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 maximun width of head in the middle, but other soldiers have a tear-drop head with the maximun 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 C_1 and C_2 (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'-CCGGTCTGAACTCAGATCACGT-3' (forward) and 3'-CGCCTGTTTAACAAAAACAT-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 C_1 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 56^0C 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 sdH$_2$O, 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 94^0C for 45s, (94^0C for 1 min, 50^0C for 1 min, 72^0C for 2 mins) x 39 cycles, 72^0C for 10 mins and keep production of 4^0C). 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 serveys

	Ratio of collection in serveys	
	2005	2013
Cryptotermes domesticus	4.7	19.4
Coptotermes formosanus	51.8	
Coptotermes gestroi	28.2	66.7
Coptotermes curvignathus	9.4	
Coptotermes emersoni	4.7	13.8
Coptotermes dimophus	1.2	

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.
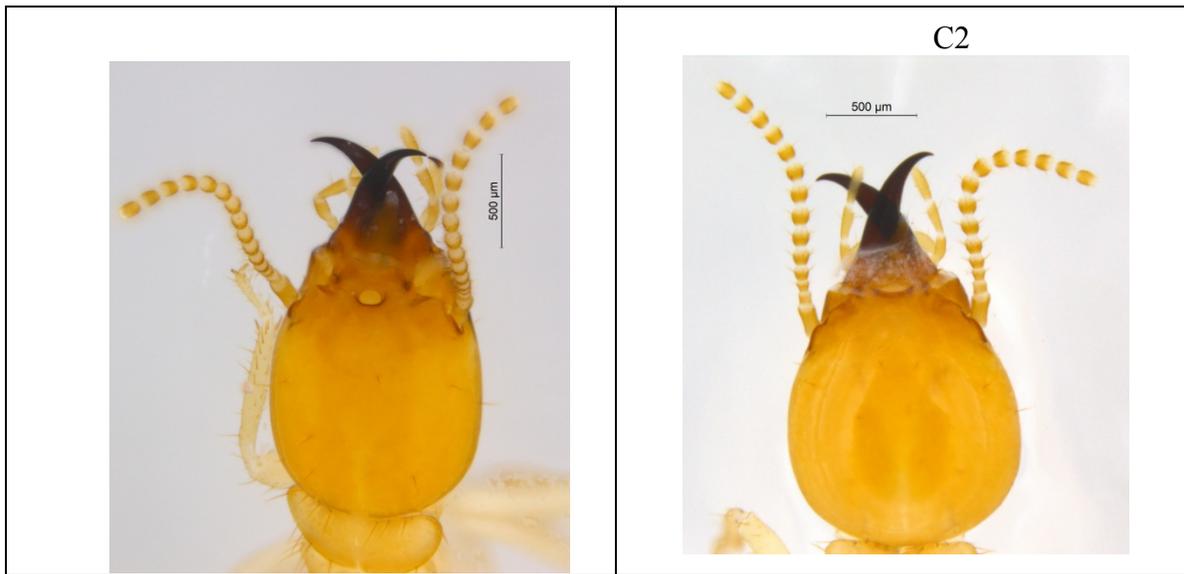

Fig.1. Head of soldier *C. gestroi* with two shapes. Shape C1: Head elongate oval (sample HN2), shape C2: head broadly oval (sample HN3)

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.

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, C_1 and C_2, 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

	Measurement	
	Shape C1	Shape C2
Length of head to side base of mandibles	1.30-1.45	1.25-1.35
Width of head at side base of mandibles	0.65-0.7	0.65-0.7
Width of head at posterolateral ends of antennal carinae	0.85-0.9	0.90-0.95
Maximun width of head	1.15-1.25	1.20-1.35
Length of left mandibles	0.75-0.85	0.8-0.875

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).
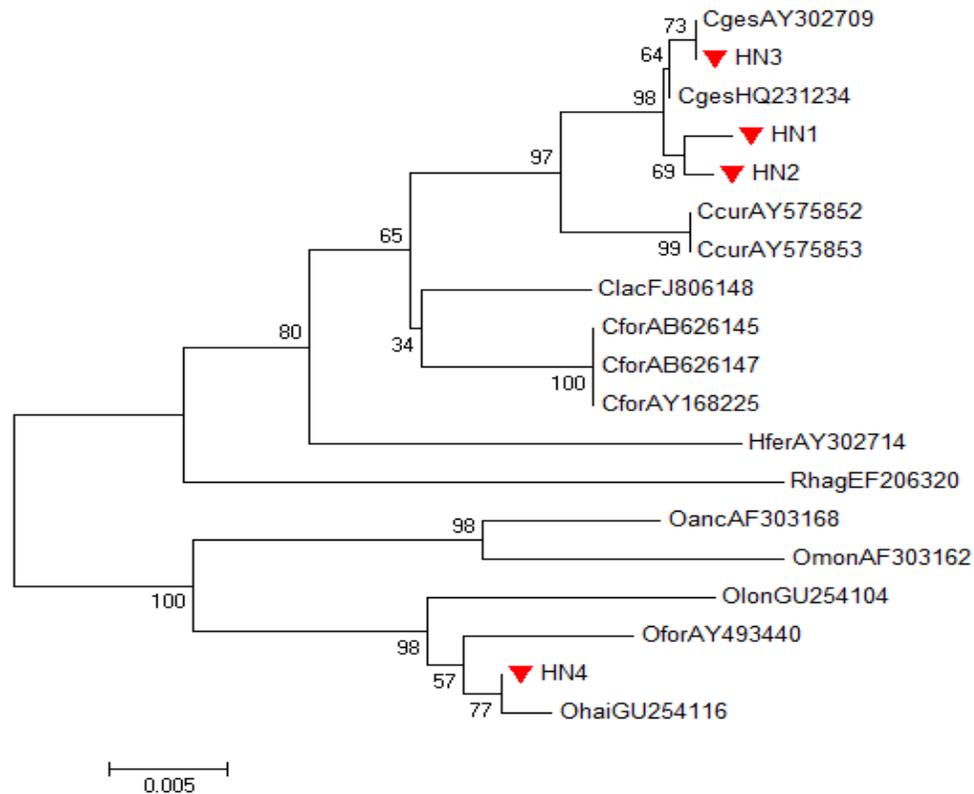
Figure 2: Relative relationship between *Coptotermes gestroi* collected in The Old Quarter of Hanoi and other *Coptotermes* species and a comparison with 3 genus: *Odontotermes, Heterotermes and Reticulitermes*

(CGES, CCur, Cfr, + CLAC, Hfer, Ohai, For, Colon, Omon Oanc stand for *C.gestroi*, *C.curvignathus*, *C.formosanus*, *C.lacteus*, *Heterotermes ferox*, *Odontotermes hainanensis*, *O.formosanus*, *O.longignathus*, *O.montanus*, *O.anceps*, *Reticulitermes hagen*)

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.

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APPENDIX 1

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

	Measurement

	Nguyen Duc Kham et al, 2007		Ahmad, 1965		Hoang Fu Sheng, 2000	
	C. formosanus	*C. gestroi*	*C. formosanus*	*C. gestroi*	*C. formosanus*	*C. gestroi*
Length of head to side base of mandibles	1.45 – 1.53	1.45 – 1.51		1.40 – 1.51	1.43 – 1.68	1.40 – 1.51
Width of head at side base of mandibles	0.75 – 0.78	0.68 – 0.75		0.68 – 0.75		0.68 – 0.75
Width of head at posterolateral ends of antennal carinae	1.02 – 1.05					
Maximun width of head	1.2 – 1.3	1.15 – 1.24		1.15 – 1.24	1,07 – 1,25	1.15 – 1.24
Length of left mandibles	0.95 – 1.03	0.82 – 0.93		0.82 – 0.93	0.9 – 1.05	0.82 – 0.93

APPENDIX 2

Gene sequence of research samples

>HN1

GTATGGCCTGCCCGCTGACATTTGGTGTTGAAGGGCCGCGGTATTTTGACCGTGCAAAGGT
AGCATAATCATTAGTTCTTTAATTGTGATCTGGAATGAATGGCTTGACGAGGCACGGGCTG
TCTTAGTGTTGAATGTTTTATTGAATTTGGTCTTTGAGTTAAAATTCTTAGATGTTTTTGT
GGGACGAGAAGACCCTATAGAGTTTGACATTTGGTTTATTTACTTGTTTGTTGTTTGTTTA
TTTTTGGTGAGTGGGCTTTTTGTTTTGTTGGGGTGATGGGAGGAATGTACTTAACTCCTCT
TAGTTTTGGTTATATTGATTTATAATTATTTGATCCAGTTATTTTGATTGTAAGACTAAAT
TACCTTAGGGATAACAGCGTTATCTTCCTTGAGAGTTCTTATCGGCAGGGGGGTTTGCGAC
CTCGATGTTGGATTAAG

>HN2

GTATGGCCTGCCCGCTGACATTTGGTGTTGAAGGGCCGCGGTATTATGACCGTGCAAAGGT
AGCATAATCATTAGTTCTTTAATTGTGATCTGGAATGAATGGCTTGACGAGGCACGGGCTG
TCTTAGTGTTGAATGTTTTATTGAATTTGGTCTTTGAGTTAAAATTCTTAGATGTTTTTGT
GGGACGAGAAGACCCTATAGAGTTTGACATTTGGTTTATTTACTTGTTTGTTGTTTGTTTA

TTTTTGGTGAGTGGGCTTTTTGTTTTGTTGGGGTGATGGGAGGAATGTACTTAACTCCTCT
TAGTTTTGGTTATATTGATTTATAATTGTTTGATCCATTTATTTTGATTGTAAGACTAAAT
TACCTTAGGGATAACAGCGTTATCTTCCTTGAGAGTTCTTATCGGCAGGGGGGTTTGCGAC
CTCGATGTTGGATTAAG

>HN3

GTATGGCCTGCCCGCTGACATTTGGTGTTGAAGGGCCGCGGTATTTTGACCGTGCAAAGGT
AGCATAATCATTAGTTCTTTAATTGTGATCTGGAATGAATGGCTTGACGAGGCACGGGCTG
TCTTAGTGTTGAATGTTTTATTGAATTTGGTCTTTGAGTTAAAATTCTTAGATGTTTTTGT
GGGACGAGAAGACCCTATAGAGTTTGACATTTGGTTTATTTACTTGTTTGTTGTTTGTTTA
TTTTTGGTGAGTGGGCTTTTTGTTTTGTTGGGGTGATGGGAGGAATGTACTTAACTCCTCT
TAGTTTTGGTTATATTGGTTTATAATTGTTTGATCCATTTATTTTGATTATAAGACTAAAT
TACCTTAGGGATAACAGCGTTATCTTCCTTGAGAGTTCTTATCGGCAGGGGGGTTTGCGAC
CTCGATGTTGGATTAA

>CgesHQ231234
GTATGGCCTGCCCGCTGACATTTGGTGTTGAAGGGCCGCGGTATTTTGACCGTGCAAAGG
TAGCATAATCATTAGTTCTTTAATTGTGATCTGGAATGAATGGCTTGACGAGGCACGGGC
TGTCTTAGTGTTGAATGTTTTATTGAATTTGGTCTTTGAGTTAAAATTCTTAGATGTTTT
TGTGGGACGAGAAGACCCTATAGAGTTTGACATTTGGTTTATTTACTTGTTTGTTGTTTG
TTTATTTTTGGTGAGTGGGCTTTTTGTTTTGTTGGGGTGATGGGAGGAATGTACTTAACT
CCTCTTAGTTTTGGTTATATTGATTTATAATTGTTTGATCCATTTATTTTGATTATAAGA
CTAAATTACCTTAGGGATAACAGCGTTATCTTCCTTGAGAGTTCTTATCGGCAGGGGGGT
TTGCGACCTCGATGTTGGATTAAG

>CgesAY302709

GTATGGCCTGCCCGCTGACATTTGGTGTTGAAGGGCCGCGGTATTTTGACCGTGCAAAGGT
AGCATAATCATTAGTTCTTTAATTGTGATCTGGAATGAATGGCTTGACGAGGCACGGGCTG
TCTTAGTGTTGAATGTTTTATTGAATTTGGTCTTTGAGTTAAAATTCTTAGATGTTTTTGT
GGGACGAGAAGACCCTATAGAGTTTGACATTTGGTTTATTTACTTGTTTGTTGTTTGTTTA
TTTTTGGTGAGTGGGCTTTTTGTTTTGTTGGGGTGATGGGAGGAATGTACTTAACTCCTCT
TAGTTTTGGTTATATTGGTTTATAATTGTTTGATCCATTTATTTTGATTATAAGACTAAAT
TACCTTAGGGATAACAGCGTTATCTTCCTTGAGAGTTCTTATCGGCAGGGGGGTTTGCGAC
CTCGATGTTGGATTAAG

Table 2. Compare gene similar of 16S between research samples (HN1 sample) and samples refered to Genbank (CgesHQ231234 and CgesAY302709)

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