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Genetic Variation and Phylogenetics of *Reticulitermes* (Isoptera: Rhinotermitidae) from the American Great Plains

by

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ABSTRACT

A molecular genetics study involving DNA sequencing of a portion of the mitochondrial 16S rRNA gene was undertaken to determine the extent of genetic variation with *Reticulitermes* and the distribution of *Reticulitermes* subterranean termites in the American Great Plains States extending from Montana to North Dakota, southwards through Nebraska and Kansas to Texas. One Hundred and eighty-one samples were sequenced and evaluated, representing 91 locations from 9 of 10 Great Plains States. A total of 160 *R. flavipes*, 18 *R. tibialis*, two *R. virginicus*, and one *R. hageni* were identified. The corresponding numbers of haplotypes for represented taxa include one haplotype of *R. hageni*, two haplotypes of *R. virginicus*, 10 haplotypes for *R. tibialis*, and 32 for *R. flavipes*. Among the 36 *R. flavipes* haplotypes, 21 nucleotides were variable and genetic variation ranged from 0 to 1.2%. Phylogenetic analysis did not reveal any specific geographic relationships among the *R. tibialis* and *R. flavipes* haplotypes. This study notes the first positive identification of *R. virginicus* residing in northern Nebraska, and expands our current knowledge of its western distributions. Additionally, this study adds important census data, corroborates identifications due to the unavailability of diagnostic castes, and confirms the known phyletic relationships of *Reticulitermes* from this region, discussing environmental restrictions which likely explain the observed occurrences of species there.

Keywords: 16S rRNA, *Reticulitermes*, termites, American Great Plains, DNA sequence.

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INTRODUCTION

There have been recent research efforts to identify the population boundaries of different Nearctic *Reticulitermes* species throughout North America applying various genetic markers. The application of genetic markers are preferred because of the clarity and number of unambiguous characters resulting in more resolute identification, particularly in instances where diagnostic castes are unavailable (Szalanski *et al.* 2003, Foster *et al.* 2004). Studies focusing on the south central United States (Austin *et al.* 2004a, b, c) and western United States (Copren *et al.* 2005, Tripodi *et al.* 2006, Szalanski *et al.* 2006) have recently been reported. Still further studies which are looking at cryptic species within the genus are forthcoming (JWA, unpublished), and comprehensive studies of individual species (Austin *et al.* 2005a) are now providing important insight into their distribution and genetic constitutions. However, there are presently no current studies which document *Reticulitermes* distribution in the American Great Plains (Fig. 1) which include Nebraska and Kansas, both central to this region of transitional topography and resource availability. As was observed in studies focusing on the south central United States (Szalanski *et al.* 2003, Austin *et al.* 2004a, b, c), there appear to be four distinct termite species occupying Nebraska and Kansas: *R. flavipes* (Kollar), *R. virginicus* (Banks), *R. tibialis* Banks, and on occasion *R. hageni* Banks.

In Nebraska, there are two *Reticulitermes* species cited in literature indicating the presence of both *R. flavipes* and *R. tibialis*. It is generally reported that *R. tibialis* prefers the western portion of the state due to its arid habitat preferences, while *R. flavipes* prefers a higher general humidity (Krishna & Weesner 1970); however, there are reports which show that its distribution is continuous throughout the state, residing in the sand dunes of central Nebraska (Light & Pickens 1934) and as far east as Lincoln, Nebraska (Compton 1951). *Reticulitermes flavipes* is likely to be found throughout both these states, as its range extends further west than previous reports (Light & Pickens 1934, Nutting *et al.* 1990, Krishna & Weesner 1970, Austin *et al.* 2005a). Limited information exists which document the respective occurrences of these termites within these two states. In Nebraska, collections deposited in the 2002 National termite survey show that *R. flavipes* has been recovered from Alliance, Auburn, Dawson, Holdrege, Lincoln, Omaha, and Wahoo (Messenger 2002).

In Kansas, *R. flavipes* has been recovered from Alton, Augusta, Baldwin, Caney, Chanute, Corning, Douglass, Fredonia, Hudson, Hutchinson, Independence, Lawrence, Leavenworth, Leoti, Lincoln, McPherson, Olathe, Parsons, Plainville, Rose Hill, Russell, Sabetha, Scott City, Silver Lake, Stockton, Sylvan Grove, Topeka, and Wichita (Messenger 2002). This same collection shows that no samples of *R. tibialis* were recovered from Nebraska, but that several were collected from Buhler, Halstead, Hutchinson, Russell, and Wichita, Kansas. Reported flight dates for *R. tibialis* from 1966 and 1967 in Kansas have been recorded in January (Krishna & Weesner 1970). Snyder (1948) suggests that alate morphology from this region is highly variable.

Another *Reticulitermes* species less frequently observed in this region is *R. virginicus* (Banks), with collection sites in Kansas from Augusta, Beloit, Buhler, Ellsworth, and Great Bend (Messenger 2002). There are no current records of *R. virginicus* in Nebraska; however, there is evidence of this species on the Nebraska-Missouri border (Messenger 2002), and from personal collections in north central Missouri (JWA, unpublished). Although not found in Nebraska, *R. hageni* Banks has been documented to occur in Topeka, Kansas. Flight records of alates from Kansas during 1966-1967 suggested that *R. hageni*'s western limits likely occur around Kansas City, Kansas, where it was first reported by Hungerford (1935) and in Atchison (Krishna & Weesner 1970).

The purpose of this research was to update known occurrences of the species occupying this region of the Nearctic, corroborate identities applying molecular markers and measure their genetic variation compared to other studies of this type, and to compare their phyletic relationships.

MATERIALS AND METHODS

Termites were collected from various locations in Nebraska and Kansas and preserved in 100% ethanol (Table 1). We solicited the assistance of Pest Management Professionals (PMPs) throughout these states for the purpose of interpreting the predominant species recovered from infested structures and from samples obtained during the 2002 National Termite Survey (Messenger 2002). PMPs were provided with collection kits and samples were mailed to our laboratory for analysis. Morphological characters from *Reticulitermes* were used for identification applying the keys of Krishna & Weesner

Table 1. Collection data, haplotypes and number of samples for Colorado, Kansas, Montana, Nebraska, New Mexico, North Dakota, Oklahoma, South Dakota, Texas, and Wyoming *Reticulitermes* and outgroup taxa.

State	(number of samples)	Species	City or County (Haplotype-number from that location)
Colorado	(5)	<i>R. flavipes</i>	Arvada (L), Broomfield (SS), Lakewood (QQ), Manitou Springs (RR), Pueblo (RR)
	(1)	<i>R. tibialis</i>	Ft. Collins (T27)
Kansas	(56)	<i>R. flavipes</i>	Augusta (F, II, P, TT), Buhler (J), Carbondale (C), Corning (II), Douglas (F23, KS2), Ellsworth (L), Eureka (KS3), Great Bend (L), Hudson (II, M), Hutchison (M-3, R, UU), Independence (P), Lawrence (F23, Q), Liberal (F, KS5, L), Lincoln (GG), Netawaka (P), Park City (L), Plainville (II), Topeka (F, KS3, L, M, P, TT, U), Rose hill (F, KS1), Russell (H), Sabetha (KS4, TT), Savonburg (TT), Silver Lake (F23), Stockton (F23, GG), Sylvan Grove (L), Wichita (DD-2, II-2, KS1, KS3-2, L, M-2, P, XX-2)
	(5)	<i>R. tibialis</i>	Butler (T7), Halstead (T36-2), Hutchison (T33), Russell (T32)
Montana	(0)	----	----
North Dakota	(1)*	<i>R. tibialis</i>	Golden Valley (no sequence)
Nebraska	(18)	<i>R. flavipes</i>	Dannebrog (F), Dawson (J), Forest Hills (Z), Holdrege (FF), Lincoln (II-2, M-3, P), Omaha (F, GG, II-2, M, P), Waco (J), Wakefield (F5)
	(1)	<i>R. tibialis</i>	Sidney (T2)
	(1)	<i>R. virginicus</i>	Jackson (V1)
New Mexico	(1)	<i>R. flavipes</i>	Roy (BB)
Oklahoma	(36)	<i>R. flavipes</i>	Ardmore (L), Edmond (L), Glenpool (H), Greer Co. (P), Harra (G), Mangum (P), Mannford (H, L, T), Marietta (H), Oklahoma City (H, L-3, S, T), Owasso (T-2), Payne Co. (E), Ponca City (L), Sapouh (L), Stillwater (C, E, F, G, L), Tulsa (H, J, L, T-4), Wagoner Co. (T), Westville (P)
	(7)	<i>R. tibialis</i>	Ardmore (T2), Goodwell (T8), Mangum (T2), Stillwater (T5), Tulsa (T8), Owasso (T8)
	(1)	<i>R. virginicus</i>	Jenks (V2)
South Dakota	(1)	<i>R. flavipes</i>	North Sioux (M)

*Specimen identified morphologically, with no genetic analysis (provided courtesy of North Dakota State University, Department of Entomology. Collected 17 June 1968 by B. Goodfellow).

†All states sampled may have substantially more termites than presented here, as the intent was to only observe *Reticulitermes* from the American Great Plains, and not merely from states which are inclusive to this geographic designation as depicted in Fig. 1.

Table 1 continued. Collection data, haplotypes and number of samples for Colorado, Kansas, Montana, Nebraska, New Mexico, North Dakota, Oklahoma, South Dakota, Texas, and Wyoming *Reticulitermes* and outgroup taxa.

State	(number of samples)	Species	City or County (Haplotype-number from that location)
Texas	(33)	<i>R. flavipes</i>	Addison (L), Amarillo (H), Arlington (G), Austin (E, F), Carrollton (C), Dallas (F-2, G), Del Rio (B, G), Dumas (M), Fritch (L), Granbury (E), Grapevine (C), Irving (L-2), Lewisville (F), Lubbock (G1, I, L-2), Midland (G, L), Odessa (F), Post (M), Richardson (J), Round Rock (F), Rowlett (G, L), San Angelo (C-2, KS1, KS2, L-2), San Antonio (C), Stephenville (G), Taylor (C), Waco (D)
	(1)	<i>R. hageni</i>	Lewisville (H1)
	(4)	<i>R. tibialis</i>	Ft. Worth (T1), Brackettville (T2), Happy (T5), DeSoto (T6)
Wyoming	(1)	<i>R. flavipes</i>	Torrington (U)

*Specimen identified morphologically, with no genetic analysis (provided courtesy of North Dakota State University, Department of Entomology. Collected 17 June 1968 by B. Goodfellow).

†All states sampled may have substantially more termites than presented here, as the intent was to only observe *Reticulitermes* from the American Great Plains, and not merely from states which are inclusive to this geographic designation as depicted in Fig. 1.

(1970), Scheffrahn & Su (1994), and Hostettler *et al.* 1995. Two additional taxa (Table 1) were included as outgroup taxa to corroborate relationships within the genus for our phylogenetic analysis. Voucher specimens preserved in 100% ethanol are maintained at the Arthropod Museum, Department of Entomology, University of Arkansas, Fayetteville, AR and at the Center for Urban and Structural Entomology, Department of Entomology, Texas A&M University, College Station, TX.

Alcohol-preserved specimens were allowed to dry on filter paper, and DNA was extracted according to Liu & Beckenbach (1992) on individual worker termites with the Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, MN). Extracted DNA was resuspended in 50 µl of Tris:EDTA and stored at -20°C. Polymerase chain reaction (PCR) of a 436bp fragment of the 16S rRNA gene was conducted using the primers LR-J-13007 (forward: 5'-TTA CGC TGT TAT CCC TAA-3') (Kambhampati & Smith 1995), and LR-N-13398 (reverse: 5'-CGC CTG TTT ATC AAA AAC AT-3') (Simon *et al.* 1994).

The PCR reactions were conducted with 1 µl of the extracted DNA (Szalanski *et al.* 2000), having a profile consisting of 35 cycles of 94°C for 45 s, 46°C for 45 s and 72°C for 60 s. Amplified DNA from individual termites

was purified and concentrated with minicolumns (Wizard PCRpreps, Promega) according to the manufacturer's instructions. Samples were sent to the DNA core sequencing facility at The University of Arkansas Medical, Little Rock for direct sequencing in both directions. GenBank accession numbers were DQ515973 to DQ515982 for termites subjected to DNA sequencing in this study. DNA sequences were aligned using the BioEdit software (Hall 1999) and adjusted manually. Mitochondrial DNA haplotypes were aligned using MacClade v4 (Sinauer Associates, Sunderland, MA). Additional DNA sequences were obtained from prior investigations which included some locations within the American Great Plains (Austin *et al.* 2004a, b, 2005a, b).

The distance matrix option of PAUP* 4.0b8 (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 termite, *Coptotermes formosanus* Shiraki, and *Heterotermes aureus* (Snyder) were added to the *Reticulitermes* DNA sequences to act as outgroup taxa. Maximum likelihood and unweighted parsimony analysis on the alignments were conducted with PAUP* 4.0b8 (Swofford 2001). Gaps were treated as missing data. The reliability of trees was tested with a bootstrap test (Felsenstein 1985). Parsimony bootstrap analysis included 1,000 resamplings and used the Branch and Bound algorithm of PAUP*. For maximum likelihood analysis, the default likelihood parameter settings were used (HKY85 6-parameter model of nucleotide substitution, empirical base frequencies, and transition/transversion ratio set to 2:1). These parameters were used to carry out a heuristic search using PAUP*, by applying either the single most parsimonious tree as the starting tree or by step-wise addition.

RESULTS

DNA sequencing of the 16S rRNA amplicon revealed that it averaged 436 bp in size. The average base frequencies were A = 0.39, C = 0.23, G = 0.14, and T = 0.24. The aligned DNA data matrix, including the outgroup taxa resulted in a total of 436 characters. Of these characters, 33 (7.5%) were variable and 55 (12.6%) were phylogenetically informative. From the phylogenetic analysis of *Reticulitermes* from 91 locations spanning the American Great Plains (Fig. 1), a total of 160 *R. flavipes*, 18 *R. tibialis*, two *R. virginicus*, and one *R. hageni* were identified (Table 1). One *R. tibialis* from Golden



Fig. 1. Map of the American Great Plains of the Nearctic.

Valley, North Dakota was only evaluated morphologically as we were unable to amplify DNA from the provided material. The sole *R. hageni* recovered in the present study (haplotype H1) is from Lewisville, Texas. This study identified two *R. virginicus* samples, haplotypes V1 and V2, from Jackson, NE and Jenks, OK, respectively. Both *R. flavipes* and *R. tibialis* were found contiguously throughout the Great Plains region (Fig. 1) with significantly more samples represented in the present study from Kansas and Nebraska, at the center of this geographic zone (Fig. 2). There was no difference in the respective topologies while conducting either the Maximum Likelihood or maximum parsimony analyses, therefore only the results of the maximum parsimony analysis is discussed.

This dataset had only one most parsimonious tree (Fig. 3), (length = 164, CI = 0.604, RI = 0.739), as documented using the Branch and Bound search algorithm of PAUP*. Bootstrap analysis of the aligned *Reticulitermes* species and the outgroup taxa resulted in a consensus tree with all branches

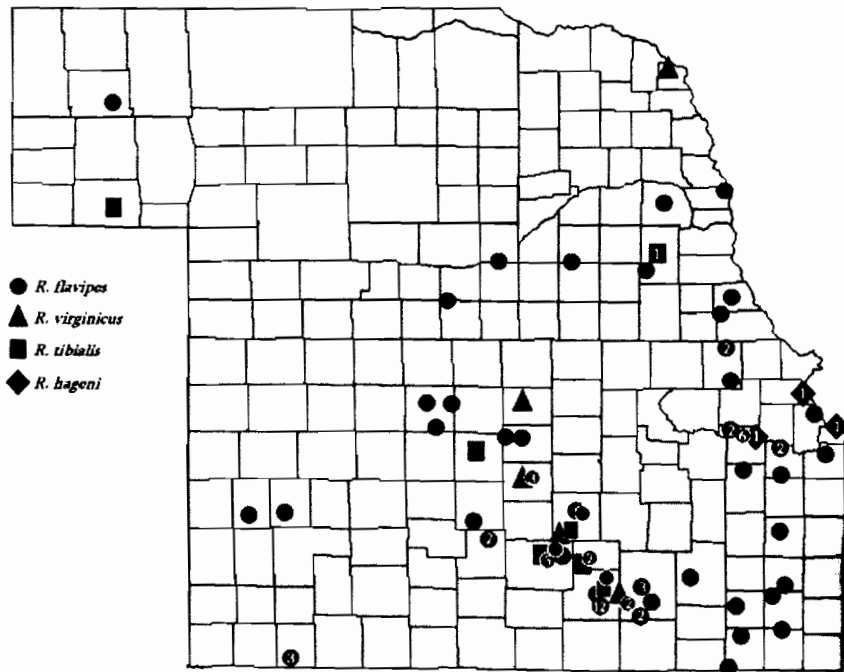


Fig. 2. Distribution of *Reticulitermes* species from Nebraska and Kansas. Numbers within icons represent the number of specimens recovered from that location.

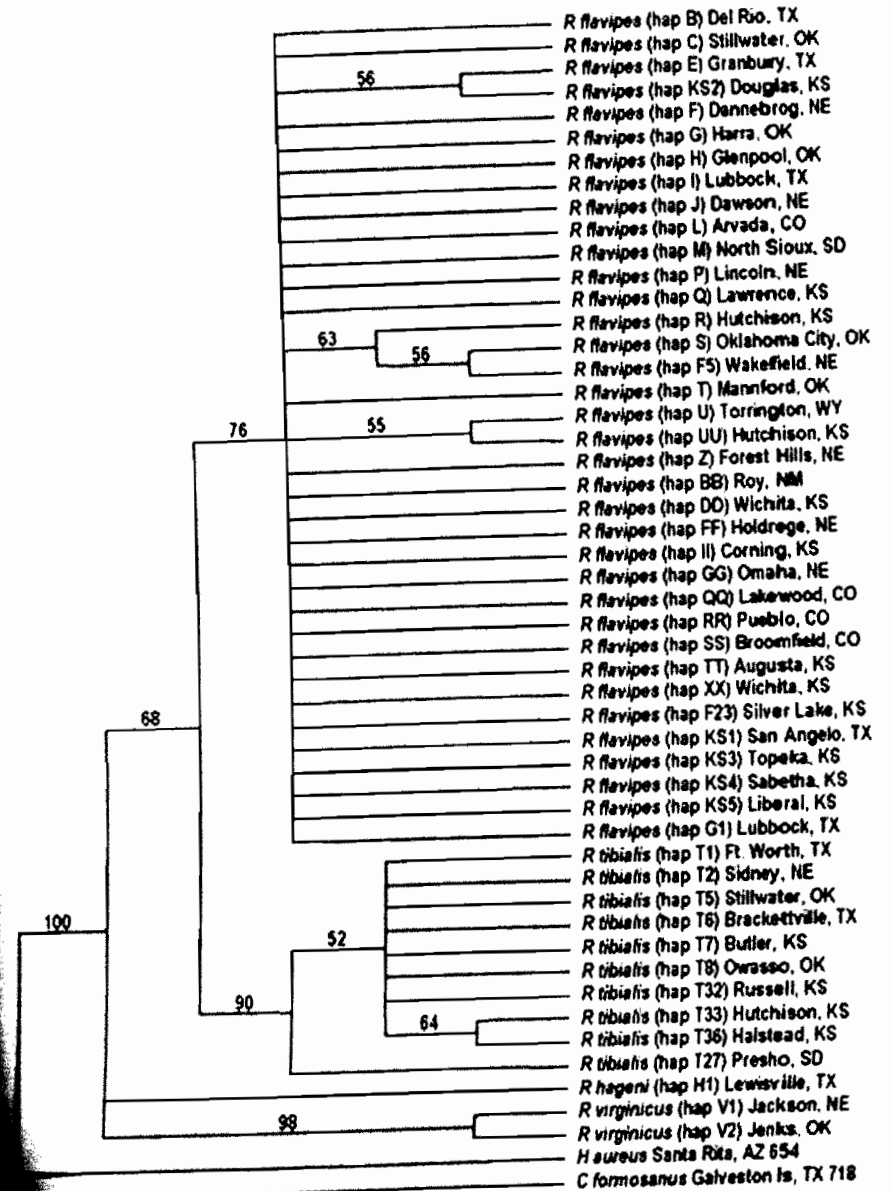


Fig. 3. 16S rRNA single most parsimonious tree for *Reticulitermes* from the American Great Plains using a branch and bound search using PAUP*. Bootstrap values for 1,000 replicates are listed above branches supported at $\geq 50\%$.

demonstrating the monophyletic nature of species within the genus, and the resolution of the 16S rRNA marker for accurate identification of species from the Nearctic. These distinct clades included: *R. flavipes*, *R. hageni*, *R. virginicus*, and *R. tibialis*. These relationships have been observed repeatedly in other genetic studies using both Cytochrome Oxidase II gene sequences (Austin *et al.* 2002, Jenkins *et al.* 1998, 1999), and 16S rRNA (Austin *et al.* 2004a, b, c). There was no apparent genetic structure associated with geographic occurrences based on such a small sample size for the observed haplotypes in the present study.

Pairwise Tajima-Nei distances (Tajima & Nei 1984) among *Reticulitermes* taxa ranged from 2.3 to 3.5 % between *R. flavipes* and *R. hageni*, to 1.8 to 4.0 % between *R. flavipes* and *R. tibialis*. Neither *R. virginicus* or *R. hageni* provide much insight into their respective variations from the region given their small sample sizes. Variation within *R. flavipes* ranged from 0 to 1.2 %. Within *R. flavipes*, a total of 21 nucleotides were variable and 36 haplotypes were identified (Table 2). The variation observed among *Reticulitermes* groups is slightly lower than in previous studies from the south central United States (Austin *et al.* 2004a, b), but well within the range anticipated.

DISCUSSION

Within the American Great Plains region (Fig. 1) the distribution of *Reticulitermes* species from some states has only recently been determined (Austin *et al.* 2004a, b; 2005a, b). In the southernmost part of the Great Plains, Texas and Oklahoma have four residing species of *Reticulitermes*: *R. flavipes*, *R. virginicus*, *R. hageni*, and *R. tibialis* (Austin *et al.* 2004a, b, c). Of these species, *R. flavipes* and *R. tibialis* appear to have the broadest distributions recorded. This appears equally true for the states of Kansas and Nebraska (Fig. 2). A recent investigation of the distribution of *R. flavipes* in North America demonstrates that *R. flavipes* can be found in almost all of the states which encompass the American Great Plains. However, the diversity of species at the northern range of the Great Plains may be more restricted. This is likely due to the nature of cold weather there, which has been demonstrated to confine them to a zone below an adequate source of food, which is usually near the ground surface (Ebeling 1975). For example, Esenther (1961) demonstrated that *Reticulitermes* from Wisconsin, could sustain expanded periods of tem-

Table 2. Nucleotide differences for 36 *R. flavipes* populations from the American Great Plains¹ from a 436 bp fragment of the 16S rRNA gene.

HAP	55	62	80	96	122	132	144	160	161	165	173	174	175	186	213	243	269	278	279	282	292	
B	G	T	T	T	A	G	G	A	G	G	G	T	-	C	T	C	-	T	T	A	G	
C	*	*	*	*	*	A	*	*	*	*	A	*	*	*	T	*	*	*	*	*	*	*
E	*	*	*	*	*	A	*	*	A	*	A	*	*	*	*	*	*	*	*	*	G	*
F	*	*	*	*	*	A	*	*	*	*	*	*	A	*	*	*	*	*	*	*	G	*
G	*	*	*	*	T	*	*	*	*	*	A	*	*	*	*	*	*	*	*	C	G	*
H	A	*	*	*	*	A	*	*	*	*	A	*	*	*	*	*	*	*	*	*	G	*
I	A	*	*	*	*	A	*	*	*	*	A	*	*	T	C	*	*	*	*	*	G	*
J	*	*	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	G	*
K	*	*	*	*	*	A	*	*	*	*	A	*	*	T	*	*	*	*	*	*	G	*
L	*	*	*	*	*	A	*	*	*	*	A	*	*	*	*	*	*	*	*	*	G	*
M	*	*	*	*	*	A	*	*	*	*	A	*	*	*	*	*	*	*	*	C	G	*
P	*	*	*	*	*	A	*	*	*	*	*	*	*	T	*	*	*	*	*	*	G	*
Q	*	*	*	*	*	A	*	*	*	*	A	*	*	*	*	*	*	*	*	*	G	A
R	*	*	*	*	*	A	*	*	*	A	A	*	*	*	*	*	*	*	*	*	G	A
S	*	*	*	*	*	A	*	*	*	*	A	*	*	*	*	*	*	C	C	G	G	*
T	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	*	*	C	*	G	*	*
U	*	*	*	C	*	A	*	*	*	*	A	*	*	A	*	*	*	*	*	C	G	*
Z	*	*	*	*	*	A	*	*	*	*	A	*	*	*	*	*	*	*	*	C	G	*
BB	*	*	*	*	*	A	A	*	*	*	A	*	*	*	*	*	*	*	*	*	G	*
DD	*	*	*	*	*	A	A	*	*	*	A	*	*	C	*	*	*	*	C	G	*	*
FF	*	*	*	*	*	A	*	*	*	*	*	*	G	*	*	*	*	*	*	*	G	*
H	*	*	*	*	*	A	*	*	*	*	*	*	G	*	*	*	*	*	*	*	G	*
GG	*	*	*	*	*	A	*	*	*	*	A	*	*	*	*	*	*	*	*	*	G	*
QQ	*	*	*	*	*	A	T	*	*	*	*	*	G	*	*	*	*	*	*	*	G	*
RR	*	*	*	*	*	A	*	*	*	*	*	*	G	*	*	*	*	*	*	*	G	*
SS	*	*	*	*	*	A	*	*	*	*	*	*	A	*	*	*	*	*	*	*	G	*
TT	*	*	*	*	*	A	*	*	*	*	*	*	G	*	*	*	*	C	*	G	*	*
UU	*	*	*	*	*	A	*	*	*	*	A	A	*	T	*	*	T	*	*	*	G	*
XX	*	*	*	*	*	A	*	*	*	A	A	*	*	*	*	*	*	*	*	*	G	A
F5	*	*	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	C	G	*	*
F23	*	*	*	*	*	A	*	*	*	*	A	*	*	T	*	*	*	*	*	*	G	*
KS1	*	*	*	*	*	A	*	A	*	*	A	*	*	*	*	A	*	*	*	*	G	*
KS2	*	C	*	*	*	A	*	*	*	*	*	*	G	*	*	*	*	*	*	*	G	*
KS3	*	*	C	*	*	A	*	*	*	*	*	*	G	T	*	*	*	*	*	*	G	*
KS4	A	*	*	*	*	A	*	*	*	*	*	*	G	*	*	*	*	*	*	*	G	*
KS5	*	*	*	*	*	*	*	*	*	*	A	*	*	*	*	A	*	*	*	*	*	*

¹Nucleotides within a given column followed by asterisks are not different from Haplotype B (Del Rio, Texas).

perature near freezing and noted that soil temperatures, although varied according to location, were seldom below -3.8 °C (25 °F) for some cities throughout the winter. He postulated that the approximate northern limit for the eastern subterranean termite, *R. flavipes*, coincides with an annual minimum isotherm of -30 °C (-22 °F). Similarly, recorded soil temperatures

in Lincoln, NE, during the months of December, January, and February at a depth of 91.4 cm from the years 1894-1902 (Swezey 1903) revealed that temperatures rarely dropped below 0°C. Continuous feeding and movement into structures in the midst of winter has been routinely observed in Omaha and Lincoln, NE (JWA, personal observation) and periods of slow cold acclimation by *R. flavipes* can prolong their survival at suboptimal temperatures (Davis & Kamble 1994). Assuming that soil temperature profiles have changed very little over the past 100 years, it seems unlikely that *R. flavipes* colonies are ever exposed to sub-zero temperatures during winter if they move >100 cm underground (Cabrera & Kamble 2001).

Only two species, *R. flavipes* and *R. tibialis*, have been collected from South Dakota and North Dakota (JWA unpublished). However, based on the identification of a newly recorded cryptic *Reticulitermes* species residing in the Western United States and Southern British Columbia (Szalanski *et al.* 2006), there remains a possibility of this new, yet unidentified species, occurring at the western range of the American Great Plains in Montana as well. The western boundaries of the Great Plains states of eastern New Mexico, Colorado, and Wyoming are all represented with samples of *R. flavipes* and *R. tibialis*, but to date we have no samples for evaluation from Montana. However, Castle (1944) lists the specific occurrences of *Reticulitermes* species in Montana as occurring in: Miles City, Harding, Billings, Havre, Dillon, Terry, Powderville, Three Forks, Florence, and Miller Creek Canyon south of Missoula. Furthermore, Castle suggests that although prior to his report (1944) all accounts of *Reticulitermes* were recorded in extreme western and Southwestern Montana, but that *R. tibialis* likely could be found extending over the entire eastern half of the state and in the drier valleys at lower elevations in western Montana, frequently associated with sage-brush and rabbit-brush or other plants in semi-arid environments. This fact would coincide with the collection of *R. tibialis* from the western areas of North Dakota where our sole sample for evaluation was collected from Golden Valley, ND. Future collecting efforts should try to establish the current distributions and abundance of species there, which may have changed over the past 60 years with greater human involvement and urban expansion. In fact, we have considerably more material from all of these states, but they do not occur in areas currently defined as the Great Plains (Fig. 1).

The northern range of *R. virginicus* and *R. hageni* had historically been depicted as occurring through the central Missouri valley expanding westward towards southeastern Kansas south towards the Texas-Louisiana border areas (Light & Pickens 1934). However, results in the present study may require a reassessment of this distribution. We positively identified *R. virginicus* from Dakota County Nebraska, which must be either an extreme northern limit for this species or an accidental introduction of anthropogenic origin. Interestingly, recent confirmation of the presence of *R. virginicus* in central Colorado near Longmont has been proposed, and alate samples were taken from infested structures there and compared to type specimens at the Smithsonian for positive identification (L. Bjostad, personal communication). Accounts from unpublished historical records in Colorado for *R. virginicus*, *R. flavipes*, and *R. hageni* in the southeast corner of the state are known to have been collected by Dr. Francis Weesner Lechleitner (W. Cranshaw, personal communication). *Reticulitermes tibialis* is present in all of the Great Plains states based on our findings and published records.

Hungerford (1935) submitted a short note in 1936 which described *R. hageni* infesting a home in Lawrence, Kansas in early October. He sent the sample to Drs. N. Banks and T.E. Snyder at the Bureau of Entomology and Plant Quarantine, U.S. Department of Agriculture, where its identity was confirmed. This swarming time would be consistent with other accounts of swarming alate flights which have been documented as early as August 25 in that region of Kansas (Atchison and Wyandotte counties) (Krishna & Weesner 1970). More recent anecdotal information about the continued presence of *R. hageni* in the Kansas City area has been recorded (MTM, unpublished). It is important to keep in mind that these dates, while they may be reflective of a chronological species isolation phenomenon, are largely influenced by precipitation and the accumulation of thermal units which trigger the biological release mechanism that drive *Reticulitermes* (such as *R. hageni*) to initiate swarming behavior (Furman 2000), and as such, may vary considerably. Previous collecting efforts to determine *Reticulitermes* distribution patterns over the south central United States have appeared to elucidate an east to west transition zone for *R. tibialis* (Austin *et al.* 2004c), and recorded accounts within the present study are no different. This is equally true as an apparent transition zone occurs too, for two largely southeastern species: *R. virginicus*

and *R. hageni*. From an environmental perspective, the transition of species in this region likely is associated with numerous geological, meteorological, and vegetative patterns which demark east from west here.

The warm, moist tropical maritime air flowing in from the Gulf of Mexico, the prime contributor of moisture to the Plains, commonly curves up the Mississippi Valley and then moves northeast, missing much of the western Great Plains entirely, resulting in the marked westward decline in average precipitation amounts. For instance, in Kansas, average annual precipitation varies from a moist 105 centimeters in the southeast to a semiarid 40 centimeters in the southwest (Birdsall & Florin 1998). This would seem to coincide with collection information of species from Kansas in the present study (Fig. 2). In central and northwestern Nebraska, the Sand Hills offer a dense, intricate pattern of grass-covered sand dunes, many of which are well over 30 meters high. The dunes were created by sand blowing along the southern margins of continental glaciers during the Pleistocene (Birdsall & Florin 1998). Glacial refugium theories are among the more recent explanations for the distribution of *Reticulitermes* from the Palearctic (Marini & Mantovani 2002, Uva *et al.* 2004, Luchetti *et al.* 2004, 2005) and the Near and Middle East (Austin *et al.* 2002, 2006), and their corresponding topographies would seem to support the most logical explanation for the occurrences of Nearctic species within the genus.

One of the more interesting observations in terms of distribution was the documentation of *R. virginicus* in northern Nebraska. This is the first record of this species in the state, and significantly furthers its known distribution range. It remains unclear as to whether these populations represent natural residing populations that are only now being documented, or if they have anthropogenic origins as observed in other studies from this region (Austin *et al.* 2004a, b). One of the principle culprits in these occurrences may be due to irrigation practices. The High Plains is a major agricultural region, providing, for example, two-fifths of America's sorghum, one-sixth of its wheat, and one-quarter of its cotton. Irrigated lands here produce 45 percent more wheat, 70 percent more sorghum, and 135 percent more cotton than neighboring nonirrigated areas (Birdsall & Florin 1998). Groundwater withdrawals have more than tripled since 1950, to more than 20 million acre-feet annually (Birdsall & Florin 1998). It would seem logical that as irrigation patterns

change, so too would the restrictions for species such as *R. virginicus* and *R. hageni*, enabling them to expand to more western and northern areas within this region.

Because the present study has relied heavily on material submitted by PMPs, a clear bias associated with the frequency of occurrences from various *Reticulitermes* species which attack structures is reflected. The Eastern subterranean termite *R. flavipes* is the predominant species observed in the study and generally throughout the United States (Austin *et al.* 2005a). In all probability its northern range within the Great Plains region is more extensive than even reported herein. This species appears to have the greatest environmental plasticity of all *Reticulitermes*, with a continental distribution in the United States (Austin *et al.* 2005a) and establishing itself around the world in extreme locations ranging from South America (Su *et al.* 2006) to the Caribbean (Sheffrahn *et al.* 1999) and to Europe (Austin *et al.* 2005b).

It is generally accepted that exotic introduction of *Reticulitermes* in Europe have implicated human involvement, particularly trade and its respective corridors (Lainè 2002). Likewise, the isolation and lack of additional collected specimens of *R. virginicus* from Nebraska and Kansas may also reflect this phenomenon. The discontinuity of some species suggests the likelihood of population fragmentation of *Reticulitermes* is indeed associated with human occupation and land management practices.

This study represents the first attempt to collectively update the current geographic distribution and genetically categorize the genus *Reticulitermes* from the American Great Plains. Most of the inferred relationships had strong quantitative support as indicated by bootstrap analysis. The relationships among taxa inferred from maximum parsimony and maximum likelihood analyses were congruent with presently accepted groupings. Samples which could be identified morphologically did not contradict our molecular findings, but it should be noted that there is significant morphological variation which frequently precludes the application of many existing dichotomous keys for the identification of *Reticulitermes* species from the region.

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