



Faculty of Resource Science and Technology

Molecular and Morphometric Analyses of *Coptotermes* spp. (Blattodea: Rhinotermitidae) with a Description of Novel Microsatellite Markers

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Molecular and Morphometric Analyses of *Coptotermes* spp. (Blattodea:
Rhinotermitidae) with a Description of Novel Microsatellite Markers

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DECLARATION

I hereby declare that, except as acknowledged in the text, the work presented in the thesis is entirely my own. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

(Norsyarizan binti Jamil)

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Date:

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ABSTRACT

Coptotermes genus, a subterranean termite from the family Rhinotermitidae, is among one of the most destructive pests in Malaysia as it can cause more than 90% damage in buildings and structures. An accurate *Coptotermes* spp. identification is essential for proper pest control and management. However, this is hampered by difficulty in distinguishing the within and between population variations of the species under this genus. The relationship of the *Coptotermes* spp. were identified by using three mitochondrial DNA sequences of 12S, 16S and cytochrome oxidase subunit II (COII) and were supported by morphometric measurement data. A total of 193 individuals consist of four different species of *Coptotermes* (*C. curvignathus*, *C. sepangensis*, *C. kalshoveni* and *C. travians*) were used in a morphometric analyses. The species grouping evaluated from morphometric result showed many overlapping morphological characters between *C. sepangensis* and *C. kalshoveni* and both species had difficulties in being differentiated based on Discriminant Function Analyses (DFA). The morphological ambiguities between *C. sepangensis* and *C. kalshoveni* and the relationships of *Coptotermes* spp. were then validated by using mitochondrial DNA sequences (12S, 16S and COII gene). The phylogenetic relationship of the five putative *Coptotermes* spp. (*C. curvignathus* Holmgren 1913, *C. travians* Haviland 1898, *C. sepangensis* Krishna 1956, *C. kalshoveni* Kemner 1934, *C. gestroi* Wasmann 1896) were used and the available sequences for these species also were included in the analyses. “Neighbour Joining (NJ)”, “Maximum Parsimony (MP)” and “Maximum Likelihood (ML)” were constructed for each genes. The phylogenetic trees resulted four major clades: I (*C. curvignathus*), II (*C. gestroi*), III (mixture of *C. sepangensis* and *C. kalshoveni*) and IV (*C. travians*). The genetic distance by “Kimura 2-parameter” model between *C. sepangensis* and *C. kalshoveni* was 0% to 3.2% in COII, 0% to 4.2% in 16S and 0% to 2.2% in 12S gene.

The discriminant function analyses (DFA) results were corresponded well with molecular phylogenetic tree constructed for *C. sepangensis* and *C. kalshoveni*. It can be proposed that *C. sepangensis* and *C. kalshoveni* might be possible synonyms based on morphometric and molecular data. A mitochondrial DNA sequences used in the current analyses showed limited genetic variation at inter- and intra- colonial level of *Coptotermes* spp.. Therefore, a more sensitive DNA markers such as polymorphic genetic marker is required to elucidate the details of colony organization and colony breeding structure of an important urban pest termites for identifying the origin for groups of foragers. The rubber termite, *Coptotermes curvignathus* is a common subterranean termite which often causes structural damages to the built environment. The incidence of this species infestation in Malaysia occurred in the early 1900's and was reported to infest on rubber trees, *Hevea brasilliensis*. Consequently, this xylophagous insect is considered, by far to be a destructive pest to buildings in urban dwelling as well as a major agricultural pest especially to oil palm plantations in its native range in Southeast Asia. Therefore, we isolated and characterize novel polymorphic microsatellite markers from the genome of *C. curvignathus* in order to understand their cryptic population genetic structure and breeding strategies in oil palm plantation that would further enhance our knowledge on the infestation dynamics of this pest species. A modest volume of 454 next generation pyrosequencing technique generated 47,462 reads whereby 1996 (4.2%) of the reads contain microsatellites with di-, tri- and tetra- nucleotide repeat motifs. Sixty primer pairs were randomly selected for preliminary test of polymorphism across five individual of *C. curvignathus* from distinct geographic sample locations collected within the Sarawak region in Malaysia. Ten of 30 primer sets tested were found to be polymorphic with 4-15 alleles per locus and were subsequently assigned into four multiplex groups for future population genetic studies. Observed and expected

heterozygosities ranged between 0.19 to 0.86 and 0.44 to 0.92, respectively. No linkage disequilibrium was found between any pair of loci and all loci do not deviate from Hardy-Weinberg equilibrium. The high degree of polymorphism among these 10 microsatellite loci will be useful as a sensitive tool to investigate the colony and population genetic structure of *C. curvignathus* in this region. To further validate the current findings, more extensive sample collection from peninsular Malaysia and Borneo region should be done and includes type of specimens (if available) with the original descriptions to provide evidence for a more robust phylogenetic positions of each species. Morphological identification based on the soldier's caste among *Coptotermes* spp. can be improved by comparing with the reproductive caste, i.e. alates, king, queen. The advances in termite taxonomy when combined with conventional methods and molecular tools are important to validate many species names as real biological taxa. An accurate species identification can have important implications for control practices to avoid duplicative testing of termite management strategies which were thought to be a different species in different geographical areas. Therefore, it will save times, resources and finances for pest management strategies. Meanwhile, a more sensitive microsatellite markers will enable understanding of termite colony social organization and their breeding system. Results from this study are hopefully applied for future identification and in turn important for effective pest management.

Keywords: *Coptotermes*, morphometric, phylogenetic relationship, polymorphic

**Molekular dan Analisis Morfometrik *Coptotermes* spp. (Blattodea: Rhinotermitidae)
dengan Deskripsi Baru Penanda Mikrosatelit**

ABSTRAK

Genus *Coptotermes*, anai-anai bawah tanah adalah daripada keluarga *Rhinotermitidae*, merupakan di antara serangga yang paling merosakkan di Malaysia kerana ia menyebabkan lebih 90% kerosakan ke atas bangunan dan struktur. Identifikasi *Coptotermes* spp. yang tepat adalah penting untuk pengawalan dan pengurusan serangga perosak yang betul. Namun begitu, terdapat masalah untuk membezakan di dalam dan di antara variasi populasi spesies di bawah genus ini. Hubungan *Coptotermes* spp. dikenalpasti dengan menggunakan tiga rangkaian mitokondria DNA 12S, 16S dan cytochrome oxidase subunit II (COII) dan disokong dengan data ukuran morfometrik. Sebanyak 193 individu yang merangkumi empat jenis *Coptotermes* spesies (*C. curvignathus*, *C. sepangensis*, *C. kalshoveni* dan *C. travians*) telah digunakan di dalam analisis morfometrik. Penilaian spesies kumpulan daripada keputusan morfometrik menunjukkan banyak pertindihan karakter morfologi antara *C. sepangensis* dan *C. kalshoveni* dan kedua-dua spesies mempunyai kesukaran untuk dibezakan berdasarkan “Discriminant Function Analyses (DFA)”. Kesamaran morfologi antara *C. sepangensis* dan *C. kalshoveni* dan hubungan dalam *Coptotermes* spp. kemudian disahkan dengan menggunakan rangkaian mitokondria DNA (gen 12S, 16S dan COII). Hubungan filogenetik antara lima “putative” *Coptotermes* spp. (*C. curvignathus* Holmgren 1913, *C. travians* Haviland 1898, *C. sepangensis* Krishna 1956, *C. kalshoveni* Kemner 1934, *C. gestroi* Wasmann 1896) telah digunakan dan rangkaian DNA yang tersedia daripada spesies ini juga dimasukkan di dalam analisis. “Neighbour Joining (NJ)”, “Maximum

Parsimony (MP)”, dan “*Maximum Likelihood (ML)*” telah dibina untuk setiap gen. Pokok filogenetik menghasilkan empat cabang utama: I (*C. curvignathus*), II (*C. Gestroi*), III (campuran *C. sepangensis* dan *C. kalshoveni*) dan IV (*C. travians*). Jarak genetik oleh model “*Kimura 2-parameter*” antara *C. sepangensis* dan *C. kalshoveni* adalah 0% hingga 3.2% dalam COII, 0% hingga 4.2% dalam 16S dan 0% hingga 2.2% dalam 12S gen. Keputusan “*Discriminant function analyses (DFA)*” sejajar dengan pokok filogenetik molekul yang dibina untuk *C. sepangensis* dan *C. kalshoveni*. Ini boleh dicadangkan bahawa *C. sepangensis* dan *C. kalshoveni* berpotensi untuk menjadi sinonim berdasarkan morfometrik dan data molekul. Namun begitu, rangkaian DNA mitokondria yang digunakan di dalam analisis menunjukkan variasi genetik yang terhad di peringkat koloni di dalam dan di antara *Coptotermes spp.*. Oleh itu, penanda DNA yang lebih sensitif seperti penanda genetik “*polymorphic*” di perlukan untuk menjelaskan perincian organisasi koloni dan struktur pembiakan koloni serangga perosak anai-anai di bandar. Anai-anai pokok getah, *Coptotermes curvignathus* adalah kebiasaan anai-anai bawah tanah yang selalu menyebabkan kerosakan struktur dalam persekitaran pembangunan. Kejadian serangan spesies ini di Malaysia berlaku di awal 1900’s dan telah dilaporkan telah menyerang pokok getah, *Hevea brasiliensis*. Natiujahnya, serangga “*xylophagous*” ini telah dipertimbangkan sebagai serangga yang paling merosakkan setakat ini kepada bangunan kediaman rumah di bandar dan juga serangga perosak major terutamanya kepada pokok kelapa sawit dalam lingkungan asal di Asia Tenggara. Oleh itu, kami mengasingkan dan mencirikan penanda baru mikrosatelit “*polymorphic*” daripada genom *C. curvignathus* untuk memahami rahsia struktur populasi genetik dan strategi pembiakan di ladang kelapa sawit yang seterusnya dapat meluaskan pengetahuan tentang serangan dinamik spesies serangga perosak ini. Teknik “*454 next generation pyrosequencing*” telah menghasilkan 47,462 bacaan di mana

1996 (4.2%) daripada bacaan mengandungi mikrosatelit dengan motif ulangan di-, tri- dan tetra-. Enam puluh pasangan primer telah dipilih secara rawak daripada ujian saringan awal “polymorphism” ke atas lima individu *C. curvignathus* yang di kutip daripada pelbagai lokasi geografi dalam kawasan Sarawak di Malaysia. Sepuluh daripada 30 primer yang diuji telah dijumpai sebagai “polymorphic” dengan 4-15 alele setiap lokus dan seterusnya di tentukan kepada 4 kumpulan “multiplex” untuk kajian populasi genetik pada masa hadapan. Pemerhatian dan jangkakan “heterozygosities” berada dalam lingkungan 0.19 hingga 0.86 dan 0.44 hingga 0.92 masing-masing. Tiada “linkage disequilibrium” dijumpai antara pasangan loci dan semua loci tidak menyimpang daripada “Hardy-Weinberg equilibrium”. Kadar “polymorphism” yang tinggi antara 10 loci mikrosatelit akan berguna sebagai alat sensitif untuk menyiasat koloni dan populasi struktur genetik *C. curvignathus* dalam kawasan ini. Untuk membuktikan hasil kajian ini, koleksi sampel yang lebih meluas daripada Semenanjung Malaysia dan kawasan Borneo perlu dilakukan dan memasukkan jenis spesimen (sekiranya ada) dengan deskripsi asal untuk menyediakan posisi filogenetik setiap spesies dengan lebih kuat. Identifikasi morfologi berdasarkan kasta askar antara *Coptotermes* spp. boleh dipertingkatkan dengan membandingkan kasta pembiakan, i.e. kelkatu, raja, permaisuri. Kemajuan dalam taksonomi anai-anai apabila digabungkan dengan kaedah konvensional dan alat molekul adalah penting untuk membuktikan banyak nama spesies sebagai “taxa” biologi sebenar. Spesies identifikasi yang tepat penting untuk implikasi pengamalan kawalan untuk mengelakkan pengulangan ujian strategi pengurusan anai-anai yang dianggap adalah spesies berbeza di setiap kawasan yang berbeza. Oleh itu, ini dapat menjimatkan masa, sumber dan kewangan untuk strategi pengurusan serangga perosak. Sementara itu, penanda mikrosatelit yang lebih sensitif akan dapat memahami organisasi social koloni dan sistem pembiakan mereka. Hasil

daripada kajian ini diharap dapat dimanfaatkan untuk identifikasi pada masa depan dan penting untuk pengurusan serangga perosak secara efektif.

Kata kunci: *Coptotermes, morfometrik, hubungan filogenetik, “polymorphic”*

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LIST OF ABBREVIATIONS

°C	Celcius
ANOVA	Analysis of Variance
bp	Base pair (s)
COII	Cytochrome oxidase subunit II
ddH ₂ O	Double distilled water
DFA	Discriminant Function Analysis
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphates
MgCl ₂	Magnesium Chloride
min	Minute (s)
mtDNA	Mitochondrial DNA
PCA	Principle Component Analysis
PCR	Polymerase Chain Reaction
rpm	Revolutions per minute
rRNA	Ribosomal RNA
sec	Second (s)
spp.	species

Taq	<i>T.aquaticus</i>
TBE	Tris-Borate EDTA
TEMED	Tetramethylethylenediamine
tRNA	Transfer RNA
gDNA	Genomic DNA

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CHAPTER 1

INTRODUCTION

Termites (Blattodea) are eusocial insect that live together in large communities of several hundred to several million of individuals. These communities are composed of different individuals that perform different labours either as defender, foragers or reproducer. They are primarily wood feeder that adapted to consume wood and dry plants, but they also feed on variety of other organic substances such as living trees, leaf litter, lichens and animal faeces (Kirton, 2005).

The species of termite that cause damage to buildings vary according to geographical region. In temperate and subtropical areas of the world, the pest species are more readily identified and better documented due to the low termite diversity in those areas. In tropical areas, where the diversity of termites is high, a complex of different species have usually been recorded causing damage in buildings, but there is usually little data on the relative importance of these species. In addition, problems in the taxonomy and identification of the diverse tropical termite fauna have sometimes led to confusion over the pest species associated with households in the tropics.

Taxonomy is the science of recognizing and classifying biological life-forms. Taxonomy is the basis for all meaningful studies on biodiversity, pest management, medicine, bioprospecting, fisheries, quarantine and defense. Before initiating any kind of studies, it is absolutely essential to know the correct name of the organism on which the studies are initiated. This is important because the correct scientific name of the organism is a functional label, using which various pieces of information concerning that organism, including all the past work done on it, can be retrieved and stored ensuring easy reference

(Narendran, 2000). If there is taxonomic confusion, the wrong name is applied to the species, then the pest management decisions we make could be based on misleading information or may not make use of the full body of information available about that species (Kirton, 2004). Thus, the confusion filters down to the level of industry, and may have serious economic consequences or, in extreme cases, even direct effects on the well being of human beings. Fundamental taxonomic research can, therefore, have far reaching impacts on science, industry and society across the world.

In Chapter Two, the pest species of *Coptotermes* affecting buildings, crops and trees in Sarawak are reviewed, and problems in the taxonomy and identification of the species are highlighted. Specific reference is made to the Asian Subterranean Termite, for which recent taxonomic work has clarified a paradox that existed with regard to its pest status, and this is used to represent the importance of accurate taxonomy in the broader perspective of termite management.

Krishna *et al.* (2013) listed 69 *Coptotermes* names that were regarded as valid in the taxonomic literature and took into account various subjective synonymies over the past century. While the nomenclatural work of Krishna *et al.* (2013) provides the biogeographical details concerning all known *Coptotermes* species names, there is still research needed to clarify the biological reality of each *Coptotermes* species name.

In Chapter Three, the molecular techniques is introduced to aid in the taxonomy of this *Coptotermes* genus. The morphology-based species delineation has been complemented by DNA sequence data, leading to an integrative taxonomy. By implementing the molecular tools, synonymisation can be minimised and the species name can be validated which can have important implications for control practices, as highlighted by Kirton (2005). Since

there were no molecular information about *Coptotermes* spp. in Sarawak, such information can be pooled with a molecular data from peninsular Malaysia into a common body of knowledges about *Coptotermes* species. The goal in Chapter 3 is to investigate the current status of all described *Coptotermes* spp. names in Malaysia as a step toward a comprehensive taxonomy of the genus.

Microsatellite genotyping has been used to track colonies and assess the efficacy of bait treatments (Vargo, 2003; Aluko and Husseneder, 2007; Husseneder *et al.*, 2007) and liquid treatments (Parman and Vargo, 2010). The true test of potential colony-level effects of termite's treatment requires that colonies exposed to field treatments be identifiable and followed over time. In many situations, the method of choice for identifying individual subterranean termite colonies and tracking their fate is genetic fingerprinting using microsatellite markers (Vargo and Husseneder, 2009). Therefore, Chapter 4 in this dissertation focused on the development of polymorphic microsatellite markers in *C. curvignathus*, one of the important pest among *Coptotermes* species. When compared to mitochondrial DNA sequences that show limited genetic variation at the intercolonial level (Yeap et al. 2007), polymorphic genetic markers can be used to elucidate the details of colony organization, population structure, and relationships among introduced and native populations. Results of this preliminary study employing microsatellite markers isolated from *C. curvignathus* in oil palm plantation will provide greater sensitivity to distinguish neighbouring colonies, which is useful to test the effectiveness of termite's baiting treatment on infested oil palm tree.

CHAPTER 2

LITERATURE REVIEW

2.1 Genus *Coptotermes* of Rhinotermitidae Family

Genus *Coptotermes* of the family Rhinotermitidae is known as the most widespread family of termites throughout the tropical, subtropical and temperate regions of the world (Abe *et al.*, 2000). Subterranean termites make their colony below the ground together with the holes present in their nest (Wood and Johnson, 1986). They are aboveground foragers which forage on above ground vegetation such as tree, decaying wood and bushes in nature. Subterranean termite of family Rhinotermitidae was initially classified by Holmgren in 1912 as part of Mesotermitidae. Then, it is later modified by Emerson in 1941 and established as family by Synder in 1949 (Austin *et al.*, 2004).

Termites can be classified into lower and higher groups (Krishna, 1970) (Figure 2.1). As showed by Figure 2.1, the lower groups consist of six families while, the higher groups consist of one family (Zhang and Leadbetter, 2012) which the majority of termite species is belong to this group by 80% of all species (Yeap *et al.*, 2009). The differences between the lower and higher group were based on their gut microbial content and diet. The lower group has diverse populations of both prokaryotes and flagellated protists in their gut. Contrary to higher group, they also has diverse array of prokaryotes, but the gut lack of flagellated protists. In terms of the diet, the lower termite restricted to wood or grass, while the higher termite can be soil feeder (humivorous), wood and grass feeder (xylophagous) and fungus cultivator (Noirot, 1992).

The Rhinotermitidae has six subfamilies: Coptotermitinae; Heterotermitinae; Psammotermitinae; Termitogetoninae; Stylotermitinae; and Rhinotermitinae. The

Coptotermitinae which includes the single genus of *Coptotermes* is especially important as intermediate taxa between the lower and higher termites as suggested by Vargo and Husseneder (2009).

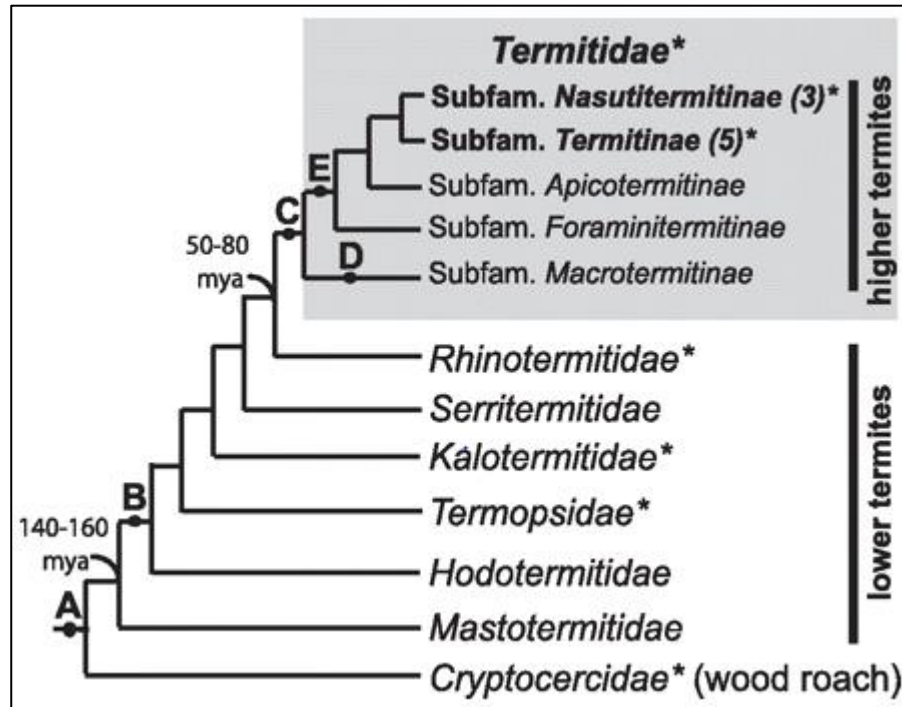


Figure 2.1: Schematic cladogram of major termite families and higher termite subfamilies (Zhang and Leadbetter, 2012)

New phylogenetic analyses described by Inward *et al.* (2007) showed the clade of the Rhinotermitidae is suggested to be a sister group with the the higher termites (Termitidae) and the most derived family under the lower termites. *Reticulitermes* and *Coptotermes* of Rhinotermitidae family are important as transitional taxa between the lower and higher termites for three reason. First, these genera are likely the sister group of the Termitidae, which can be explained that *Reticulitermes* and *Coptotermes* have a closed relationship with the higher termites. Second, these genera contain more species than any other genus of subterranean termites (Kambhampati and Eggleton, 2000). Third, they exhibit

features intermediate between the lower and higher termites (Inward *et al.*, 2007) such as 1) feeding behaviour of lower termites, 2) have nesting habits intermediate of lower termites and higher termites between the single-site nesting and the central-site nesting and 3) the presence of true works, a trait primarily associated with higher termites. The single-site nesting of more basal lower termites in which colonies use a single piece of wood as host nesting site and food source and the central-site nesting of higher termites in which colonies use multiple sources of food away from the nest site (Shellman-Reeve, 1997). The understanding basic features of the life history, behaviour and ecology of *Coptotermes* can provide insights into the evolution and remarkable radiation of the higher termites (Vargo and Husseneder, 2009). In Malaysia and Borneo forest, *Coptotermes* is one of the commonly found genus (Thapa, 1981; Collins, 1988; Tho, 1992).

The occurrence of termites is determined by environmental factors mainly temperature and moisture. These environmental factors are known to affect the geographical distribution of subterranean termites (Kofoid, 1934). The distribution of the subterranean termites is restricted to highly humid environment with temperature range from 24°C to 35°C (Potter, 2013). However, Bouillon (1969) described they do not have specific niches and have an ability to modify their surroundings and nest areas in a way that meet their habitat requirements.

2.2 The Caste System of Termites

The social termite's castes are group of individuals which cooperate to perform a specific colony tasks which characterised by a reproductive division of labour. They are cooperating to rear offspring and share the resources such as food and shelter (Miura, 2004). This caste system is a result of phenotypic plasticity (Hamilton, 1964; Crozier and Pamilo,

1996; Evans and Wheeler, 1999) and the caste differentiation also can be determined by extrinsic factors such as pheromones and hormones (Krishna, 1970). Generally, the caste is categorised into worker caste, soldier caste and the reproductive caste as shown in Figure 2.2.

The worker termites or also known as pseudergates, false worker or helpers (Roisin, 2000) are physically wingless, white and the most numerous castes. According to Weesner (1987), they can be either sterile male or female. The workers are typically the first termites seen when the nest or mounds in the infested structural woods or infested logs is breached. The workers are lack of compound eyes and ocelli (Ross, 1956; Thorne, 1996; Pearce, 1997) and probably depend on light intensity of surrounding.

The workers perform most of the labour work for colony maintenance. They locate and colonise food resources, build and repair the mound and mud tubes (Harris, 1957) feeding, grooming and caring for juvenile termite, feed the reproductive termite and soldiers (Thorne, 1996). Laine and Wright (2003) described *Coptotermes* workers have the possibility (1) to undergo status quo worker to worker molts, (2) differentiate into presoldiers (immediately followed by soldier differentiation) or (3) differentiate into apterous (wingless) and eyeless third-form reproductive, or “ergatoid neoténics” (Laine and Wright, 2003).

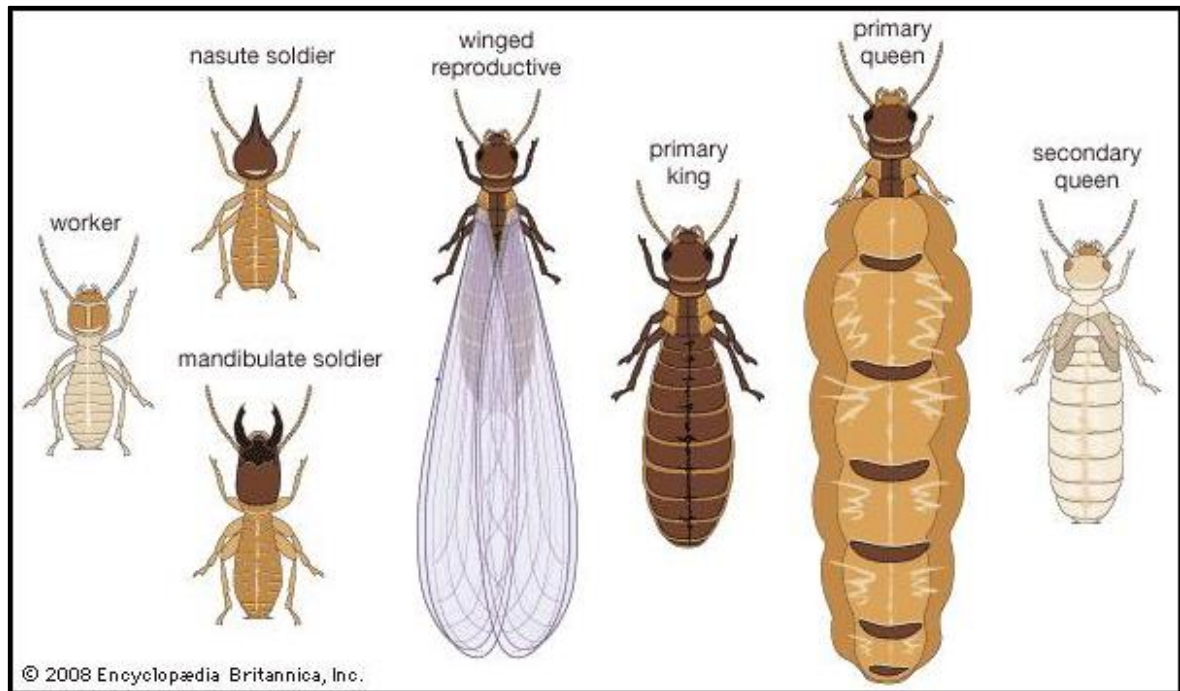


Figure 2.2: The caste system of termites consist of worker, soldier (mandibulate and nasute) and reproductive (winged reproductive, primary king, primary queen and secondary queen) (adapted from Krishna, 2014).

The soldier castes are different from worker by the presence of large mandible, larger in size and have red to brownish head part. Like a worker, the soldier also without wing and can be either infertile male or female (Weesner, 1987) and lack of compound eyes and ocelli (Ross, 1956; Thorne, 1996; Pearce, 1997). However, their number only represent 10% of the population at most (Harris, 1957) not as many as worker's number. The major role of the soldier is for defensive purposes (Bignell and Eggleton, 1998) to protect the colony from their enemy such as ants and other termites from another colony. Among of the unique features of *Coptotermes*, they exhibit a frontal gland that releases a self-protective secretion which can be poisonous or repellent to invaders (Richard and Davies, 1978).

2.3 The Significance of Subterranean Termites in Ecosystem

In the tropical ecosystem, termites act as decomposer in soil-litter system of organic matter (Peakins and Josens, 1978; Wood and Johnson, 1986). The termite's decomposition process is thought involved by two main attribute, their feeding behaviour or capability to displace soil during tunnel excavation. Their food which is cellulose based enable them to decompose the dead plant material that is rich in cellulose (Abe, 1995). The termite's decomposition process able to improve the fertility of the soil from its by-products of nitrogen and methane gas (Lee and Wood, 1971; Wood and Sands, 1978; Collins, 1984; Wood, 1988).

2.4 The economic importance of *Coptotermes* in Malaysia

The Coptotermitinae, which included the single genus of *Coptotermes*, is the most primitive subfamily of the Rhinotermitidae, with 71 described species (Kambhampati and Eggleton, 2000). *Coptotermes* spp. are widely distributed in the tropical and subtropical regions of the world (Figure 2.3). There are six recorded species in Malaysia and Borneo region so far including *C. curvignathus*, *C. kalshoveni*, *C. sepangensis*, *C. travians*, *C. bentongensis* and *C. gestroi* (Chouvenc *et al.*, 2015).

Austin *et al.* (2004) described the certain species in Rhinotermitidae as destructive termite species associated with their feeding preferences which are wood and their by-products. Su (2002) estimated that more than \$20 billion annually were accounted for worldwide damage caused by termites. In Malaysia, *Coptotermes* spp. cause more than 90% damages in buildings and structure (Lee, 2002a; Lee, 2007). Among the most, important species of *Coptotermes* recognised are *C. travians*, *C. curvignathus*, *C. havilandi*, *C.*

kalshoveni and *C. sepangensis* (Lee, 2002a). In the urban and suburban area, *C. traviants*, *C. curvignathus*, *C. havilandi* and *C. kalshoveni* are important to the pest control industry (Sajap and Wahab, 1997). *C. traviants* and *C. curvignathus* are found as the most aggressive species in urban areas while *C. sepangensis* found to infest suburban and urban structures (Lee, 2002a).



Figure 2.3: Distribution of *Coptotermes* spp. (grey areas) in tropical and subtropical regions of the world. Modified from Bourguignon *et al.* (2016).

The pest study in termites was initiated indirectly in the early 1900s when the rubber trees (*Hevea brasiliensis*) which had been introduced into Malaya were seriously attacked by termite. In other cases, inspection of 32 buildings in peninsular Malaysia by pest control companies in 1998 showed that *C. traviants* caused 53% damages to buildings, followed by *C. curvignathus* and the remaining by the other three *Coptotermes* spp. and another species of *Macrotermes gilvus*, *M. carbonarius*, *Globitermes sulphureus*, *M. pakistanicus*, *Odontotermes* spp. and *Microcerotermes crassus*.

It can be concluded that termites caused a major infestation problem to residential buildings in Malaysia as a result from reusing the ex-rubber and oil palm plantations area for residential housing projects. Thus, the professional pest control operators and good pest control strategies are required to control the population of pest termites in Malaysia. The rule of thumb to make good strategy of pest management must be based on the knowledges especially on the pest behaviours and reliable species identification.

2.5 Morphological Approach for *Coptotermes* Identification

Morphological identification is the most common, standard method to identify species based on their external characters and have contribute largely to the systematics of the insects. For eusocial insect (i.e. termites, ants, bees) that consist of different features and sizes among each caste, it is quite challenging to recognise a single species based on the diagnostic character.

A notable diagnostic features exhibit by soldiers and alates of termites make them useful for termite identification. Furthermore, they are the most accessible individuals in their colony. However, in *Coptotermes* spp., most of the identification work of termite and species descriptions solely dependent on the soldier caste only due to seasonal occurrences that may prevent simultaneous collection and description of alate caste (Jones *et al.*, 2005; Yang and Li, 2012). Based on the characters of the soldiers, the *Coptotermes* key feature is it has fontanelle opening far in front with short tubular elongation as shown in Figure 2.4.

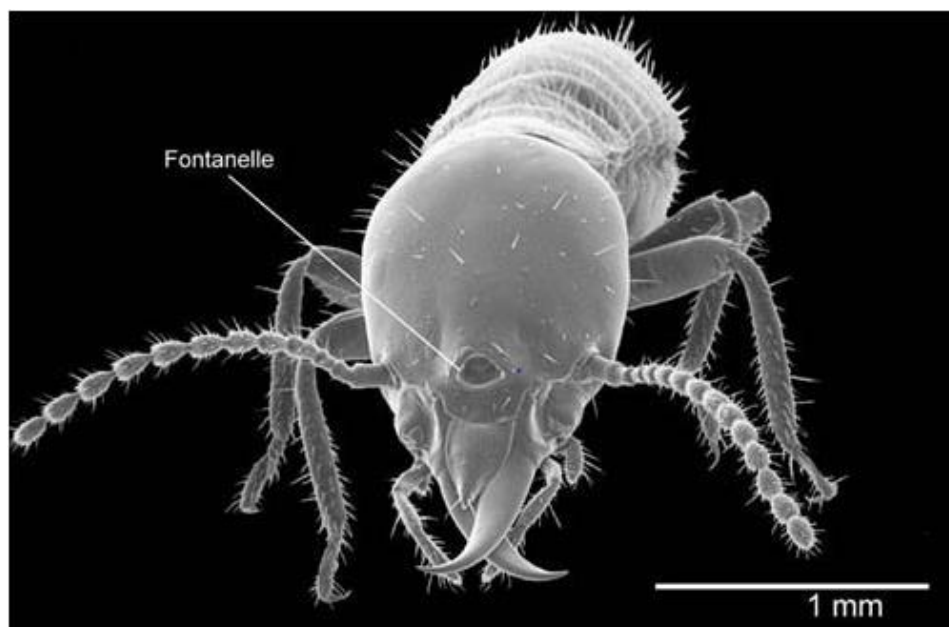


Figure 2.4: The fontanelle opening on its head which characterize the *Coptotermes* spp. Photo credits: Rudolf H. Scheffrahn, University of Florida.

As stated by Kirton and Brown (2003), the head is commonly pear shaped, laterally rounded and armed with slender and slightly incurved mandible. No teeth are apparent on the mandibles. Another notable character among *Coptotermes* is the distinctly large and conspicuous fontanelle or frontal gland in soldier caste. Thus, making it easier to compare with other genus of Rhinotermitidae family.

Figure 2.5 shown the diagnostic characters of the soldier caste that are commonly used in termite identification. Although still commonly used, the overdependence on the soldier morphology presenting a major limitation for single species recognition because of the intra-variation in soldiers (Emerson, 1971; Husseneder and Grace, 2001) and lack of distinguishing diagnostic features among the species of *Coptotermes* (Li *et al.*, 2010). The phenotypic plasticity and genetic variability in the characters which commonly occurred in *Coptotermes* may give problems for routine task of species identification thus may lead to misidentification.

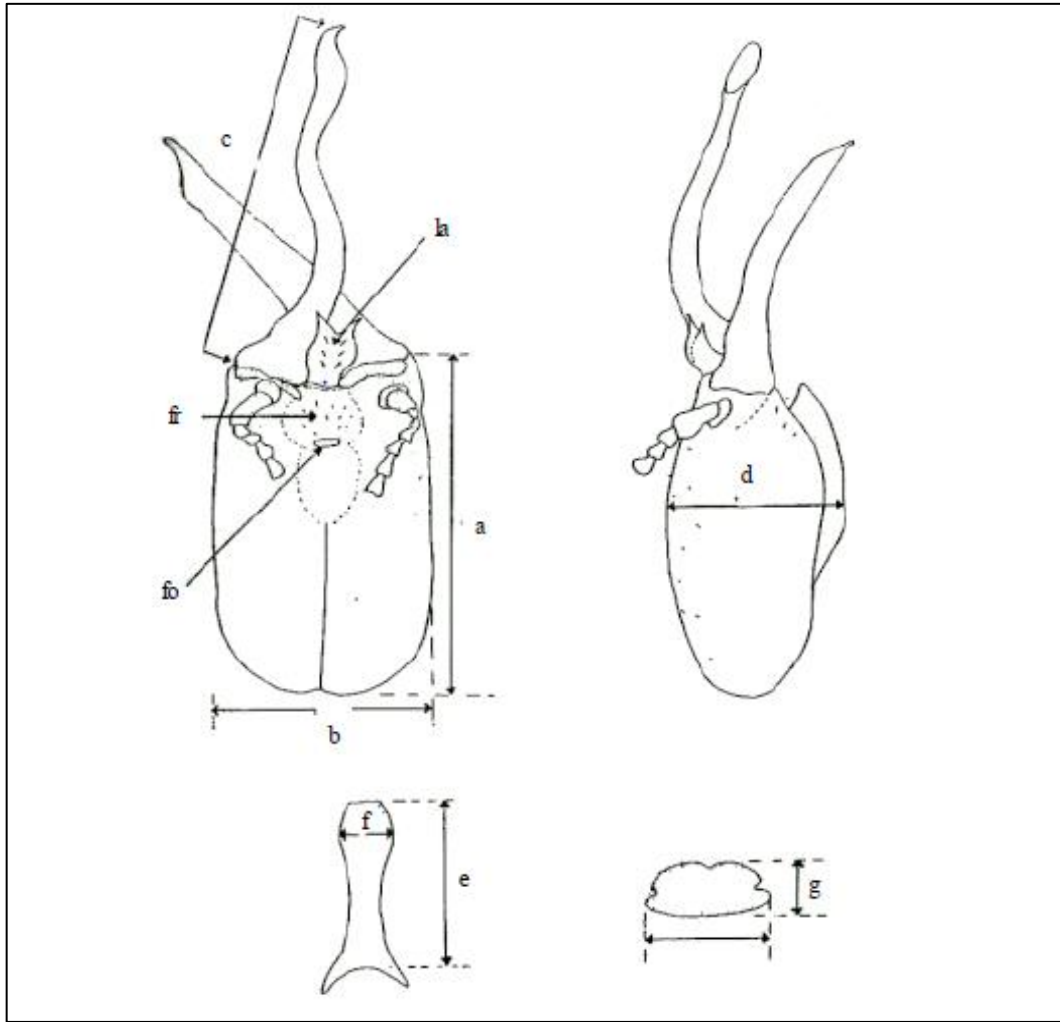


Figure 2.5: Dorsal and profile view of soldier heads, postmentum and pronotum: (a) head length to lateral base of mandibles, (b) maximum head width, (c) mandible length to lateral base, (d) height of head including postmentum, (e) length of postmentum, (f) maximum width of postmentum, (g) length of pronotum, (h) width of pronotum, fr=frons, fo=fontanelle, la=labrum (adapted from Rahman and Tawatao, 2003)

Yeap *et al.* (2009) reported that most of the *Coptotermes* identification using morphological characters has overlapping range and only several key characters are reliable. They observed the variation in the characters still occur within the same species. For example, *C. gestroi* collected from the forests had distinct larger size compared to those collected from urban areas. Scheffrahn *et al.* (2005) explained the environmental factors, i.e. habitat, colony's age, can influenced the termite morphology. Yeap *et al.* (2009) concluded

there are difficulties and limited reliability for morphological identification of *Coptotermes* samples at species level.

The researchers in Vietnam also had confusion in distinguishing between *C. formosanus* and *C. gestroi* based on morphological measurement and characters. After 2010, the identification of *Coptotermes* spp. is more reliable and significantly improved with a range of supporting systematic keys. Scheffrahn and Su (2000) highlighted several key features to distinguish between *C. gestroi* and *C. formosanus* using the number of bristles around the fontanelle and flexures of soldier head behind fontanelle when observed from a lateral view.

The limitation on this conventional identification for discriminating species thus make multivariate morphometric approach using statistical analyses such as PCA and DFA seems to be better tools for termite identification (Cadrins, 2000). Classical or traditional morphometric that involves direct measurement of various characters that are then analysed using multivariate methods often successful at separating different species, even there is overlap characters between individuals (Yeap *et al.*, 2009; Lumley and Sperling, 2010).

PCA replaces many original characters with only a few most significant principal components (PCs) and does not require *priori* grouping. The most significant principal components (PCs) are selected based on combinations of closely correlated original characters which are referred to as patterns of covariation in size and shape in morphometric analysis (Wiley, 1981). DFA is a multivariate extension of ANOVA that can be used to observe the variation and covariation among studied groups (Fisher, 1936). In contrary with PCA, the DFA requires a *priori* grouping and it is used to classify individuals into the predetermined groups (Turan, 1999) based on the examined morphological variables.

DFA is a multivariate technique for describing a mathematical function that will distinguish among predefined groups of samples. As an eigenanalysis method, DFA has a strong connection to multiple regression and principal components analysis. In addition, DFA is the counterpart to ANOVA and MANOVA.

The chosen morphometric analysis or approaches depends on research questions that arised in the research such as, 1) can the group's member be discriminated? 2) If so, how well the groups are different? 3) Which variables are the most important in discriminating the groups? 4) Can group membership be predicted for 'unknown individual'?

One approach would be to test for differences among the species by performing a series of t-tests or ANOVAs, but this would be tedious, especially if there are many variables. Another approach might be a principal components analysis to see how the groups plot in multidimensional space, and this is often a good exploratory approach. DFA takes a similar approach to PCA, but DFA seeks a linear function that will maximize the differences among the groups. The function will show how well the species can be distinguished, as well as where the classification is robust and where it may fail (Davis, 2002).

2.6 Phylogenetic Studies in Termite by Using Mitochondrial Genetic Marker

Termite mitochondrial genomes are about 16 kb, of which 15 kb is phylogenetically informative that consist of 37 genes including two rRNA genes, 22 tRNA genes and 13 protein coding gene (Cameron and Whiting, 2007). MtDNA has been widely used in systematic and phylogenetic studies of termites (Liu and Beckenbach, 1992; Frati *et al.*, 1997; Miura *et al.*, 1998; Austin *et al.*, 2004; Ye *et al.*, 2004; Jenkins *et al.*, 2007). MtDNA is an ideal genetic marker because it is haploid that shares the genetic history of lineages (Wilson *et al.*, 1985) and reliable to infer the phylogenetic relationship among closely related

extant populations (Hillis *et al.*, 1996). As it is known to evolve more rapidly than nuclear gene, the mitochondrial gene resulted to have higher mutation rate which give significant variation in mtDNA sequence between species and within species, make it as a good marker for analysing species level relationships within a genus, and for population studies (Miura *et al.*, 2000).

The non-protein coding regions of mtDNA including rRNAs; 12S rRNA and 16S rRNA are frequently used to infer evolutionary relationship among species and phylogenetic studies. They are considered as a best target marker for phylogenetic relationship because it is universally present in all life forms that have a protein-synthesizing system and highly conserved (George *et al.*, 1977).

The protein coding genes of mitochondrial consist of 13 protein coding genes (Arif and Khan, 2009) are used in evolutionary study in families, genera and species. As compared to ribosomal gene, protein coding gene are often used at species level due to their faster evolutionary rates than rRNA gene (Arif and Khan, 2009). The COII gene is often used compared to other molecular marker of protein coding gene to evaluate population structures, gene flow, phylogeny, and taxonomy of termites as it has proved to be a good marker and has been extensively used in the past 10 years (Kambhampati *et al.*, 2000; Thompson *et al.*, 2000; Ohkuma *et al.*, 2004).

A first phylogenetic study on termites based on mitochondrial 16S rRNA gene had successfully solved a relationship of ten genera of termites (Kambhampati *et al.*, 1996) which contradict from the previous study based on morphological characters (Krishna & Weesner, 1970). They discovered that Mastotermitidae is the basal lineage among the five

termites (Figure 2.6) and did not form a sister group relationship to Kalotermitidae families as proposed by Krishna (1970).

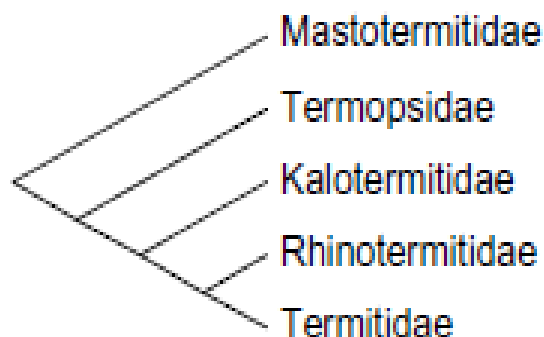


Figure 2.6: Family level phylogenies by Kambhampati *et al.* (1996)

This phylogenetic relationship of termites was improved over the years when Inward *et al.* (2007) conducted a first comprehensive study by using both molecular genes of COII, 12S and 28S and morphological phylogenetic analysis. They successfully revealed the phylogenies of family level in termite by using these both approaches and showed the Hodotermitidae, Kalotermitidae and Termitidae was monophyletic group, while Termopsidae and Rhinotermitidae were both paraphyletic.

The phylogenetic studies based on the molecular tools have become a great potential to study molecular ecology and population biology on the termite's pest. Rise of economic damages caused by termites have become a major concern worldwide to the development of pesticide to suppress this pest from infesting the wooden structure, crops and buildings. Subterranean termite, i.e. *Reticulitermes* spp. and *Coptotermes* spp., are the most destructive and economically important pest of wood and other cellulose product, thus make the taxonomic studies of these species becoming crucial to determine the current status of these

pest and their distribution throughout the world, while the correct identification of termite species was a key requirement for their control (Yeap *et al.*, 2009).

Moreover, several studies took into single genus or limited group of genera. The recent study by Yeap *et al.* (2009) in Malaysia had successfully used combination of partial sequence of mitochondrial genes of 12S, 16S and COII to assess the synonymies between *C. gestroi* and *C. heimi*, and concluded that *C. heimi* is a junior synonym of *C. gestroi*.

2.7 Microsatellite

Microsatellites or literally knows as variable number of tandem repeats (VNTRs) or short tandem repeats (STRs) are found the most in eukaryotic and lesser in prokaryotic genome. STR is a short sequences of DNA nucleotide repeated numerous times in DNA code, for example CACACACA and are categorized as mononucleotide, dinucleotide, trinucleotide, tetranucleotide, pentanucleotide and hexanucleotide repeats. As a name applied, the repeated stretches of STR are short range from one to six nucleotide in length with only two to ten base pairs long. Since the number of repeated sequences in given STR varies from individuals of the same species and highly unique to each individual, this variant can be useful for DNA fingerprinting.

Microsatellite are commonly located in non-coding region which does not usually coded for protein in most cases unlike gene and are therefore called “junk DNA”. They do not influence the phenotypic characteristics of the organism as long as it is located in non-coding region such as the 5'- untranslated regions (UTRs) or introns. However, if they located in a coding region, it may activate the gene function and may influenced the phenotypic characteristics through expression of the protein (Wang *et al.*, 2009). The

microsatellite is described as “mutator alleles” when it disturbs a normal genetic function with “mutation rate ranging from 10^6 to 10^2 ” (Schlotterer, 2000).

The “mutator alleles” of non-coding regions can accumulate mutations over the generation and give rise to variation of individuals. They are more likely to retain in population because it is not under selective pressure. Contrary to the “mutator alleles” which located in gene, they are usually harmful to an individual. Hence, the individual may select it against the population and typically do not survive to reproduce or pass the genetic material to the next generation. But, considering the microsatellite mutation does not influence the fitness of the individual, the “mutator alleles” can retain in the population as it is not selected against, and resulted to have many mutated microsatellite regions in the population.

The features of PCR driven, codominant, abundant (Wang *et al.*, 2009) economic, robust and reproducible (Weber and May 1989; Schuelke 2000) in microsatellite marker make it a powerful genetic marker compared to other marker system for insects and other eukaryotic species to do genome mapping, genetic diversity, marker-assisted diagnosis or selection, population and evolutionary studies (Behura, 2006).

Microsatellite can be targeted by designing a specific primer flanking with the repeat units. This can be done by PCR amplification from a small genomic of DNA, and the product of PCR will be observed on polyacrylamide gel electrophoresis (PAGE) to determine the differences in the length of microsatellite within and between population of species. The application of microsatellite as molecular genetic markers can improve our understanding on the basic features of subterranean termite colonies. Recent studies had applied microsatellite genetic tools to track the fate of colonies after insecticide treatment. This approach is proven to overcome all of the limitations by using fat soluble dyes for identifying

individual colonies in the field and delimiting colony foraging areas (Su and Scheffrahn, 1988).

Vargo (2003) conducted a study to determine the ability of hexaflumuron bait (Sentricon ®) to eliminate colonies around an apartment complex in Raleigh, North Carolina. To test the efficacy of the hexaflumuron bait for colony suppression, highly variable microsatellite markers were used to “fingerprint” and to track the fate of colonies. He managed to test the efficacy of the bait in discovering the successive declines in both “hit” monitoring station and by the number of colonies detected. All of the colonies (35 *R. flaviceps* and 1 *R. virginicus*) were suppressed and likely eliminated by baiting treatment during the course of study.

Another study by Parman and Vargo (2008) used microsatellite markers to determine the colony identity of termites present on 20 heavy infested residential properties by subterranean termite in central North Carolina. All the collected samples were genotyped at 10 microsatellite loci after the methods of Vargo (2000) and Dronnet *et al.* (2004). They identified 188 distinct colonies belonging to three species: *R. flaviceps* (89.9% of all colonies), *R. hageni* (7.4%) and *R. virginicus* (2.7%).

More recently, Thoms *et al.* (2009) had applied microsatellite DNA analysis to determine a specific colony of *Retitculitermes* spp. and *C. formosanus*. The genetic data in the study is important to know the efficacy of noviflumuron bait as trials to fulfil the newly revised state rules for termiticide efficacy in Florida before it is registered. They observed that the reappearance of termites in the bait stations was not due to bait failure, but rather due to reinvasion of new colonies to the successfully eliminated colony areas. The noviflumuron bait was successfully treat colonies in all cases and were not detected again.

After the first bait cycle, 4% showed new termite activity. The DNA analysis revealed that the present colonies were new and had not been previously baited.

2.8 Problem statement and rationale

Coptotermes is one of the most economically important subterranean termite genera but the taxonomy validity of many named *Coptotermes* spp. remains unclear. The great size variation and lack of distinguishing morphological features between *Coptotermes* spp. cause difficulties for rapid identification in the field. A similar study was done by Yeap *et al.* (2009), using a specimen from East Asia and Australia. In this study, we focus on the specimen from Sarawak, Malaysia with extension to peninsular Malaysia to reveal the phylogenetic relationship of four *Coptotermes* spp. in Borneo (Thapa, 1981). So far no study was reported previously particularly in Sarawak. The published termite identification book by Tho (1992) and Thapa (1981) should be revised especially on *Coptotermes* spp.. Thapa (1981) recorded that there were four species of *Coptotermes* in Sabah, *C. borneensis*, *C. curvignathus*, *C. kalshoveni* and *C. sepangensis*, while Tho (1992) recorded seven *Coptotermes* spp. in peninsular Malaysia, *C. curvignathus*, *C. kalshoveni*, *C. travians*, *C. havilandi*, *C. bentongensis* and *C. sinabangensis*. There were lack morphological descriptions on *C. bentongensis* and *C. sinabangensis*. *C. havilandi* was reported later to be a junior synonym to *C. gestroi* (Kirton and Brown, 2003). There were almost similar description and diagnostic features between *C. travians* and *C. borneensis* that might be synonym between these two species.

Therefore, it is crucial to evaluate the interspecific and intraspecific relationship of *Coptotermes* spp. in Malaysia. The analysis of morphological characters in subterranean social insect can be difficult since some features may not accurately be identified. The

molecular phylogeny reconstructed using only one gene somehow cannot provide a robust relationship between examined species (Barrett *et al.*, 1995). Thus, a combination of both molecular and morphological data from the same material will provide a much better basis to resolve the taxonomy status of *Coptotermes* (Yeap *et al.*, 2009).

The evaluation of termites baiting efficacy at colony level especially on important pest of *Coptotermes* is also important and require more sensitive molecular marker such as microsatellite marker. In the study, we isolated and developed a polymorphic microsatellite marker of *C. curvignathus* which had become a serious threat to crops such as oil palm plantation and rubber plantation in Sarawak. No studies on development of polymorphic microsatellite markers for *C. curvignathus* was reported previously in Sarawak.

The findings of morphometric and molecular approaches will have significant interest in worldwide studies of *Coptotermes*, especially in Malaysian Borneo. The morphometric outcomes could be useful for pre-identification of species as an alternative to molecular studies which required less technical and material costs, yet molecular studies still essential for showing the evolutionary relationships of genetically related species.

2.9 Objectives and hypothesis

The aim of this project is to carry out molecular analyses and compare these findings with morphological assessment of the soldier caste in genus *Coptotermes* to resolve the identification problem by morphological diagnostic features alone. This study will cover the following objectives:

- i. To identify diagnostic morphological character(s) for species identification and classification of *Coptotermes* spp.

- ii. To elucidate the phylogenetic relationships of *Coptotermes* spp. based on morphology and molecular analysis using three mitochondrial genes (12S, 16S and COII).
- iii. To isolate and characterize a set of novel microsatellite marker from *Coptotermes curvignathus*.

It is hypothesized that the classification of morphometric data will be congruent with the phylogenetic reconstruction of the molecular DNA sequencing data. These two different approaches would be helpful to confirm the validity of the identified species and reveal the relationships among and between the examined species of *Coptotermes*, particularly in Malaysia. Meanwhile, a polymorphic microsatellite markers from *C. curvignathus* were developed and selected for an analysis for allele diversity and polymorphism screening. It is hypothesized that all developed polymorphic microsatellite markers did not deviate from Hardy-Weinberg equilibrium, no linkage disequilibrium, no presence of null alleles and no large allelic dropout which make a good as genetic marker to study the population structure of *C. curvignathus*.

CHAPTER 3

MORPHOMETRIC ANALYSES OF *Coptotermes* SPP. (BLATTODEA: RHINOTERMITIDAE) IN SARAWAK, MALAYSIA

3.1 Introduction

The termite genus of *Coptotermes* from the family of Rhinotermitidae is an invasive pest in urban and suburban areas. The genus is very destructive to wood and wooden material in the world (Takematsu *et al.*, 2000) and has wide distribution throughout Asia, Australia, Africa and the New World. In Peninsular Malaysia, widespread infestation of the termite was reported on rubber trees (*Hevea brasiliensis*) before it was considered as a serious pest in the early 1900s. Today, the species is highly regarded as a destructive pest as it is highly destructive to buildings and its furnishings. It is also an agricultural nuisance in places such as oil palm plantations, garden landscapes and dwellings (Lee, 2002) and brings damage to any cellulose materials such as books, papers, blankets, window frames and furniture (Chao *et al.*, 1989).

Termite taxonomy especially *Coptotermes* spp. is challenging and problematic. The inherent difficulties in *Coptotermes* taxonomy include lack of distinguishing features, high degree of morphological variation among intraspecific populations and difficulty in matching soldiers and alates of the same species (Kirton, 2005). The overdependence on the soldier morphology presenting a major limitation for single species recognition because of the intraspecific variation in morphological characters in soldiers (Emerson, 1971; Husseneder & Grace, 2001) and lack of distinguishing diagnostic features among the species of *Coptotermes* (Li *et al.*, 2010) but yet the identification based on soldier morphology considered as a best option so far compared to alates due to seasonal occurrences that prevent

simultaneous collection and description of this caste (Jones *et al.*, 2005; Yang & Li, 2012).

Recently, the identification of the species is not solely depending on the morphology diagnostic features. Normal classical identification procedure as practised by experienced zoologist in the field, may still encounter some misidentification problem (Sazali *et al.*, 2012). Therefore, by using statistical analysis which combined with morphological data, rapid assessment and evaluation of the species can be done in more reliable and convincing way.

The morphometric analyses of *Coptotermes* spp. aim to assess the morphological variation between species of the genus particularly in soldier caste and therefore would aid in identifying diagnostic morphological character(s) for species identification and classification.

3.2 Materials and Method

3.2.1 Collection, Identification and Measurements of Samples

Termites were collected from 21 sites across Sarawak (Figure 3.1) from September 2015 through December 2016 (Table 3.1) to represent geographical variation and these samples also were used in Chapter Three. Soldiers (one species found from one section was considered as one colony) were collected following a standard method established by Jones *et al.* (2005), with few modifications. The 100- m belt transect consisted of 20 plots of 5 × 2 m² that were sampled sequentially. Each plot was sampled for 30 min by two workers and collection of samples were made in the following microsites: forest litter, dead wood, tree trunks and buttress roots, mounds, soil surface to 5 cm depth (10–15 points) and runways to 2 m height in the vegetation. The samples were preserved in 70% alcohol and brought to the laboratory for identification. Additional samples were provided by cooperating NLC

General Pest Company based in Kuching, Sarawak. All specimens have been stored in the UNIMAS Insect Reference Collection, Faculty of Resource Science and Technology (FRST), Universiti Malaysia Sarawak (UNIMAS).

The identification of termites to the species level was done using taxonomic keys published by Thapa (1981), Tho (1992), Ahmad (1965) and Krishna *et al.* (2013). In this study, a minimum of five individual soldiers were collected for each colony. Eight colonies of *C. curvignathus* and *C. sepangensis* respectively, two colonies of *C. kalshoveni* and three colonies of *C. travians* were successfully collected in this study as shown in Table 3.1. The photographs of the soldiers were taken using a Motic SMZ-16B Series stereomicroscope attached to a Moticom 2000 camera and then the image sequences were combined with Helicon Focus 6 software. Calibrated measurements were taken by using Motic Image Plus 2.0 software.

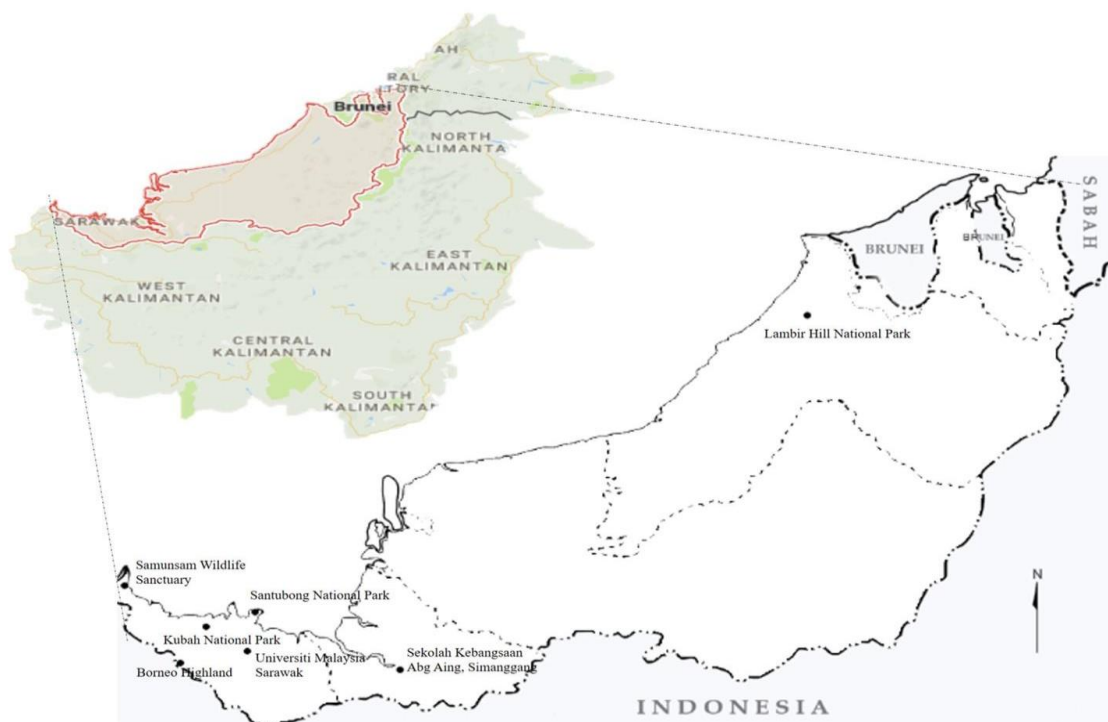


Figure 3.1: Sample collection in Sarawak. Source: Modified from Australian National University CartoGIS CAP00-120

Table 3.1: Samples collected and used in this study.

Sample Id#	Species Name	Collection sites	Number of soldiers collected
Samples from this study			
CC02UNS	<i>C. curvignathus</i> (Holmgren)	Secondary forest, UNIMAS	10
CC05UNS	<i>C. curvignathus</i> (Holmgren)	Secondary forest, UNIMAS	10
CC04SWS	<i>C. curvignathus</i> (Holmgren)	Primary forest, Samunsam Wildlife Sanctuary	10
CC01TB	<i>C. curvignathus</i> (Holmgren)	Secondary forest, Tanjung Bijat, Sri Aman	10
CC01SKAG	<i>C. curvignathus</i> (Holmgren)	Sekolah Kebangsaan Abg Aing, Sri Aman	10
CC02SKAG	<i>C. curvignathus</i> (Holmgren)	Sekolah Kebangsaan Abg Aing, Sri Aman	10
CC01BHR	<i>C. curvignathus</i> (Holmgren)	Borneo Highland, Padawan	10
CC02BHR	<i>C. curvignathus</i> (Holmgren)	Borneo Highland, Padawan	10
CS01UNS	<i>C. sepangensis</i> (Krishna)	Secondary forest, UNIMAS	10
CS02KNP	<i>C. sepangensis</i> (Krishna)	Primary forest, Kubah National Park	7
CS01BHW	<i>C. sepangensis</i> (Krishna)	Borneo Highland, Padawan	10
CS02BHW	<i>C. sepangensis</i> (Krishna)	Borneo Highland, Padawan	10
CS03BHW	<i>C. sepangensis</i> (Krishna)	Borneo Highland, Padawan	10
CS03SWS	<i>C. sepangensis</i> (Krishna)	Primary forest, Samunsam Wildlife Sanctuary	10
CS05SWS	<i>C. sepangensis</i> (Krishna)	Primary forest, Samunsam Wildlife Sanctuary	10
CK03UNS	<i>C. kalshoveni</i> (Kemner)	Secondary forest, UNIMAS	10
CK04UNS	<i>C. kalshoveni</i> (Kemner)	Secondary forest, UNIMAS	10
CT01SNP	<i>C. travians</i> (Haviland)	Primary forest, Santubong National Park	10
CT01KNP	<i>C. travians</i> (Haviland)	Primary forest, Kubah National Park	6
CT01LHNP	<i>C. travians</i> (Haviland)	Primary forest, Lambir Hill National Park, Miri	10
Total			193

3.2.2 Morphometric Analysis

A total of 80 soldier's individuals of *C. curvignathus*, 67 individuals of *C. sepangensis*, 20 individuals of *C. kalshoveni*, and 26 individuals of *C. travians* were examined and measured. A minimum of 17 external characters were measured for each specimen (Figure 3.2). The following 17 morphometric characters were measured : (1) total length (TL) (2) total length without head (TLH) (3) length of head at base of mandibles (TLM) (4) length to fontanelle (LF) (5) maximum width of head (WH) (6) width of head at

base of mandibles (WHM) (7) length of antennae, segment 1 (AL1) (8) width of antennae, segment 1 (WA1) (9) length of antennae, segment 2 (AL2) (10) width of antennae, segment 2 (AW2) (11) length of labrum (LLb) (12) width of labrum (WLb) (13) width of pronotum (WPr) (14) length of pronotum (LPr) (15) maximum width of postmentum (MWPt) (16) minimum width of postmentum (MnWPt) (17) length of postmentum (LPt).

The indices for body index (BI) and head index (HI) were calculated: (1) Body index (WH/TL) (2) head index (WH/WHM) (3) pronotum index (WPr/LPr) (4) postmentum index (MxWPt/MnWPt).

These data were subjected to ANOVA, and means were separated by Tukey's HSD (Honest Significant Difference) using STATISTIX Version 10.0. Next, morphometric data were $\log_{10}X + 1$ transformed using Statistical Package for Social Sciences (SPSS) version 21 (SPSS Inc., 2006) to reduce the heterogeneity in variance (Smith *et al.*, 2009) and then the transformed data were subjected to DFA. This procedure identifies which variables that help in group differentiation (McGarigal *et al.*, 2000).

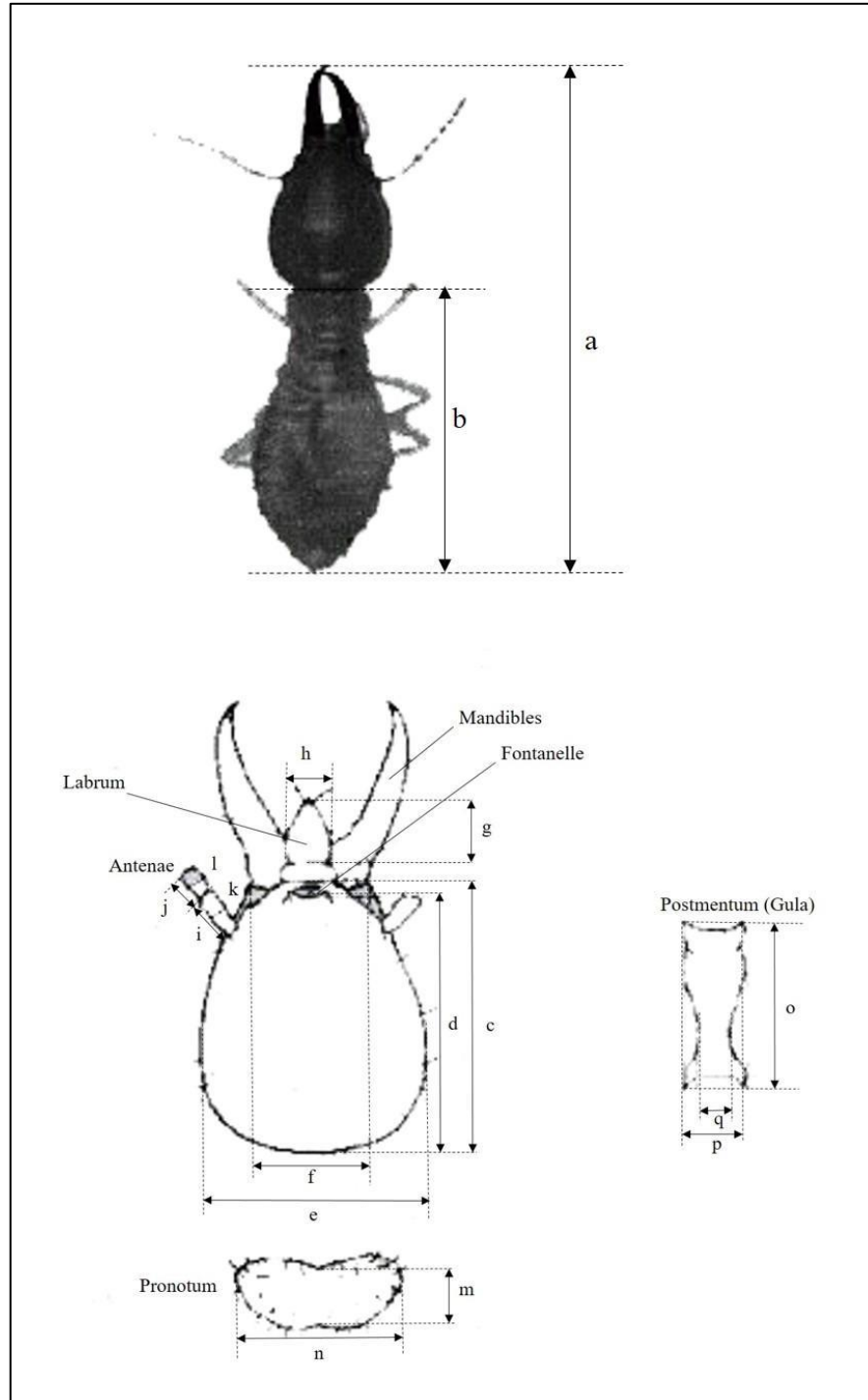


Figure 3.2: The external morphological characters for *Coptotermes* identification. Note: (a) total length (TL) (b) total length without head (TLH) (c) length of head at base of mandibles (TLM) (d) length to fontanelle (LF) (e) maximum width of head (WH) (f) width of head at base of mandibles (WHM) (g) length of labrum (LLb) (h) width of labrum (WLb) (i) length of antennae, segment 1 (AL1) (j) length of antennae, segment 2 (AL2) (k) width of antennae, segment 1 (WA1) (l) width of antennae, segment 2 (WA2) (m) length of pronotum (LPr) (n) width of pronotum (WPr) (o) length of postmentum (LPt) (p) maximum width of postmentum (MxWPt) (q) minimum width of postmentum (MnWPt)

3.3 Results

3.3.1 Diagnostic Features Examination

Comparison was made between the *Coptotermes* spp. based on the diagnostic features such as head capsule (Figure 3.3), shape of the mandibles (Figure 3.4), shape of pronotum (Figure 3.5), shape of postmentum (Figure 3.6) and their fontanelle (Figure 3.7) under various magnification.

Head capsule: As shown in Figure 3.3, the head capsule of *C. curvignathus* was observed to have very rounded to broadly rounded lateral margin, and most of the colour of collected specimens were generally yellow. *C. curvignathus* was noted to be distinctively different from other three *Coptotermes* spp. based on the head capsule. *C. sepangensis* and *C. kalshoveni* was noted to have pear-shaped head capsule and both of the species were difficult to be distinguished based on the head capsule shaped alone. *C. travians* was differed from *C. sepangensis* and *C. kalshoveni* where the head of soldiers is ovoid, much longer than wide.

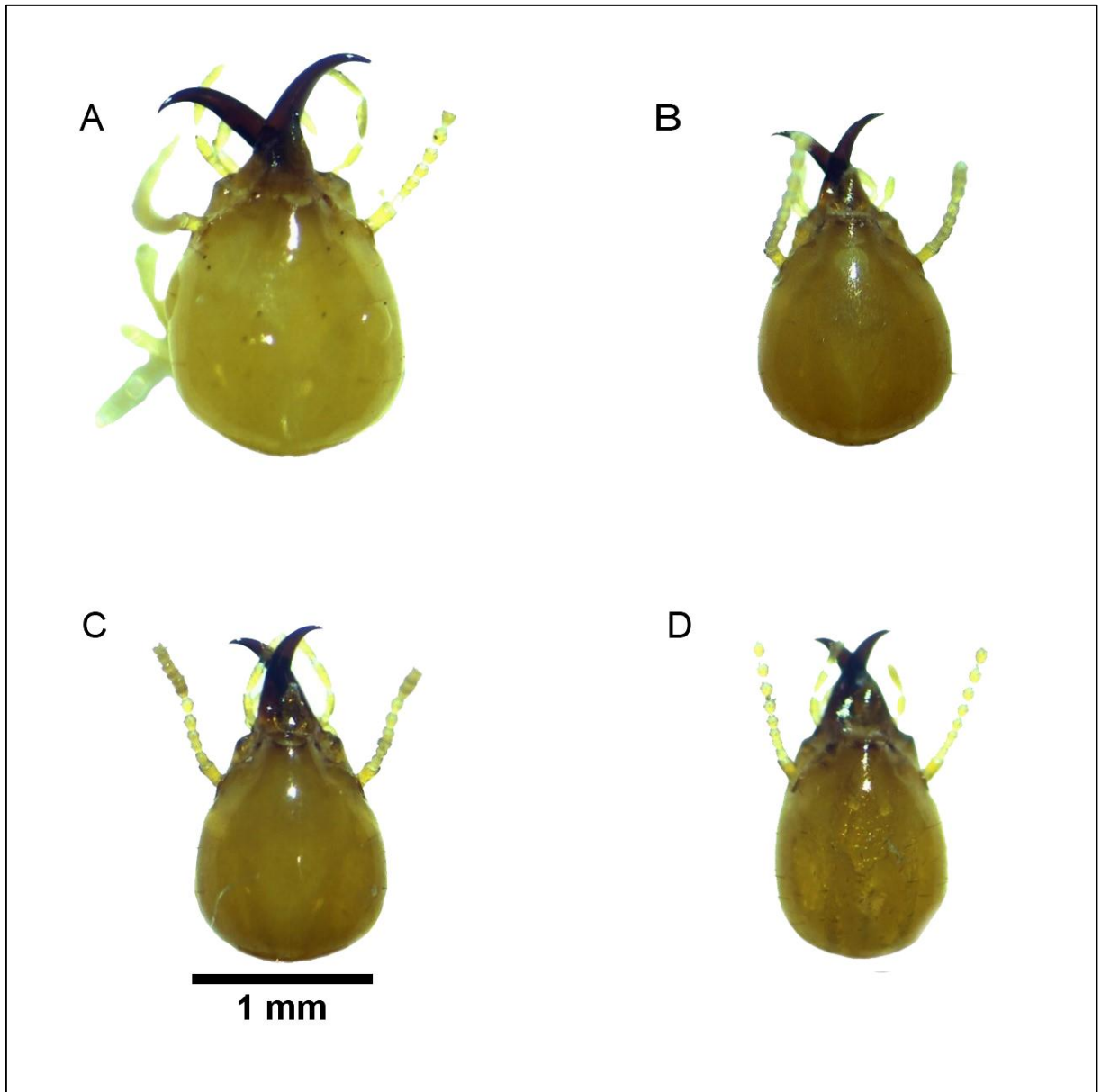


Figure 3.3: Dorsal view of soldier head of *Coptotermes* under 30x magnification. **A:** *C. curvignathus*; **B:** *C. sepangensis*; **C:** *C. kalshoveni*; **D:** *C. travians*. Scale bar same for each image (=1 mm).

Shape of mandibles: As shown in Figure 3.4, *C. curvignathus* and *C. sepangensis* have strongly curved, slender mandibles and the curvature begins at the middle of its length. The soldier's mandibles of *C. curvignathus* are distinctive in being large compared to the other mandibles of *Coptotermes* spp.. *C. kalshoveni* and *C. travians* have less curved mandibles and the curvature begins at the anterior thirds of its length.

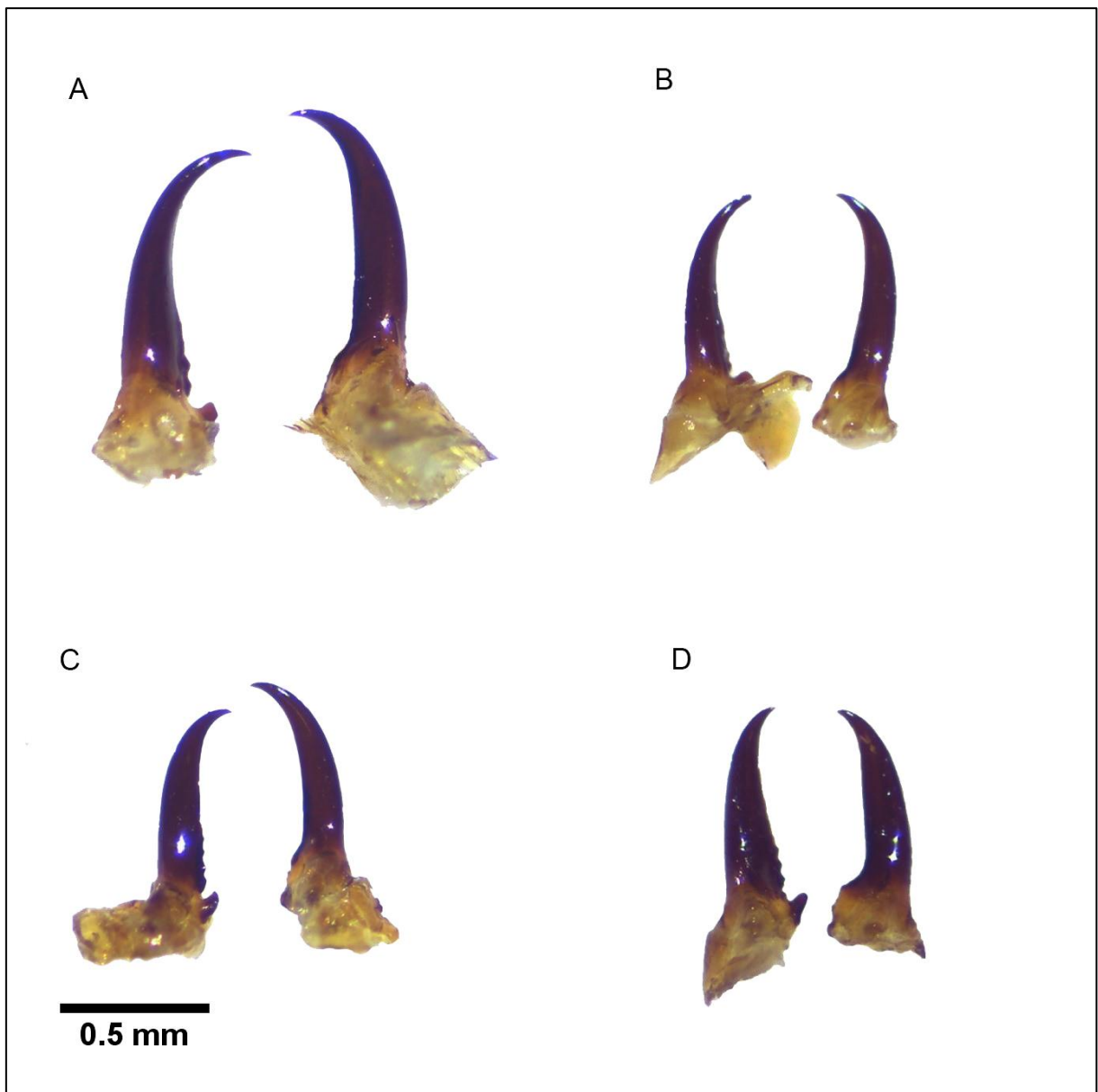


Figure 3.4: The shape of mandibles of *Coptotermes* under 50x magnification. **A:** *C. curvignathus*; **B:** *C. sepangensis*; **C:** *C. kalshoveni*; **D:** *C. travians*. Scale bar same for each image (=0.5 mm).

Pronotum: As shown in Figure 3.5, it was difficult to distinguish between *C. kalshoveni*, *C. sepangensis* and *C. travians* based on the pronotum shape. The pronotum shape between *C. travians* and *C. kalshoveni* almost similar; lateral margin weakly convex and anterolateral corners narrowly rounded, compared to *C. sepangensis* which have strongly convex lateral margin and appeared in small pronotum size.

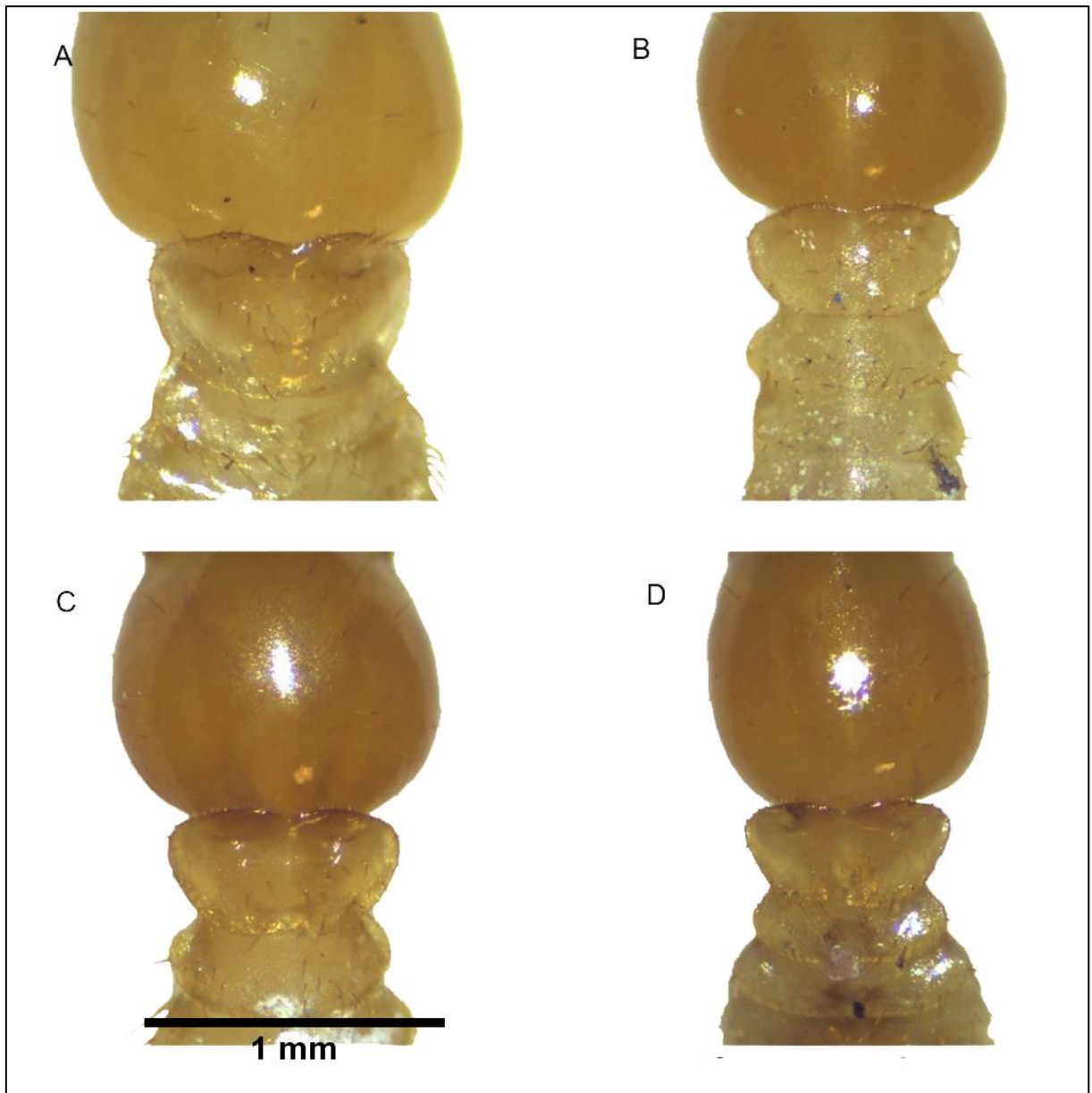


Figure 3.5: Dorsal view of pronotum of *Coptotermes* under 50x magnification. **A:** *C. curvignathus*; **B:** *C. sepangensis*; **C:** *C. kalshoveni*; **D:** *C. travians*. Scale bar same for each image (=1 mm).

Postmentum: As shown in Figure 3.6, *C. travians* was noted to have different shape postmentum compared to other *Coptotermes* spp.; long postmentum with more than twice as long as its maximum width; posterior waist slightly less wide compared to anterior waist. *C. kalshoveni*, *C. sepangensis* and *C. curvignathus* was observed to have almost similar shape of pronotum yet *C. curvignathus* was the largest compared to other two *Coptotermes* spp..

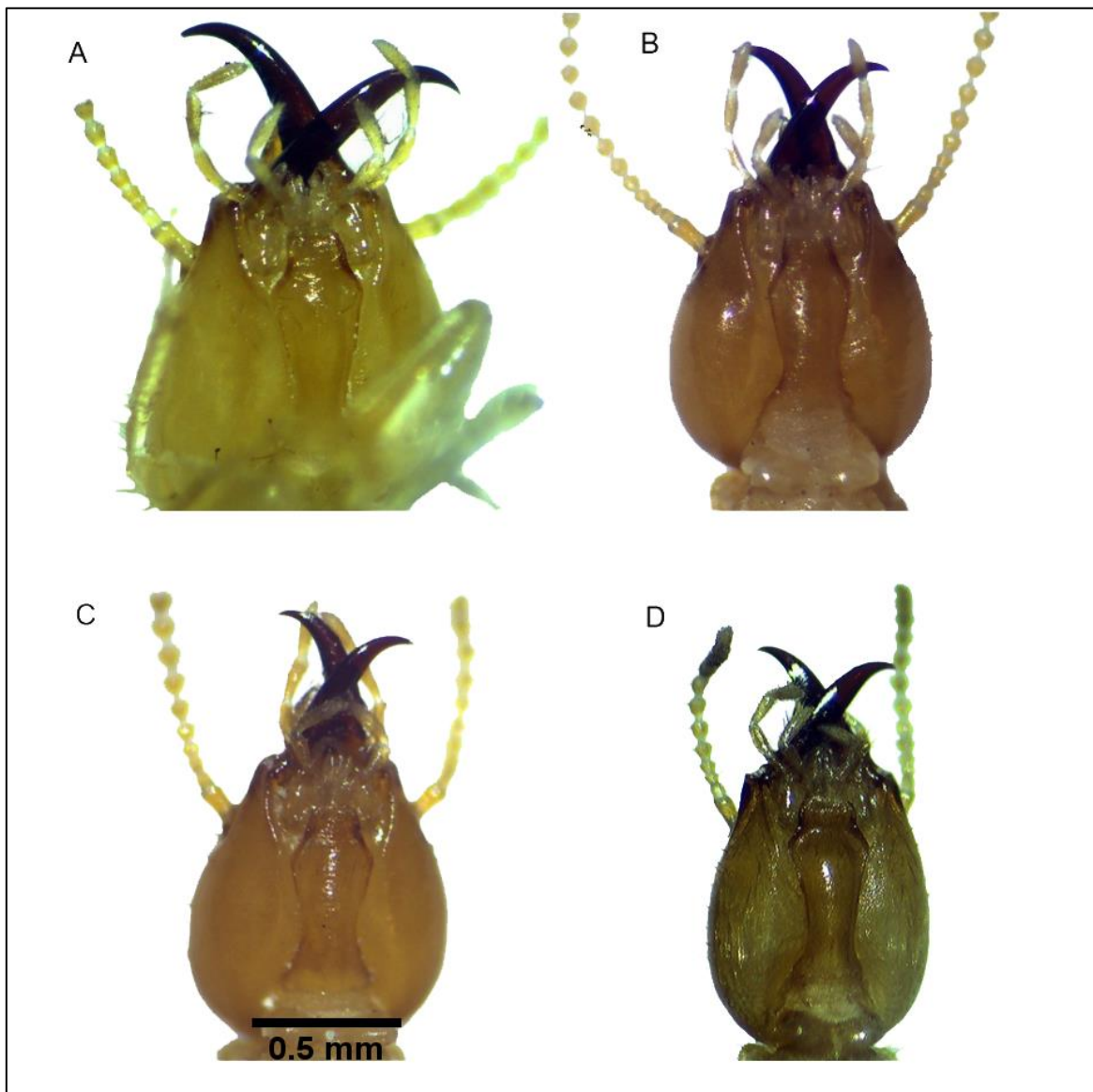


Figure 3.6: Ventral view of postmentum of *Coptotermes* under 40x magnification. **A:** *C. curvignathus*; **B:** *C. sepangensis*; **C:** *C. kalshoveni*; **D:** *C. travians*. Scale bar same for each image (=0.5 mm).

Fontanelle: As shown in Figure 3.7, *C. travians* observed to have distinctively small in fontanelle size compared to other three *Coptotermes* spp. with ovoid shape. *C. curviganthus*, *C. sepangensis* and *C. kalshoveni* have rounded fontanelle shape yet *C. curviganthus* have the largest size of fontanelle compared to other *Coptotermes* spp. analysed in this study.

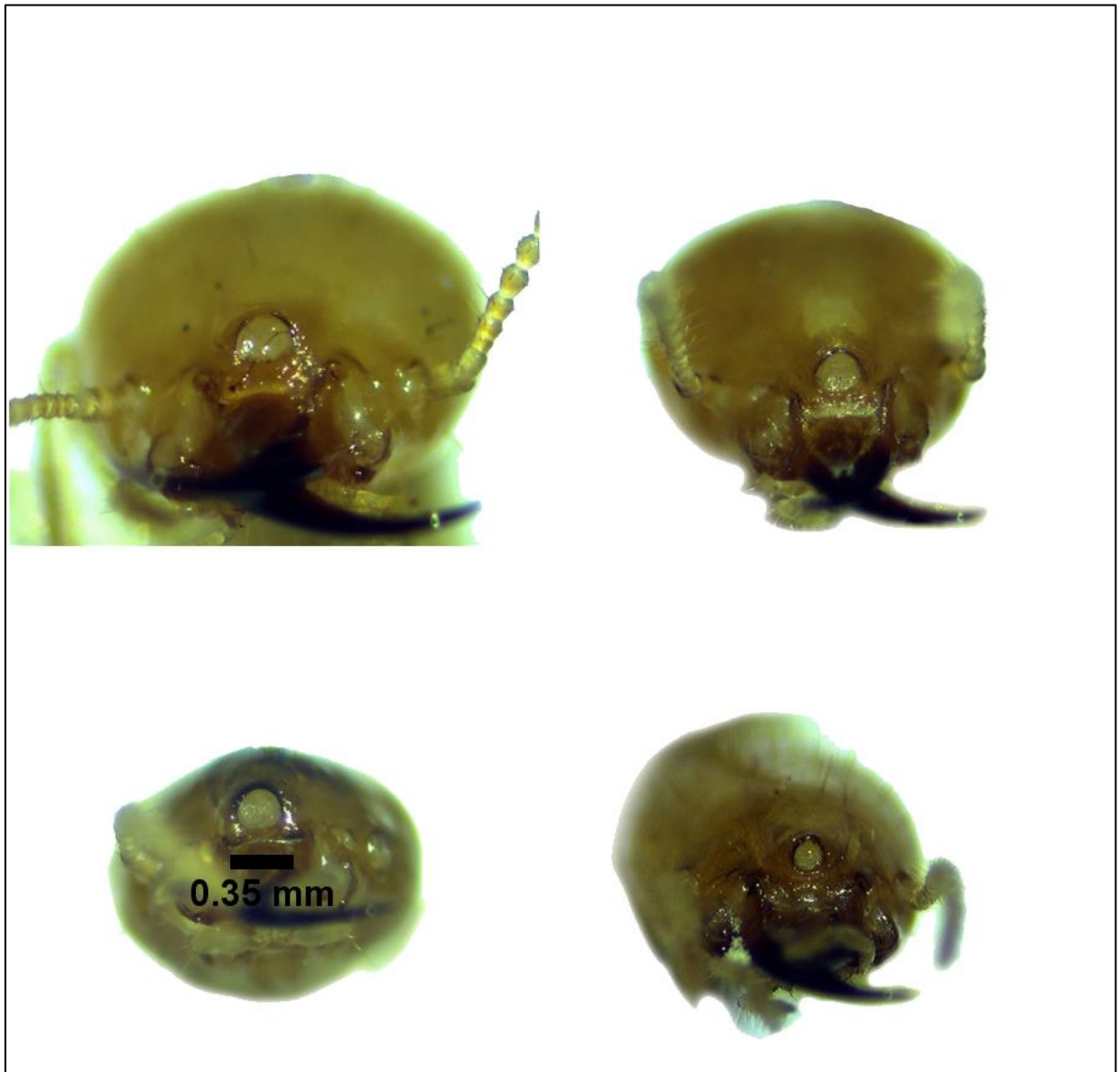


Figure 3.7: Anterior view of fontanelle of *Coptotermes* under 50x magnification. **A:** *C. curviganthus*; **B:** *C. sepangensis*; **C:** *C. kalshoveni*; **D:** *C. travians*. Scale bar same for each image (=0.35 mm).

Morphologically, it is difficult to distinguish soldiers of *C. kalshoveni*, *C. sepangensis* and *C. travians*. Soldiers of *C. curvignathus* are readily distinguishable by relatively wide head, most incurved mandibles and sharp mandibles. The ANOVA F-statistics indicated the presence of significant difference between the *Coptotermes* spp. for all the characters except for pronotum index. Tukey-Kramer testing of individual characteristics provided groupings that indicate significant pairwise population differences. The means, standard deviation and range (minimum and maximum) are summarised in Table 3.2.

The current measurement data were compared with the several measurement character from Thapa (1981), Tho (1992), Ahmad (1965), locotype and holotype (Table 3.3 and Table 3.4). The current measurement of *C. curvignathus* for mean total length of head at base of mandibles (TLM) and mean length of pronotum (LPr) fell within the mean measurement from locotype (mean for TLM=1.48 and LPr=0.49). In addition, the current measurement character for total length of head at base of mandibles (TLM), width of head (WH), width of pronotum (WPr) and length of pronotum (LPr) of *C. sepangensis* also closed to measurement from holotype and Thapa (1981). However, no measurement characters for *C. travians* were closed to measurement from Thapa (1981) and Tho (1992) which indicates a size variation had occurred for *C. travians*'s soldier specimens.

Table 3.2: Descriptive statistics of studied population of *Coptotermes* spp.

Species	<i>C. sepangensis</i> (n=67)			<i>C. curvignathus</i> (n=80)			<i>C. travians</i> (n=26)			<i>C. kalshoveni</i> (n=20)		
Char.	M±SD	Min	Max	M±SD	Min	Max	M±SD	Min	Max	M±SD	Min	Max
TL	4.02±0.55a	2.71	5.27	5.11±0.85b	2.91	6.48	4.76±0.79b	3.63	6.07	4.18±0.45a	3.61	5.35
TLH	2.36±0.39a	1.54	3.32	2.99±0.57b	1.57	4.1	2.72±0.59b	1.89	3.69	2.67±0.44ab	2.05	3.61
TLM	1.17±0.13a	0.91	1.56	1.46±0.18b	0.79	1.7	1.39±0.22b	1.09	1.96	1.06±0.12c	0.84	1.3
LF	1.17±0.10a	0.98	1.43	1.34±0.12b	1.01	1.56	1.36±0.21b	1.11	1.89	1.11±0.07a	0.96	1.21
WH	1.08±0.10ac	0.93	1.33	1.33±0.15b	0.93	1.53	1.13±0.17c	0.91	1.58	1.00±0.06a	0.93	1.11
WHM	0.39±0.10a	0.22	0.61	0.54±0.12b	0.25	0.81	0.56±0.10b	0.37	0.82	0.32±0.06a	0.22	0.42
AL1	0.15±0.03ac	0.07	0.22	0.18±0.04b	0.11	0.27	0.17±0.02ab	0.13	0.22	0.13±0.02c	0.1	0.18
WA1	0.08±0.01ac	0.05	0.12	0.10±0.01b	0.07	0.14	0.09±0.03ab	0.06	0.23	0.08±0.01c	0.06	0.09
AL2	0.07±0.01a	0.05	0.12	0.09±0.02b	0.05	0.14	0.08±0.02b	0.06	0.11	0.07±0.01a	0.04	0.09
WA2	0.06±0.01a	0.04	0.09	0.07±0.01b	0.05	0.1	0.07±0.02b	0.05	0.12	0.06±0.004a	0.05	0.06
LLb	0.22±0.04a	0.1	0.31	0.35±0.09b	0.1	0.5	0.29±0.05c	0.22	0.41	0.19±0.06a	0.11	0.33
WLb	0.22±0.04a	0.1	0.36	0.30±0.06b	0.14	0.48	0.27±0.10bc	0.08	0.6	0.22±0.07ac	0.12	0.33
MxWPt	0.33±0.03a	0.28	0.39	0.40±0.05b	0.28	0.46	0.40±0.05b	0.32	0.47	0.31±0.01a	0.29	0.34
MnWPt	0.23±0.03a	0.16	0.3	0.24±0.03b	0.19	0.3	0.22±0.02a	0.19	0.26	0.21±0.01a	0.19	0.25
LPt	0.67±0.11a	0.41	0.85	0.91±0.19b	0.39	1.5	0.88±0.13b	0.65	1.18	0.62±0.08a	0.48	0.75
LPr	0.37±0.04a	0.29	0.46	0.50±0.13b	0.1	1.09	0.36±0.05c	0.25	0.46	0.34±0.03a	0.3	0.41
WPr	0.73±0.08a	0.46	0.91	0.93±0.15b	0.6	1.73	0.81±0.11a	0.65	0.95	0.69±0.07a	0.62	0.91
BI	0.27±0.03a	0.23	0.37	0.26±0.02a	0.21	0.34	0.24±0.03b	0.21	0.37	0.24±0.02b	0.2	0.27
HI	2.96±0.73a	1.85	5.32	2.57±0.46b	1.84	4.24	2.02±0.22c	1.65	2.46	3.20±0.49a	2.44	4.5
PI	1.96±1.32a	1.44	2.29	2.00±0.92a	0.55	9.8	2.24±0.18a	1.94	2.64	2.04±0.13a	1.79	2.33
GI	1.45±0.10a	1.07	1.75	1.68±0.13b	1.34	2.0	1.83±0.12c	1.60	2.05	1.48±0.10a	1.28	1.63

Char.: Character; Means (M), within rows, followed by the different letter (a, b, c, d) are significantly different ($P < 0.05$; Turkey's HSD). TL: Total length; TLH= Total length without head; TLM= Length of head at base of mandibles; LF= Length to fontanelle; WH= Maximum width of head; WHM= Width of head at base of mandibles; LLb= Length of labrum; WLb= Width of labrum; AL1= Length of antennae, segment 1; AL2= Length of antennae, segment 2; WA1= Width of antennae, segment 1; WA2= Width of antennae, segment 2; LPr= Length of pronotum; WPr= Width of pronotum; LPt= Length of postmentum; MxWPt= Maximum width of postmentum; MnWPt= Minimum width of postmentum; BI= Body index; HI= Head index; PI= Pronotum index; GI= Postmentum index

Table 3.3: Measurements of *C. curvignathus* and *C. sepangensis* from type and previous studies.

Measure	<i>C. curvignathus</i>				<i>C. sepangensis</i>		
(in mm) of:	(locotype)	(Thapa, 1981)	(Tho, 1992)	(Ahmad, 1965)	(holotype)	(Thapa, 1981)	(Tho, 1992)
Length of head to side base of mandibles	1.47-1.50 (1.48*)	1.45-1.65 (1.55*)	1.51-1.85 (1.68*)	1.65-1.76 (1.69*)	1.13-1.16 (1.14*)	1.12-1.25 (1.16*)	1.08-1.31 (1.23*)
Maximum width of head	1.27-1.30 (1.29*)	1.25-1.38 (1.33*)	1.28-1.57 (1.34*)	1.40-1.63 (1.53*)	0.98-1.01 (0.99*)	1.00-1.07 (1.03*)	0.85-1.08 (0.93*)
Width of head at side base of mandibles	0.76-0.77 (0.77*)	0.72-0.83 (0.78*)	0.65-0.82 (0.71*)	0.86-0.90 (0.87*)	-	0.52-0.60 (0.56*)	0.54-0.65 (0.61*)
Width of pronotum	0.85-0.91 (0.99*)	0.87-0.98 (0.93*)	-	1.04-1.18 (1.12*)	0.71-0.75 (0.72*)	0.68-0.75 (0.70*)	-
Length of pronotum	0.47-0.50 (0.49*)	0.52-0.55 (0.54*)	-	0.54-0.63 (0.57*)	0.38-0.41 (0.38*)	0.37-0.42 (0.39*)	-

Note: * Mean for each measurement

Table 3.4: Measurements of *C. kalshoveni* and *C. travians* from previous studies.

Measure	<i>C. kalshoveni</i>			<i>C. travians</i>	
(in mm) of:	(Thapa, 1981)	(Tho, 1992)	(Ahmad, 1965)	(Thapa, 1981)	(Tho, 1992)
Length of head to side base of mandibles	0.95-1.05 (1.01*)	1.02-1.20 (1.14*)	1.00-1.15 (1.07*)	1.25-1.32 (1.29*)	1.25-1.34 (1.29*)
Maximum width of head	0.85-0.93 (0.90*)	0.80-1.02 (0.91*)	0.90-0.99 (0.93*)	0.97-1.03 (1.01*)	0.68-0.77 (0.72*)
Width of head at side base of mandibles	0.47-0.51 (0.48*)	0.45-0.50 (0.45*)	0.46-0.52 (0.49*)	0.65-0.67 (0.65*)	0.63-0.68 (0.65*)
Width of pronotum	0.58-0.65 (0.60*)	-	0.61-0.72 (0.65*)	0.72-0.81 (0.77*)	-
Length of pronotum	0.31-0.37 (0.33*)	-	0.27-0.34 (0.30*)	0.37-0.42 (0.40)	-

Note: * Mean for each measurement

3.3.2 Morphometric Analysis

Discriminant function analysis using morphometric identification formed four groups as shown in Figure 3.8. *C. travians* and *C. curvignathus* were clearly distinguished from *C. sepangensis* and *C. kalshoveni*. However, an overlapping characters were notable between a group of *C. sepangensis* and *C. kalshoveni*. In the DFA analysis, three significant

functions were determined; the Function 1, 2, and 3 explained with 56.7%, 36.3% and 7.0% of the variance respectively (Table 3.5). Through Function 1 and 2, it can be observed that about 56.7% had been explained for the variability in the 17 character measurement and 93% had been explained in Function 2. The Function 1 has higher variability of characters in the analysis. The Wilk's lambda statistic (Table 3.6) for the tests of Function 1 through 3 function (Wilk's lambda=0.06), Function 2 through 3 function (Wilk's lambda=0.243) and Function 3 (Wilk's lambda=0.724) have a probability of $p=0.000$ respectively.

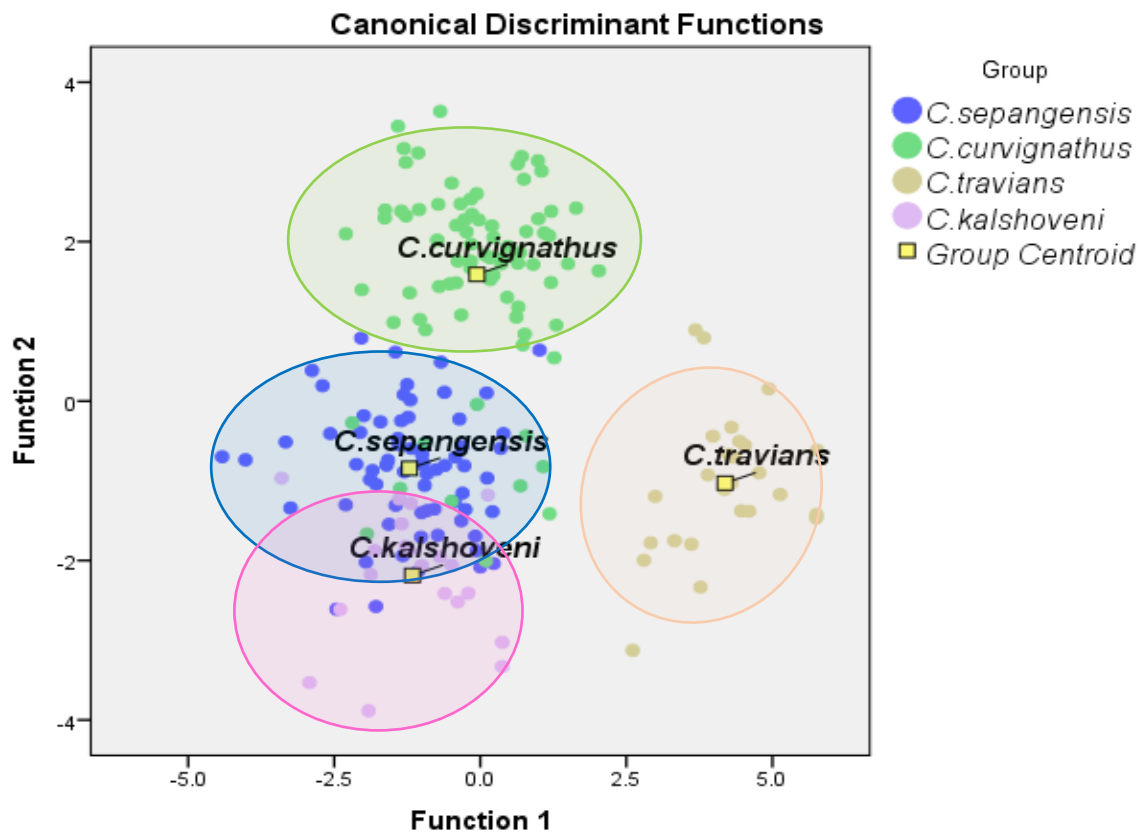


Figure 3.8: CVA plot of Functions 1 and 2 of four selected *Coptotermes*.

Table 3.5: Eigenvalues for DFA of four selected *Coptotermes*.

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	3.078 ^a	56.7	56.7	.869
2	1.973 ^a	36.3	93.0	.815
3	.382 ^a	7.0	100.0	.526

Note: (a) First 3 canonical discriminant functions were used in the analysis.

Table 3.6: Wilks' Lambda for DFA of four selected *Coptotermes*.

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 3	.060	511.602	51	.000
2 through 3	.243	256.467	32	.000
3	.724	58.718	15	.000

From Table 3.7, the highest character loadings observed in Function 1 is the maximum width of postmentum (MxPt), while character for Function 2 is the width of head (WH) and Function 3 is the total length to the mandible (TLM). It was suggested that MxPt (1.677) and WH (-2.261) as showed in Table 3.5 played a prominent role in separating *Coptotermes* spp. through Function 1 (56.7% of trace). Meanwhile, in Function 2 (36.3% of trace), a separation of *Coptotermes* spp. was driven primarily by opposing contributions of WH (1.563) and TLH (-0.866).

Table 3.7: Standardised Canonical Discriminant Function coefficients of four selected *Coptotermes*.

Character	Function		
	1	2	3
TL	.524	.200	.095
TLH	-.619	-.866	-1.114
TLM	.662	-.207	.841*
LF	.986	-.744	.306
WH	-2.261	1.563*	-.295
WHM	.386	-.124	.695
AL1	.052	.175	.412
AW1	-.007	-.083	.142
AL2	.085	.163	-.266
AW2	.077	.029	-.169
WLb	-.147	.106	.021
LLb	.040	.419	-.049
MnWPt	-.835	-.543	.579
MxWPt	1.677*	.123	-.802
LPt	-.182	-.037	-.432
WPr	-.153	.153	.098
LPr	-.081	.375	.017

Note: * Diagnostic character in each function. TL: Total length; TLH: Total length without head; TLM: Length of head at base of mandibles; LF: Length to fontanelle; WH: Maximum width of head; WHM: Width of head at base of mandibles; LLb: Length of labrum; WLb: Width of labrum; AL1: Length of antennae, segment 1; AL2: Length of antennae, segment 2; WA1: Width of antennae, segment 1; WA2: Width of antennae, segment 2; LPr: Length of pronotum; WPr: Width of pronotum; LPt: Length of postmentum; MxWPt: Maximum width of postmentum; MnWPt: Minimum width of postmentum

3.4 Discussion

C. curvignathus (Holmgren) is a larger species compared to other three species of *C. sepangensis*, *C. kalshoveni* and *C. travians*. In addition, *C. curvignathus* can be easily distinguished from others by having the most incurved mandible, slender and saber shaped. The notable diagnostic features to differentiate *C. curvignathus* with other *Coptotermes* spp. besides the mandibles are the large and oval shaped head capsule, pronotum, postmentum and large fontanelle size. Similarly, *C. sepangensis* (Krishna) has mandible with saber shaped and incurved mandible but the total body is much smaller than *C. curvignathus*. When comparison was made among *C. travians*, *C. kalshoveni* and *C. sepangensis*, it was observed that *C. sepangensis* closely resembled *C. kalshoveni* in most of the morphological

characters evaluated in this study. However, among *C. travians*, *C. sepangensis* and *C. kalshoveni*, *C. travians* had much longer than wide head capsule, less curved mandibles and small fontanelle than the other two species.

Based on the mandible features, *C. travians* have nearly straight mandibles and the curvature begins at the anterior third, which is almost similar with mandible of *C. kalshoveni*. In addition, *C. travians* and *C. kalshoveni* can be differentiated by the shape of head capsule, postmentum shape and the fontanelle size and it was agreed with the descriptions from termites of Peninsular Malaysia and Sabah (Thapa, 1981; Tho, 1992) and morphological study of *Coptotermes* from Sarawak (Norsyarizan and Wan Nurainie, 2016). As shown in Figure 3.3, *C. travians* head capsule is slightly longer than broad compared to *C. kalshoveni*. From the observation, it is suggested that size of fontanelle may become useful in distinguishing among *C. kalshoveni*, *C. travians* and *C. sepangensis* as shown in Figure 3.7.

According to Thapa (1981) and Tho (1992), *C. kalshoveni* and *C. sepangensis* likely resembled each other and the only useful characters to differentiate them is the shape of mandibles where the *C. sepangensis* are strongly curved and the curvature begins at the halfway along their length, while *C. kalshoveni* curvature begins at the anterior third. These diagnostic features given by Thapa (1981) and Tho (1992) were noted from the comparison made between these two species as shown in Figure 3.4.

The concept of discriminant function analyses (DFA) used classification of individuals into the predetermined groups. A current result showed that predetermined group's individuals for *C. curvignathus* and *C. travians* were successfully predicted based on the 17 characters of measurement. However, it was observed that there are overlap of morphological characters and several outliers for *C. sepangensis* and *C. kalshoveni*. The

possible reasons might be due to size variation of morphological characters which influenced by the age of the colony, combined effect of environment (Bonabeau *et al.*, 1998; Minter *et al.*, 2012) and the size of colony (Buhl *et al.*, 2004; Toffin *et al.*, 2009). The variability of the observed distributions of canonical discriminant scores can be caused by measurement error, preservation and storage effects (e.g, shrinkage and deformation) (Smith *et al.*, 2009). Generally, the DFA conducted in this study suggested the analysis of size corrected morphometric data was successful in distinguishing species belong to genus *Coptotermes*.

From the 17 sizes of corrected characters, width of head is the single most useful discriminant proved by canonical discriminant function. It is recommended to include additional features such as shape of mandibles and the shape of fontanelle to facilitate field identification. Table 3.8 summarised the ten most useful characters that provide the best discrimination among groups based on the canonical discriminat function, while Table 3.9 summarised which diagnostic features is useful to separate the *Coptotermes* spp.. Based on the diagnostic feature, shape of mandible is the most useful diagnostic feature to differentiate all the *Coptotermes* spp. and can be used as well as preliminary identification in the field.

Table 3.8: Summary of the most useful characters that were used to discriminate *Coptotermes* spp. based on discriminat function analyses.

Characters	Measurement range			
	<i>C. curvignathus</i>	<i>C. sepangensis</i>	<i>C. kalshoveni</i>	<i>C. travians</i>
WH	0.93-1.53 (1.33*)	0.93-1.33 (1.08*)	0.93-1.11 (1.00*)	0.91-1.58 (1.13*)
MxWPt	0.28-0.46 (0.40*)	0.28-0.39 (0.33*)	0.29-0.34 (0.31*)	0.32-0.47 (0.40*)
LF	1.01-1.56 (1.34*)	0.98-1.43 (1.17*)	0.96-1.21 (1.11*)	1.11-1.89 (1.36*)
MnWPt	0.19-0.30 (0.24*)	0.16-0.30 (0.23*)	0.19-0.25 (0.21*)	0.19-0.26 (0.22*)
TLM	0.79-1.70 (1.46*)	0.91-1.56 (1.17*)	0.84-1.30 (1.06*)	1.09-1.96 (1.39*)
TLH	1.57-4.10 (2.99*)	1.54-3.32 (2.36*)	2.05-3.61 (0.44*)	1.89-3.69 (2.72*)
TL	2.91-6.48 (5.11*)	2.71-5.27 (4.02*)	3.61-5.35 (4.18*)	3.63-6.07 (4.76*)
WHM	0.25-0.81 (0.54*)	0.22-0.61 (0.39*)	0.22-0.42 (0.32*)	0.37-0.82 (0.56*)
LPt	0.39-1.50 (0.91*)	0.41-0.85 (0.67*)	0.48-0.75 (0.62*)	0.65-1.18 (0.88*)
WPr	0.60-1.73 (0.93*)	0.46-0.91 (0.73*)	0.62-0.91 (0.69*)	0.65-0.95 (0.81*)

Note: * Mean for each measurement

Table 3.9: Summary of diagnostic features that can be used in the field to discriminate *Coptotermes* spp.

Diagnostic Features	Species			
	<i>C. curvignathus</i>	<i>C. travians</i>	<i>C. sepangensis</i>	<i>C. kalshoveni</i>
Shape of mandibles	√	√	√	√
Shape of head capsule	√	√	x	x
Shape of pronotum	√	x	x	x
Shape of postmentum	√	x	x	x
Shape of fontanelle	√	√	x	x

Note: √: Useful feature; x: Useless feature

Our survey summary reported that *C. curvignathus* is the most common species encountered (40% of the samples), and can be found in primary, secondary forest and as a pest infesting wood structures in urban areas. The other species, *C. kalshoveni* and *C. travians* encountered for <15%. *C. curvignathus* commonly found species in forest areas or urban areas because the species are very aggressive among oriental *Coptotermes* spp. (Thapa, 1981; Bit and Lim, 2001), and were observed to be able to occupy the food sources from other termites as reported by Jing Ee *et al.* (2016). The species is well known to become a major pest to rubber and oil palm in Sarawak (Bong and WeiHong, 2011; Bong *et al.*, 2012) because it is well adapted to peat areas with a lot of woody material and high water moisture.

There is no representative for *C. gestroi* in this study. It was not encountered throughout the sampling conducted in Sarawak. Moreover, the *Coptotermes* samples collection from this study was similar with Bong *et al.* (2012) with no record of *C. gestroi* from Sarawak. It is unknown whether an absence of *C. gestroi* in the present study is associated to bias sampling from the forest areas or *C. gestroi* can be found only in urban areas.

Several ranges of diagnostic characters of *C. kalshoveni* and *C. travians* collected in Sarawak did not agree with the samples described from peninsular Malaysia (Tho, 1992)

and Sabah (Thapa, 1981). According to Tho (1992), the soldier's width of head at base of mandibles of *C. kalshoveni* was 0.42-0.50 mm (50 soldiers) and Thapa (1981) reported 0.47-0.51 mm based on 10 soldiers; but the present study recorded was less than 0.42 mm (20 soldiers). In addition, the soldier's width of head of *C. travians* in the present data was larger (0.91-1.58 mm based on 26 soldiers) compared with those described from peninsular Malaysia (Tho, 1992); 0.68-0.77 mm based on 25 soldiers.

However, no description of *C. travians* was written by Thapa (1981). Based on the description and the morphology of the soldiers from Tho (1992), it can be found that *C. travians* description is similar with *C. borneensis* as described by Thapa (1981), i.e. ovoid head, mandible nearly straight, mandible curvature just beyond the mid-point of its length. The key ranges of diagnostic characters, i.e. length of head at base of mandibles and width of head at base of mandibles, for these two species are agreed from both study. It is suggested that the *C. travians* and *C. borneensis* are synonym. The synonymies between these two species also was supported by Kirton and Brown (2003), thus concluded the *C. borneensis* from Borneo is a junior synonym to *C. travians*.

Interspecies variation was observed in the soldier morphology. A previous study suggested that size variation in the soldiers and workers may influence by its habitat and the availability of the food source (Yeap *et al.*, 2009). The termites found in the large dead woods or timber tend to have larger in size compared to those whom found in small dead trunk or woods. The cause of the size variation has been reported by Waller and Page (1988) as well and highlighted this variation may be related to differences in nutrition and seasonal changes. The latter factors might be confounded by the age of colony (Grace *et al.*, 1995) and the degree of inbreeding (Husseneder *et al.*, 2005).

3.5 Summary and Conclusion

In summary, morphological keys for the identification of *Coptotermes* spp. have certain limitations such as lack of quantitative description of diagnostic characters. Yet, these keys provided a quick and accurate method of identification. By incorporating a multivariate analysis of discriminant function analysis provide a better identification of *Coptotermes* spp..

It can be concluded that a diagnostic feature of mandible's shape can be used as a preliminary identification to predict the species group membership and the feature can provide a quick identification in the field. This preliminary identification effort is important later to proceed with the DFA. Meanwhile, the DFA resulted a width of head is the most useful character to discriminate the *Coptotermes* spp.. It can be observed there are morphological ambiguities and overlapping characters observed during discriminant function analyses between *C. sepangensis* and *C. kalshoveni* in the current study. The morphological identification and any ambiguities based on morphology diagnostic features were then validated by using molecular analyses in Chapter Four.

CHAPTER 4

PHYLOGENETIC RELATIONSHIP OF *Coptotermes* SPP. (BLATTODEA: RHINOTERMITIDAE) INFERRED FROM MITOCHONDRIAL GENES OF 12S, 16S AND CYTOCHROME OXIDASE II

4.1 Introduction

Molecular genetic methods provide new insights into the biology of subterranean termites. In addition to elucidating such basic processes as development and caste differentiation, molecular techniques give a picture into the breeding structure, as well as colony and population dynamics of the cryptic nesting and foraging habits characteristic of these species. Genus *Reticulitermes* and *Coptotermes* had become a major study interest under family of Rhinotermitidae because these genera contain the most economically important termites in many parts of the world, especially temperate and subtropical regions.

According to Kirton (2005), subterranean termite of the genus *Coptotermes* are considered so far impacted the economy at the greatest rate on urban residence. In Malaysia, the termite control efforts accounted for 50% of total business turnover and was estimated at RM40 million annually (Lee, 2002a). Due to the circumstances, the economic impacts of *Coptotermes* on many by-products, products of wood, building and sector of plantations had become a major concern worldwide (Scheffrahn and Su, 2000).

The variation in size and shape within the single species of termites (Kirton and Brown, 2003) creates a problem in the taxonomy and identification of the diverse tropical termite fauna that led to confusion and ambiguities of the urban pest species (Kirton, 2005) particularly for an important pest species such as *Coptotermes*. Therefore, the morphological

approach is not sufficient to establish proper identification of these termite-related pest species because of inadequate information about distinguishing features, high degree of morphological variation and difficulty of matching soldiers and alates of the same species (Li *et al.*, 2010). These made morphology identification alone are sometimes unreliable because morphological traits are determined by both genetic and environmental influences such as climate and diet.

Today, the modern taxonomist has access to tools that were not available to predecessors in this field. The advent of molecular techniques has provided taxonomists with an invaluable tool for studying populations and species at the genetic level, as has been demonstrated for a few termite genera, particularly *Reticulitermes* (Clement *et al.*, 2001; Austin *et al.*, 2002; Szalanski *et al.*, 2003). Through these techniques, taxonomists are now able to match castes reliably and better determine species boundaries. It has also provided a supplementary tool to study relationships between species and to test the strength of classification systems.

The application of molecular genetic techniques to clarify species relationships has provided PCR-based tools for species identification. PCR-restriction fragment length polymorphism methods have been developed for distinguishing among *Reticulitermes* species in the south-central United States (Szalanski *et al.*, 2003) and for differentiating *C. formosanus* from other species of *Coptotermes* (Szalanski *et al.*, 2004). The advantages of such diagnostic methods are (a) they remove much of the ambiguity of identification based on morphological keys, (b) they can be used with any caste or developmental stage, and (c) they can be performed on a single individual. These techniques have allowed for more extensive studies of species ranges, for detection of species introduced into locations outside

their native ranges, and for the determination of the relative abundance of species in particular geographic areas (Austin *et al.*, 2004; Szalanski *et al.*, 2003).

Morphological technique provides a relatively convenient and inexpensive technique for identification and description of species. However, when morphological studies combined with genetic structure, it provides a powerful tool in the recognition, characterisation and classification of species (Clement *et al.*, 2001). Together with the increasing degree of networking and increasing ease of communication between scientists in different parts of the world, molecular taxonomy coupled with conventional methods are set to enable great advances in current understanding of the pest species of termites in the world.

A current study used mtDNA sequences of 12S, 16S and COII to elucidate the relationship among *Coptotermes* spp. populations collected from Sarawak. *Coptotermes* spp. from East Malaysia also were collected and obtained from GenBank and included into the current phylogenetic analysis.

4.2 Methods and Materials

4.2.1 Sample collection

A total of 41 populations of five putative *Coptotermes* spp. were studied. *Coptotermes* samples were collected and preserved in 100% ethanol (Table 4.1). Preserved soldier specimens were identified up to species level based on morphometric characters using Motic SMZ-16B Series stereomicroscope attached to a Moticom 2000 camera and then the image sequences were combined with Helicon Focus 6 software. The sequences for the outgroup species, *Globitermes sulphureus* (Haviland) (Li *et al.*, 2009) was retrieved from

GenBank. A DNA sequences of *Coptotermes* spp. from previous study also were used in a current analysis to provide additional information in the phylogenetic analyses. (Table 4.2).

Table 4.1: *Coptotermes* spp. and populations included in the present study.

Sample Id#	Species Name	Locality
Samples from this study		
CC02UNS	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Kota Samarahan, UNIMAS
CC05UNS	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Kota Samarahan, UNIMAS
CC08UNS	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Kota Samarahan, UNIMAS
CC04SWS	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Lundu, Samunsam Wildlife Sanctuary
CC01TB	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Sri Aman
CC01SKAG	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Sri Aman
CC02SKAG	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Sri Aman
CC01KL	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Sri Aman, Engkelili
CC02KL	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Sri Aman, Engkelili
CC01KLKG	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Sri Aman, Engkelili
CC02KLKG	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Sri Aman, Engkelili
CC03KLKG	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Sri Aman, Engkelili
CC01BHR	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Kuching, Borneo Highland
CC02BHR	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Kuching, Borneo Highland
CC01SNR	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Kuching, Samajaya Nature Reserve
CC01MA	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Kuching, Matang
IK19R29P2	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Sibu
IK19R31P4	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Sibu
IK19R31P5	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Sibu
IL19R16P1	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Sibu
IL19R17P1	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Sibu
IL19R18P1	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Sibu
IL19R19P2	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Sibu
IL19R19P1	<i>C. curvignathus</i> (Holmgren)	Malaysia, Sarawak, Sibu
CS01UNS	<i>C. sepangensis</i> (Krishna)	Malaysia, Sarawak, Kota Samarahan, UNIMAS
CS06UNS	<i>C. sepangensis</i> (Krishna)	Malaysia, Sarawak, Kota Samarahan, UNIMAS
CS07UNS	<i>C. sepangensis</i> (Krishna)	Malaysia, Sarawak, Kota Samarahan, UNIMAS
CS02KNP	<i>C. sepangensis</i> (Krishna)	Malaysia, Sarawak, Kuching, Kubah National Park
CS01BHW	<i>C. sepangensis</i> (Krishna)	Malaysia, Sarawak, Kuching, Borneo Highland
CS02BHW	<i>C. sepangensis</i> (Krishna)	Malaysia, Sarawak, Kuching, Borneo Highland
CS03BHW	<i>C. sepangensis</i> (Krishna)	Malaysia, Sarawak, Kuching, Borneo Highland
CS04BHW	<i>C. sepangensis</i> (Krishna)	Malaysia, Sarawak, Kuching, Borneo Highland
CS03SWS	<i>C. sepangensis</i> (Krishna)	Malaysia, Sarawak, Lundu, Samunsam Wildlife Sanctuary
CS05SWS	<i>C. sepangensis</i> (Krishna)	Malaysia, Sarawak, Lundu, Samunsam Wildlife Sanctuary
CK03UNS	<i>C. kalshoveni</i> (Kemner)	Malaysia, Sarawak, Kota Samarahan, UNIMAS
CK04UNS	<i>C. kalshoveni</i> (Kemner)	Malaysia, Sarawak, Kota Samarahan, UNIMAS
CT01SNP	<i>C. travians</i> (Haviland)	Malaysia, Sarawak, Kuching, Santubong National Park
CT01KNP	<i>C. travians</i> (Haviland)	Malaysia, Sarawak, Kuching, Kubah National Park
CT01LHNP	<i>C. travians</i> (Haviland)	Malaysia, Sarawak, Miri, Lambir Hills National Park
CG01JBS	<i>C. gestroi</i> (Wasmann)	Malaysia, Johor, Skudai

Table 4.2: *Coptotermes* spp. from previous studies used in current analyses.

Species name	Locality	Genebank accession no.			References
		12S	16S	COII	
Published GenBank sequences used in this study					
<i>C. curvignathus</i>	Malaysia, Penang, USM	FJ235110	FJ376686	FJ384634	Yeap <i>et al.</i> , 2007
<i>C. curvignathus</i>	Malaysia, Penang	FJ235111	FJ376680	FJ384635	Yeap <i>et al.</i> , 2007
<i>C. gestroi</i>	Malaysia, Penang, USM	EF379982	EF379963	EF379945	Yeap <i>et al.</i> , 2007
<i>C. gestroi</i>	Malaysia, Kuala Lumpur, Bangsar	EF379987	EF379969	EF379951	Yeap <i>et al.</i> , 2007
<i>C. gestroi</i>	Malaysia, Johor, Muar	EF379988	EF379970	EF379952	Yeap <i>et al.</i> , 2007
<i>C. kalshoveni</i>	Malaysia, Penang, USM	FJ235118	FJ376684	FJ384652	Yeap <i>et al.</i> , 2007
<i>C. kalshoveni</i>	Malaysia, Penang, Pantai, Keracut	FJ235119	FJ376685	-	Yeap <i>et al.</i> , 2007
<i>C. sepangensis</i>	Malaysia, Perak, Bagan Dato Estate	-	FJ376679	-	Yeap <i>et al.</i> , 2007
<i>C. sepangensis</i>	Malaysia	-	-	AJ854163	unpublished
<i>C. sepangensis</i>	Malaysia	-	-	AJ854165	unpublished
<i>C. travians</i>	Malaysia, Perak, Teluk Intan	FJ915823	FJ915824	-	Yeap <i>et al.</i> , 2007
<i>C. travians</i>	Malaysia	-	-	AJ854169	unpublished
<i>C. travians</i>	Malaysia	-	-	AJ854170	unpublished
<i>G. sulphureus</i>	Malaysia, Penang, USM	EF379993	EF379975	EF379957	Yeap <i>et al.</i> , 2007

4.2.2 DNA Extraction

The total genomic DNA was extracted from single individual and purified by using DNeasy tissue kit (QIAGEN GmbH, Hilden, Germany). Alcohol-preserved specimens was allowed to dry on filter paper. Then, the samples were put into 1.5 ml microcentrifuge tube with 180 µl buffer ATL, 20 µl Proteinase K and incubated in water bath at 56°C for 45 min. 200 µl buffer AL then was added and the solution was precipitated with 100% ethanol. Mixtures was centrifuged at 8000 rpm for 1 min in Dneasy Mini spin column, following by adding 500 µl each of buffer AW1 and AW2. Then, it was centrifuged at 8000 rpm for 1 min

and 14000 rpm for 1 min respectively. Total DNA obtained was added with 200 µl buffer AE, centrifuged at 8000 rpm for 1 min and stored at -20°C for further analyses.

4.2.3 Polymerase Chain Reaction (PCR)

PCR primers targeting mtDNA genes used in this study was conducted using the primers used from the previous study (Kambhampati and Smith, 1995; Miura *et al.*, 1998; Yeap *et al.*, 2009) as shown in Table 4.3.

Table 4.3: Primer used for PCR amplification and sequencing.

Gene	Name	Sequence (5' – 3')
16S	LR-J-13007	TTA CGC TGT TAT CCC TAA
	LR-N-13398	CGC CTG TTT ATC AAA AAC AT
12S	12SF	TAC TAT GTT ACG ACT TAT
	12SR	AAA CTA GGA TTA GAT ACC C
COII	C2F2	ATA CCT CGA CGW TAT TCA GA
	TKN3785	GTT TAA GAG ACC AGT ACT TG

PCR amplification was performed in a standard 25ul reaction and carried out in a thermal cycler machine (BIO-RAD My Cycler) with the reagents as follow: 15.5 µl of ddH₂O, 1.5 mM of MgCl₂ (Promega), 0.2 mM of dNTP, 10 µM of each primer, 1X PCR buffer, 1 U of *Taq polymerase* (Promega) and approximately 100 ng of template DNA. PCR cocktail was prepared according to the recipe below (Table 4.4). Then, the PCR was conducted using the following profile in thermal cycler under the following conditions (Table 4.5). The amplified products were loaded in 1% agarose gel containing ethidium bromide, run for approximately 30 min at 90V and photographed under UV illumination. Fragment sizes of the amplified products were estimated using a low range DNA ladder (Promega, 100bp).

Table 4.4: Components of PCR master mix.

Component	1X reaction	10X reactions
ddH ₂ O	14.3 µL	143 µL
5X reaction buffer	5.0 µL	50 µL
dNTP mix	0.5 µL	5 µL
MgCl ₂	1.5 µL	15 µL
Primer forward	1.25 µL	12.5 µL
Primer reverse	1.25 µL	12.5 µL
<i>Taq</i> polymerase	0.2 µL	-
Template DNA	1.0 µL	-
Total reaction	25 µL	

Table 4.5: PCR Profile.

Step	Temp.	Time	No cycles
Pre Denaturation	94°C	3 min	1
Denaturation	94°C	1 min	35
Annealing	46°C	1 min	
Extension	72°C	1 min	
Post Extension	72°C	10 min	1
Soak	4°C	∞	

4.2.4 DNA Sequencing

Purification of amplified products were done by using purification kit (Fermentas) following the protocol provided from the manufacturer. Purified products were later sent to private laboratory (1st Base Laboratories, Selangor, Malaysia) for DNA sequencing services, both forward and reverse strands were sequences using the ABI ® 377 DNA automated sequencer with the ABI PRISM BigDye® Terminator version 3.0 Cycle Sequencing Kit.

4.2.5 Phylogenetic Analysis

CHROMAS program version 2.5.1 (McCarthy, 1998) was used to display fluorescence nucleotide bases of DNA sequence results. Multiple alignments of the nucleotide sequences were generated in Clustal X program version2.0 (Thompson *et al.*, 1997) and subsequently aligned and saved as the clustal and nexus formats. The aligned

sequences of mitochondrial protein coding genes, i.e. COII, was translated into amino acids by Molecular Evolutionary Genetic Analysis (MEGA) version 7.0 (Kumar *et al.*, 2015) and the occurrences of stop codons were removed. Base compositional analyses and pairwise genetic distance was used to calculate the genetic distance according to the Kimura 2-parameter model of sequence evolution (Kimura, 1980) using MEGA version 7 (Kumar *et al.*, 2015).

Maximum parsimony (MP) analysis was conducted with “tree bisection-reconnection (TBR)” branch swapping and 10 random taxon addition replicates under a heuristic search in PAUP 4.0b10. Neighbour joining (NJ) analysis was conducted using the Kimura 2 parameter distance option. All characters were unordered and weighted equally. Sites with missing data or gaps were treated as missing characters for all analyses. To estimate branch support on the recovered topology, nonparametric bootstrap values were assessed with 1000 bootstrap pseudo-replicates (Felsenstein, 1985).

Modeltest 3.7 was used to find the best of DNA substitution model (Posada and Crandall, 1998) prior to maximum likelihood (ML) analysis. The phylogenetic reconstruction for maximum likelihood was based on the best-fit model, which was selected by “Akaike information criterion (AIC)” (Akaike, 1974) and TBR branch for “heuristic” search swapping was performed in PAUP 4.0b10 (Swofford, 2002). A maximum of “1000 pseudo-replicates” for ML nodal support was predicted using the “non-parametric bootstrap” (Felsenstein, 1985). All trees were rooted with *G. sulphureus*.

Combined data were analysed by MP, NJ, and ML for phylogenetic reconstruction. Congruence among tree topologies generated with 12S, 16S and COII sequences was tested with the “incongruence length difference test (ILD)” in the “partition homogeneity test (PHT) in PAUP* (Mickeyvich and Farris, 1981).

4.3 Results

4.3.1 Characterization of the Nucleotide Data

A complete alignment of the partial COII gene sequences from the 34 *Coptotermes* spp. specimens and 10 specimens from NCBI resulted in a fragment containing 478 characters of which 272 positions (56.9%) are variable and 87 positions (18.2%) are parsimoniously informative.

The percentage of nucleotide composition at each codon position and the rate of nucleotide substitutions varied (Table 4.6). The mean frequency of nucleotides was biased towards A +T (Thymine (T) 20.0%, Cytosine (C) 31.6%, Adenine (A) 35.2% and Guanine (G) 13.2%). The A+T content at codon positions 3, 2, and 1 were 60.2%, 45.4% and 60.1% respectively.

The multiple sequence alignment for the 12S gene from 40 specimens of *Coptotermes* spp. and 8 specimens retrieved from NCBI resulted 337 characters, of which 37 positions (10.98 %) are variable and 36 positions (7.72%) are parsimoniously informative, while the complete alignment of 16S gene using 41 specimens of *Coptotermes* spp. and 7 specimens from NCBI had 321 characters of which 44 positions (13.71%) are variable and 36 positions (11.21%) are parsimoniously informative.

The mean frequencies of nucleotide were biased towards A+T for both 12S and 16S gene (62.7% A+T in 12S and 60.6% A+T in 16S) as summarised in Table 4.6.

Table 4.6: Summary of nucleotide composition of *Coptotermes* spp. based on 12S, 16S and COII.

			12S	16S	COII			
					All sites	Codon Position 1	Codon Position 2	Codon Position 3
Adenine (A)	+		62.7%	60.6%	55.2%	60.1%	45.4%	60.2%
Thymine (T)								
Cytosine (C)	+		37.4%	39.3%	44.8%	40%	54.6%	39.8%
Guanine (G)								

The genetic pairwise distance of 12S, 16S and COII were calculated using the model of Kimura 2-parameter among the species of *C. curvignathus*, *C. kalshoveni*, *C. sepangensis*, *C. travians* and *C. gestroi*.

Genetic diversity of COII gene showed intraspecies variation of *C. curvignathus* was ranged from 0% to 0.009% in COII gene (Table 4.9), from 0% to 1% in 16S gene (Table 4.8) and 0% to 0.01% in 12S gene (Table 4.7), while *C. gestroi* showed no genetic diversity in COII and 12S gene and from 0% to 0.3% in 16S gene. The genetic diversity of *C. sepangensis* ranged from 0% to 0.09% in COII (Table 4.9), from 0% to 0.019 12S gene (Table 4.7) and from 0% to 0.024% in 16S gene (Table 4.8). *C. kalshoveni* has genetic diversity ranged from 0.004% to 0.023% in COII gene (Table 4.9), from 0% to 0.032% in 16S gene (Table 4.8), and from 0% to 0.09% in 12S gene (Table 4.7), while the genetic diversity of *C. travians* from Sarawak showed high genetic distance with *C. travians* from Penang with 0.113% in COII gene, 0.061% in 16S gene and 0.035% in 12S gene. The genetic variation among *C. travians* in Sarawak was 0.3% in 12S gene and 0.7% in 16S gene. All sequences were separated by outgroup, *G. sulphureus* with value range from 0.2% to 1%.

4.3.2 Partition Homogeneity Test (PHT)

The partition homogeneity test (Farris *et al.*, 1994) conducted in this study showed that the combined datasets of three genes (12S, 16S and COII) had significant of p-

value=0.01. Therefore, the genes were assumed to have relatively different evolutionary rates and the analysis of the combined genes cannot proceed for phylogenetic reconstruction.

Table 4.7: Genetic pairwise distance specimens of rRNA 12S gene using Kimura 2 parameter model.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1																				
2	0.000																			
3	0.025	0.025																		
4	0.022	0.022	0.003																	
5	0.022	0.022	0.003	0.000																
6	0.022	0.022	0.003	0.000	0.000															
7	0.029	0.029	0.009	0.006	0.006	0.006														
8	0.000	0.000	0.025	0.022	0.022	0.022	0.029													
9	0.035	0.035	0.016	0.013	0.013	0.013	0.019	0.035												
10	0.003	0.003	0.029	0.025	0.025	0.025	0.032	0.003	0.039											
11	0.003	0.003	0.029	0.025	0.025	0.025	0.032	0.003	0.039	0.000										
12	0.000	0.000	0.025	0.022	0.022	0.022	0.029	0.000	0.035	0.003	0.003									
13	0.006	0.006	0.025	0.022	0.022	0.022	0.029	0.006	0.035	0.003	0.003	0.006								
14	0.003	0.003	0.022	0.019	0.019	0.019	0.025	0.003	0.032	0.006	0.006	0.003	0.003							
15	0.003	0.003	0.022	0.019	0.019	0.019	0.025	0.003	0.032	0.006	0.006	0.003	0.003	0.000						
16	0.006	0.006	0.025	0.022	0.022	0.022	0.029	0.006	0.035	0.003	0.003	0.006	0.000	0.003	0.003					
17	0.000	0.000	0.025	0.022	0.022	0.022	0.029	0.000	0.035	0.003	0.003	0.000	0.006	0.003	0.003	0.006				
18	0.000	0.000	0.025	0.022	0.022	0.022	0.029	0.000	0.035	0.003	0.003	0.000	0.006	0.003	0.003	0.006	0.000			
19	0.000	0.000	0.025	0.022	0.022	0.022	0.029	0.000	0.035	0.003	0.003	0.000	0.006	0.003	0.003	0.006	0.000	0.000		
20	0.006	0.006	0.032	0.029	0.029	0.029	0.035	0.006	0.042	0.003	0.003	0.006	0.006	0.009	0.009	0.006	0.006	0.006	0.006	
21	0.003	0.003	0.029	0.025	0.025	0.025	0.032	0.003	0.039	0.000	0.000	0.003	0.003	0.006	0.006	0.003	0.003	0.003	0.003	0.003
22	0.049	0.049	0.032	0.029	0.029	0.029	0.032	0.049	0.042	0.052	0.052	0.049	0.049	0.045	0.045	0.049	0.049	0.049	0.049	0.055
23	0.022	0.022	0.003	0.000	0.000	0.000	0.006	0.022	0.013	0.025	0.025	0.022	0.022	0.019	0.019	0.022	0.022	0.022	0.022	0.029
24	0.000	0.000	0.025	0.022	0.022	0.022	0.029	0.000	0.035	0.003	0.003	0.000	0.006	0.003	0.003	0.006	0.000	0.000	0.000	0.006
25	0.006	0.006	0.032	0.029	0.029	0.029	0.035	0.006	0.042	0.009	0.009	0.006	0.013	0.009	0.009	0.013	0.006	0.006	0.006	0.013
26	0.045	0.045	0.029	0.025	0.025	0.025	0.032	0.045	0.038	0.049	0.049	0.045	0.045	0.042	0.042	0.045	0.045	0.045	0.045	0.052
27	0.000	0.000	0.025	0.022	0.022	0.022	0.029	0.000	0.035	0.003	0.003	0.000	0.006	0.003	0.003	0.006	0.000	0.000	0.000	0.006
28	0.003	0.003	0.029	0.025	0.025	0.025	0.032	0.003	0.039	0.000	0.000	0.003	0.003	0.006	0.006	0.003	0.003	0.003	0.003	0.003
29	0.000	0.000	0.025	0.022	0.022	0.022	0.029	0.000	0.035	0.003	0.003	0.000	0.006	0.003	0.003	0.006	0.000	0.000	0.000	0.006
30	0.022	0.022	0.003	0.000	0.000	0.000	0.006	0.022	0.013	0.025	0.025	0.022	0.022	0.019	0.019	0.022	0.022	0.022	0.022	0.029
31	0.019	0.019	0.006	0.003	0.003	0.003	0.009	0.019	0.016	0.022	0.022	0.019	0.025	0.022	0.022	0.025	0.019	0.019	0.019	0.025
32	0.022	0.022	0.003	0.000	0.000	0.000	0.006	0.022	0.013	0.025	0.025	0.022	0.022	0.019	0.019	0.022	0.022	0.022	0.022	0.029

Table 4.7 continued

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
33	0.022	0.022	0.003	0.000	0.000	0.000	0.006	0.022	0.013	0.025	0.025	0.022	0.022	0.019	0.019	0.022	0.022	0.022	0.022	0.029
34	0.035	0.035	0.032	0.029	0.029	0.029	0.035	0.035	0.042	0.038	0.038	0.035	0.035	0.032	0.032	0.035	0.035	0.035	0.035	0.042
35	0.003	0.003	0.029	0.025	0.025	0.025	0.032	0.003	0.039	0.000	0.000	0.003	0.003	0.006	0.006	0.003	0.003	0.003	0.003	0.003
36	0.003	0.003	0.029	0.025	0.025	0.025	0.032	0.003	0.039	0.000	0.000	0.003	0.003	0.006	0.006	0.003	0.003	0.003	0.003	0.003
37	0.022	0.022	0.003	0.000	0.000	0.000	0.006	0.022	0.013	0.025	0.025	0.022	0.022	0.019	0.019	0.022	0.022	0.022	0.022	0.029
38	0.003	0.003	0.022	0.019	0.019	0.019	0.025	0.003	0.032	0.006	0.006	0.003	0.003	0.000	0.000	0.003	0.003	0.003	0.003	0.009
39	0.025	0.025	0.013	0.009	0.009	0.009	0.016	0.025	0.022	0.029	0.029	0.025	0.025	0.022	0.022	0.025	0.025	0.025	0.025	0.032
40	0.025	0.025	0.013	0.009	0.009	0.009	0.016	0.025	0.022	0.029	0.029	0.025	0.025	0.022	0.022	0.025	0.025	0.025	0.025	0.032
41	0.003	0.003	0.029	0.025	0.025	0.025	0.032	0.003	0.039	0.006	0.006	0.003	0.009	0.006	0.006	0.009	0.003	0.003	0.003	0.009
42	0.003	0.003	0.029	0.025	0.025	0.025	0.032	0.003	0.039	0.006	0.006	0.003	0.009	0.006	0.006	0.009	0.003	0.003	0.003	0.009
43	0.029	0.029	0.022	0.019	0.019	0.019	0.025	0.029	0.032	0.032	0.032	0.029	0.029	0.025	0.025	0.029	0.029	0.029	0.029	0.035
44	0.032	0.032	0.029	0.025	0.025	0.025	0.032	0.032	0.038	0.035	0.035	0.032	0.032	0.029	0.029	0.032	0.032	0.032	0.032	0.038
45	0.032	0.032	0.029	0.025	0.025	0.025	0.032	0.032	0.038	0.035	0.035	0.032	0.032	0.029	0.029	0.032	0.032	0.032	0.032	0.038
46	0.032	0.032	0.029	0.025	0.025	0.025	0.032	0.032	0.038	0.035	0.035	0.032	0.032	0.029	0.029	0.032	0.032	0.032	0.032	0.038
47	1.015	1.015	1.053	1.048	1.048	1.048	1.048	1.015	1.066	1.015	1.015	1.015	1.031	1.031	1.031	1.031	1.015	1.015	1.015	1.026
21	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
22	0.052																			
23	0.025	0.029																		
24	0.003	0.049	0.022																	
25	0.009	0.042	0.029	0.006																
26	0.049	0.003	0.025	0.045	0.039															
27	0.003	0.049	0.022	0.000	0.006	0.045														
28	0.000	0.052	0.025	0.003	0.009	0.049	0.003													
29	0.003	0.049	0.022	0.000	0.006	0.045	0.000	0.003												
30	0.025	0.029	0.000	0.022	0.029	0.025	0.022	0.025	0.022											
31	0.022	0.032	0.003	0.019	0.025	0.029	0.019	0.022	0.019	0.003										
32	0.025	0.029	0.000	0.022	0.029	0.025	0.022	0.025	0.022	0.000	0.003									
33	0.025	0.029	0.000	0.022	0.029	0.025	0.022	0.025	0.022	0.000	0.003	0.000								
34	0.038	0.045	0.029	0.035	0.042	0.042	0.035	0.038	0.035	0.029	0.032	0.029	0.029							

Table 4.7 continued

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
35	0.000	0.052	0.025	0.003	0.009	0.049	0.003	0.000	0.003	0.025	0.022	0.025	0.025	0.038						
36	0.000	0.052	0.025	0.003	0.009	0.049	0.003	0.000	0.003	0.025	0.022	0.025	0.025	0.038	0.000					
37	0.025	0.029	0.000	0.022	0.029	0.025	0.022	0.025	0.022	0.000	0.003	0.000	0.000	0.029	0.025	0.025				
38	0.006	0.045	0.019	0.003	0.009	0.042	0.003	0.006	0.003	0.019	0.022	0.019	0.019	0.032	0.006	0.006	0.019			
39	0.029	0.038	0.009	0.025	0.032	0.035	0.025	0.029	0.025	0.009	0.013	0.009	0.009	0.032	0.029	0.029	0.009	0.022		
40	0.029	0.038	0.009	0.025	0.032	0.035	0.025	0.029	0.025	0.009	0.013	0.009	0.009	0.032	0.029	0.029	0.009	0.022	0.000	
41	0.006	0.045	0.025	0.003	0.003	0.042	0.003	0.006	0.003	0.025	0.022	0.025	0.025	0.038	0.006	0.006	0.025	0.006	0.029	0.029
42	0.006	0.052	0.025	0.003	0.009	0.049	0.003	0.006	0.003	0.025	0.022	0.025	0.025	0.038	0.006	0.006	0.025	0.006	0.029	0.029
43	0.032	0.035	0.019	0.029	0.035	0.032	0.029	0.032	0.029	0.019	0.022	0.019	0.019	0.032	0.032	0.032	0.019	0.025	0.009	0.009
44	0.035	0.042	0.025	0.032	0.039	0.039	0.032	0.035	0.032	0.025	0.029	0.025	0.025	0.003	0.035	0.035	0.025	0.029	0.029	0.029
45	0.035	0.042	0.025	0.032	0.039	0.039	0.032	0.035	0.032	0.025	0.029	0.025	0.025	0.003	0.035	0.035	0.025	0.029	0.029	0.029
46	0.035	0.042	0.025	0.032	0.039	0.039	0.032	0.035	0.032	0.025	0.029	0.025	0.025	0.003	0.035	0.035	0.025	0.029	0.029	0.029
47	1.015	1.031	1.048	1.015	1.015	1.015	1.015	1.015	1.015	1.048	1.031	1.048	1.048	1.036	1.015	1.015	1.048	1.031	1.078	1.078
	41	42	43	44	45	46	47													
41																				
42	0.006																			
43	0.032	0.032																		
44	0.035	0.035	0.029																	
45	0.035	0.035	0.029	0.000																
46	0.035	0.035	0.029	0.000	0.000															
47	1.015	1.031	1.072	1.025	1.025	1.025														

Note: 1=CC01BHR; 2=CC02BHR; 3=CS01BHW; 4=CS02BHW; 5=CS03BHW; 6=CS04BHW; 7=CS03SWS; 8=CC04SWS; 9=CS05SWS; 10=IK19R29P2; 11=IK19R31P4; 12=IK19R31P5; 13=IL19R16P1; 14=IL19R17P1; 15=IL19R19P1; 16=IL19R19P2; 17=CC01KL; 18=CC02KL; 19=CC01KLKG; 20=CC02KLKG; 21=CC03KLKG; 22=CT01KNP; 23=CS02KNP; 24=CC01SKAG; 25=CC02SKAG; 26=CT01SNP; 27=CC01SNR; 28=CC01TB; 29=CC05UNS 30=CS06UNS; 31=CS07UNS; 32=CK04UNS; 33=CK03UNS; 34=C01JBS; 35=CC01MA; 36=CC02UNS; 37=CS01UNS; 38=IL19R18P1; 39=CKFJ235118.1; 40=CKFJ235119.1; 41=CCFJ235110.1; 42=CCFJ235111.1; 43=CTFJ915823; 44=CGEF379982.1; 45=CGEF379987.1; 46=CGEF379988.1; 47=GSEF379993.1

Table 4.8: Genetic pairwise distance specimens of rRNA 16S gene using Kimura 2 parameter model.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1																				
2	0.043																			
3	0.043	0.000																		
4	0.043	0.000	0.000																	
5	0.039	0.043	0.043	0.043																
6	0.039	0.043	0.043	0.043	0.000															
7	0.043	0.046	0.046	0.046	0.003	0.003														
8	0.003	0.039	0.039	0.039	0.035	0.035	0.039													
9	0.007	0.035	0.035	0.035	0.032	0.032	0.035	0.003												
10	0.007	0.035	0.035	0.035	0.032	0.032	0.035	0.003	0.000											
11	0.007	0.035	0.035	0.035	0.039	0.039	0.043	0.003	0.007	0.007										
12	0.003	0.039	0.039	0.039	0.035	0.035	0.039	0.000	0.003	0.003	0.003									
13	0.000	0.043	0.043	0.043	0.039	0.039	0.043	0.003	0.007	0.007	0.007	0.003								
14	0.003	0.039	0.039	0.039	0.035	0.035	0.039	0.000	0.003	0.003	0.003	0.000	0.003							
15	0.000	0.043	0.043	0.043	0.039	0.039	0.043	0.003	0.007	0.007	0.007	0.003	0.000	0.003						
16	0.007	0.035	0.035	0.035	0.032	0.032	0.035	0.003	0.000	0.000	0.007	0.003	0.007	0.003	0.007					
17	0.007	0.035	0.035	0.035	0.039	0.039	0.043	0.003	0.007	0.007	0.000	0.003	0.007	0.003	0.007	0.007				
18	0.003	0.039	0.039	0.039	0.035	0.035	0.039	0.000	0.003	0.003	0.003	0.000	0.003	0.000	0.003	0.003	0.003			
19	0.007	0.035	0.035	0.035	0.032	0.032	0.035	0.003	0.000	0.000	0.007	0.003	0.007	0.003	0.007	0.000	0.007	0.003		
20	0.007	0.035	0.035	0.035	0.039	0.039	0.043	0.003	0.007	0.007	0.000	0.003	0.007	0.003	0.007	0.007	0.000	0.003	0.007	
21	0.007	0.035	0.035	0.035	0.032	0.032	0.035	0.003	0.000	0.000	0.007	0.003	0.007	0.003	0.007	0.000	0.007	0.003	0.000	0.007
22	0.007	0.035	0.035	0.035	0.032	0.032	0.035	0.003	0.000	0.000	0.007	0.003	0.007	0.003	0.007	0.000	0.007	0.003	0.000	0.007
23	0.007	0.035	0.035	0.035	0.032	0.032	0.035	0.003	0.000	0.000	0.007	0.003	0.007	0.003	0.007	0.000	0.007	0.003	0.000	0.007
24	0.043	0.000	0.000	0.000	0.043	0.043	0.046	0.039	0.035	0.035	0.035	0.039	0.043	0.039	0.043	0.035	0.035	0.039	0.035	0.035
25	0.062	0.054	0.054	0.054	0.028	0.028	0.032	0.058	0.054	0.054	0.062	0.058	0.062	0.058	0.062	0.054	0.062	0.058	0.054	0.062
26	0.065	0.057	0.057	0.057	0.032	0.032	0.035	0.061	0.058	0.058	0.065	0.061	0.065	0.061	0.065	0.058	0.065	0.061	0.058	0.065
27	0.065	0.057	0.057	0.057	0.032	0.032	0.035	0.061	0.058	0.058	0.065	0.061	0.065	0.061	0.065	0.058	0.065	0.061	0.058	0.065
28	0.076	0.072	0.072	0.072	0.042	0.042	0.046	0.073	0.069	0.069	0.076	0.073	0.076	0.073	0.076	0.069	0.076	0.073	0.069	0.076
29	0.065	0.065	0.065	0.065	0.032	0.032	0.035	0.061	0.058	0.058	0.065	0.061	0.065	0.061	0.065	0.058	0.065	0.061	0.058	0.065
30	0.073	0.069	0.069	0.069	0.039	0.039	0.042	0.069	0.065	0.065	0.073	0.069	0.073	0.069	0.073	0.065	0.073	0.069	0.065	0.073
31	0.069	0.069	0.069	0.069	0.035	0.035	0.039	0.065	0.061	0.061	0.069	0.065	0.069	0.065	0.069	0.061	0.069	0.065	0.061	0.069
32	0.069	0.069	0.069	0.069	0.035	0.035	0.039	0.065	0.061	0.061	0.069	0.065	0.069	0.065	0.069	0.061	0.069	0.065	0.061	0.069

Table 4.8 continued

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
33	0.065	0.057	0.057	0.057	0.032	0.032	0.035	0.061	0.058	0.058	0.065	0.061	0.065	0.061	0.065	0.058	0.065	0.061	0.058	0.065
34	0.069	0.061	0.061	0.061	0.035	0.035	0.039	0.065	0.061	0.061	0.069	0.065	0.069	0.065	0.069	0.061	0.069	0.065	0.061	0.069
35	0.062	0.054	0.054	0.054	0.028	0.028	0.032	0.058	0.054	0.054	0.062	0.058	0.062	0.058	0.062	0.054	0.062	0.058	0.054	0.062
36	0.065	0.057	0.057	0.057	0.032	0.032	0.035	0.061	0.058	0.058	0.065	0.061	0.065	0.061	0.065	0.058	0.065	0.061	0.058	0.065
37	0.046	0.058	0.058	0.058	0.058	0.058	0.061	0.043	0.046	0.046	0.046	0.043	0.046	0.043	0.046	0.046	0.046	0.043	0.046	0.046
38	0.046	0.058	0.058	0.058	0.058	0.058	0.061	0.043	0.046	0.046	0.046	0.043	0.046	0.043	0.046	0.046	0.046	0.043	0.046	0.046
39	0.046	0.057	0.057	0.057	0.057	0.057	0.061	0.043	0.046	0.046	0.046	0.043	0.046	0.043	0.046	0.046	0.046	0.043	0.046	0.046
40	0.007	0.035	0.035	0.035	0.032	0.032	0.035	0.003	0.000	0.000	0.007	0.003	0.007	0.003	0.007	0.000	0.007	0.003	0.000	0.007
41	0.007	0.035	0.035	0.035	0.032	0.032	0.035	0.003	0.000	0.000	0.007	0.003	0.007	0.003	0.007	0.000	0.007	0.003	0.000	0.007
42	0.007	0.035	0.035	0.035	0.032	0.032	0.035	0.003	0.000	0.000	0.007	0.003	0.007	0.003	0.007	0.000	0.007	0.003	0.000	0.007
43	0.007	0.035	0.035	0.035	0.032	0.032	0.035	0.003	0.000	0.000	0.007	0.003	0.007	0.003	0.007	0.000	0.007	0.003	0.000	0.007
44	0.007	0.035	0.035	0.035	0.032	0.032	0.035	0.003	0.000	0.000	0.007	0.003	0.007	0.003	0.007	0.000	0.007	0.003	0.000	0.007
45	0.007	0.035	0.035	0.035	0.032	0.032	0.035	0.003	0.000	0.000	0.007	0.003	0.007	0.003	0.007	0.000	0.007	0.003	0.000	0.007
46	0.007	0.035	0.035	0.035	0.032	0.032	0.035	0.003	0.000	0.000	0.007	0.003	0.007	0.003	0.007	0.000	0.007	0.003	0.000	0.007
47	0.014	0.043	0.043	0.043	0.039	0.039	0.043	0.010	0.007	0.007	0.014	0.010	0.014	0.010	0.014	0.007	0.014	0.010	0.007	0.014
48	0.966	0.973	0.973	0.973	0.942	0.942	0.954	0.985	0.966	0.966	1.005	0.985	0.966	0.985	0.966	0.966	1.005	0.985	0.966	1.005
21	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	
22	0.000																			
23	0.000	0.000																		
24	0.035	0.035	0.035																	
25	0.054	0.054	0.054	0.054																
26	0.058	0.058	0.058	0.057	0.003															
27	0.058	0.058	0.058	0.057	0.003	0.007														
28	0.069	0.069	0.069	0.072	0.021	0.024	0.024													
29	0.058	0.058	0.058	0.065	0.010	0.014	0.014	0.010												
30	0.065	0.065	0.065	0.069	0.017	0.021	0.021	0.003	0.007											
31	0.061	0.061	0.061	0.069	0.014	0.017	0.010	0.014	0.003	0.010										
32	0.061	0.061	0.061	0.069	0.014	0.017	0.010	0.014	0.003	0.010	0.000									
33	0.058	0.058	0.058	0.057	0.003	0.007	0.000	0.024	0.014	0.021	0.010	0.010								
34	0.061	0.061	0.061	0.061	0.007	0.010	0.010	0.028	0.017	0.024	0.021	0.021	0.010							
35	0.054	0.054	0.054	0.054	0.000	0.003	0.003	0.021	0.010	0.017	0.014	0.014	0.003	0.007						

Table 4.8 continued

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
36	0.058	0.058	0.058	0.057	0.003	0.007	0.000	0.024	0.014	0.021	0.010	0.010	0.000	0.010	0.003				
37	0.046	0.046	0.046	0.058	0.062	0.065	0.065	0.084	0.073	0.081	0.077	0.077	0.065	0.069	0.062	0.065			
38	0.046	0.046	0.046	0.058	0.062	0.065	0.065	0.084	0.073	0.081	0.077	0.077	0.065	0.069	0.062	0.065	0.007		
39	0.046	0.046	0.046	0.057	0.062	0.065	0.065	0.077	0.073	0.080	0.077	0.077	0.065	0.069	0.062	0.065	0.007	0.007	
40	0.000	0.000	0.000	0.035	0.054	0.058	0.058	0.069	0.058	0.065	0.061	0.061	0.058	0.061	0.054	0.058	0.046	0.046	0.046
41	0.000	0.000	0.000	0.035	0.054	0.058	0.058	0.069	0.058	0.065	0.061	0.061	0.058	0.061	0.054	0.058	0.046	0.046	0.046
42	0.000	0.000	0.000	0.035	0.054	0.058	0.058	0.069	0.058	0.065	0.061	0.061	0.058	0.061	0.054	0.058	0.046	0.046	0.046
43	0.000	0.000	0.000	0.035	0.054	0.058	0.058	0.069	0.058	0.065	0.061	0.061	0.058	0.061	0.054	0.058	0.046	0.046	0.046
44	0.000	0.000	0.000	0.035	0.054	0.058	0.058	0.069	0.058	0.065	0.061	0.061	0.058	0.061	0.054	0.058	0.046	0.046	0.046
45	0.000	0.000	0.000	0.035	0.054	0.058	0.058	0.069	0.058	0.065	0.061	0.061	0.058	0.061	0.054	0.058	0.046	0.046	0.046
46	0.000	0.000	0.000	0.035	0.054	0.058	0.058	0.069	0.058	0.065	0.061	0.061	0.058	0.061	0.054	0.058	0.046	0.046	0.046
47	0.007	0.007	0.007	0.043	0.061	0.065	0.065	0.069	0.065	0.072	0.069	0.069	0.065	0.069	0.061	0.065	0.054	0.054	0.046
48	0.966	0.966	0.966	0.973	0.998	0.985	1.011	1.018	1.011	1.011	1.024	1.024	1.011	1.004	0.998	1.011	1.018	1.018	1.005
	40	41	42	43	44	45	46	47	48										
40																			
41	0.000																		
42	0.000	0.000																	
43	0.000	0.000	0.000																
44	0.000	0.000	0.000	0.000															
45	0.000	0.000	0.000	0.000	0.000														
46	0.000	0.000	0.000	0.000	0.000	0.000													
47	0.007	0.007	0.007	0.007	0.007	0.007	0.007												
48	0.966	0.966	0.966	0.966	0.966	0.966	0.966	0.985											

Note: 1=CCFJ376686.1; 2=CGEF379963.1; 3=CGEF379969.1; 4=CGEF379970.1; 5=CKFJ376684.1; 6=CKFJ376685.1; 7=CTFJ915824.1; 8=CC001MA; 9=CC01BHR; 10=CC01KL; 11=CC01KLKG; 12=CC01SKAG; 13=CC01SNR; 14=CC01TB; 15=CC02BHR; 16=CC02KL; 17=CC02KLKG; 18=CC02SKAG; 19=CC02UNS; 20=CC03KLKG; 21=CC04SWS; 22=CC05UNS; 23=CC08UNS; 24=CG01JB; 25=CK03UNS; 26=CK04UNS; 27=CS01BHW; 28=CS01UNS; 29=CS02BHW; 30=CS02KNP; 31=CS03BHW; 32=CS03SWS; 33=CS04BHW; 34=CS05SWS; 35=CS06UNS; 36=CS07UNS; 37=CT01KNP; 38=CT01LHNP; 39=CT01SNP; 40=IK19R29P2; 41=IK19R31P4; 42=IK19R31P5; 43=IL19R16P1; 44=IL19R17P1; 45=IL19R18P1; 46=IL19R19P1; 47=IL19R19P2; 48=GSEF379993.1

Table 4.9: Genetic pairwise distance specimens of COII gene using Kimura 2 parameter model.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1															
2	0.007														
3	0.007	0.000													
4	0.093	0.100	0.100												
5	0.095	0.103	0.103	0.009											
6	0.098	0.095	0.095	0.004	0.013										
7	0.093	0.100	0.100	0.000	0.009	0.004									
8	0.000	0.009	0.009	0.099	0.098	0.104	0.099								
9	0.093	0.090	0.090	0.023	0.032	0.018	0.023	0.101							
10	0.002	0.009	0.009	0.090	0.093	0.095	0.090	0.000	0.090						
11	0.004	0.002	0.002	0.098	0.100	0.093	0.098	0.004	0.088	0.007					
12	0.007	0.004	0.004	0.095	0.098	0.090	0.095	0.004	0.085	0.004	0.002				
13	0.004	0.002	0.002	0.098	0.100	0.093	0.098	0.004	0.088	0.007	0.000	0.002			
14	0.000	0.007	0.007	0.093	0.095	0.098	0.093	0.000	0.093	0.002	0.004	0.007	0.004		
15	0.007	0.004	0.004	0.095	0.098	0.090	0.095	0.004	0.085	0.004	0.002	0.000	0.002	0.007	
16	0.007	0.004	0.004	0.095	0.098	0.090	0.095	0.004	0.085	0.004	0.002	0.000	0.002	0.007	0.000
17	0.009	0.007	0.007	0.098	0.100	0.093	0.098	0.006	0.088	0.007	0.004	0.002	0.004	0.009	0.002
18	0.004	0.002	0.002	0.098	0.100	0.093	0.098	0.004	0.088	0.007	0.000	0.002	0.000	0.004	0.002
19	0.002	0.009	0.009	0.090	0.093	0.095	0.090	0.000	0.090	0.000	0.007	0.004	0.007	0.002	0.004
20	0.002	0.009	0.009	0.090	0.093	0.095	0.090	0.000	0.090	0.000	0.007	0.004	0.007	0.002	0.004
21	0.002	0.009	0.009	0.090	0.093	0.095	0.090	0.000	0.090	0.000	0.007	0.004	0.007	0.002	0.004
22	0.002	0.009	0.009	0.090	0.093	0.095	0.090	0.000	0.090	0.000	0.007	0.004	0.007	0.002	0.004
23	0.002	0.009	0.009	0.090	0.093	0.095	0.090	0.000	0.090	0.000	0.007	0.004	0.007	0.002	0.004
24	0.100	0.098	0.098	0.007	0.011	0.002	0.007	0.106	0.020	0.098	0.095	0.093	0.095	0.100	0.093
25	0.093	0.100	0.100	0.002	0.007	0.007	0.002	0.099	0.025	0.090	0.098	0.095	0.098	0.093	0.095
26	0.093	0.100	0.100	0.002	0.007	0.007	0.002	0.099	0.025	0.090	0.098	0.095	0.098	0.093	0.095
27	0.004	0.002	0.002	0.098	0.100	0.093	0.098	0.004	0.088	0.007	0.000	0.002	0.000	0.004	0.002
28	0.009	0.007	0.007	0.098	0.100	0.093	0.098	0.006	0.088	0.007	0.004	0.002	0.004	0.009	0.002
29	0.007	0.004	0.004	0.095	0.098	0.090	0.095	0.004	0.085	0.004	0.002	0.000	0.002	0.007	0.000
30	0.007	0.004	0.004	0.095	0.098	0.090	0.095	0.004	0.085	0.004	0.002	0.000	0.002	0.007	0.000
31	0.009	0.007	0.007	0.098	0.100	0.093	0.098	0.006	0.088	0.007	0.004	0.002	0.004	0.009	0.002
32	0.007	0.000	0.000	0.100	0.103	0.095	0.100	0.009	0.090	0.009	0.002	0.004	0.002	0.007	0.004

Table 4.9 continued

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
33	0.007	0.004	0.004	0.095	0.098	0.090	0.095	0.004	0.085	0.004	0.002	0.000	0.002	0.007	0.000
34	0.098	0.095	0.095	0.004	0.013	0.000	0.004	0.104	0.018	0.095	0.093	0.090	0.093	0.098	0.090
35	0.095	0.093	0.093	0.009	0.013	0.004	0.009	0.101	0.023	0.093	0.090	0.088	0.090	0.095	0.088
36	0.117	0.114	0.114	0.103	0.111	0.098	0.103	0.113	0.098	0.114	0.112	0.109	0.112	0.117	0.109
37	0.117	0.114	0.114	0.103	0.111	0.098	0.103	0.113	0.098	0.114	0.112	0.109	0.112	0.117	0.109
38	0.109	0.106	0.106	0.119	0.121	0.114	0.119	0.102	0.119	0.106	0.104	0.101	0.104	0.109	0.101
39	0.109	0.106	0.106	0.119	0.121	0.114	0.119	0.102	0.119	0.106	0.104	0.101	0.104	0.109	0.101
40	0.109	0.106	0.106	0.119	0.121	0.114	0.119	0.102	0.119	0.106	0.104	0.101	0.104	0.109	0.101
41	0.098	0.096	0.096	0.022	0.027	0.018	0.022	0.104	0.013	0.096	0.093	0.090	0.093	0.098	0.090
42	0.093	0.090	0.090	0.022	0.032	0.018	0.022	0.101	0.013	0.090	0.088	0.085	0.088	0.093	0.085
43	0.231	0.228	0.228	0.224	0.227	0.218	0.224	0.233	0.228	0.234	0.225	0.228	0.225	0.231	0.228
16	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
17	0.002														
18	0.002	0.004													
19	0.004	0.007	0.007												
20	0.004	0.007	0.007	0.000											
21	0.004	0.007	0.007	0.000	0.000										
22	0.004	0.007	0.007	0.000	0.000	0.000									
23	0.004	0.007	0.007	0.000	0.000	0.000	0.000								
24	0.093	0.095	0.095	0.098	0.098	0.098	0.098	0.098							
25	0.095	0.098	0.098	0.090	0.090	0.090	0.090	0.090	0.009						
26	0.095	0.098	0.098	0.090	0.090	0.090	0.090	0.090	0.009	0.000					
27	0.002	0.004	0.000	0.007	0.007	0.007	0.007	0.007	0.095	0.098	0.098				
28	0.002	0.000	0.004	0.007	0.007	0.007	0.007	0.007	0.095	0.098	0.098	0.004			
29	0.000	0.002	0.002	0.004	0.004	0.004	0.004	0.004	0.093	0.095	0.095	0.002	0.002		
30	0.000	0.002	0.002	0.004	0.004	0.004	0.004	0.004	0.093	0.095	0.095	0.002	0.002	0.000	
31	0.002	0.004	0.004	0.007	0.007	0.007	0.007	0.007	0.095	0.098	0.098	0.004	0.004	0.002	0.002
32	0.004	0.007	0.002	0.009	0.009	0.009	0.009	0.009	0.098	0.100	0.100	0.002	0.007	0.004	0.004
33	0.000	0.002	0.002	0.004	0.004	0.004	0.004	0.004	0.093	0.095	0.095	0.002	0.002	0.000	0.000
34	0.090	0.093	0.093	0.095	0.095	0.095	0.095	0.095	0.002	0.007	0.007	0.093	0.093	0.090	0.090

Table 4.9 continued

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
35	0.088	0.090	0.090	0.093	0.093	0.093	0.093	0.093	0.007	0.007	0.007	0.090	0.090	0.088	0.088
36	0.109	0.112	0.112	0.114	0.114	0.114	0.114	0.114	0.100	0.103	0.103	0.112	0.112	0.109	0.109
37	0.109	0.112	0.112	0.114	0.114	0.114	0.114	0.114	0.100	0.103	0.103	0.112	0.112	0.109	0.109
38	0.101	0.098	0.104	0.106	0.106	0.106	0.106	0.106	0.116	0.119	0.119	0.104	0.098	0.101	0.101
39	0.101	0.098	0.104	0.106	0.106	0.106	0.106	0.106	0.116	0.119	0.119	0.104	0.098	0.101	0.101
40	0.101	0.098	0.104	0.106	0.106	0.106	0.106	0.106	0.116	0.119	0.119	0.104	0.098	0.101	0.101
41	0.090	0.093	0.093	0.096	0.096	0.096	0.096	0.096	0.020	0.020	0.020	0.093	0.093	0.090	0.090
42	0.085	0.088	0.088	0.090	0.090	0.090	0.090	0.090	0.020	0.025	0.025	0.088	0.088	0.085	0.085
43	0.228	0.231	0.225	0.234	0.234	0.234	0.234	0.234	0.221	0.224	0.224	0.225	0.231	0.228	0.228
	31	32	33	34	35	36	37	38	39	40	41	42	43		
31															
32	0.007														
33	0.002	0.004													
34	0.093	0.095	0.090												
35	0.090	0.093	0.088	0.004											
36	0.112	0.114	0.109	0.098	0.095										
37	0.112	0.114	0.109	0.098	0.095	0.000									
38	0.104	0.106	0.101	0.114	0.116	0.117	0.117								
39	0.104	0.106	0.101	0.114	0.116	0.117	0.117	0.000							
40	0.104	0.106	0.101	0.114	0.116	0.117	0.117	0.000	0.000						
41	0.093	0.096	0.090	0.018	0.018	0.101	0.101	0.122	0.122	0.122					
42	0.088	0.090	0.085	0.018	0.023	0.108	0.108	0.119	0.119	0.119	0.013				
43	0.225	0.228	0.228	0.218	0.221	0.245	0.245	0.237	0.237	0.237	0.218	0.227			

Note: 1=CC02UNS; 2=CC03KLKG; 3=CC01KLKG; 4=CS01BHW; 5=CS02KNP; 6=CS02BHW; 7=CS03BHW; 8=CT01SNP; 9=CKFJ384652.1; 10=CS05SWS; 11=CC01MA; 12=IK19R29P2; 13=CC01SNR; 14=CC01TB; 15=IL19R17P1; 16=CC05UNS; 17=CCFJ384635.1; 18=CC01SKAG; 19=CC02SKAG; 20=IK19R31P5; 21=IK19R31P4; 22=IL19R19P1; 23=CC01KL; 24=CS04BHW; 25=CS06UNS; 26=CS07UNS; 27=CC04SWS; 28=CCFJ384634.1; 29=CC02KL; 30=CC02BHR; 31=CC01BHR; 32=CC02KLKG; 33=CC08UNS; 34=CK04UNS; 35=CK03UNS; 36=CTAJ854170.1; 37=CTAJ854169.1; 38=CGEF379952.1; 39=CGEF379945.1; 40=CGEF379951.1; 41=CSAJ854163.1; 42=CSAJ854165.1; 43=GSEF379957.1

4.3.3 Phylogenetic Analysis

In this study, phylogenetic analyses of *Coptotermes* based on the DNA sequence of a partial mitochondrial COII, 16S and 12S gene is presented. Phylogenies derived using distance, maximum likelihood, and maximum parsimony showed similar topology for all genes used.

Maximum parsimony analysis of COII, 12S and 16S nucleotide matrices resulted in a total of 100 (initial Max Trees setting = 100) equally most parsimonious tree respectively (Figure 4.1: length = 205, consistency index (CI) = 0.7092, retention index (RI) = 0.9394; Figure 4.2: length = 210, consistency index (CI) = 0.9143, retention index (RI) = 0.9427; Figure 4.3: length = 251, consistency index = 0.8845, retention index (RI) = 0.9134). The best model for the maximum likelihood analysis of the 12S gene was “TrN+G” with the following parameter settings: base = (0.3453, 0.1782, 0.1715), Nst=6, Rmat = (1.0000, 4.6925, 1.0000, 1.0000, 10.0137), rates = gamma shape = 0.6950 and pinvar = 0. For the 16S gene, the selected model was “HKY+G” with the following parameter settings: base = (0.3317, 0.2158, 0.1522), Nst = 2, TRatio = 2.4318, rates = gamma shape = 2.6230 and pinvar = 0. For the COII gene, the “TIM2+I+G” model was selected with the following parameter settings: base = (0.3497, 0.3221, 0.1369), Nst = 6, Rmat = (2.6590, 13.2788, 2.6590, 1.0000, 47.5519), rates = gamma shape = 0.3380, and pinvar = 0.2280. A single tree was recovered for 12S and 16S gene (-Ln L= 1166.21409 for 16S and -Ln L=1150.22234 for 12S). Five trees were recovered for COII gene (-Ln L=1533.02565). The most parsimonious trees with topology similar to that generated by the ML and NJ analysis were chose.

Four major clades within the *Coptotermes* spp. were apparent in COII, 12S and 16S gene (Figure 4.1, Figure 4.2 and Figure 4.3 respectively). All three gene trees consist of

Clade I: *C. curvignathus*, Clade II: *C. gestroi*, Clade III: *C. sepangensis* and *C. kalshoveni* and Clade IV: *C. travians*. Clade I composed of all individuals that were identified morphologically as *C. curvignathus* in current study were clustered together and this cluster was consistent with the morphometric analyses conducted in the Chapter Two. A common Asian subterranean termite, *C. gestroi* in clade II formed a sister clade with rubber tree termite, *C. curvignathus* in all MP trees. Clade III consists of combined individuals of *C. sepangensis* and *C. kalshoveni*, and the cluster was found inconsistent to DFA analyses result in which two different cluster with the overlapping measurement characters were formed for both species as shown in Chapter Two. Clade IV consist of all individuals of that were morphologically identified as *C. travians* and was parallel with the phylogenetic analyses in current chapter and DFA analyses in Chapter Two. Under Clade III (Figure 4.2 and Figure 4.3), one individual of *C. travians* was grouped with other individuals of *C. sepangensis* and *C. travians*. It may have been misidentified since the individual contain almost similar sequences to *C. sepangensis* and *C. kalshoveni* rather than to *C. travians* in clade IV.

Close relationship was observed between *C. sepangensis* and *C. kalshoveni* (Clade III) in COII gene and 16S gene with high bootstrap value of 100% and 74% respectively, while moderate bootstrap value of 52% in 12S gene. Interestingly, two sub-clades under Clade III of *C. sepangensis* and *C. kalshoveni* were generated for all the genes, in which individuals from Sarawak and individuals from Peninsular Malaysia were cluster separately.

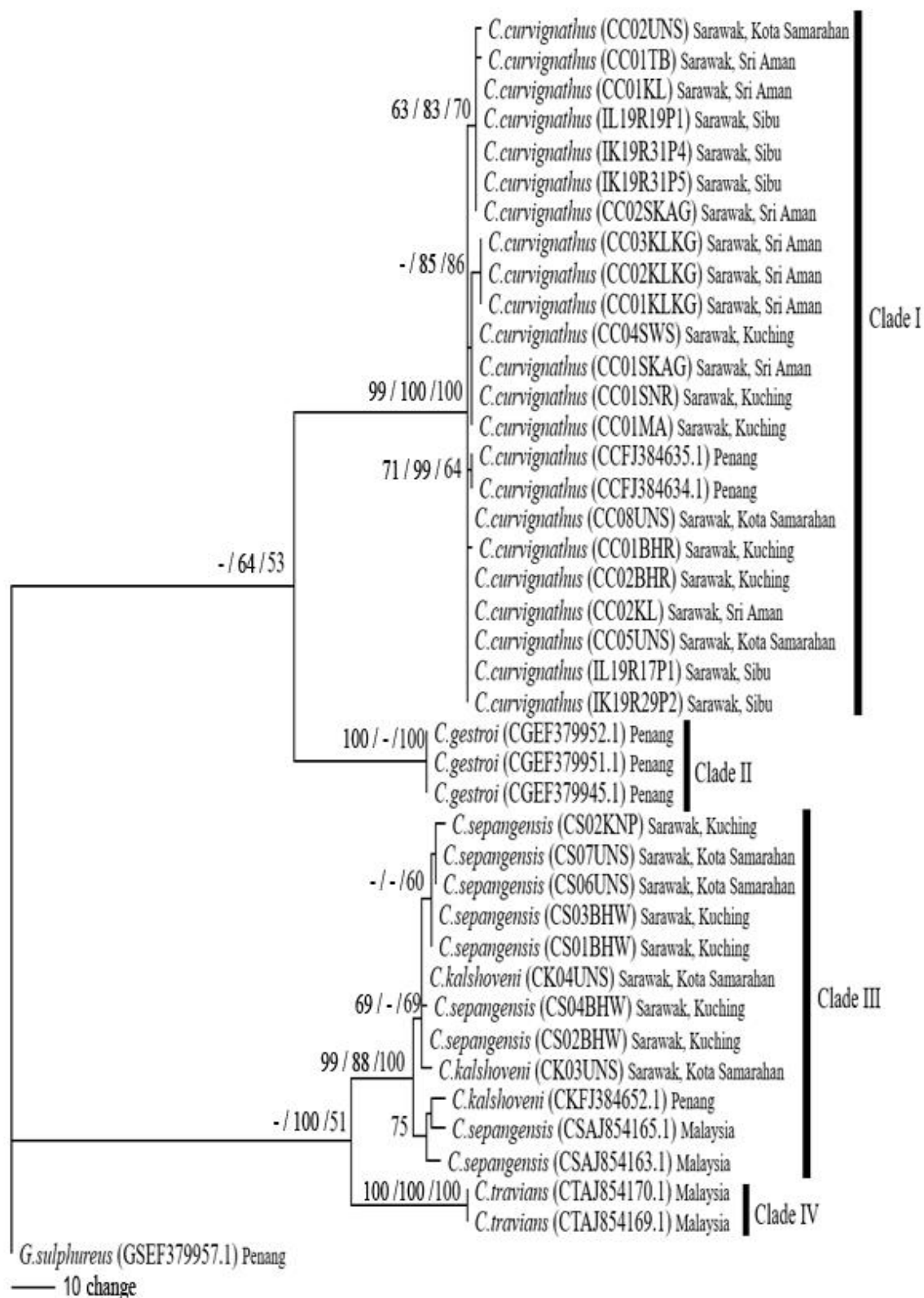


Figure 4.1: The best parsimonious tree of COII gene. Support values at $\geq 50\%$ only were selected and shown at above of the branches. The bootstrap value was stated in the order of maximum likelihood, neighbour joining and maximum parsimony.

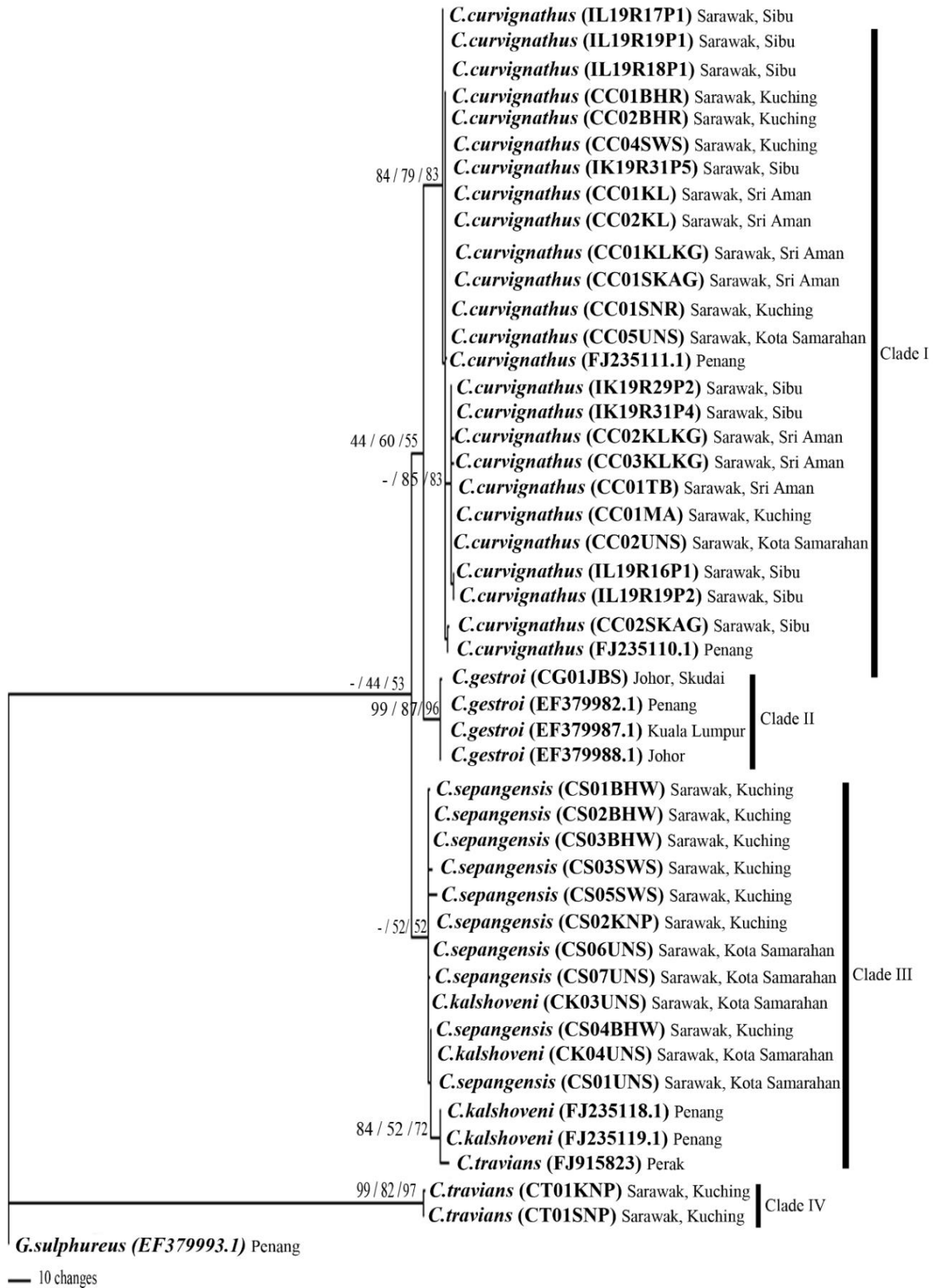


Figure 4.2: The best parsimonious tree of 12S gene. Support values at $\geq 50\%$ only were selected and shown at above of the branches. The bootstrap value was stated in the order of maximum likelihood, neighbour joining and maximum parsimony.



Figure 4.3: The best parsimonious tree of 16S gene. Support values at $\geq 50\%$ only were selected and shown at above of the branches. The bootstrap value was stated in the order of maximum likelihood, neighbour joining and maximum parsimony.

4.4 Discussion

Genus *Coptotermes* are notably recognized as aggressive as it have high tendency to become a major pest to residential, plantations and nuisance to humans. Current available treatment in pest management for this urban and plantation pest is by using chemical pesticide and baits becomes favourable to suppress and eliminate the colony termites. However, the good termite management only can be achieved when suitable and right treatment is chosen which depends on the type of the pest species involved. Therefore, identification of termite pest especially subterranean termite pest using morphology and molecular will facilitate the development of termite control and may improve termite pest response to currently available termite control.

In this study, three mitochondrial genes of 12S, 16S and COII were used to infer the *Coptotermes* relationship at the species level. From the phylogenetic analyses, it can be concluded that four clades in the *Coptotermes* spp. which includes: (1) *C. curvignathus*, (2) *C. gestroi*, (3) *C. sepangensis* and *C. kalshoveni* and (4) *C. travians* from three individual mitochondrial gene sequences in this study.

All three mitochondrial genes were A+T rich (62.7% A+T in 12S, 60.6% A+T in 16S and 55.2% A+T in COII) as shown in Table 4.6. Of the three gene fragments, COII gene was observed to have the most variable and informative site and 12S gene was the most conserved. The base composition content in the current study was biased towards A+T content in ribosomal and protein coding gene. The biases in base composition of this taxa very similar with other taxa of insects, with a high proportion of A+T nucleotide (Sun *et al.*, 2009).

C. curvignathus was described as the largest *Coptotermes* spp. based on the body size and readily distinguishable by having the most incurved mandibles and have the largest

body size. *C. curvignathus* specimens collected in this study agreed with the original description of Thapa (1981) and Tho (1992). The DNA sequences of *C. curvignathus* presented here were clearly indicated the group separation of this species with other species and was supported with the previous morphological description as discussed in Chapter Two. A current findings showed a great size variation had occurred among the samples of *C. curvignathus* inter- and intra- colony. Therefore, it can be suggested that size variation may create ambiguities for taxonomy studies from morphological identification. (Takematsu *et al.*, 2006; Yeap *et al.*, 2009).

Morphological analysis and molecular phylogenetic analyses suggested that *C. sepangensis* have a potential to become a junior synonym to *C. kalshoveni*. The morphology of *C. sepangensis* was parallel with *C. kalshoveni* and being hardly distinguished by morphological characters and overlapped greatly based on morphometric analyses in Chapter Two. The genetic distances proved a closed relationship between these two species with low genetic diversity (0% to 3.2% in COII, 0% to 4.2% in 16S and 0% to 2.2% in 12S gene) and formed a common clade with strong bootstrap support (100% in COII gene). The closed relationships between *C. sepangensis* and *C. kalshoveni* implied from these three genes sequences were largely in agreement with results previously obtained by using similar DNA mitochondrial gene whereby *C. sepangensis* and *C. kalshoveni* was clustered together although they represented from varied locality (Yeap *et al.*, 2009; Lee *et al.*, 2015). A morphology identification by using an alates samples instead of single soldier caste might be necessary to further resolve the relationship between these two species (Yeap *et al.*, 2009). Furthermore, the original description and type specimens (if available) should be included to provide evidence for a more robust identification of each species.

The samples of *C. sepangensis* and *C. kalshoveni* from West Malaysia (Penang) were found to be closely related and fall into same sub-clade under clade III, yet it observed to diverged and formed subdivision with represented species of *C. sepangensis* and *C. kalshoveni* from East Malaysia (Sarawak). The South China Sea boundary existed between Borneo and Peninsular Malaysia could be the factor of this separation. The previous study had found that *Coptotermes* spp. from Peninsular Malaysia is closely related to the other *Coptotermes* spp. from Thailand and Singapore (Yeap *et al.*, 2009). Thailand, Malaysia and Singapore shared a well-developed transportation such as road highway system, railway and port system (Jenkins *et al.*, 2007). These numerous modes of transportation can provide coincidental distribution of *Coptotermes* spp. across Thailand, Malaysia and Singapore. However, between Sarawak and Peninsular, the mode of transportation only restricted to the sea and air transportation. The high inspection of cargo in port may lead to difficulties of this spreading across the two regions which may explain the intraspecies genetic variation among *C. kalshoveni*, *C. sepangensis* and *C. travians* samples from Sarawak and peninsular Malaysia. However, this will certainly require more samples of *C. sepangensis*, *C. kalshoveni* and *C. travians* from East Malaysia and West Malaysia to further substantiate the current findings.

As discussed earlier in Chapter Three, the separation of *Coptotermes* spp. was noted from the similarities and differences of morphological features. These molecular data make it possible to study the aspect of ecological differences of the species which providing some insights into their basic biology. *C. curvignathus* and *C. gestroi* represent as structural and forestry pest in Southeast Asia and both species apparently have a wide distribution overlap in their native range (Chouvenc *et al.*, 2016). The current molecular data showed that *C. curvignathus* and *C. gestroi* have a closed genetic relationship compared to the others

Coptotermes spp.. It may be suggested that this relationship can be related to similar behavioural and biological as explained by the previous findings from Yeoh and Lee (2007) on the tunnelling activity, wood activity and survivorship of the *C. gestroi*, *C. curvignathus* and *C. kalshoveni* in the laboratory. *C. curvignathus* and *C. gestroi* was reported to have same tunnelling patterns which were wide and highly branched, and both species are practicing aggressive searching pattern. In other words, they are still searching for other food sources even after they had encountered their first food source. These indirectly explained why these species were more frequently found to be infesting building and tree crops (Kirton and Azmi, 2005).

At the baseline of current data, it can be deduced that COII gene generates a more informative tree topology compared to 16S and 12S DNA sequences. The mitochondrial protein coding gene have faster evolutionary rates compared to ribosomal RNA genes, thus it has been used to examine phylogenetic relationship in relatively lower categorical levels such as families, genera, species or populations (Mandal *et al.*, 2014). As comparison of 12S and 16S gene, the current study showed that 12S gene was much more conserved than 16S gene. The highly conserved 12S gene was more suitable to illustrate the phylogeny of higher categorical levels such as in phyla or subphyla (Ballard *et al.*, 1992) while 16S gene usually used for phylogenetic studies at mid-categorical levels such as in families or genera (Kambhampati *et al.*, 1996).

4.5 Summary and Conclusion

The present results from this study can be summarized by the following four subclades with strong relationship among the *Coptotermes* spp, from Sarawak and East Malaysia and explained based on three mitochondrial genes: (I) *C. curvignathus*, (II) *C. gestroi* (III) *C. sepangensis* and *C. kalshoveni* and (IV) *C. travians*.

As conclusion, through a combination of mitochondrial markers and morphological characters, we identified that the species cluster in DFA analyses corresponded well with the molecular phylogenetic tree constructed except for *C. sepangensis* and *C. kalshoveni*. The phylogenetic tree generated from the three mitochondrial genes revealed that the populations of *C. sepangensis* and *C. kalshoveni* could be divided into two geographical groups: I: East Malaysia populations and II: West Malaysia populations. Based on the Chapter Two and Three, phylogenetic analyses with the support of morphometric data suggested that *C. sepangensis* has potential to become a junior synonym to *C. kalshoveni*. However, a further investigation should be performed to relegate the junior synonym status, such using an alates samples instead of single soldier caste for morphology (Yeap *et al.*, 2009). Furthermore, the original description and type specimens (if available) should be included to provide evidence for a more robust identification of each species.

CHAPTER 5

**IDENTIFICATION AND DEVELOPMENT OF POLYMORPHIC
MICROSATELLITE MARKERS OF *Coptotermes curvignathus* (HOLMGREN)
(BLATTODEA: RHINOTERMITIDAE)
IN OIL PALM PLANTATION**

5.1 Introduction

Rubber termite (*Coptotermes curvignathus*) are relatively typical among subterranean termite which often cause damage to the built environment. The incidence of this species infestation in Malaysia occurred in the early 1900's and was reported to infest on rubber trees (*Hevea brasiliensis*). Consequently, this xylophagous insect are considered, by far to be a destructive pest to buildings in urban dwelling and also agricultural nuisance to oil palm plantation. The species is indigenous from Vietnam and Indonesia (Krishna *et al.*, 2013), but also can be found throughout South East Asia (SEA). Despite their economic importance, many fundamental features of the ecology and population biology of subterranean termites remain poorly understood. The main obstacle to progress in these areas has been the difficulty of identifying and delineating individual colonies by using traditional field methods.

The application of high resolution microsatellite molecular marker to study infestation dynamics and population genetic structure of *C. curvignathus* can be a great tool to understand their infestation history and patterns (Paupy *et al.*, 2008; Perez de Rosas *et al.*, 2008; Yoon *et al.*, 2008; Piccinali *et al.*, 2009; Zhu *et al.*, 2010). The use of microsatellite molecular marker in applied studies to identify large numbers of individual colonies and

track them over time allows for more rigorous field evaluations of insecticide treatments than was previously possible (Evans *et al.*, 1999; , Husseneder *et al.*, 2007; , Vargo *et al.*, 2003). By comparing the genotypic profiles of colonies present before and after treatment, we can determine whether termites that reinfest treated areas or bait stations are remnants of targeted colonies, invading neighboring colonies, or previously undetected hidden colonies. For example, studies of *C. formosanus* found that foraging areas of colonies eliminated by baiting with chitinase inhibitors were often reinvaded, and that the source of reinfesting termites was either known neighboring colonies or hidden colonies (Husseneder *et al.*, 2007; Messenger, 2005). Similar results were obtained in baiting studies with *Reticulitermes* spp. (Vargo, 2003). Although reinfestation after treatment is common in areas with high colony density, reinfestation rates decline with repeated treatments (Husseneder, 2007; Vargo, 2003), indicating such treatments have a population-level effect, at least on a small scale.

To date, microsatellite markers within the genus (for Asian species) are only available for *C. formosanus* Shiraki (Vargo and Henderson, 2000) and *C. gestroi* Wasmann (Yeap *et al.*, 2009) and no available microsatellite marker was reported so far specifically for *C. curvignathus*. However, primers developed for one species will often work for other species within the same genus (Pamilo *et al.*, 1997; Vargo & Husseneder, 2011). For example, microsatellites identified in *Zootermopsis nevadensis*, were successfully amplified in both *Zootermopsis augusticolis* and *Zootermopsis nuttinginuttingi* (Aldrich & Kambhampati, 2004; Booth *et al.*, 2012). It has even been noted that occasionally amplification is possible across different families. Goodisman *et al.* (2001) was able to successfully amplify loci developed for *Macrotermes darwinensis*, in *Coptotermes lacteus*, *Cryptotermes dudleyi*, *Neotermes insularis*, *Porotermes adamsoni* and *Hodotermes mossambicus*.

In this present study, a cross species amplification from existing microsatellite markers was not deployed, but a novel and species-specific microsatellite polymorphic markers is developed from *C. curvignathus*. One drawback of cross-species amplification is that success rates decline with evolutionary distance between the target species and source species (Primmer *et al.*, 2005; Dawson *et al.*, 2005).

The isolation of microsatellite markers was done via Next Generation Sequencing (NGS) technique based on the Roche 454 GS-FLX Titanium pyrosequencing platform as it holds great promises in terms of less time consumption, low cost and high throughput compared to enrichment protocol (Rothberg and Leamon, 2008)

5.2 Materials and methods

5.2.1 Samples collection, preservation and identification

C. curvignathus soldier's termite were collected from oil palm plantation area in Sibul, Sarawak in. About 10 to 30 individuals of soldier's termite from infested oil palm tree were collected by using a soft forceps and were preserved in 100% ethanol.

The identification of termites to the species level was done using taxonomic keys published by Thapa (1981), Tho (1992), Ahmad (1965) and Krishna *et al.* (2013).

5.2.2 Development of novel molecular markers from the genome of *C. curvignathus* using next generation sequencing Roche 454

The development of novel molecular markers from the genome of *C. curvignathus* was performed as follows: (1) DNA extraction of pool genomic DNA (2) Assessment of genomic DNA quality and (3) 454 pyrosequencing.

5.2.2.1 DNA Extraction of pool genomic DNA

The total genomic DNA was extracted on the head and abdomen of 15 worker termite per colony by using DNeasy tissue kit (QIAGEN GmbH, Hilden, Germany). Alcohol-preserved specimens was allowed to dry on filter paper. Then, the samples were put into 1.5ml microcentrifuge tube with 180 µl buffer ATL, 20 µl Proteinase K and incubated in water bath at 56°C for 45 min. 200 µl buffer AL then was added and the solution was precipitated with 100% ethanol. Mixtures was centrifuged at 8000 rpm for 1 min in Dneasy Mini spin column, following by adding 500 µl each of buffer AW1 and AW2. Then, it was centrifuged at 8000 rpm for 1 min and 14000 rpm for 1 min respectively. Total DNA obtained was added with 200 µl buffer AE, centrifuged at 8000 rpm for 1 min and stored at -20°C for further analyses.

5.2.2.2 Assessment of genomic DNA Quality

Assessment of DNA yield and quality was measured through (1) UV spectrophotometer (2) UV fluorometry (3) Agarose gel electrophoresis and (4) Microfluidic gel electrophoresis.

5.2.2.2.1 UV spectrophotometer by NanoDrop 2000 UV Spectrophotometer

The optical density (OD) and concentration of pooled gDNA were measured using NanoDrop 2000 Spectrophotometer V1.0 (Thermo Fisher Scientific, USA). Blank solution was established by using 1.5 µl of Buffer CE. It was then followed by pipetting 1.5 µl of samples. A good DNA quality has an OD_{260}/OD_{280} ratio of 1.8 – 2.0, meanwhile concentration of 10.0 ng/µl is sufficient enough for DNA product of termite to ensure a success downstream application.

5.2.2.2.2 UV fluorometry by Qubit dsDNA BR Assay

The concentration of gDNA were determined using UV absorbance performed by Qubit dsDNA BR Assay. All assay reagents including dye, Qubit® reagent and Qubit®buffer were incubated at room temperature for 30 min prior usage. Two assay tubes for standards (Standard 1 and Standard 2) and one assay tube for sample were set up. Qubit® working solution were prepared by diluting Qubit® reagent 1:200 in Qubit®buffer. Two sets of 190 µl and one set of 198 µl of working solution were prepared for standard assay tubes and sample assay tube respectively. All assay tubes were vortexed for 2 to 3-s. The tubes were then incubated for 2 min at room temperature in dark. The tubes were inserted in the Qubit® Fluorometer and readings were taken.

5.2.2.2.3 Agarose gel electrophoresis

1.0% (w/v) was prepared by dissolving 0.35 g of agarose powder in 35 ml of 1.0X TAE buffer (Biotechnology Grade, Fisher Scientific, New Jersey) followed by heating for 1 min and 30-s in a microwave oven until it was fully dissolved. The dissolved mixture was let to cool about 50 °C before addition of 0.4 µl of SYBR® Green I nucleic acid gel stain 10 000X (Invitrogen, USA). Dissolved solution was then poured into a gel chamber and let to set for about 30 min. 2 µl of genomic DNA was mixed with 1 µl of glycerol loading dye [0.25 M EDTA, 0.1 % (w/v) SDS, 0.01 % (w/v) bromophenol blue and 50 % glycerol] and each sample was loaded individually into the wells of the agarose gels. GeneRuler™ 1 Kb (Fermentas, USA) was used in size determination of the DNA fragments. Electrophoresis was carried out using Mini-Sub Cell ® GT (Bio-Rad Laboratories, California) at 90 V (BIO-RAD PowerPac™ Basic, Singapore) for 1 h in 1.0X TAE (Biotechnology Grade, Fisher Scientific, New Jersey) buffer. Subsequently, the resolved genomic DNA bands were

visualized under UV light on the UV transilluminator (BIO-RAD Universal Hood II, California).

5.2.2.2.4 Microfluidic gel electrophoresis by HS DNA Chip Bioanalyzer

A new High Sensitivity DNA chip was placed on the priming station. 9 µl of the gel-dye mix was pipetted in each of the wells of the chip. 5 µl of green-capped High Sensitivity DNA marker was then pipetted into the well which was marked with the ladder symbol and each of the 11 sample wells. It was followed by pipetting 1 µl of yellow-capped High Sensitivity DNA ladder into the well which marked with the ladder symbol. 1 ul of samples were then pipetted into the sample wells. The chip was placed horizontally in the adapter of vortex mixer and was ensured that no damage was made to the buldge that fixed the chip during vortexing. The chip was vortexed for 60 s at 2400 rpm. The prepared chip was then run on a Bioanalyzer instrument (HS DNA Bioanalyzer Agilent) under default settings. The results were then saved and recorded.

5.2.2.3 454 Pyrosequencing

The pooled genomic gDNA sample that passed the quality assurance issued by First Base Laboratory Sdn. Bhd was carefully sealed and sent to Macrogen Inc, Korea for high-throughput sequencing in a microfabricated high-density picolitre reactor. The sample was subjected to shotgun sequencing using the Roche 454 Genome Sequencer FLX+ (Roche Applied Science, Penxberg, Germany), performed by Macrogen Inc, South Korea. The raw sequence reads in FASTA format from NGS output were then screened for microsatellite repeat motifs.

5.2.3 Microsatellite identification and designation of primer

The microsatellite repeats of ≥ 8 , ≥ 6 and ≥ 5 for each dinucleotide, trinucleotide, and tetranucleotide were identified respectively by using the program Msatcommander version 0.8.2 (Faircloth, 2008). Primers were designed using Primer3 version 0.4.0 (Rozen and Skaletsky, 1999) in-built within MSATCOMMANDER program. Primer pairs were designed based on following characteristics as suggested by Faircloth (2008), i.e. (i) amplification product between 100 to 500bp, (ii) an optimal T_M of 60.0 °C (range 57.0 – 62.0 °C) (iii) possess no greater than 5.0 °C difference in T_M (iv) optimal condition of 50% GC content (v) have at least a 1-bp GC clamp (vi) low levels of self- or pair complementarity and (vii) have maximum end-stability (ΔG) of 8.0.

The primer pairs were checked for its GC content and stability to enhance rate of success amplification. Primer pairs which have maximum end-stability (ΔG) of 8.0 and GC content of $\geq 50\%$ were screened and chosen. Complementarity of primer pairs and hairpin formation were also checked using an online software <http://biotools.nubic.northwestern.edu/OligoCalc.html>. The desired primer pairs with GC content of $\geq 50\%$, maximum-end stability (ΔG) of 8.0, no self- or pair complementarity and hairpin formation were finally subjected to BLAST nucleotide search. The primers that fulfilled all criteria were then purchased.

5.2.4 Microsatellite Primer screening and optimization

Sixty final designed primer pairs were selected for preliminary test of polymorphism from four different sample locations collected within the Sarawak region (Sri Aman, Sibul, Kuching and Kota Samarahan) in Malaysia. PCR were carried out in 12.5- μ l total volumes, each containing ≈ 50 ng of genomic DNA; 1X Master Mix (Kapa HiFi Hotstart PCR); 0.1 to

0.2 μ M of forward and reverse primers, and sdH₂O added up to final volume (Table 5.1). PCR Amplification was conducted on thermal cycler (The Applied Biosystems Veriti™) with following parameters as shown in Table 5.2.

Table 5.1: PCR recipe.

Solution	Initial	Final	0.1 of primer μl	0.2 of primer μl
Sterile distilled water, sdH ₂ O	NA	Up to 12.5 μ l	5.5	5.25
KAPA	2X	1X	6.25	6.25
Forward primer	10 μ M	0.1 – 0.2 μ M	0.125	0.25
Reverse primer	10 μ M	0.1 – 0.2 μ M	0.125	0.25
DNA		~10 ng	0.5	0.5
Total			12.5	12.5

Table 5.2: PCR profile.

Step	Temp.	Time	No cycles
Pre Denaturation	95°C	3 min	1
Denaturation	95°C	30 sec	35
Annealing	55°C to 65°C	30 sec	
Extension	72°C	1 min	
Final extension	72°C	5 min	1
Soak	4°C	∞	

5.2.5 Electrophoresis on 6% Polyacrylamide Gel

The amplified PCR products were tested prior to fragment analysis by electrophoresis on 6.0% non-denaturing polyacrylamide gels to check its DNA polymorphism. 6.0% non-denaturing polyacrylamide gel was prepared using materials as shown in Table 5.3. The reagents were mixed and poured carefully between two glass plates. A comb was inserted between the glass plates and was left standing for 40 minutes until it was polymerized completely. Next, the glass plates were placed into vertical electrophoresis unit (Fisherbrand, USA) without a comb. Then, 1X TBE buffer was poured inside the chamber until it reached the maximum level. 5 μ l of 20 bp DNA ladder (Lonza Rockland,

Inc., USA) was used as a marker and 6 µl of the PCR product was loaded into each well and was run at 160V for 5 hours. A post-staining with ethidium bromide (EtBr) was conducted afterwards to detect the PCR products under UV transilluminator 325. Primer pairs that produced scorable, unambiguous PCR products of the expected size, and that demonstrated allelic variation were selected for further testing.

Table 5.3: Reagent and their concentration used for preparation of 6.0% non-denaturing polyacrylamide gels.

Reagent	Volume
Sterile distilled water, sdH ₂ O	25.56 ml
Acrylamide/Bisacrylamide 19:1	6.0 ml
5X TBE	8.0 ml
10 % (w/v) Ammonium persulfate (APS)	400 µl
TEMED	40 µL
Total	40 ml

5.2.6 Multiplex PCR group designation and optimization

Total of 15 markers were identified as polymorphic using 6% PAGE. Ten out of 15 markers were selected for multiplex PCR (mPCR) groupings according to the size and pair complementarity to enhance amplification efficiency.

An online software, Multiple Primer Analyzer (Thermo Fisher Scientific) was used to assess the possibility of efficient mPCR groupings.

Primer pairs were multiplexed into three multiplex groups as shown in Table 5.4. The resulting polymorphic loci were evaluated across a total of 63 individuals (Table 5.5) from Sibü (five colonies) and Kuching (one colony) by using fluorescently labelled primers that incorporates the dyes 6-FAM, HEX or TAMRA-S.

Table 5.4: Multiplex group and labelled forward or reverse primers with fluorescent dye.

Multiplex Group	Primer	Size (bp)	Label fluorescent dye
A	T08	323	Forward-HEX
	T46	225	Reverse-6-FAM
	T25	160	Forward-HEX
B	T34	410	Forward-HEX
	T04	375	Forward-6-FAM
	T14	196	Forward-HEX
C	T49	307	Reverse-HEX
	T44	246	Forward-6-FAM
	T40	170	Forward-HEX
	T19	167	Forward-TAMRA-S

Table 5.5: Localities, population Id and total number of individuals used.

Localities	Population Id	No of individuals
Sibu	B20R79P13	10
Sibu	B20R45P03	10
Sibu	D20R159P04	11
Sibu	D21R143P06	11
Sibu	D22R128P15	11
Kuching	BHR2	10
Total		63

Amplification of mPCR groupings were done in a total reaction of 20 µl reaction consisting of sdH₂O, 1X KAPA HiFi Hotstart Readymix (KAPABIOSYSTEMS, Massachusetts), 0.1 – 0.2 µM of primers and ~10 ng of DNA template. PCR conditions for these loci were outlined in Table 5.6 .Quality of PCR products were assessed on an agarose gel electrophoresis and 6.0% polyacrylamide gel electrophoresis.

Table 5.6: PCR profile.

Step	Temp.	Time	No cycles
Denaturation	95°C	3 min	1
Denaturation	95°C	30 sec	35
Annealing	65°C ^a , 60°C ^b	30 sec	
Extension	72°C	1 min	
Final extension	72°C	5 min	1
Soak	4°C	∞	

Note: ^a Annealing temperature used for multiplex group A (T25, T46, T08) and group C (T49, T44, T40, T19)

^b Annealing temperature used for multiplex group B (T14, T04, T34)

5.2.7 Spectral optimization and fragment analysis

Amplified mPCR products of three plex groups were sealed carefully and submitted to First BASE Laboratories Sdn. Bhd. (Selangor, Malaysia) for spectral optimization which determined the dilution factor of each grouping. The dilution factors for each mPCR group were necessary for fragment analysis.

A total of 189 individuals were genotyped for population genetics (Table 5.7). The PCR samples were prepared in 96-well PCR plate and sent to First BASE Laboratories Sdn. Bhd. (Selangor, Malaysia) for fragment analysis.

Table 5.7: Total number of individuals used for genotyping.

Localities	Population Id	No individuals used for genotyping			
		Mplex A	Mplex B	Mplex C	Total
Sibu	B20R79P13	10	10	10	30
Sibu	B20R45P03	10	10	10	30
Sibu	D20R159P04	11	11	11	33
Sibu	D21R143P06	11	11	11	33
Sibu	D22R128P15	11	11	11	33
Kuching	BHR2	10	10	10	30
Total individuals					189

The results of fragment analysis were then scored using scored using Peak Scanner version 1.0 (Applied Biosystems Inc., Foster City, CA).

5.2.8 Microsatellite Data Analysis

The allelic scoring was performed in PeakScanner software version 1.0 (Applied Biosystems Inc., Foster City, CA) by using GS500 (-250) as a size standard. The highest peak was determined as actual peak while a frequently repeating and ambiguous shorter peaks were considered as stutter (Tautz, 1989). The software CONVERT (Glaubitz, 2004) was used to convert specific data formats for different softwares. The MICRO-CHECKER 2.2.1 software (Van Oosterhout *et al.*, 2004) was used to identify possible genotyping errors and null alleles (1000 randomizations). GENEPOP (<http://genepop.curtin.edu.au/>) was used to test the deviations from Hardy-Weinberg equilibrium (HWE) for each locus and linkage disequilibrium between all pairs (exact test, 1000 iterations). Cervus 3.0 software (Kalinowski *et al.*, 2007) was used to calculate the observed (H_o) and expected (H_e) heterozygosity and number of alleles.

5.3 Results

5.3.1 Development of microsatellite marker

5.3.1.1 Quantification and qualification of gDNA samples

The assessment of DNA integrity and DNA quality is a critical first step in obtaining meaningful data prior to NGS. Two different parts of termite were used in a standard DNA extraction method for the starting materials, i) head and ii) abdomen. The quantity and quality of genomic DNA samples were evaluated by using UV spectrophotometry and UV absorbance. Then, the integrity of the samples were evaluated by agarose gel electrophoresis and capillary electrophoresis.

NanoDrop 2000 Spectrophotometer V1.0 (Thermo Fisher Scientific, USA) was used to assess the quantity and quality of gDNA by UV spectrophotometry. The range of OD260/OD280 for gDNA samples were 1.94 and 1.79, whereas the concentration were 117.1 and 241.8 ng/μl (Table 5.8). These results depicted that DNA extracted from the head had low level of protein contamination compared to abdomen. NanoDrop concentration values were higher than Qubit for both head and abdomen samples. The concentration of gDNA from the both samples for Nanodrop and Qubit readings were considerably high and followed the quality control issued by the service provider.

Table 5.8: Comparison of gDNA according to NanoDrop and Qubit quantification.

No	Sample	Nanodrop 2000		Qubit (ng/μl)	Yield (μg)
		Purity (OD)	Concentration (ng/μl)		
1	Head	1.94	117.1	74.4	2.604
2	Abdomen	1.79	241.8	238.0	8.610

To confirm this observation, the extraction products were loaded on agarose gel and microfluidic gel electrophoresis to check the integrity of the head and abdomen extracts. All gDNA samples were quantified on 1.0% (w/v) agarose gel pre-stained with SYBR® Green I nucleic acid gel stain 10 000X (Invitrogen, USA) before observing under UV transilluminator. The agarose results showed that both of head and abdomen samples had potential of DNA degradation which resulted in smearing (Figure 5.1). To verify these results, the samples were assessed with HS DNA Chip Bioanalyzer.

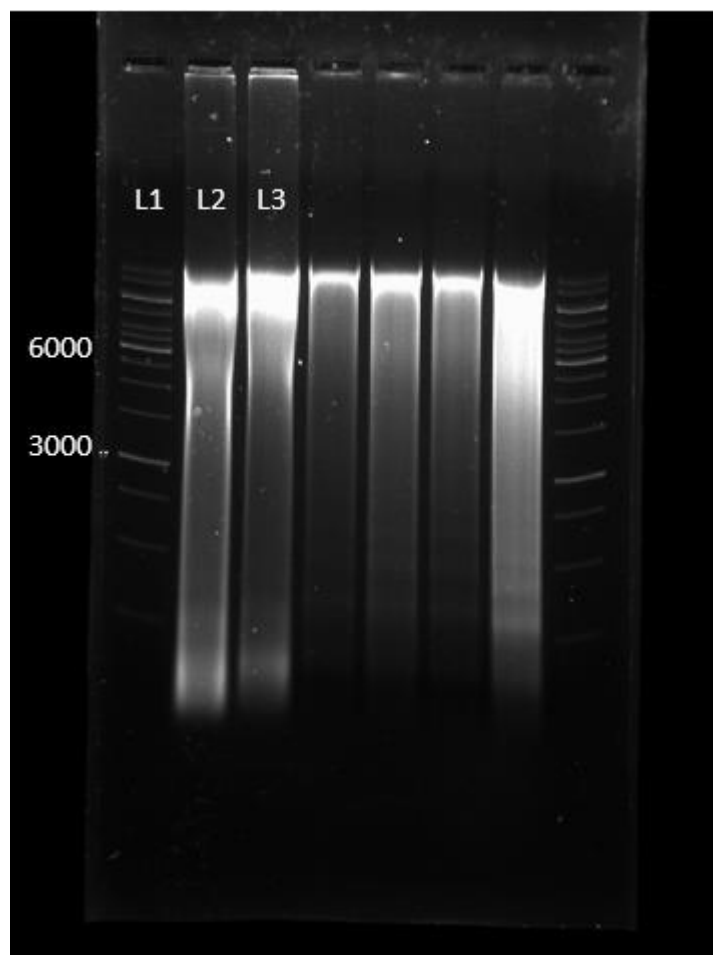


Figure 5.1: Gel agarose electrophoresis image 90V of *C. curvignathus*. **Lane 1:** GeneRuler™ 1Kb (Fermentas, USA). **Lane 2:** DNA extraction product from head. **Lane 3:** DNA extraction product from abdomen.

Bioanalyzer results showed different interpretation from standard 1.0% agarose gel. The result of head sample showed size of relatively 2000 to 3000 bp. The abdomen sample showed no single intact of DNA with significant smearing on the gel preparation (Figure 5.2).

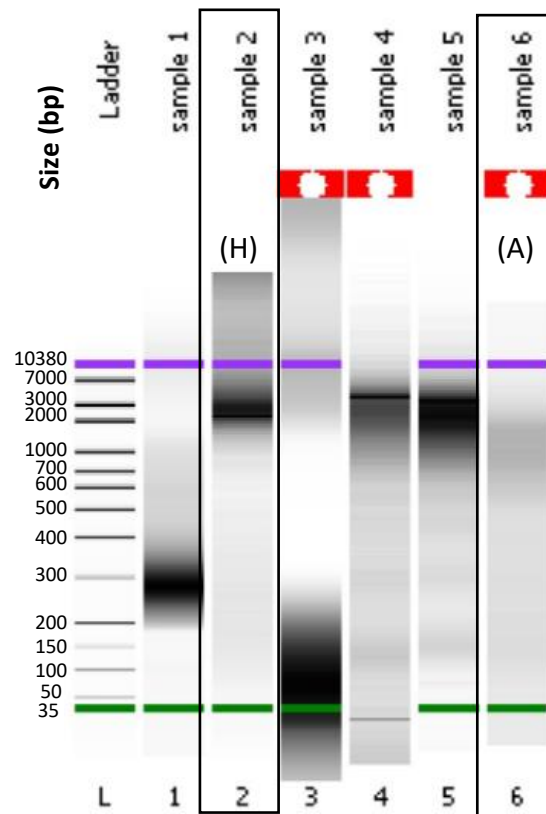


Figure 5.2: Electrophoresis by using HS DNA Chip Bioanalyzer. **Sample 2 (H):** Head. **Sample 6 (A):** Abdomen. Note: Sample 1, 3, 4 and 5 not *C. curvignathus*'s samples.

The electropherogram of the head samples showed presence of mild DNA degradation but it still have a very high DNA quality compared to abdomen samples (Figure 5.3). No clear DNA band was observed for abdomen samples on electrophoresis (Figure 5.2) suggesting that the DNA might have degraded. An irregular baseline with low signals was also observed for abdomen samples (Figure 5.3; Sample 6). It indicated a low concentration of DNA samples, which may probably due to degradation of gDNA. A spike was observed for abdomen sample in electropherogram result which could be due to interference in the electrical current while the instrument is running. A contaminated sources such as dust, or other particle in the tube may also cause the spike.

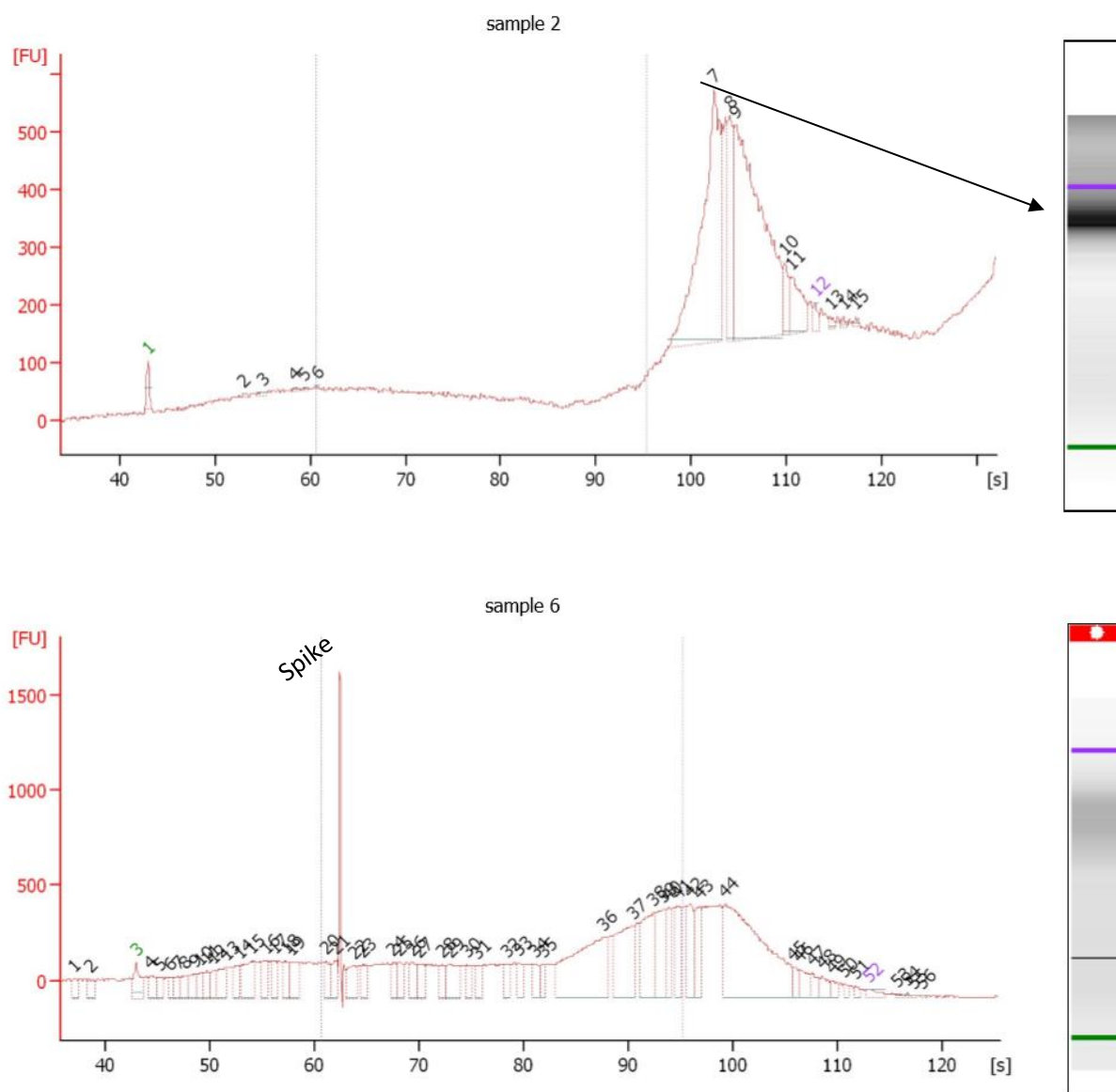


Figure 5.3: Electropherogram of Sample 2 (Head) and Sample 6 (Abdomen) by HS DNA Chip Bioanalyzer

5.3.1.2 454 pyrosequencing

A modest sequencing volume generated 47,462 reads with 1996 (4.2%) reads contains di-, tri- and tetra-nucleotide repeat motifs, with an average length of 389.931 bp (Table 5.9 and Figure 5.4).

Table 5.9: Raw data of 454 sequencing.

Read counts	Total bases	Average read length
47,462	18,506,883	389.931

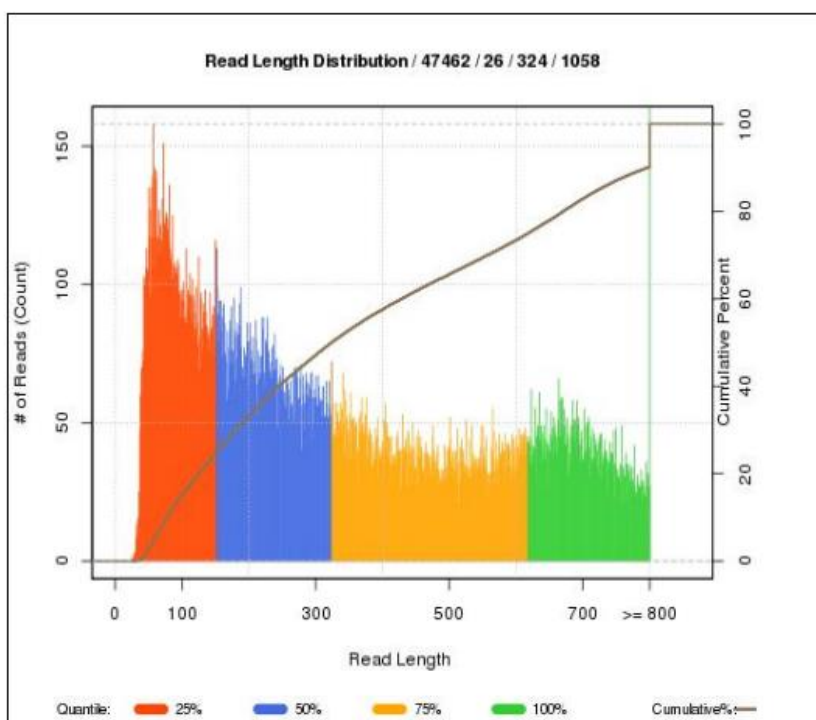


Figure 5.4: Read length distribution

The search using MSATCOMMANDER yielded 129 sequences suitable for primer design consist of 47 di- (81.77%), 43 tri- (12.42%) and 39 tetra-nucleotide (5.81%) repeat motifs. Out of 129 sequences, 20 primers consist of each di-, tri- and tetra-nucleotide repeats were selected for primer design (Table 5.10).

Table 5.10: The number of repeat, primer contained microsatellite repeat motifs and designed primer.

Type of repeats	Total number of repeats	Total number of primers containing repeats	Total designed primer
Dinucleotide	1016	47	20
Trinucleotide	562	43	20
Tetranucleotide	418	39	20
	1996	129	60

5.3.1.3 Primer design

A total of 60 primers out of 129 primers were designed as final primers pairs (Table 5.11). The main selection was prioritized to the primer sequences that matched to the sequences of termites and insects in National Centre for Biotechnology Information (NCBI) after a BLAST search. The sequences found in non-coding region only were highlighted especially sequences that hit microsatellite sequences.

Table 5.11: 60 sequences of primers pairs.

Locus	Primer Sequence (5' – 3')	Repeat motif	Locus Size (bp)	Sequence Region	BLAST Similarity Search
T01	CGCCTAACTTCAGGATGCC TCTGGCTTATTTGCTGTAAC TTC	(AC) ⁸	224	Nuclear	<i>Macrotermes gilvus</i>
T02	CAGCCATGCCTCCTCAATAG TTAGCGGACAGTTGTGAACC	(CT) ⁸	203	Msat sequence	<i>Odontotermes sp.</i>
T03	AGTGGCGGGATCTGTGAAC CGTGCCGTCTCAAGTGTAG	(AC) ¹⁶	425	Retrotransposons	<i>Reticulitermes lucifugus lucifugus</i>
T04	CGAGTTGTCTGCATAAACCG CCCTCAGCGTAGACGGAC	(AT) ⁸	375	mRNA	<i>Reticulitermes flavipes</i>
T05	ACAGCAGGTGTCGAGGTTG AAAGCATTGATTGGCGTGG	(CT) ¹¹	408	Msat sequence	<i>Coptotermes gestroi</i>

Table 5.11 continued

T06	ACTCAAATTCCGCCGTGTC GTGTGAACACCGATCCACG	(CT) ¹⁵	365	Msat sequence	<i>Coptotermes formosanus</i>
T07	AAGAACTTAAACAGGTTGC GG TAAACGCAATGTGGGCACC	(GT) ¹³	351	mRNA	<i>Odontotermes formosanus</i>
T08	CCAGACTGTGTTATGCGCC TGCCGTGTCCCGGTTAATG GCGATACGACAAAGTGTG	(GT) ⁹	323	Msat sequence	<i>Odontotermes sp.</i>
T09	C ATATGGCCGGGTTGCTCTG CCATTATGAGGTCCTTCTTC	(GT) ⁹	242	Msat sequence	<i>Coptotermes gestroi</i>
T10	CG CCATGTTGCTACGGCACG	(AG) ⁹	396	Msat sequence	<i>Coptotermes formosanus</i>
T11	GGTTAACGCACCTAAAGAG GC GGAAAGACAAGTTTCACAG TACC	(AC) ²⁷	161	Retrotran sposons	<i>Reticulitermes lucifugus lucifugus</i>
T12	TGGTGGCGATGAGCTGTC ACTGTGGGCCTGATGGAAC	(GT) ⁸	222	mRNA	<i>Coptotermes formosanus</i>
T13	GACGACGTGGAGACCTTGG TTGAGTTGTTCAATGGGACG G	(AC) ²¹	312	Msat sequence	<i>Reticulitermes lucifugus lucifugus</i>
T14	AATACGGGTGACTGCTTGTC TGTCCAGGGCTGTAATGGG	(AC) ⁸	196	Retrotran sposons	<i>Reticulitermes lucifugus lucifugus</i>
T15	GCCCCAAGTATGGATCTGG ACGTAAAGTTACCGCTAGGC	(AT) ⁹	155	Msat sequence	<i>Coptotermes formosanus</i>
T16	AACAACGGCTGCGAGATTG AAGAGGAAACCTGCCTGGG	(AC) ¹⁰	350	Msat sequence	<i>Odontotermes sp.</i>
T17	GTAGACGCACTTGTGACGC AGCAGAATTACCAAAGGCC G	(AC) ⁹	385	Msat sequence	<i>Amitermes meridionalis</i>
T18	TTTCTCTTGCGACACCTCG TGGCTTATTTGCTGTAAGTT CCC	(AC) ⁸	199	Msat sequence	<i>Macrotermes michaelseni</i>

Table 5.11 continued

T19	TGCATGCCACAGCAACTTC TGGACGTGAGAACATTCCA G	(GT) ¹⁴	167	Msat sequence	<i>Macrotermiteae</i> sp.
T20	CGCTTGATTGCTGGGATCG TGGCCCGTCTCACTGTTG	(GT) ¹¹	202	mRNA	<i>Reticulitermes</i> <i>flavipes</i>
T21	GATGAAGGCAAGTGGCGG TGAACGCAATGTGCAGAGC	(ATT) ⁹	340	Msat sequence	<i>Coptotermes</i> <i>formosanus</i>
T22	ATCGCTTGAACACGAAGGC TTCCCGGTACGCTGCTC	(ATT) ¹⁰	446	Msat sequence	<i>Coptotermes</i> <i>formosanus</i>
T23	CCGGTTTACTTACATATACA AGGG AGCACCATAACCAGGAAGGC	(ATT) ¹⁰	311	Msat sequence	<i>Coptotermes</i> <i>formosanus</i>
T24	CACATACTGCGGGAAGTGC GGAACCCAACGACTGTTCTG	(AAT) ¹²	271	Msat sequence	<i>Coptotermes</i> <i>formosanus</i>
T25	AGCTCGTCTTCCTTGAGGTG GTGGCTGTGCCCAATATAGC	(GAT) ⁸	160	Msat sequence	<i>Coptotermes</i> <i>formosanus</i>
T26	TAAACGCAATGTGGGCACC AAGGCAAACGCGACAAAGG GATGTCCGGTTTACTGTTGG	(AAT) ⁹	230	Msat sequence	<i>Coptotermes</i> <i>formosanus</i>
T27	G CGATCGCAGGCTATTTGGC	(ATT) ¹⁰	157	Msat sequence	<i>Coptotermes</i> <i>formosanus</i>
T28	GCATTCCCGAAAGTGCTCC GGTGGACGTGTGTGCTTAAC	(GAT) ⁹	205	Msat sequence	<i>Reticulitermes</i> <i>santonensis</i>
T29	AACGTTCTGTACGGCAGGG AGTGGCAAGTTACGAAGCG	(GTT) ¹¹	286	Msat sequence	<i>Coptotermes</i> <i>formosanus</i>
T30	CCCTATGCCTTTATGCCGTG ACTGCGGGAAGTGCTAATG	(ATT) ¹³	318	Msat sequence	<i>Coptotermes</i> <i>formosanus</i>
T31	CAGGAATCTCCAGGACGGG GGGTGAACTGCCAATACGT G	(ATT) ¹¹	352	Msat sequence	<i>Macrotermes</i> <i>gilvus</i>
T32	ACGTGGTTTGTTCAGGCAC CGCTTCCAGAATGGTCCAAG	(AAT) ⁸	413	Msat sequence	<i>Coptotermes</i> <i>formosanus</i>
T33	AGGTCTCCCTTTGACAGCC TCACGAAATGTTTCAGTAAGAAGC	(ATT) ⁹	224	Msat sequence	<i>Macrotermes</i> <i>gilvus</i>

Table 5.11 continued

T34	ACACGAAATGTCGCACAGG GTCGCAAGTACGGCAACTG	(AAT) ⁹	410	Msat sequence	<i>Coptotermes formosanus</i>
T35	TGATGGCGATGGTTCAGTTA G TCAGGCTGTTGGCTTGGAC	(AAT) ¹⁴	294	Msat sequence	<i>Incisitermes minor</i>
T36	TGCAATTTGTGCTGTTCCG CTCACCCTAGGACTGTGGC	(AAT) ⁹	406	Msat sequence	<i>Macrotermes gilvus</i>
T37	CGTGCCTTCCTCGCTAATG GGATCGCCGTTGGTTCAAG	(ATT) ⁸	316	Msat sequence	<i>Nasutitermes corniger</i>
T38	CGCATTTCGATTCCCGATGG ACGCTGAACTACTGTCTTGC	(AAT) ¹⁰	364	Msat sequence	<i>Coptotermes formosanus</i>
T39	CTGATTGGCCCGCTCAAAG GTCTGTACTGGTGTAAAGACT GC	(ATT) ⁸	209	Msat sequence	<i>Macrotermes gilvus</i>
T40	ATAGGGACACGTGGTGGAC GCAAGAGGCTACGGACCTG	(AAT) ⁸	170	Msat sequence	<i>Macrotermes gilvus</i>
T41	ATGTGGTAAGGGACCTCCG TCATTGGGAGTGCTGACCG	(AGTT) ¹ 1	323	Msat sequence	<i>Coptotermes formosanus</i>
T42	CGGAAGCCTCAAATCACGC AGGATAGGGACAGGTTGGC	(AACT) ¹ 0	257	Msat sequence	<i>Coptotermes formosanus</i>
T43	AAAGTTCTACGTGGTGCCC CCTTGGAATCGCAGTTGGTC	(ATGT) ¹ 1	415	Msat sequence	<i>Coptotermes formosanus</i>
T44	AAAGCGACTATTCCTTCGT C AACGTACATCCGTCTTGGTG	(CCGT) ¹ 2	246	Msat sequence	<i>Reticulitermes santonensis</i>
T45	CAGGATAGGGACAGGTGGC AGTACTAAGCCAGGGCACG	(AGTT) ¹ 0	338	Msat sequence	<i>Coptotermes formosanus</i>
T46	GGCGGGATCTTGTGAATGC TTCTGACCTCTGTTCTACGT G	(AGTT) ⁸	225	Msat sequence	<i>Coptotermes formosanus</i>
T47	TTGCCGCTCGTATTGACTG GCTGGCTCTGGATAGGGAC	(AACT) ¹ 2	438	Retrotran posons	<i>Reticulitermes lucifugus lucifugus</i>

Table 5.11 continued

T48	AATGCACACCCGTCTGAAC TGCGGTAATGAACCTTTGGG CCGTCCGGCGAGAGTTG	(AACT) ¹ 9	352	Msat sequence	<i>Coptotermes formosanus</i>
T50	GGCAAGGATGCTGTGTGAA C TGTGGAGAACTCGCTGTGG	(CATT) ⁸	173	Msat sequence	<i>Macrotermes gilvus</i>
T51	AGGGTAACAGGGCAGTTGG CAGGATAGGGACAGGTGGC	(ACTC) ² 2	357	Msat sequence	<i>Macrotermitinae sp.</i>
T52	ACCTCGACATGGAGAAGGC GCTGGCTCAGGATAGGGAC	(AACT) ¹ 2	326	Msat sequence	<i>Coptotermes formosanus</i>
T53	AGGGTGATTACAGTGCGGG CAGGATAGGGACAGGTGGC	(AACT) ⁹	225	Msat sequence	<i>Macrotermitinae sp.</i>
T54	CCCAGAAGTTTGACGCACC GCCCATCCAGTAGGAGCC	(CCGT) ⁸	159	Msat sequence	<i>Reticulitermes santonensis</i>
T55	TCGTTCGATGGGTAGTGAGG TCACCGCGTTAACAACTGC	(AACT) ¹ 3	341	Msat sequence	<i>Macrotermes gilvus</i>
T56	GGGATGAACGCAGATTGCA C TTGGGCTGTTGTGGACACG	(AACT) ⁸	205	Msat sequence	<i>Coptotermes formosanus</i>
T57	AACGCAGCAATGAACCTCC GTGGAGTACAGAAATGGGC AC	(AGTT) ⁸	206	Msat sequence	<i>Coptotermes formosanus</i>
T58	CCCATGACTTGGACCTGGC GACTGTGTGGAGAACTCGC	(CATT) ¹⁶	191	Msat sequence	<i>Macrotermes gilvus</i>
T59	ATGGACAATGCTCGGACTG TGACCGGATATCTTCGCTAC TG	(CATT) ¹¹	161	mRNA	<i>Reticulitermes lucifugus lucifugus</i>
T60	GCTAAGGGCAAAGGTGCG GCTGGCTCAGGATAGGGAC	(AACT) ² 3	333	Msat sequence	<i>Coptotermes formosanus</i>

Note: Msat: Microsatellite

5.3.1.4 Microsatellite primer screening and PCR multiplexing

Sixty final designed primer pairs were selected for preliminary test of polymorphism. The amplified PCR products of these 60 primers pairs were then visualized on 6% polyacrylamide gel electrophoresis (PAGE). Out of 60 primer pairs tested, only 38 loci produced unambiguous amplification within expected allele size range. Of these, 15 loci showed allelic variation, whereas 23 loci were found monomorphic when tested across individuals from multiple comparisons from Kota Samarahan, Sibul, Kuching and Sri Aman (preliminary test result by using 6% polyacrylamide gel electrophoresis). When evaluated within a single population (Sibu), 10 loci were found to be polymorphic and therefore were selected for PCR multiplexing (Table 5.12) and further visualized on 6% PAGE (Figure 5.5)

Table 5.12: PCR multiplex group.

Multiplex Group	Primer	Size (bp)	Temperature (°C)	Primer Concentration (μM)	Fluorescent dye labels
A	T08	323	65	0.1	Forward-HEX
	T46	225	65	0.16	Reverse-6-FAM
	T25	160	65	0.1	Forward-HEX
B	T34	410	60	0.1	Forward-HEX
	T04	375	60	0.2	Forward-6-FAM
	T14	196	60	0.1	Forward-HEX
C	T49	307	65	0.1	Reverse-HEX
	T44	246	65	0.1	Forward-6-FAM
	T40	170	65	0.1	Forward-HEX
			65	0.1	Forward-
	T19	167			TAMRA-S

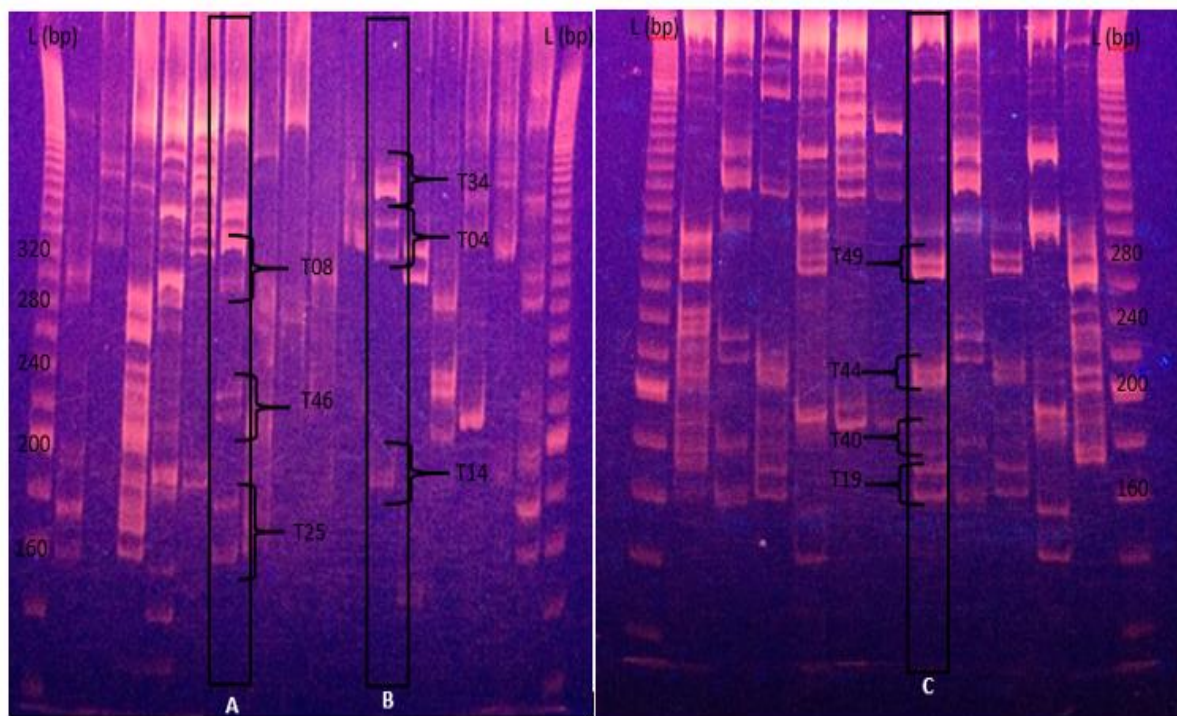


Figure 5.5: Visualization of PCR Multiplex Group on 6% PAGE. **A:** Multiplex Group A. **B:** Multiplex Group B. **C:** Multiplex Group C. **L:** 20 bp DNA ladder (Lonza)

5.3.2 Microsatellite Data Analysis

Each of the ten microsatellite loci were polymorphic, with four to 15 alleles observed per locus with a total of 91 alleles across all loci (Table 5.13). The observed heterozygosity (H_e) per locus ranged from 0.19 to 0.86, and the expected heterozygosity (H_o) ranged from 0.44 to 0.92. The highest number of allele ($N_a=16$) was detected at locus T46 and the lowest number of allele ($N_a=5$) was detected at locus T14. There was no significant deviations from Hardy-Weinberg equilibrium (HWE) observed across all loci after multiple Bonferroni correction. No significant linkage disequilibrium after Bonferroni correction was detected suggesting that all ten loci segregate independently of each other. There was no evidence for large allele dropout, null alleles for any of the loci tested and stutter according to MICROCHECKER 2.23 (Van Oosterhout *et al.*, 2004). For the 10 polymorphic loci of *C.*

curvignathus, eight are highly polymorphic with PIC value more than 0.5, while only two (T14 and T34) are moderately polymorphic ($0.25 < \text{PIC} < 0.5$).

Table 5.13: Characteristics of ten polymorphic microsatellite loci in *Coptotermes curvignathus*, number of alleles (Na), mean expected heterozygosity (MHe) and mean observed heterozygosity (MHo).

Locus	Repeat Motif	Primer Sequence (5'-3')	Ta (°C)	Mplex group	Size (bp)	Na	Ho	He	PIC
T25	(GAT) ⁸	Fo: AGCTCGTCTTCCTTGAGGTG Re: GTGGCTGTGCCCAATATAGC	65	A	160	13	0.86	0.89	0.872
T46	(AGTT) ⁸	Fo: GCGGGATCTTGTGAATGC Re: TTCTGACCTCTGTTCTACGTG	65	A	225	15	0.84	0.92	0.905
T08	(GT) ⁹	Fo: CCAGACTGTGTTATGCGCC Re: TGCCGTGTCCCGGTTAATG	65	A	323	11	0.59	0.89	0.870
T14	(AC) ⁸	Fo: AATACGGGTGACTGCTTGTC Re: TGTCCAGGGCTGTAATGGG	60	B	196	4	0.32	0.46	0.392
T04	(AT) ⁸	Fo: CGAGTTGTCGCATAAACCG Re: CCCTCAGCGTAGACGGAC	60	B	375	9	0.41	0.67	0.649
T34	(AAT) ⁹	Fo: ACACGAAATGTCGCACAGG Re: GTCGCAAGTACGGCAACTG	60	B	410	5	0.19	0.44	0.414
T49	(CTGT) ¹⁰	Fo: GGATCCCGTCAAAGGAGTG Re: CCGTCCGGCGAGAGTTG	65	C	307	10	0.61	0.86	0.834
T44	(CCGT) ¹²	Fo: AAAGCGACTATTTTCCTTCGTC Re: AACGTACATCCGTCTTGGTG	65	C	246	7	0.62	0.74	0.693
T40	(AAT) ⁸	Fo: ATAGGGACACGTGGTGGAC Re: GCAAGAGGCTACGGACCTG	65	C	170	7	0.82	0.78	0.738
T19	(GT) ¹⁴	Fo: TGCATGCCACAGCAACTTC Re: TGGACGTGAGAACATTCCAG	65	C	167	10	0.58	0.84	0.810
Mean						91	0.58	0.75	

Note: Fo: forward; Re: reverse; Ta: Annealing temperature; Mplex: Multiplex; N: number of individuals; Na: number of alleles; Ho: observed heterozygosity; He: expected heterozygosity; PIC: polymorphic information content

5.4 Discussion

Next Generation Sequencing (NGS) requires the input of high molecular weight genomic DNA (gDNA) to construct quality libraries for large scale sequencing projects. The DNA integrity of the samples is very crucial and critical as a first step in obtaining meaningful data for successful library construction. It is because the next step in library preparation for automated sequencing is DNA shearing, which needed high molecular weight of gDNA (Quail *et al.*, 2008, Wilkening *et al.*, 2013).

UV spectrophotometer analysis by using NanoDrop instrument, dSDNA-specific flurometry assay based on Qubit and quantitative PCR (qPCR) are the most commonly used reliable and accurate methods to do DNA quantification (Glaser, 1995, Robertson, 2003). In this study, the performance of the most widespread DNA quantification methods (NanoDrop and Qubit) was tested on different parts of termite body which are head and abdomen. The NanoDrop result showed the OD_{260}/OD_{280} of head was higher than the abdomen. The result indicated that DNA samples by using head as a starting material have a better purity of DNA compared to abdomen. Meanwhile, when compared the NanoDrop with the Qubit reading, the measurement of DNA concentrations of head and abdomen samples by NanoDrop was higher than those of Qubit readings.

However, over estimation for DNA concentration is possible following NanoDrop method (Okamoto *et al.*, 2000). The principle of DNA spectrophotometer with UV absorbance at 260 nm made the presence of RNA contaminants in the extract can be absorbs at 260 nm and thus can interfered the absorbance value for DNA quantification purposes (Schipor *et al.*, 2016). Therefore, more sensitive and specific assessment such as UV

fluorometry by Qubit can overcome the drawback of Nanodrop as it much less interfered with RNA contaminations (Simbolo *et al.*, 2013; Schipor *et al.*, 2016).

The combination of both technologies allows the correct quantification of DNA samples for NGS. The current results depicted that the concentration DNA extracted from the termite's abdomen from both Nanodrop and Qubit readings were higher than the head samples. However, based on the Nanodrop purity readings, the head samples had higher DNA purity compared to abdomen. The head samples showed an optimum concentration of DNA from both Nanodrop and Qubit.

The most classical method for rapid and low cost visualization of gDNA extracts are often evaluated on agarose gels. However, this approach is not sufficient enough for a high-throughput workflow and automation (Kong *et al.*, 2014). The current results by agarose gel electrophoresis proved that the gDNA extracts were difficult to estimate the size products against a ladder coupled with densitometry to determine the concentration of the gDNA extracts. The low resolution images from the current results cannot provide a detailed visual assessment of the DNA size distribution and fragments. Therefore, the Bioanalyzer was better for visual assessment compared to agarose gel electrophoresis in current study, plus it provides the gold standard in QC checks for downstream NGS analysis (Kong *et al.*, 2014).

HS DNA Chip Bioanalyzer generated an electropherogram that determines the level of sample degradation as opposed to the classical gel electrophoresis method that inadequately determines the sample integrity. The electropherogram results from head and abdomen samples revealed that the abdomen samples had higher DNA degradation compared to the head samples which cannot be differentiated by an agarose gel. The noisy

baseline showed by the abdomen samples indicated a low quality of gDNA which probably cause by contaminants.

454 pyrosequencing technology is proved to be a powerful tool for isolating microsatellite markers from the *C. curvignathus* genome, in which previous attempt by using 454 pyrosequencing method also had successfully produce functional polymorphic microsatellite loci in *Macrotermes gilvus* (Veera Singham *et al.*, 2014). A total of 47,462 sequence reads were generated by the 454 pyrosequencing in current study. Out of them, 60 clones were successful for primer design, yet in the end, only 15 primers were displayed polymorphism while the remaining was monomorphic. The mutation that occurred in the primer flanking region probably resulted in null alleles in some of individual tested or in some cases resulted in no amplification (Peatkau and Strobeck, 1995).

The commonly found repeat motif in the current study was dinucleotide, with 47 out of 1016 sequences contained suitable flanking sites for primer design. Although the most commonly found repeat motif was dinucleotide, tri- and tetranucleotide repeats motif had higher suitable flanking regions for primer design. The dinucleotide repeat motif only had 4.6% which suitable for primer design in contrast to trinucleotide repeats with 7.7% of 562 sequences had suitable flanking regions, followed by tetranucleotide with 9.3% of 418 sequences. Our current results contradicted with the findings of Gardner *et al.* (2011), Copeland *et al.* (2011) and Veera Singham *et al.* (2014), who reported that trinucleotide repeats have the highest proportion of all microsatellite motifs suitable for primer design among the invertebrates.

Most of repeat sequences among the 10 primers selected for downstream genotyping work consist of dinucleotide repeat motifs (T08, T14, T04, and T19) followed by

trinucleotide (T25, T34 and T40) and tetranucleotide (T44, T46 and T49). This combination is ideal because most of the trinucleotide and tetranucleotide showed highly variable number of alleles. Tri- and tetranucleotide repeats loci seems to offer more advantages over dinucleotide repeats as they are often easier to score (Edwards *et al.*, 1991; Hearne *et al.*, 1992) and the repeats were relatively free multiple alleles artifact (Chin *et al.*, 1996).

The population studied displayed a fairly high level of genetic diversity (allelic diversity, $A = 9.1$ alleles per locus and gene diversity, h [Nei, 1973] = 0.75 compared with other studies of insect population using microsatellite data. Veera Singham *et al.* (2011) reported microsatellite data in *Macrotermes gilvus* with $A = 2.9$ and $h = 0.3$), Booth *et al.*, (2008) reported $h = 0.60$ and $A = 4.7$ in a drywood termite while Vargo and Henderson (2000) reported $h = 0.65$ and $A = 6.1$ in a lower subterranean termite.

5.5 Summary and Conclusion

The primary aim of this study was to identify a set of polymorphic microsatellite loci in *C. curvignathus*, and develop corresponding optimised primers to provide researchers with tool that can be used to elucidate the population genetic structure of this important species. From the results of the basic descriptive statistics performed on five small populations, novel set of loci characterized and described here is sufficiently polymorphic for studies of fine scale population genetic structure. A cross amplification in other Rhinotermitidae species is suggested, which is a worthwhile avenue for future research.

The next generation sequencing (NGS) approached was used to develop and design the PCR primers flanking the microsatellites loci while at the same time discovered a set of functional polymorphic microsatellite markers in *C. curvignathus*. This preliminary assessment of genetic diversity were successful on both counts, whereby 15 of 60

microsatellite showed polymorphism after a screening and finally, ten were chosen for multiplex primer sets to be used in preliminary assessment. The current results provide a valuable database with over 100 sequences containing suitable flanking regions for development of additional microsatellite loci in future. The successful isolation of polymorphic loci from *C. curvignathus* can be a useful tool for investigating the colony and population genetic structure of *C. curvignathus* in SEA.

CHAPTER 6

GENERAL CONCLUSION

Coptotermes is one of the most economically important subterranean termite genera but the taxonomy validity of many named *Coptotermes* spp. remains unclear. The great size variation and lack of distinguishing morphological features between *Coptotermes* spp. cause difficulties for rapid identification in the field. Over the last decades, revision of the systematics of *Coptotermes* has been conducted in a more comprehensive way with more advanced tool. This dissertation focus on the specimen from Sarawak, Malaysia with extension to peninsular Malaysia to reveal the phylogenetic relationship of only four *Coptotermes* spp. in Borneo (Thapa, 1981) which is no study was reported previously particularly in Sarawak. The findings of morphometric and molecular approaches will have significant interest in worldwide studies of *Coptotermes*, especially in Malaysian Borneo. The morphometric outcomes could be useful for pre-identification of species as an alternative to molecular studies which required less technical and material costs, yet molecular studies still essential for showing the evolutionary relationships of genetically related species.

Taxonomy provides the baseline for all comparative biology. Accurate species identification is crucial among *Coptotermes* spp. as a basis to understand its biological, ecological and behavioural studies. To further validate the current findings, more extensive sample collection from peninsular Malaysia and Borneo region should be done to provide more informative phylogenetic tree based on molecular data. Morphological identification based on the soldier's caste among *Coptotermes* spp. can be improved by comparing with the reproductive caste, i.e. alates, king, queen. The advances in termite taxonomy when

combined with conventional methods and molecular tools will enable understanding of termite colony structure, ecology and social evolution in a larger scope which in turn, important for pest management in an effective way.

Many aspects of the management and control of termites are dependent on reliable and accurate information on the taxonomy of the pest species of termites targeted. This is illustrated in the case of the Asian Subterranean Termite, which has been shown to be a single important pest species in Southeast Asia, where it was previously thought that three pest species existed. The taxonomy of the pest species of termites is constrained by inherent difficulties in the morphology of termites, as well as by historical, geographical and political factors. However, modern tools available to the taxonomist, such as numerical, chemical and molecular techniques, and increased inter-regional cooperation, are enabling advances in our understanding of the taxonomy of termite pest species, when combined with conventional methods. There remain unresolved questions as to the status of some termite pest species, particularly in the genus *Coptotermes*. Resolving these questions will require genetic and morphological variation in populations of the species complex to be studied on a wide geographic scale.

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APPENDICES

Appendix A. The aligned sequences of the rRNA 12S gene for each specimen. Positions are given at the end of line.

DataType=Nucleotide
NSeqs=47 NSites=319
Identical=, Missing=? Indel=-;

#CC01BHR_12S	GAGAGCGACG GGCGATGTGT ACATATCTTA GAACCCATAT CACGAAAACA ATCTTCAAGA CATTACAATC AAATCCAATT [80]
#CC02BHR_12S	GAGAGCGACG GGCGATGTGT ACATATCTTA GAACCCATAT CACGAAAACA ATCTTCAAGA CATTACAATC AAATCCAATT [80]
#CS01BHW_12S	GAGACCGACG GGCGATGTGT ACATATCTTA GAACCTATAT CACGAAAACA ATCTTCAAGA CATTACAATC AAATCCAATT [80]
#CS02BHW_12S	GAGAGCGACG GGCGATGTGT ACATATCTTA GAACCTATAT CACGAAAACA ATCTTCAAGA CATTACAATC AAATCCAATT [80]
#CS03BHW_12S	GAGAGCGACG GGCGATGTGT ACATATCTTA GAACCTATAT CACGAAAACA ATCTTCAAGA CATTACAATC AAATCCAATT [80]
#CS04BHW_12S	GAGAGCGACG GGCGATGTGT ACATATCTTA GAACCTATAT CACGAAAACA ATCTTCAAGA CATTACAATC AAATCCAATT [80]
#CS03SWS_12S	GAGCGCGAGG GGCGATGTGT ACATATCTTA GAACCTATAT CACGAAAACA ATCTTCAAGA CATTACAATC AAATCCAATT [80]
#CC04SWS_12S	GAGAGCGACG GGCGATGTGT ACATATCTTA GAACCCATAT CACGAAAACA ATCTTCAAGA CATTACAATC AAATCCAATT [80]
#CS05SWS_12S	GAGAGCGACG GGCGATGTGT ACATATCTTA GAACCTATAT CACGAAAACA ATCTTCAAGA CATTACAATC AAATCCAATT [80]
#IK19R29P2_12S	GAGAGCGACG GGCGATGTGT ACATATCTTA GAACCCATAT CACGAAAACA ATCTTCAAGA CATTACAATC AAATCCAATT [80]
#IK19R31P4_12S	GAGAGCGACG GGCGATGTGT ACATATCTTA GAACCCATAT CACGAAAACA ATCTTCAAGA CATTACAATC AAATCCAATT [80]
#IK19R31P5_12S	GAGAGCGACG GGCGATGTGT ACATATCTTA GAACCCATAT CACGAAAACA ATCTTCAAGA CATTACAATC AAATCCAATT [80]
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Appendix A. continued

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Appendix A. continued

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Appendix A. continued

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Appendix A. continued

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#IL19R18P1_12S ATAACGAGGC AGATTCCTCT GAATGGAATA AGATACCGCC AAATTCCTTGT TTCAAGAACA TACCTACTAC TCAAGCACA [319]
#C.kalshoveni_FJ235118.1 ATAACGAGAC AGATTCCTCT GAACGGAATA AGATACCGCC AAATTCCTTGT TTCAAGAACA TACCTACTAC TCAAGCACA [319]
#C.kalshoveni_FJ235119.1 ATAACGAGAC AGATTCCTCT GAACGGAATA AGATACCGCC AAATTCCTTGT TTCAAGAACA TACCTACTAC TCAAGCACA [319]
#C.curvignathus_FJ235110.1 ATAACGAGGC AGATTCCTCT GAATGGAATA AGATACCGCC AAATTCCTTGT TTCAAGAACA TACCTACTAC TCAAGCACA [319]
#C.curvignathus_FJ235111.1 ATAACGAGGC AGATTCCTCT GAATGGAATA AGATACCGCC AAATTCCTTGT TTCAAGAACA TACCTACTAC TCAAGCACA [319]
#C.travians_FJ915823 ATAACGAGAC AGATTCCTCT GAACGGAATA AGATACCGCC AAATTCCTTGT TTCAAGAACA TACCTACTAC TCAAGCACA [319]
#C.gestroi_EF379982.1 ATAACGAGAC AGATTCCTCT GAACGGAATA AGATACCGCC AAATTCCTTGT TTCAAGAACA ---CTACTAC TCAAGCATA [319]
#C.gestroi_EF379987.1 ATAACGAGAC AGATTCCTCT GAACGGAATA AGATACCGCC AAATTCCTTGT TTCAAGAACA ---CTACTAC TCAAGCATA [319]
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#Gsulphureus_EF379993.1 ATTACAGGAT TGTTTGTTT GAATGAAATT GGATGTTGTT ---TGAGGT TTCTATGATA TTGGTTCTAT ATGTACACA [319]

Appendix B. The aligned sequences of the rRNA 16S gene for each specimen. Positions are given at the end of line.

DataType=Nucleotide
NSeqs=48 NSites=321
Identical=, Missing=? Indel=-;

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#C.curvignathusMY_FJ376686.1  TGGATCAAAC AACTAATCAA TATAACCAAA ACCAAGAGGA GAACATATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#C.gestroi_EF379963.1      TGGATCAAAC AATTAATCAA TATAACCAAA ACTAAGAGGA GAGTACATTC CTCCCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#C.gestroi_EF379969.1      TGGATCAAAC AATTAATCAA TATAACCAAA ACTAAGAGGA GAGTACATTC CTCCCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#C.gestroi_EF379970.1      TGGATCAAAC AATTAATCAA TATAACCAAA ACTAAGAGGA GAGTACATTC CTCCCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#C.kalshoveni_FJ376684.1    TGGATCAAAC AACTAATAAA TATAACCAAA ACAAAGAAGA GAATACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#C.kalshoveni_FJ376685.1    TGGATCAAAC AACTAATAAA TATAACCAAA ACAAAGAAGA GAATACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#C.traviansMY_FJ915824.1    TGGATCAAAC AACTAATCAA TATAACCAAA ACAAAGAAGA GAATACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#CC001MA                   TGGATCAAAC AACTAATCAA TATAACCAAA ACCAAGAGGA GAACATATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#CC01_BHR                  TGGATCAAAC AACTAATCAA TATAACCAAA ACCAAGAGGA GAACACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#CC01_KL                   TGGATCAAAC AACTAATCAA TATAACCAAA ACCAAGAGGA GAACACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#CC01_KLKG                 TGGATCAAAC AACTAATCAA TATAACCAAA ACCAAGAGGA GAACATATTC CTCCCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#CC01_SKAG                 TGGATCAAAC AACTAATCAA TATAACCAAA ACCAAGAGGA GAACATATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#CC01_SNR                  TGGATCAAAC AACTAATCAA TATAACCAAA ACCAAGAGGA GAACATATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#CC01_TB                   TGGATCAAAC AACTAATCAA TATAACCAAA ACCAAGAGGA GAACATATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#CC02_BHR                  TGGATCAAAC AACTAATCAA TATAACCAAA ACCAAGAGGA GAACATATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#CC02_KL                   TGGATCAAAC AACTAATCAA TATAACCAAA ACCAAGAGGA GAACACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#CC02_KLKG                 TGGATCAAAC AACTAATCAA TATAACCAAA ACCAAGAGGA GAACATATTC CTCCCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#CC02_SKAG                 TGGATCAAAC AACTAATCAA TATAACCAAA ACCAAGAGGA GAACATATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#CC02_UN                   TGGATCAAAC AACTAATCAA TATAACCAAA ACCAAGAGGA GAACACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#CC03_KLKG                 TGGATCAAAC AACTAATCAA TATAACCAAA ACCAAGAGGA GAACATATTC CTCCCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#CC04_SWS                  TGGATCAAAC AACTAATCAA TATAACCAAA ACCAAGAGGA GAACACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#CC05_UN                   TGGATCAAAC AACTAATCAA TATAACCAAA ACCAAGAGGA GAACACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#CC08_UN                   TGGATCAAAC AACTAATCAA TATAACCAAA ACCAAGAGGA GAACACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
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#CK03_UN                   TGGATCA--- AACTAATAAA TATAACCAAA ATAAAGAAGA GACTACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCT [ 80]
#CK04_UN                   TGGATCA--- AACTAATAAA TATAACCAAA ATAAAGAAGA GACTACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCT [ 80]
#CS01_BHW                  TGGATCA--- AACTAATAAA TATAACCAAA ATAAAGAAGA GTCTACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCT [ 80]
#CS01_UN                   TGGATCAAAA ACATAA---- ----ACCAA ATAAAGAAGA GACTACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCT [ 80]
#CS02_BHW                  TGGATCA--- AACTAATAAA TATAACCAAA ATAAAGAAGA GACTACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCT [ 80]
#CS02_KNP                  TGGATCAAAA ACATAA---- ----ACCAA ATAAAGAAGA GACTACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCT [ 80]
#CS03_BHW                  TGGATCA--- AACTAATAAA TATAACCAAA ATAAAGAAGA GTCTACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCT [ 80]
#CS03_SWS                  TGGATCAAAA ACATAA---- ----ACCAA ATAAAGAAGA GTCTACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCT [ 80]
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#CS05_SWS                  TGGATCA--- AACTAATAAA TATAACCAAA ATAAAGAAGA GACTACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCT [ 80]
#CS06_UN                   TGGATCA--- AACTAATAAA TATAACCAAA ATAAAGAAGA GACTACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCT [ 80]
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Appendix B. continued

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#CS07_UNG      TGGATCA--- AACTAATAAAA TATAACCAAAA ATAAAGAAGA GTCTACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCT [ 80]
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#CT01_LHNP     TGGATCAAAC AACTAATCAA TATAACCAAAA ACCAAGAGGA GATTATATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#CT01_SNP      TGGATCAAAC AACTAATCAA TATAACCAAAA ACCAAGAGGA GATTATATTC CTCTCATCAC CCCAACAAAA CAAAAAACCC [ 80]
#IK19R29P2     TGGATCAAAC AACTAATCAA TATAACCAAAA ACCAAGAGGA GAACACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#IK19R31P4     TGGATCAAAC AACTAATCAA TATAACCAAAA ACCAAGAGGA GAACACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#IK19R31P5     TGGATCAAAC AACTAATCAA TATAACCAAAA ACCAAGAGGA GAACACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#IL19R16P1     TGGATCAAAC AACTAATCAA TATAACCAAAA ACCAAGAGGA GAACACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#IL19R17P1     TGGATCAAAC AACTAATCAA TATAACCAAAA ACCAAGAGGA GAACACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#IL19R18P1     TGGATCAAAC AACTAATCAA TATAACCAAAA ACCAAGAGGA GAACACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#IL19R19P1     TGGATCAAAC AACTAATCAA TATAACCAAAA ACCAAGAGGA GAACACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#IL19R19P2     TGGATCAAAC ACAAATCAA TATAACCAAAA ACCAAGAGGA GAACACATTC CTCTCATCAC CCCAACAAAA CAAAAAGCCC [ 80]
#Gsulphureus_EF379993.1 TGGATTAATT GTTAAAGTAG TATTATTGAA ACTAAGAAGG TATCTCATTC CTCTCGTTAT C---GAAGTC CGCGGATCTT [ 80]

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#CC08_UNG      ACTCACCAAC AAAACAAACA AACAACCAAC AGGAAATAAAA CCAAATGTCA AACTCTATGG TCTTCTCGTC CCACAAAAAC [160]
#CG01_JB       ACTCACCAAC AAAATAAACA AACAACCAAC AAGAAATAAAA CCAAATGTCA AACTCTATGG TCTTCTCGTC CCACAAAAAC [160]
#CK03_UNG      ATTCACCAAC AAAATAAACA AACAACCAAC AAAAAATAAAA CCAAATGTCA AACTCTATGG TCTTCTCGTC CCAAAACA-- [160]
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#CS05_SWS      ATTCACCAAC AAAATAAACA AACAACCAAC AAAAAATAAAA CCAAATGTCA AACTCTATGG TCTTCTCGTC CCAAAACA-- [160]
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#CS07_UNG      ATTCACCAAC AAAATAAACA AACAACCAAC AAAAAATAAAA CCAAATGTCA AACTCTATGG TCTTCTCGTC CCAAAACA-- [160]
#CT01_KNP      ACTTACCAAC AAAACAAACA AACAACCAAG AAAAAATAAAA TCAAATGTCA AACTCTATGG TCTTCTCATC CCAAAACA-- [160]
#CT01_LHNP     ACTTACCAAC AAAACAAACA AACAACCAAG AAAAAATAAAA TCAAATGTCA AACTCTATGG TCTTCTCGTC CCAAAACA-- [160]
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#IK19R29P2     ACTCACCAAC AAAACAAACA AACAACCAAC AGGAAATAAAA CCAAATGTCA AACTCTATGG TCTTCTCGTC CCACAAAAAC [160]
#IK19R31P4     ACTCACCAAC AAAACAAACA AACAACCAAC AGGAAATAAAA CCAAATGTCA AACTCTATGG TCTTCTCGTC CCACAAAAAC [160]
#IK19R31P5     ACTCACCAAC AAAACAAACA AACAACCAAC AGGAAATAAAA CCAAATGTCA AACTCTATGG TCTTCTCGTC CCACAAAAAC [160]
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#IL19R17P1     ACTCACCAAC AAAACAAACA AACAACCAAC AGGAAATAAAA CCAAATGTCA AACTCTATGG TCTTCTCGTC CCACAAAAAC [160]
#IL19R18P1     ACTCACCAAC AAAACAAACA AACAACCAAC AGGAAATAAAA CCAAATGTCA AACTCTATGG TCTTCTCGTC CCACAAAAAC [160]
#IL19R19P1     ACTCACCAAC AAAACAAACA AACAACCAAC AGGAAATAAAA CCAAATGTCA AACTCTATGG TCTTCTCGTC CCACAAAAAC [160]
#IL19R19P2     ACTCACCAAC AAAACAAACA AACAACCAAC AGGAAATAAAA CCAAATGTCA AACTCTATGG TCTTCTCGTC CCACAAAAAC [160]

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Appendix B. continued

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#C.gestroi_EF379963.1 ATCTAAGAAT TTAACTCAA AGACCAAATT CAATAAAACA TTCAACACGA CAGCCCGTGC CTCGTCAAGC CATTCAATTCC [240]

#C.gestroi_EF379969.1 ATCTAAGAAT TTAACTCAA AGACCAAATT CAATAAAACA TTCAACACGA CAGCCCGTGC CTCGTCAAGC CATTCAATTCC [240]

#C.gestroi_EF379970.1 ATCTAAGAAT TTAACTCAA AGACCAAATT CAATAAAACA TTCAACACGA CAGCCCGTGC CTCGTCAAGC CATTCAATTCC [240]

#C.kalshoveni_FJ376684.1 ATTTAAGAAT TTAACTCAA AGACCAAATT CAATAAAATA TTCAACACGA CAGCTCGTGC CTCGTCAAGC CATTCAATTCC [240]

#C.kalshoveni_FJ376685.1 ATTTAAGAAT TTAACTCAA AGACCAAATT CAATAAAATA TTCAACACGA CAGCTCGTGC CTCGTCAAGC CATTCAATTCC [240]

#C.traviansMY_FJ915824.1 ATTTAAGAAT TTAACTCAA AGACCAAATT CAATAAAATA TTCAACACGA CAGCTCGTGC CTCGTCAAGC CATTCAATTCC [240]

#CC001MA ATTTAAGAAT TTAACTCAA AGACCAAATT CAATAAAACA TTCAACACGA CAGCCCGTGC CTCGTCAAGC CATTCAATTCC [240]

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#CC01_KL ATTTAAGAAT TTAACTCAA AGACCAAATT CAATAAAACA TTCAACACGA CAGCCCGTGC CTCGTCAAGC CATTCAATTCC [240]

#CC01_KLKG ATTTAAGAAT TTAACTCAA AGACCAAATT CAATAAAACA TTCAACACGA CAGCCCGTGC CTCGTCAAGC CATTCAATTCC [240]

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#CT01_LHNP -TCTAAGAAT TTAACTCAA AAACCAAATT CAATAAAACA TTCAACACGA CAGCCCGTGC CTCGTCAAGC CATTCAATTCC [240]

Appendix B. continued

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#IK19R31P4     ATTTAAGAAT TTAACTCAA AGACCAAATT CAATAAAACA TTCAACACGA CAGCCCGTGC CTCGTCAAGC CATTCAATTCC [240]
#IK19R31P5     ATTTAAGAAT TTAACTCAA AGACCAAATT CAATAAAACA TTCAACACGA CAGCCCGTGC CTCGTCAAGC CATTCAATTCC [240]
#IL19R16P1     ATTTAAGAAT TTAACTCAA AGACCAAATT CAATAAAACA TTCAACACGA CAGCCCGTGC CTCGTCAAGC CATTCAATTCC [240]
#IL19R17P1     ATTTAAGAAT TTAACTCAA AGACCAAATT CAATAAAACA TTCAACACGA CAGCCCGTGC CTCGTCAAGC CATTCAATTCC [240]
#IL19R18P1     ATTTAAGAAT TTAACTCAA AGACCAAATT CAATAAAACA TTCAACACGA CAGCCCGTGC CTCGTCAAGC CATTCAATTCC [240]
#IL19R19P1     ATTTAAGAAT TTAACTCAA AGACCAAATT CAATAAAACA TTCAACACGA CAGCCCGTGC CTCGTCAAGC CATTCAATTCC [240]
#IL19R19P2     ATTTAAGAAT TTAACTCAA AGACCAAATT CAATAAAACA TACAACACGA CAGCCCGTGC CTCGTCAAGC CATTCAATTCC [240]
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#C.curvignathusMY_FJ376686.1 AGATCACAAT TAAAGAACTT ATGCTACCTT TGCACGGTCA AAATACCGCG GCCCTTCAAC ACTCAATGTC AGCGGGCAGG [320]
#C.gestroi_EF379963.1 AGATCACAAT TAAAGAACTT ATGCTACCTT TGCACGGTCA AAATACCGCG GCCCTTCAAC ACCAAATGTC AGCGGGCAGG [320]
#C.gestroi_EF379969.1 AGATCACAAT TAAAGAACTT ATGCTACCTT TGCACGGTCA AAATACCGCG GCCCTTCAAC ACCAAATGTC AGCGGGCAGG [320]
#C.gestroi_EF379970.1 AGATCACAAT TAAAGAACTT ATGCTACCTT TGCACGGTCA AAATACCGCG GCCCTTCAAC ACCAAATGTC AGCGGGCAGG [320]
#C.kalshoveni_FJ376684.1 AGATCACAAT TAAAGAACTT ATGCTACCTT TGCACGGTCA AAATACCGCG GCCCTTCAAC ACTCAATGTC AGCGGGCAGG [320]
#C.kalshoveni_FJ376685.1 AGATCACAAT TAAAGAACTT ATGCTACCTT TGCACGGTCA AAATACCGCG GCCCTTCAAC ACTCAATGTC AGCGGGCAGG [320]
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#CC01_BHR     AGATCACAAT TAAAGAACTT ATGCTACCTT TGCACGGTCA AAATACCGCG GCCCTTCAAC ACTCAATGTC AGCGGGCAGG [320]
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#CC01_KLKG    AGATCACAAT TAAAGAACTT ATGCTACCTT TGCACGGTCA AAATACCGCG GCCCTTCAAC ACTCAATGTC AGCGGGCAGG [320]
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#CK04_UN      AGATCACAAT TAAAGAACTT ATGCTACCTT TGCACGGTCA AAATACCGCG GCCCTTCAAC ACTCAATGTC AGCGGGCAGG [320]
#CS01_BHW     AGATCACAAT TAAAGAACTT ATGCTACCTT TGCACGGTCA AAATACCGCG GCCCTTCAAC ACTCAATGTC AGCGGGCAGG [320]
#CS01_UN      AGATCACAAT TAAAGAACTT ATGCTACCTT TGCACGGTCA AAATACCGCG GCCCTTCAAC ACTCAATGTC AGCGGGCAGG [320]
#CS02_BHW     AGATCACAAT TAAAGAACTT ATGCTACCTT TGCACGGTCA AAATACCGCG GCCCTTCAAC ACTCAATGTC AGCGGGCAGG [320]
#CS02_KNP     AGATCACAAT TAAAGAACTT ATGCTACCTT TGCACGGTCA AAATACCGCG GCCCTTCAAC ACTCAATGTC AGCGGGCAGG [320]
#CS03_BHW     AGATCACAAT TAAAGAACTT ATGCTACCTT TGCACGGTCA AAATACCGCG GCCCTTCAAC ACTCAATGTC AGCGGGCAGG [320]

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Appendix B. continued

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#IK19R31P5     AGATCACAAT TAAAGAACTT ATGCTACCTT TGCACGGTCA AAATACCGCG GCCCTTCAAC ACTCAATGTC AGCGGGCAGG [320]
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#CC02_UN      C [321]
#CC03_KLKG     C [321]
#CC04_SWS      C [321]
#CC05_UN      C [321]
#CC08_UN      C [321]
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Appendix B. continued

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#CS04_BHW	C [321]
#CS05_SWS	C [321]
#CS06_UN	C [321]
#CS07_UN	C [321]
#CT01_KNP	C [321]
#CT01_LHNP	C [321]
#CT01_SNP	C [321]
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#IK19R31P4	C [321]
#IK19R31P5	C [321]
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#IL19R18P1	C [321]
#IL19R19P1	C [321]
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Appendix C. The aligned sequences of the COII gene for each specimen. Positions are given at the end of line.

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#CS01BHW      CCC CCA TAC AAC TCT TTT TCC ATG ACC ATG TAC TAT ACC ACC ACA ACC GTA TTA TAC ATT ACC CTC CGA AAT CAA ACC [ 78]
#CS02KNP      CCC CCA TAC AAC TCT TTT TCC ATG ACC ATG TAC TAT ACC ACC ACA ACC GTA TTA TAC ATT ACC CTC CGA AAT CAA ACC [ 78]
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#CC02SKAG     CCC CCA TAC AAT TCT TCT TCC ACG ACC ACG TAC TAT ATT ACT ACA ACC GTA TCA TAC ATC ACC CTC CGA AAC CAA ACC [ 78]
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#CGEF379952.1 CCC CCA TAC AAC TCT TCT TTC ACG ACC ATG TAC TAT ACT ACA ACA ACC GTA TCA TAC ATT ACC CTC CGA AAT CAA ACA [ 78]
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Appendix C. continued

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Appendix C. continued

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#CK04UNS ACT CCT ATA TCA CGA AGT CCA CAA CCC CAC ATC ACA AGC AGT AGG ACA CCA ATA ATA ATA CTC AGA TTT CAC ACA ATT [234]
#CK03UNS ACT CCT ATA TCA CGA AGT CCA CAA CCC CAC ATC ACA AGC AGT AGG ACA CCA ATA ATA ATA CTC AGA TTT CAC ACA ATT [234]
#CTAJ854170.1 ACT TCT ATA CCA CGA GGT CAA --- --- --- ACC GCA GGC AGT CGG ACA CCA ATA ATA ATA TTC AGA CTT CAC ACA ATT [234]
#CTAJ854169.1 ACT TCT ATA CCA CGA GGT CAA --- --- --- ACC GCA GGC AGT CGG ACA CCA ATA ATA ATA TTC AGA CTT CAC ACA ATT [234]
#CGEF379952.1 ACT TCT ATA CCA CGA AGT TCA CAA CCC AAC ACC ACA AGC AGT TGG GCA CCA ATA TTA GTA TTC AGA CTT TAC ACA ATT [234]

Appendix C. continued

#CGEF379945.1 ACT TCT ATA CCA CGA AGT TCA CAA CCC AAC ACC ACA AGC AGT TGG GCA CCA ATA TTA GTA TTC AGA CTT TAC ACA ATT [234]
 #CGEF379951.1 ACT TCT ATA CCA CGA AGT TCA CAA CCC AAC ACC ACA AGC AGT TGG GCA CCA ATA TTA GTA TTC AGA CTT TAC ACA ATT [234]
 #CSAJ854163.1 ACT CCT ATA TCA CGA AGT CCA CAA CCC CAC ATC ACA AGC AGT AGG ACA CCA ATA ATA ATA CTC AGA TTT CAC ACA ATT [234]
 #CSAJ854165.1 ACT CCT ATA TCA CGA AGT CCA CAA CCC CAC ATC ACA AGC AGT AGG ACA CCA ATA ATA ATA CTC AGA TTT CAC ACA ATT [234]
 #GSEF379957.1 ACT ATT ATA CCA TGA AAT CCA CAA CCC AGC GAC ACA AGC TGT TGG ACA TCA ATA CTA ATA CTC AGA CTT TAC ACA ATT [234]
 #CC02UNS CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #CC03KLKG CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #CC01KLKG CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #CS01BHW CGA CTC ATA TAT CCC CCA AGA AGA ACA ACA AGA AAG AAC ATT TCG ACT ACA CAC AGA CCG AAT TGT CCT ACC AAA CTC [312]
 #CS02KNP CGA CTC ATA TAT CCC CCA AGA AGA ACA ACA AGA AAG AAC ATT TCG ACT ACA CAC AGA CCG AAT TGT CCT ACC AAA CTC [312]
 #CS02BHW CGA CTC ATA TAT CCC CCA AGA AGA ACA ACA AGA AAG AAC ATT TCG ACT ACA CAC AGA CCG AAT TGT CCT ACC AAA CTC [312]
 #CS03BHW CGA CTC ATA TAT CCC CCA AGA AGA ACA ACA AGA AAG AAC ATT TCG ACT ACA CAC AGA CCG AAT TGT CCT ACC AAA CTC [312]
 #CKFJ384652.1 CGA CTC ATA TAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ACA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #CC01MA CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #IK19R29P2 CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #CC01SNR CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #CC01TB CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #IL19R17P1 CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #CC05UNS CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #CCFJ384635.1 CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #CC01SKAG CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #CC02SKAG CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #IK19R31P5 CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #IK19R31P4 CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #IL19R19P1 CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #CC01KL CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #CS04BHW CGA CTC ATA TAT CCC CCA AGA AGA ACA ACA AGA AAG AAC ATT TCG ACT ACA CAC AGA CCG AAT TGT CCT ACC AAA CTC [312]
 #CS06UNS CGA CTC ATA TAT CCC CCA AGA AGA ACA ACA AGA AAG AAC ATT TCG ACT ACA CAC AGA CCG AAT TGT CCT ACC AAA CTC [312]
 #CS07UNS CGA CTC ATA TAT CCC CCA AGA AGA ACA ACA AGA AAG AAC ATT TCG ACT ACA CAC AGA CCG AAT TGT CCT ACC AAA CTC [312]
 #CC04SWS CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #CCFJ384634.1 CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #CC02KL CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #CC02BHR CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #CC01BHR CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #CC02KLKG CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #CC08UNS CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ATA CAC AGA CCG AAT CGT CCT ACC AAA CTC [312]
 #CK04UNS CGA CTC ATA TAT CCC CCA AGA AGA ACA ACA AGA AAG AAC ATT TCG ACT ACA CAC AGA CCG AAT TGT CCT ACC AAA CTC [312]
 #CK03UNS CGA CTC ATA TAT CCC CCA AGA AGA ACA ACA AGA AAG AAC ATT TCG ACT ACA CAC AGA CCG AAT TGT CCT ACC AAA CTC [312]
 #CTAJ854170.1 TGA CTC ATA TAT CCC ACA AGA AGA ACA ACA AGA AAG AAC ATT CCG ACT ACA CAC AGA CCG AGT CGT TTT ACC AAA CTC [312]
 #CTAJ854169.1 TGA CTC ATA TAT CCC ACA AGA AGA ACA ACA AGA AAG AAC ATT CCG ACT ACA CAC AGA CCG AGT CGT TTT ACC AAA CTC [312]

Appendix C. continued

#CGEF379952.1 CGA CTC ATA CAT TCC TCA AGA AGA ACA ACA AGA AAG AAC ATT CCG ACT ACA CAC AGA CCG AAT CGT TCT ACC AAA CTC [312]
 #CGEF379945.1 CGA CTC ATA CAT TCC TCA AGA AGA ACA ACA AGA AAG AAC ATT CCG ACT ACA CAC AGA CCG AAT CGT TCT ACC AAA CTC [312]
 #CGEF379951.1 CGA CTC ATA CAT TCC TCA AGA AGA ACA ACA AGA AAG AAC ATT CCG ACT ACA CAC AGA CCG AAT CGT TCT ACC AAA CTC [312]
 #CSAJ854163.1 CGA CTC ATA TAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ACA CAC AGA TCG AAT TGT CCT ACC AAA CTC [312]
 #CSAJ854165.1 CGA CTC ATA CAT CCC CCA AGA AGA ACA GCA AGA AAG AAC ATT TCG ACT ACA CAC AGA CCG AAT TGT CCT ACC AAA CTC [312]
 #GSEF379957.1 CGA CTC ATA CAT ACA ACA AGA GGA CCA ACA AAC TGA TAC ATT TCG ACT ACA TAC AGA TCG AAT TGT ACT ACC AAA TTC [312]
 #CC02UNS ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #CC03KLKG ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #CC01KLKG ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #CS01BHW CCC AAT CCG AAC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGA ACA ATC CCA AGA CTA GAA ACA GAT GCC ACA CCC GGA [390]
 #CS02KNP TCC AAT CCG AAC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGA ACA ATC CCA AGA CTA GAA ACA GAT GCC ACA CCC GGA [390]
 #CS02BHW CCC AAT CCG AAC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGA ACA ATC CCA AGA CTA GAA ACA GAT GCC ACA CCC GGA [390]
 #CS03BHW CCC AAT CCG AAC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGA ACA ATC CCA AGA CTA GAA ACA GAT GCC ACA CCC GGA [390]
 #CKFJ384652.1 CCC AAT CCG AAC GTT ACA GCA GCA GAT GTC CTA CAC TCA TGA ACA ATC CCA AGA CTA GAA ACA GAT GCC ACA CCC GGA [390]
 #CC01MA ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #IK19R29P2 ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #CC01SNR ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #CC01TB ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #IL19R17P1 ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #CC05UNS ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #CCFJ384635.1 ACC AAT CCG ATT GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #CC01SKAG ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #CC02SKAG ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #IK19R31P5 ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #IK19R31P4 ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #IL19R19P1 ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #CC01KL ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #CS04BHW CCC AAT CCG AAC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGA ACA ATC CCA AGA CTA GAA ACA GAT GCC ACA CCC GGA [390]
 #CS06UNS TCC AAT CCG AAC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGA ACA ATC CCA AGA CTA GAA ACA GAT GCC ACA CCC GGA [390]
 #CS07UNS TCC AAT CCG AAC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGA ACA ATC CCA AGA CTA GAA ACA GAT GCC ACA CCC GGA [390]
 #CC04SWS ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #CCFJ384634.1 ACC AAT CCG ATT GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #CC02KL ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #CC02BHR ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #CC01BHR ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #CC02KLKG ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #CC08UNS ACC AAT CCG ATC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGG ACA ATC CCA AGA CTG GAA ACA GAT GCC ACA CCG GGG [390]
 #CK04UNS CCC AAT CCG AAC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGA ACA ATC CCA AGA CTA GAA ACA GAT GCC ACA CCC GGA [390]
 #CK03UNS TCC AAT CCG AAC GTT ACA GCA GCA GAC GTC CTA CAC TCA TGA ACA ATC CCA AGA CTA GAA ACA GAT GCC ACA CCC GGA [390]
 #CTAJ854170.1 ACC AAT CCG ACC GTC ACA GCA GCA GAT GTT TTA CAC TCA TGA ACA ATC CCA AGA CTT GAA ACA GAT GCC ACA CCA GGA [390]
 #CTAJ854169.1 ACC AAT CCG ACC GTC ACA GCA GCA GAT GTT TTA CAC TCA TGA ACA ATC CCA AGA CTT GAA ACA GAT GCC ACA CCA GGA [390]

Appendix C. continued

#CGEF379952.1 ACC AAT TCG ACT GTA ACA GCA GCA GAC GTC CTA CAC TCA TGA ACT ATC CCA AGA CTG GAA ACA GAT GCC ACG CCA GGA [390]
 #CGEF379945.1 ACC AAT TCG ACT GTA ACA GCA GCA GAC GTC CTA CAC TCA TGA ACT ATC CCA AGA CTG GAA ACA GAT GCC ACG CCA GGA [390]
 #CGEF379951.1 ACC AAT TCG ACT GTA ACA GCA GCA GAC GTC CTA CAC TCA TGA ACT ATC CCA AGA CTG GAA ACA GAT GCC ACG CCA GGA [390]
 #CSAJ854163.1 TCC AAT CCG AAC GTT ACA GCA GCA GAT GTC CTA CAC TCA TGA ACA ATC CCA AGA CTA GAA ACA GAT GCC ACA CCA GGA [390]
 #CSAJ854165.1 CCC AAT CCG AAC GTT ACA GCA GCA GAT GTC CTA CAC TCA TGA ACA ATC CCA AGA CTA GAA ACA GAT GCC ACA CCC GGA [390]
 #GSEF379957.1 ACC AAT TCG ATC GTC ACA GCA GCT GAC GTA TTA CAC TCT TGA ACA GTA CCT AGA CTT GAA ACA GAT GCC ACA CCA GGC [390]
 #CC02UNS CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #CC03KLKG CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #CC01KLKG CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #CS01BHW CGA CAT CAA ACA AGA TTC TCA ATT AGT CGT CCG GGT ATC CTA TAC GGA CAA TGC TCA GAA ATT TGT GGA GCA AAT CAC [468]
 #CS02KNP CGA CAT CAA ACA AGA TTC TCA ATT AGC CGT CCG GGT ATC CTA TAC GGA CAA TGC TCA GAA ATT TGT GGA GCA AAT CAC [468]
 #CS02BHW CGA CAT CAA ACA AGA TTC TCA ATT AGT CGT CCG GGT ATC CTA TAC GGA CAA TGC TCA GAA ATT TGT GGA GCA AAT CAC [468]
 #CS03BHW CGA CAT CAA ACA AGA TTC TCA ATT AGT CGT CCG GGT ATC CTA TAC GGA CAA TGC TCA GAA ATT TGT GGA GCA AAT CAC [468]
 #CKFJ384652.1 CGA CAT CAA ACA AGA TTC TCA ATC AGT CGT CCG GGT ATC CTA TAC GGA CAA TGC TCA GAA ATT TGT GGA GCA AAT CAC [468]
 #CC01MA CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #IK19R29P2 CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #CC01SNR CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #CC01TB CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #IL19R17P1 CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #CC05UNS CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #CCFJ384635.1 CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #CC01SKAG CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #CC02SKAG CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #IK19R31P5 CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #IK19R31P4 CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #IL19R19P1 CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #CC01KL CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #CS04BHW CGA CAT CAA ACA AGA TTC TCA ATT AGT CGT CCG GGT ATC CTA TAC GGA CAA TGC TCA GAA ATT TGT GGA GCA AAT CAC [468]
 #CS06UNS CGA CAT CAA ACA AGA TTC TCA ATT AGT CGT CCG GGT ATC CTA TAC GGA CAA TGC TCA GAA ATT TGT GGA GCA AAT CAC [468]
 #CS07UNS CGA CAT CAA ACA AGA TTC TCA ATT AGT CGT CCG GGT ATC CTA TAC GGA CAA TGC TCA GAA ATT TGT GGA GCA AAT CAC [468]
 #CC04SWS CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #CCFJ384634.1 CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #CC02KL CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #CC02BHR CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #CC01BHR CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #CC02KLKG CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #CC08UNS CGA CAC CAA ACA AGA TTC TCA ATC AGC CGT CCC GGC ATC CTA TAT GGA CAA TGC TCA GAA ATT TGT GGA GCA AAC CAC [468]
 #CK04UNS CGA CAT CAA ACA AGA TTC TCA ATT AGT CGT CCG GGT ATC CTA TAC GGA CAA TGC TCA GAA ATT TGT GGA GCA AAT CAC [468]
 #CK03UNS CGA CAT CAA ACA AGA TTC TCA ATT AGT CGT CCG GGT ATC CTA TAC GGA CAA TGC TCA GAA ATT TGT GGA GCA AAT CAC [468]
 #CTAJ854170.1 CGA CAT CAA ACG AGA TTC TCA ATT AGT CGT CCT GGT ATC CTA TAC GGA CAA TGC TCA GAA ATT TGT GGG GCA AAC CAC [468]
 #CTAJ854169.1 CGA CAT CAA ACG AGA TTC TCA ATT AGT CGT CCT GGT ATC CTA TAC GGA CAA TGC TCA GAA ATT TGT GGG GCA AAC CAC [468]

Appendix C. continued

#CGEF379952.1 CGA TAC CAA ACA AGA TTC TCA ATT AGC CGT CCT GGC ATC TTA TAT GGA CAA TGT TCA GAA ATT TGC GGG GCA AAC CAC [468]
#CGEF379945.1 CGA TAC CAA ACA AGA TTC TCA ATT AGC CGT CCT GGC ATC TTA TAT GGA CAA TGT TCA GAA ATT TGC GGG GCA AAC CAC [468]
#CGEF379951.1 CGA TAC CAA ACA AGA TTC TCA ATT AGC CGT CCT GGC ATC TTA TAT GGA CAA TGT TCA GAA ATT TGC GGG GCA AAC CAC [468]
#CSAJ854163.1 CGA CAT CAA ACA AGA TTC TCA ATC AGT CGT CCG GGT ATC CTA TAC GGA CAA TGC TCA GAA ATT TGC GGA GCA AAT CAC [468]
#CSAJ854165.1 CGA CAT CAA ACA AGA TTC TCA ATC AGT CGT CCG GGT ATC CTA TAT GGA CAA TGC TCA GAA ATT TGC GGA GCA AAT CAC [468]
#GSEF379957.1 CGA CAC CAA GTA AGA TTT TCA ATT AAC CGA CCA GGC CTC CTA TAC GGA CAA TGC TCG GAG ATT TGC GGA GCA AAC CAC [468]

#CC02UNS AGA TTC ATA [477]
#CC03KLKG AGA TTC ATA [477]
#CC01KLKG AGA TTC ATA [477]
#CS01BHW AGA TTC ATG [477]
#CS02KNP AGA TTC ATG [477]
#CS02BHW AGA TTC ATG [477]
#CS03BHW AGA TTC ATG [477]
#CKFJ384652.1 AGA TTC ATG [477]
#CC01MA AGA TTC ATA [477]
#IK19R29P2 AGA TTC ATA [477]
#CC01SNR AGA TTC ATA [477]
#CC01TB AGA TTC ATA [477]
#IL19R17P1 AGA TTC ATA [477]
#CC05UNS AGA TTC ATA [477]
#CCFJ384635.1 AGA TTC ATA [477]
#CC01SKAG AGA TTC ATA [477]
#CC02SKAG AGA TTC ATA [477]
#IK19R31P5 AGA TTC ATA [477]
#IK19R31P4 AGA TTC ATA [477]
#IL19R19P1 AGA TTC ATA [477]
#CC01KL AGA TTC ATA [477]
#CS04BHW AGA TTC ATG [477]
#CS06UNS AGA TTC ATG [477]
#CS07UNS AGA TTC ATG [477]
#CC04SWS AGA TTC ATA [477]
#CCFJ384634.1 AGA TTC ATA [477]
#CC02KL AGA TTC ATA [477]
#CC02BHR AGA TTC ATA [477]
#CC01BHR AGA TTC ATA [477]

Appendix C. continued

#CC02KLKG	AGA TTC ATA [477]
#CC08UNS	AGA TTC ATA [477]
#CK04UNS	AGA TTC ATG [477]
#CK03UNS	AGA TTC ATG [477]
#CTAJ854170.1	AGA TTC ATG [477]
#CTAJ854169.1	AGA TTC ATG [477]
#CGEF379952.1	AGA TTC ATA [477]
#CGEF379945.1	AGA TTC ATA [477]
#CGEF379951.1	AGA TTC ATA [477]
#CSAJ854163.1	AGA TTC ATG [477]
#CSAJ854165.1	AGA TTC ATG [477]
#GSEF379957.1	AGA TTC ATA [477]

Appendix D. Nucleotide composition of *Coptotermes* spp. based on COII gene.

Species name	Accession number	All sites					Codon position 1			
		T(U)	C	A	G	Total	T-1	C-1	A-1	G-1
<i>C.curvignathus</i>	CC02UNS	19.7	32.1	35.0	13.2	477.0	16.4	28.3	44.0	11.3
<i>C.curvignathus</i>	CC03KLKG	19.7	31.7	35.2	13.4	477.0	15.7	28.3	44.7	11.3
<i>C.curvignathus</i>	CC01KLKG	19.7	31.7	35.2	13.4	477.0	15.7	28.3	44.7	11.3
<i>C.curvignathus</i>	CC01MA	19.5	32.1	35.0	13.4	477.0	15.7	28.3	44.7	11.3
<i>C.curvignathus</i>	IK19R29P2	19.3	32.3	35.0	13.4	477.0	15.1	28.9	44.7	11.3
<i>C.curvignathus</i>	CC01SNR	19.5	32.1	35.0	13.4	477.0	15.7	28.3	44.7	11.3
<i>C.curvignathus</i>	CC01TB	19.7	32.1	35.0	13.2	477.0	16.4	28.3	44.0	11.3
<i>C.curvignathus</i>	IL19R17P1	19.3	32.3	35.0	13.4	477.0	15.1	28.9	44.7	11.3
<i>C.curvignathus</i>	CC05UNS	19.3	32.3	35.0	13.4	477.0	15.1	28.9	44.7	11.3
<i>C.curvignathus</i>	CCFJ384635.1	19.5	32.1	35.0	13.4	477.0	15.1	28.9	44.7	11.3
<i>C.curvignathus</i>	CC01SKAG	19.5	32.1	35.0	13.4	477.0	15.7	28.3	44.7	11.3
<i>C.curvignathus</i>	CC02SKAG	19.5	32.3	35.0	13.2	477.0	15.7	28.9	44.0	11.3
<i>C.curvignathus</i>	IK19R31P5	19.5	32.3	35.0	13.2	477.0	15.7	28.9	44.0	11.3
<i>C.curvignathus</i>	IK19R31P4	19.5	32.3	35.0	13.2	477.0	15.7	28.9	44.0	11.3
<i>C.curvignathus</i>	IL19R19P1	19.5	32.3	35.0	13.2	477.0	15.7	28.9	44.0	11.3
<i>C.curvignathus</i>	CC01KL	19.5	32.3	35.0	13.2	477.0	15.7	28.9	44.0	11.3
<i>C.curvignathus</i>	CC04SWS	19.5	32.1	35.0	13.4	477.0	15.7	28.3	44.7	11.3
<i>C.curvignathus</i>	CCFJ384634.1	19.5	32.1	35.0	13.4	477.0	15.1	28.9	44.7	11.3
<i>C.curvignathus</i>	CC02KL	19.3	32.3	35.0	13.4	477.0	15.1	28.9	44.7	11.3
<i>C.curvignathus</i>	CC02BHR	19.3	32.3	35.0	13.4	477.0	15.1	28.9	44.7	11.3
<i>C.curvignathus</i>	CC01BHR	19.5	32.1	35.0	13.4	477.0	15.1	28.9	44.7	11.3
<i>C.curvignathus</i>	CC02KLKG	19.7	31.7	35.2	13.4	477.0	15.7	28.3	44.7	11.3
<i>C.curvignathus</i>	CC08UNS	19.3	32.3	35.0	13.4	477.0	15.1	28.9	44.7	11.3
<i>C. gestroi</i>	CGEF379952.1	21.8	29.6	35.2	13.4	477.0	21.4	23.9	42.8	11.9

Appendix D. continued

<i>C. gestroi</i>	CGEF379945.1	21.8	29.6	35.2	13.4	477.0	21.4	23.9	42.8	11.9
<i>C. gestroi</i>	CGEF379951.1	21.8	29.6	35.2	13.4	477.0	21.4	23.9	42.8	11.9
<i>C. kalshoveni</i>	CKFJ384652.1	20.1	31.4	35.4	13.0	477.0	15.1	30.2	43.4	11.3
<i>C. kalshoveni</i>	CK04UNS	20.3	31.2	35.8	12.6	477.0	15.1	30.2	44.7	10.1
<i>C. kalshoveni</i>	CK03UNS	20.3	31.2	35.8	12.6	477.0	15.7	29.6	44.7	10.1
<i>C. sepangensis</i>	CS01BHW	20.5	31.2	35.8	12.4	477.0	15.7	30.2	44.0	10.1
<i>C. sepangensis</i>	CS02KNP	19.9	31.8	35.9	12.4	468.0	16.0	30.1	44.2	9.6
<i>C. sepangensis</i>	CS02BHW	20.3	31.2	35.8	12.6	477.0	15.1	30.2	44.7	10.1
<i>C. sepangensis</i>	CS03BHW	20.5	31.2	35.8	12.4	477.0	15.7	30.2	44.0	10.1
<i>C. sepangensis</i>	CS05SWS	19.5	32.3	35.0	13.2	477.0	15.7	28.9	44.0	11.3
<i>C. sepangensis</i>	CS04BHW	20.1	31.2	35.8	12.8	477.0	15.1	30.2	44.7	10.1
<i>C. sepangensis</i>	CS06UNS	20.8	31.0	35.8	12.4	477.0	16.4	29.6	44.0	10.1
<i>C. sepangensis</i>	CS07UNS	20.8	31.0	35.8	12.4	477.0	16.4	29.6	44.0	10.1
<i>C. sepangensis</i>	CSAJ854163.1	20.5	30.8	35.6	13.0	477.0	16.4	28.9	43.4	11.3
<i>C. sepangensis</i>	CSAJ854165.1	20.1	31.2	35.4	13.2	477.0	14.5	30.2	44.0	11.3
<i>C. travians</i>	CT01SNP	19.3	32.8	34.5	13.3	472.0	22.2	31.0	29.7	17.1
<i>C. travians</i>	CTAJ854170.1	21.2	29.9	34.8	14.1	468.0	16.7	26.9	43.6	12.8
<i>C. travians</i>	CTAJ854169.1	21.2	29.9	34.8	14.1	468.0	16.7	26.9	43.6	12.8
<i>G. sulphureus</i>	GSEF379957.1	22.9	29.4	34.2	13.6	477.0	20.1	25.8	40.3	13.8
Avg. (with outgroup)		20.0	31.5	35.2	13.2	476.3	16.3	28.6	43.8	11.4
Avg. (Ingroup)		20.0	31.6	35.2	13.2	476.2	16.2	28.7	43.9	11.3

Appendix D. continued

Species name	Accession number	Codon position 2				Codon position 3			
		T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
<i>C. curvignathus</i>	CC02UNS	21.4	34.6	23.3	20.8	21.4	33.3	37.7	7.5
<i>C. curvignathus</i>	CC03KLKG	21.4	34.0	23.9	20.8	22.0	32.7	37.1	8.2
<i>C. curvignathus</i>	CC01KLKG	21.4	34.0	23.9	20.8	22.0	32.7	37.1	8.2
<i>C. curvignathus</i>	CC01MA	21.4	34.6	23.3	20.8	21.4	33.3	37.1	8.2
<i>C. curvignathus</i>	IK19R29P2	21.4	34.6	23.3	20.8	21.4	33.3	37.1	8.2
<i>C. curvignathus</i>	CC01SNR	21.4	34.6	23.3	20.8	21.4	33.3	37.1	8.2
<i>C. curvignathus</i>	CC01TB	21.4	34.6	23.3	20.8	21.4	33.3	37.7	7.5
<i>C. curvignathus</i>	IL19R17P1	21.4	34.6	23.3	20.8	21.4	33.3	37.1	8.2
<i>C. curvignathus</i>	CC05UNS	21.4	34.6	23.3	20.8	21.4	33.3	37.1	8.2
<i>C. curvignathus</i>	CCFJ384635.1	21.4	34.6	23.3	20.8	22.0	32.7	37.1	8.2
<i>C. curvignathus</i>	CC01SKAG	21.4	34.6	23.3	20.8	21.4	33.3	37.1	8.2
<i>C. curvignathus</i>	CC02SKAG	21.4	34.6	23.3	20.8	21.4	33.3	37.7	7.5
<i>C. curvignathus</i>	IK19R31P5	21.4	34.6	23.3	20.8	21.4	33.3	37.7	7.5
<i>C. curvignathus</i>	IK19R31P4	21.4	34.6	23.3	20.8	21.4	33.3	37.7	7.5
<i>C. curvignathus</i>	IL19R19P1	21.4	34.6	23.3	20.8	21.4	33.3	37.7	7.5
<i>C. curvignathus</i>	CC01KL	21.4	34.6	23.3	20.8	21.4	33.3	37.7	7.5
<i>C. curvignathus</i>	CC04SWS	21.4	34.6	23.3	20.8	21.4	33.3	37.1	8.2
<i>C. curvignathus</i>	CCFJ384634.1	21.4	34.6	23.3	20.8	22.0	32.7	37.1	8.2
<i>C. curvignathus</i>	CC02KL	21.4	34.6	23.3	20.8	21.4	33.3	37.1	8.2
<i>C. curvignathus</i>	CC02BHR	21.4	34.6	23.3	20.8	21.4	33.3	37.1	8.2
<i>C. curvignathus</i>	CC01BHR	22.0	34.0	23.3	20.8	21.4	33.3	37.1	8.2
<i>C. curvignathus</i>	CC02KLKG	21.4	34.0	23.9	20.8	22.0	32.7	37.1	8.2

Appendix D. continued

<i>C. curvignathus</i>	CC08UNS	21.4	34.6	23.3	20.8	21.4	33.3	37.1	8.2
<i>C. gestroi</i>	CGEF379952.1	20.8	35.2	23.3	20.8	23.3	29.6	39.6	7.5
<i>C. gestroi</i>	CGEF379945.1	20.8	35.2	23.3	20.8	23.3	29.6	39.6	7.5
<i>C. gestroi</i>	CGEF379951.1	20.8	35.2	23.3	20.8	23.3	29.6	39.6	7.5
<i>C. kalshoveni</i>	CKFJ384652.1	22.0	33.3	24.5	20.1	23.3	30.8	38.4	7.5
<i>C. kalshoveni</i>	CK04UNS	22.6	32.7	23.9	20.8	23.3	30.8	39.0	6.9
<i>C. kalshoveni</i>	CK03UNS	22.6	32.7	23.9	20.8	22.6	31.4	39.0	6.9
<i>C. sepangensis</i>	CS01BHW	22.6	32.7	23.9	20.8	23.3	30.8	39.6	6.3
<i>C. sepangensis</i>	CS02KNP	21.2	33.3	24.4	21.2	22.4	32.1	39.1	6.4
<i>C. sepangensis</i>	CS02BHW	22.6	32.7	23.9	20.8	23.3	30.8	39.0	6.9
<i>C. sepangensis</i>	CS03BHW	22.6	32.7	23.9	20.8	23.3	30.8	39.6	6.3
<i>C. sepangensis</i>	CS05SWS	21.4	34.6	23.3	20.8	21.4	33.3	37.7	7.5
<i>C. sepangensis</i>	CS04BHW	22.0	32.7	23.9	21.4	23.3	30.8	39.0	6.9
<i>C. sepangensis</i>	CS06UNS	22.6	32.7	23.9	20.8	23.3	30.8	39.6	6.3
<i>C. sepangensis</i>	CS07UNS	22.6	32.7	23.9	20.8	23.3	30.8	39.6	6.3
<i>C. sepangensis</i>	CSAJ854163.1	22.0	33.3	23.9	20.8	23.3	30.2	39.6	6.9
<i>C. sepangensis</i>	CSAJ854165.1	22.0	33.3	23.9	20.8	23.9	30.2	38.4	7.5
<i>C. travians</i>	CT01SNP	17.2	33.8	38.2	10.8	18.5	33.8	35.7	12.1
<i>C. travians</i>	CTAJ854170.1	21.2	34.0	23.1	21.8	25.6	28.8	37.8	7.7
<i>C. travians</i>	CTAJ854169.1	21.2	34.0	23.1	21.8	25.6	28.8	37.8	7.7
<i>G. sulphureus</i>	GSEF379957.1	23.9	30.8	25.2	20.1	24.5	31.4	37.1	6.9
Avg. (with outgroup)		21.6	34.0	23.9	20.6	22.3	32.1	38.0	7.7
Avg. (Ingroup)		21.5	34.0	23.9	20.6	22.2	32.1	38.0	7.7

Appendix E. Nucleotide composition of *Coptotermes* spp. based on 12S gene.

Species Name	Accession Number	T(U)	C	A	G	Total
<i>C. curvignathus</i>	CC01BHR	19.6	23.7	43	13.6	337
<i>C. curvignathus</i>	CC02BHR	19.6	23.7	43	13.6	337
<i>C. curvignathus</i>	CC04SWS	19.6	23.7	43	13.6	337
<i>C. curvignathus</i>	IK19R29P2	19.6	23.7	43	13.6	337
<i>C. curvignathus</i>	IK19R31P4	19.6	23.7	43	13.6	337
<i>C. curvignathus</i>	IK19R31P5	19.6	23.7	43	13.6	337
<i>C. curvignathus</i>	IL19R16P1	19.9	23.4	43	13.6	337
<i>C. curvignathus</i>	IL19R17P1	19.9	23.4	43	13.6	337
<i>C. curvignathus</i>	IL19R19P1	19.9	23.4	43	13.6	337
<i>C. curvignathus</i>	IL19R19P2	19.9	23.4	43	13.6	337
<i>C. curvignathus</i>	CC01KL	19.6	23.7	43	13.6	337
<i>C. curvignathus</i>	CC02KL	19.6	23.7	43	13.6	337
<i>C. curvignathus</i>	CC01KLKG	19.6	23.7	43	13.6	337
<i>C. curvignathus</i>	CC02KLKG	19.9	23.7	42.7	13.6	337
<i>C. curvignathus</i>	CC03KLKG	19.6	23.7	43.3	13.4	337
<i>C. curvignathus</i>	CC01SKAG	19.6	23.7	43	13.6	337
<i>C. curvignathus</i>	CC02SKAG	19.6	23.7	43	13.6	337
<i>C. curvignathus</i>	CC01SNR	19.6	23.7	43	13.6	337
<i>C. curvignathus</i>	CC01TB	19.6	23.7	43	13.6	337
<i>C. curvignathus</i>	CC05UNS	19.6	23.7	43	13.6	337
<i>C. curvignathus</i>	CC01MA	19.6	23.7	43	13.6	337
<i>C. curvignathus</i>	CC02UNS	19.6	23.7	43	13.6	337
<i>C. curvignathus</i>	IL19R18P1	19.9	23.4	43	13.6	337
<i>C. curvignathus</i>	FJ235110.1	19.6	23.7	42.7	13.9	337

Appendix E. continued

<i>C. curvignathus</i>	FJ235111.1	19.3	24	43	13.6	337
<i>C. gestroi</i>	C01JBS	20	23.3	43.6	13.1	335
<i>C. gestroi</i>	EF379982.1	19.9	23.1	43.9	13.1	337
<i>C. gestroi</i>	EF379987.1	19.9	23.1	43.9	13.1	337
<i>C. gestroi</i>	EF379988.1	19.9	23.1	43.9	13.1	337
<i>C. kalshoveni</i>	CK04UNS	19	24.6	43	13.4	337
<i>C. kalshoveni</i>	CK03UNS	19.3	24.3	43	13.4	337
<i>C. kalshoveni</i>	FJ235118.1	20.2	23.7	42.7	13.4	337
<i>C. kalshoveni</i>	FJ235119.1	20.2	23.7	42.7	13.4	337
<i>C. sepangensis</i>	CS01BHW	19.3	24.6	43	13.1	337
<i>C. sepangensis</i>	CS02BHW	19.3	24.3	43	13.4	337
<i>C. sepangensis</i>	CS03BHW	19.3	24.3	43	13.4	337
<i>C. sepangensis</i>	CS04BHW	19	24.6	43	13.4	337
<i>C. sepangensis</i>	CS03SWS	19.3	24.3	42.7	13.6	337
<i>C. sepangensis</i>	CS05SWS	19.6	24.6	41.8	13.9	337
<i>C. sepangensis</i>	CS02KNP	19.3	24.3	43	13.4	337
<i>C. sepangensis</i>	CS06UNS	19.3	24.3	43	13.4	337
<i>C. sepangensis</i>	CS07UNS	19	24.6	43	13.4	337
<i>C. sepangensis</i>	CS01UNS	19	24.6	43	13.4	337
<i>C. travians</i>	CT01KNP	19.3	23.7	43.3	13.6	337
<i>C. travians</i>	CT01SNP	19.3	23.7	43.6	13.4	337
<i>C. travians</i>	FJ915823	20.2	23.4	43	13.4	337
<i>G. sulphureus</i>	EF379993.1	43.2	10.8	23.8	22.2	324
Avg. (with outgroup)		20.1	23.6	42.7	13.7	336.7
Avg. (Ingroup)		19.6	23.9	43.1	13.5	337

Appendix F. Nucleotide composition of *Coptotermes* spp. based on 16S gene.

Species name	Accession number	T(U)	C	A	G	Total
<i>C. curvignathus</i>	FJ376686.1	19	28.7	40.5	11.8	321
<i>C. curvignathus</i>	CC001MA	19	28.7	40.8	11.5	321
<i>C. curvignathus</i>	CC01BHR	18.7	29	40.8	11.5	321
<i>C. curvignathus</i>	CC01KL	18.7	29	40.8	11.5	321
<i>C. curvignathus</i>	CC01KLKG	18.7	29	40.8	11.5	321
<i>C. curvignathus</i>	CC01SKAG	19	28.7	40.8	11.5	321
<i>C. curvignathus</i>	CC01SNR	19	28.7	40.5	11.8	321
<i>C. curvignathus</i>	CC01TB	19	28.7	40.8	11.5	321
<i>C. curvignathus</i>	CC02BHR	19	28.7	40.5	11.8	321
<i>C. curvignathus</i>	CC02KL	18.7	29	40.8	11.5	321
<i>C. curvignathus</i>	CC02KLKG	18.7	29	40.8	11.5	321
<i>C. curvignathus</i>	CC02SKAG	19	28.7	40.8	11.5	321
<i>C. curvignathus</i>	CC02UNS	18.7	29	40.8	11.5	321
<i>C. curvignathus</i>	CC03KLKG	18.7	29	40.8	11.5	321
<i>C. curvignathus</i>	CC04SWS	18.7	29	40.8	11.5	321
<i>C. curvignathus</i>	CC05UNS	18.7	29	40.8	11.5	321
<i>C. curvignathus</i>	CC08UNS	18.7	29	40.8	11.5	321
<i>C. curvignathus</i>	IK19R29P2	18.7	29	40.8	11.5	321
<i>C. curvignathus</i>	IK19R31P4	18.7	29	40.8	11.5	321
<i>C. curvignathus</i>	IK19R31P5	18.7	29	40.8	11.5	321
<i>C. curvignathus</i>	IL19R16P1	18.7	29	40.8	11.5	321
<i>C. curvignathus</i>	IL19R17P1	18.7	29	40.8	11.5	321
<i>C. curvignathus</i>	IL19R18P1	18.7	29	40.8	11.5	321

Appendix F. continued

<i>C. curvignathus</i>	IL19R19P1	18.7	29	40.8	11.5	321
<i>C. curvignathus</i>	IL19R19P2	18.1	29	41.4	11.5	321
<i>C. gestroi</i>	EF379963.1	19	28.3	41.1	11.5	321
<i>C. gestroi</i>	EF379969.1	19	28.3	41.1	11.5	321
<i>C. gestroi</i>	EF379970.1	19	28.3	41.1	11.5	321
<i>C. gestroi</i>	CG01JB	19	28.3	41.1	11.5	321
<i>C. kalshoveni</i>	FJ376684.1	20.2	26.8	42.4	10.6	321
<i>C. kalshoveni</i>	FJ376685.1	20.2	26.8	42.4	10.6	321
<i>C. kalshoveni</i>	CK03UNS	20.6	27	41.6	10.8	315
<i>C. kalshoveni</i>	CK04UNS	21	27	41.3	10.8	315
<i>C. sepangensis</i>	CS01BHW	21	27	41.3	10.8	315
<i>C. sepangensis</i>	CS01UNS	19.1	27.2	42.7	11	309
<i>C. sepangensis</i>	CS02BHW	20.3	26.7	42.2	10.8	315
<i>C. sepangensis</i>	CS02KNP	19.4	27.2	42.4	11	309
<i>C. sepangensis</i>	CS03BHW	20.6	26.7	41.9	10.8	315
<i>C. sepangensis</i>	CS03SWS	20.4	26.7	42.1	10.7	318
<i>C. sepangensis</i>	CS04BHW	21	27	41.3	10.8	315
<i>C. sepangensis</i>	CS05SWS	21	27.3	41.3	10.5	315
<i>C. sepangensis</i>	CS06UNS	20.6	27	41.6	10.8	315
<i>C. sepangensis</i>	CS07UNS	21	27	41.3	10.8	315
<i>C. travians</i>	CT01KNP	20.1	27.7	41.8	10.4	318
<i>C. travians</i>	CT01LHNP	20.1	27.7	41.2	11	318
<i>C. travians</i>	CT01SNP	19.8	27.7	41.8	10.7	318
<i>C. travians</i>	FJ915824.1	20.2	26.8	42.4	10.6	321
<i>G. sulphureus</i>	EF379993.1	43.4	11.5	22.7	22.4	304
Avg. (with outgroup)		19.9	27.8	40.8	11.5	318.8
Avg. (Ingroup)		19.4	28.1	41.2	11.2	319.1

Appendix G. Allele scoring from fragment analysis result

pop	Allele A	Allele B	Allele A	Allele B	Allele A	Allele B	Allele A	Allele B	Allele A	Allele B
B20R79P13	Locus T25 (Tri:160)		Locus T46 (Tetra:225)		Locus T08 (Di:323)		Locus T14 (Di:196)		Locus T04 (Di:375)	
B20R79P13(1)	167	173	217	233	331	331	191	191	340	360
B20R79P13(2)	167	170	217	233	321	331	191	191	340	360
B20R79P13(3)	167	167	237	241	321	321	191	191	340	340
B20R79P13(4)	167	173	233	241	331	331	191	193	340	340
B20R79P13(5)	167	173	217	233	321	321	191	191	340	340
B20R79P13(6)	170	173	233	241	345	345	191	193	340	340
B20R79P13(7)	167	170	237	241	321	331	191	191	340	360
B20R79P13(8)	170	170	217	233	331	331	191	191	340	360
B20R79P13(9)	170	170	217	237	321	321	191	191	340	340
B20R79P13(10)	170	170	217	233	321	331	193	193	360	360
B20R45P03(1)	151	157	220	244	350	350	191	191	340	340
B20R45P03(2)	157	175	220	244	322	322	191	191	340	340
B20R45P03(3)	151	157	232	232	330	350	191	193	340	340
B20R45P03(4)	154	157	232	232	330	350	191	193	340	340
B20R45P03(5)	157	175	220	232	350	350	191	191	340	340
B20R45P03(6)	151	175	220	232	350	350	191	193	340	340
B20R45P03(7)	151	175	220	244	330	350	191	191	340	340
B20R45P03(8)	154	157	232	244	322	330	191	191	340	340
B20R45P03(9)	151	175	220	244	350	350	191	191	340	340
B20R45P03(10)	157	175	220	244	322	322	191	193	360	360
D20R159P03(1)	161	167	211	215	335	343	191	191	340	340
D20R159P03(2)	167	167	211	215	335	353	191	191	360	360
D20R159P03(3)	157	160	219	231	335	343	191	191	340	340
D20R159P03(4)	161	167	211	215	345	353	191	191	360	360

Appendix G. continued

D20R159P03(5)	167	167	211	231	345	353	191	191	340	340
D20R159P03(6)	167	167	219	231	345	353	191	191	340	368
D20R159P03(7)	161	167	215	219	343	343	191	191	340	340
D20R159P03(8)	161	167	211	215	343	343	191	191	340	368
D20R159P03(9)	161	167	211	215	335	343	191	191	340	340
D20R159P03(10)	158	167	211	215	335	343	191	191	368	368
D20R159P03(11)	158	161	211	215	345	353	191	191	340	340
D21R143P05(1)	151	157	215	231	321	343	193	203	340	340
D21R143P05(2)	0	0	0	0	0	0	191	193	340	340
D21R143P05(3)	151	172	211	211	321	343	191	193	340	350
D21R143P05(4)	151	172	211	211	321	321	191	193	340	350
D21R143P05(5)	166	166	211	231	345	353	191	191	340	340
D21R143P05(6)	151	172	211	211	321	321	191	191	340	340
D21R143P05(7)	151	172	211	231	321	321	191	193	350	350
D21R143P05(8)	151	151	211	215	0	0	191	191	340	340
D21R143P05(9)	151	172	211	211	321	343	191	193	350	350
D21R143P05(10)	151	172	215	231	321	343	191	193	350	350
D21R143P05(11)	151	172	211	231	321	321	191	193	368	368
D22R128P15(1)	157	160	221	233	343	343	193	193	340	364
D22R128P15(2)	157	160	233	241	335	345	193	209	340	364
D22R128P15(3)	157	160	221	241	335	343	193	193	340	364
D22R128P15(4)	157	160	221	233	335	335	193	209	340	364
D22R128P15(5)	160	172	221	233	343	343	193	193	340	364
D22R128P15(6)	157	160	221	233	335	343	193	209	340	364
D22R128P15(7)	157	160	233	241	343	343	193	209	340	364
D22R128P15(8)	157	160	221	221	335	345	193	193	340	364

Appendix G. continued

D22R128P15(9)	157	160	221	221	335	345	193	209	340	364
D22R128P15(10)	160	172	221	221	343	343	193	209	340	364
D221R28P15 (11)	160	172	221	221	335	335	193	193	340	364
BHR2(1)	157	169	209	213	345	357	191	191	337	337
BHR2(2)	169	175	209	213	355	357	191	191	327	339
BHR2(3)	157	172	229	233	355	357	191	191	339	353
BHR2(4)	157	172	209	213	345	355	191	191	337	353
BHR2(5)	157	169	209	229	345	357	191	191	337	337
BHR2(6)	157	169	209	229	355	357	191	191	339	353
BHR2(7)	169	175	229	233	345	357	191	191	337	337
BHR2(8)	157	169	209	229	345	357	191	191	337	353
BHR2(9)	157	172	209	229	355	357	191	191	337	353
BHR2(10)	157	172	229	233	345	357	191	191	339	353

Appendix G. continued

pop	Allele A	Allele B	Allele A	Allele B	Allele A	Allele B	Allele A	Allele B	Allele A	Allele B
B20R79P13	Locus T34 (Tri:410)		Locus T49 (Tetra:307)		Locus T44 (Tetra:246)		Locus T40 (Tri:170)		Locus T19 (Di:167)	
B20R79P13(1)	414	414	282	282	0	0	166	166	188	196
B20R79P13(2)	414	414	282	318	0	0	166	172	190	190
B20R79P13(3)	414	414	282	282	0	0	166	172	190	196
B20R79P13(4)	414	414	282	282	0	0	166	166	188	188
B20R79P13(5)	414	414	270	270	0	0	166	172	190	196
B20R79P13(6)	414	414	270	318	0	0	166	166	190	196
B20R79P13(7)	414	414	282	282	0	0	166	166	190	196
B20R79P13(8)	414	414	282	282	0	0	166	166	188	196

Appendix G. continued

B20R79P13(9)	414	414	282	282	0	0	166	166	190	190
B20R79P13(10)	414	414	270	318	0	0	166	172	188	196
B20R45P03(1)	414	414	272	272	224	224	160	172	188	188
B20R45P03(2)	414	414	272	284	224	236	160	172	188	188
B20R45P03(3)	414	414	272	272	224	224	163	175	192	192
B20R45P03(4)	414	414	272	284	224	224	160	175	188	196
B20R45P03(5)	414	414	272	284	224	236	163	175	188	196
B20R45P03(6)	414	414	272	272	224	236	163	175	192	198
B20R45P03(7)	414	414	284	284	224	224	160	175	188	196
B20R45P03(8)	414	414	272	284	224	224	163	175	188	196
B20R45P03(9)	414	414	272	272	224	224	163	175	192	198
B20R45P03(10)	414	414	292	292	224	224	0	0	0	0
D20R159P03(1)	414	420	272	280	224	236	160	169	192	192
D20R159P03(2)	420	420	280	284	0	0	160	169	192	192
D20R159P03(3)	414	420	284	312	224	236	166	169	192	192
D20R159P03(4)	420	420	284	312	224	236	166	169	192	192
D20R159P03(5)	414	420	284	312	0	0	166	169	192	192
D20R159P03(6)	414	414	272	312	0	0	166	169	192	192
D20R159P03(7)	414	414	272	280	0	0	160	169	192	192
D20R159P03(8)	414	420	272	312	0	0	166	169	192	192
D20R159P03(9)	414	420	280	284	0	0	166	169	192	192
D20R159P03(10)	414	420	280	284	224	236	160	169	192	192
D20R159P03(11)	414	420	272	312	224	236	160	160	192	192
D21R143P05(1)	0	0	272	272	236	236	160	169	0	0
D21R143P05(2)	414	414	272	272	236	244	160	169	176	188
D21R143P05(3)	414	414	272	284	236	268	160	169	188	188

Appendix G. continued

D21R143P05(4)	414	414	320	320	236	268	169	169	188	188
D21R143P05(5)	0	0	280	292	236	244	160	169	176	188
D21R143P05(6)	0	0	280	292	236	244	160	160	176	176
D21R143P05(7)	414	414	284	320	236	268	169	172	188	188
D21R143P05(8)	0	0	272	284	0	0	160	160	0	0
D21R143P05(9)	414	414	284	320	236	268	169	169	172	188
D21R143P05(10)	414	414	284	320	0	0	160	169	0	0
D21R143P05(11)	414	414	272	284	236	268	169	172	176	188
D22R128P15(1)	414	414	280	292	224	236	160	169	164	176
D22R128P15(2)	414	414	280	292	224	236	160	169	164	172
D22R128P15(3)	414	414	280	280	224	236	160	166	164	176
D22R128P15(4)	414	414	292	292	0	0	160	169	0	0
D22R128P15(5)	414	414	0	0	236	244	160	166	0	0
D22R128P15(6)	414	414	284	324	244	244	160	166	172	184
D22R128P15(7)	414	414	292	292	0	0	160	169	184	184
D22R128P15(8)	414	414	292	292	224	224	166	169	0	0
D22R128P15(9)	414	414	292	292	224	224	160	166	172	184
D22R128P15(10)	414	414	284	292	224	236	166	169	172	172
D22R128P15 (11)	414	414	280	292	236	244	160	166	164	172
BHR2(1)	419	419	280	280	236	244	160	181	176	188
BHR2(2)	407	419	280	280	244	244	160	181	176	188
BHR2(3)	416	416	284	312	236	244	160	166	176	194
BHR2(4)	407	419	280	280	236	240	160	166	176	194
BHR2(5)	419	419	280	284	236	240	160	181	176	194
BHR2(6)	407	416	284	312	244	244	160	166	0	0
BHR2(7)	419	419	280	312	240	244	160	181	176	188

Appendix G. continued

BHR2(8)	407	419	280	312	236	240	160	166	176	188
BHR2(9)	419	419	284	312	240	244	160	166	176	188
BHR2(10)	416	416	280	284	236	240	160	181	176	188

Appendix H. List of Publications and Seminars

Norsyarizan Jamil, Wan Nurainie Wan Ismail, Siti Shamimi Abidin, Mazdan Ali Amaran and Ratnawati Hazali (2017). A preliminary survey of species composition of termites (Insecta: Isoptera) in Samunsam Wildlife Sanctuary, Sarawak. *Tropical Life Sciences Research*, 28(2), 211–212.

Norsyarizan Jamil and Wan Nurainie Wan Ismail (2016). Morphological Variation of Selected Species of *Coptotermes* (Isoptera: Rhinotermitidae) in Western Sarawak. Entomology Postgraduate Symposium (ENTOPOST), 29th-30th March, Hotel Bangi Putrajaya.

Norsyarizan Jamil and Wan Nurainie Wan Ismail. Morphological Variation of Selected Species of *Coptotermes* (Isoptera: Rhinotermitidae) in Western Sarawak (2017). *Serangga*, 21(1), 1-38.