

DISTRIBUTION AND GENETIC VARIATION OF *RETICULITERMES* (ISOPTERA: RHINOTERMITIDAE) IN OKLAHOMA

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ABSTRACT

Sequencing of a portion of the mitochondrial DNA 16S gene was undertaken to determine genetic variation and distribution of *Reticulitermes* in Oklahoma. From 16 Oklahoma counties, 43 *R. flavipes*, four *R. hageni*, one *R. virginicus*, and seven *R. tibialis* samples were collected, identified and subjected to DNA sequencing. No genetic variation was observed in *R. virginicus*, while two haplotypes were observed in *R. hageni*, four in *R. tibialis*, and 10 for *R. flavipes*. Among the 10 *R. flavipes* haplotypes, nine nucleotides were variable and genetic variation ranged from 0.2 to 1.4%. Phylogenetic analysis revealed several minor relationships within *R. tibialis* and *R. flavipes*; however, there was no apparent geographical association to the haplotypes. The high amount of genetic variation, but a lack of geographically distinct haplotypes in *R. flavipes*, indicate that this termite species has been distributed randomly in Oklahoma by humans due to its association with structures.

Key Words: 16S, DNA sequence, genetic variation, population genetics, *Reticulitermes*, termite.

RESUMEN

Se llevo a cabo la secuenciación del una porción del gen 16S de ADN mitocondrial para determinar la variación genética y distribución de *Reticulitermes* en Oklahoma. De los 16 condados de Oklahoma, 43 *R. flavipes*, cuatro *R. hageni*, un *R. virginicus*, y siete *R. tibialis* muestras fueron recolectadas, identificadas y sujetas a la secuenciación de ADN. Ningún variación genética fue observada en *R. virginicus*, mientras que dos haplotipos fueron observados en *R. hageni*, cuatro en *R. tibialis*, y 10 en *R. flavipes*. Entre los 10 haplotipos de *R. flavipes*, nueve nucleótidos varían y la variación genética fue de 0.2 hasta 1.4%. Un análisis filogenético reveló una relación menor entre *R. tibialis* y *R. flavipes*; sin embargo, no había una asociación geográfica aparente entre los haplotipos. La cantidad mas alta de variación genética, junta con la falta de haplotipos distintos geográficos en *R. flavipes*, indica que esta especie de termita ha sido distribuida al azar en Oklahoma por humanos debido a su asociación con estructuras.

Subterranean termites in the genus *Reticulitermes* Holmgren belong to the Isopteran family Rhinotermitidae and contain some of the most destructive and damaging termite species with respect to their wood feeding preference. The four principal subterranean termite species in the United States are the eastern subterranean termite *Reticulitermes flavipes* (Kollar), the arid subterranean termite *R. tibialis* Banks, and the dark-southern subterranean termite *R. virginicus* (Banks). Ninety percent of the termite control business in the United States involves these four *Reticulitermes* species plus *Coptotermes formosanus* (Shiraki) (Forschler & Lewis 1997). In Oklahoma, subterranean termites (Isoptera: Rhinotermitidae) are found throughout the state and cause millions of dollars in structural damage every year. The probability that termites will attack a wooden structure within 10 to 20 years after construction is greater than 70% in Oklahoma (Criswell & Pinkston 2001). While the total economic impact of *Reticulitermes* spp. in Oklahoma

is uncertain, anecdotal accounts of their presence and destructive activities within urban areas have been documented (Affeltranger et al. 1987; Anonymous 2001a).

Recently, Brown et al. (2004) conducted a study involving species identification and distribution, and wood consumption rates of termites collected from over 200 sites in Oklahoma using in-ground and surface-ground boards. The most abundant naturally occurring termite species found were in the genus *Reticulitermes*. *Reticulitermes flavipes*, the light-southern subterranean termite *R. hageni* Banks, *R. tibialis*, and *R. virginicus* are known from Oklahoma (Weesner 1965). Presently, the K.C. Emerson Entomology Museum at Oklahoma State University, Stillwater, Oklahoma has 25 *Reticulitermes* specimens of which only 13 have been identified to species (all *Reticulitermes flavipes*). *Reticulitermes* spp. have been reported from less than 25 counties in Oklahoma (out of 75 total) (Anonymous 1999; Anonymous 2000; Anonymous 2001b; Anonymous 2002).

Oklahoma probably has a similar complement of *Reticulitermes* species as found in states with which it has contiguous borders and where surveys have or will be conducted given that they are in the same geographical area with similar climates and habitats with no geographical barriers (Howell et al. 1987; Wang & Powell 2001; Messenger et al. 2002; Austin et al. 2004).

Correctly identifying termites is important because different control methods and strategies may be used depending on the target species. Identifying termite workers to species is difficult, and identifying soldiers is sometimes inaccurate because precise measurements are required and overlap may occur between species (Scheffrahn & Su 1994). Difficulties can arise in species determination from individual collection sites because colonies consist mostly of the worker caste while soldiers are less abundant. Alates are found less frequently in collections given their seasonal occurrences and unpredictable swarming. Soldiers represent only 1-3% of the total population of *Reticulitermes* colonies and are morphologically variable; use of this caste alone for identification can result in equivocal species determinations. Subtle clinal variations imposed by geographic boundaries can influence morphology making correct species determinations difficult.

In contrast, molecular genetic methods are able to differentiate species regardless of caste (Szalanski et al. 2003). Also, genetic information obtained from existing collections can be an integral component to phylogenetic studies as a whole, reflecting potential changes in species distributions over time. The extent of genetic variation and subsequent gene flow in *Reticulitermes* spp. from Oklahoma has never been studied. Previous genetic studies have focused on *Reticulitermes* spp. from the southeastern United States and Western Europe (Jenkins et al. 1998; Jenkins et al. 2001; Marini & Mantovani 2002; Uva et al. 2003). Recently, Austin et al. (2004) conducted the first comprehensive genetic survey of *Reticulitermes* in Texas and found 13 haplotypes of *R. flavipes*, seven *R. tibialis* haplotypes, and one haplotype each for *R. virginicus* and *R. hageni*.

Identification to the species level of specimens from existing collections with molecular techniques as outlined in this study may add significant information on species distribution and gene flow. Genetic variation and gene flow information may elucidate existing patterns of spread, possible hybridization, and general speciation of *Reticulitermes* spp. in Oklahoma. In this study, we investigated the extent of genetic variation within and among Oklahoma *Reticulitermes*, evaluated the utility of genetic markers used for identifying species, expanded the known geographical distribution of these taxa within Oklahoma, and determined if *Reticulitermes* distributions are influenced by human activity.

MATERIALS AND METHODS

Termites were collected from several locations in Oklahoma (Table 1) and preserved in 100% ethanol. In addition to our own collecting efforts, we solicited the assistance of Pest Management Professionals (PMPs) throughout the state to determine the predominant species found in infested structures. PMPs were provided with collection kits and samples were mailed to our laboratory. Fifty-five samples, representing various geographic zones were used for molecular analysis. When available, *Reticulitermes* alates or soldiers were also morphologically identified to species with keys by Krishna & Weesner (1969), Scheffrahn & Su (1994), Hostettler et al. (1995), Donovan et al. (2000). For samples consisting only of workers, species identification was conducted by using mtDNA 16S sequences (Szalanski et al. 2003). Voucher specimens preserved in 100% ethanol are maintained at the Arthropod Museum, Department of Entomology, University of Arkansas, Fayetteville, AR.

Alcohol-preserved specimens were allowed to dry on filter paper, and DNA was extracted from whole individual termites with the Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, MN) per Austin et al. (2002). Extracted DNA was resuspended in 50 μ l of Tris:EDTA and stored at -20°C. Polymerase chain reaction was conducted with primers LR-J-13007 (5'-TTACGCTGTTATCCTAA-3') (Kambhampati & Smith 1995) and LR-N-13398 (5'-CGCCTGTTTATCAAAACAT-3') (Simon et al. 1994). These PCR primers amplify an approximately 428 bp region of the mtDNA 16S rRNA gene. PCR reactions were conducted with 1 μ l of extracted DNA (Szalanski et al. 2000) and a profile consisting of 35 cycles of 94°C for 45 s, 46°C for 45 s and 72°C for 60 s. Amplified DNA from individual termites was purified and concentrated with minicolumns (Wizard PCRpreps, Promega Corp., Madison, WI) according to the manufacturer's instructions. Samples were sent to the University of Arkansas DNA Sequencing Facility (Fayetteville, AR) for direct sequencing in both directions. GenBank accession numbers were AY538739 to AY538744 for the termite haplotypes new to this study and not present in Austin et al. (2004). DNA sequences were aligned by the PILEUP command of GCG (Accelrys, San Diego, CA). Mitochondrial DNA haplotypes were aligned by using MacClade v4 (Sinauer Associates, Sunderland, MA).

The distance matrix option of PAUP* 4.0b10 (Swofford 2001) was used to calculate genetic distances according to the Kimura 2-parameter model of sequence evolution (Kimura 1980). Mitochondrial 16S sequences from the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (GenBank AY558910), and the desert subterranean termite *Heterotermes aureus* (Sny-

TABLE 1. COLLECTION DATA, AND HAPLOTYPES FOR OKLAHOMA *RETICULITERMES* SPP. AND OUTGROUP TAXA.

Species	City	County	Haplotype	N
<i>R. flavipes</i>	Stillwater	Payne	E	1
	—	Payne	E	1
	Spiro	LeFlore	F	1
		McCurtain	F	1
	Norman	Cleveland	F	1
	Stillwater	Payne	F	1
	Grove	Delaware	F	1
	Harrah	Oklahoma	G	1
	Stillwater	Payne	G	1
	Glenpool	Tulsa	H	1
	Mannford	Pawnee	H	1
	Marietta	Love	H	1
	Oklahoma City	Oklahoma	H	1
	Tulsa	Tulsa	H	1
	Tulsa	Tulsa	J	1
	Ardmore	Carter	L	1
	Edmond	Oklahoma	L	2
	Oklahoma City	Oklahoma	L	3
	Slapout	Beaver	L	1
	Stillwater	Payne	L	1
	Tulsa	Tulsa	L	1
	—	McCurtain	L	1
	Oklahoma City	Oklahoma	O	1
	Magnum	Greer	N	1
	—	Greer	N	1
	Colcord	Delaware	P	1
	Edmond	Oklahoma	P	1
	Glenpool	Tulsa	P	1
	Grove	Delaware	P	1
	Mannford	Pawnee	P	1
	Oklahoma City	Oklahoma	P	2
	Owasso	#Tulsa	P	2
	Tulsa	Tulsa	P	4
—	Wagoner	P	1	
Monkey Island	Ottawa	Q	1	
<i>R. hageni</i>	Fort Towson	Choctaw	H1	1
	Grove	Delaware	H1	1
	Jay	Delaware	H2	1
	Fort Towson	Choctaw	H2	1
<i>R. virginicus</i>	Jenks	Tulsa	V1	1
	<i>R. tibialis</i>	Ardmore	Carter	T2
Magnum		Greer	T2	1
Stillwater		Payne	T5	1
Grove		Delaware	T7	1
Goodwell		Texas	T8	1
Owasso		Tulsa	T8	1
Tulsa		Tulsa	T8	1
<i>Coptotermes formosanus</i>		Baton Rouge, LA	outgroup	
<i>Heterotermes aureus</i>	Santa Rita, AZ	outgroup		

der) (GenBank AY380299) were added to the *Reticulitermes* DNA sequences to act as outgroup taxa. The DNA sequences were aligned by the PILEUP program in GCG (Genetics Computer Group, Madison, WI) and adjusted manually. Maximum parsimony analysis on the alignments were conducted with PAUP* 4.0b10 (Swofford

2001). Gaps were treated as missing data. The reliability of trees was tested with a bootstrap test (Felsenstein 1985). Parsimony bootstrap analysis included 1,000 resamplings and used the Branch and Bound algorithm of PAUP*. Because there are few published accounts of the occurrence of *Reticulitermes* spp. in Oklahoma, we compiled all

available data from existing sources and noted them on our distribution map (Fig. 1).

RESULTS

DNA sequencing of the 16S rDNA 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. The aligned DNA data matrix, including the outgroup taxa, resulted in a total of 433 characters. Of these characters, 86 (20%) were variable and 46 (11%) were phylogenetically informative. From the DNA sequence analysis of *Reticulitermes* spp. samples from 16 Oklahoma counties, a total of 43 *R. flavipes*, four *R. hageni*, one *R. virginicus*, and seven *R. tibialis* were identified based on species diagnostic nucleotide sites (Szalanski et al. 2003) (Table 1, Fig. 1). Morphological identifications yielded the same species identification as the DNA sequences. An additional 10 counties, were included from published anecdotal accounts, bringing the total number of reported counties in Oklahoma to 24 (Fig. 1).

No genetic variation was observed in *R. virginicus*, while two unique haplotypes were found in *R. hageni*, four in *R. tibialis* and 10 in *R. flavipes* (Table 1). Pairwise Tajima-Nei distances (Tajima & Nei 1984) among *Reticulitermes* taxa ranged from 5.7% between *R. flavipes* and *R. hageni*, to 8.3% between *R. flavipes* and *R. tibialis*. A total of nine nucleotide sites varied among the 10 *R. flavipes* haplotypes (Table 2) and genetic variation among the *R. flavipes* haplotypes ranged from 0.2 to 1.4% (Table 3). The most com-

mon haplotypes were L and P with 10 and 14 representatives, respectively. Within *R. tibialis*, a total of three sites varied among the four haplotypes and variation ranged from 0.2 to 0.7%. Within *R. hageni*, one nucleotide site was variable between the two haplotypes.

Bootstrap analysis of the aligned *Reticulitermes* spp. and the outgroup taxa resulted in a consensus tree with several distinct branches (Fig. 2). These distinct clades included *R. flavipes*, *R. hageni* and *R. virginicus*; and *R. tibialis*. Within *R. flavipes*, haplotypes Q and F formed a distinct clade. For *R. tibialis*, haplotypes T8 and T5 formed a distinct clade relative to the two other haplotypes. There was no genetic structure observed among the *R. hageni* haplotypes in the present study.

DISCUSSION

This study updates the geographic distribution of, and genetically classifies, the genus *Reticulitermes* in Oklahoma. However, it does not represent a comprehensive survey of *Reticulitermes* spp. in Oklahoma. Rather, it documents new occurrences of *Reticulitermes* spp. in Oklahoma over a large geographic area. In the present study, genetic divergence values were similar to genetic divergence detected in a study of *Reticulitermes* in Texas (Austin et al. 2004). In terms of population structure, a weak relationship was observed between *R. flavipes* haplotypes Q and F. Haplotype F is distributed throughout the central and eastern portions of the state, while haplotype Q was only observed in Ottawa County, which is located in

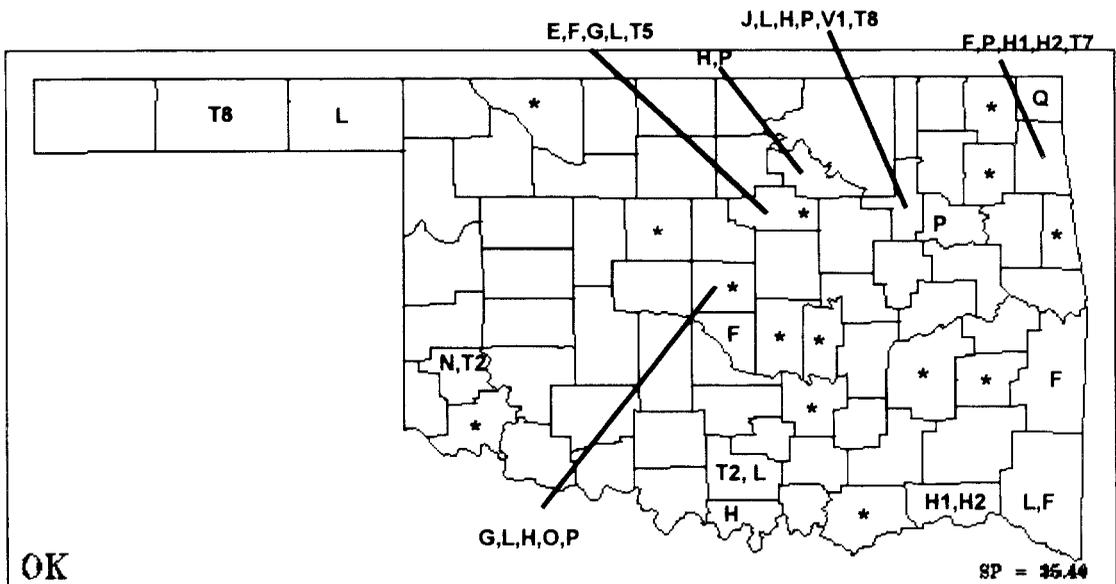


Fig. 1. Distribution of *Reticulitermes* spp. and haplotypes in Oklahoma. Counties designated with an asterisk represent reported cases of *Reticulitermes* spp. but were not used in our genetic analysis and are merely included to update the current distribution of the genus in Oklahoma.

TABLE 2. HAPLOTYPE VARIATION AT NINE NUCLEOTIDE SITES AMONG *RETICULITERMES FLAVIPES* FROM OKLAHOMA.

Haplotype	130	131	158	162	168	179	236	271	274
E	A	A	A	G	A	C	A	T	G
F	*	*	G	*	G	*	C	*	*
G	*	G	G	*	*	*	C	C	*
H	*	*	G	*	*	*	C	*	*
J	*	G	G	*	*	*	C	*	*
L	*	*	G	*	*	G	C	*	*
N	*	*	G	A	*	T	C	*	A
O	T	C	G	A	*	*	C	*	*
P	*	*	G	*	*	*	C	C	*
Q	*	A	G	*	G	T	C	*	G

the northeast corner of Oklahoma. For *R. tibialis* haplotypes T5 and T8 formed a distinct clade, and were collected from two non adjacent counties. In general, there was no population structure for *Reticulitermes* spp. based on genetic haplotypes. Likely reasons for this could be attributed to anthropogenic origins, a lack of nestmate agonism (mixing with non-nestmates imposed by foraging traffic in complex colonies with multiple reproductive centers) (Bulmer & Traniello 2002), or from mixing between different colonies (Clément 1986). In fact, for *R. flavipes*, six of the 10 observed haplotypes (E, F, G, H, J, and L) are shared with Texas. More thorough sampling including termite specimens from more counties are desirable to reveal any existing genetic patterns.

Both *R. virginicus* (Jenks, OK) and *R. hageni* (Grove, OK) were found only in the eastern part of the state where two-thirds of Oklahoma's forest ecosystem consisting of over two million ha of Oklahoma's timberlands (Lewis 2001). This distribution of *R. virginicus* and *R. hageni* in Oklahoma was also observed by Brown et al. (2004). These species are generally found in areas of minimal human disturbance, which may account for their respective occurrences in this study and previous studies. For example, in Arkansas, *R. virginicus* and *R. hageni* are more prevalent in undisturbed habitats (JWA, unpublished). Similarly, the abundance of these species in eastern

Oklahoma may indicate central and western Oklahoma represent an east to west transition zone which delimits the westernmost occurrence of *Reticulitermes* species not commonly known from western U.S. states. Interestingly, Oklahoma *R. tibialis* from Ardmore and Stillwater share haplotypes with *R. tibialis* from Texas (T2 and T5, respectively). Also, two of the three *R. hageni* samples are identical to the only southern subterranean termite haplotype observed in Texas (Austin et al. 2004).

Competition between ecologically similar termite species can lead to coexistence through resource partitioning (Houseman et al. 2001). Given *Reticulitermes* ability to hybridize (Clément 1979) and fuse colonies, the opportunity to observe greater genetic diversity is probable, particularly in sympatric zones, where otherwise strong species isolation mechanisms (behavioral, chemical, or temporal) are inadequate to prevent hybridized mating (Austin et al. 2002). Because colony structure and the spatial organization of foraging *Reticulitermes* spp. is less understood, population studies such as this are important in understanding the complex ecology of subterranean termites and *Reticulitermes* spp. in general. By expanding our genetic investigations of *Reticulitermes* spp. from additional geographic zones, the ecological interactions of this genus can be better understood.

TABLE 3. GENETIC DIVERGENCE AMONG *RETICULITERMES FLAVIPES* HAPLOTYPES (HAP) FROM OKLAHOMA.

Hap	Q	F	P	H	J	O	E	G	N
Q	—								
F	0.002	—							
P	0.007	0.005	—						
H	0.005	0.002	0.002	—					
J	0.007	0.005	0.005	0.002	—				
O	0.009	0.007	0.007	0.005	0.005	—			
E	0.009	0.007	0.007	0.005	0.007	0.009	—		
G	0.012	0.009	0.005	0.007	0.005	0.009	0.012	—	
N	0.007	0.009	0.009	0.007	0.009	0.007	0.012	0.014	—

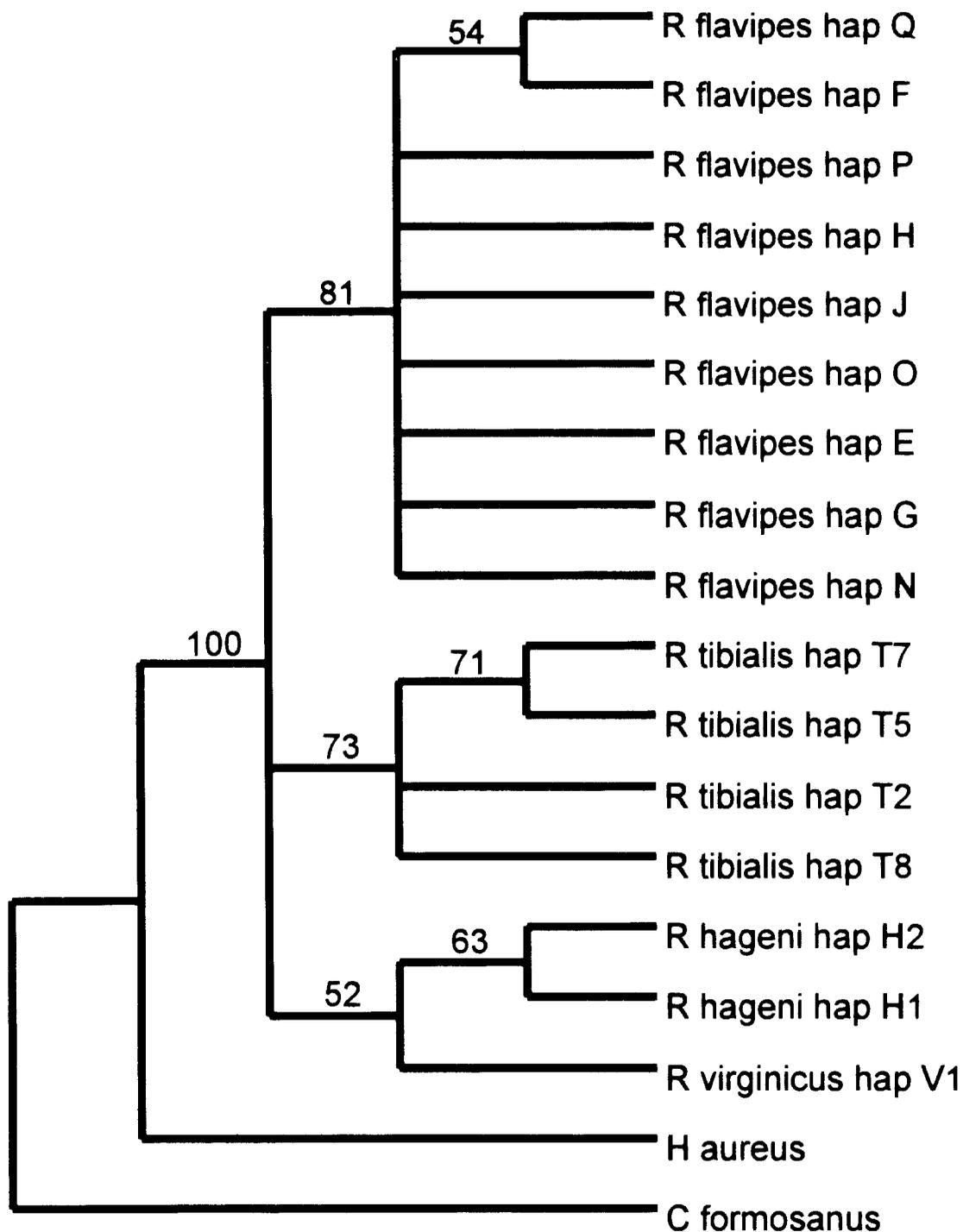


Fig. 2. Single most parsimonious tree during a branch and bound search with PAUP*. Bootstrap values for 1,000 replicates are listed above the branches supported at $\geq 50\%$. Tree length = 121, CI = 0.777.

ACKNOWLEDGMENTS

We thank contributors who provided specimens, particularly the PMPs of Oklahoma. Special thanks are given to Martyn Hafley (FMC, Philadelphia, PA), Matt

Messenger (Dow Agrosociences, Indianapolis, IN) and Paul Baker (University of Arizona) for their contribution of samples. This research was supported in part by the University of Arkansas, Arkansas Agricultural Experiment Station.

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