Molecular Diagnostic Technique for the Differentiation of the Formosan Subterranean Termite, Coptotermes formosanus (Isoptera: Rhinotermitidae) from Other Subterranean Termites by Multiplex-PCR

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Molecular diagnostic technique for the differentiation of the Formosan subterranean termite, *Coptotermes formosanus* (Isoptera: Rhinotermitidae) from other subterranean termites by multiplex-PCR

*Mark A. Janowiecki and Allen L. Szalanski**

The Formosan subterranean termite (FST), *Coptotermes formosanus* Shriaki (Isoptera: Rhinotermitidae), is an invasive termite that was introduced into the continental United States in the 1950s (Evans et al. 2013). Since its introduction, it has spread to the southeastern US (including North Carolina, South Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana, and Tennessee) and Texas (Evans et al. 2013). This termite is particularly damaging since it can also feed on the heartwood of living trees, rather than solely dead wood (Lai et al. 1983). Annually, Americans spends over $1 billion in preventing and treating this termite (Lax & Osbrink 2003). Furthermore, damage from this species cost Americans an additional $1 billion each year (Raina 2004).

Termite identification is difficult and various methods have previously been used to identify this pest species (Scheffrahn & Su 1994; Szalanski et al. 2003, 2004; Smith et al. 2010). Traditionally, taxonomic keys based on morphological traits are used to identify termite species (Scheffrahn & Su 1994), but these keys are developed for soldiers or alates, which are not always collected. Thus, small samples or samples lacking these castes are problematic. DNA sequence data have been used to identify FST (Smith et al. 2010). This process is time consuming and expensive requiring that every sample be sequenced. A molecular diagnostic technique to identify FST was developed by Szalanski et al. (2004), but still this method requires 2 polymerase chain reactions (PCRs), because the oligonucleotide primers annealing temperatures are incompatible. This duplication made the process more time consuming and increased the chance for mistakes.

The objective of this study was to develop a multiplex PCR protocol that could be used to identify FST regardless of life stage. The technique, requiring only a single PCR reaction, is simpler than previous molecular methods, and will facilitate monitoring of this invasive termite.

Termites were collected from locations in North America, South America, Africa, Asia, Australia, and the Middle East (Table 1). Identification was conducted using the keys of Scheffrahn & Su (1994). Voucher specimens are housed in the Arthropod Museum, Department of Entomology, University of Arkansas, Fayetteville, Arkansas, USA.

Samples preserved in ethanol were dried on filter paper. DNA was extracted using DNeasy® (Qiagen Sciences, Germantown, Maryland), resuspended in 10mM Tris-HCL (pH 8.0), and stored at -20 °C. Universal termite oligonucleotide primers were designed using composite termite sequences in Geneious (v6.1.7, Invitrogen Corp., Grand Island, New York): 16S 104F (5'-CCTCYCATCRCCCCAACRAA-3') and 16S 368R (5'-TTGAAGGGCCGCGGTATYTT-3'). A 16S FST specific primer was also used: FST-F (5'-TAAAACAAACAAACAACAAACAAAC-3') (Szalanski et al. 2004). Polymerase chain reaction was performed at 94 °C for 2 min; followed by 40 cycles at 94 °C for 45 s, 50 °C for 45 s, and 72°C for 60 s. The final extension at 72 °C f was for 5 min.

Table 1. *Coptotermes, Reticulitermes*, and *Heterotermes* samples subjected to DNA sequencing and PCR analysis for Formosan subterranean termite specific PCR diagnostic analysis.

Species	Number PCR Screened	Number Sequence Screened
C. formosanus	9	57
C. gestroi	2	19
C. michaelseni	1	2
C. lacteus	1	6
C. testaceus	1	29
C. intermedius	1	1
C. curvignathus	1	2
C. heimi	1	2
R. flavipes	16	747
R. virginicus	15	108
R. tibialis	12	95
R. hageni	4	82
R. hesperus	1	75
R. malletei	4	31
H. aureus	1	1
H. tenius	0	10
H. cardini	0	18
H. convexinotatus	0	21

The new method was validated by screening a broad geographical sampling of FST and *Reticulitermes* species from the US. Samples were visualized on a 2% agarose gel with ethidium bromide staining (Fig. 1). The FST samples yielded 2 amplicons of 262 and 221 bp in size. Other Nearctic termite species north of Mexico [*Coptotermes gestroi* Wasmann, *Reticulitermes flavipes* (Kollar), *R. virginicus* Banks, *R. tibialis* Banks, *R. hageni* Banks, *Heterotermes aureus* (Snyder), *R. hesperus* Banks, and *R. malletei* Howard and Clement] were used (Table 1) and generated only a single amplicon of 262 bp. Additional *Coptotermes*

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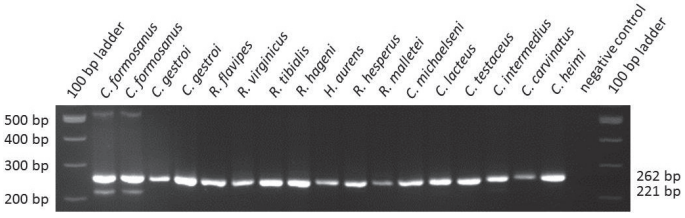

Fig. 1. Ethidium bromide-stained agarose gel (2%) illustrating a common amplicon of 262 bp from the mtDNA 16S gene for various termite species and unique amplicon of 221 bp specific for the Formosan subterranean termite.

spp. [*C. michaelseni* Silvestri, *C. lacteus* (Froggatt), *C. testaceus* (L.), *C. intermedius* Silvestri, *C. heimi* (Wasmann)] produced only the single universal amplicon of 262 bp. The negative control did not produce a detectable amplicon, indicating no contamination. A total of 1,373 16S sequences from 9 *Coptotermes*, 6 *Reticulitermes*, and 4 *Heterotermes* species from GenBank and from our DNA sequence database (ALS unpublished) (Table 1) were analyzed using Geneious software to confirm that the FST oligonucleotide primers would be specific for the *C. formosanus* (FST) sequences and not the other taxa for PCR amplification.

The results show that the universal primers produced a 262 bp band in all species tested (Table 1) whereas the FST specific primer produces an additional band (221 bp) only in FST (Fig. 1), indicating that this primer combination and PCR reaction successfully distinguish FST from other termites in this study.

This new molecular method simplifies previous methods of identification (Szalanski et al. 2004; Evans et al. 2013), in that it can be completed in a single PCR reaction and allows identification of worker specimens that cannot be keyed morphologically to species. Proper identification that is simple and economical can be useful for monitoring the spread of this invasive to new areas.

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Summary

The Formosan subterranean termite *Coptotermes formosanus* Shriaki; (Isoptera: Rhinotermitidae), is a major pest that is spreading throughout the southeastern United States. Morphological identification of worker specimens is not possible using available taxonomic keys based on morphological traits. A multiplex PCR protocol was developed that can differentiate the Formosan subterranean termite from other termite species in a single PCR reaction. This multiplex PCR protocol simplifies previous molecular diagnostic techniques.

Key Words: *Coptotermes formosanus*, molecular genetics, invasive termite, subterranean termite

Sumario

La termita subterránea de Formosa, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), es una plaga importante que se está extendiendo desde el sureste de los Estados Unidos. La identificación morfológica de los especimenes de trabajadores no es posible usando las claves basadas en características morfológicas. Un protocolo de PCR múltiple fue desarrollado que puede diferenciar la termita subterránea de Formosa en una reacción de PCR. Este protocolo PCR multiplex reemplaza las técnicas diagnósticas moleculares anteriores, que requieren múltiples reacciones de PCR.

Palabras Clave: *Coptotermes formosanus*, genética molecular, termitas invasoras, termita subterránea

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