

# Phylogenetic relationship of the Asian subterranean termite, *Coptotermes gestroi* (Wasmann) and Philippine milk termite, *Coptotermes vastator* Light (Isoptera: Rhinotermitidae) as inferred from 16S mitochondrial DNA

by

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## Abstract

The Asian subterranean termite, *Coptotermes gestroi* (Wasmann) and the Philippine milk termite, *Coptotermes vastator* Light were compared using molecular phylogenetic technique. Partial sequence of ribosomal RNA large subunit 16S was obtained from 7 colonies of *C. gestroi* and 4 colonies of *C. vastator*. In addition, 4 colonies of *C. formosanus* Shiraki with *Globitermes sulphureus* (Haviland) were used as the outgroups. DNA sequencing of the 16S ribosomal DNA amplicon revealed an average size of 428 bp. Consensus sequences were obtained and aligned using the BioEdit v7.0.5 software. *C. vastator* and *C. gestroi* were likely synonymous based on the DNA sequence with differences detected at only 3 base pairs across the partial 16S gene. On the basis of partial 16S rDNA sequences determined, phylogenetic trees were constructed using maximum parsimony, likelihood, and distance methods. The results revealed 2 minor subclades of *C. gestroi* and *C. vastator* within a major clade. The interspecific pairwise sequence divergence, base on uncorrected “p” distance between *C. gestroi* and *C. vastator*, varied up to only 0.79%. Other findings that further support the synonymy are discussed.

**Keywords:** *Coptotermes gestroi*, *Coptotermes vastator*, 16-S ribosomal DNA, molecular phylogenetic, synonymous.

## Introduction

Amongst the termite genera within Rhinotermitidae, the genus *Coptotermes* is probably regarded as one of the most important genera. Several species of *Coptotermes* including the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, and the Asian subterranean termite, *Coptotermes gestroi* (Wasmann) have been known for their destructive nature to buildings and structures in the subtropical and tropical regions, respectively. Globally, *C. formosanus* accounted a substantial amount of the USD 22 billion of termite damage yearly (Su 2002). In Malaysia, Thailand and Singapore, *C. gestroi* contributed more than 85% of the total termite damage in buildings and structures in the urban area (Lee 2002; Lee *et al.* 2003). Despite being located in the tropics, *Coptotermes vastator* Light is the primary subterranean termite species of urban environment in the Philippines, and not *C. gestroi*. *C. vastator* is a serious structural pest that accounted for >90% of the termite damages to timber and wooden structures. These damages cost nearly USD 1 million to residential and commercial properties of the Mariana Islands and between USD 8 and 10 million for the damages in and around Manila (Yudin 2002). Both *C. gestroi* and *C. vastator* are very similar based on analysis of morphological characteristics, and it was long suspected that *C. vastator* is a junior synonym of *C. gestroi* (Kirton & Brown 2003; Kirton 2005). However, no attempt has been executed so far to address this issue from molecular phylogenetic perspective. Molecular phylogenetic analyses are able to reveal the relationship among the populations and differentiate species regardless of the termite caste (Szalanski *et al.* 2003). In this study, we used 16S mitochondrial gene to elucidate the relationship between *C. gestroi* and *C. vastator*.

## Materials and methods

*Morphology:* Samples were collected or obtained as shown in Table 1. They were preserved in absolute ethanol (Table 1). Micrographs pictures were made of the heads, mandibles, and whole bodies for each species. Morphometric measurements of the length of mandible, maximum width of head, and length of head were measured for 10 soldier termites for all 7 colonies of *C. gestroi*, 4 colonies of *C. vastator*, and 4 colonies of *C. formosanus*.

**Table 1. Summary of the samples and information used in this study.**

| Sample code/<br>Gene bank<br>accession no. | Species              | Collection site                        | Collector    |
|--|----------------------|--|--------------|
| CG001MY                                    | <i>C. gestroi</i>    | Malaysia, Penang, USM.                 | B.K. Yeap    |
| CG004MY                                    | <i>C. gestroi</i>    | Malaysia, Kuala Lumpur, Bangsar.       | K.T. Koay    |
| *CG005MY                                   | <i>C. gestroi</i>    | Malaysia, Muar.                        | C.Y. Lee     |
| CG001SG                                    | <i>C. gestroi</i>    | Singapore, Serenity Terr.              | SPMA         |
| CG002SG                                    | <i>C. gestroi</i>    | Singapore, Serangoon.                  | SPMA         |
| CG001TH                                    | <i>C. gestroi</i>    | Bangkok, Thailand1.                    | V. Charunee  |
| CG002TH                                    | <i>C. gestroi</i>    | Bangkok, Thailand1.                    | V. Charunee  |
| CF001JP                                    | <i>C. formosanus</i> | Japan, Wakayama.                       | T. Yoshimura |
| CF002JP                                    | <i>C. formosanus</i> | Japan, Wakayama.                       | T. Yoshimura |
| CF003JP                                    | <i>C. formosanus</i> | Japan, Okayama.                        | T. Yoshimura |
| CF001HW                                    | <i>C. formosanus</i> | USA, Hawaii, Oahu.                     | J. Yates III |
| CV001HW                                    | <i>C. vastator</i>   | USA, Hawaii, Oahu.                     | J. Yates III |
| CV001PHI                                   | <i>C. vastator</i>   | Los Banos, Laguna Philippines, colony1 | C. Garcia    |
| CV002PHI                                   | <i>C. vastator</i>   | Los Banos, Laguna Philippines, colony2 | C. Garcia    |
| CV003PHI                                   | <i>C. vastator</i>   | Los Banos, Laguna Philippines, colony3 | C. Garcia    |
| GS001MY                                    | <i>G. sulphurues</i> | Malaysia, Penang, USM.                 | B.K. Yeap    |
| AY302709                                   | <i>C. gestroi</i>    | Thailand, Bangkok.                     |              |
| AY558907                                   | <i>C. gestroi</i>    | USA: Miami, Florida                    |              |
| AY558906                                   | <i>C. gestroi</i>    | Turks and Caicos Islands, Grand Turk.  |              |
| AY558905                                   | <i>C. gestroi</i>    | Antigua and Barbuda                    |              |
| AY302713                                   | <i>C. vastator</i>   | Philippines: Wedgewood                 |              |
| AY302712                                   | <i>C. vastator</i>   | Philippines: Manila                    |              |
| AY302711                                   | <i>C. vastator</i>   | USA: Honolulu, Hawaii                  |              |

\*Dried specimens.

*DNA Extraction:* The specimen preserved in absolute ethanol was washed with distilled water and dried on a filter paper. Total genomic DNA was extracted from single termite using Dneasy tissue kit manufactured by QIAGEN (Valencia, CA). Extracted genomic DNA from each sample was used as polymerase chain reaction (PCR) template. PCR amplification was performed in a standard 25-  $\mu$ l reaction volume with 2  $\mu$ l of total genomic DNA, 1 pmol of each primer, 1.5 mM MgCl<sub>2</sub>, 2 mM dNTPs, and 5U/ $\mu$ l Taq DNA polymerase. Amplification was accomplished in a MJ Research PTC-200, Peltier Thermol Cycle, with a profile consisting of a precycle denaturation at 94°C for 2 min, a postcycle extension at 72°C for 10 min, and 35 cycles of a standard three-step PCR (53.1°C annealing). Reaction conditions were optimized with respect to MgCl<sub>2</sub> concentration and annealing temperature. Primer set of LR-J-13007 (TTA CGC TGT TAT CCC TAA) and LR-N-13398 (CGC CTG TTT ATC AAA AAC AT) was used to amplify mtDNA 16S gene. Two  $\mu$ l of each PCR product was visualized by UV transillumination on a 1.2% agarose gel containing 0.5 mg/ml ethidium bromide. Double-stranded PCR products were purified using SpinClean Gel Extraction Kit (column) and subjected for direct sequencing.

**Data Analysis:** BioEdit v7.0.5 software was used to edit individual electropherograms and to form contigs. Multiple consensus sequences were aligned using CLUSTAL X. The alignment results were adjusted manually for obvious alignment errors. The data were imported into PAUP4.0 (Swofford 2000) and analyzed to generate maximum likelihood, neighbour-joining (NJ), and parsimony bootstrapped trees, based on nucleotide data. A bootstrap test was used to test the reliability of trees (Felsenstein 1985). Using the heuristic search option, 1000 replicates were performed and 50% majority rule consensus trees were generated. Gaps were treated as missing data.

## Results and Discussion

**Morphology:** The soldier termites were used for morphological studies. *C. formosanus* was readily distinguished from *C. vastator* and *C. gestroi* with two pairs of setae projecting dorso-laterally from the base of the fontanelle, compared with only a pair of setae in the latter two species. It is difficult to distinguish *C. vastator* from *C. gestroi* by the size, shape of its postmentum and head, as they are highly parallel. As reported in Kirton and Brown (2003), there is a continuous variation in size and shape of a single species. The morphology of termites can be influenced by the age and state of the colony, or the environment of the habitat. Furthermore, additional variability in coloration may be attributed to sample age and storage condition (Scheffrahn *et al.* 2005).

**Nucleotide analyses:** Average amplicon size of 16S gene resulting from DNA sequencing was approximately 428 basepairs (bp). Thirty five bp from the 5' end of the amplicon was excluded in the analysis to facilitate genetic comparisons with existing GenBank DNA sequences. The average nucleotide compositions among *Coptotermes* species (excluding GenBank sequences) for 16S gene for A, C, G and T are 42.82%, 24.61%, 10.57%, and 22.00%, respectively. The multiple sequences alignment for 16S gene, including the outgroup taxon resulted in a data matrix with 385 characters, of which 326 are constant and 20 parsimony-informative. The interspecific pairwise sequence divergence based on uncorrected "p" distance between *C. gestroi* and *C. vastator* ranges from 0 to 0.79% across the entire 16S gene. On the other hand, the divergence values between the *Coptotermes* species and the outgroup vary from 12.45 to 13.75% in 16S gene. From the aligned data matrix, there were only 3 characters (at base -39, -103, and -135) in the 16S of *C. vastator* which were different from those of *C. gestroi*. The divergence values and the high similarity in the sequences suggested that *C. gestroi* and *C. vastator* are conspecific.

**Phylogenetic relationships inferred from 16S gene:** Phylogenies derived using maximum parsimony, neighbour-joining and maximum likelihood methods showed the same tree topology. Only the tree from maximum parsimony analysis is shown here (Fig.1). The parsimony analysis using the heuristic search algorithm of the aligned sequences yielded a single maximally parsimonious tree with 68 evolutionary steps, index of consistency (CI) of 0.971 and 0.976 retention index (RI). The robustness of the trees was tested by bootstrapping with 1000 replicates. Nodes with less than 50% support were collapsed. The strict consensus tree (Figure 1) consisted of two clades with strong bootstrap support of 99% and 100%. The first clade comprises *C. gestroi* and *C. vastator* from various populations. There is only one or two changes along the branches between *C. gestroi* and *C. vastator* which falls within the same clade. The second clade is composed of *C. formosanus* from Japan and Hawaii. *C. vastator* has been previously suggested to be closely related to the *C. gestroi* based on morphological characters under the *C. gestroi* complex (Kirton and Brown 2003; Kirton 2005). The results of the analysis of mitochondrial gene sequences in this study further support these earlier reports.

**Geographical distribution:** *C. gestroi* is a native species of Asia. Zoogeographical regions of this species include Nearctic, Neotropical, and Oriental. In the New World tropics, *C. gestroi* was first reported in Brazil in 1923 and in Barbados in 1937. Later, the distribution of this species is endemic to Antigua and Barbuda, Grand Cayman, Grand Turk, Jamaica (Montego Bay and Port Antonio), Little Cayman, Montserrat, Nevis, Providenciales, Puerto Rico (San Juan), St. Kitts, and United States. It has also been collected in southern Mexico (Scheffrahn and Su 2000). There is limited information on the distribution of *C. vastator*. Thus far, it is a notably pest in Guam and the Philippines as well as Hawaii.



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