Survey and Identification of Termites (Isoptera: Rhinotermitidae) in Indiana

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ABSTRACT In total, 289 termite samples were collected from 45 counties in Indiana during 2002–2004. Approximately 89% of the collection sites were associated with artificial structures, and almost half of the samples were from inside homes. The rest of the samples were from forested areas. Termite samples were identified based on their morphological characteristics, molecular characteristics, or both. Five species from the genus *Reticulitermes* were identified, and the relative abundance (percentage of the total collections) of these five species was *Reticulitermes flavipes* (Kollar) (90.0), *Reticulitermes virginicus* (Banks) (7.6), *Reticulitermes arenincola* (Goellner) (1.0), *Reticulitermes tibialis* (Banks) (1.0), and *Reticulitermes hageni* Banks (0.3). Based on the distribution map, *R. flavipes* was the dominant and the most widely distributed species in Indiana (44 counties); followed by *R. virginicus* (13 counties). The three other *Reticulitermes* species, *R. arenincola*, *R. tibialis*, and *R. hageni*, were encountered in only five counties. *R. arenincola* is considered a rare species and its distribution has been limited to sand dunes near Lake Michigan. However, in this study, two of the three *R. arenincola* samples were collected outside of its type location. *R. tibialis* was found in three counties, whereas *R. hageni* was only found in Evansville, IN. To complement the morphological identifications, a 389-bp region of the mitochondrial DNA (mtDNA) 16S rRNA gene was amplified and sequenced from all five *Reticulitermes* species. Based on species-specific polymorphisms exhibited in mtDNA sequences, a polymerase chain reaction-restriction fragment length polymorphism–based diagnostic tool was developed to identify samples lacking of diagnostic morphological characters.

KEY WORDS termite, survey, 16S rRNA, polymerase chain reaction-restriction fragment length polymorphism, *Reticulitermes*

Termites are the most important and most efficient lignocellulose (e.g., dead wood) decomposers (Sugimoto et al. 2000). They play a vital role in recycling wood and plant materials, modifying soil condition, improving soil composition and fertility, and providing food for other animals (Lee and Wood 1971). However, because of their large colony size, nesting behavior, and feeding preferences, termites can cause considerable damage to artificial structures and commodities. Su (2002) estimated that the overall damage and cost to control termites exceeds $20 billion annually worldwide. Wood has long been the primary building material in the United States because it has been readily available in most parts of the country. With sufficient moisture, these wood materials become the ideal food sources for subterranean termites. Drywood termites and subterranean termites are the two major termite groups that have the greatest economic impacts in North America, with subterranean termites causing >80% of the damage, and drywood termites causing the other 20% (Su and Scheffrahn 1990). Subterranean termites from the genera *Reticulitermes* and *Coptotermes* arguably are the most economically important species. Furthermore, *Reticulitermes* is the most widespread termite genus in North America, with seven described species, including *Reticulitermes flavipes* (Kollar), *Reticulitermes virginicus* (Banks), *Reticulitermes tibialis* (Banks), *Reticulitermes arenincola* Goellner, *Reticulitermes hesperus* Banks, *Reticulitermes hageni* Banks, and *Reticulitermes malletei* (nomen nudum) (Austin et al. 2007).

Termite species vary in their basic biology and ecology, including colony size, nesting, feeding, swarming, and reproductive behavior. Proper identification of

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termite species and knowledge of their distribution are the first steps in developing environmentally compatible/sustainable integrated pest management (IPM) strategies. Of the 2,600 known termite species, only a fraction (≈40 species) have been identified in the United States (Kambhampati and Eggleton 2000). Identification of termite species is a challenging task due to the ambiguity in their morphological characters, the difficulty of collecting morphologically distinct castes (e.g., soldiers and alates), geographical variations, and the overall lack of termite systematic studies. This is especially true for the genus *Reticulitermes* that notoriously lacks discrete morphological characters and rightfully deserves a thorough systematic revision (Nutting 1990, Scheffrahn and Su 1994). Developmental end points, such as soldier and alate castes, have been used extensively in the morphological identification of *Reticulitermes* species. In contrast, the worker caste is indistinguishable morphologically.

Molecular techniques can circumvent the limitations of morphological assessments, and have been adopted for the study of termite classification/identification, phylogenetic analyses (Kambhampati et al. 1996; Miura et al. 1998; Clément et al. 2001; Austin et al. 2004; Szalanski et al. 2003, 2004) and population genetics (Jenkins et al. 1998, 2001; Marini and Mantovani 2002; Austin et al. 2002, Ye et al. 2004). Mitochondrial genes are known to evolve more rapidly and their DNA to be more abundant relative to nuclear genes; therefore, they are the most widely used marker in molecular identifications (Simon et al. 1994). Polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) techniques readily separate closely related taxons by their taxon-specific mitochondrial DNA (mtDNA) markers and have been used extensively in molecular systematic and phylogenetic analyses, especially after the introduction of universal primers (Simon et al. 1994, Loxdale and Lushai 1998). rRNA (e.g., 12S and 16S), tRNA, and protein coding genes (e.g., cytochrome oxidase [COI] I and II) are the primary mtDNA markers used in molecular identification of *Reticulitermes* species (Austin et al. 2002, Szalanski et al. 2003, Ye et al. 2004). Among them, 16S rRNA has been proven to be an accurate, reliable, repeatable, and inexpensive mtDNA marker for the termite identification, especially for *Reticulitermes* species (Szalanski et al. 2003; Austin et al. 2005a,b; Tripodi et al., 2006). With this technique, Szalanski et al. (2003) identified four *Reticulitermes* species from south central United States. In the current study, we adopted the 16S rRNA marker to further develop the PCR-RFLP technique to accommodate all the *Reticulitermes* species in Indiana.

Our current knowledge of identity and distribution of existing termite species is far from satisfying. In 2002, New Orleans Mosquito and Termite Control Board launched a nationwide survey (http://www.termitesurvey.com) to update the last U.S. termite survey conducted in 1965 (Weesner 1965). A few regional termite surveys have been carried out, including Texas (Howell et al. 1987), Florida (Scheffrahn et al. 1988), South Carolina (Hathorne et al. 2000), Georgia (Scheffrahn et al. 2001), Mississippi (Wang and Powell 2001), Louisiana (Messenger et al. 2002), Ohio (Jones and Nuss 2002), Arkansas (Austin et al. 2004), Oklahoma (Brown et al. 2004), Nebraska (Husen et al. 2006), and Missouri (Pinzon and Houseman 2009). There is no termite survey data available for Indiana. To fill this gap we have 1) investigated the diversity, distribution, and abundance of the termite species in Indiana; 2) developed a termite identification key with quantifiable morphological characters; and 3) developed a PCR-RFLP–based molecular diagnostic tool to complement morphological identifications.

Materials and Methods

Termite Survey. Survey letters and collection kits were sent to pest management companies in Indiana with help from the Indiana Pest Management Association in 2002 and 2003. Each collection kit included general instructions, 10 collection vials containing 100% ethanol and two postage-paid return envelopes. Each vial was individually sealed in a plastic zip-lock bag. Each bag was provided with a label for filling in the collection information, including the collection date, county, mailing address, habitat type, and the collector's name. In total, 62 collection kits were sent out in April 2002 and 82 kits were sent in February 2003. In total, 262 samples (vials) were returned. In addition, the authors collected 27 samples. Among these 27 samples, 11 were from dead wood materials at Dune Acres, Porter County, and 16 were from infested structures in Tippecanoe County or surrounding counties. Dune Acres is located at the center of the *R. arenincola* distribution area described by Goellner (1931). Each collection (vial) returned was assigned a number for future reference. Subsequently, half of the termites in each vial were transferred to a vial containing 75% ethanol for morphological identification, and the other half were transferred to a vial containing 100% ethanol for molecular identification.

Morphological Identification. Collections containing soldiers, alates, or both were identified to species by their morphological characters under an SZ 60 dissecting microscope (Olympus, Vienna, Austria). Species determination was based on previous descriptions by Goellner (1931), Weesner (1965), Nutting (1990), and Scheffrahn and Su (1994). Majority of the samples with soldiers or alates were easily identified using these keys because most of the samples belonged to *R. flavipes.* Samples containing only workers, or ambiguous soldiers and/or alates, were subjected to molecular identification (see next section). Sample measurements that fell between two species were subject to PCR-RFLP analysis. Voucher specimens from this study are deposited in the Insect Collection, Department of Entomology, Purdue University, West Lafayette, IN.

Genomic DNA Extraction. Ethanol-preserved termite samples were air-dried on filter paper and genomic DNA of individual termites was extracted

and precipitated using a standard proteinase K/phenol-chloroform/ethanol procedure as described by Sambrook et al. (1989) with minor adjustments. The resulting genomic DNA pellets were air-dried at room temperature and resuspended in 20–30 μl of TE buffer (1 mM Tris-HCl and 0.1 mM EDTA, pH 7.0). Genomic DNA samples were stored at −20°C for further analysis by PCR-RFLP.

PCR Amplification and mtDNA Sequencing. To develop PCR-RFLP technique, five *Reticulitermes* species—*R. flavipes*, *R. virginicus*, *R. arenincola*, *R. tibialis*, and *R. hageni*—identified by soldier/alate morphology, were subjected to mtDNA sequencing. A fragment of the mtDNA 16S rRNA was amplified using a universal primer set, LR-J-13007 (forward primer, 5′-TTACGCTGTTATCCCTAA-3′) (Kambhampati and Smith 1995) and LR-N-13398 (reverse primer, 5′-CGCCTGTTTATCAAAAACAT-3′) (Simon et al. 1994). PCR was carried out in a MyCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA) following Szalanski et al. (2003) in a final reaction volume of 20 μl. Amplified PCR products were cleaned by a QIAquick gel extraction kit (QIAGEN, Valencia, CA) following the manufacturer's protocol. The purified PCR products were sent to the Purdue University Genomics Core Facility for direct sequencing. DNA sequences were obtained from five to nine individuals per termite species (*R. arenincola* colony was collected from the type location, Dune Acres, Porter County, IN). The partial sequences of *Reticulitermes* 16S rRNA were assembled using SeqMan software (DNA Star, Madison, WI) and aligned with ClustalW (Thompson et al. 1994) using default settings.

Digestion with Restriction Endonucleases. Restriction maps for all five *Reticulitermes* species were generated by the web-based software NEBCutter 2.0 (Vincze et al. 2003), and from these, three restriction enzymes (TspRI, BsmAI, and Ms1I) were selected. Restriction enzyme digestions were carried out following the manufacturer's instructions (New England Biolabs, Ipswich, MA). The PCR-RFLP–based molecular identification initiated with TspRI digestion, followed by either BsmAI or Ms1I digestion. The digestion profile was visualized on agarose gel (2%) stained with ethidium bromide, and the actual size of each digested fragment was determined by both 20- and 100-bp DNA ladder (Takara Bio Inc., Shiga, Japan).

GIS Data Analysis. Data from both morphological and molecular identifications was entered into an Excel file (Microsoft, Redmond, WA). Locations of each collection were plotted using ArcView GIS 3.3 (Environmental Systems Research Institute, Inc., Redlands, CA).

Results

Morphological Identification. In total, 289 samples were collected throughout Indiana during the 2-yr span. Among them, 131 samples had soldiers, 52 samples had alates, 29 samples had both soldiers and alates, and 77 samples did not have soldiers or alates. In total, 178 collections with either soldiers or alates or both were identified to species based on morphology. Traditionally, head size and shape, mandible shape, pronotum width, and labrum shape are useful diagnostic characters for soldier caste (Scheffrahn and Su 1994, Hostettler et al. 1995), whereas color, size, and ocelli position are usually used to compare alates (Weesner 1965). In this study, gula shape (maximal width/minimum width), mandible shape, pronotum width, and head width were used for soldier caste comparisons, whereas size, color, and ocelli position were used when comparing alates. Labrum shape was not used because of regional variations. The following are proposed morphological keys for Indiana termites (*Reticulitermes* species) by either alate or soldier caste.

Morphological Identification Keys to Indiana Termites

Alates

1. Tibia color much darker than tarsi. . . . . *Reticulitermes tibialis*
   Tibia color as pale as tarsi 2
2. Color yellowish brownish; total length = 9.0 mm (light southern subterranean termite) . . . . . *Reticulitermes hageni*
   Color dark brown to black; total length usually >9.2 mm 3
3. Ocelli are at least fully their diameter away from the eye; total length usually >10 mm; wing length usually >8.5 mm (eastern subterranean termite) . . . . . *Reticulitermes flavipes*
   Ocelli less than their diameter away from the eye; total length usually <10 mm; wing length usually <8.5 mm . . . . . *Reticulitermes arenincola* and *R. virginicus*

Soldiers

1. Gula less than twice as wide in front (maximum width) as in middle (minimal width); head color reddish; tip of mandibles slender (Fig. 1) . . . . . *Reticulitermes tibialis*
   Gula at least twice as wide in front as in middle; head less reddish 2
2. Pronotum width <0.85 mm; small species; tip of mandibles slender (Fig. 1) . . . . . *Reticulitermes hageni*
   Pronotum width ≥0.85 mm; if not, then mandibles not as the above 3
3. Pronotum width always <0.85 mm; head length 2.5–2.7 mm; head width 1.1–1.3 mm . . . . . *Reticulitermes virginicus*
   Pronotum width usually ≥0.85 mm . . . . . *Reticulitermes flavipes* and *R. arenincola*

Soldiers of *R. tibialis* are readily distinguishable by their reddish head color, relatively wide gula (maximum/minimum <2), and sharp mandibles. *R. tibialis* alates have dark blackish tibia, which is different from the other *Reticulitermes* species. *R. hageni* soldiers overlap with *R. virginicus* in their pronotum width (*R.*

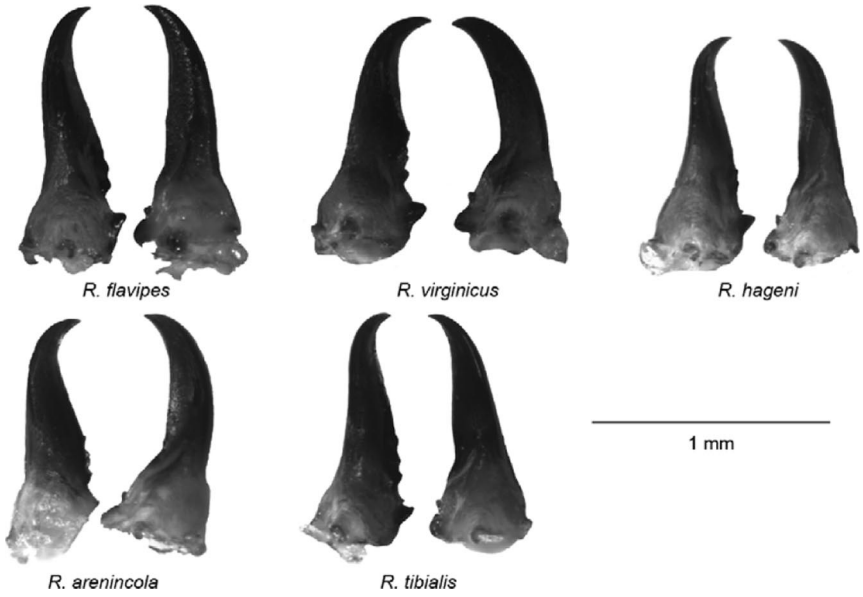

Fig. 1. Morphology of soldier mandibles of *Reticulitermes* species in Indiana.

hageni, 0.75–0.85 mm; *R. virginicus*, 0.77–0.85 mm), but *R. hageni* soldier mandibles are more slender than those of *R. virginicus* (Fig. 1). The best diagnostic morphological characters exist in alates, in which *R. hageni* alates have light yellowish brown color and small size. For *R. flavipes*, a threshold of >0.85-mm pronotum width has been used to separate it from *R. virginicus* (Hostetller et al. 1995). If soldier pronotum widths were <0.85 mm, then alate morphology would be necessary for the correct identification.

Among the three *R. arenincola* collections, one was from the type location in northwest Indiana (Dune Acres, Porter County, IN), whereas the other two were from Dubois County in southwestern Indiana. One of the two Dubois County samples was consistent with the type specimen morphologically, whereas the other one collected in mulch with four soldiers and five alates varied greatly in soldier sizes. Their pronotum width ranged from 0.80 to 1.04 mm. Minimum gula width ranged from 0.15 to 0.225 mm. Head length ranged from 2.65 to 3.20 mm. These characters overlapped with *R. flavipes.* However, alate ocelli were <1 diameter away from the eye, which agreed with the original descriptions of the type specimen (Goellner 1931). Because of the small number of *R. arenincola* samples and overlapping measurements, the keys only partially separate *R. flavipes*, *R. arenincola*, and *R. virginicus* soldiers and alates.

Molecular Identification. The partial sequences of *Reticulitermes* 16S rRNAs have been deposited in the GenBank database under accessions DQ278593–DQ278597 (Table 1), and their respective restriction

Table 1. RFLP analysis of 16S rRNAs from *Reticulitermes* in Indiana

Restriction enzyme	Species[a]	Restriction Site	Fragment(s)	Pattern
TspR1 (CAGTG)	*R. flavipes*	354	37/354	A
	R. tibialis	350	37/350	A
	R. virginicus	121	121/267	B
	R. hageni	112	112/277	B
	R. arenincola	120/352	37/120/232	C
BsmA1 (GAGAC)	*R. flavipes*	145	145/246	A
	R. tibialis			B
	R. virginicus	144	144/245	A
	R. hageni	145	145/244	A
MslI (CATATAAATG)	*R. arenincola*	144	144/245	A
	R. flavipes			A
	R. tibialis			A
	R. virginicus			A
	R. hageni	150	150/239	B
	R. arenincola			A

[a] The partial sequences of *Reticulitermes* 16S rRNAs (≈389 bp without primer sequences) have been deposited in the GenBank database under accessions DQ278593 (*R. flavipes*), DQ278594 (*R. tibialis*), DQ278595 (*R. virginicus*), DQ278596 (*R. hageni*), and DQ278597 (*R. arenincola*).

Fig. 2. Alignment of 16S rRNA partial sequences from five *Reticulitermes* species. Sequence alignments were generated by Clustal W. Shaded areas denote sequence differences, boxes enclose the predicted restriction enzyme cutting sites in *Reticulitermes*, and arrows denote the specific cutting sites recognized by restriction enzymes.

enzyme digestion sites are shown in Fig. 2. Multiple *Reticulitermes* samples with distinct soldier/alate morphology [*R. flavipes* (3), *R. virginicus* (3), *R. hageni* (3), *R. tibialis* (3), and *R. arenincola* (1, from the type location, Dune Acres, Porter County, IN)] were used to develop PCR-RFLP technique. Digestion of PCR products with selected restriction enzymes yielded diagnostic patterns for all five *Reticulitermes* in Indiana, regardless of castes (Table 1). Thereafter, three RFLP markers, TspRI, BsmAI, and Ms1I, were used sequentially to identify a total of 118 termite collections (113 from Indiana, four from Mississippi, and one from Michigan). The inclusion of Mississippi samples (*R. virginicus and R. hageni*) and Michigan (*R. tibialis*) samples were intended to validate the PCR-RFLP technique. Among them, 77 samples collected from Indiana did not have morphologically diagnostic castes (soldiers and/or alates), and their identities were determined solely by PCR-RFLP technique. Conversely, the rest of the 41 collections were identified by both morphological and molecular identifications. Species identifications by both morphological and molecular methods were generally congruent (matched 38 of 41 samples). However, three samples identified as *R. virginicus* based on morphological characteristics were determined as *R. flavipes* by PCR-RFLP later on.

Termite Distribution in Indiana. The current termite survey covered 45 of 92 counties in Indiana (Fig. 3A). Among the 289 collections, 256 were associated with artificial structures (residences, barns, fences), of which 112 were from inside structures; 71 from landscaping materials; 37 from termite monitoring stations installed around houses; and 36 from trees, stumps, and wood piles near the structures. The relative abundance of each species was: *R. flavipes* (90.0%), *R. virginicus* (7.6%), *R. arenincola* (1.0%), *R. tibialis* (1.0%), and *R. hageni* (0.3%). *R. flavipes* was by far the most commonly collected termite species throughout the state of Indiana (44 counties), followed by *R. virginicus* (13 counties) (Fig. 3B and C). Three *R. arenincola* samples were located in Porter and Dubois counties, and only one sample was collected from the type location (Dune Acres, Porter County, IN), whereas the other two were found in residential areas in Dubois County. *R. tibialis* was identified in Porter, Starke, and Tippecanoe counties, whereas *R. hageni* was only found in an infested house in Evansville, IN (Fig. 3D). Among the 11 samples collected from Dune Acres, Porter County, IN (the type location of *R. arenincola*), only one was identified as *R. arenincola*, whereas the rest were determined to be *R. flavipes* (nine samples) and *R. tibialis* (one sample).

Discussion

Survey Summary. The overall taxonomic diversity of termites in Indiana is relatively low. All 289 termite samples collected throughout the state of Indiana belong to the genus *Reticulitermes.* In contrast, southern and western states tend to have a higher diversity of termite genera (Howell et al. 1987; Scheffrahn et al. 1988, 2001; Hathorne et al. 2000; Messenger et al., 2002; Brown et al. 2004). *R. flavipes* is the most common species in each of these states, and also in Indiana (90% of the samples). It is unknown whether this dominance is related to the bias of sampling from the urban environment. The distribution of *R. virginicus* identified in this survey extended further northward than described by Snyder (1954). In total, *R. flavipes* and *R. virginicus* represented 97.6% of the termite samples in Indiana. The other species, *R. arenincola*, *R. tibialis*, and *R. hageni*, accounted for <3%. It is no surprise that *R. flavipes* is the most common species in the state

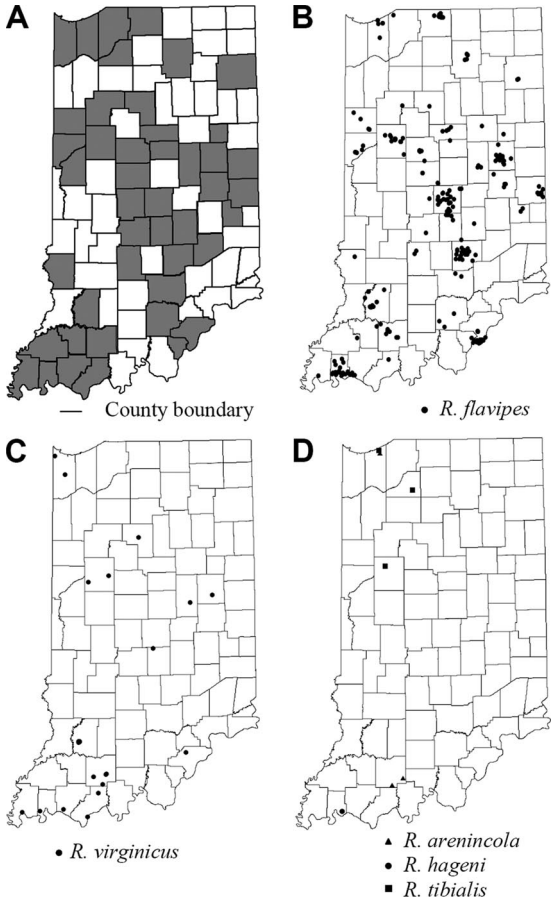

Fig. 3. Termite distribution maps in Indiana. Statewide survey covers 45 (shaded in gray) of 92 counties in Indiana (A). *Reticulitermes* was the only genus found in the current survey, and the exact distribution maps of the dominant species, *R. flavipes* (B) and *R. virginicus* (C), and the marginal species, *R. arenincola*, *R. tibialis*, and *R. hageni* (D) were plotted using ArcView GIS 3.3 (Environmental Systems Research Institute, Inc.). Some records are not shown due to incomplete address information.

because of its wide distribution throughout eastern North America.

Generally, based on our findings, *R. flavipes* seems less tolerant of dry soil than *R. tibialis*, but more tolerant than *R. arenincola*. *R. arenincola* is a rare species and previously has only been reported from its type collection location (sand dunes in the Upper Indiana and Lower Michigan alongside shores of Lake Michigan) (Goellner 1931, Ye et al. 2004). The new *R. arenincola* locations (Dubois County) discovered in this study and the dominance of *R. flavipes* among the termite samples from Dune Acres indicate that *R. arenincola* is not necessarily specific to sandy areas. Both *R. virginicus* and *R. hageni* are considered southern species with a northern distribution boundary at Indiana. However, the spatial distribution profile suggests *R. virginicus* (covered ≈30% of the surveyed counties) is well adapted to the climate in Indiana. Additional findings of *R. virginicus* and *R. hageni* in the American Great Plains region (Austin et al. 2006) demonstrated that these two species can have much wider distributions than previously thought. *R. tibialis* (arid land subterranean termite) ranges from eastern prairies of the Central West to Oregon (Snyder 1954). *R. tibialis* had been recorded previously in northern Indiana (Park 1929, Goellner 1931) and central Indiana (Ye et al. 2004), and the current survey reverified the existence of *R. tibialis* in Indiana, which is considered its eastern distribution limit.

Termite Identification Strategy. Among the five *Reticulitermes* species in Indiana, *R. tibialis* is the easiest to identify, whereas the rest all have somewhat similar morphology. For this reason, morphological keys (including those proposed in this paper) for the identification of *Reticulitermes* species have certain limitations such as lack of quantitative descriptions of diagnostic characters. Yet, these keys provided a quick and accurate method of identification of many of the samples. Incorporating more morphological characteristics and using multivariate analysis may provide better identifications.

Keys based on regional collections do not match very well with specimens from the other regions due to geographical variations (Scheffrahn and Su 1994), or possible environmental influences on morphology (Ye et al. 2004). The ranges of diagnostic characters of Indiana termites differ considerably from other regions. The only *R. hageni* sample collected in Indiana has a very large size compared with that described from Florida samples (Scheffrahn and Su 1994). Soldier pronotum widths are 0.75–0.85 mm (based on six soldiers). Alate lengths are 8.6–9.0 mm. In addition, the total length and wing length of alates from *R. virginicus* and *R. arenincola* did not agree with previous reports (Snyder 1954, Weesner 1965, Nutting 1990). According to Weesner (1965), the wing length (from suture to tip) of *R. hageni* was <6 mm, but the sample collected from Dubois County of Indiana had wing lengths (from suture to tip) of 6.7–7.4 mm. Total length of *R. virginicus* alates was ≈8 mm according to Nutting (1990), whereas the majority of the alates in the current collections were >9 mm in length.

The ambiguity of morphological characters was particularly evident in the case of *R. arenincola*. Large variations existed in soldier morphology, even within the same sample. Typically, *R. arenincola* is smaller than *R. flavipes*. However, soldiers from a sample collected in Dubois County had pronotum widths of 0.80–1.04 mm, which overlapped with that of both *R. flavipes* and *R. virginicus*. The morphological ambiguities, in general, can be resolved by the complementary molecular identifications. In the case of *R. arenincola*, RFLP profiling and 16S rRNA sequence generated from this study supports *R. arenincola* as a distinct species. However, they were not congruent with a previous report (Ye et al., 2004). A thorough search of 16s rRNA sequences from genus *Reticulitermes* in GenBank retrieved in total 391 entries, including 132 sequences from *R. flavipes*. Among them, only one other North American *Reticulitermes* species,

R. hesperus, has the same RFLP-TspRI polymorphism displayed in *R. arenincola* from this study, i.e., both species have two TspRI recognition sites at nucleotide positions 120 and 352, respectively. Given that hundreds of haplotypes exist in the genus of *Reticulitermes*, a larger sample size and additional genetic information from mitochondrial DNA markers such as COI and COII are needed for *R. arenincola* and the subsequent phylogenetic analysis to shed light on its current *nomen dubium* status (Austin et al., 2007, Vargo and Husseneder, 2009).

Our 16S rRNA based PCR-RFLP diagnostic tool was developed to accommodate all five *Reticulitermes* species in Indiana, and molecular identifications are consistent with morphological identifications. The discrepancy between molecular and morphological identification exhibited in this study and others are not without precedent. Noël et al. (2004) used two mtDNA markers, COI and COII, to differentiate the myiasis-causing flies *Cuterebra grisea* (Coquillet) and *Cuterebra fontinella* (Clark) (Diptera: Oestridae). The molecular diagnostic tools readily separated *C. grisea* and *C. fontinella*, although there were a few disagreements between molecular and morphological identifications. Our survey provides new information on distribution patterns of *Reticulitermes* species, geographical variations of termite morphology, and a molecular technique assisting in the identification of termites. The results further testify to the significant geographic variations in termite morphology and the overlapping of morphological characteristics among species. Use of multiple samples with morphological and molecular techniques will provide the best species determination.

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