dimeC23[b]	1.02 (0.36)	1.54 (0.37)	0.82 (0.21)	0.05 (0.04)	0.19 (0.23)		0.55 (0.08)
C24	0.87 (0.37)	1.83 (0.68)	1.68 (0.85)	0.71 (0.39)	1.05 (0.15)	0.67 (0.16)	1.26 (0.26)
12-; 11-; 10-; 9-meC24	1.39 (0.32)	2.31 (0.53)	0.46 (0.08)		0.06 (0.11)	0.15 (0.07)	1.94 (0.22)
9,11-dimeC24	0.20 (0.07)						
8-meC24				0.25 (0.28)			
6-meC24				0.16 (0.15)			0.54 (0.10)
5-meC24				0.23 (0.22)	1.22 (0.23)	0.26 (0.10)	0.54 (0.10)
2-meC24; C25:2; C25:1; 9,13-dimeC24; 3-meC24[c]	6.87 (1.33)	7.85 (1.60)	8.82 (1.49)	3.14 (1.57)	4.84 (0.89)	1.00 (0.28)	3.75 (0.71)
6,16-dimeC24				0.13 (0.11)			
C25:1 (ECL=24.70)	0.13 (0.11)	0.02 (0.08)		0.07 (0.06)			
C25:1 (ECL=24.80)	0.18 (0.15)	0.03 (0.12)		0.05 (0.09)			
C25	5.86 (1.83)	7.81 (2.49)	10.55 (7.16)	6.29 (2.13)	7.76 (1.26)	4.96 (1.22)	5.81 (1.04)
13-; 11-; 9-meC25; C25:2[d]	28.56 (4.87)	24.94 (5.39)	8.75 (1.07)	4.75 (2.95)	11.91 (1.65)	4.10 (1.13)	21.17 (1.33)
7-meC25; 11,13-dimeC25[e]			0.83 (0.22)	2.48 (1.74)	1.77 (2.07)	0.17 (0.05)	1.59 (0.10)
C25:2 (ECL=25.50)	0.80 (0.23)	1.17 (0.92)					
5-meC25				11.30 (2.78)	11.22 (2.66)	7.78 (0.51)	7.65 (1.79)
9,11-dimeC25; C25:2	**1.26 (0.84)**	**0.02 (0.09)**					
9,11,13-trimeC25	**0.52 (0.33)**						
11,15-; 9,13-dimeC25; 2-meC25[f]	12.03 (3.52)	8.86 (2.20)	4.14 (2.38)	0.97 (0.31)	2.19 (0.42)	1.03 (0.13)	2.71 (0.61)
3-meC25	4.16 (0.74)	4.49 (1.11)	4.84 (3.32)	2.02 (0.69)	1.82 (0.43)	1.81 (0.18)	2.46 (0.26)
7,17-dimeC25				0.89 (0.51)			
5,15-; 5,9-dimeC25					**3.22 (0.20)**	**1.96 (0.19)**	
5,17-dimeC25				**7.68 (5.55)**	**1.00 (0.19)**	**1.10 (0.18)**	**12.54 (2.71)**
C26:1 (ECL=25.70)		0.08 (0.10)					
C25:3 (ECL=25.98)[g]	**1.36 (0.43)**	**2.75 (2.71)**		**1.44 (2.96)**	**1.00 (0.18)**		
C26		0.25 (0.34)	1.48 (0.68)		0.66 (0.25)	1.36 (0.31)	
3,7-dimeC25		0.11 (0.15)		0.41 (0.19)			0.08 (0.20)
C25:3 (ECL=26.07)[g]	**9.47 (3.60)**	**3.42 (3.94)**		**0.13 (0.22)**	**0.61 (0.05)**		**2.66 (1.55)**
5,9,17-trimeC25				0.57 (0.28)	0.88 (0.14)		0.39 (0.18)
C27:1 (ECL=26.30)	0.14 (0.09)			0.02 (0.03)			
13-; 12-; 11-meC26; C26:2	0.50 (0.08)	0.50 (0.18)	1.98 (0.73)	0.14 (0.13)	0.68 (0.11)	1.22 (0.16)	1.09 (0.05)
C25:3 (ECL=26.47)	0.33 (0.31)	0.20 (0.23)		0.12 (0.33)			
6-meC26				1.09 (0.26)	1.57 (0.19)	1.79 (0.14)	0.99 (0.23)
5-meC26				0.12 (0.08)	0.25 (0.17)	0.37 (0.05)	0.10 (0.02)
4-meC26				0.15 (0.05)	0.19 (0.01)	0.26 (0.05)	0.14 (0.02)
9,13-dimeC26; 2-meC26; C27:2; C27:1[h]	0.33 (0.17)	0.39 (0.21)	11.23 (1.18)	0.47 (0.21)	1.27 (0.08)	1.50 (0.27)	0.31 (0.05)
C27:2 (ECL=26.70)				0.05 (0.05)			
3-meC26		0.04 (0.06)				0.15 (0.01)	
6,18-;5,17-dimeC26				1.35 (0.28)	1.01 (0.28)	1.79 (0.18)	0.87 (0.18)
4,16-; 4,18-dimeC26				0.27 (0.08)	0.27 (0.21)	0.43 (0.05)	0.20 (0.03)
C26:3 (ECL=26.81)		0.06 (0.08)					
C27	0.21 (0.19)	0.42 (0.27)	3.22 (1.24)	0.33 (0.17)	1.11 (0.33)	2.49 (0.51)	0.17 (0.03)

Table 2 (continued)

Hydrocarbon[a]	SC-A *N*=18	CA-A/A' *N*=16	CA-C *N*=4	SC-B *N*=23	SC-B' *N*=4	CA-B *N*=5	CA-D *N*=6
C26:3 (ECL=27.15)	0.05 (0.09)	0.06 (0.08)					
4,8,16-trimeC26				0.12 (0.08)			
13-; 11-meC27; C27:2; 11,13-dimeC27[i]	**0.17 (0.19)**	**0.74 (0.38)**	**12.89 (2.60)**	**0.66 (0.36)**	**2.22 (0.27)**	**7.93 (1.67)**	**0.71 (0.10)**
7-meC27				0.62 (0.23)	1.09 (0.08)	1.37 (0.19)	0.17 (0.02)
C27:2 (ECL=27.50)	0.04 (0.08)	0.06 (0.10)	0.62 (0.22)				
5-meC27				**2.34 (0.88)**	**3.46 (0.47)**	**4.67 (0.85)**	**0.65 (0.07)**
11,15-dimeC27; 2-meC27[j]		**0.10 (0.09)**	**6.66 (2.24)**			**0.52 (0.15)**	
3-meC27		0.10 (0.09)	0.39 (0.06)				
5,17-dimeC27				**27.32 (10.74)**	**9.84 (1.41)**	**18.45 (2.00)**	**1.51 (0.40)**
C28		0.11 (0.23)	0.40 (0.21)			0.53 (0.33)	
C27:3 (ECL=28.03)	0.41 (0.21)	0.10 (0.13)	0.27 (0.22)				0.63 (0.60)
3,7-dimeC27						0.30 (0.05)	
C27:3 (ECL=28.24)	0.43 (0.26)	0.11 (0.15)	0.59 (0.48)				0.21 (0.12)
5,9,17-trimeC27				0.71 (0.45)	0.34 (0.10)	0.85 (0.11)	0.18 (0.06)
14-; 13-; 12-;10-meC28			0.77 (0.15)			0.31 (0.06)	
11,15-dimeC28			0.35 (0.31)				
6-meC28						0.29 (0.06)	
3-meC28; C29:2; C29:1			0.28 (0.19)				
6,18-dimeC28						0.83 (0.16)	
C29:3 (ECL=28.65)	0.02 (0.05)						
C29			0.18 (0.17)			0.43 (0.20)	
15-; 13-; 11-meC29; C29:2[k]			0.44 (0.30)			0.23 (0.04)	
C29:2 (ECL=29.50)			0.22 (0.15)				
5-meC29						0.27 (0.06)	
5,17-dimeC29						0.48 (0.06)	
C30			0.12 (0.09)			0.24 (0.11)	
C31			0.06 (0.07)			0.19 (0.09)	
C32			0.04 (0.05)			0.13 (0.05)	
5,17-dimeC32				0.14 (0.14)			
C33			0.05 (0.06)			0.13 (0.04)	
13-; 11-meC33					0.08 (0.17)	1.17 (0.27)	
13,17-dimeC33					0.14 (0.29)		
5,19-; 5,17-dimeC33[l]				**4.55 (3.32)**	**4.60 (0.71)**	**2.47 (0.47)**	
14-meC34					0.13 (0.16)	0.22 (0.04)	
6,18; 5,17-dimeC34				0.55 (0.40)	1.62 (2.43)	0.43 (0.06)	
15-; 13-; 11-meC35	**1.37 (0.49)**	**2.59 (1.04)**	**1.91 (0.42)**		**2.68 (0.37)**	**1.14 (0.20)**	**0.35 (0.09)**
13,17-; 11,15-dimeC35	**0.55 (0.34)**	**3.32 (1.23)**	**0.46 (0.32)**		**1.07 (0.35)**		**0.01 (0.03)**
7-meC35							0.08 (0.03)
5-meC35							0.09 (0.03)
5,17-dimeC35				**5.16 (2.72)**	**3.94 (0.57)**	**3.61 (0.63)**	**1.68 (0.44)**
5,9,17-trimeC35				0.03 (0.15)	0.07 (0.14)	0.84 (0.16)	0.15 (0.08)
12-; 11-meC36	0.76 (0.20)	0.39 (0.12)	0.31 (0.21)	0.02 (0.08)	0.46 (0.07)	0.30 (0.04)	0.30 (0.03)
12,16-; 11,15-dimeC36	0.15 (0.14)	0.59 (0.27)					0.05 (0.04)
6,18-; 5,17-dimeC36				0.41 (0.27)	0.19 (0.18)	0.55 (0.09)	0.48 (0.14)
15-; 13-; 11-meC37	**5.07 (0.97)**	**4.15 (0.75)**	**2.18 (0.39)**	**0.03 (0.12)**	**1.63 (0.30)**	**2.54 (0.60)**	**2.91 (0.58)**
13,17-; 11,15-dimeC37	**3.84 (1.62)**	**5.10 (1.32)**	**1.21 (0.42)**		**0.72 (0.27)**		**0.78 (0.65)**
5,17-dimeC37				**2.45 (1.25)**	**1.60 (0.30)**	**3.37 (0.48)**	**2.78 (0.58)**
5,9,17-trimeC37				0.02 (0.07)	0.07 (0.13)	0.79 (0.19)	0.28 (0.05)
12-; 11-meC38	0.15 (0.12)	0.20 (0.14)	0.16 (0.11)				0.26 (0.04)
12,16-; 11,15-dimeC38	0.52 (0.12)	0.28 (0.18)	0.19 (0.13)				0.05 (0.07)
6,18-; 5,17-dimeC38				0.40 (0.35)		0.35 (0.10)	0.45 (0.12)
15-; 13-; 11-meC39	0.51 (0.21)	0.98 (0.43)	1.15 (0.19)	0.02 (0.05)	0.14 (0.16)	0.71 (0.18)	0.89 (0.17)
13,17-dimeC39	**3.36 (0.88)**	**2.04 (0.84)**	**2.16 (0.48)**		**0.26 (0.18)**		**0.47 (0.14)**
5,17-dimeC39				2.65 (1.49)	1.17 (0.19)	2.18 (0.48)	1.39 (0.27)

Table 2 (continued)

Hydrocarbon[a]	SC-A *N*=18	CA-A/A' *N*=16	CA-C *N*=4	SC-B *N*=23	SC-B' *N*=4	CA-B *N*=5	CA-D *N*=6
5,9,17-trimeC39						0.16 (0.06)	
12-; 11-meC40	0.01 (0.03)	0.04 (0.05)	0.09 (0.06)				
12,16-dimeC40	0.01 (0.03)	0.07 (0.10)	0.17 (0.12)				
5,17-dimeC40						0.16 (0.06)	0.37 (0.24)
15-; 13-; 11-meC41	0.21 (0.19)	0.26 (0.19)	0.89 (0.14)				0.49 (0.19)
13,17-dimeC41	0.39 (0.18)	0.74 (0.40)	1.88 (0.35)		0.07 (0.14)		0.72 (0.24)
5,17-dimeC41				1.00 (0.81)	0.61 (0.10)	1.92 (0.32)	3.26 (0.54)
12-; 11-meC42		0.01 (0.03)					
12,16-; 11,15-dimeC42		0.01 (0.04)					
15-; 13-; 11-meC43						0.17 (0.10)	
15-; 13-; 11-meC43	0.03 (0.06)	0.03 (0.07)	0.33 (0.22)		0.37 (0.25)		
13,17-dimeC43	0.71 (0.32)	0.10 (0.15)	0.87 (0.35)				0.43 (0.22)
5,17-dimeC43				1.59 (0.95)	2.73 (0.39)	2.20 (0.76)	1.25 (0.31)

[a] Presented in order of elution. Co-eluting compounds are listed together. *ECL* equivalent chain length (approximate). **Bold type** indicates diagnostic peaks.
[b] 9,13-dimeC23 is only present in SC-A and CA-D.
[c] 9,13-dimeC24 is present only in SC-A, CA-A/A', CA-D; only 2-meC24 is present in CA-B.
[d] C25:2 is absent in CA-C and CA-B.
[e] 11,13-dimeC25 is present only in CA-C.
[f] Only 2-meC25 is present in CA-C, SC-B and CA-B.
[g] These trienes are not found in CA-A; their absence is diagnostic for this phenotype.
[h] 9,13-dimeC26 is present only in SC-A and CA-A/A'; C27:2 and C27:1 are present only in CA-C.
[i] C27:2 is present only in CA-A/A', CA-C, and CA-D; 11,13-dimeC27 is present only in CA-C.
[j] 11,15-dimeC27 is present only in CA-A/A' and CA-C.
[k] C29:2 is present only in CA-C.
[l] 5,19-dimeC33 is present with 5,17-dimeC33 only in CA-B.

Phenotype CA-C is distinguished from phenotypes CA-A/A′ and SC-A by the presence of large quantities of C27:1, C27:2, 11-; 13-meC27, and 11,15-dimeC27 (Fig. 2e; Table 2). A phylogenetic analysis based on the COII mtDNA gene also separated this phenotype from all others reported in this paper (Copren et al. 2005). Thus far, this phenotype appears to be rare and has been found only at our site in Placerville, CA.

Cluster analysis of the lineage II *Reticulitermes* resulted in four clusters (Fig. 3), two with all the southern California samples and two with all the northern California samples. Twenty-three samples from southern California comprise phenotype SC-B, while the smaller cluster is composed of four samples we designated as SC-B′ (Table 2). The two remaining clusters included one with five samples from northern California that had been previously designated as CA-B, and one with six samples that had been previously designated as CA-D (Table 2) (Haverty and Nelson 1997).

CHC phenotype SC-B is similar to phenotypes CA-B and CA-D from northern California (Fig. 4a-c; Table 2). The most abundant compound in the majority of phenotype SC-B samples was 5,17-dimeC27, and there was a homologous series of similar compounds, with carbon chain length from 33 to 43. This pattern is comparable to that of CA-B and CA-D. However, several monomethylalkanes present in CA-B and CA-D, such as 11-meC35 and 11-; 13-meC37, were absent in most samples of SC-B (Fig. 4a–c; Table 2). CA-D produced less 5,17-dimeC27 than SC-B or CA-B, and more internally branched monomethylalkanes, such as 11- and 13-meC25 and 11- and 13-meC37 (Fig. 4a-c; Table 2).

Two variants of SC-B were seen in San Diego County. In one variant, which included samples SC-SF1 and SC-SF2, 5,17-dimeC27 was less abundant, and 5,17-dimeC33 was absent (Fig. 4d; Table 2). These two samples grouped together with another, SC-TP, within the main SC-B cluster. Closer examination of the profile of SC-TP indicated that it is more similar to the other SC-B samples in the main cluster than to the two SC-SF samples, as it contained a large amount of 5,17-dimeC27 (19.2%), and 5,17-dimeC33 was present (3.4%).

The other variant (samples SC-MR2, -3, -4, and SC-PTF) had significant quantities of internally branched mono- and dimethylalkanes with chain lengths of 35 to 39 carbons, and smaller amounts of 5,17-dimeC27 (Fig. 4e; Table 2). Because the four samples formed a separate cluster and had other distinguishing features in common, they were designated as SC-B′ and are possibly a separate taxon.

We indicate in bold type the diagnostic CHCs that distinguish the phenotype clusters in Table 2. It is noted

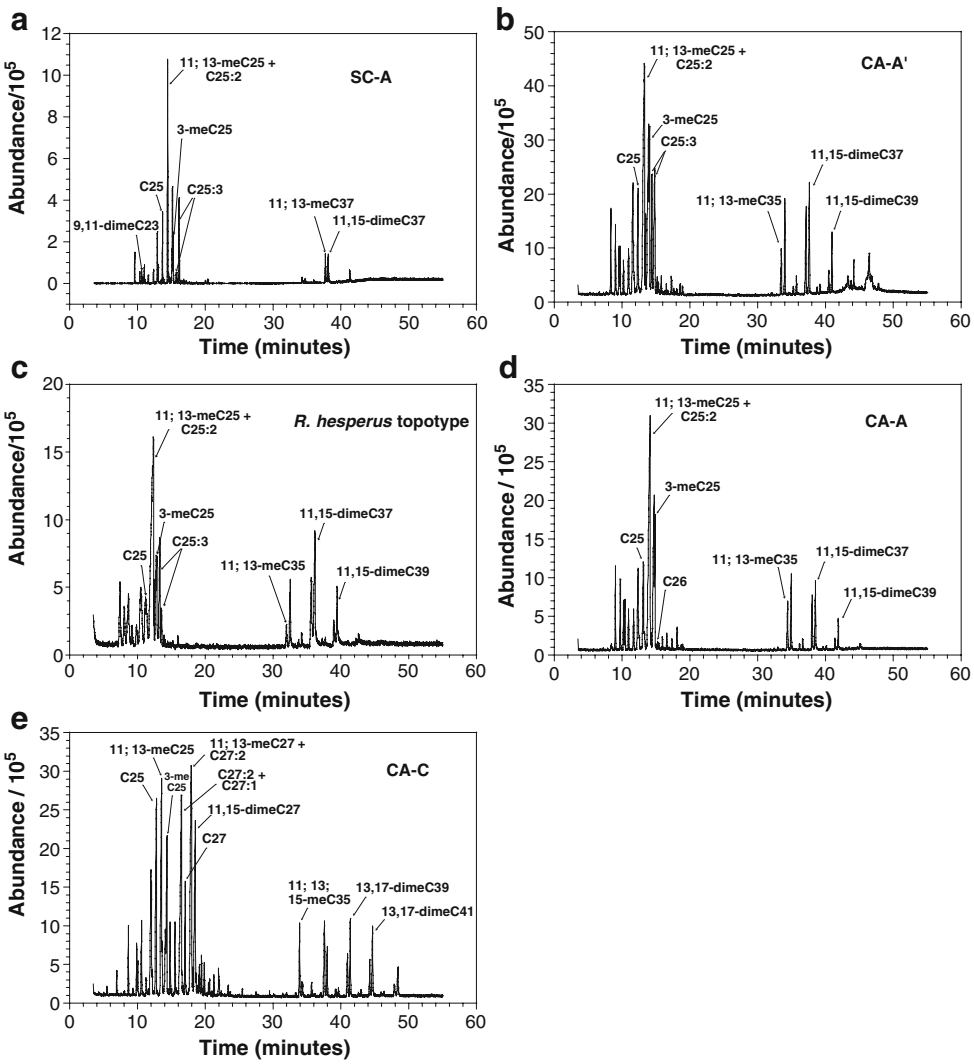

Fig. 2 Total ion chromatograms of cuticular hydrocarbons from *Reticulitermes* workers collected from **a** Goleta, Santa Barbara Co., CA (SC-A); **b** Novato, Marin Co., CA (CA-A′); **c** Lake Arrowhead, San Bernardino Co., CA (*R. hesperus* topotype); **d** Placerville, El Dorado Co., CA (CA-A); **e** Placerville, El Dorado Co., CA (CA-C)

that since phenotypes CA-A and CA-A′ clustered together, their hydrocarbon data were combined in Table 2. However, they can be distinguished easily from each other by the absence of the two isomers of C25:3 in CA-A, which is not apparent in the table, but can be seen in Fig. 2b and d. Haverty et al. (1991) proposed that if CHCs are to be reliable as taxonomic characters, they should be abundant components (at least 1%, but preferably 5% or more of the total hydrocarbon mixture). They should also be unique or present in only a few of the species, or conversely, they should be common in most of the species yet completely absent or in insignificant quantities in one or a few species. These are the criteria that we used to select the diagnostic CHCs listed in bold in Table 2.

Soldier Defense Secretions Soldiers of CHC phenotypes from southern California generally produced substantial amounts of germacrene A in the SDS (Table 3). The defense secretions of soldiers taken from colonies with CHC phenotype SC-A consisted almost entirely of germacrene A (>95%), except for one sample (SC-111) that had 79.0% germacrene A and greater amounts of γ-cadinene (15.0%) than the rest. Soldiers from the Lake Arrowhead collections (SC-115, -116, -117, and -118) produced mainly γ-cadinene (>78%) in their SDS, similar to the samples of CA-A and CA-A′ (Nelson et al. 2001). Other compounds, such as 3-octanol, *Z,E*-germacrene A, and δ-amorphene, were present in the Lake Arrowhead samples in significant amounts (0.5–9.9%, 5.0–11.5%, 1.2–2.9%, respectively), but were either absent or present in trace quantities in CA-A and CA-A′. The latter two compounds were previously identified in the SDS of CA-B soldiers (Table 3; Nelson et al. 2001).

The SDS of phenotype SC-B also contained significant amounts of germacrene A; however, there was variation in the relative quantities, ranging from 16.2% to 97.2%. Most of the samples had >62% germacrene A, along with moderate amounts of γ-cadinene (Table 3). Two collections

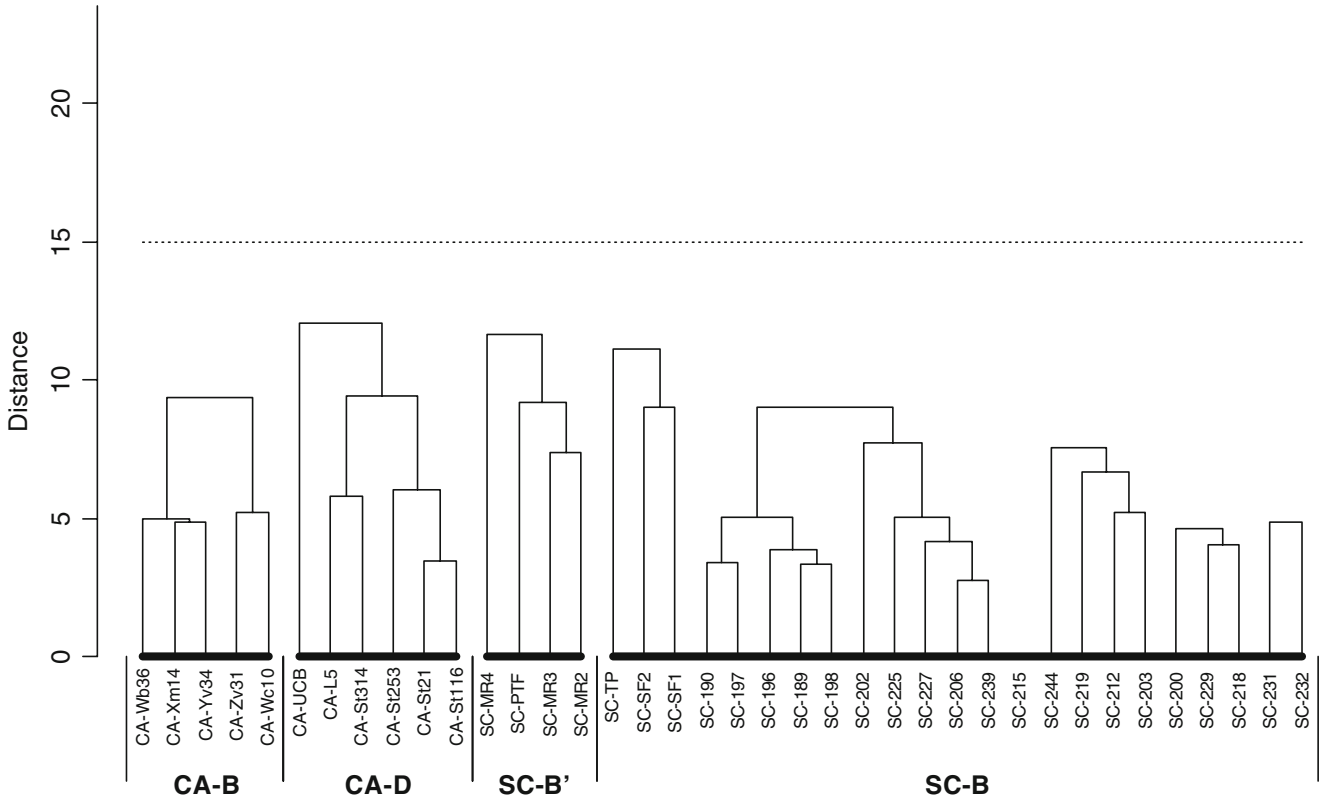

Fig. 3 Dendrograms from cluster analyses based on Euclidean distance of cuticular hydrocarbons extracted from samples of *Reticulitermes* workers from California. These samples contain 5-methylalkanes and 5,X-dimethylalkanes. See Tables 1 and 2 for data pertaining to individuals and phenotypes

from San Diego County (SC-SF1 and SC-SF2) contained much less germacrene A and substantially more geranyl linalool (Table 3). These same samples had notable differences in their hydrocarbon mixture when compared with other SC-B samples (Fig. 4a,d).

Collections from Riverside County, which were designated as hydrocarbon phenotype SC-B′, contained predominantly germacrene A (56.7–98.7%), and γ-cadinene (0.8–16.6%; Table 3). Samples MR2, MR4, and PTF1 contained modest amounts of dauca-8,11-diene and dauca-4(11),8-diene (1.4 to 5.8% and 0.3 to 2.2%, respectively). These compounds have not been reported before from *Reticulitermes* soldiers. A small amount of dauca-8,11-diene (0.6%) was observed also in SC-SF1, and dauca-4(11),8-diene was found in SC-SF1 and SC-TP. Several other components (daucene, β-santalene, zingiberene, β-sesquiphellandrene) were found in these samples as well. Small amounts of unknowns designated as 204f and 204g were seen in SC-SF1, SC-MR2, SC-MR4, SC-PTF1, and 204g was also found in SC-TP from San Diego Co. (Table 3).

Zingiberene was reported in trace amounts in *Reticulitermes* phenotype AZ-C(I) from Arizona (Nelson et al. 2001). Daucene, 3-octanol, β-santalene, (*Z*,*E*)-α-farnesene, and β-sesquiphellandrene also have not been reported previously from *Reticulitermes* soldiers, although 3-octanol was reported in pentane extracts from workers of four European *Reticulitermes* species (Reinhard et al. 2003). Nerolidol was found in one sample of SC-A (0.4%) and two samples of SC-B (<0.03%). This compound was reported previously, by Quintana et al. (2003), in soldiers of *R. lucifugus* and *R. lucifugus corsicus*.

Discussion

Before addressing the particular results of the current study, it is helpful to review the current state of taxonomic research on *Reticulitermes*.

We are troubled by several things in the literature on termite taxonomy: exclusive reliance on morphological determination of species, with use (or misuse) of inadequate keys; use of samples from the GenBank sequence database that may or may not be correctly identified to species; and misrepresentation of the value of chemical characters in delimiting species in termites. We also agree with Uva et al. (2004) who, in a study of *Reticulitermes* from southern Europe, warned against the following assumptions: (1) the classification of termites is definitive, and (2) that identification of termites can be made based on geographic origin alone. Unfortunately, these cautions, and other difficulties

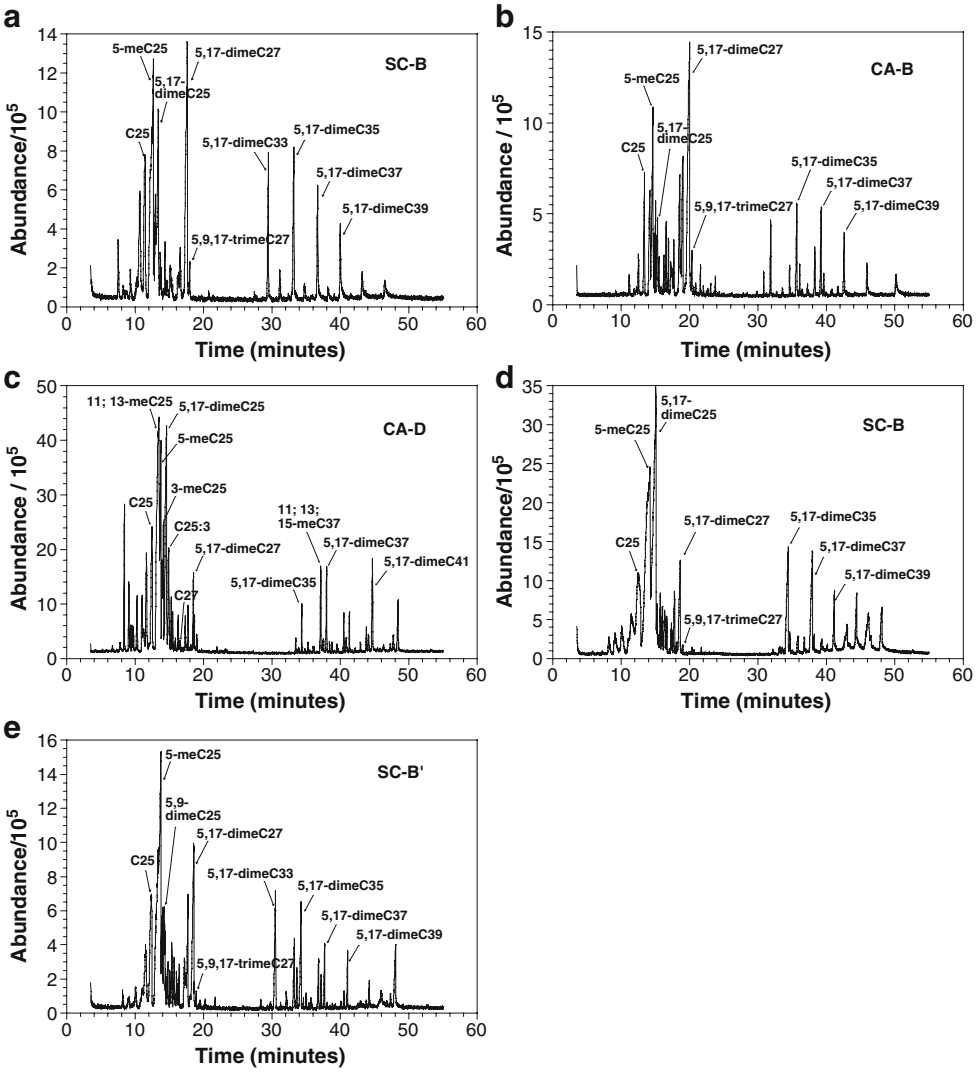

Fig. 4 Total ion chromatograms of cuticular hydrocarbons from *Reticulitermes* workers collected from **a** Carpinteria, Santa Barbara Co., CA (SC-B); **b** Placerville, El Dorado Co., CA (CA-B); **c** Novato, Marin Co., CA (CA-D); **d** Scissors Crossing, San Diego Co., CA (SC-B); **e** Motte Rimrock Reserve, Riverside Co., CA (SC-B′)

inherent in the taxonomic study of *Reticulitermes*, often have been overlooked.

Taxonomy of North American Reticulitermes Much of the biogeographical and taxonomic information on *Reticulitermes* was developed in the first half of the past century (Banks and Snyder 1920; Light 1934; Pickens 1934a, b; Banks 1946; Miller 1949; Snyder 1954). Banks and Snyder (1920) provided the first "descriptions" of species within this genus, and Snyder (1949) established the number of species of *Reticulitermes* in North America at six: *R. arenincola* Goellner, *R. flavipes* (Kollar), *R. hageni* Banks, *R. hesperus* Banks, *R. tibialis* Banks, and *R. virginicus* (Banks). Snyder (1954) reiterated that there are only six species of *Reticulitermes* in North America, and this has been affirmed by Weesner (1965, 1970) and Nutting (1990) and assumed by most termite biologists (Haverty et al. 1999b). However, the growing body of literature on the diversity of *Reticulitermes* suggests that there are more species than the six currently recognized. Our own chemotaxonomic analyses (Haverty et al. 1996a, 1999b; Haverty and Nelson 1997, 2007; Jenkins et al. 2000; Nelson et al. 2001; Copren et al. 2005) and ethological studies (Haverty et al. 1999a, 2003; Delphia et al. 2003) indicated that undescribed species of *Reticulitermes* occur in the United States. Su et al. (2006) reported two undescribed species from California, based on mtDNA sequences, as did Tripodi et al. (2006). Szalanski et al. (2006) named a new species, *R. okanaganensis*, from the western United States and Canada, based on the mtDNA 16S gene. Austin et al. (2007) provided genetic and other evidence to confirm the existence of *R. malletei*, first described by Clément et al. (1986), in Georgia and several other states. Correspondence of genetic data with those from CHC, biometric, and ethological analyses support resurrecting this species name, which had never been formally accepted. Taken together, studies like these underscore the outdated and incomplete nature of North

American *Reticulitermes* taxonomy. As Weesner (1970) put it, "...this genus is woefully in need of a critical taxonomic study..."

Identification of Reticulitermes *Specimens* Assigning insect specimens to the correct species has been done traditionally by using dichotomous keys and/or surmised by the geographic location of the collection. In practice, there are serious problems and pitfalls with both methods.

The morphological keys most commonly used and cited for identification of *Reticulitermes* species are those by Snyder (1954), Weesner (1965), and Nutting (1990). These keys are based on the original keys in Banks and Snyder (1920), with additional characters added and the acceptance of the synonomies presented by Snyder (1949). The keys developed by Scheffrahn and Su (1994) and Hostettler et al. (1995) were intended for identifying termites only from Florida, but have been cited in numerous papers as useful for identifying specimens of *Reticulitermes* from Arkansas, Louisiana, Missouri, and Virginia (Szalanski et al. 2003), Texas (Austin et al. 2004b; Foster et al. 2004), Oklahoma (Austin et al. 2004c), California (Tripodi et al. 2006), Oregon (McKern et al. 2006, 2007), and Delaware (King et al. 2007). In their genetic analysis of *Reticulitermes*, including samples from Europe, Asia, and North America, Austin et al. (2002) stated that they identified species in their study by using keys by Krishna and Weesner (1969), Scheffrahn and Su (1994), and Donovan et al. (2000). There are no keys for identification of species in Krishna and Weesner (1969), nor in Donovan et al. (2000). Furthermore, Scheffrahn and Su (1994) only have keys to three species: *R. flavipes*, *R. virginicus*, and *R. hageni*, and they restrict their focus to Florida specimens. It is unclear how specimens from China, Israel, Japan, South Korea, Arizona, or California can be identified by using these references.

In their phylogenetic analyses of the family Rhinotermitidae, Austin et al. (2004a) claimed to have identified termites collected from North America and the Caribbean by applying keys by Goellner et al. [sic] (1931), Scheffrahn and Su (1994), and Hostettler et al. (1995). Again, the last two references include termite species known to occur only in Florida. It appears that all but three samples [*R. arenicola* (sic), *Coptotermes formosanus* Shiraki, and *Heterotermes cardini*] were gathered from GenBank and not collected by the authors. It is questionable how the *H. cardini* sample was identified by using any of these keys.

In their study of the phylogeography of *Reticulitermes* in California, Tripodi et al. (2006) claim that "*R. flavipes* and *R. tibialis* were morphologically identified when alates were available by using the keys of Krishna and Weesner (1969), Banks and Snyder (1920), and Hostettler et al. (1995)." As mentioned earlier, there is no key in Krishna and Weesner (1969). The key in Banks and Snyder (1920) is timeworn, and if it is agreed that the genus needs revision, reliance on such a key is inherently problematic. The keys to *Reticulitermes* of Florida in Hostettler et al. (1995) do not include *R. tibialis*. We have serious reservations about morphological identifications of *Reticulitermes* species by using geographically inappropriate keys, i.e., that do not include all the species. Equally suspect are identifications that claim to use sources (such as Krishna and Weesner 1969 or Donovan et al. 2000) that do not contain keys to species (Austin et al. 2002, 2004b, c, 2005a; Szalanski et al. 2003; Tripodi et al. 2006, McKern et al. 2007).

A major problem with existing morphological keys to *Reticulitermes* species is the complication of the caste used for identification. If only workers of *Reticulitermes* are collected, identification to species is all but impossible, as there are no morphological keys to workers available. For imagoes (alates) and soldiers, the most common approach is to have separate keys (see Snyder 1954; Weesner 1965; Nutting 1990; Scheffrahn and Su 1994). However, the great majority of *Reticulitermes* collections involve either foraging groups, which rarely contain imagoes, or flights of imagoes (alates) without the associated soldiers. Weesner (1965) lacks a key to the soldiers for *Reticulitermes* from the Pacific Coastal region, and thus makes species identification of *Reticulitermes* foraging groups impossible. The problem is exacerbated when both castes are present in the same sample, and keys to alates indicate a species different from that indicated by the keys to soldiers (Haverty et al. 1999b; King et al. 2007).

While ignoring geography can lead to dubious identifications of specimens, uncritical reliance on the location of a collection in its identification is also hazardous. In their discussion of phylogeography of *Reticulitermes* in California, Tripodi et al. (2006) stated that their "... experience with this group shows that it consistently has been misidentified because of prejudices or assumptions about their respective distributions (Austin et al. 2002, 2005b)." Weesner (1970) gave detailed geographic information on the distribution of *Reticulitermes* species that could lead to erroneous conclusions. For example, if one collected a *Reticulitermes* sample anywhere in Arizona, USA, and referred to Weesner (1970) or Nutting (1990), the conclusion would be that it could only be *R. tibialis*. Now, we know that there are at least four CHC phenotypes of *Reticulitermes* in Arizona, that we believe represent four different species, only one (or none) of which could actually be *R. tibialis* (Haverty and Nelson 2007). Identification of *Reticulitermes* in California has proven difficult (Haverty and Nelson 1997; Tripodi et al. 2006), especially considering the possible occurrence of exotic species, such as *R. flavipes* (Kollar), or undescribed species (Haverty and Nelson 1997; Su et al. 2006; Tripodi et al. 2006). The assumed species distributions in extant keys for California would allow only two choices, *R. hesperus* or *R. tibialis*.

Table 3 Terpenoid percent composition of soldier defense secretions from *Reticulitermes* from southern and northern California[a]

Terpenoid[b]	SC-104 SC-A	SC-106 SC-A	SC-107 SC-A	SC-108 SC-A	SC-109 SC-A	SC-110 SC-A	SC-111 SC-A	SC-112 SC-A	SC-113 SC-A	SC-114 SC-A	SC-124 SC-A	SC-ALP2 SC-A
(—)-α-Pinene												
(—)-β-Pinene												
Myrcene							0.6		0.0	0.1	0.1	
Daucene												
(—)-Limonene	0.0	0.0				0.0	0.1		0.0	0.0	0.0	
(3-Octanol)	0.0	0.1	0.1	0.1	0.3	0.2	0.8	0.2	0.2	0.1	0.1	
(Z)-Ocimene	0.0	0.1				0.0	0.2		0.0	0.0	0.0	
(E)-Ocimene	0.0	0.1				0.0	0.2		0.0	0.0	0.0	
Unk. 204b												
δ−Elemene	0.0	0.1	0.1		0.1	0.1	0.4	0.1	0.1	0.1	0.1	
(+)-α-Himachalene												
(*E*)-β-Farnesene	0.1	0.4	0.2	0.1	0.2	0.1	1.2	0.2	0.2	0.2	0.7	
β−Santalene												
Unk. 204f												
Unk. 204g												
α−Humulene												
cis-Muurola-4(14),5-diene												
Unk. 204c												
γ−Himachalene					0.0							
γ−Humulene	0.0	0.1	0.1	0.0	0.1	0.1	0.8	0.2	0.1	0.0	0.3	
(—)-β-Selinene												
(—)-α-Selinene												
β−Himachalene												
(Zingiberene)												
***(Z,E)*-α-Farnesene**	0.1	0.4				0.1	1.4	0.3	0.2	0.2	0.6	
dauca-8,11-diene												
β−Bisabolene												
((*Z,E*)-Germacrene A)												
(—)-Germacrene A	98.3	95.7	98.9	98.5	96.7	97.5	79.0	95.5	96.1	98.1	90.9	99.6
δ−Amorphene												
(+)-γ-Cadinene	1.3	2.3	0.3	0.9	1.5	1.3	14.7	3.2	2.6	0.9	6.9	0.3
δ−Cadinene												
β−Sesquiphellandrene												
dauca-4(11),8-diene												
Unk. 204e												
α−Cadinene		0.1			0.0	0.0	0.2	0.1	0.1	0.0	0.3	
Nerolidol					0.4							
Germacrene B												0.0
((*E*)-γ-Bisabolene)												
(+)-γ-Cadinenal												
Unk. 272a												
Unk. 238a												
Unk. 290a												
Geranyl linalool	0.1	0.5	0.4	0.3	0.7	0.5	0.5	0.3	0.3	0.2		

[a] Values of 0.0 indicate the compound was detected, but at a level <0.05% of the total SDS.
[b] Terpenoids are listed in GC elution order. Terpenoids in **bold type** have not previously been reported for termite soldiers. Compound name in parentheses or brackets indicates identification is tentative. Unknowns are designated by an apparent molecular weight and an identifying suffix letter.
[c] The terpene phenotypes for these samples are reported in Nelson et al. 2001.

All these difficulties have led us, as well as other researchers, to investigate chemical or molecular characters as a better means of distinguishing termite taxa based on soldiers or workers (pseudergates; (Haverty and Nelson 1997; Takematsu 1999; Takematsu and Yamaoka 1999; Jenkins et al. 2000, 2001; Nelson et al. 2001; Austin et al. 2002, 2004a, b, c, 2005a, b, 2007; Szalanski et al. 2003, 2006; Foster et al. 2004; Uva et al. 2004; Ye et al. 2004; Copren et al. 2005; Su et al. 2006; Tripodi et al. 2006; King et al. 2007).

Chemical Taxonomy of Reticulitermes Howard and Blomquist pioneered the use of CHCs as taxonomic characters for delimiting species or taxa of *Reticulitermes* (Howard et al.

SC-ALP3 SC-A	SC-101 SC-B	SC-103 SC-B	SC-105 SC-B	SC-119 SC-B	SC-120 SC-B	SC-121 SC-B	SC-123 SC-B	SC-TP SC-B	SC-SF1 SC-B	SC-SF2 SC-B	SC-MR2 SC-B'	SC-MR3 SC-B'	SC-MR4 SC-B'
									0.1				0.0
0.0										0.0	0.1		
											0.4		2.5
	0.1	0.8	0.5	0.1	0.4		0.7						
	0.1		0.1	0.0	0.0		0.0						
0.3	1.6	0.3	0.6	0.1	0.1	0.1	0.1	0.8	0.2	0.2	0.2	0.1	0.5
											0.2		1.0
									0.3		0.5		1.8
								0.2	0.3		0.3		1.0
										0.1			
	0.7												
0.3	0.7	0.7	0.4	0.9	0.7	0.8	0.6	0.4	0.8	1.1	0.6		0.5
										0.1			
											0.8		2.8
	1.4	0.1	0.8	0.1									
									0.6		1.4		5.8
	1.7												
98.2	71.3	76.4	84.6	66.3	66.3	62.4	76.8	82.5	22.7	16.2	74.3	97.2	56.7
1.1	20.3	20.9	11.7	27.8	21.1	23.3	18.5	12.1	18.2	31.6	16.6	2.0	16.1
									0.3		0.5		2.2
								0.1	0.2		1.3		6.4
0.0	0.8	0.4	0.3	0.7	0.6	0.7	0.6	0.4	0.6	0.9	0.5	0.1	0.7
	0.0		0.0										
0.0											0.0	0.0	0.0
									4.1	0.1			
				2.3	10.2	7.2	1.4	3.3		10.9	1.6	0.4	0.6
	1.3	0.4	1.1	1.7	0.5	5.6	1.3	0.2	51.4	38.7	0.6	0.1	1.5

1978, 1982; Howard and Blomquist 1982). With a few exceptions, most termites have species-specific mixtures of CHCs (Kaib et al. 1991; Howard 1993; Page et al. 2002). CHCs are homologous characters; they represent hierarchical distributions of shared characters and are independent characters with discrete states (Page et al. 2002). Termites synthesize most, if not all, of their complement of CHCs de novo (Blomquist and Dillwith 1985). As such, hydrocarbon composition is an expression of a taxon's genotype, and therefore, we can assume there is a genetic basis for different hydrocarbon composition among species (Page et al. 2002).

Use of CHCs to sort specimens has led to identification of new species and has resulted in morphological descriptions supplemented by chemical data (Takematsu 1999; Takematsu

Table 3 (continued)

Terpenoid[b]	SC-PTF1 SC-B'	SC-PTF2 SC-B'	SC-115 CA-A'	SC-117 CA-A'	SC-118 CA-A'	CA-A (N=16) Mean[c]	CA-A' (N=10) Mean[c]	CA-B (N=6) Mean[c]	CA-C (N=6) Mean[c]	CA-D (N=10) Mean[c]
(—)-α-Pinene						0.1	0.0	0.0		0.0
(—)-β-Pinene	0.0					0.1	0.1	0.0	0.0	0.0
Myrcene	0.0					0.1		0.0	0.0	0.0
Daucene	1.2									
(—)-Limonene						0.1	0.0		0.0	0.0
(3-Octanol)			9.9	4.8	8.3					
(Z)-Ocimene										
(E)-Ocimene										
Unk. 204b								0.7		
δ−Elemene										
(+)-α-Himachalene								5.0		
(*E*)-β-Farnesene	0.2	0.1							0.2	0.2
β−Santalene	0.5									
Unk. 204f	1.3									
Unk. 204g	0.6									
α−Humulene			0.3	0.1	0.2			0.4		
cis-Muurola-4(14),5-diene						0.0	0.1	0.0		0.0
Unk. 204c						0.0				0.0
γ−Himachalene			0.3	0.3	0.3			13.4		
γ−Humulene	0.2		0.4	0.1		1.6	1.8	1.2	0.0	0.7
(—)-β-Selinene									0.2	0.9
(—)-α-Selinene									2.0	9.1
β−Himachalene								4.9		
(Zingiberene)	1.7									
***(Z,E)*-α-Farnesene**										
dauca-8,11-diene	3.3									
β−Bisabolene						1.1	0.9			
((*Z,E*)-Germacrene A)			5.0	11.5	9.1	0.1	0.2	6.8		
(—)-Germacrene A	76.8	98.7				1.3	1.0		92.8	33.0
δ−Amorphene			1.2	2.9	2.3			8.5		
(+)-γ-Cadinene	8.5	0.8	81.6	79.8	78.2	90.0	94.3	39.6	3.7	17.4
δ−Cadinene						0.2		0.7		
β−Sesquiphellandrene	1.4									
dauca-4(11),8-diene	3.6									
Unk. 204e								1.4		
α−Cadinene	0.4		1.0	0.6		1.6	0.8	0.8	0.0	0.3
Nerolidol										
Germacrene B										
((*E*)-γ-Bisabolene)						0.3				
(+)-γ-Cadinenal	0.2									
Unk. 272a		0.3						0.0		
Unk. 238a						0.2		0.0	0.1	
Unk. 290a										0.3
Geranyl linalool	0.0	0.1	0.4		1.7	3.1	0.8	16.7	0.7	38.0

and Yamaoka 1999). These studies examined the CHCs of six species of *Reticulitermes* from Japan, Korea, and Taiwan, observed nine different hydrocarbon phenotypes, and recommended that four subspecies be elevated to species status. They concluded that the nine species had unique compounds that can be used to separate species and that hydrocarbons are of value for classification of *Reticulitermes* species. These studies demonstrated that *R. speratus* is a complex of three species, and the investigators found new morphological characters for separating species. Similarly, important phenotypic differences in CHC profiles (Bagnères et al. 1988, 1991) and SDS composition (Parton et al. 1981; Bagnères et al. 1990; Quintana et al. 2003) were found between the different species of *Reticulitermes* in Europe.

Two recent studies (Szalanski et al. 2006; Tripodi et al. 2006)[1] have, in our opinion, misrepresented the value of CHCs for taxonomy, even though two of the authors used CHCs (data from *P. Uva*) in an earlier study to validate the species status of *R. lucifugus* from Turkey (Austin et al. 2002) and to confirm the identity of samples of *R. malletei* in the eastern United States (Austin et al. 2007). Szalanski et al. (2006) stated "Recently, Copren et al. (2005) found evidence for as many as seven new species of *Reticulitermes* from the western United States based upon cuticular hydrocarbon phenotypes, but resolved to designate them as putative new species after attempting to corroborate their relationship with molecular phylogenetics and reproductive flight dates. These discrepancies likely are attributable to the environmental plasticity of cuticular hydrocarbons and stresses (sic) the need for fixed character states for species identification such as mtDNA sequences." Tripodi et al. (2006) echoed this line of thought. Actually, there were no discrepancies; this is a misinterpretation of Copren et al. (2005) as she and her colleagues corroborated the species groups based on CHCs and COII mtDNA sequences. Thus, taxa determined by CHC analyses agreed with taxa determined by mtDNA sequences. The "environmental plasticity" caveat has been carefully considered, and the references provided by Szalanski et al. (2006) and Tripodi et al. (2006) are not relevant to this analysis. Our phenotype designations are based on numerous, repeatable qualitatively different profiles. There can be minor quantitative variation among collections of the same phenotype, in which case, we do not distinguish them as separate phenotypes (Haverty et al. 1991, 1996b).

Tripodi et al. (2006) state "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 (Kaib et al. 1991), variation between groups may be more greatly attributable to environmental differences and available food sources (Liang and Silverman 2000)." The study by Liang and Silverman (2000) involved colonies, not of termites but of the Argentine ant, *Linepithema humile* (Mayr), which were feeding on and mixing with various species of cockroaches. It is clear that predatory insects feeding on waxy organisms, such as laboratory-reared cockroaches, will acquire foreign hydrocarbons that could easily affect kin recognition. This study, however, has no relevance with regard to the alleged "plastic nature" of termite CHC mixtures.

In their discussion on the effects of diet on aggression in *Coptotermes formosanus* Shiraki, Tripodi et al. (2006) claim 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 among colonies of termites, considerable variation of hydrocarbons across small spatial distances within an apparently single morphological species may occur and should alert taxonomists to interpret cuticular hydrocarbon patterns with care." Florane et al. (2004) do not show effects of diet on CHC composition, but rather on volatile compounds (collected by headspace solid-phase microextraction of workers) that possibly affected kin recognition and intercolonial aggression.

In summary, we find no valid objections to the use of CHC profiles in *Reticulitermes* taxonomy. The extensive body of knowledge demonstrating the taxonomic value of termite CHCs allows us to accept distinct, repeatable CHC profiles as descriptive of distinct taxa.

Reticulitermes soldiers, like soldiers from other termite genera, synthesize their defense secretions *de novo* (Prestwich 1979, 1983). It follows that these SDS mixtures, like CHCs, could be useful as taxonomic characters. Several studies have characterized and compared SDSs from *Reticulitermes* species. Parton et al. (1981) compared SDS mixtures from populations of *R. lucifugus* and *R. santonensis* in Europe, finding geographic variation in the *lucifugus* complex, while *R. santonensis* was homogeneous. In the southeastern United States, Clément et al. (1985) identified two SDS phenotypes in *R. flavipes*, one in *R. virginicus* and another in *R. malletei*. Bagnères et al. (1990) examined chemical characters from *R. flavipes* in the southeastern United States and *R. santonensis* in Europe, and reported six SDS phenotypes in *R. flavipes*, while *R. santonensis* was, again, homogeneous. In a study of the same characters from *Reticulitermes* samples from Georgia, Arizona, and northern California, Nelson et al. (2001) reported that some SDS phenotypes pair with more than one CHC phenotype; however, with two exceptions, each hydrocarbon phenotype is associated with only one SDS phenotype. Samples that keyed to *R. flavipes* were represented by three SDS phenotypes, similar to ones previously reported by Clément et al. (1985) and Bagnères et al. (1990). Quintana et al. (2003) described species-

[1] There are several incorrect references in these papers. Szalanski et al. (2006) acknowledged the "identification of new species from northern California ...reported by Haverty and Nelson (1997), Haverty et al. (1999) [our Haverty et al. 1999b] applying CHCs, and further investigated with ethological data (Getty et al. 2000a, b)." However, there was no new information on species of *Reticulitermes* from northern California reported in the cited Haverty et al. (1999) reference. This paper (Haverty et al. 1999b) reported a number of new CHC phenotypes from Georgia, New Mexico, Arizona, and Nevada. Furthermore, the ethological information that supports species designations is not found in the Getty et al. (2000a, b) papers, but in Haverty et al. (1999a) and Delphia et al. (2003) in which we examined agonistic behavior; and in Haverty et al. (2003) we studied flight phenology as a reproductive isolating mechanism. This same referencing error was repeated by Tripodi et al. (2006).

specific SDS phenotypes for seven European *Reticulitermes* species.

Our findings for *Reticulitermes* from southern California show that the two primary CHC phenotypes (SC-A and SC-B) have qualitatively similar SDS mixtures, although they are quantitatively dissimilar (Table 3). Possible explanations for this lack of correspondence include (a) different rates of evolution among the character sets and/or (b) this particular SDS phenotype is adaptive to the conditions of southern California. However, the samples collected at Lake Arrowhead, which is the type locality for *R. hesperus*, had the same SDS profile as CA-A/A′ from northern California.

Taken together, CHC and SDS profiles appear to be complementary character sets that have similar, although not always identical, sensitivity in discriminating putative *Reticulitermes* taxa.

Genetics of Reticulitermes Within the past decade, numerous genetic analyses have been published that indicate additional species or taxa, potential synonymies, and the degree of genetic variation within a species. Copren et al. (2005) used the COII gene in a phylogenetic analysis that indicated at least six clades or species of *Reticulitermes* in California, including the two CHC phenotypes presented here from southern California. Szalanski et al. (2006) and Tripodi et al. (2006) provided genetic evidence of an undescribed species in western North America and a phylogenetic analysis of *Reticulitermes* by applying the 16S mtDNA gene, and validated the prior genetic analysis by Copren et al. (2005). Furthermore, Tripodi et al. (2006) and Su et al. (2006) identified an apparent exotic species, *R. flavipes*, in California via genetic analyses. McKern et al. (2006, 2007) identified *R. flavipes* and *R. hageni* in Oregon by using the 16S mtDNA, which is the first report of these species in that state.

Recent genetic evaluations of North American, European, and Chilean *Reticulitermes* have resulted in intriguing conclusions: (1) North American *R. flavipes* and *R. arenincola*, and European *R. santonensis* are likely conspecific (Ye et al. 2004), and (2) the invasive *Reticulitermes* in Chile and Uruguay is *R. flavipes* (Austin et al. 2005b; Su et al. 2006). One Indiana population of *R. arenincola* shares identical DNA sequences with one *R. santonensis* population from France and one *R. flavipes* population from Indiana (Ye et al. 2004). Even though *R. flavipes* = *R. arenincola* by DNA sequence, there appeared to be morphological differences between the respective soldier castes, and the authors decided that the synonymy of these two species would be premature (Ye et al. 2004). Su et al. (2006) demonstrated that all 13 samples of Chilean *Reticulitermes* termites collected from four cities were *R. flavipes* and had identical gene sequences for all loci examined, thus suggesting a single geographic introduction. A similar conclusion was reached by Austin et al. (2005b).

As an adjunct to population studies of *Reticulitermes* in northern California, Copren (2007) found that three microsatellite loci, specifically developed for *R. hesperus*, did not amplify with the three other species of *Reticulitermes* from northern California. Finding fixed genetic differences among cryptic species that result in reciprocal monophyly, particularly among sympatric species, provides information useful for taxonomic studies (Copren 2007).

We think that GenBank is an important tool for phylogenetic analyses, but its use is not without problems. The use of GenBank data submitted by others requires one to accept the submitter's species designations as accurate or authoritative. With refractory genera, such as *Reticulitermes* or *Heterotermes*, GenBank sequences should be used with caution.

Use of Multiple Characters in Reticulitermes *Taxonomy* In a difficult genus like *Reticulitermes*, taxonomic conclusions are greatly strengthened when two or more features, CHC mixtures, SDS mixtures, genetic markers, morphology, behavioral characteristics (such as agonism or flight times), etc., correlate. The more differences that exist among groups of organisms, the greater the likelihood of their being separate species (Futuyma 1998). Jenkins et al. (2000) and Copren et al. (2005) demonstrated the association of CHC phenotypes and mtDNA haplotypes, illustrating that CHCs are useful in separating known species, determining new species, and understanding termite evolution, and adding robustness to the phylogenetic analyses (Jenkins et al. 2000; Page et al. 2002). Additional examples of multiple, independent data sets for determining species of *Reticulitermes* include the CHC and morphological work of Takematsu and Yamaoka (1999), the CHC and SDS study of Nelson et al. (2001), and the use of microsatellites to assess sympatric populations of *R. hesperus* and cryptic species of *Reticulitermes* in northern California (Copren 2007).

In contrast to this approach, Ye et al. (2004) relied heavily on morphological information and did not address contradictory genetic information. Genetic data gathered from three mitochondrial DNA genes (COII, 16S, and 12S) indicated that *R. flavipes*, *R. santonensis*, and *R. arenincola* are conspecific. Others reached the same conclusion about *R. flavipes* and *R. santonensis* (Austin et al. 2005b; Su et al. 2006). However, Ye et al. (2004) felt it was premature to synonymize *R. arenincola* and *R. flavipes* due to the morphological separation of *R. arenincola* and *R. flavipes* by soldier characteristics.

Reticulitermes *in Southern California: the Current Study* In early studies of Californian termites, *Reticulitermes* was

determined by various authors as consisting of just two species, *R. hesperus* and *R. tibialis* (Light and Pickens 1934; Pickens 1934a, b; Snyder 1949, 1954). There is a persistent assumption that these are the only *Reticulitermes* species in California and that they have distributions that are allopatric over most of their ranges (Weesner 1970).

We were among the first to question the number of species of *Reticulitermes* in California, and we reported five CHC phenotypes of *Reticulitermes* (CA-A, CA-A′, CA-B, CA-C, and CA-D) in northern California (Haverty and Nelson 1997). In the current study, we report a wider distribution of previously described phenotypes SC-A and SC-B and a variant of one of them (SC-B′) that occurs in Riverside County. Correlation of CHC phenotypes with mtDNA (COII) genotypes supported elevating phenotypes CA-B, CA-C, CA-D, and SC-B to the status of new species, but suggested that CA-A, CA-A′, and SC-A represent one species, which we postulated to be *R. hesperus*, with the CA and SC phenotypes comprising geographic subspecies (Copren et al. 2005).

Banks and Snyder (1920) originally described *R. hesperus* based on samples from California, Nevada, Oregon, and Washington and designated the type locality as Little Bear Lake, San Bernardino Mountains, California (Snyder 1949). Many of these sites are distant from the Pacific Coast and east of the Cascade Range and Sierra Nevada. In a case like this, with an old species designation based on morphology of a limited number of specimens (Banks and Snyder 1920), it is difficult to associate chemical or genetic determinations of taxa with the species in question. We collected at the type locality of *R. hesperus* so that we could associate one of our CHC phenotypes with a species name. The type specimen of *R. hesperus* (winged, Cat. No. 21864, U.S.N.M.) was reported from Little Bear Lake, CA, now known as Lake Arrowhead (Robinson 1989), in the San Bernardino Mountains (see Banks and Snyder 1920; Snyder 1949). Haverty et al. (2003) and Copren et al. (2005) reported that topotype samples from Lake Arrowhead all had one CHC phenotype, CA-A′. Copren et al. (2005) used the geographic nomenclature SC-A to describe this phenotype, but the lack of 9,11-dimethylalkanes, the presence of C25:3, and γ-cadinene in the SDSs puts it in the same group as CA-A′ from northern California. Thus, this phenotype was postulated to represent *R. hesperus*.

Studies by Szalanski et al. (2006) and Tripodi et al. (2006) that used 16S mtDNA, have identified a genetically distinct species in the western United States, which they have named *R.* n. sp. "*R. okanaganensis*." We contend that is incorrect, and the collections designated as *R.* n. sp. "*R. okanaganensis*" are actually the common, and widely distributed, *R. hesperus*. Austin et al. (2002) were likely correct, and the revision by Szalanski et al. (2006) and Tripodi et al. (2006) simply confused the status of *Reticulitermes* in California. Szalanski et al. (2006) and Tripodi et al. (2006) apparently disregarded the value of collections at the type locality. Instead, they designated a sample from Lake Arrowhead as their putative new species, *R. okanaganensis*, but did not find what they now consider to be *R. hesperus* at the type locality. Actually, the distribution presented for *R. okanaganensis* (Szalanski et al. 2006) closely resembles the original distribution map of *R. hesperus* reported by Banks and Snyder (1920) and reinforced by Weesner (1970) and Nutting (1990).

One particular mitochondrial DNA sequence is key to our study, especially as it relates to Tripodi et al. (2006): a fragment of the mtDNA cytochrome oxidase (COII) from *R. hesperus* [GenBank AF525329] (Austin et al. 2002, 2004a; Copren et al. 2005). The sequence for *R. hesperus* from Los Angeles, California [GenBank AF525329] was useful for the demonstration of the correlation of species determinations based on CHCs and COII sequences, as this sequence was similar to that of the collections from the type locality for *R. hesperus* (Copren et al. 2005). Now, based on the mtDNA 16S sequence, Tripodi et al. (2006) state that the sample identified by Austin et al. (2002) as *R. hesperus* [GenBank AF525329] was a mistake, and it truly represents a new species, "*R. okanaganensis*." We think that Tripodi et al. (2006) are wrong and that Austin et al. (2002) attached the correct species name to sequence AF525329, possibly relying on location to assign the species name since the references they cited did not have keys or had keys that were inappropriate for specimens from California. The confusion would be compounded if, based on Tripodi et al. (2006), someone "corrected" the species name associated with AF525329.

If our analysis is correct, *R. hesperus* is the most common subterranean termite throughout California. It is found sympatrically in northern California with three unnamed species: *R.* sp. CA-B, *R.* sp. CA-C, and *R.* sp. CA-D. *R.* sp. CA-B and *R.* sp. CA-C are rare compared to *R. hesperus* and *R.* sp. CA-D (Copren 2007). In southern California, *R. hesperus* (SC-A) occurs sympatrically with *R.* sp. SC-B over most of their distributions. Furthermore, in Arizona and Nevada, we collected samples (phenotype AZ-D) that resemble specimens (designated CA-A′) from the type locality of *R. hesperus*. Therefore, it is likely that *R. hesperus* also occurs in northwestern Arizona and mountainous areas of southern Nevada, but is somewhat rare (Haverty and Nelson 2007). The CA-A/A′, SC-A, and AZ-D phenotypes may each represent a geographic subspecies of *R. hesperus*, as the CHCs are similar, but the SDSs are dissimilar, with AZ-D having SDSs consisting of mostly geranyl linalool (Haverty and Nelson 2007).

Today, the distribution, or even presence, of *R. tibialis* in California is not as clear as it apparently was to early researchers (Banks and Snyder 1920; Light and Pickens 1934; Pickens 1934b; Snyder 1954). Tripodi et al. (2006) only reported samples of *R. tibialis* in the southern portion of California in areas that border the Mojave Desert. Likewise, Copren et al. (2005) found specimens in Inyo County, California, on the xeric east side of the Sierra Nevada in the northern portion of the Mohave Desert that closely resembled (by COII mtDNA) the sample of "*R. tibialis*" from Cochise County, Arizona, USA, (GenBank AF525355) reported by Austin et al. (2002).

The type specimen for *R. tibialis* (winged, Cat. No. 21861 U.S.N.M.) was collected in Beeville, Texas (Banks and Snyder 1920; Snyder 1949). This species is purported to be the most widely distributed species, covering diverse habitats in North America (Banks and Snyder 1920; Snyder 1934; Weesner 1970). However, Weesner (1970) recognized that many of the alate specimens collected from the range of the species (Colorado, Illinois, Kansas, Missouri, Oklahoma, and Texas) were "typical" for *R. tibialis*, yet additional collections from the same area, particularly Texas, were extremely variable and could not be designated as *R. tibialis*. This suggests that there is more than one species in this distribution.

One of the GenBank COII sequences assigned to *R. tibialis* [AF525355] has been used in many studies (Austin et al. 2002, 2004a; Szalanski et al. 2003; Copren et al. 2005) and was collected from Cochise County, Arizona. This specimen was collected from an area that is likely to be the same as the species designated as our CHC phenotype AZ-B because only this phenotype has been found at the lower elevations in this region of Arizona. AZ-B is the most common CHC phenotype/species found in Arizona from Fairbank in Cochise County (≈1,200 m) to the North Rim of the Grand Canyon in Coconino County (≈2,500 m; Haverty and Nelson 2007). Using this sequence, *R. tibialis* [AF525355] is similar to, yet significantly different from, other *Reticulitermes* taxa in California (Copren et al. 2005).

Other COII sequences assigned to *R. tibialis* [GenBank AY168206 & AY168207, see Ye et al. 2004; GenBank AY808094, see Su et al. 2006] have not been used together with AF525355 in a comparative study and have likely been assigned to *R. tibialis* based on the location of the collection, as neither present a source for identification of any species (Ye et al. 2004) or the *R. tibialis* samples (Su et al. 2006). Furthermore, none of the samples identified as *R. tibialis* have been collected within 1500 km of the type locality of this species or in habitats resembling the type locality. As we see it, the actual genotype(s) or CHC phenotype of *R. tibialis* remains unknown.

Other Reticulitermes *Taxa in California* Su et al. (2006) and Tripodi et al. (2006) reported finding *R. flavipes* in California on the basis of mtDNA. We, too, have found *R. flavipes* in California based on CHC and SDS composition (Haverty and Nelson, unpublished), as have Ripa et al. (2002). Tripodi et al. (2006) were not able to confirm this based on morphology, whereas Su et al. (2006) identified their California population from Sacramento as *R. flavipes* by using the key in Snyder (1954). One unexpected finding was that the *R. flavipes* sample collected from Sacramento, California, closely resembled the introduced Chilean *R. flavipes* in combined mitochondrial DNA (COII, 16S, and 12S) sequences (Su et al. 2006). Su et al. (2006) hypothesized that the Chilean *R. flavipes* may have been introduced from California, or vice versa, or that both Chilean and Californian *R. flavipes* populations may have the same origin in North America. The well-established *R. flavipes* infestation in Sacramento suggests the ability of this east coast species to survive in a Mediterranean climate and that it could be more widespread in California than is known (Su et al. 2006). Tripodi et al. (2006) opined that the establishment of *R. flavipes* in Sacramento and El Cajon probably represents either extreme western distributions of the species or accidental introductions from anthropogenic sources. We believe the latter explanation is more likely.

Additional exotic or undescribed species of *Reticulitermes* are likely present in southern California given the numerous coastal, montane, and desert habitats. Based on mtDNA sequences, Su et al. (2006) found two populations of *Reticulitermes* from California (US27 and US42) that were similar to a sample labeled *R. tibialis* (US5), but one (US27) differed from it by 61 bp, the other (US42) by 52 bp, and they differed from each other by 34 bp. These samples may thus represent two undescribed species. Furthermore, Tripodi et al. (2006) also reported two undescribed species from southern California; one from Arrowbear, San Bernardino County, and another from Mission Gorge, San Diego County.

To summarize, the total count of probable species of *Reticulitermes* in California is at least seven: (1) *R. hesperus* (our hydrocarbon phenotypes CA-A, CA-A′, and SC-A); (2) *R.* sp. CA-B; (3) *R.* sp. CA-C; (4) *R.* sp. CA-D; (5) *R.* sp. SC-B [and SC-B′]; (6) "*R. tibialis*" = *R.* unknown sp. (Copren et al. 2005); (7) *R. flavipes* (Su et al. 2006, Tripodi et al. 2006); as well as *R.* unknown I (Su et al. 2006) and *R.* unknown II (Su et al. 2006). It is possible that the two unknown species identified by Tripodi et al. (2006) are the same as those found by Su et al. (2006). Genetic comparison of these samples will resolve this question. It is also possible that one of the unknown species of Su et al. (2006) and Tripodi et al. (2006) equates with our SC-B/B′.

Reproductive isolation is the most convincing if populations are sympatric (Freeman and Herron 1998). Exam-

ples of valid species of *Reticulitermes* with sympatric distributions have been reported numerous times for *R. flavipes*, *R. virginicus*, and *R. hageni* (Weesner 1970 and others), as well as *R. banyulensis* and *R. grassei* (Uva et al. 2004). Haverty et al. (2003) provided evidence of reproductive isolation in sympatric populations of different CHC phenotypes of *Reticulitermes* in northern California. Alates of phenotype CA-A/A′ (identified here as *R. hesperus*) take flight in the spring, whereas alates of phenotype CA-D fly only in the fall. These disparate flight times make reproductive isolation, and thus species status, complete. In our sampling of *Reticulitermes* in southern California, alates with the hydrocarbon phenotype SC-A were collected in March 2001 and 2002, while SC-B alates were collected in September and November 2001. This sampling is not extensive and warrants further corroboration, but the trend matches earlier reports for *Reticulitermes* species in northern California.

We (MIH and LJN) and our colleagues have been cautious, perhaps too cautious, in delineating and naming new termite species. We have taken this approach in order to avoid further confusing the taxonomy of *Reticulitermes* in North America before appropriate suites of taxonomic characters are agreed upon, and the status of previously published species is resolved. We do not propose naming species based on CHC phenotypes alone, but once taxa are sorted by this method, we can corroborate or refute the groupings with other evidence, such as morphological, chemical, behavioral, and genetic data. When there is agreement of two or more character sets, we can be more confident assigning species status to the phenotypes. The evidence for two predominant species, *R. hesperus* and *R.* sp. SC-B, in southern California is strong, and we believe our inference regarding *R. hesperus* and its type locality is correct. Finally, we urge researchers in this field to be cautious in applying species names based on inappropriate, unspecified, or incorrectly cited reference sources, and to take care in accurately describing both their own methodology and research performed by others.

Acknowledgments We thank Brian Forschler, Barbara Thorne, J. Kenneth Grace, Vernard Lewis, and Srini Kambhampati for their helpful comments on an earlier draft of this manuscript. Gail Getty, Kirsten Copren, and Casey Delphia assisted in collecting and preparing many samples used in this study. The number of samples collected was increased by the cooperation of Dow AgroSciences personnel in their extensive evaluation of the Sentricon® Termite Colony Elimination System in southern California. Jim Baldwin assisted with the cluster analyses.

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