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

Norsyarizan binti Jamil

A thesis submitted

In fulfilment of the requirements for the degree of Master of Science

(Molecular Ecology)

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2018

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.

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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 di sokong 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. sepangesis 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 di masukkan 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 kebiasan anai-anai bawah tanah yang selalu menyebabkan kerosakan stuktur dalam persekitaran pembangunan. Kejadian serangan spesies ini di Malaysia berlaku di awal 1900's dan telah dilaporkan telah menyerang pokok getah, Hevea brasilliensis. Natijahnya, 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 jangkaan "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 stuktur 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 kovensional 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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