Applying molecular technology to identify termite species of the genus Coptotermes in the Hanoi Old Quarter

by

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Abstract

Research on the termites damaging buildings in the Hanoi Old Quarter determined 3 species, belonging to 2 genera and 2 families. In which, 2 species of Coptotermes were found to be abundant and the main pest damaging various structures in this area, although their individuals are different in soldier head shape and size. Furthermore, this study might be the premise for further termite taxonomy in Vietnam, using molecular technology.

Key words: Coptotermes gestroi, Coptotermes formosanus, Odontotermes hainanensis

Introduction

Previous studies showed that there were more than 101 termite species in Vietnam. All these species were formerly identified by morphological keys, thus the results are still controversial. Because, one termite species could have a range of variation in morphological shapes or different termite species may have the same morphological description. Therefore, there exist a few “tricks” interfering with termite identification in Vietnam.

On the other hand, Hanoi Old Quarter is in the central business district of Thanglong- Hanoi royal-capital for over thousand years. The Old Quarter covers an area of almost 100ha having low and narrow houses and very high building density (more than 90%). Most of these structures and their furniture are made from wood, bamboo and other cellulosic materials, that are favorite food of termites. Hence, termite attacks are quite common in this area.

In a recent survey, many termite specimens were observed to have the same features as C. gestroi. In some systematic key references, the head of C. gestroi soldiers are egg (or round-oval) shaped with maximum width of head in the middle, but other soldiers have a tear-drop head with the maximum width of head slightly behind the middle point of the head and were recognized as C. formosanus Shiraki, according to Sheng et al. (2000). Also, there were still individuals having the shapes of these two types described above.

In this study, morphological features of C. gestroi with two types of head shapes were differentiated. In addition, the exact identification of Coptotermes species in the Old Quarter of Hanoi was performed, using molecular technology.
Materials and Methods

Materials

56-termite samples and pair of mitochondrial marker primers for the 16S gene were used as materials of this research. Termite samples belong to 2 morphological groups C₁ and C₂ (based on 11 morphological features) were collected from different buildings in the Hanoi Old Quarter in some main streets, such as: Lan Ong, Hang Bac, Thuoc Bac, Hang Thiec, Hang Chieu... Mitochondrial marker primer sequences were: 5'-'CCGGTCTGAACATCGATCAT-3' (forward) and 3'-'CGCCTGTTTACAAAAACAT-5' (reverse), amplifying the DNA fragments which characterized the genus Coptotermes.

All specimens were morphologically characterized using systematic keys of termite, primarily Roonwal (1969), Ahmad (1958), Sheng et al. (2000) and Nguyen et al. (2007). Eleven morphological characters were described in detail. Based on the category grouping of these authors, Coptotermes soldiers with a tear-drop head shape were assumed to be C₁ group and others with a round-oval/egg head shape were placed in the C₂ group. Selected termite specimens were captured using Leica application Suite (LAS).

Molecular identification

DNA extraction

Total DNA was extracted from termite worker’s head, using the DNA isolation kit. Alcohol-preserved specimens were allowed to dry on filter paper. After that, samples were put into a 1.5ml microcentrifuge tube with 180 µl buffer ATL, 20 µl Proteinase K and incubated in water bath at 56°C for 45 mins. 200 µl buffer AL then added and the solution were precipitated with ethanol 100%. Mixtures were centrifuged at 8000 rpm for 1 min in Dneasy Mini spin column, following by adding 500 µl each of buffer AW1 and AW2 and centrifuging at 8000 rpm for 1 min and 14.000 rpm for 1 min, respectively. Total DNA was obtained by 200 µl buffer AE, centrifuged at 8000 rpm for 1 min and stored at -20ºC.

PCR reaction

Components of PCR reaction were: 33.8 µl sdH₂O, 5 µl buffer PCR, 1 µl dNTP, 4 µl for each primer, 2 µl for each DNA sample, 0.2 µl Taq polymerase. PCR reactions were then conducted with a profile consisting of 94ºC for 45s, (94ºC for 1 min, 50ºC for 1 min, 72ºC for 2 mins) x 39 cycles, 72ºC for 10 mins and keep production of 4ºC). After that, the product was tested with 1% agarose by gel electrophoresis method.

Purifying and sequencing DNA

PCR products were purified by using QIAquick Gel Extraction Kit (GmbH Qiagen, Hilden, Germany) and sequenced by ABI PRISM 310 Genetic Analyzer (PE Applied Biosystem, USA). After
that, the nucleotide sequences were analyzed using FastPCR program and aligned using BLAST software (http://www.ncbi.nlm.nih.gov). The sequences represented for high similarity and different areas were collected to build a consensus tree by MEGA5 software. The distance matrix was calculated by two parameters method of Kimura (Kimura’s 2-parameter model) [6].

Results and discussion

Termite identification by morphological characters

From 56 termite samples, there were 2 species of Coptotermes recorded. The frequency of appearance of these two species was noted and compared with the old data from Nguyen et al. (2005) (Table 1). The result showed that the frequency of Coptotermes species in the Old Quarter of Hanoi has been reduced and this might due to a their less abundance in living environment.

Table 1. Composition of termite collected in Hanoi’s old quarter in surveys

<table>
<thead>
<tr>
<th>Termite Species</th>
<th>Ratio of collection in surveys</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2005</td>
</tr>
<tr>
<td>Cryptotermes domesticus</td>
<td>4.7</td>
</tr>
<tr>
<td>Coptotermes formosanus</td>
<td>51.8</td>
</tr>
<tr>
<td>Coptotermes gestroi</td>
<td>28.2</td>
</tr>
<tr>
<td>Coptotermes curvignathus</td>
<td>9.4</td>
</tr>
<tr>
<td>Coptotermes emersoni</td>
<td>4.7</td>
</tr>
<tr>
<td>Coptotermes dimophus</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Particularly, there is only one Coptotermes species, *C. gestroi*, commonly invading the Old Quarter while this number was 5 in 2005. Significantly, there was no *C. formosanus* collected when this was the major pest damaging most of structures in Hanoi as well as in Vietnam in general. From the Table 1, in 2005, one species *C. formosanus* invaded half of the structures (51.8%) in this area, greater than the total of the other species. A lack of 3 species including *C. formosanus*, *C. curvignathus* and *C. dimophus*, but especially *C. formosanus* is a surprisingly finding.

Before 2008, the researchers in Vietnam mainly used some systematic key of Sheng et al. (2000), Nguyen et al. (2007), Ahmad (1958, 1965) etc. for termite identification. Reviewing all these keys on *Coptotermes spp.*, there is no total difference between *C. formosanus* and *C. gestroi*, except their head size. However, measuring width and length of the soldier head from them showed no significant difference for visually observation (Appendix 1). Therefore, if only based on morphological measurement and characters, it leads to confusion in distinguishing between those two Coptotermes species in Vietnam. With a tricky character of head shape and fontanelle, the morphological
identification of *C. gestroi* and *C. formosanus* did challenge Vietnamese termite researchers. As a result, this causes a mistaken identification in *Coptotermes* spp. in Hanoi.

After 2010, the exact identification of *Coptotermes* spp. has been significantly improved with a range of supporting systematic keys. Notably, Scheffrahn & Su (2011) highlighted several main morphological characters used to differentiate between *C. gestroi* and *C. formosanus*. They include the number of bristles around the fontanelle and flexures of soldier head behind fontanelle when observed from a lateral view.

![Fig.1. Head of soldier *C. gestroi* with two shapes. Shape C1: Head elongate oval (sample HN2), shape C2: head broadly oval (sample HN3)](image)

Among the 56 termite samples collected, *C. gestroi* was abundant, accounting for over 80% of the total, the remaining belong to *C. emersoni* (only 13.8%) (Scheffrahn & Su, 2011). The slight difference in the soldier head shape could be a distinction between these two groups of *Coptotermes*. From then, individuals were divided into 2 groups: C1 with tear-drop head shape (about 67.8%) and C2 with egg/round-oval head shape (32.2%), as illustrated in Figure 1.

![Table 3](image)

The detail measurements of *Coptotermes* soldier heads are provided in Table 3. As shown in this table, the measurement values partly overlapped, hence the two morphological-distinguished groups, C1 and C2, could only be the variation in flexure of head sides within the same species. Also, there is a requirement of applying an appropriate tool in termite identification, molecular analysis, to understand any difference in gene sequence between the two groups.
Table 2: Measurements (in millimeters) of soldier of Coptotermes

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Shape C1</th>
<th>Shape C2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of head to side base of mandibles</td>
<td>1.30-1.45</td>
<td>1.25-1.35</td>
</tr>
<tr>
<td>Width of head at side base of mandibles</td>
<td>0.65-0.7</td>
<td>0.65-0.7</td>
</tr>
<tr>
<td>Width of head at posterolateral ends of antennal carinae</td>
<td>0.85-0.9</td>
<td>0.90-0.95</td>
</tr>
<tr>
<td>Maximum width of head</td>
<td>1.15-1.25</td>
<td>1.20-1.35</td>
</tr>
<tr>
<td>Length of left mandibles</td>
<td>0.75-0.85</td>
<td>0.8-0.875</td>
</tr>
</tbody>
</table>

*Termite identification by molecular method*

Based on morphological observation, 6 termite samples that represented different collection sites and two head shapes were collected. They included: HN1, HN2 and HN5 (tea-drop head shape) and HN3, HN6 and HN12 (egg/round-oval head shape) and were run through DNA analysis.

Research of identification by molecular biology method showed that the gene fragment of 16S was sequenced and identified to be 516bp in length, almost identical to the sequence segment of 16S *C. gestroi* HQ231234, AY302709 which was published in Genbank (Appendix 1).

Because the results of four populations: HN2, HN5, HN6 and HN12 had a similar sequence we only chose the HN2 population for construction of a consensus tree. The consensus tree was based on three gene sequences from termite populations: (HN1), (HN2), (HN3), including reference to the 16S sequence of *Odontotermes hainanensis* which was collected in the Bavi mountainous area of Hanoi (HN4).
According to sequence consensus and previous references, all of the C. gestroi populations had a common phylogenetic tree. Therefore, Coptotermes samples collected in The Hanoi Old Quarter and Hanoi general area were C. gestroi but not C. formosanus. In other words, C. gestroi is a major termite pest in this district.

Conclusions

There was agreement in termite identification of all Coptotermes samples collected in The Old Quarter of Hanoi as Coptotermes gestroi although the individuals were different in soldier head capsule shape and size. C. gestroi were found to be abundant with a large percentage (67%) of the total sample and a main pest damaging various structures in this area. Furthermore, this study might be the basis for further termite taxonomy in Vietnam, using molecular technology.
References


Ahmad, M. 1965. Termites (Isoptera) of Thailand. Bulletin of the AMNH; v. 131, article 1.


http://entomology.ifas.ufl.edu/pestalert/asian_termite.htm.

APPENDIX 1

Table 1. Measurements (in millimeters) of soldier of *C. formosanus* and *C. gestroi* from identification documents

<p>| Measurement |</p>
<table>
<thead>
<tr>
<th></th>
<th>Nguyen Duc Kham et al, 2007</th>
<th>Ahmad, 1965</th>
<th>Hoang Fu Sheng, 2000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>C. formosanus</em></td>
<td><em>C. gestroi</em></td>
<td><em>C. formosanus</em></td>
</tr>
<tr>
<td>Length of head to side base of</td>
<td>1.45 – 1.53</td>
<td>1.45 – 1.51</td>
<td>1.40 – 1.51</td>
</tr>
<tr>
<td>mandibles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Width of head at side base of</td>
<td>0.75 – 0.78</td>
<td>0.68 – 0.75</td>
<td>0.68 – 0.75</td>
</tr>
<tr>
<td>mandibles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Width of head at posterolateral</td>
<td>1.02 – 1.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ends of antennal carinae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum width of head</td>
<td>1.2 – 1.3</td>
<td>1.15 – 1.24</td>
<td>1.15 – 1.24</td>
</tr>
<tr>
<td>Length of left mandibles</td>
<td>0.95 – 1.03</td>
<td>0.82 – 0.93</td>
<td>0.82 – 0.93</td>
</tr>
</tbody>
</table>

**APPENDIX 2**

Gene sequence of research samples

>HN1

GTATGGCCTGCCCGCTGACATTTTGGTGTGAAAGGCGGCGGTATTTTTGACCGTGCAAGGCT
AGCTAATCGTATCTTTATTTTGTATCCTGGAATGCGCCCTGGCTGAGGCGCCGC
TCTTTATTTTTGCTTTACTTTTGACGAGGCACGGGCGT
GGGACGAGAAAGCCTATAGAGTTGGACATTTTTGTTTTATATTTTATTGTGTTTTTGA
TTTTTTGGTGAGTGCTTTTTTGTGTTTTTTGATTTTTTTTATGTTTATGTTTATG
TACCTTACAGCTATCTGTTTGAGAGTCTTATCGGCAGGGGGTTTGCAGC
CTTGATGTTGGATTAAG

>HN2

GTATGGCCTGCCCGCTGACATTTTGGTGTGAAAGGCGGCGGTATTTTGAGCCTGCAAGGCT
AGCTAATCGTATCTTTATTTTGTATCCTGGAATGCGCCCTGGCTGAGGCGCCGC
TCTTTATTTTTGCTTTACTTTTGACGAGGCACGGGCGT
GGGACGAGAAAGCCTATAGAGTTGGACATTTTTGTTTTATATTTTATTGTGTTTTTGA
TTTTTTGGTGAGTGCTTTTTTGTGTTTTTTGATTTTTTTTATGTTTATGTTTATG
TACCTTACAGCTATCTGTTTGAGAGTCTTATCGGCAGGGGGTTTGCAGC
CTTGATGTTGGATTAAG
TTTTTGGTGAGTGGGCTTTTTGTTTTGTTGGGGTGATGGGAGGAATGTACTTAACTCCCT
TAGTTTTGGTTATATTGATTTATAATTGTTTGATCCATTTATTTTGATTATAAGACTAAAT
TAGTTTTGGTTATATTGATTTATAATTGTTTGATCCATTTATTTTGATTATAAGACTAAAT
TACCTTAGGGGATAACAGCGTTATCTTCTGAGAGTCTTATCAGACTGAGGTTTGGTGAC
CTCGATGTTGGATTAAG

>CgesHQ231234
GTATGGCCTGCCCCGTACATTTGTGTGAAGGCGCGGATATTTTGACCCTGCAAGGT
TAGCATAATCATATTCTTAAATTTGTGATCCTGGAATGAGGCTTGACGACCAGCAGG
TTGTTTGGTTGATGTTTGTGTATTTTTGGTTATATTGATTTATAATTGTTTGATCCATTTATTTTGATTATAAGACTAAAT
TACCTTAGGGGATAACAGCGTTATCTTCTGAGAGTCTTATCAGACTGAGGTTTGGTGAC
CTCGATGTTGGATTAAG

>CgesAY302709
GTATGGCCCTGCCCCGTACATTTGTGTGAAGGCGCGGATATTTTGACCCTGCAAGGT
TAGCATAATCATATTCTTAAATTTGTGATCCTGGAATGAGGCTTGACGACCAGCAGG
TTGTTTGGTTGATGTTTGTGTATTTTTGGTTATATTGATTTATAATTGTTTGATCCATTTATTTTGATTATAAGACTAAAT
TACCTTAGGGGATAACAGCGTTATCTTCTGAGAGTCTTATCAGACTGAGGTTTGGTGAC
CTCGATGTTGGATTAAG
Table 2. Compare gene similar of 16S between research samples (HN1 sample) and samples refered to Genbank (CgesHQ231234 and CgesAY302709)

<table>
<thead>
<tr>
<th>Species</th>
<th>Code</th>
<th>Level of gene cover (%)</th>
<th>Ratio of similar nucleotide (%)</th>
<th>Geographical position</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. gestroi</td>
<td>HQ231234</td>
<td>100</td>
<td>99%</td>
<td>Italy</td>
</tr>
<tr>
<td>C. gestroi</td>
<td>AY302709</td>
<td>100</td>
<td>99%</td>
<td>United States of America</td>
</tr>
<tr>
<td>C. curvignathus</td>
<td>AY575852</td>
<td>100</td>
<td>96</td>
<td>Malyasia</td>
</tr>
<tr>
<td>C. formosanus</td>
<td>AB626147</td>
<td>100</td>
<td>95</td>
<td>Japan</td>
</tr>
<tr>
<td>C. formosanus</td>
<td>AY168225</td>
<td>100</td>
<td>95</td>
<td>United States of America</td>
</tr>
<tr>
<td>C. lacteus</td>
<td>FJ806148</td>
<td>100</td>
<td>94</td>
<td>United States of America</td>
</tr>
<tr>
<td>Heterotermes ferox</td>
<td>AY302714</td>
<td>100</td>
<td>92</td>
<td>United States of America</td>
</tr>
</tbody>
</table>