

# Phylogenetic Analysis of *Reticulitermes speratus* using the Mitochondrial Cytochrome C Oxidase Subunit I Gene<sup>\*1</sup>

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## ABSTRACT

*Reticulitermes speratus* is commonly found in Asia, including Korea and Japan. We recently analyzed the 5' region of mitochondrial cytochrome c oxidase subunit I to perform a phylogenetic analysis of *R. speratus* KMT1, isolated in Seoul, Korea. Our results, using COXI, suggest that the taxonomy of *R. speratus* should be reconsidered with regard to the subgenus group. A similar phylogenetic analysis by COXI and COXII demonstrated the reliability of COXI genetic information in a molecular phylogenetic analysis of termites.

**Keywords :** *Reticulitermes speratus*, Cytochrome c oxidase subunit I, phylogeny, taxonomy

## 1. INTRODUCTION

The termite is an economically significant insect in the wood industry. New Orleans, USA, spends over US \$300 million annually for damages of wooden structures that are caused mostly by *Coptotermes formosanus* (Alan and Weste, 2003). Korean traditional wooden houses in Seoul, Korea also had a termite damage which might be accelerated by modern temperature control facilities (Son and Lee, 2008). It is expected that in Korea, the termite damage on wooden structures will be expanded and intensified in consequence of global warming. In East Asia, more than 70 species of *Reticulitermes* have been reported, and 5 species of *Reticulitermes* have been identified in Japan (Take-

matsu, 1999). *R. speratus* is the termite species found in South Korea (Lee *et al.*, 2001; Ra *et al.*, 2005; Son and Lee, 2008) and has been suggested to have migrated from China through southern Japan (Park *et al.*, 2006).

Morphological observations have been used to classify termites (Takematsu, 1999; Kitade and Hayashi, 2002; Koshikawa *et al.*, 2004). New genetic approaches have been adopted in the last decade and have modified and strengthened older termite taxonomy. Termite, the order Isoptera, is consisted with 7 families. The family Rhinotermitidae has extremely heterogeneous genera with 17 genus and 350 species (Lee and Ryu, 2003). Analysis of the mitochondrial cytochrome c oxidase subunit II (COXII) gene showed that the family Rhinotermiti-

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tidae is polyphyletic (Miura *et al.*, 1998; Thompson *et al.*, 2000; Ohkuma *et al.*, 2004). In Rhinotermitidae, the genera *Coptotermes* and *Reticulitermes* can be found in temperate zones (Lee and Ryu, 2003).

We analyzed the phylogenetic classification of *R. speratus* in the genus *Reticulitermes* in a molecular phylogenetic study using the COXII gene and the 5' region of the mitochondrial cytochrome c oxidase subunit I (COXI) gene. The 5' region of COXI has been useful for the phylogenetic analysis of many invertebrate taxa (Folmer *et al.*, 1994).

## 2. EXPERIMENTAL

### 2.1. Termites and DNA Extraction

*Reticulitermes speratus* KMT1 was collected from Bukhan Mountain in Seoul, Korea. Fifty individual termites (workers) were selected randomly. After the guts were extracted using tweezers, the entire body was frozen and finely ground with a mortar and pestle in liquid nitrogen. Total DNA was purified with the Genomic DNA Prep Kit for animal tissue and fungi according to the manufacturer's instructions (SolGent Co., LTD., Daejeon, Korea).

### 2.2. DNA Amplification and Sequencing

One primer set, LCO1490 (5'-GGTCAACAA-ATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'), was used to amplify a partial region of the 5' area of mitochondrial COXI (Folmer *et al.*, 1994). The universal insect primer pair TK-N-3785 (5'-GT-TTAAGAGACCAGTACTTG-3') and TL2-J-3037 (5'-ATGGCAGATTAGTGCAATGG-3') was used to amplify the entire mitochondrial COXII gene (Liu and Beckenbach, 1992; Simons *et al.*, 1994).

The target regions were amplified by polymerase chain reaction (PCR), and products were puri-

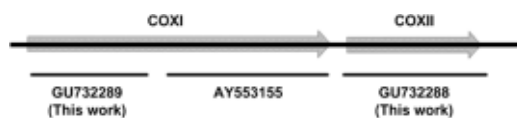


Fig. 1. Genetic locations of sequenced genes in the mitochondrial genome of *R. speratus*. Arrow shows an open reading frame. Sequenced regions are indicated by underlines with the NCBI GenBank accession number. COXI: cytochrome c oxidase subunit I; COXII: cytochrome c oxidase subunit II.

fied by using the QIAquick PCR Purification Kit according to the manufacturer's instructions (Qiagen Korea, LTD., Seoul, Korea). The PCR products were sequenced using HCO2198 and TL2-J-3037 for subunit I and subunit II, respectively, at SolGent Co., LTD.

### 2.3. Data Analysis

To evaluate the phylogeny of the COXI and COXII genes, 10 termite sequences were obtained from GenBank (<http://www.ncbi.nlm.nih.gov/>) (Benson *et al.*, 2008). Sequences were aligned using AlignX in Vector NTI Advance 11.0 (Invitrogen Korea Co., Seoul, Korea). Phylogenetic and molecular evolutionary analyses were performed using MEGA version 4 using the neighbor-joining method and bootstrap phylogeny test with 500 replications (Tamura *et al.*, 2007).

## 3. RESULTS and DISCUSSION

The entire mitochondrial COXII gene of *Reticulitermes speratus* KMT1 was sequenced and submitted to GenBank (accession number: GU732288). The sequence was identical to the COXII gene (AB193239, AB109530, EF016101, and DQ493739) of many *R. speratus* species (data not shown). This result confirms that the termites that we collected from Bukhan Mountain in Seoul, Korea were *R. speratus*.

EF206320 (1503)	TCTCAACAAATCATAAAGACATTGGAACACTATACTTTGTATTTCGGTGCCGTGATCAGGAATGGTCGGAACATCACTCAGA	(1582)
GU732289 (1)	GGTCAACAAATCATAAAGATATTGGAACATTATACTTTGTATTTCGGTGCCGTGATCAGGAATGGTCGGGACATCACTCAGA	
Consensus	TCAACAAATCATAAGA ATTGGAACA TATACTTTGTATTTCGGTGCCGTGATCAGGAATGGTCGG ACATCACTCAGA	
EF206320 (1583)	ATACTAATTCGAACAGAATTAGGACAACCTGGATCTCTAATTGGGGATGACCAGATCTACAATGTTATCGTTACTGCCCA	(1662)
GU732289 (81)	ATGCTAATTCGAACAGAATTAGGACAACCTGGATCTTTAATTGGAGATGACCAATCTACAACGTTATCGTTACTGCCCA	
Consensus	AT CTAATTCGAACAGAATTAGGACAACCTGGATCT TAATTGG GATGACCA ATCTACAA GTTATCGTTACTGCCCA	
EF206320 (1663)	CGCATTTTGTATGATTTTCTTTATGGTTATACCAATCATAATTGGTGGATTTCGGGAACGACTAGTACCACCTTATGCTAG	(1742)
GU732289 (161)	CGCATTTTGTATGATTTTCTTTATGGTTATACCAATCATAATTGGTGGATTTCGGGAACGACTAGTACCACCTTATGCTAG	
Consensus	CGCATTTGT AT ATTTTCTTTATGGTTATACCAATCATAATTGGTGGATTTCGGGAACGACTAGTACCACCTTAT T G	
EF206320 (1743)	GAGCCCCGATATGGCATTCCACGAATAAACAACATAAGATTTTGATTACTCCACCATCACTAACACTACTACTTACT	(1822)
GU732289 (241)	GAGCTCCAGATATGGCATTCCACGAATAAACAACATAAGATTTTGATTACTCCACCATCACTAACACTACTACTTACT	
Consensus	GAGC CC GATATGGCATTCCACGAATAAACA ATAAGATT TGATTACT CCACCATCA TAACACT CTACTTACT	
EF206320 (1823)	AGTAGAACAGTAGAAAGTGGGCGGGGACTGGATGAACAGTTTACCCCTCTTGCAAGAGGGATTGCCCATGCGAGGAC	(1902)
GU732289 (321)	AGTAGAACAGTAGAAAGTGGTGCAGGGACAGGTGGACAGTTTACCCCTCTTGCAAGAGGGATTGCCCATGCGAGGAC	
Consensus	AGTAGAACAGTAGAAAGTGG GC GGGAC GG TG ACAGTTTACCCCTCTTGCAAGAGGGATTGCCCA GC GGAGC	
EF206320 (1903)	ATCCGTAGACCTAGCCATCTTTCTTTACACTTAGCAGGTGTATCATCAATTCTGGGAGCAGTAAATTTTATCTCAACAA	(1982)
GU732289 (401)	ATCCGTAGACCTAGCAATCTTCTCTTACACTTAGCAGGTGTATCATCAATTCTGGGAGCAGTAAATTTTATCTCAACAA	
Consensus	ATCCGTAGAC TAGC ATCTT TC TTACAC TAGCAGGTGTATCATCAATTCTGGGAGCAGTAAA TTTATCTCAACAA	
EF206320 (1983)	CAATCAACATAAAGCCAAAAATATAAAACCCGAGCGAATTCCTACTTTGTATGATCCGTCGCCATCACAGCCCTACTC	(2062)
GU732289 (481)	CAATCAACATAAAGCCAAAAACATAAAACCCGAAACGAAATCCCACTATTGTATGGTCTGTGCGCCATCACAGCCCTACTT	
Consensus	CAATCAACATAAA CCAAAAAA ATAAACCCGA CGAAT CCACTATTGTATG TC GTCGCCATCACAGCCCTACT	
EF206320 (2063)	CTATTACTATCCCTACCAGTATTGGCCGGA	(2092)
GU732289 (561)	CTTCTACTGTCCCTGCCAGTATTGGCAGGA	
Consensus	CT TACT TCCCT CCAGTATTGGC GGA	

Fig. 2. Genetic sequence comparison of COXI 5' region from *R. speratus* (GU732289) with the homologous region in the mitochondrial genome from *R. hageni* (EF206320). The consensus nucleic acids are indicated on the lowest line, which is separated by a line. Expect = 0.0; identities = 544/589 (92%); gaps = 2/589 (0%).

The 5' region of mitochondrial COXI was sequenced and submitted to GenBank (accession number: GU732289). The 3' region of COXI has been sequenced (Lo *et al.*, 2004) and used to classify the genus *Reticulitermes* in family Rhinotermitidae (Lo *et al.*, 2004).

Fig. 1 shows the location of the sequenced regions in the mitochondrial genome of *R. speratus*. A BLAST search (National Center for Biotechnology Information) showed that the mitochondrial genome of *R. hageni* isolate IS198 was most similar among the NCBI database (Fig. 2). This result indicates a lack of phylogenetic studies on *R. speratus* using the 5' region of COXI, which has been useful for phylogenetic analyses of many invertebrate taxa (Folmer *et al.*, 1994).

The classification of *R. speratus* in the genus *Reticulitermes* was analyzed using COXI and

COXII sequences (Fig. 3). *Macrotermes subhyalinus* and *Pseudacanthotermes spiniger* were separated at the top of the tree in both COXI and COXII analyses. Because they belong to the family Termitidae, this early separation suggested good reliability of both analyses in Fig. 3.

The next species to separate was *Coptotermes lacteus*, which was in the same family as *Reticulitermes* but a different genus. In the genus *Reticulitermes*, *R. speratus* diverged first from the other *Reticulitermes* spp., by COXII gene analysis (Fig. 3B), but two *R. virginicus* strains separated first, based on COXI sequences (Fig. 3A).

In the taxonomy of the National Center for Biotechnology Information (NCBI), *R. speratus* belongs to the subgenus *Frontotermes* and the remaining *Reticulitermes* spp. belong to the subgenus *Reticulitermes*. The taxonomy of NCBI is

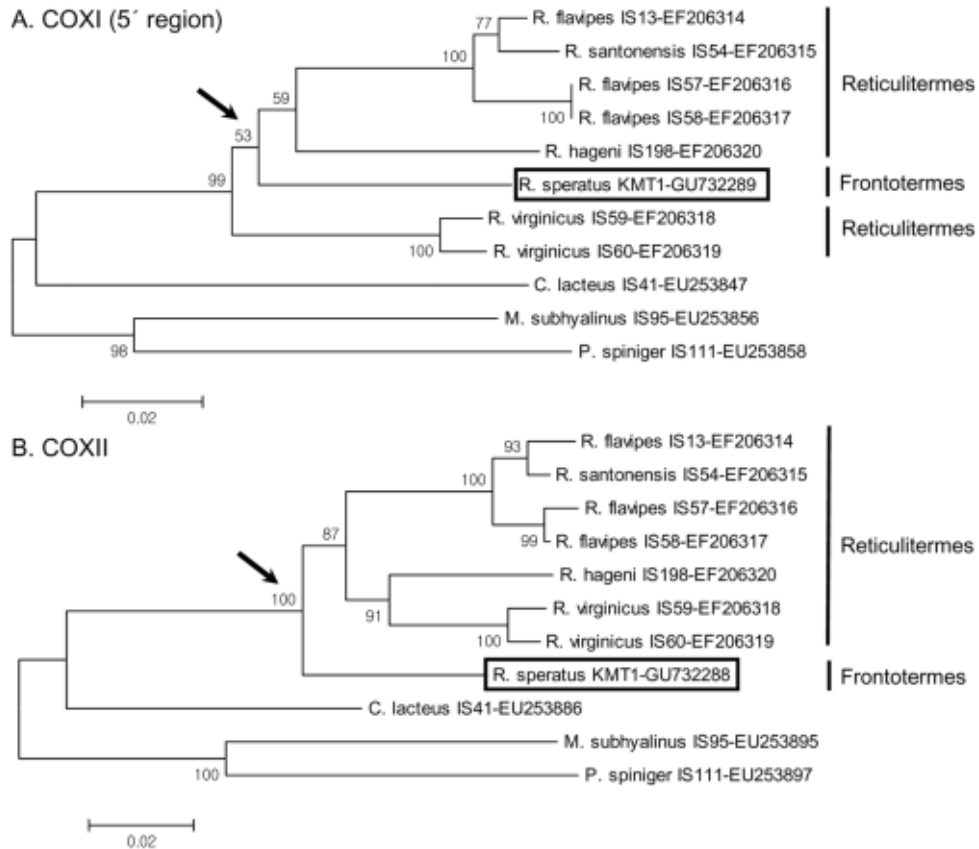


Fig. 3. Phylogenetic tree of *R. speratus* using COXI (A) and COXII (B). MEGA 4.0 using the neighbor-joining method with 1000 replicates for the bootstrap test was used. The *R. speratus* is boxed, and its branch separation is indicated by an arrow. Values at nodes indicate percentage of replicates. The evolutionary distances were computed using the maximum composite likelihood method and are expressed in base substitutions per site. The subgenus name is listed on the right-hand side of the tree. The genus names are R. for *Reticulitermes*, C. for *Coptotermes*, M. for *Macrotermes*, and P. for *Pseudacanthotermes*.

supported by the phylogenetic analysis of the COXII gene (Fig. 3B) but not with the COXI gene (Fig. 3A). The phylogenetic analysis with COXI suggests that *R. speratus* KMT1 is closer to another subgenus, *Reticulitermes*, than the current subgenus (*Frontotermes*).

## 4. CONCLUSIONS

The phylogenetic analysis with COXI showed

that *R. speratus* is closer to certain species in the subgenus *Reticulitermes* than other species in the same subgenus. This analysis encourages the taxonomic reconsideration of *R. speratus* for the subgenus group. A similar phylogenetic analysis of a different family and genus using COXI and COXII demonstrated the reliability of COXI genetic information with regard to the molecular phylogenetic analysis of termites.

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