A Case Study of the Formosan Subterranean Termite, *Coptotermes formosanus* (Isoptera: Rhinotermitidae) Transported with a Non-Cellulosic Commercial Carrier in South Mississippi

by

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ABSTRACT

This report provides new evidence that the spread of *C. formosanus* in Mississippi is also attributed to commercial activities with some non-wood carriers moved from infested coastal areas. Sequencing of mitochondrial cytochrome oxidase subunit II indicated that the genotype of a termite colony collected from a boat located in south Mississippi, (originally purchased in New Orleans, Louisiana by the owner) has not been previously documented in the southeastern mainland of the United States. Preliminary sequence alignment analysis exhibited that this termite sample from Mississippi was genetically more similar to colonies documented in southeastern Asia.

Key words: Invasive subterranean termites, Distribution, Mississippi, cytochrome oxidase, COII

INTRODUCTION

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is one of the most destructive structural pests in the world. A native to China and Japan, this species has been transported worldwide. During the 1950’s, the first record of *C. formosanus* in the continental United States was reported in Charleston, South Carolina (Cabrera *et al*. 2005). Approximately ten years later, *C. formosanus* was found in a shipyard in Texas, and a few years later established colonies were also being reported in New Orleans, Louisiana (Cabrera *et al*. 2005). During the 1980’s, *C. formosanus* was reported in

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Alabama, Mississippi, and Florida (Woodson et al. 2001). Since that time, this invasive species has become established in eleven states.

It is commonly accepted that the Formosan subterranean termite arrived in the United States at shipping ports via shipping containers and boats (Su 2003, Sun et al. 2007). Any type of cellulose material used in shipping objects such as shipping containers, pallets or crates can provide the groundwork of a new introduction of *C. formosanus* along port cities (Su & Tamashiro 1987). However, *C. formosanus* is often transported inland from port cities along the Gulf Coast areas by commercial activities involving cellulose material as a carrier (Su 2003). Many local surveys and reports over the past decade in Mississippi and other states in the US support the hypothesis that the spread of *C. formosanus* may be primarily enhanced by commercial activities from port areas to inland areas of a state. Any type of cellulose material or cellulosic commercial products that has been in contact with the soil in an area known to have *C. formosanus* infestations has the potential to be moved inadvertently via commercial activities and contribute to the spread of this pest. Infested cellulose products can be easily transported from one location to another without the knowledge that it may be holding termite colonies because *C. formosanus* are evasive and do not disclose themselves with distinct signs of activity (Jenkins et al. 2002). Cellulosic materials, such as railway cross ties, wood-based mulches, infested tree debris, etc. have been the focus of the movement of *C. formosanus* throughout the areas infested by *C. formosanus* in the US (Woodson et al. 2001, Sun 2007). However, the primary focus of many investigations remains on the reuse of railroad ties regarding the spread of *C. formosanus* (Lax & Osbrink 2003). As home owners continue to use these railway cross ties around their home as landscape timbers, fence posts, etc. the spread of *C. formosanus* will persist. Until this time, non-cellulosic commercial products have often been overlooked as a potential medium to carry this invasive structural pest across a long distance. This report offers compelling evidence that the spread of *C. formosanus* in Mississippi is also attributed to commercial activities using non-wood-based carriers.

**CASE DESCRIPTION**

Early reports indicate that *C. formosanus* can be spread inland from port cities via the transportation of infested wood products, such as landfill ma-
 materials, lumber, organic mulches, and recycled railroad cross ties. Railroad cross ties have traditionally been used as an inexpensive alternative to landscape timbers (Woodson et al. 2001, Su and Tamashiro 1987, Hardy 1988, Henderson 2001), and are considered the primary means for spreading the Formosan subterranean termite to inland areas of the United States. Until recently, the spread of *C. formosanus* was thought to be limited to the use of wood-based products.

In February 2008, a large population of *C. formosanus* was reported aboard a privately owned 14-ft fiberglass fishing boat in Poplarville, Mississippi (Fig. 1). The boat was purchased from an individual in New Orleans, Louisiana, in 2002, and had been rested on a boat trailer in a residential area in Poplarville. After the purchase of the boat, the owners never used the vessel. Further investigations determined that there was no direct contact between the soil and the boat, and the only moisture source was rainwater pooled in the bottom of the hatch. The termites had constructed a very large carton nest measuring 80×50×70 cm inside the hatch of the boat (Fig. 2A-B).

In the hatch of the boat, there were several buoys made of a rubber-like material which had been extensively damaged by the termites (Fig. 2C-D). A large book placed inside the boat by the new owner was utilized by the termites as a food source (Fig. 3A). Several nontraditional cellulosic items inside the cabin of the boat, such as life jackets and seat cushions, were tunneled through by the termites, but not extensively. It appears that these items did not serve as the main food sources for the termites (Fig. 3B).

The plywood used to reinforce the boat seats had been attacked and significantly damaged by this termite colony to a point where they had completely fallen over (Fig. 3C). However, the wooden accessories that were made from teak wood (*Tectona* sp.) had less than 1% termite damage due to its natural resistance (Fig. 4, Wolcott 1950, Rudman et al. 1967, Grace & Yamamoto 1994). This colony had

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Fig. 1. Fiberglass fishing boat inadvertently purchased from New Orleans, Louisiana, with a colony of *C. formosanus*. The boat rested on a trailer at a residential area in Poplarville, Mississippi.
enough moisture from rain to maintain survival but had what appeared to be a very limited amount of cellulosic food available inside the boat; as a result, *C. formosanus* were forced to hunt and attack those non-traditional cellulose products available inside the boat.

Although there seemed to be a limited amount of cellulosic food available to the termites in this boat, the colony remained active and healthy. In order to determine if the termite colony was under the stress of starvation, following

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**Fig. 2.** (A) Carton nest found in boat. (B) Nest inside hatch of boat. (C) Termite-damaged buoy prior to cleaning. (D) Termite-damaged buoy after cleaning.

**Fig. 3.** (A) A book located inside boat and infested by termites. (B) Life jackets and seat cushions infested by termites. (C) Boat seats reinforced by plywood completely destroyed by termites.
colony collection, 10 termite workers were randomly sampled from the colony for intestinal dissection. The number of intestinal protozoa community in the termite’s hindgut would generally reflect the status of food they ingested. These symbiotic flagellates exclusively rely on the consumption of cellulosic substrates for survival (Tanaka et al. 2006). An estimate of the total number of cellulolytic protozoa per termite worker was ~ 2650, of which the counts of each protozoa species in the hindgut were 450 ± 149, 1000 ± 224, and 1200 ± 426/termite for *Pseudotrichonympha grassi* Koidzumi, *Holomastigotoides hartmanni* Koidzumi, and *Spirotrichonympha leidyi* Koidzumi, respectively. Clearly, the presence of all three principal cellulolytic protozoa species at regular levels in the hindguts of termites suggested that this termite colony was not under serious starvation stress due to lack of cellulosic food. After further investigation of possible cellulosic food sources that were utilized by the termites, copious amounts of plant litter that had fallen from overhead trees was found along the floor of the boat (Fig. 5). Thus, the possible food sources utilized by this colony from the boat may consist of books, plywood, life jackets, seat cushions, as well as the plant litter dropped to the boat.

The size of the termite population in this boat was estimated to be more than 150,000. There were numerous nymphs (with wing-buds) observed in the colony and it did produce a significant swarm in May 2008 after being maintained in the laboratory for three months. Because of the presence of nymphs and the swarming

![Teak Wood](image)

Fig. 4. Wooden boat accessory made from teak wood (*Tectona* sp.).
of this colony, as an indication of maturity, and because of no soil contact with this colony in the boat, it is clear that the colony was established before the boat was purchased from New Orleans five years ago. It seems obvious that it would not be possible for a colony of this size and maturity to develop from an incipient colony that had naturally swarmed to the boat from local alates during the period of time after the boat was purchased from New Orleans (although _C. formosanus_ had been first reported in the town of Poplarville, Mississippi in a year prior to the purchase of the boat (Fig. 6)). Therefore, the only way to explain the origin of this colony is that the vessel’s owner had inadvertently purchased the boat from New Orleans with an established colony of _C. formosanus_ already present.

In order to understand the possible origin of the termite colony in this boat, the termite samples collected from the infested boat were further subjected to a genotype analysis to estimate their phylogenetic relationships with other termite colonies collected in New Orleans.

**MATERIALS AND METHODS**

The cytochrome oxidase subunit II (CO-II) gene is one of the best-known mitochondrial genes. Because it contains both highly conserved and variable regions, the mitochondrial COII gene sequence has been widely used to estimate phylogenetic relationships at different taxonomic levels among insects (Pruess _et al._ 2000). In this study, we explored COII sequence to determine potential genotype and origin of the termite colony collected in Pearl River County, Mississippi.

![Fig. 5. Plant litter located on floor of the boat.](image-url)
Isolation of Genomic DNA.

DNA of the termite samples was extracted by using the method of Sambrook et al. (1989) with modification. The abdomen of the workers, potentially containing symbiont DNA, was excised and discarded, and only head and thorax tissues from single workers were selected as samples for DNA extraction. The samples were placed individually in 0.5-ml microcentrifuge tubes and homogenized using a motorized homogenizer (Glas-Col, Terre Haute, IN) in 100 µl of isolation buffer (0.1 M NaCl, 0.2 M sucrose, 0.1 M Tris-HCl (pH 9.1), 0.05 M EDTA, 1% SDS). The homogenate was spun briefly and incubated for 40 min at 65°C. Twenty µl of 8 M potassium acetate were added to the tube and mixed by tapping. The tube containing homogenate was incubated on ice for 30 min, and then centrifuged for 15 min at 10,000 X g, after which the supernatant was transferred to a new tube. DNA was precipitated overnight at -20°C, pelleted by centrifugation, and resuspended in 100 µl distilled water.

PCR Amplification and Sequencing of cytochrome oxidase II (COII) DNA.

The COII DNA fragment was amplified using the forward primer CO2F (5’-ATGGGAGATTAGTGCAATGG-3’) and reverse primer CO2R (5’-GTGATAGGACCAGTACTTG-3’). PCR product (5 µl) was verified on agarose gel for single band with expected size (c.a. 790 bp), and the rest of PCR product (45 µl) was cleaned using Qiagen MinElute PCR purification kit (Qiagen, Santa Clarita, CA). Two primers, tCO2F: 5’-ATGAAGATTCTCAAACCCACT-3’ and tCO2R: 5’-GGTCAATTGGGATGATTGAT-3’, were used to sequence the COII DNA fragment from both directions using an automated sequencer (ABI Prism 3730XL). The COII sequence from the termite colony collected in Pearl River County was subjected to multiple sequence alignment (Clustal-W) with 31 COII sequences of C. formosanus downloaded from GenBank.

Results and Discussion

The COII gene fragment sequenced in this study from the colony collected from the boat located in Pearl River County in southern Mississippi showed that the amplified putative COII fragment contained 790 nucleotides. GenBank similarity search confirmed that the DNA fragment was part of the gene coding for cytochrome oxidase subunit II.
The DNA sequence was confirmed as partial mitochondrial cytochrome oxidase sub-unit II gene through Blastn similarity search of GenBank nucleotide databases (National Center for Biotechnology Information, Bethesda, Maryland). Results revealed two polymorphic nucleotides at position 290 and position 365 (positions were numbered based on 790-bp COII from this study). The COII sequence from the termite colony collected in Mississippi contained A and T at positions 290 and 365, which were different from those (290/365=G/A) collected from southeastern region of the United State (Austin et al. 2004; Jenkins et al. 2002; and Ye et al. 2004). The A/T (290/365) type COII gene from the termite colony located inside the boat was more similar to those COII sequences of *C. formosanus* distributed mainly in southeast Asian countries, including China (Long et al. 2009; Fang 2006) and Japan (Yashiro & Matsuura 2007, Ohkuma et al. 2004; Kitada et al. 2006). The A/T genotype termites have not been reported previously in the southeast US, except a sample collected in Hawaii (Austin et al. 2004) and a sample collected from Ft. Worth, TX (Jenkins et al. 2002). It is very likely that *C. formosanus* in southern Mississippi originated from New Orleans. Because of the disjunction with Texas and Hawaii and no previous report of the A/T genotype in the regions near New Orleans (LA, US), we suggest that the A/T type termites are substantially less dominant than G/A type termites in the United States. It is also suggested that the *C. formosanus* colony collected from the boat in Poplarville had potentially come from southeast Asian countries via shipment with woody materials. Although this colony was first reported and documented in south Mississippi, it seems that the original invasive landing port was not in Mississippi due to a subsequent commercial transaction of this boat from New Orleans to Poplarville in 2002.

Commercial products, both cellulosic and non-cellulosic, may potentially serve as carrier media to help spread *C. formosanus* accidentally by human activities. The natural spread of *C. formosanus* occurs by the seasonal dispersal of the adult caste members (alates). These members pair male to female and initiate an incipient colony if food and environmental conditions are met. However, the natural spread of *C. formosanus* by alates is less significant due to a weak flight capability (< 1000 m, Messenger & Mullins 2005); therefore, the spread of colonies to a remote location is mainly accounted for by an artificial factor from commercial activities (Su & Tamashiro 1987, Sun et al. 2007). This report provides new
evidence that the spread of *C. formosanus* inland from port cities, such as New Orleans (Louisiana), Charleston (South Carolina), Galveston (Texas), Pensacola (Florida), Mobile (Alabama), and the Mississippi Gulf Coast could be a direct result of commercial activities. The town of Poplarville, Mississippi is located approximately 50 miles inland from the nearest port city along the Gulf of Mexico, and 75 miles inland from the Port of New Orleans (Fig. 6). This study

Fig. 6. Distribution of *C. formosanus* in Mississippi and the year first reported.
documented the only known case in Mississippi where a fully-developed colony of *C. formosanus* could be moved commercially by a non-wood carrier from a termite-infested area. It also alerts us that *C. formosanus* can be spread through various means and materials, other than with wood products only.

Further investigations are urgently needed to determine the extent of non-cellulose based commercial movements of this invasive species. Mississippi has experienced a very rapid expansion of *C. formosanus* over the past decade. A distribution study conducted by Mississippi State University reported that *C. formosanus* had successfully established from coastal to central regions of Mississippi, indicating that this termite species has been transported far inland in this state by human activity in recent years (Sun *et al.* 2007). Based on the very slow natural spread of *C. formosanus*, approximately 64 km in 50 years (Sun *et al.* 2007), the state of Mississippi is approximately one hundred years ahead of the natural spread of *C. formosanus* via alate swarming and foraging activity. Clearly, these rapid inland infestations in Mississippi can only be explained by human transportation with a variety of carry media. As commercial activities continue to contribute to the rapid spread of this species, economic losses associated with termite damage will continue to increase as well. Research shows that there have been no documented cases of eradication from any city once *C. formosanus* is established (Su 2003).

It appears that more efforts have to be made to educate people on how to reduce the risk of the spread of this invasive structural pest into other areas of Mississippi by human activities. As one of the efforts in Mississippi, the Mississippi Department of Agriculture and Commerce Bureau of Plant Industry issued a quarantine policy for *C. formosanus* in 2002 that regulates the movement of cellulose materials. This quarantine states that cellulose material that has been in contact with the soil (such as firewood, utility poles, and railroad cross ties) cannot be transported from a county or parish with known *C. formosanus* infestations to any part of Mississippi that has no reported infestations of this termite. However, this quarantine is very difficult to enforce in Mississippi (Hardy 1988, Sun *et al.* 2007). Based on the findings documented in this case study, other types of commercial activities involving non-cellulosic carriers should also be monitored and regulated.
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