

PHYLOGEOGRAPHY, INTERSPECIFIC COMPETITION, AND CONTROL OF  
*Coptotermes formosanus* AND *Coptotermes gestroi* (ISOPTERA: RHINOTERMITIDAE)  
IN TAIWAN

By

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To my parents for their unconditional love and support

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Abstract of Dissertation Presented to the Graduate School  
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Tokuichi Shiraki and Masamitsu Oshima were the first entomologists who studied termites in Taiwan in the early 1900s. The identification of *Coptotermes* species in Taiwan and southern Japan was a controversial issue that involved several Japanese entomologists as well as European authorities of termite taxonomy. The key point of the debate was whether the common termite pest species in Taiwan was a new species, *Coptotermes formosanus* Shiraki, or *C. gestroi* (Wasmann) of southeast Asia. Recent evidence indicated that both species existed in Taiwan at the time. *Coptotermes formosanus* was found to be distributed throughout the island whereas *C. gestroi* was limited to the south. I speculated that entomologists involved in the debate in the early 1900s had collected or obtained *Coptotermes* samples from different localities of Taiwan and Japan. These collections might include either one of the two species or both species; hence, each researcher drew different conclusions. By analyzing partial mitochondrial sequences of COII, 12S rRNA, and 16S rRNA, I found that most Taiwanese *C. formosanus* populations were closely related to Japanese and some Chinese populations, and that Taiwanese *C. gestroi* populations were most closely

related to those from the Philippines and Hawaii rather than populations from Thailand, Malaysia, Singapore and Indonesia. The intraspecific variation of *C. formosanus* was 0.7-0.8% for three genes among seven Taiwanese populations whereas all Taiwanese *C. gestroi* populations had identical sequences. The results support that Taiwan may be a center of origin for *C. formosanus*, but it is a recent introduction site for *C. gestroi*.

The distributions of the two species currently overlap in Taiwan, and interspecific competition is likely to occur. The results of the foraging arena bioassay supported the long-held assumption that interspecific competition is an important regulatory factor in maintaining termite colonial territories. Tunnel interception of the two species resulted in minor fights and then both species quickly buried the connected tunnel at multiple locations, which prevented further combat and heavy losses. Termite cadavers resulting from agonistic behavior are a stimulus for tunnel blocking and a deterrent for reopening these blockages. I speculate that tunnel interceptions would offer information on conditions of neighboring colonies in the field. If both neighboring colonies are active, the agonistic interactions resulting from tunnel interception would delineate the colonial boarder. If one colony is dead or weak, the healthy colony could invade the vacated territory quickly through the existing tunnel system.

Of the 17 named termite species found in Taiwan, *C. formosanus* and *C. gestroi* were the most destructive and responsible for > 87% of termite structural infestations. More than 75% of infested buildings were residential premises. The liquid termiticide, containing the active ingredient fipronil, accounted for ≈75% market share, and baiting accounted for ≈20% market share. The revenue of termite control industry in 2008 was estimated as ≈4 million USD.

CHAPTER 1  
TOKUICHI SHIRAKI, MASAMITSU OSHIMA, AND THE DISCOVERY OF  
*COPTOTERMES FORMOSANUS* AND *COPTOTERMES GESTROI* IN TAIWAN

**Introduction**

After the First Sino-Japanese War between the Qing Dynasty of China and the Meiji period of Japan, Taiwan became a Japanese territory in 1895 under Treaty of Shimonoseki till the end of WWII in 1945. Over this fifty-year period, the main economic policy of the Japanese government was to develop industry in Japan and agriculture in Taiwan. As a result of this policy, many outstanding Japanese agricultural experts began to work in Taiwan, resulting in rapid modernization. Entomology was one of most important subjects in the era due to the need for crop protection, the prevention of insect-borne diseases, and urban pest management.

Since most of Japan has a temperate climate and termites are primarily subtropical /tropical pests, Japanese construction techniques were not adapted for termite prevention. Thus, construction built in Taiwan during the early Japanese colonial period suffered from severe termite damage. Termite control became a priority of the Governor of Taiwan in the early 1900s. In 1907, both Tokuichi Shiraki (1882-1970) (Figure 1-1A) and Masamitsu Oshima (1884-1965) (Figure 1-1B) started to work in Taiwan and became the leading figures in termite research on the island. Their major control target was known at the time colloquially as “house termites,” what we now know to be *Coptotermes* species. There was controversy over the identification and naming of the Taiwanese *Coptotermes* species between 1909 and 1912. Many influential Japanese entomologists and European termite experts were involved in this debate. One hundred years after the controversy, I reviewed this historical story, clarified the

controversial issues based on the current study, and offered solutions to avoid similar problems in the future.

## **Termite Research of Shiraki and Oshima in Taiwan**

### **Early Life of Tokuichi Shiraki and Masamitsu Oshima**

Tokuichi Shiraki (Figure 1-1A) was born in Hakodate, Hokkaido, Japan on 9 March 1882 (Issiki 1971). He was the eldest of six children in a wealthy family (Ou 2006). His father was a president of the Hakodate Normal School (now Hokkaido University of Education, Hakodate) and his mother was an elementary school teacher. Shiraki moved around Japan with his family as his father moved through a series of jobs. He stayed in Hakodate until he was 8 years old, and then spent the next two years in Tokyo and Kumamoto. In 1892, he moved back to Hokkaido and went to middle and high school in Sapporo. He planned to enter medical school in Tokyo after high school, but when his father passed away in 1899, he lost the financial support. Instead, Shiraki entered Sapporo Agriculture College (now Hokkaido University) with a full scholarship in 1900, and he taught math in a night school as a part-time job to support his family. In college, he studied taxonomy of Orthoptera and Dermaptera under Shounen Matsumura (1872-1960). Matsumura was the most influential entomologist in Japan at the time. Shiraki earned his B.S. degree in July 1906 and then worked in the same college as an assistant professor for a year. Shiraki had to teach courses not related to entomology such as mineralogy, forest zoology, and agricultural product processing, and he was dissatisfied with the appointment. Meanwhile Shunji Miyao (1868-1937), chair of the Bureau of Productive Industries, Government-General of Taiwan invited him to work in Taiwan, and his mentor, Matsumura, recommended him to take the job. In 1907 July,

Shiraki began his 40-years career in Taiwan. His first position was the director of the Insect Department of the Agriculture Experimental Station, Taipei, Taiwan.

The background of Masamitsu Oshima (Figure 1-1B) parallels Shiraki's in many ways. Oshima was born in Sapporo, Hokkaido, Japan on 21 June 1884 (Hasegawa 1967). His father was also a well known educator who had been a professor and high school president. Oshima is also the eldest son of his family. Oshima moved around Japan with his family in his childhood due to changes of his father's employment. He went to elementary school in Sapporo and Kyoto, middle school in Nara, high school in Tokyo, and then entered Tokyo Imperial University in 1904 (Hasegawa 1967). In July 1907, his uncle, Hannpei Nagao (1865-1936), the chair of the Bureau of Civil Engineering, Government-General of Taiwan, invited him to conduct termite inspections in Taiwan. In July 1908, Oshima earned his B.S. degree in zoology and officially started to work in the Bureau of Civil Engineering on termite control.

### **Controversial Issues in the Identification of *Coptotermes* spp. in Taiwan**

Shiraki and Oshima cooperated on termite control and taxonomy during the first two years of their careers in Taiwan. Shiraki (1909) briefly described *C. formosanus* Shiraki, in Japanese, based on Oshima's collection from many places in Taiwan. The article included descriptions of the morphology of several castes, including the male and female imago, nymph, soldier, worker, and queen, for which no figure was offered. The authority, type locality, and type specimen of *C. formosanus* was not mentioned either. The species name was mentioned twice but incorrectly spelled as *Captotermes formosanus* [sic] and *Coptotermes formosanus* [sic]. In the same year, based on Shiraki's description, Oshima (1909) offered a more detailed examination of the morphological characteristics of *C. formosanus*. This included drawings of the dealate,

soldier, worker, fore and hind wings (Figure 1-2), and pictures of nests. Twelve collection locations in Taiwan and the Penghu islands were also described (Figure 1-3A). Oshima vividly and comprehensively described damage caused by *C. formosanus*, its tunneling behavior, and soldier defensive behaviors such as head banging and secreting from the fontanelle.

In the following year, Oshima (1910a) questioned the validity of several termite species named by Shiraki (1909), including *C. formosanus*. Oshima (1910a) emphasized that there was no personal animosity between Shiraki and himself, and they worked together and shared references, books, and termite samples with each other. He had no wish to offend Shiraki, but he believed that the correct identification of termite species was a key requirement for their control. Oshima mentioned that the soldiers of *C. formosanus* superficially resembled those of *C. gestroi* described by Haviland in 1898 (Oshima 1910b, 1911). Oshima did not compare the imagoes of these two species, probably due to absence of imago description of *C. gestroi* (Wasmann 1896, Haviland 1898). Since Taiwan was geographically close to southeast Asia where *C. gestroi* occurred, and no significant morphological difference was found, Oshima proposed that *C. formosanus* was a junior synonym of *C. gestroi*. Oshima also provided 12 collection locations of *C. gestroi/ formosanus* in Taiwan and the Penghu islands (Oshima 1911) (Figure 1-3A).

In addition to Shiraki and Oshima, several other Japanese researchers such as Munemoto Yano (1883-1970) of Forestry Experimental Station, Tokyo, Shozaburo Watasé (1862-1929) and his student, Sanji Hozawa (1885-1947) of the Zoological Institute, Science College, Tokyo Imperial University also became involved in

identification of *Coptotermes* spp. of Taiwan and southern Japan. Yano (1911) mentioned that Watasé sent Japanese specimens to a German termite taxonomist, Eric Wasmann (1859-1931) for identification. Wasmann believed that these samples were *C. gestroi* which he named in 1896. However, Yano still had some doubt about Wasmann's opinion. Yano mentioned that the description of *C. gestroi* by Wasmann was too vague to differentiate it from other *Coptotermes* spp. Hence, Yano further compared Japanese/ Taiwanese *Coptotermes* samples with the description of *C. gestroi* of Haviland (1898). He thought the two species were similar, but that their soldiers could be distinguished by the ratio of head width and length. The Japanese/ Taiwanese soldier samples had elongated heads (1.5 mm in length and 1.2 mm in width) while *C. gestroi* as described by Haviland (1898) had more circular heads (1.4 mm in length and 1.3 mm in width). Additionally, Yano sent the Japanese and Taiwanese *Coptotermes* samples to a Swedish termite taxonomist, Nils Holmgren (1877-1954), through Chiyomatsu Ishikawa (1861-1935) of Tokyo Imperial University and another German termite researcher, Karl Escherich (1871-1951). Holmgren examined Yano's specimens and described a new species, *Coptotermes formosae* Holmgren (Holmgren 1911) and compared it with other *Coptotermes* spp. of Sri Lanka. Based on Holmgren's opinion and his own measurements, Yano concluded that the *Coptotermes* sp. collected in Japan and Taiwan was not *C. gestroi*. Yano thought it should be appropriate to adopt Holmgren's nomenclature, *C. formosae*, because Holmgren was a recognized authority on termite taxonomy. Yano also criticized Shiraki's description of *C. formosanus* (Shiraki 1909) in which no figures were offered, the format was informal, and the description was in Japanese. Oshima (1912) severely criticized Yano's (Yano 1911) attempt to invalidate

*C. formosanus* based on the publication format and language used for description instead of the rules of zoological nomenclature and the content of the work. Oshima listed the original description of *C. formosae* (Holmgren 1911) in The Third Official Report on Termites (Oshima 1912) to show that Holmgren only offered five measurements of alates which was less informative than the first description of *C. formosanus* (Shiraki 1909), and no picture was offered by Holmgren either.

Oshima (1912) was torn between the conflicting opinions of the two internationally recognized authorities on termite taxonomy, Wasmann and Holmgren. He agreed with a part of Yano's opinions (1911) that there was a difference between Japanese/ Taiwanese samples and *C. gestroi* in the ratio of head width and head length of soldier. However, he thought it was insufficient to name a new species (herein *C. formosanus*) because the difference might be due to the intraspecific variation. This was the major reason why he doubted the validity of *C. formosanus* (Oshima 1910b and 1911).

To solve this controversial issue, Oshima collected more samples and measured head length and width with a more accurate method based on a clear definition. He had seven soldiers from two locations in southern Japan and 11 soldiers from three locations in Taipei, Taiwan (Oshima 1912, Figure 1-3A). He removed the soldier heads and placed them on a glass plate in a natural position, and measured the distance between medium base of the labrum to hindmost margin of the head-capsule. The data was rounded to two decimal places. Oshima found the head length (1.66-1.72 mm) of Japanese/ Taiwanese were much longer than that of *C. gestroi* (1.4 mm) as described by Haviland 1898. No significant difference was found between Japanese and Taiwanese samples (Taipei only), and the variation of these samples in head length

was only 0.03 mm. Based on the new measurements, Oshima confidently believed *Coptotermes* samples collected in Japan and Taiwan were not *C. gestroi*. It is worthy of note that the head length of soldiers reported by Oshima in 1910b and 1911 was 1.5 mm but it increased to 1.66-1.72 mm when he made another measurement in 1912. Oshima attributed the difference to different measuring methods. In addition, Oshima thought that the alate of *C. formosae* described by Holmgren (1911) was similar to *C. formosanus* described by Shiraki (1909). Since *C. formosanus* was described first, *C. formosae* should be a junior synonymy. After The Third Official Report on Termites was published by Oshima in 1912, most termite researchers such as Holmgren (1913), Watasé, and Hozawa (1915) accepted his opinion that *C. formosanus* as the only *Coptotermes* sp. in southern Japan and Taiwan.

### **Shiraki's and Oshima's Careers in Taiwan**

Although Shiraki only conducted termite research during his first 5 years in Taiwan (1907-1912), six of 17 currently named termite species in Taiwan were described by him. These include: *C. formosanus*, *Odontotermes formosanus* (Shiraki), *Pericapritermes nitobei* (Shiraki), *Neotermes koshunensis* (Shiraki) (Shiraki 1909), *Nasutitermes parvonasutus* (Shiraki), and *Na. takasagoensis* (Shiraki) (Nawa 1911). Shiraki himself mentioned that a failure in the maintenance of termite colonies in his laboratory caused him to cease further experiments on termites (Ou 2006). Following Shiraki's research on termites, he focused his studies on agricultural pests. In 1909, Shiraki introduced Vedalia beetles, *Rodolia cardinalis* (Mulsant), a natural enemy of cottony cushion scales, *Icerya purchasi* Maskell, into Taiwan for biological control, and he achieved success within three years (Chu 2005). He earned his Ph.D. degree from Hokkaido Imperial University in 1917 based on the research of a rice pest, the yellow

stem borer, *Scirpophaga incertulas* (Walker), in Taiwan. Shiraki worked in Taiwan for almost 40 years until 1947. He served as director of the Applied Zoology Division (previously the Insect Department) in the Agriculture Research Institute (previously the Agricultural Experimental Station) for 35 years (1907-1942). Shiraki was also involved in the establishment of the Plant Quarantine Station, Taiwan Governor Museum, and the curriculums of Entomology and Sericulture, Taihoku Imperial University. He was a founder of the Society of Natural Sciences and the Society of Entomology in Taiwan and served its president for several years. Shiraki was the leading figure in the development of Entomology in Taiwan (Chu 2005).

Oshima focused on termite research for around ten years (1907-1917) and served as an official in the Civil Engineering Bureau and then the chair of the Zoology Department, Institute of Science. Oshima published over 20 articles related to termites in his life time and described over 40 termite species. To date, four Taiwanese termite species were named by Oshima, including *Sinocapritermes mushae* (Oshima and Maki), *Reticulitermes flaviceps* (Oshima), *Glyptotermes fuscus* (Oshima) and *Incisitermes inamurae* (Oshima). Oshima did a comprehensive termite biology study in Taiwan including taxonomy, ecology, and control which was published in six official reports, as well as the Philippine Journal of Science, and Japanese zoological magazines. His research started with Japanese and Taiwanese termites, and then extended to termite fauna of Hong Kong, Singapore, and the Philippines, where he named 13 Philippine termite species (Snyder and Francia 1960). About 1917, Oshima changed his research subject to vertebrates including fishes, birds, and snakes. He earned his Ph.D. degree on the taxonomy of freshwater fishes of Taiwan in 1920 and returned to Japan in 1924.

## Current Taxonomic Research of *C. formosanus* and *C. gestroi*

### *Coptotermes gestroi* in Taiwan

After 1912, *C. formosanus* was believed the only *Coptotermes* species in Taiwan. *Coptotermes gestroi* in Taiwan had not been mentioned until 2003. Based on soldier morphology, the new record of *C. gestroi* in Taiwan was proposed again (Tsai and Chen 2003). During 2005 and 2009, *Coptotermes* spp. samples were collected by the author and local pest control operators from every county in Taiwan both in urban areas and natural environments. Li et al. (2009a) confirmed that *C. gestroi* is present in southern Taiwan by using mitochondrial gene sequences and morphology. In total, 220 *Coptotermes* samples including museum specimens were identified and used for mapping their distribution (Figure 1-3B). *Coptotermes* spp. were mostly collected in lowland area (< 500m). *Coptotermes formosanus* was distributed throughout the island of Taiwan, while *C. gestroi* was only collected in the tropical zone.

Oshima (1909, 1911) collected *Coptotermes* samples at 12 places in Taiwan (Figure 1-3A), and seven of them were in the tropical zone where both *C. gestroi* and *C. formosanus* can be currently collected. The drawing of a dark-brownish dealate (Figure 1-2A, Oshima 1909) resembled *C. gestroi* instead of a light brown-yellowish dealate of *C. formosanus*. The drawing of soldier head capsule (Figure 1-2B and Figure 1-4A, Oshima 1909) was somewhat rounded and also similar to *C. gestroi* (Figure 1-4C) and its head length was recorded as 1.5 mm. Three years later in 1912, Oshima collected *Coptotermes* samples only in northern Taiwan (Taipei city) (Figure 1-3A) and in Japan, where only *C. formosanus* is currently found. The image of soldier head capsule (Figure 1-4B, Oshima 1912) was elongated and similar to *C. formosanus* (Figure 1-4D). The head lengths of soldiers reported by Oshima was 1.66-1.72 mm in 1912. Soldiers

collected in 1909 and 1911 might include both *C. formosanus* and *C. gestroi*; hence, the average of their head length was shorter than that of *C. formosanus* collected only from Taipei in 1912. Oshima (1912) emphasized twice there was only one *Coptotermes* sp. in Taiwan. However, no effort was made to prove all the *Coptotermes* samples in Taiwan were the same species (Oshima 1909, 1910b, 1911). Based on this false assumption, *Coptotermes* samples collected at many places in Taiwan were lumped together and represented each morphological measurement as a mean of the two species. When Holmgren and Wasmann identified Japanese/ Taiwanese *Coptotermes* samples as *C. formosae* and *C. gestroi*, respectively (Yano 1911), Oshima and Yano were confused but they did not suspect there might be two *Coptotermes* spp. in Taiwan. Shiraki, Oshima, Yano (Oshima 1909, 1910b, 1911, 1912; Shiraki 1909, Yano 1911) drew their conclusions based on termite samples collected in different places in Taiwan and Japan, which may be the source of the controversy in early 1900s.

### **Discovery of Syntypes of *C. formosanus***

In order to investigate the controversial issue caused by identification of *C. formosanus* and *C. gestroi* in the early 1900s, the museum specimens preserved in six major insect collections in Taiwan were examined to search for the collections used by Shiraki and Oshima:

- Taiwan Agriculture Research Institute, Insect and Mite Collection, Wufeng, Taichung, Taiwan, ROC (TARI)
- Taiwan Forestry Research Institute, Insect Collection, Taipei, Taiwan, ROC (TFRI)
- National Museum of Natural Science, Taichung, Taiwan, ROC
- National Taiwan University, Department of Entomology, Insect Collection, Taipei, Taiwan, ROC

- National Chung-Hsing University, Department of Entomology, Insect Collection, Taichung, Taiwan, ROC
- National Pingtung University of Science and Technology, Plant Protection Department, Insect Collection, Neipu, Pingtung, Taiwan, ROC

A set of specimens (Figure 1-5A) labeled “*Coptotermes formosanus* Shiraki” was found in TARI where Shiraki worked between 1907 and 1942. The autograph on the label (Figure 1-5B) is very similar to Shiraki’s handwriting in an unpublished manuscript (Figure 1-5C) offered by Wen-Jer Wu (National Taiwan University). Termites soldiers preserved in vials were identified as *C. formosanus* with two setae on each side of fontanelle (Figure 1-5D) (Scheffrahn and Su 1990), but no collection information of these soldiers was found. Three alate samples were labeled, but all of them were damaged to some degree. The best preserved sample (Figure 1-5E) was collected on June 23<sup>rd</sup>, 41<sup>th</sup> year of Meiji era (1908) from a location called “old farm” which is unknown to me. The other two severely damaged samples were collected by Inao Nitobe (1883-1915), Shiraki’s assistant, on April 23<sup>rd</sup>, 41<sup>th</sup> year of Meiji era (1908) from Pingtung county, Gangkou research station of TFRI (Figure 1-3A). One of Nitobe’s surviving samples has only wings, and the other one only head and thorax remained, which were not sufficient to be identified to species. Shiraki might have used these samples collected in 1908 for description of *C. formosanus* in 1909. Hence, these collections should be assigned as syntypes. There is no doubt that Shiraki obtained some real *C. formosanus* samples for his initial description and naming of *C. formosanus*, but none of Oshima’s collection was found.

### **Difficulty and Solution on Identification of *Coptotermes* spp.**

Soldiers and winged imagoes are the two main castes used for termite identification and taxonomy. Soldiers of *Coptotermes* spp. can be collected year round

but they are superficially similar. To differentiate the soldiers of *C. gestroi* and *C. formosanus* required a microscopic examination. *Coptotermes formosanus* soldiers have two pairs of setae near the rim of the fontanelle, while in *C. gestroi*, one pair originated around the fontanelle. Imagoes offer more differentiable characters for species identification. The dark brownish head, pronotum, and dorsal abdomen of *C. gestroi* gives it a much darker appearance than *C. formosanus* in general (Scheffrahn and Su 2007). The winged imagoes of *C. formosanus* are larger than those of *C. gestroi* (Figure 1-6). However, imagoes are only present in mature colonies seasonally for a short period of time. Even in the dispersal flight season, imagoes only dwell in specific areas of nests. Rarely were both imagoes and soldiers collected from the same colony; hence, intraspecific imagoes and soldiers might be described as two species, such as *C. gestroi* and *C. havilandi* Holmgren (Kirton and Brown 2003).

The first description of *C. formosanus* (Shiraki 1909) was based on some of Oshima's collection that might include *C. gestroi* collected in tropical Taiwan. In addition, the syntypes of *C. formosanus* preserved in TARI were damaged to some degree and its collection information was incomplete. To prevent any further confusion in identification of *C. formosanus*, it would be better to re-describe *C. formosanus* based on both soldier and winged imago morphology, and included genetic sequence data for comparison. To assign alates and soldier castes collected from the same colony at northern Taiwan for a neotype and neoparatypes would be a practical method to prevent mismatching castes of *C. formosanus* with that of other *Coptotermes* spp.

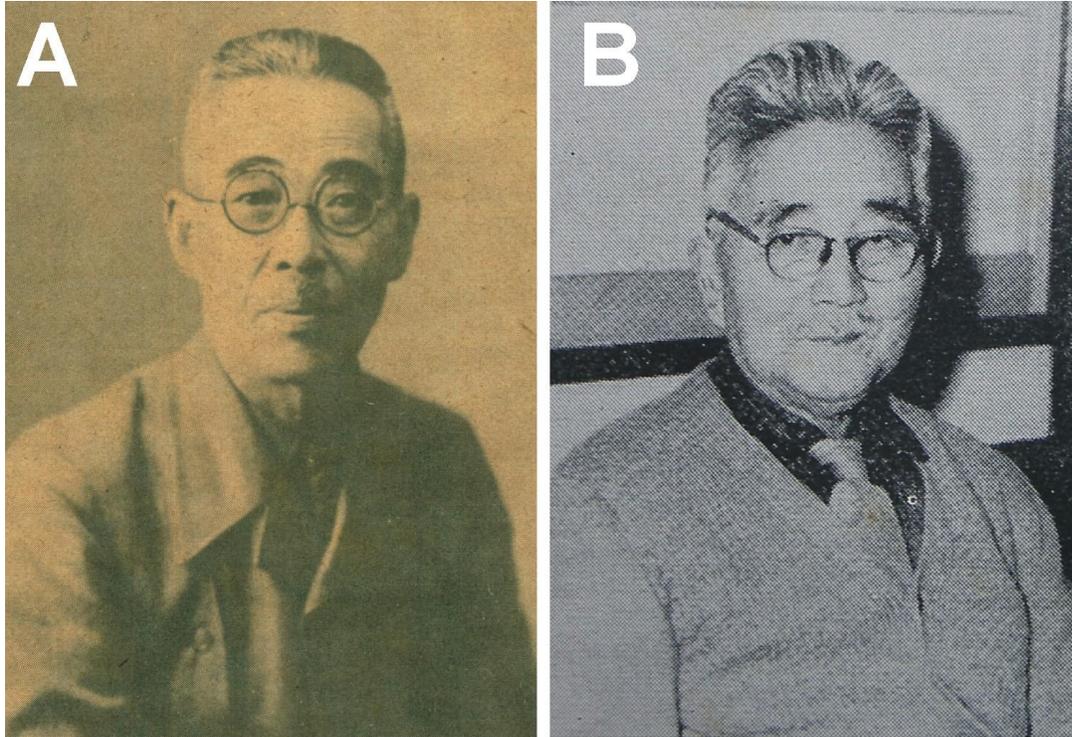


Figure 1-1. Tokuichi Shiraki (1882-1970) (A) (Photo provided by the Entomology Dept. of the National Taiwan University); Masamitsu Oshima (1884-1965) (B) (Photo provided by the Entomological Society of Japan)

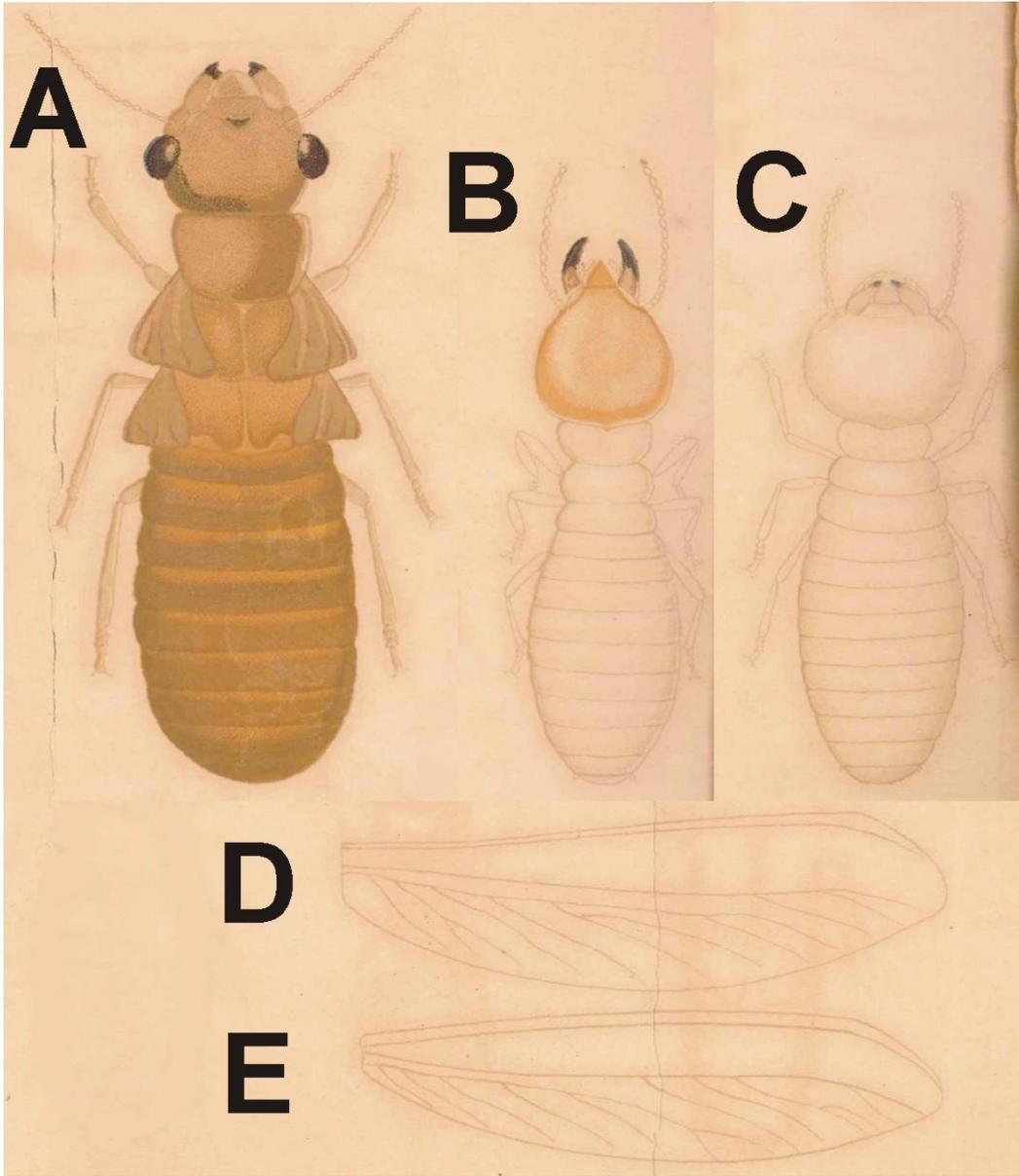


Figure 1-2. *Coptotermes formosanus* drawn by Oshima in 1909 provided by the National Taiwan University Library. Dealate (A); soldier (B); worker (C); front (D) and hind (E) wings.

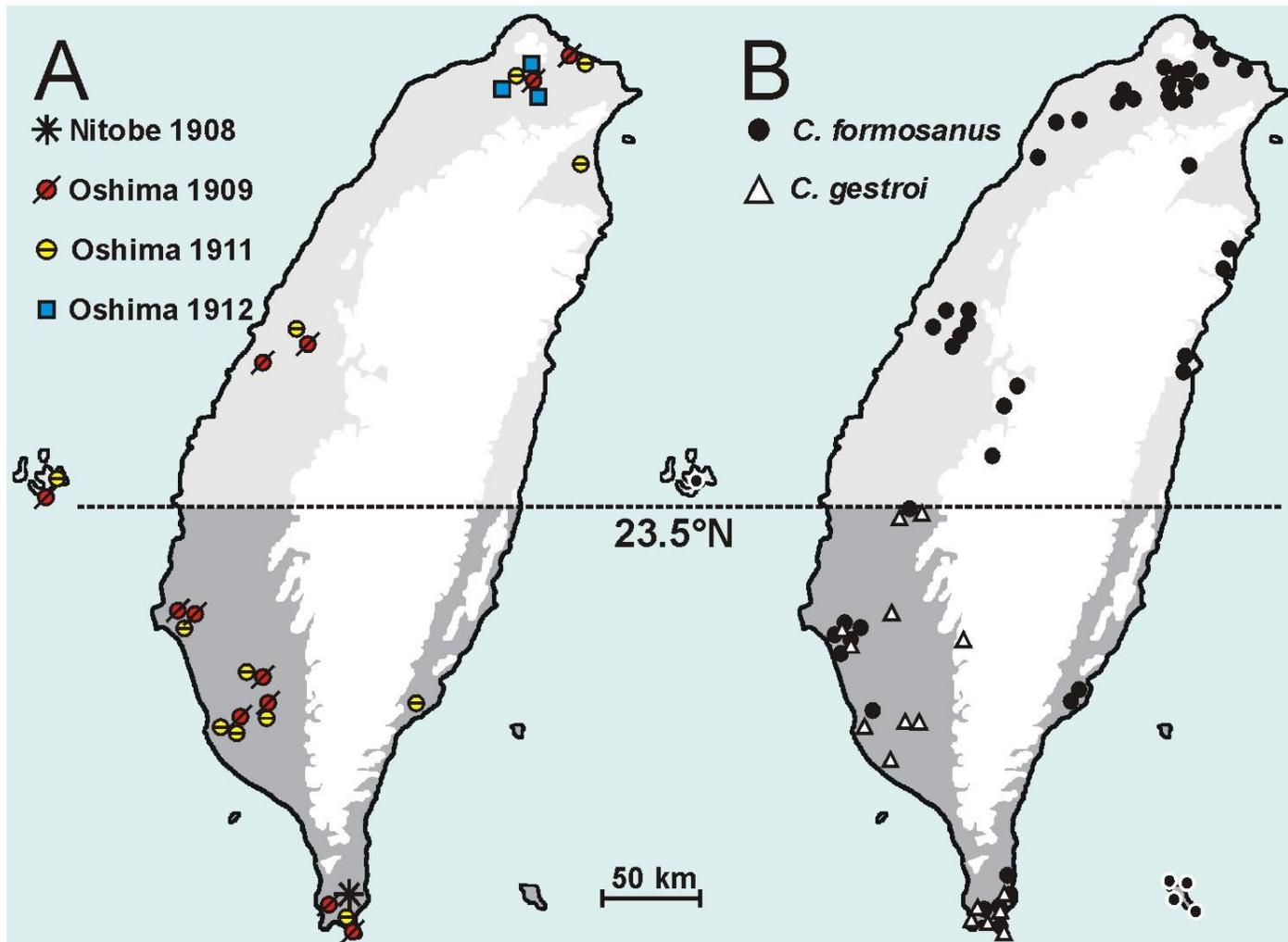


Figure 1-3. *Coptotermes* spp. collection sites in early 1900s (A) and in current study (B). White area, altitude > 500 m; light gray area, subtropical lowland; dark gray area, tropical lowland.

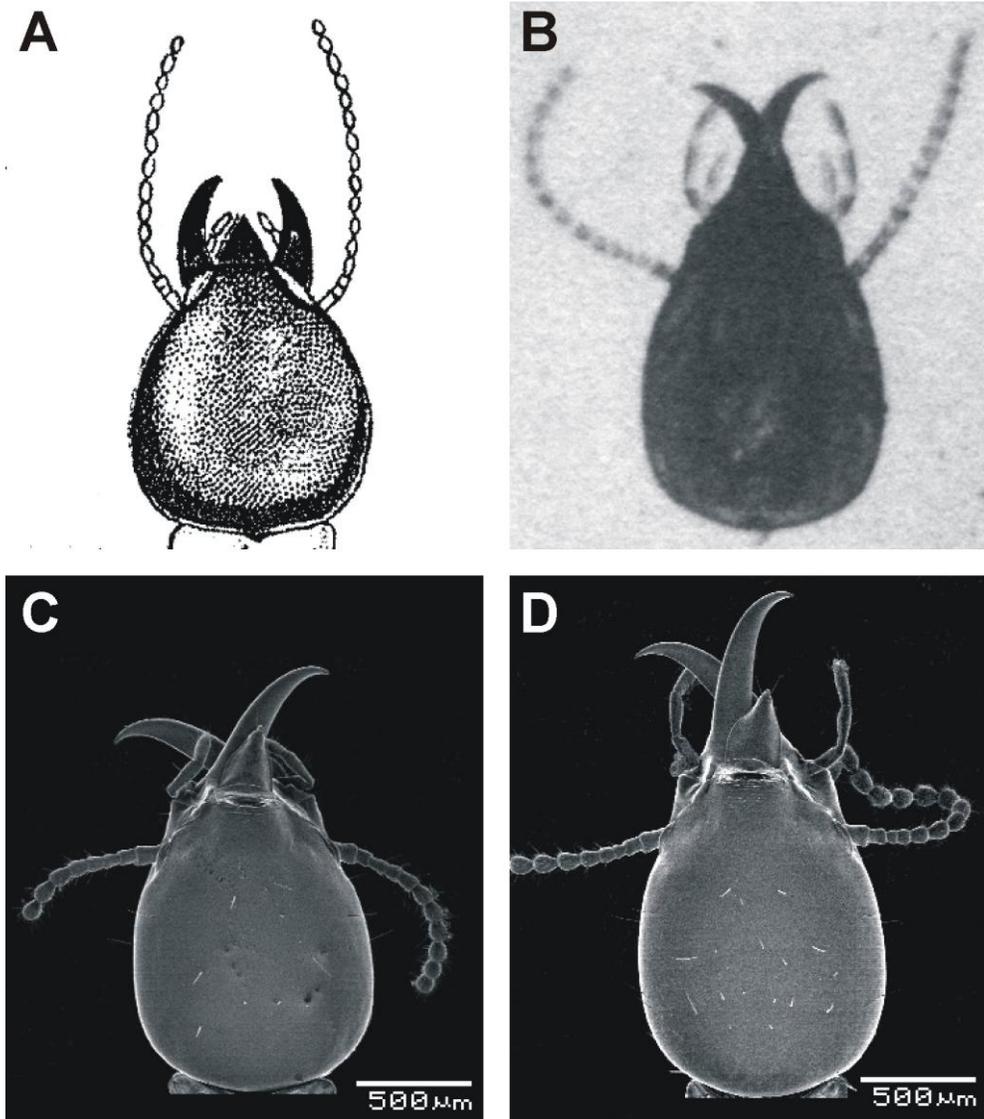


Figure 1-4. Soldier head capsules of *Coptotermes* spp. A soldier collected in Taiwan by Oshima in 1909 resembled *C. gestroi* (A) (Photo provided by the National Taiwan University Library); *C. formosanus* collected in Taipei or southern Japan by Oshima in 1912 (Photo provided by the Entomology Dept. of the National Taiwan University) (B); SEM pictures of *C. gestroi* (C) and *C. formosanus* (D) collected in southern and northern Taiwan, respectively, in current study.

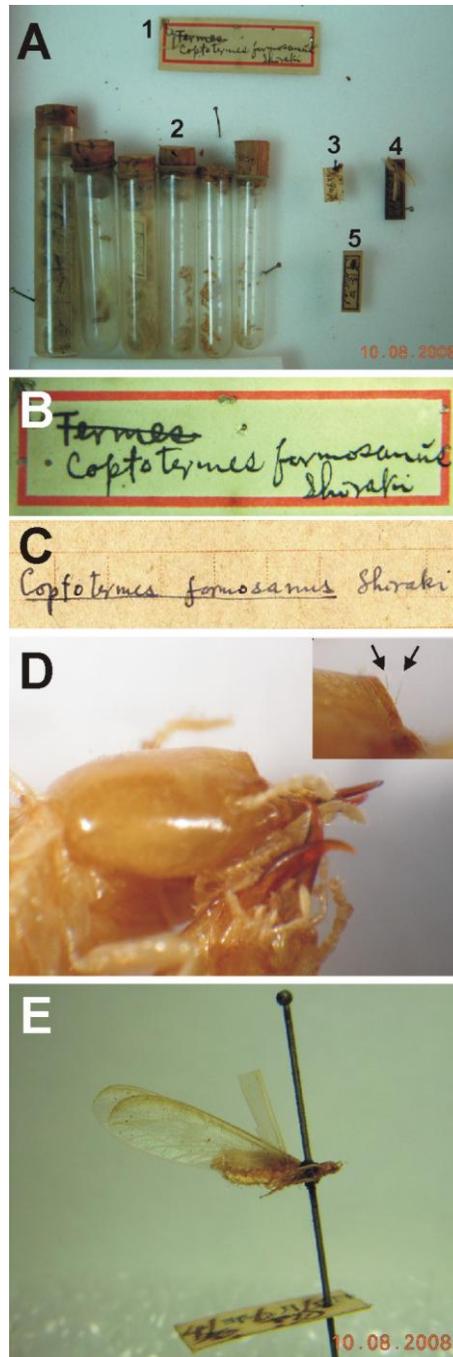


Figure 1-5. Syntypes of *C. formosanus*. Shiraki's *C. formosanus* collection preserved in Taiwan Agriculture Research Institute (A); the autography of the label (B) was similar to Shiraki's handwriting in an unpublished manuscript (C); soldiers preserved in vials (D) with two setae (inset) on one side of fontanelle; the most well preserved *C. formosanus* imago of the syntypes collected in 1908 (E). 1, label; 2, soldiers preserved in vials; 3, the best preserved imago; 4, imago collected by Nitobe, only wings remaining; 5, imago collected by Nitobe, head and thorax remaining.

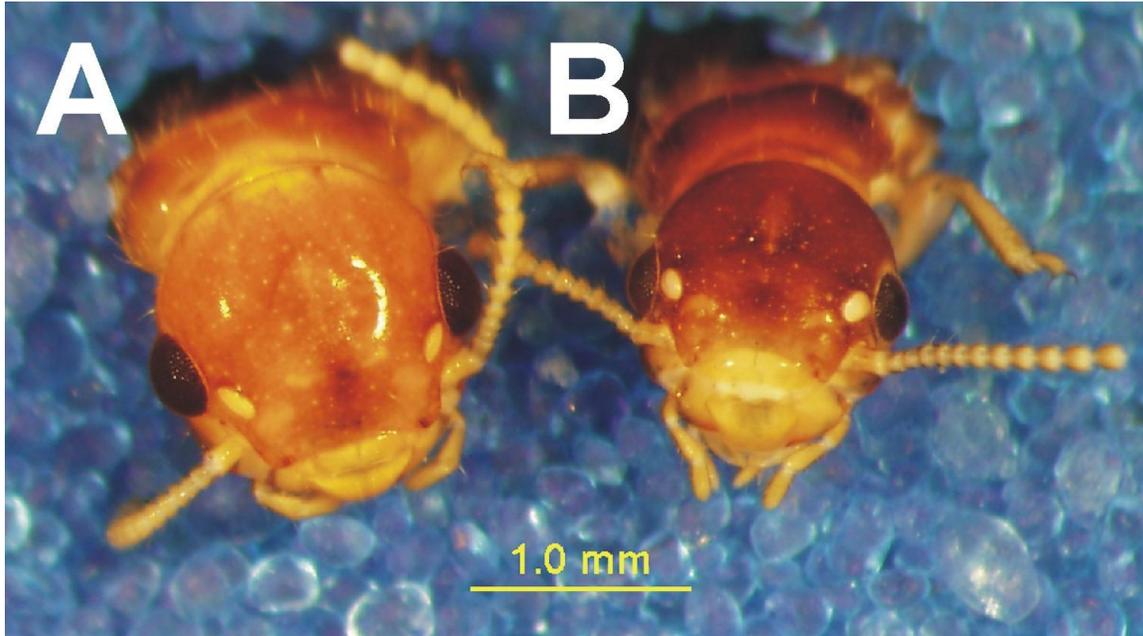


Figure 1-6. Winged imagoes of *C. formosanus* (A) and *C. gestroi* (B)

## CHAPTER 2 PHYLOGEOGRAPHY OF *COPTOTERMES FORMOSANUS* AND *COPTOTERMES GESTROI* IN TAIWAN

### Introduction

*Coptotermes formosanus* is a widely distributed pest species and found in several subtropical and temperate areas, including southern China, Taiwan, Japan, Hawaii, the southern United States, and South Africa (Su 2003). *Coptotermes gestroi* is another important structural pest, but it is primarily found in tropical regions. The distribution of *C. gestroi* extends from southeast Asia (Assam of India, Myanmar, Laos, Cambodia, Vietnam, Thailand, Peninsular Malaysia, Singapore, and the Indonesian archipelago) through the Philippines, Taiwan, and Hawaii, to the New World, including Florida, West Indies, Mexico, and Brazil (Wasmann 1896, Light 1929, Kirton and Brown 2003, Ferraz and Méndez-Montiel 2004, Yeap et al. 2007). The three regions of distributional overlap of these two species are Florida (Scheffrahn and Su 2005), Taiwan (Shiraki 1909, Tsai and Chen 2003), and Hawaii (Swezey 1914, Weesner 1965).

Several studies of the origin and dispersal routes of *C. formosanus* and *C. gestroi* have been conducted by using multiple methods. Studies on termitophilous beetles suggested that *C. formosanus* is endemic to southern China (Kistner 1985) and some islands of southern Japan (Maruyama and Iwata 2002, Tsunoda 2006). Genetic studies by using mitochondrial COII gene sequences (Austin et al. 2006) indicated *C. formosanus* populations in the United State were introduced from China and Japan. Research on population genetic structure (Vargo et al. 2003) by using microsatellite markers indicated that *C. formosanus* has been established in Japan for more than 300 years. With the same method, Vargo et al. (2006) concluded that *C. formosanus* invaded the United States mainland at least from two different sources. Jenkins et al.

(2007) used the mitochondrial COII and 16S gene sequences and revealed that *C. gestroi* collected in Ohio and Florida are closely related to populations from Singapore and Malaysia, respectively. These previous studies mainly focused on populations in the United States, China, and Japan. Few samples were collected from the historically and geographically important locality of Taiwan.

Taiwan is the type locality of *C. formosanus* (Shiraki 1909). The existence of *C. gestroi* in Taiwan had been suspected by Oshima (1911, 1912), but was only recently confirmed by Tsai and Chen (2003). The objectives of this study were to examine the geographic distribution of the two species in the subtropical island of Taiwan and to investigate the phylogeographic relationship between Taiwanese populations and other distant populations.

## **Materials and Methods**

### **Termite Samples and DNA Extraction**

During 2005 and 2007, *Coptotermes* spp. samples were collected from every county in Taiwan at urban areas and natural environments including several ecological reserves and national parks. Specimens were identified to species by using soldier or alate morphology. For phylogenetic study, 23 *Coptotermes* samples including 14 samples from Taiwan, five samples from Hainan Island, China, and four samples from Florida, United States, were identified first by using morphological characters (Shiraki 1909, Oshima 1911, 1912; Light 1929, Tsai and Chen 2003). These samples were preserved in > 95% ethanol before DNA extraction (Table 2-1). The voucher specimens are deposited in the University of Florida Termite Collection, Fort Lauderdale Research and Education Center. Total genomic DNA from the same colony was extracted from three individual termites by using a DNeasy Tissue Kit (Qiagen Inc., Valencia, CA),

following the manufacturer's protocol, and stored at -20 °C before polymerase chain reaction (PCR) attempts.

### **PCR and Purification**

Primers for mitochondrial COII, 12S, and 16S, and references are listed in Table 2-2. The 50- $\mu$ l PCR mixture (with final concentrations) contained 35  $\mu$ l water, *Taq* DNA polymerase incubation buffer (1X), dNTP mixture (0.2 mM), forward and reverse primers (0.2  $\mu$ M each), 2  $\mu$ l of termite DNA template, and 1.25 U of AmpliTaq Gold (Applied Biosystems, Foster City, CA). The thermal cycling program for all PCR was as follows: a precycle denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min, and a postcycle extension at 72 °C for 10 min. The PCR products were cleaned by using Montage PCR centrifugal filter devices (Millipore, Billerica, MA), following the manufacturer's protocol.

### **Sequencing and Phylogenetic Inferences**

PCR products were sequenced in both directions at the University of Florida DNA Sequencing Core Laboratory using ABI Prism Big Dye Terminator cycle sequencing protocols developed by Applied Biosystems (part number 4303153, PerkinElmer, Foster City, CA). The sequences in this study were submitted to the GenBank database, and accession numbers are shown in Table 2-1. Sequences from other studies including Yeap et al. (2007), Fang et al. (2008), and Tsai (2003) were downloaded from GenBank and added for phylogeny analysis. Two *R. flaviceps* samples from Lanyu Island, Taiwan were included as the outgroup taxa (Li et al. 2008) (Table 2-1). Base compositional analyses were conducted using a computer program MEGA version 4.0.1 (Tamura et al. 2007). The pairwise analyses of base substitutions per site were conducted by using the

p-distance model and the number of differences model in MEGA (Tamura et al. 2004, Tamura et al. 2007). DNA sequences were aligned by using ClustalW (Thompson et al. 1994). A chi-square test was performed in PAUP\* 4.0b10 (Swofford 2002) to check for homogeneity in base frequencies. The model of base substitution in the COII, 12S rRNA, 16S rRNA genes sequences, and combined data sets was evaluated using MODELTEST 3.7 (Posada and Crandall 1998). The Akaike-supported model, the base frequencies, the proportion of invariable sites and the gamma distribution shape parameters and substitution rates were used in phylogenetic analyses.

The tree topology of each gene and their combined data set was performed by using Bayesian analysis (MrBayes 3.1.2, Huelsenbeck and Ronquist 2001) running the chain for 1,000,000 generations and setting the burnin at 1,000 after checking the saturation curve. The Markov Chain Monte Carlo method within a Bayesian framework was used to estimate the posterior probabilities of the phylogenetic trees (Larget and Simon 1999) using 50% majority-rule. Sites with missing data or gaps were treated as missing characters for all analyses. The first, second, and third codon positions were partitioned for COII protein-coding gene.

## **Results**

### **Distribution and Dispersal Flight Season**

Thirty-five *C. formosanus* samples and seven *C. gestroi* samples were collected from 32 locations in Taiwan. Their distribution was mapped based on the combined data of current study, Tsai (2003), and Tu (1955) (Figure 2-1A). *Coptotermes formosanus* is distributed throughout the island of Taiwan, and *C. gestroi* was found only in the southwestern part of the island (Figure 2-1A). *Coptotermes formosanus* was mostly found in man-made structures, but *C. gestroi* was found in both man-made structures

and natural wood sources such as tree stumps, dead branches, and dead tree trunks. Neither species was found in the mountainous area (> 500m) of Taiwan. A dispersal flight of several *C. gestroi* alates (TW8) in Chiayi City was observed on May 22, 2006 ≈6:00 PM, which is the first record of *C. gestroi* alates in Taiwan. During dusk the next day, numerous *C. formosanus* alates swarmed at the same location. This observation suggests an overlap of the dispersal flight seasons of the *C. formosanus* and *C. gestroi* in Taiwan.

### **Genetic Analysis**

Partial fragments consisting of 660-663 bp of COII, 358-362 bp of 16S rRNA, and 396-401 bp of 12S rRNA were sequenced for the 23 samples obtained in the current study. In addition, 17 samples from Yeap et al. (2007), seven samples from Fang et al. (2008), and one sample from Tsai (2003) were obtained from GenBank and included for phylogenetic analysis (Table 2-1). All three mitochondrial genes of all ingroup taxa were A + T rich (62.91% in COII, 65.93% in 12S, and 65.5% in 16S) and had a base use with an excess of A's. Among the three gene fragments, COII was the most variable (78.1% constant characters) and informative (19.3% informative sites), whereas 16S was the most conserved (89.9% constant characters) and the least informative (7.9% informative sites). Based on Chi-square tests for base frequency homogeneity among taxa, the base frequency distribution of the three gene fragments and their combined data set were homogenous ( $P = 1.0$ ). Sequence divergence of combined COII, 12S rRNA, and 16S rRNA genes varied from 5.2 to 5.8 % (73-82 bp) between *C. gestroi* and *C. formosanus* populations, and from 13.2 to 14.2 % (186-200 bp) between *Coptotermes* spp. and *R. flaviceps* (Table 2-3). Intraspecies variation of *C. gestroi* ranged from 0 to 1% (0-14 bp), and ranged from 0 to 0.3 % (0-4 bp) for *C. formosanus*

except for TW55, which was 0.7-0.8 % (10-11 bp) different from all of the other *C. formosanus* samples in this study.

In Taiwan, the seven *C. gestroi* samples had identical COII, 12S rRNA, and 16S rRNA sequences. The COII gene sequences of Taiwanese *C. gestroi* in this study were also identical with the sample collected in Taiwan by Tsai (2003) (AY295078). Among the seven populations of *C. formosanus* distributed throughout Taiwan, the genetic diversity ranged from 0-1.2% in COII, 0-0.7% in 16S, but was identical for 12S.

Two different DNA fragments were amplified (>70 bp, ≈10% difference) for each of eight *C. gestroi* samples from Taiwan and Florida by using two pairs of COII gene primers, A-tLeu and B-tLys (Jenkins et al. 2007), and C2F2 and B-tLys (Yeap et al. 2007) (Table 2-2) using the same PCR conditions. The sequence amplified by using A-tLeu and B-tLys primers has a single nucleotide deletion relative to the sequence obtained using C2F2 and B-tLys primers. After comparing the two sequences with other COII sequences of *C. gestroi* deposited in GenBank, it was considered that the sequence with a deletion nucleotide was a pseudogene and not used in tree construction. However, DNA fragments of the 15 *C. formosanus* samples in this study amplified by using the two pairs of COII primers were not different.

### **Phylogenetic Analysis and Population Structure**

Bayesian trees showed the phylogenetic relationships inferred from COII (Figure 2-2) and combination of COII, 12S rRNA, and 16S rRNA (Figure 2-3). The 12S and 16S trees can be viewed at <http://flrec.ifas.ufl.edu/su/hou-feng-li.shtml>. Using *R. flaviceps* as the outgroups, *C. gestroi* and *C. formosanus* were well separated in two distinct clades. Among *C. gestroi* populations, the COII and 12S rRNA trees placed Indonesian into a

distinct clade with support of 100% (COII) and 85% (12S). The 16S rRNA gene tree offered few clues to population structure of these *C. gestroi* populations. The tree generated from three mitochondrial gene sequences combined (Figure 2-3) revealed that the 21 samples of *C. gestroi* could be divided into three geographical groups: group I: Taiwan, the Philippines, and Hawaii populations (support of 88%); group II: Thailand, Malaysia, and Singapore population (support of 65%); group III: Indonesian populations (support of 98%). The Florida sample was inferred to be close to group II.

Among *C. formosanus* populations, there are more samples available in COII, making it more informative for investigating population structure with this gene than 12S rRNA and 16S rRNA trees. The COII tree supported that Taiwanese, Japanese, and some Chinese samples (haplotype B and G) were grouped into a clade (support of 96%). Samples from Florida, Hawaii, and China (haplotype D) were separated into another clade (support of 83%). The Chinese haplotype A, C, E, and F and samples from Hainan Island were not resolved. There was only 0-3 bp differences (<0.5%) between the 26 COII sequences (Figure 2-2) except for TW55 collected from southeastern Taiwan (6-8 bp,  $\approx$ 1% difference). Although TW55 was separated into another clade in the 16S rRNA tree, it was not separated from the others in COII and 12S rRNA trees, and no morphological differences were found between TW55 and other samples.

## **Discussion**

### **Distribution**

Among East Asian archipelagos, *C. formosanus* has been reported from the southern part of Japan including southern Honshu ( $\approx$ 35°N), Shikoku, and Kyushu,

through Ryukyu islands (Mori 1987) to the southern tip of Taiwan ( $\approx 22^{\circ}\text{N}$ ) (Tu 1955, Tsai 2003). *Coptotermes gestroi* has been reported from the middle of Taiwan (Taichung City) ( $\approx 24^{\circ}\text{N}$ ) (Tsai and Chen 2003) through the Philippines to the Java islands ( $\approx 8^{\circ}\text{S}$ ) (Yeap et al. 2007). The zone of overlap of the two species lies between  $22$  and  $24^{\circ}\text{N}$  in Taiwan (Figure 2-1A). A similar distribution pattern of the two species in America has also been reported. *Coptotermes formosanus* has been reported from North Carolina ( $\approx 35^{\circ}\text{N}$ ) (Su 2003) to Florida City, Florida ( $\approx 25.5^{\circ}\text{N}$ ) (Scheffrahn and Su 2005). *Coptotermes gestroi* has been reported from Riviera Beach, Florida ( $\approx 27^{\circ}\text{N}$ ) (Scheffrahn and Su 2007) to the Caribbean islands such as Little Cayman Island, Turks and Caicos Islands (Scheffrahn and Su 1990, Su et al. 2000). The overlapping area of the two species lies between  $25.5$  and  $27^{\circ}\text{N}$  in Florida (Figure 2-1B).

Low temperature is believed to be a limiting factor for termite activity (Sponsler and Appel 1991, Fei and Henderson 2002). Even though Florida is further north than Taiwan, the average January temperature of the two overlapping zones are similar (Figure 2-1) (Henry et al. 1994, Lee et al. 1997) because of the warming effects of the Gulf Stream that passes coastal southeastern Florida. The average temperature of the overlapping zone ranged from  $14$  to  $20^{\circ}\text{C}$  in January. Neither *C. formosanus* nor *C. gestroi* have so far been found in the central mountainous area of Taiwan and the middle wetland area of south Florida, which suggests the distribution of the two species is also limited by geography.

### **Phylogeography**

Before early 1900s, the reported distribution of *C. formosanus* was restricted to China, Taiwan, and Japan (Su 2003). Most Taiwanese are immigrants from south

China, and frequent shipping between China and Taiwan has been recorded since the Ming Dynasty (1600s) (Su 1986). Japan has imported many agricultural products such as sugar cane, rice, tea, and logs from Taiwan since the late 1800s (Su 1986). The frequent transportation of infested materials between these three areas may have increased the gene flow among *C. formosanus* populations. Genetic data in this study support that most Taiwanese populations of *C. formosanus* are closely related to Japanese and some Chinese populations which is consistent with human mediated movement of these termites.

In contrast to *C. formosanus*, which showed a high level of divergence within Taiwan, the seven *C. gestroi* colonies distributed over 170 km in southwest Taiwan had identical sequences of COII, 12S rRNA, and 16S rRNA. Results suggest that the *C. gestroi* invaded Taiwan recently from a limited or point source. I hypothesize that the Taiwanese population of *C. gestroi* was introduced from the Philippines because it was closest to the Philippine populations both geographically and genetically. The frequent shipping between Taiwan and the Philippines could be traced back to the 17th century when Spanish colonized in both areas and Dutch colonized in southern Taiwan (Andrade 2008). The tendency of *C. gestroi* to infest boats and ships may have contributed to its dispersion (Scheffrahn and Su 2005). Jenkins et al. (2007) did not include Philippine and Hawaiian samples for determining the source of Taiwanese *C. gestroi*, partially because that the Philippine and Hawaiian populations were thought to be another species, *C. vastator* Light, which was proved to be a junior synonymy to *C. gestroi* recently (Yeap et al. 2007).

When Light (1929) described *C. vastator* (= *C. gestroi*) in the Philippines, he reported *C. gestroi* was already a major pest to manmade structures there. However, *C. gestroi* was not recorded in Hawaii until 1963 (Weesner 1965). In this study, the Hawaiian populations of *C. gestroi* were also closely related to the Philippine populations. Based on the historical records, geographic distance, and genetic data, I speculate the *C. gestroi* in Hawaii originated from the Philippines. Guam is located at midway among Taiwan, the Philippines, and Hawaii; hence, although the genetic data was not available for the present study, the *C. gestroi* (= *C. havilandi*) populations in Guam (Su and Scheffrahn 1998a) may be also close to those from these three areas.

#### **Origin of *C. formosanus* and *C. gestroi***

Southern China is considered the origin of *C. formosanus* because of high species diversity of *Coptotermes* (24 species) (Li 2000) and the association of termitophilous beetles in *C. formosanus* nests (Kistner 1985). Populations of endemic species generally have higher genetic diversity in the center of origin than introduced areas, and this principle is applicable to termites (Tsutsui et al. 2000, Austin et al. 2006). However, Fang et al. (2008) analyzed COII gene sequences of 35 *C. formosanus* colonies from six provinces in China and reported a low level of genetic variation (0-0.5%) compared with other termite species in the same area. Fang et al. (2008) considered that the low level of genetic variation was caused by frequent human activity and also pointed out that higher genetic diversity was found in areas with low transportation development in China such as Guangxi province. *Coptotermes formosanus* is highly adapted to the urban environment, and has been dispersed by railway and ships (Austin et al. 2008, Jenkins et al. 2002, Scheffrahn and Su 2005) through 10 southeastern U.S. states in the past 50 years (Su 2003). The rapid expansion suggests a possibility that some of

the 12 provinces in China may have been infested relatively recently through human activity.

Taiwan and mainland China were connected during the Pleistocene (Ota 1998, Voris 2000), and high genetic variation of Taiwanese populations found in this study suggests that *C. formosanus* could be endemic to Taiwan. In the current study, the COII gene variation among Taiwanese populations (0-1.2%) was higher than that among Chinese populations (0-0.5%) due to sample TW55, which was collected in southeast Taiwan from an isolated area surrounded by mountains (Figure 2-1A). The 16S rRNA gene sequences of TW55 also showed difference from other samples to a certain degree (0.7-1.0%). More *C. formosanus* samples collected from east Taiwan for genetic analysis could further confirm the high genetic variation among Taiwanese populations. *Coptotermes gestroi* populations possesses higher genetic variation among several geographic areas than *C. formosanus* (Table 2-3), which supports the hypothesis that southeast Asian countries including the Philippines, Malaysia, Singapore, and Indonesia are the center of origin for *C. gestroi*. Obtaining genetic sequences from samples collected in other southeast Asian countries, such as Vietnam, Laos, Cambodia, and the type locality, Myanmar, will be helpful to infer the specific origin.

In conclusion, *C. formosanus* was found to be distributed throughout Taiwan, whereas *C. gestroi* was limited to the south. The zone of overlap for the two species was between 22 and 24°N with average temperatures of 14-20°C in January. The genetic data support that Taiwan is one of the endemic areas for *C. formosanus*, but *C. gestroi* is an introduced species from the Philippines.

Table 2-1. Termite species, localities, and GenBank accession numbers

Species	Code	Location	GenBank Accession No.		
			COII <sup>a</sup>	12S	16S
Samples from this study					
<i>C. gestroi</i>	TW8	Taiwan, Chiayi City, West District	EU805750	EU805704	EU805727
<i>C. gestroi</i>	TW11	Taiwan, Chiayi City, East District	EU805751	EU805705	EU805728
<i>C. gestroi</i>	TW19	Taiwan, Pingtung County, Pingtung City	EU805752	EU805706	EU805729
<i>C. gestroi</i>	TW21	Taiwan, Pingtung County, Hengchun Township	EU805753	EU805707	EU805730
<i>C. gestroi</i>	TW24	Taiwan, Tainan City, East District	EU805754	EU805708	EU805731
<i>C. gestroi</i>	TW29	Taiwan, Tainan City, North District	EU805755	EU805709	EU805732
<i>C. gestroi</i>	TW30	Taiwan, Tainan City, North District	EU805756	EU805710	EU805733
<i>C. gestroi</i>	FLCG	U.S.A., Florida, Key West	EU805757	EU805711	EU805734
<i>C. formosanus</i>	TW49	Taiwan, Taoyuan County, Taoyuan City	EU805758	EU805712	EU805735
<i>C. formosanus</i>	TW50	Taiwan, Taichung City, Situn District	EU805759	EU805713	EU805736
<i>C. formosanus</i>	TW51	Taiwan, Tainan City, South District	EU805760	EU805714	EU805737
<i>C. formosanus</i>	TW52	Taiwan, Yilan County, Nan-ao Township	EU805761	EU805715	EU805738
<i>C. formosanus</i>	TW53	Taiwan, Yilan County, Nan-ao Township	EU805762	EU805716	EU805739
<i>C. formosanus</i>	TW54	Taiwan, Hualien County, Hualien City	EU805763	EU805717	EU805740
<i>C. formosanus</i>	TW55	Taiwan, Taitung County, Taitung City	EU805764	EU805718	EU805741
<i>C. formosanus</i>	H1	China, Hainan Province, Sanya City	EU805765	EU805719	EU805742
<i>C. formosanus</i>	H2	China, Hainan Province, Sanya City	EU805766	EU805720	EU805743
<i>C. formosanus</i>	H3	China, Hainan Province, Sanya City	EU805767	EU805721	EU805744
<i>C. formosanus</i>	H4	China, Hainan Province, Qionghai City	EU805768	EU805722	EU805745
<i>C. formosanus</i>	H5	China, Hainan Province, Qionghai City	EU805769	EU805723	EU805746
<i>C. formosanus</i>	WBBR	U.S.A., Hallandale Beach, Broward County, Florida	EU805770	EU805724	EU805747
<i>C. formosanus</i>	LNDN	U.S.A., Hallandale Beach, Broward County, Florida	EU805771	EU805725	EU805748
<i>C. formosanus</i>	437GI	U.S.A., Hallandale Beach, Broward County, Florida	EU805772	EU805726	EU805749
Yeap et al. 2007					
<i>C. gestroi</i>	CG1MY	Malaysia, Penang	EF379945	EF379982	EF379963
<i>C. gestroi</i>	CG4MY	Malaysia, Kuala Lumpur	EF379951	EF379987	EF379969
<i>C. gestroi</i>	CG5MY	Malaysia, Muar	EF379952	EF379988	EF379970
<i>C. gestroi</i>	CG1SG	Singapore, Serenity Terr.	EF379946	EF379983	EF379964
<i>C. gestroi</i>	CG2SG	Singapore, Serangoon	EF379949	EF379985	EF379967
<i>C. gestroi</i>	CG1TH	Thailand, Bangkok	EF379947	EF379977	EF379965
<i>C. gestroi</i>	CG2TH	Thailand, Bangkok	EF379950	EF379986	EF379968

<i>C. gestroi</i>	CG1IN	Indonesia, Cibinong	EF379944	EF379981	EF379962
<i>C. gestroi</i>	CG2IN	Indonesia, Bogor	EF379948	EF379984	EF379966
<i>C. gestroi</i> (= <i>C. vastator</i> )	CV1HW	U.S.A., Hawaii, Oahu	EF379953	EF379990	EF379971
<i>C. gestroi</i> (= <i>C. vastator</i> )	CV1PH	Philippines, Los Banos, Laguna	EF379954	EF379989	EF379972
<i>C. gestroi</i> (= <i>C. vastator</i> )	CV2PH	Philippines, Los Banos, Laguna	EF379955	EF379991	EF379973
<i>C. gestroi</i> (= <i>C. vastator</i> )	CV3PH	Philippines, Los Banos, Laguna	EF379956	EF379992	EF379974
<i>C. formosanus</i>	CF1JP	Japan, Wakayama	EF379941	EF379978	EF379959
<i>C. formosanus</i>	CF2JP	Japan, Wakayama	EF379942	EF379979	EF379960
<i>C. formosanus</i>	CF3JP	Japan, Okayama	EF379943	EF379980	EF379961
Li et al. 2008					
<i>R. flaviceps</i>	TW223	Taiwan, Taitung County, Lanyu Township	EU627782	EU627778	EU627780
<i>R. flaviceps</i>	TW224	Taiwan, Taitung County, Lanyu Township	EU627783	EU627779	EU627781

<sup>a</sup> COII gene sequences from GenBank used for analysis include seven haplotypes (A-G) of *C. formosanus* from mainland China, EF056702, EF056705, EF056706, EF056709, EF056714, EF056729, and EF056738 (Fang et al. 2008), and one *C. gestroi* sample, AY295078, from Taiwan (Tsai 2003).

Table 2-2. PCR and sequencing primers used in the present study

Name	Gene	Orientation	Sequence	Reference <sup>a</sup>
A-tLeu <sup>b</sup>	COII	Forward	5' ATGGCAGATTAGTGCAATGG 3'	1, 2, 3, 4
C2F2 <sup>c</sup>	COII	Forward	5' ATACCTCGACGWTATTCAGA 3'	1, 5, 6
B-tLys	COII	Reverse	5' GTTTAAGAGACCAGTACTTG 3'	1, 2, 4, 5, 6
16Sar	16S	Forward	5' CGCCTGTTTAACAAAAACAT 3'	1, 7
16Sbr	16S	Reverse	5' CCGGTCTGAACTCAGATCACGT 3'	1, 7
12SF	12S	Forward	5' TACTATGTTACGACTTAT 3'	1, 5, 8
12SR	12S	Reverse	5' AAAGTAGGATTAGATACCC 3'	1, 5, 8

<sup>a</sup> References: (1) Simon et al. 1994; (2) Miura et al. 1998; (3) Liu and Bechenbach 1992; (4) Jenkins et al. 2007; (5) Yeap et al. 2007; (6) Hayashi et al. 2003; (7) Marini and Mantovani 2002; (8) Kambhampati 1995.

<sup>b</sup> Forward primer was used for amplifying COII gene of *C. formosanus*.

<sup>c</sup> Forward primer was used for amplifying COII gene of *C. gestroi*.

Table 2-3. Distance matrix (percentage, above diagonal) and number of difference of nucleotides (below diagonal) of combined mitochondrial COII, 12S rRNA, and 16S rRNA gene sequences. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). There were a total of 1406 positions in the final dataset. The rectangle with solid lines and dotted lines indicate the intraspecific difference of *C. gestroi* and *C. formosanus*, respectively.

No. species, country, code	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1 <i>C. gestroi</i> Taiwan <sup>a</sup>		0.4	0.4	0.4	0.3	0.6	0.6	0.7	0.6	0.6	0.9	0.8	5.7	5.8	5.4	5.7	5.8	5.7	5.7	5.8	14.2	14.1
2 <i>C. gestroi</i> USA Hawaii CV1HW	5		0.0	0.1	0.5	0.7	0.7	0.8	0.7	0.7	0.9	0.9	5.5	5.6	5.3	5.5	5.6	5.5	5.5	5.6	13.9	13.8
3 <i>C. gestroi</i> Philippines CV1PH	5	0		0.1	0.5	0.7	0.7	0.8	0.7	0.7	0.9	0.9	5.5	5.6	5.3	5.5	5.6	5.5	5.5	5.6	13.9	13.8
4 <i>C. gestroi</i> Philippines CV2PH	6	1	1		0.6	0.8	0.8	0.9	0.8	0.8	1.0	0.9	5.5	5.5	5.2	5.5	5.5	5.5	5.5	5.5	13.9	13.8
5 <i>C. gestroi</i> Philippines CV3PH	4	7	7	8		0.8	0.8	0.9	0.8	0.8	1.0	0.9	5.7	5.8	5.4	5.7	5.8	5.7	5.7	5.8	14.1	13.9
6 <i>C. gestroi</i> USA Florida FLCG	9	10	10	11	11		0.1	0.2	0.1	0.1	0.5	0.6	5.5	5.6	5.3	5.5	5.6	5.5	5.5	5.6	13.9	13.8
7 <i>C. gestroi</i> Malaysia <sup>b</sup>	9	10	10	11	11	2		0.1	0.0	0.0	0.5	0.6	5.5	5.6	5.3	5.5	5.6	5.5	5.5	5.6	13.8	13.7
8 <i>C. gestroi</i> Singapore CG1SG	10	11	11	12	12	3	1		0.1	0.1	0.6	0.6	5.6	5.7	5.3	5.6	5.7	5.6	5.6	5.7	13.9	13.7
9 <i>C. gestroi</i> Singapore CG2SG	9	10	10	11	11	2	0	1		0.0	0.5	0.6	5.5	5.6	5.3	5.5	5.6	5.5	5.5	5.6	13.8	13.7
10 <i>C. gestroi</i> Thailand <sup>c</sup>	9	10	10	11	11	2	0	1	0		0.5	0.6	5.5	5.6	5.3	5.5	5.6	5.5	5.5	5.6	13.8	13.7
11 <i>C. gestroi</i> Indonesia CG1IN	12	13	13	14	14	7	7	8	7	7		0.1	5.7	5.8	5.4	5.7	5.8	5.7	5.7	5.8	13.9	13.7
12 <i>C. gestroi</i> Indonesia CG2IN	11	12	12	13	13	8	8	9	8	8	1		5.8	5.8	5.5	5.8	5.8	5.8	5.8	5.8	13.8	13.7
13 <i>C. formosanus</i> Taiwan <sup>d</sup>	80	78	78	77	80	78	78	79	78	78	80	81		0.1	0.7	0.0	0.1	0.1	0.1	0.2	13.7	13.4
14 <i>C. formosanus</i> Taiwan TW50	81	79	79	78	81	79	79	80	79	79	81	82	1		0.8	0.1	0.1	0.2	0.2	0.3	13.7	13.4
15 <i>C. formosanus</i> Taiwan TW55	76	74	74	73	76	74	74	75	74	74	76	77	10	11		0.7	0.8	0.7	0.7	0.8	13.9	13.7
16 <i>C. formosanus</i> Japan <sup>e</sup>	80	78	78	77	80	78	78	79	78	78	80	81	0	1	10		0.1	0.1	0.1	0.2	13.7	13.4
17 <i>C. formosanus</i> Japan CF3JP	81	79	79	78	81	79	79	80	79	79	81	82	1	2	11	1		0.2	0.2	0.3	13.7	13.4
18 <i>C. formosanus</i> USA Florida <sup>f</sup>	80	78	78	77	80	78	78	79	78	78	80	81	2	3	10	2	3		0.1	0.1	13.7	13.4
19 <i>C. formosanus</i> China, Hainan <sup>g</sup>	80	78	78	77	80	78	78	79	78	78	80	81	2	3	10	2	3	2		0.2	13.5	13.2
20 <i>C. formosanus</i> Hawaii CF1HW	81	79	79	78	81	79	79	80	79	79	81	82	3	4	11	3	4	1	3		13.7	13.4
21 <i>R. flaviceps</i> TW223	200	196	196	196	198	196	194	195	194	194	195	194	192	192	196	192	193	192	190	193		1.1
22 <i>R. flaviceps</i> TW224	198	194	194	194	196	194	192	193	192	192	193	192	188	188	192	188	189	188	186	189	15	

<sup>a</sup> Samples include TW8, TW11, TW19, TW21, TW24, TW29, and TW30.

<sup>b</sup> Samples include CG1MY, CG4MY, and CG5MY.

<sup>c</sup> Samples include CG1TH and CG2TH.

<sup>d</sup> Samples include TW49, TW51, TW52, TW53, and TW54.

<sup>e</sup> Samples include CF1JP and CF2JP.

<sup>f</sup> Samples include WBBR, LNDN, and 437GI.

<sup>g</sup> Samples include H1, H2, H3, H4, and H5



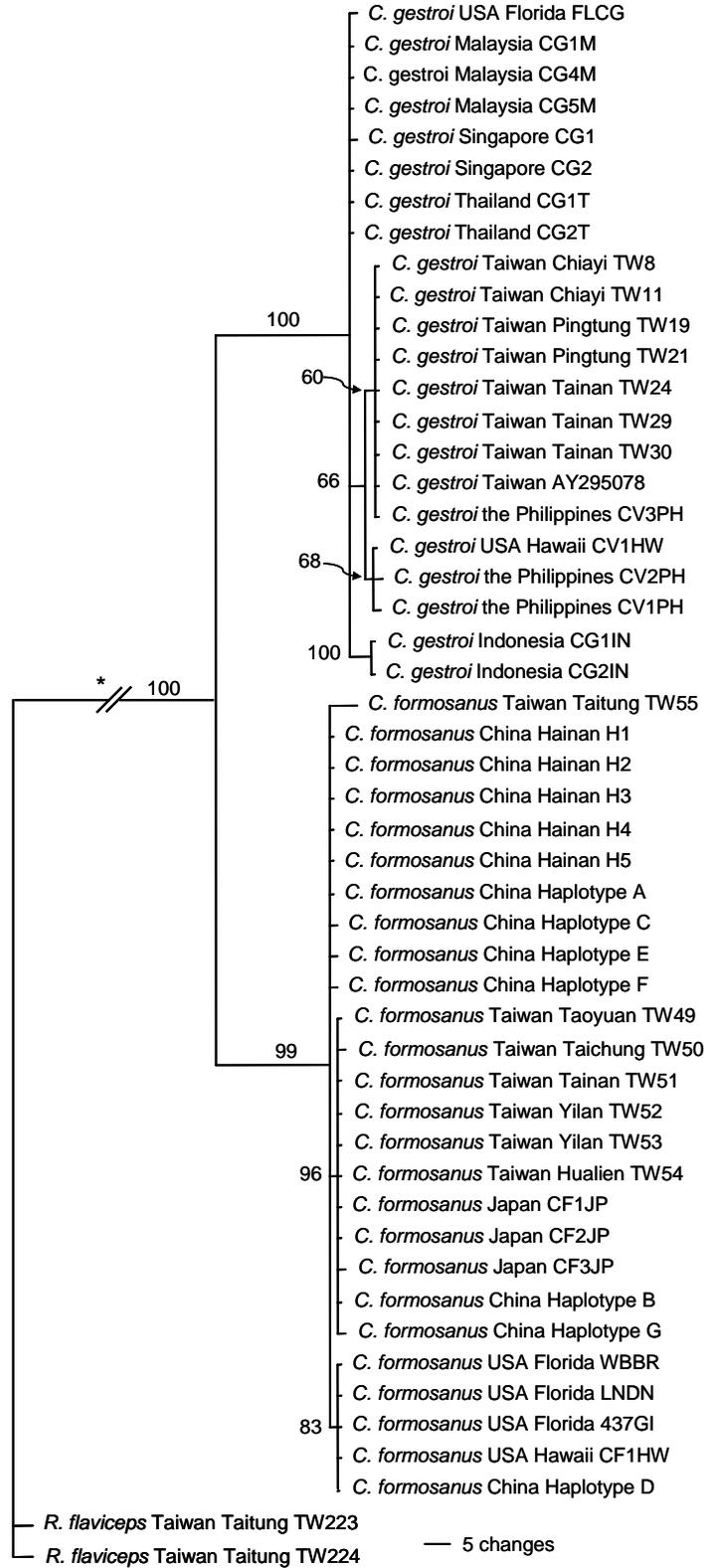


Figure 2-2. The 10001st Bayesian tree inferred from COII gene sequences. Posterior probability values exceeding 50 are given on appropriate clades. \*, >75 changes.

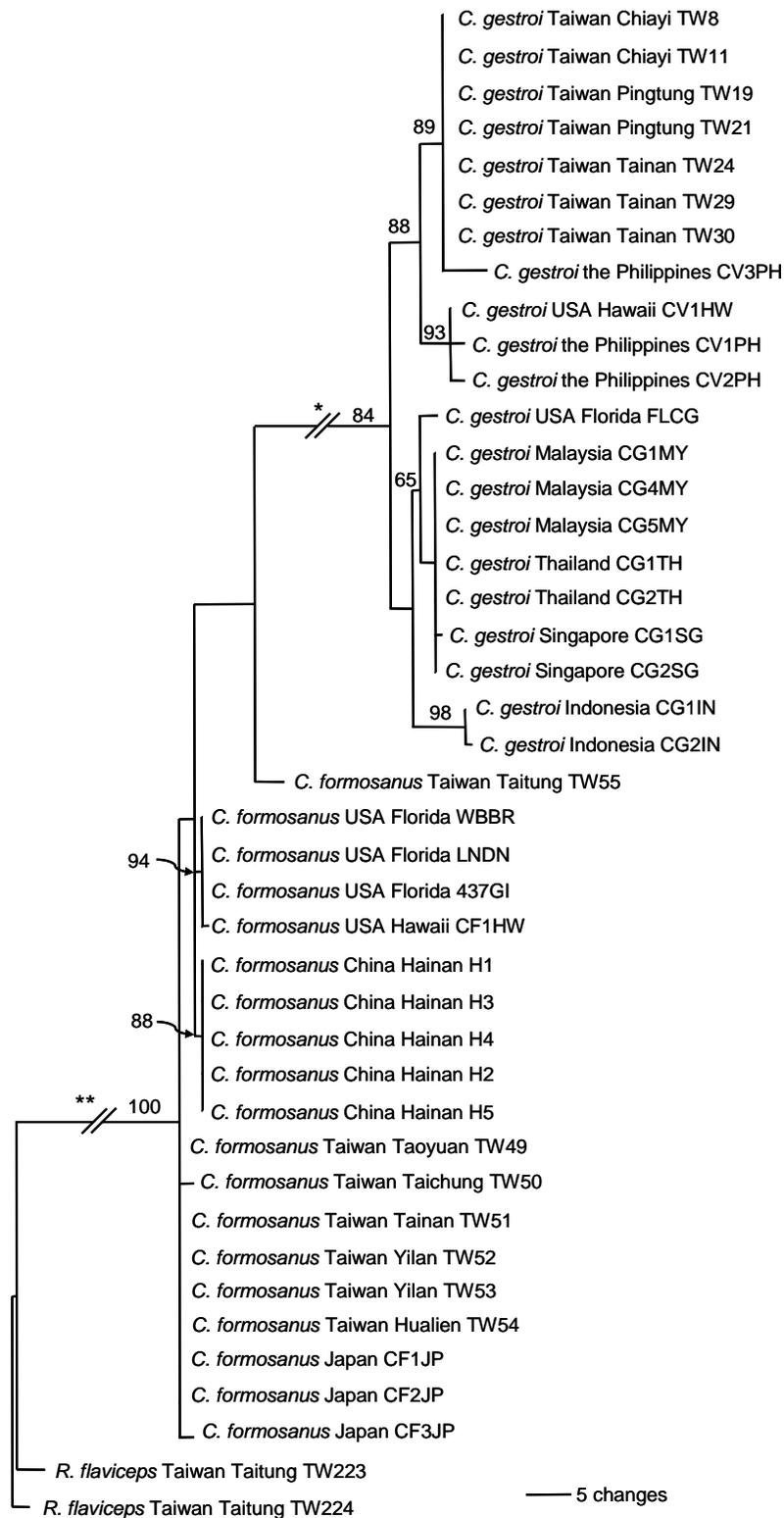


Figure 2-3. The 10001st Bayesian tree inferred from combined COII, 12S rRNA, and 16S rRNA gene sequences. Posterior probability values exceeding 50 are given on appropriate clades. \*, >65 changes, \*\*, >170 changes.

CHAPTER 3  
INTERSPECIFIC COMPETITION AND TERRITORY DEFENSE MECHANISMS OF  
*COPTOTERMES FORMOSANUS* AND *COPTOTERMES GESTROI*

**Introduction**

*Coptotermes formosanus* and *C. gestroi* originated in Asia and have been dispersed to North America, South America, and Africa by human transportation in the past century (Su 2003, Scheffrahn and Su 2005, Austin et al. 2006, Jenkins et al. 2007). To date *C. formosanus* is primarily found in the subtropics and warm temperate zones, and *C. gestroi* is reported mostly from the tropics (Su 2003). Their distribution overlaps in some subtropical areas such as south Florida (Scheffrahn and Su 2005), Hawaii (Weesner 1965), and Taiwan (Li et al. 2009a). *Coptotermes formosanus* colonized these three areas earlier than *C. gestroi* did. *Coptotermes formosanus* is endemic to Taiwan (Li et al. 2009a) while *C. gestroi* is an invasive species first recorded in 1911 by Oshima (Oshima 1911). In Hawaii, *C. formosanus* and *C. gestroi* (= *C. vastator*) were first found in 1913 (Swezey 1914) and in 1963 (Bess 1970), respectively. In south Florida *C. formosanus* and *C. gestroi* (= *C. havilandi*) were first reported in 1980 (Koehler 1980) and in 1996 (Su et al. 1997), respectively. Both species are subterranean termites and structural pests. Their ecological niches probably overlap in the sympatric areas. Termites are territorial, and intra- and inter-specific competition has been considered to be an important regulatory factor to maintain colonial territories (Jones and Trosset 1991, Levings and Adams 1984, Jmhasly and Leuthold 1999). The outcome of interspecific competition between *C. formosanus* and *C. gestroi* may affect their distribution and overlapped zone.

Field studies on subterranean termites typically use above- or in-ground monitoring stations in combination with mark-recapture methods or molecular tools to

delineate colony boundary and abundance (Haverty et al. 1975, Su and Scheffrahn 1988b, Jones 1990, Su et al. 1993, Vargo 2003, Messenger et al. 2005). These field investigations revealed that more than one species of subterranean termite colony could maintain their neighboring territories for a long period of time (years) and keep their territories in distance from each other. However, monitoring stations are inadequate to serve as a window in the field for observing interactions among subterranean termite species in real time. Only a few field observations based on serendipitous discovery were reported (Jones and Trosset 1991, Jmhasly and Leuthold 1999), and the mechanism forming territory spatial distribution remains unknown. To date, most experiments related to interspecific competition of termites were conducted in Petri-dish bioassays (no-choice bioassay) (Thorne and Haverty 1991, Polizzi and Forschler 1998, Šobotník et al. 2008) and most of them focused on immediate agonistic behaviors resulting from encounters of two subterranean termite species. These laboratory experiments usually resulted in high mortality within hours, which contradicts field observations in which several neighboring intra- or interspecific colonies might survive for years.

The objective of this study was to examine the territory defense behavior between *C. formosanus* and *C. gestroi* in a foraging arena bioassay which allowed us to observe the tunnel progress and encounters of these two species in real time. The results generated from a foraging arena bioassay were further compared with that of a Petri-dish bioassay.

## Materials and Methods

### Termite Species

Individuals of three field colonies of both *C. formosanus* and *C. gestroi* were used in this study. *Coptotermes formosanus* were collected in New Orleans, Louisiana (colonies F1 and F2) and Hallandale, Florida (F3). *Coptotermes gestroi* were collected in Broward County, Florida (G1), Miami-Dade County, Florida (G2), and Monroe County, Florida (G3). Before testing, termites were kept at 28°C in one-liter cylindrical plastic jars with pieces of moist wood. Voucher specimens were preserved in absolute ethanol and deposited in the University of Florida Termite Collection, Fort Lauderdale Research and Education Center.

Three colonies each of the two species were used to create a 3 x 3 combination of interspecifically paired interaction. To differentiate species in each pair, two species of termites were fed on non-dyed or dyed filter paper containing 0.1% Nile blue (wt/wt) for 5 days before each test (Su et al. 1991) resulting in 18 (3 x 3 x 2) possible pairs. Each pair was tested three times in the Petri-dish bioassay and once in the foraging arena bioassay resulting in 54 and 18 tests, respectively. During the experiments, the room temperature was maintained at ≈28°C.

### Petri-dish Bioassay

One group of nine workers and one soldier each of *C. formosanus* and *C. gestroi* were placed in a Petri-dish (60 x 15 mm) lined with moist filter paper. The initial behavioral response was observed and recorded through a camcorder (Sony DCR-TRV9, Tokyo, Japan) for 1 min, and the number of surviving individuals of each species was counted at 24 h. The results were separated into three categories, “no fight,” where more than 8 individuals of both species survived, “severe fight,” where less than 2

individuals of both species survived, and the rest of results were in the “one species dominant” category. In the last category, the species with more survivors was classified as the winner. A chi-square test was used to examine the dye and species effects on interspecies competition.

### **Foraging Arena Bioassay**

The experimental arena (Figure 3-1A) was constructed of two sheets of transparent Plexiglas (60 x 60 x 0.6 cm in thickness) separated from each other by Plexiglas laminates (5 cm in width and 0.15 cm in thickness on each side) placed between the outer margins to form a 0.15-cm gap of 50 x 50 cm and held together with screws (Figure 3-1A). In the arena, five pieces of wood (3.7 x 3.7 x 0.15 cm) were fastened at each of two corners, by injecting glue into a 0.6-cm diameter hole in its center, which encompassing a square space (8.4 x 8.4 x 0.15 cm) at each of the two corners. Sand was used to fill the rest of space between the two Plexiglas sheets. The top Plexiglas sheet had four 1-cm-diameter access holes in the center for injecting water to moisten sand. A 5-cm-diameter disc placed in center of the arena served as a rotatable switch of the central access holes and also maintained the arena gap. Two hundred termites (180 workers and 20 soldiers) were introduced into the square space encompassed by wood pieces through a 0.6-cm-diameter access hole on top Plexiglas at each of the two corners. Termites accessed the foraging sand through the four 0.5-cm-width gateways between wood pieces. All access holes on top Plexiglas were closed and plugged immediately after the sand was moistened and termites were introduced.

After introducing termites into the arena, back-lit digital images of the entire arena were taken every five minutes for five days yielding 1,440 images per test. The images

of each test were combined into a video for quick review by using Windows Movie Maker version 6.0 (Microsoft Corp. Redmond, WA) and were converted into a filmstrip for detail analysis by using Acrobat 6.0 Professional (Adobe Systems Inc. San Jose, CA). Tunnel interceptions of the two species, and the subsequent agonistic behavior and tunnel blockage patterns were reviewed (Figure 3-1). The distance between the tunnel junction and resultant tunnel blockages, and time length from tunnel interception to the formation of tunnel blockages, were measured. Live termites of each species were counted on the fifth day from the image on the computer monitor.

## Results

### Petri-dish Bioassay

Immediately after releasing termites of the two species into Petri-dishes, termites expressed agonistic behavior toward each other in most cases. Soldiers of both species moved rapidly and chased the individuals of the other species. Soldier mandibles flared and slashed the head and abdomen of the other species. Workers bit legs, antennae and abdomens of soldier and workers of the other species. It was frequently observed that several workers simultaneously attacked a single soldier or worker of the other species. Fierce combat was generally observed and several individuals died in the first minute.

After 24 h, based on the number of surviving individuals, only four of 54 test results were classified as “no-fight”, 18 of 54 test results were recorded as “severe-fight”, and 32 of 54 test results were recorded as “one-species-won”. The dye had no effect on interspecies competition ( $\chi^2=0$ ,  $p=1$ ). *Coptotermes gestroi* won most of the agonistic encounters over *C. formosanus* ( $\chi^2=32$ ,  $p<0.0001$ ). The overall survival rates

were  $44.44 \pm 4.30\%$  (Mean  $\pm$  SE) and  $8.15 \pm 3.37\%$  for *C. gestroi* and *C. formosanus*, respectively.

### **Tunnel Interception and Blockage**

In the foraging arena bioassay, both *C. gestroi* and *C. formosanus* excavated several tunnels through the gateways simultaneously and deposited sand into the two respective square spaces. The first encounter of tunnels of the two species occurred within 1-2 d. First interception is when each primary tunnel extended from the gateways and encountered a tunnel of the other species for the first time (Figure 3-1A), and 44 such first interceptions were observed in the 18 replications (Table 3-1) during the entire 5-d experimental period. After a tunnel interception, termites from both sides rapidly rushed into each other's tunnel which immediately caused agonistic encounters. The worker caste is the major caste involved in fights. Some individuals were wounded and died in tunnels and body fluids stained the surrounding sand. Both species buried the connected tunnel at several locations shortly after tunnel interception and agonistic encounters. One species might excavate another tunnel and bypass the blocked portion or reopened the blockage, and then encountered tunnels of the other species again, which is defined as additional interception (Table 3-1 and Figure 3-1C). In total, there were 80 tunnel interceptions, 59 tunnels of *C. gestroi* encountered the tunnels of *C. formosanus*, 17 tunnels of *C. formosanus* encountered the tunnels of *C. gestroi*, and only in four cases, leading tunnels of both species encountered each other.

*Coptotermes gestroi* was more active than *C. formosanus* in tunnel excavation in the foraging arena bioassay, but in most cases (17 of 18 replications) both species survived

to the end of experimental period. The survival rates were  $38.89 \pm 3.06\%$  and  $51.56 \pm 5.19\%$  for *C. gestroi* and *C. formosanus*, respectively.

Numbers of tunnel blockage induced by tunnel interception are shown in Table 3-2. In most cases (72 of the 80 observations), the first tunnel blockage was made within an hour after tunnel interception, which completely separated both species, and 77.5% of tunnel interceptions induced more than one tunnel blockage. On average, each tunnel interception resulted in  $2.71 \pm 0.16$  tunnel blockages, and > 60% of them were made in the first hour (Table 3-2). The average distance between tunnel interception and blockages was  $6.42 \pm 0.53$  cm, and over 78% of all blockages were within 10 cm of the interception. In the total 217 of tunnel blockages, 185 of them (85.3%) were induced in the presence of termite cadavers, and 32 of them (14.7%) were initiated without termite cadavers. Surviving termites covered cadavers with sand and then further filled a section of the tunnel. During the 5-d experimental period, only 5.4% (10 of 185) tunnel blockages containing cadavers were reopened, but 40.6% (13 of 32) blockages without cadavers were reopened, which indicates that termites avoided contact with cadavers.

## Discussion

### Interspecific Competition

Both the Petri-dish bioassay and the foraging arena bioassay showed *C. gestroi* was more aggressive than *C. formosanus*. In the Petri-dish bioassay, *C. gestroi* killed most of the *C. formosanus* within 24 h. In the foraging arena bioassay, most first tunnel interceptions (36 of 54) and most additional tunnel interceptions (23 of 26) resulted from leading tunnels of *C. gestroi* intercepting the tunnel of *C. formosanus* (Table 3-1). These results may partially explain how *C. gestroi* successfully infested the areas such as

south Florida and Hawaii where *C. formosanus* had been previously established for several decades.

Low temperature is a limiting factor for termite activity (Sponsler and Appel 1991, Fei and Henderson 2002) that affects their distributions (Li et al. 2009a). If climate changes, the distribution of *C. gestroi* and *C. formosanus* may change accordingly. The average temperature of their overlapping region in the United States ranged from 18 to 20°C in January which may shift toward north, and the distribution of *C. gestroi* may expand. Based on the current study, the presence of *C. formosanus* is not likely to be a limiting factor for further expansion of *C. gestroi*.

### **Territory Defense Mechanism**

In the foraging arena bioassay, both *C. formosanus* and *C. gestroi* quickly responded to tunnel interceptions and blocked tunnels within an hour resulting in territory separation and avoidance, which differed from the high mortality results of the Petri-dish bioassay (no-choice bioassay). The death of some individuals might have been caused by the fight and suicide cramming (Messenger and Su 2005a) in the foraging arena bioassay, and termite cadavers were usually the starting point of tunnel blockages. I speculated that chemicals released from termite cadavers may trigger a series of behaviors with multiple adaptive functions. Freshly killed termites triggered the burying behaviors by the surviving termites leading to avoidance of pathogenic organisms such as bacteria and fungi produced from such cadavers (Su 1982, Jones et al. 1996, Chouvenc 2003). Surviving termites further filled a section of tunnel with deposited sand which prevented invasion of the other species. During decomposition of the buried cadavers, the continued release of chemicals served as a deterrent which

prevented both termite species from reopening the blockages and encountering each other again through the same tunnel.

### **Territory Dynamic Equilibrium**

In light of the territory defense mechanism, I further speculate that neighboring territories of subterranean termite colonies in the field equilibrate in a dynamic state. During the active season in summer, subterranean termites actively excavated tunnels and expanded their territories (Waller and LaFage 1987, Messenger and Su 2005b). The tunnels of neighboring colonies may encounter each other and cause some agonistic responses which results in tunnel blockage. Based on the current study, the multiple tunnel blockages induced by a single tunnel interception were only 6 cm from the tunnel intercepting point on average. Tunnel blockages separated the neighboring colonies from each other by relatively short distances. Once one species bypasses the tunnel blockage and keeps excavating, an additional interception may happen. Hence, borders of neighboring territories are delineated by minor fights induced by tunnel interception. The borders may shift over time according to each tunnel intercepting point but it is not likely that one species or one colony could easily overwhelm the others.

Tunnel interceptions of two neighboring colonies also provide information on the colony status to each other. Once one colony is no longer able to maintain their tunnels, the dynamic equilibrium may shift. The stronger colony could expand their territory quickly through the tunnel systems left by the weaker colony. This observation was recently reported. After one colony was eliminated by feeding them with a chitin synthesis inhibitor, the vacated territories were occupied by neighboring colonies within days (Messenger et al. 2005, Lee et al. 2007).

In conclusion, *C. gestroi* was more aggressive than *C. formosanus* in both bioassays. *Coptotermes formosanus* is not likely to be a limiting factor for further expansion of *C. gestroi*. The results of the current study support the long-held assumption that interspecific competition is an important regulatory factor to maintain termite colonial territories. The territory defense mechanism of *C. formosanus* and *C. gestroi* is a series of tunnel blockages resulting from tunnel interceptions. Severe fights with heavy loss between neighboring colonies, as results from the no-choice Petri-dish bioassay are unlikely as long as the colonies are healthy in the field. I speculate that neighboring territories equilibrate in a dynamic state.

Table 3-1. The tunnel interception sequences between *C. gestroi* and *C. formosanus*. F, a tunnel of *C. formosanus* intercepted the tunnel of *C. gestroi*. G, a tunnel of *C. gestroi* intercepted the tunnel of *C. formosanus*. B, leading tunnels of both species encountered each other.

Primary interception	Additional interceptions					n
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	
F	-	-	-	-	-	10
F	F	-	-	-	-	1
F	F	G	-	-	-	1
F	G	-	-	-	-	1
F	G	G	G	G	G	1
G	-	-	-	-	-	23
G	F	-	-	-	-	1
G	G	-	-	-	-	10
G	G	G	G	-	-	2
B	-	-	-	-	-	3
B	G	-	-	-	-	1

Table 3-2. Tunnel blockages induced by tunnel interception of *C. gestroi* and *C. formosanus*

Time after tunnel intersecting	Number of blockages				Number (%) of blockages made in each time period <sup>a</sup>
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	≥ 4 <sup>th</sup>	
< 1 h	72	38	13	8	131 (60.4%)
1-2 h	6	14	15	3	38 (17.5%)
2-3 h	0	3	5	11	19 (8.8%)
> 3 h	1	7	8	13	29 (13.4%)
Number (%) of each sequential blockages induced by tunnel interceptions <sup>b</sup>	79 (98.8%)	62 (77.5%)	41 (51.3%)	35 (43.8%)	

<sup>a</sup> The total number of blockages observed in all replicates was 217.

<sup>b</sup> The total number of tunnel interceptions observed in all replicates was 80.

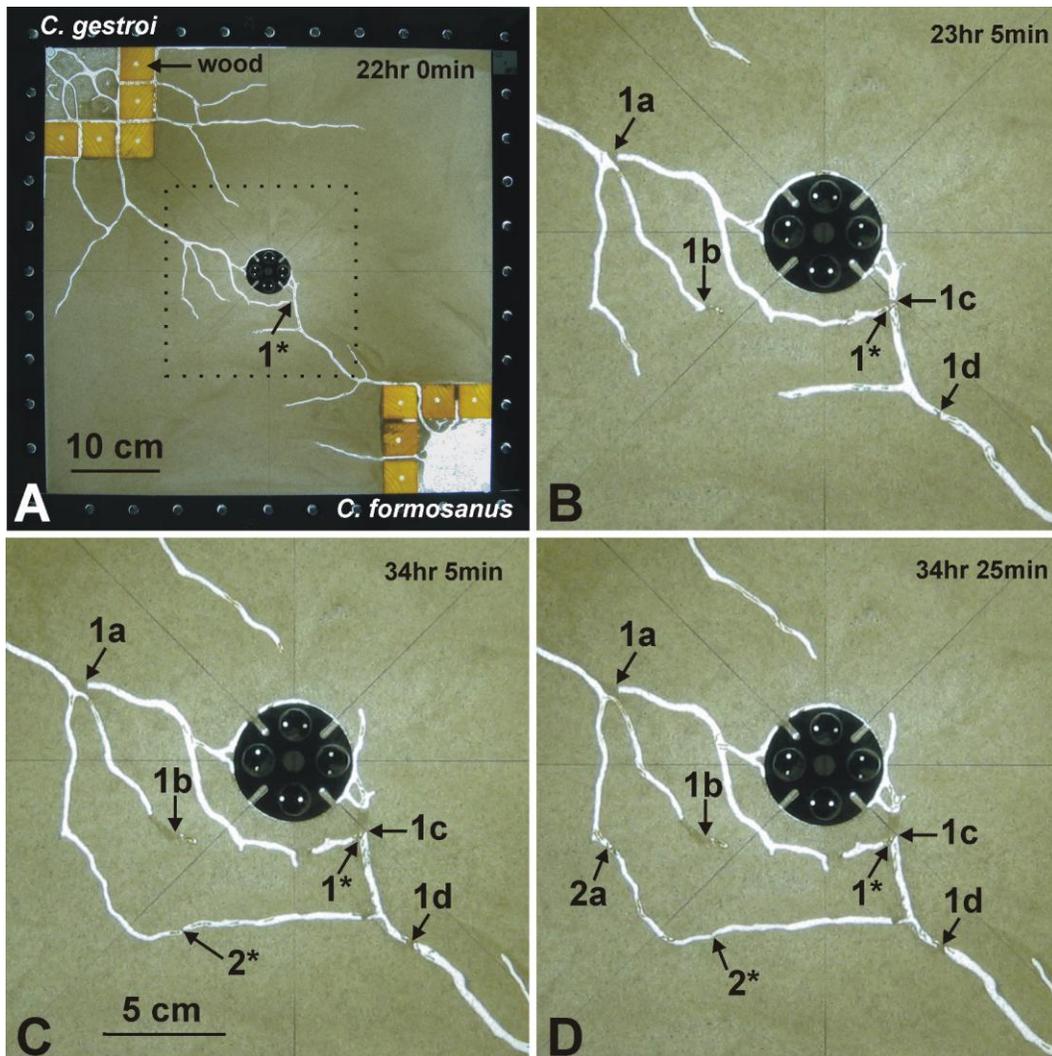


Figure 3-1. Time courses study of tunnel interceptions and consequent tunnel blockages in the foraging arena bioassay. (A) Two hundred individuals of *C. gestroi* and *C. formosanus* were released in the square space at the two corners. Termites excavated several primary tunnels from the space. After 22h, one leading tunnel of *C. gestroi* was going to intercept the tunnel of *C. formosanus*. The encounter point was denoted as 1\* and classified as a primary interception. The square with dotted lines represents the correlated position in the arena of the following three figures. (B) After the first tunnel interception, termites of both species blocked the tunnel at four locations in 65 min which were denoted as 1a, 1b, 1c, and 1d. (C) *Coptotermes gestroi* continually excavated from a branch of the same primary tunnel and then intercepted the tunnel of *C. formosanus* again. The second encounter point was denoted as 2\* herein and classified as additional interception. (D) The tunnel blockage resulting from the second tunnel interception was built within 20 min and was denoted as 2a.

## CHAPTER 4 TERMITE PESTS AND THEIR CONTROL IN TAIWAN

### Introduction

Termites are serious pests in Taiwan, which have caused severe damage to manmade structures for over 200 years (Su 2003). So far, 17 named termite species of four families and 12 genera have been recorded, including Termitidae: *Na. kinoshitae* (Hozawa), *Na. parvonasutus*, *Na. takasagoensis*, *O. formosanus*, *P. nitobei*, *S. mushae*; Rhinotermitidae: *C. formosanus*, *C. gestroi*, *Prorhinotermes japonicus* (Holmgren), *R. flaviceps*, *R. chinensis* Snyder; Kalotermitidae: *Cryptotermes domesticus* (Haviland), *G. fuscus*, *G. satsumensis* (Matsumura), *I. inamurae*, *N. koshunensis*, and Termopsidae: *Hodotermopsis sjoestedti* (Holmgren) (Chung and Chen 1994, Tsai and Chen 2003, Li et al. 2009b). Among the Taiwanese termites, *Coptotermes* spp. were considered most destructive to wooden structures, books, clothes, and furniture (Oshima 1909, Yi 1954, Tsai and Lai 2004). *Reticulitermes flaviceps* and *Cr. domesticus* were also known to damage wooden structures but their economical importance is uncertain. *Odontotermes formosanus* was most common species in Taiwan and caused damages to vegetables, tea, fruit trees, sugar cane, rice, and wooden structures at a point of wood-soil interface (Tu 1954, Yi 1954). *Nasutitermes parvonasutus* and *S. mushae* were recorded as agricultural pests of sugar cane (Yi 1954) and wild rice (Tu 1954), respectively, and *H. sjoestedti* caused damaged in silviculture (Huang 2000). In addition, it has been reported several times that *C. formosanus* and *O. formosanus* chewed through the lead sheathing of electrical and telephone cables and caused short circuits (Yi 1954, Tsai et al. 2004).

Coal-tar creosote was the first chemical used for wood treatment in Taiwan in the early 1900s (Oshima 1909, 1911, 1912). In 1940s and 1950s, arsenic compounds were widely employed for soil and wood treatment (Yi 1954). The local manufacture of chlorinated hydrocarbons, such as DDT, aldrin, lindane, and chlordane began in the mid 1950s (Yi 1954), and they became popular between 1960s and mid 1980s. After most of chlorinated hydrocarbons were banned in late 1980s, organophosphates, particularly chlorpyrifos, became the major termiticide in Taiwan. Due to the public concern over its potential contamination of groundwater in 2005, the overall use of chlorpyrifos was cancelled in the United States, and it is also being phased out from the market in Taiwan. Tsai and Lai (2004) considered that chemical treatment was still the major tool for termite control in Taiwan then, but no quantitative data on termiticide use was reported in their study. The objective of the current study includes a verification of the economic status of termites as pests, the quantification of current termiticide use, and an estimation of the annual revenue of termite control business in Taiwan.

### **Materials and Methods**

A questionnaire (Appendix A and its translated version, Appendix B) was designed for licensed pest control operators (PCOs) to quantify termite damage and the control measures used in Taiwan in 2008. Six questions were included: 1. How many cases of termite control were done by your company in each county of Taiwan in 2008? 2. Among these infested buildings, what were the percentages of residential premises, industrial buildings, commercial constructions, historical constructions, and others? 3. What kinds of termiticide were used and their ratio? 4. What was the proportion of each termiticide's cost to its corresponding service charge? 5. Was an effort made to identify termite species? 6. If termites were identified, what species were they and their ratio?

The questionnaires were delivered to PCOs through the Taiwan Environmental Pest Management Association and then phone interviews were conducted to obtain further information and answers of missed questions. All reported cases from the valid questionnaires were lumped and used for calculating the proportions of control cases from each county, proportions of infested buildings by each termite species, proportions of infested buildings of each type, and percentage of each termiticide used by PCOs. The average cost of each termiticide was based on the data offered by PCOs who used such termiticides. Posted information on the website of the Environmental Protection Administration (EPA) of Taiwan regarding permitted pesticides (Anonymous 2009a) and licensed pest control companies (Anonymous 2009b) were used for related analysis. The correlation between the number of termite control cases in each county reported from questionnaires and the number of registered PCOs in each county from EPA's database were tested by correlation analysis (SAS Institute 1985).

## **Results and Discussion**

### **Questionnaire and Registered Pest Control Companies**

The administration of the EPA of Taiwan requires PCOs to take a one-week training and a certified exam to obtain a license. There were 732 registered pest control companies in Taiwan (Anonymous 2009b), and they were primarily located in three densely populated counties, Taipei, Taichung and Kaohsiung (Table 1). A total of 17 completed questionnaires was returned for this study. The number of termite control cases in each county reported from the questionnaires was strongly correlated to the number of registered pest control companies in each county ( $r = 0.95$ ,  $P < 0.0001$ ; proc corr, SAS Institute 1985) (Table 1), which indicated that these questionnaires were representative.

## Termite Pests and Infested Buildings

Twelve of the 17 PCOs ( $\approx 70\%$ ) identified termite species when they offered termite control service. In the total 1,638 of termite control cases,  $>87\%$  was caused by *C. formosanus* or *C. gestroi*,  $\approx 6\%$  by *Cr. domestics*,  $\approx 6\%$  by *O. formosanus*, and less than 1% by *R. flaviceps*. *Coptotermes formosanus* and *C. gestroi* were usually found in lowland area ( $< 500$  m) especially in urban environments (Li et al. 2009a). The drywood termite, *Cr. domestics*, was found to be a major pest on wooden constructions, such as historical buildings and temples. *Cryptotermes domestics* distributed throughout the island while most were found in southern Taiwan. The fungus growing termite, *O. formosanus*, was the most common termite species found in both urban and natural environments under the altitude of 1,000 m throughout Taiwan (Huang 2004). *Odontotermes formosanus* may occasionally enter houses from the yards but seldom caused severe damage. *Reticulitermes flaviceps* is a minor pest and found in either northern Taiwan or in mountainous areas of southern Taiwan.

Of the total 1,934 of termite control cases reported from the questionnaires,  $\approx 75\%$  of them were conducted on residential premises,  $\approx 8\%$  on commercial buildings,  $\approx 6\%$  on industrial constructions,  $\approx 5\%$  on governmental buildings,  $\approx 5\%$  on historical constructions, and  $< 1\%$  on trees. The modern buildings were usually constructed of steel and concrete in order to survive frequent earthquakes and typhoons on the island. No specific construction code or pre-treatment is required for preventing termite damage in Taiwan; hence, PCOs primarily offered remedial control. Termites did not cause damage to frame structures of most Taiwanese buildings, but furniture, wooden floor, ceiling, and other wooden decorations were seriously threatened.

## Termiticide

Currently, 80 pesticide products were labeled for termite control use and seven of them were specialized for this purpose (Table 2). The active ingredients of the seven termiticide products are boric acid, hexaflumuron, fipronil, and cypermethrin.

Organophosphates and pyrethroids are the primary active ingredients of the non-termite-specific pesticides. Among the 80 pesticides, 56 (70%) were Restricted Use Pesticides (RUPs), which are only purchased and used by licensed PCOs. Based on the questionnaire survey, the liquid pesticide with fipronil as the active ingredient was used in  $\approx 75\%$  cases and termite bait containing hexaflumuron was employed in  $\approx 20\%$  cases. Chlorpyrifos and pyrethroids only had 4% market share. Even though 90% of the 80 termiticides were made in Taiwan, 95% market share belonged to two imported products, Termidor®, a liquid termiticide with fipronil, and Sentricon® Termite Colony Elimination System with hexaflumuron baits.

Termite bait systems were introduced into Taiwan around 1996, but its use was limited due to its high cost. The cost of bait systems accounted for  $\approx 26\%$  of total cost, but that of liquid pesticide accounted for  $\approx 19\%$  (Table 2). Recently, due to the concern of potential impact of liquid pesticides on the environment, baiting has been better accepted in subterranean termite control, especially at culturally important sites (Su and Hsu 2003). Most urban constructions are multi-story buildings without yards, requiring above-ground baiting systems to be used in  $\approx 95\%$  of cases instead of in-ground baiting stations, which were the most used type in the United States. Since termite bait systems are not effective against drywood termites and higher termites, such as *Cr. domesticus* and *O. formosanus*, PCOs applied a combination of both bait system and chemical treatment to control termites, in  $\approx 2\%$  of total cases in the current survey.

## Business Revenue of Termite Control Industry

In this study, I attempted to estimate the total business revenue of termite control industry based on all termiticide sale of Taiwan in 2008. However, there was a concern that Termidor® and other liquid termiticides might be partially used on non-termite pest control. Hence, I chose Sentricon®, which is only used to control termites, to estimate the total business revenue in 2008 based on the equation, the total business revenue = [(total sales of Sentricon®) / (Sentricon® cost /service charge)] / (market share of Sentricon®), which was estimated as ≈ 4 million USD. There is a need to further estimate the cost of termite control conducted by non-licensed PCOs and repair costs in order to obtain the total monetary expenditure associated with termite control and damage.

In conclusion, *C. formosanus* and *C. gestroi* are the most serious termite pests in Taiwan and responsible for over 87% termite control cases in 2008. Most infested buildings were residential premises (75%). The liquid termiticide with fipronil and the termite bait with hexaflumuron were the two primary products used by PCOs, and they had 75% and 20% market shares, respectively. The annual business revenue of termite control industry of Taiwan was ≈ 4 million USD.

Table 4-1. Distribution of licensed PCOs and termite control cases in Taiwan

Area	County	No. of licensed PCOs (%) <sup>a</sup>	No. of cases <sup>b</sup>
Northern Taiwan	Taipei <sup>c</sup>	315 (43.0%)	787 (40.7%)
	Taoyuan	55 (7.5%)	164 (8.5%)
	Hsinchu <sup>d</sup>	22 (3.0%)	133 (6.9%)
	Miaoli	1 (0.1%)	17 (0.9%)
Central Taiwan	Taichung <sup>e</sup>	94 (12.8%)	192 (9.9%)
	Changhua	25 (3.4%)	51 (2.6%)
	Yunlin	7 (1.0%)	5 (0.3%)
	Nantou	9 (1.2%)	12 (0.6%)
Southern Taiwan	Chiayi <sup>f</sup>	16 (2.2%)	25 (1.3%)
	Tainan <sup>g</sup>	60 (8.2%)	360 (18.6%)
	Kaohsiung <sup>h</sup>	92 (12.6%)	153 (7.9%)
	Pingtung	11 (1.5%)	21 (1.1%)
Eastern Taiwan	Yilan	2 (0.3%)	1 (0.1%)
	Hualien	10 (1.4%)	4 (0.2%)
	Taitung	8 (1.1%)	6 (0.3%)
Islands	Penghu	2 (0.3%)	2 (0.1%)
	Kinmen	2 (0.3%)	1 (0.1%)
	Lienchiang	1 (0.1%)	0 (0.0%)
total		732 (100.0%)	1934 (100.0%)

<sup>a</sup> Information was obtained from the website of the Environmental Protection Administration, ROC (Taiwan).

<sup>b</sup> Total cases of termite control of each county in 2008 reported in 17 valid questionnaires.

<sup>c</sup> Taipei City and Keelung City were included.

<sup>d</sup> Hsinchu City was included.

<sup>e</sup> Taichung City was included.

<sup>f</sup> Chiayi City was included.

<sup>g</sup> Tainan City was included.

<sup>h</sup> Kaohsiung City was included.

Table 4-2. Type, cost, and use of termiticide in Taiwan

Active ingredient category	Registered termiticides <sup>a</sup>			Termiticide use <sup>b</sup>	
	No. of termiticides (specialized for termite)	No. of RUPs (%)	No. of products made in Taiwan	No. of cases (%)	Termiticide cost/ service charge ± SE
Pyrethroids <sup>c</sup>	46 (3)	31 (67%)	44 (96%)	8 (0.39%)	20.00%
Pyrethroids + organophosphates	7 (0)	6 (86%)	7 (100%)		
Pyrethroids + Imiprothrin	3 (0)	0	1 (33%)		
Organophosphates <sup>d</sup>	18 (0)	18 (100%)	16 (89%)	66 (3.39%)	11.25 ± 1.25%
Carbamate <sup>e</sup>	2 (0)	0	2 (100%)		
Boric acid	2 (2)	0	2 (100%)		
Hexaflumuron	1 (1)	0	0	385 (19.89%)	25.98 ± 2.62%
Fipronil	1 (1)	1 (100%)	0	1446 (74.76%)	18.59 ± 2.20%
total	80 (7)	56 (70%)	72 (90%)	1905 (98.44%) <sup>f</sup>	

<sup>a</sup> Information obtained from the website of the Environmental Protection Administration of Taiwan, ROC. (Anonymous 2009a)

<sup>b</sup> Data was generated from questionnaires.

<sup>c</sup> Pyrethroids includes permethrin, bifenthrin, cypermethrin, alphacypermethrin, etofenprox, deltamethrin, phenothrin, tetramethrin, pyrethrins, and esbiothrin, d-tetramethrin.

<sup>d</sup> Organophosphates includes chlorpyrifos, pirimiphos-methyl, and fenitrothion.

<sup>e</sup> Carbamate only includes propoxur.

<sup>f</sup> No termiticide was used in 29 cases.



2. 在這些防治案件中各類型建築物所占百分比為？其平均面積？

- a. 住家：占\_\_\_\_%，平均\_\_\_\_坪。
- b. 商業建築（如：店面、辦公大樓）：占\_\_\_\_%，平均\_\_\_\_坪。
- c. 工業用建築（如：工廠、倉庫）：占\_\_\_\_%，平均\_\_\_\_坪。
- d. 政府機關單位含學校：占\_\_\_\_%，平均\_\_\_\_坪。
- e. 文化建築物（如：古蹟、寺廟）：占\_\_\_\_%，平均\_\_\_\_坪。
- f. 其它一\_\_\_\_\_（請描述場所類型）：占\_\_\_\_%，平均\_\_\_\_坪。
- g. 其它二\_\_\_\_\_（請描述場所類型）：占\_\_\_\_%，平均\_\_\_\_坪。
- h. 其它三\_\_\_\_\_（請描述場所類型）：占\_\_\_\_%，平均\_\_\_\_坪。
- i. 其它四\_\_\_\_\_（請描述場所類型）：占\_\_\_\_%，平均\_\_\_\_坪。

3. 使用的藥劑種類或組合，及其佔所有案件的比例為？

**範例：**A 公司於 2008 年進行的 100 件白蟻防治工程中，60 件僅使用特滅多乳劑，20 件僅使用滅蟻能餌劑，10 件同時使用兩種藥劑：特滅多和滅蟻能，另有 10 件同時使用三種藥劑：特滅多、滅蟻能與陶斯松。

則 A 公司的回答方式如下：

僅施用單一藥劑

- a. 特滅多乳劑：佔案件比例為 60%
- b. 滅蟻能餌劑：佔案件比例為 20%

同時施用兩種藥劑

- a. 特滅多乳劑 + 滅蟻能餌劑：佔案件比例為 10%

同時施用兩種以上藥劑

- a. 特滅多乳劑、滅蟻能餌劑、陶斯松：佔案件比例為 10%

請填貴公司的資訊於下列空格中：

僅施用單一藥劑

- a. \_\_\_\_\_（請填寫藥劑名稱）：佔案件比例為\_\_\_\_%
- b. \_\_\_\_\_（請填寫藥劑名稱）：佔案件比例為\_\_\_\_%
- c. \_\_\_\_\_（請填寫藥劑名稱）：佔案件比例為\_\_\_\_%
- d. \_\_\_\_\_（請填寫藥劑名稱）：佔案件比例為\_\_\_\_%
- e. \_\_\_\_\_（請填寫藥劑名稱）：佔案件比例為\_\_\_\_%
- f. \_\_\_\_\_（請填寫藥劑名稱）：佔案件比例為\_\_\_\_%

同時施用兩種藥劑

a. \_\_\_\_\_ + \_\_\_\_\_ : 佔案件比例為\_\_\_\_\_ %

b. \_\_\_\_\_ + \_\_\_\_\_ : 佔案件比例為\_\_\_\_\_ %

同時施用兩種以上藥劑

a. \_\_\_\_\_ (請填寫所有藥劑名稱) : 佔案件比例為\_\_\_\_\_ %

b. \_\_\_\_\_ (請填寫所有藥劑名稱) : 佔案件比例為\_\_\_\_\_ %

4. 貴公司所使用的各種藥劑成本占總收費的比例為？

**範例：**B 公司在使用特滅多乳劑進行白蟻防治案件中，平均收費 20,000 元，藥劑成本平均花費 2,000 元；在使用滅蟻能餌劑防治案件中，平均收費 20,000 元，藥劑成本平均花費 4,000 元。

則 B 公司的回答方式如下：

藥劑的成本占總收費的比例是否依不同藥劑而有所不同？

各種藥劑成本占總收費的比例相近，其為\_\_\_\_\_ %

各種藥劑成本占總收費的比例不同，其比例如下

a. 特滅多乳劑 (請填寫藥劑名稱) 約占：10 % (2,000/20,000)

b. 滅蟻能餌劑 (請填寫藥劑名稱) 約占：20 % (4,000/20,000)

請填貴公司的資訊於下列空格中：

藥劑的成本占總收費的比例是否依不同藥劑而有所不同？

各種藥劑成本占總收費的比例相近，其為\_\_\_\_\_ %

各種藥劑成本占總收費的比例不同，其比例如下

a. \_\_\_\_\_ (請填寫藥劑名稱) 約占：\_\_\_\_\_ %

b. \_\_\_\_\_ (請填寫藥劑名稱) 約占：\_\_\_\_\_ %

c. \_\_\_\_\_ (請填寫藥劑名稱) 約占：\_\_\_\_\_ %

d. \_\_\_\_\_ (請填寫藥劑名稱) 約占：\_\_\_\_\_ %

e. \_\_\_\_\_ (請填寫藥劑名稱) 約占：\_\_\_\_\_ %

5. 請問貴公司是否有對所防治白蟻進行種類之鑑定？

- 無 (問卷調查結束)
- 有 (請填下面最後一題)

6. 所防治白蟻的種類與其佔防治案件比例大約為？

- a. 家白蟻 (*Coptotermes spp.*，如：台灣家白蟻) 約占：\_\_\_\_\_ %
- b. 乾木白蟻 (*Cryptotermes spp.*，如：截頭堆砂白蟻) 約占：  
\_\_\_\_\_ %
- c. 土白蟻 (*Odontotermes spp.*，如：黑翅土白蟻) 約占：\_\_\_\_\_ %
- d. 散白蟻 (*Reticulitermes spp.*，如：黃肢散白蟻) 約占：\_\_\_\_\_ %
- e. \_\_\_\_\_ (請填寫其它白蟻種類) 約占：\_\_\_\_\_ %
- f. \_\_\_\_\_ (請填寫其它白蟻種類) 約占：\_\_\_\_\_ %
- g. \_\_\_\_\_ (請填寫其它白蟻種類) 約占：\_\_\_\_\_ %
- h. \_\_\_\_\_ (請填寫其它白蟻種類) 約占：\_\_\_\_\_ %

APPENDIX B  
TRANSLATED QUESTIONNAIRE

1. How many cases of termite control were done by your company in each county of Taiwan in 2008?

Taipei\_\_\_; Taoyuan\_\_\_; Hsinchu\_\_\_; Miaoli\_\_\_; Taichung\_\_\_; Changhua\_\_\_;  
Yunlin\_\_\_; Nantou\_\_\_; Chiayi\_\_\_; Tainan\_\_\_; Kaohsiung\_\_\_; Pingtung\_\_\_;  
Yilan\_\_\_; Hualien\_\_\_; Taitung\_\_\_; Penghu\_\_\_; Kinmen\_\_\_; Lienchiang\_\_\_.

2. Among these infested buildings, what were the percentages of the following locations?

Residential premises\_\_\_%; industrial buildings\_\_\_%; commercial constructions\_\_\_%; historical constructions\_\_\_%; others ( )\_\_\_%.

3. What kinds of termiticide were used and their ratio?

Only use one termiticide:

a. \_\_\_\_\_ (termiticide name)\_\_\_%.

b. \_\_\_\_\_ (termiticide name)\_\_\_%.

Only use two termiticides:

a. \_\_\_\_\_ + \_\_\_\_\_ (termiticide names)\_\_\_%.

b. \_\_\_\_\_ + \_\_\_\_\_ (termiticide names)\_\_\_%.

Only use multiple termiticides:

a. \_\_\_\_\_ (all termiticide names)\_\_\_%.

b. \_\_\_\_\_ (all termiticide names)\_\_\_%.

4. What was the proportion of each termiticide's cost to its corresponding service charge?

a. \_\_\_\_\_ (termiticide name) \_\_\_\_%.

b. \_\_\_\_\_ (termiticide name) \_\_\_\_%.

c. \_\_\_\_\_ (termiticide name) \_\_\_\_%.

5. Was an effort made to identify termite species?

Yes: \_\_\_\_.

No: \_\_\_\_.

6. If termites were identified, what species were they and their ratio?

a. *Coptotermes* spp.: \_\_\_\_%.

b. *Cryptotermes domesticus*: \_\_\_\_%.

c. *Odontotermes formosanus*: \_\_\_\_%.

d. *Reticulitermes* spp.: \_\_\_\_%.

e. Other termite \_\_\_\_\_ (termite name): \_\_\_\_%.

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## BIOGRAPHICAL SKETCH

Hou-Feng Li was born in Taiwan, 1979, and he was fascinated with insects during his childhood and wished to be an entomologist since he was ten years old. In high school, he founded an insect lecture in the student Biology Club, and studied entomology under Dr. How-Jing Lee in the Department of Plant Pathology and Entomology, National Taiwan University. In 1997, he graduated from high school and became a “qualified” freshman in the department. From 1997 to 2001, he was involved in physiological research on insect biological clocks. In 2001-2003, he did compulsory military service as a second lieutenant in a tank company. In 2003 summer, he returned to his previous research team as a research assistant for a year.

Since the fall of 2004, Hou-Feng studied and did research on termite biology under Dr. Nan-Yao Su in the Department of Entomology and Nematology, Fort Lauderdale Research and Education Center, University of Florida. He explored several research areas including taxonomy, ethology, phylogeography, and practical control of the destructive termite pests of Florida and Taiwan. Hou-Feng was selected as the recipient of the 2008 William L. and Ruth D. Nutting Award for outstanding achievements in basic termite biology under the North American Section of the International Union for the Study of Social Insects. He received his M.S. and Ph.D. degrees in May 2006 and in December 2009, respectively.

Hou-Feng married Rou-Ling Yang, another Ph.D. trained entomologist, in May 2007. He and his wife enjoy their lives and research together.