



***MOLECULAR GENETICS OF THE PENINSULAR MALAYSIAN TERMITE
GENUS ODONTOTERMES***

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By

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Termites of the genus *Odontotermes* (Macrotermitinae) are important decomposers in the Old World tropics. Species within the genus have overlapping size ranges and are difficult to differentiate based on morphology. As a result, the taxonomy of species within the genus is poorly resolved. In this study, the species boundaries of the Peninsular Malaysian *Odontotermes* were elucidated using mtDNA genes, namely the 16S ribosomal RNA and cytochrome oxidase subunit I. MtDNA genes defined at least eleven species of *Odontotermes* in Peninsular Malaysia, namely, *O. escherichi*, *O. hainanensis*, *O. javanicus*, *O. longignathus*, *O. malaccensis*, *O. oblongatus*, *O. paraoblongatus*, *O. sarawakensis*, and three morpho-species that have now been identified as *O. azmiensis* sp. nov, *O. minutus*

and *O. srinakarinensis* sp. nov. Microsatellite markers were also developed *de novo* to investigate the presence of cryptic species in *O. srinakarinensis* sp. nov, a clade which consisted of samples with highly divergent sequences. Shared allele trees, principal components analysis and Bayesian clustering of genotype data from 191 termite individuals from this species complex showed that samples in this clade were in fact made up of two species, that is, *O. srinakarinensis* and *O. denticulatus*. This was corroborated by morphological and distributional differences between these species. After mtDNA and microsatellite data helped elucidate the species boundaries of the Peninsular Malaysian *Odontotermes*, the soldiers of each species were morphometrically characterised. In addition, information on the host and habitat associations of species within the *Odontotermes*, and their distribution throughout the peninsula were determined.

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GENETIK MOLEKULAR ANAI-ANAI GENUS *ODONTOTERMES* DI SEMENANJUNG MALAYSIA

Oleh

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Anai-anai dari genus *Odontotermes* (Macrotermitinae) adalah agen pereputan yang penting di kawasan tropika di Asia. Spesies-spesies dalam genus ini mempunyai pertindihan dalam julat sais dan sukar untuk dikenalpasti berdasarkan morfologi. Perkara ini menyebabkan taksonomi bagi spesies-spesies dalam genus ini tidak mampu diselesaikan. Dalam kajian ini, perbezaan diantara spesies-spesies *Odontotermes* di Semenanjung Malaysia dijelaskan dengan menggunakan gen-gen DNA mitokondria (mtDNA), iaitu 16S RNA ribosomal dan sitokrom oksidase subunit I. Filogeni-filogeni mtDNA menjumpai sekurang-kurangnya sebelas spesies *Odontotermes* di Semenanjung Malaysia, iaitu *O. escherichi*, *O. hainanensis*,

O. javanicus, *O. longignathus*, *O. malaccensis*, *O. oblongatus*, *O. paraoblongatus*, *O. sarawakensis* dan tiga morfospesies yang kini dikenali sebagai *O. azmiensis* sp. nov, *O. minutus* dan *O. srinakarinensis* sp. nov. Penanda-penanda mikrosatelite telah juga dibangunkan secara *de novo* untuk memeriksa kewujudan spesies kriptik dalam *O. srinakarinensis*, satu klad yang mengandungi sampel-sampel dengan rangkaian-rangkaian DNA yang sangat berbeza. Filogeni “shared-allele”, analisa komponen prinsipal dan pengklasteran Bayesian data genotaip daripada 191 individu anai-anai dari kompleks spesies ini menunjukkan bahawa semua sampel dalam klad ini sebenarnya terbentuk daripada dua buah spesies iaitu *O. srinakarinensis* dan *O. denticulatus*. Penemuan ini telah disokong kuat oleh kewujudan perbezaan secara morfologi dan lokasi taburan diantara spesies-spesies ini. Setelah data mtDNA dan mikrosatelite menerangi sempadan spesies anai-anai *Odontotermes*, individu-individu daripada kasta askar setiap spesies dikarakterisasikan dan diperbandingkan secara morfometrik. Sebagai tambahan, maklumat tentang hos dan hubungan habitat-habitat dan lokasi taburan di seluruh semenanjung telah dikenalpasti.

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LIST OF ABBREVIATIONS

a.s.l.	Above sea level
AFLP	Amplified fragment length polymorphism
ATP	Adenosine triphosphate
BLASTn	Basic Local Alignment Search Tool (nucleotide)
bp	Base pair
BSA	Bovine serum albumin
BSC	Biological species concept
CD-Hit	Cluster Database at High Identity with Tolerance
CIPRES	Cyberinfrastructure for Phylogenetic Research
cox1	Cytochrome oxidase subunit I
cox2	Cytochrome oxidase subunit II
d.f.	Degrees of freedom
ddH ₂ O	Double distilled water
D-loop	Displacement loop
DNA	Deoxyribonucleic acid
dNTP	2' –deoxynucleoside 5' –triphosphate
EDTA	Diaminoethanetetra-acetic acid
F.R.	Forest Reserve
FASTA	Text-based format for representing nucleotide or peptide sequences
F_{IS}	Inbreeding coefficient
FRIM	Forest Research Institute Malaysia
F_{ST}	Fixation index
GDA	Genetic Data Analysis (software)
gDNA	Genomic DNA
GenBank	National Institutes of Health (U.S.) genetic sequences database
H	Hour(s)
H_e	Expected heterozygosity

H _o	Observed heterozygosity
ITS	Internal transcribed spacer
LB	Lysogeny broth
m	Minute(s)
MCMC	Monte Carlo Markov Chain
MgCl ₂	Magnesium Chloride
MISA	MicroSatellite Identification tool (software)
Mt.	Mount
mtDNA	Mitochondrial DNA
N _A	Number of alleles
NADH	Nicotinamide adenine dinucleotide
NHM	Natural History Museum
NJ	Neighbour-joining
nuDNA	Nuclear DNA
PC	Principal component
PCA	Principal components analysis
PCR	Polymerase chain reaction
PSC	Phylogenetic species concept
RAXML	Randomised Axelerated Maximum Likelihood
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
R _s	Allelic richness
s	Second(s)
SDS	Sodium dodecyl sulfate
SOC	Super optimal broth with catabolite repression
SSC	Saline-sodium citrate
SSR	Simple sequence repeat
STR	Short tandem repeat
TAE	Tris-acetate-EDTA
TE	Tris-EDTA
Tris	Tris(hydroxymethyl) aminomethane
Tris-HCL	Tris(hydroxymethyl) aminomethane hydrochloride

tRNA
UPGMA
X-gal

Transfer RNA
Unweighted pair group method with arithmetic mean
5-Bromo-4-chloro-3-indolyl- β -D-galactoside



CHAPTER 1

INTRODUCTION

1.1 Background

The *Odontotermes* is the most species-rich genus within the Macrotermitinae with a total of 176 species described from Africa and Asia (Darlington et al. 2008). The genus was established by Holmgren (1912) and is characterised by its soldiers possessing a small, rudimentary, to large and prominent tooth situated on the inner margin of the left mandible with a small corresponding tooth often found on the right mandible. Species have been separated based on this character as well as differences in the size and shape of its soldier head capsules (Ahmad 1958).

Research on the taxonomy and systematics of the *Odontotermes*, however, has primarily been on the African fauna where observations on their behaviour, nest architecture, soldier morphology and molecular phylogenetics have been used to delineate species boundaries (Darlington et al. 2008; Davison et al. 2001; Darlington 1997). In the Indo-Tropics, the genus appears to be well studied and taxonomically less diverse than in the Afrotropics, however, it abounds with cryptic species (see Tho 1991 and Ahmad 1965).

A short record of their diversity in Peninsular Malaysia can be found in the monograph on termites from Peninsular Malaysia by Tho (1991). Although no detailed figures and morphometric measurements were provided, species within the genus were noted to be morphologically similar and separated only by small differences in size. Species within the genus also tend to overlap in size with one another. Tho (1991) recorded nine morphospecies in Peninsular Malaysia although he provided drawings for ten species. Some of the morphospecies were reconciled by Tho (1991) with their conspecific taxa after comparison with described or identified collections at the Natural History Museum (NHM), London. A list of taxa found in Peninsular Malaysia is provided in Table 1.1.

Table 1.1 *Odontotermes* species in Peninsular Malaysia (Tho 1991).

No.	Species	Type locality	Note
1.	<i>O. assmuthi</i> Holmgren	Singapore	An Indian species that was recorded by John in 1925 from Singapore.
2.	<i>O. billitonii</i> Holmgren		The species was listed by Pendlebury in 1930 and Harris in 1957. But it was placed as <i>O. sundaeicus</i> f. <i>esuriens</i> Kemner by Snyder (1949).
3.	<i>O. butteli</i> Holmgren	Malacca	-
4.	<i>O. denticulatus</i> Holmgren	Singapore	A specimen of this species is in the NHM London.
5.	<i>O. dives</i> (Hagen)	Selangor	The species was recorded by John in 1925 from Selangor.
6.	<i>O. javanicus</i> Holmgren	Buitenzorg (Java)	-
7.	<i>O. grandiceps</i> Holmgren	Malacca	-

Table 1.1 (continued)

No.	Species	Type locality	Notes
8.	<i>O. longignathus</i> Holmgren	Malacca	-
9.	<i>O. malaccensis</i> Holmgren	Malacca	-
10.	<i>O. oblongatus</i> Holmgren		-
11.	<i>O. praevalens</i> (John)		The species was described by John in 1925, but Tho (1991) recognised it as being similar to <i>O. longignathus</i> .
12.	<i>O. proximus</i> Holmgren	Maxwell Hill, Taiping	The species was collected from Maxwell Hill and described based only on the imago; it could represent a known species described from soldier material, as pointed out by Tho (1991).
13.	<i>O. sarawakensis</i> Holmgren	Sarawak	-
14.	<i>O. taprobanes</i> (Walker)	Malacca	-
15.	O sp. H	-	-
16.	O sp. I	-	-

A slightly more detailed account of the *Odontotermes* in the neighbouring region of Thailand by Ahmad (1965) provides a better framework for understanding their diversity in the region. The key devised for the *Odontotermes* in Ahmad (1965) was mainly based on soldiers although a short key was also devised for the imagoes of some species. Several species were listed by Ahmad (1965), namely, *O. formosanus* (Shiraki), *O. sarawakensis* Holmgren, *O. paraoblongatus* Ahmad, *O. oblongatus* Holmgren, *O. takensis* Ahmad, *O. feae* (Wasmann) and *O. longignathus* Holmgren, *O. maesodensis* Ahmad and *O. proformosanus* Ahmad.

In Peninsular Malaysia, there is a need for revisionary taxonomic studies and phylogenetic studies on the *Odontotermes* because they serve as basic fundamental units of analysis in ecological, biogeographical and macroevolutionary studies.

1.2 Study objectives

This study aims to elucidate the taxonomy of species within this genus using traditional taxonomy, mitochondrial DNA sequences and microsatellite markers and to determine the distribution and ecology of Peninsular Malaysian *Odontotermes* (Termitidae: Macrotermitinae). The specific objectives of the study are:

1. To elucidate the species boundaries of the Peninsular Malaysian *Odontotermes* using mtDNA gene fragments, specifically the 16S ribosomal RNA and cytochrome oxidase subunit I genes;
2. To differentiate between cryptic species of *Odontotermes* using microsatellite DNA markers;
3. To determine the diversity and habitat associations of the Peninsular Malaysian *Odontotermes*.

REFERENCES

- Aanen DK, Eggleton P, Rouland-Lefevre C, Guldberg-Froslev T, Rosendahl S, Boomsma JJ. The evolution of fungus-growing termites and their mutualistic fungal symbionts. *Proc Natl Acad Sci USA* 99:14887-92 (2002).
- Aanen DK, Eggleton P. Fungus-growing termites originated in African rain forests. *Current Biology* 15:851-55 (2005).
- Agapow P-M, Bininda-Edmonds ORP, Crandall KA, Gittleman JL, Mace GM, Marshall JC, Purvis A. The impact of species concept on biodiversity studies. *The Quarterly Review of Biology* 79 (2):161-79 (2004).
- Ahmad M. Key to the Indo-Malayan termites. *Biologia* 4:33-198 (1958).
- Ahmad M. Termites (Isoptera) of Thailand. *Bulletin of the American Museum of Natural History* 131:1-114 (1965).
- Brandl R, Hyodo K, von Korff-Schmising M, Maekawa K, Miura T, Takematsu Y, Matsumoto T, Abe T, Bagine R, Kaib M. Divergence times in the termite genus *Macrotermes* (Isoptera: Termitidae). *Mol. Phylogenet. Evol.* 45:239-50 (2007).
- Brown DM, Brenneman RA, Koepfli K-P, Pollinger JP, Milá B, Georgiadis NJ, Louis EEJ, Grether GF, Jacobs DK, Wayne RK. Extensive population genetic structure in the giraffe. *BMC Biology* 5:57 (2007).
- Chhotani OB. Fauna of India—Isoptera (Termites) Vol. II. Zoological Survey of India, Calcutta; 1997. 800p.

Cifarelli RA, Gallitelli M, Cellini F. Random amplified hybridization microsatellites (RAHM): isolation of a new class of microsatellite-containing DNA clones. Nucleic Acids Research 23:3802-3 (1995).

Claridge M F, Dawah H A, Wilson M R. 1997. Practical approaches to species concepts for living organisms. In: Claridge MF, Dawah HA, Wilson MR, editors. Species: The units of biodiversity. London: Chapman and Hall; 1997. p. 1-15

Coddington JA, Griswold CE, Dávila DS, Peñaranda E, Larcher SF. Designing and testing sampling protocols to estimate biodiversity in tropical ecosystems. In: Dudley EC, editor. The unity of evolutionary biology: Proceedings of the Fourth International Congress of Systematic and Evolutionary Biology. Portland: Dioscorides Press; 1991. p. 44-60.

Collins NM. The distribution of soil macrofauna on the West Ridge of Gunung (Mount) Mulu, Sarawak. Oecologia (Berl.) 44:263-275 (1980).

Constantino R. Abundance and diversity of termites (Insecta: Isoptera) in two sites of primary rainforest in Brazilian Amazonia. Biotropica 24:420-30 (1992).

Cracraft J. Species concepts and speciation analysis. Current Ornithology 1:159-87 (1983).

Darlington JPEC. Comparison of nest structure and nest parameters of sympatric species of *Odontotermes* (Termitidae: Macrotermitinae) in Kenya. Insect Soc 44:393-408 (1997).

Darlington JPEC, Benson RB, Cook CE, Walker G. Resolving relationships in some African fungus-growing termites (Termitidae, Macrotermitinae) using molecular, morphology and field parameters. Insect Soc 55:256-265 (2008).

Darwin C. The origin of species by means of natural selection or the preservation of favoured races in the struggle for life. Reprint of the 6th edition of 1872, as reprinted in 1878. London: Oxford University Press (1859).

Davison D, Darlington JPEC, Cook CE. Species-level systematics of some Kenyan termites of the genus *Odontotermes* (Termitidae: Macrotermitinae) using mitochondrial DNA, morphology and behaviour. Insect Soc 48:138-43 (2001).

Dean MD, Ballard JW. Factors affecting mitochondrial DNA quality from museum preserved *Drosophila simulans*. Entomologia Experimentalis et Applicata. 98:279-83 (2001).

DeHeer CJ, Vargo EL. Colony genetic organization and colony fusion in the termite *Reticulitermes flavipes* as revealed by foraging patterns over time and space. Mol. Ecol. 13:431-44 (2004).

Dronnet S, Chaipusat M, Vargo EL, Bagneres AG. Genetic analysis of the breeding system of an invasive subterranean termite, *Reticulitermes santonensis*, in urban and natural habitats. Mol. Ecol. 14:1311-20 (2005).

Earl DA. Structure Harvester ver 0.6.6: Python script to harvest data from STRUCTURE results folder, 2011, http://users.soe.ucsc.edu/~dearl/software/struct_harvest/ Accessed 2011 March 7.

Eggleton PE, Williams PH, Gaston KJ. Explaining global termite diversity: productivity or history? Biodiversity and Conservation 3:318-30 (1994).

Eggleton P, Bignell DE, Sands WA, Waite B, Wood TG, Lawton JH. The species richness of termites (Isoptera) under differing levels of forest disturbance in the

Mbalmayo Forest Reserve, southern Cameroon. Journal of Tropical Ecology 11:85-98 (1995).

Eggleton P, Bignell DE, Sands WA, Mawdsley NA, Lawton JH, Wood TG, Bignell NC. The diversity, abundance, and biomass of termites under differing levels of disturbance in the Mbalmayo Forest Reserve, southern Cameroon. Philosophical Transactions of the Royal Society of London, Series B 351:51-68 (1996).

Eggleton P, Homathevi R, Jeeva D, Jones DT, Davies RG, Maryati M. The species richness and composition of termites (Isoptera) in primary and regenerating lowland dipterocarp forest in Sabah, east Malaysia. Ecotropica 3:119-28 (1997).

Eggleton P. Termite species description rates and the state of termite taxonomy. Insectes Sociaux 46:1-5 (1999).

Eggleton PE. Global patterns of termite diversity. In: Abe T, Bignell DE, Higashi M, editors. Termites: Evolution, Sociality, Symbioses, Ecology. Dordrecht: Kluwer Academic Publishers; 2000. p. 1-24.

Elmer KR, Davila JA, Lougheed SC. Cryptic diversity and deep divergence in an upper Amazonian frog, *Eleutherodactylus ockendeni*. BMC Evol Biol 7:247 (2007).

Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14:2611-20 (2005).

Felsenstein J. Inferring phylogenies. Massachusetts: Sinauer Associates; 2004. p. xix.

Förschler MI, Senar JC, Perret P, Björklund M. The species status of the Corsican finch *Carduelis corsicana* assessed by three genetic markers with different rates of evolution. *Molecular Phylogenetics and Evolution* 52:234-40 (2009).

Gathorne-Hardy FJ, Syaukani, Davies RG, Eggleton P, Jones DT. Quaternary rainforest refugia in south-east Asia: using termite (Isoptera) as indicators. *Biol. Journal of the Linn. Soc.* 75:453-66 (2002).

Gilbert MTP, Moore W, Melchior L, Worobey M. DNA Extraction from Dry Museum Beetles without Conferring External Morphological Damage. *PLoS ONE* 2 (3):e272 (2007).

Giraud T, Pedersen JS, Keller L. Evolution of supercolonies: the argentine ants of southern Europe. *Proceedings of the National Academy of Sciences USA* 99(9):6075-79 (2002).

Goldstein DB, Linares AR, Cavalli-Sforza LL, Feldman MW. An evaluation of genetic distances for use with microsatellite loci. *Genetics* 139:463-71 (1995).

Goldstein DB, Pollock DD. Launching microsatellites: A review of mutation processes and methods of phylogenetic inference. *Journal of Heredity* 88:335-42 (1997).

Goudet J. PCA-GEN for Windows version 1.2 August 1999: Computer package for Windows which performs principal component analysis on gene frequency data, 1999, <http://www2.unil.ch/popgen/softwares/> Accessed 2010 December 2.

Goudet J. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3), 2001, <http://www.unil.ch/izea/softwares/fstat.html>. Accessed 2011 January 5.

Hacker M, Kaib M, Bagine RKN, Epplen JT, Brandl R. Unrelated queens coexist in colonies of the termite *Macrotermes michaelseni*. Mol. Ecol. 14:1527-32 (2005).

Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser 41:95-98 (1999).

Hebert PDN, Cywinska A, Ball SL, DeWaard JR. Biological identifications through DNA barcodes. Proc. R. Soc. B 270:313-21 (2003).

Hey J. Genes, Categories and Species. New York: Oxford University Press. 2001.

Hoffman JI, Amos W. Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. Molecular Ecology 14:599-612 (2005).

Holmgren N. Termitenstudien. 3. Systematic der Termiten. Die Familie Metatermitidae. Kungl Svenska Vetensk-Akad Handl 48 (4):1-166 (1912).

Holmgren N. Termitenstudien. 4. Versuch einer systematischen Monographie der Termiten der orientalischen Region. Kungl Svenska Vetensk-Akad Handl 50:1-276 (1913).

Holmgren N. Wissenschaftliche Ergebnisse einer Forschungsreise nach Ostindien, ausgeführt im Auftrage der Kgl. Preuss. Akademie der

Wissenschaften zu Berlin von H. v. Buttel-Reepen. 3. Termiten aus Sumatra, Java, Malacca und Ceylon. Zoologische Jahrbücher, Abteilungen Systematik 36:229-290 (1913-14).

Hurst GDD, Jiggins FM. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. Proc. R. Soc. B. 272:1525-34 (2005).

Inward DJG, Vogler AP, and Eggleton P. A comprehensive phylogenetic analysis of termites (Isoptera) illuminates key aspects of their evolutionary biology. Molecular Phylogenetics and Evolution 44 (3):953-67 (2007).

Jones DT, Eggleton P. Sampling termite assemblages in tropical forests: testing a rapid biodiversity assessment protocol. Journal of Applied Ecology 37:191-203 (2000).

Kaib M, Husseneder C, Epplen C, Epplen JT, Brandl R. Kin-biased foraging in a termite. Proceedings of the Royal Society London B 263:1527-32 (1996).

Kaib M, Hacker M, Over I, Hardt C, Epplen JT, Baginess RK, Brandl R. Microsatellite loci in *Macrotermes michaelsoni* (Isoptera: Termitidae). Mol. Ecol. 9 (4):502-4 (2000).

Kambhampati S, Smith PT. PCR primers for the amplification of four insect mitochondrial gene fragments. Insect Mol. Biol. 4 (4):233-36 (1995).

Kambhampati S, Eggleton P. Taxonomy and phylogeny of termites. In: Abe T, Bignell DE, Higashi M, editors. Termites: Evolution, Sociality, Symbioses, Ecology. Dordrecht: Kluwer Academic Publishers; 2000. p. 1-23.

Kijas JM, Fowler JC, Garbett CA, Thomas MR. Enrichment of microsatellites from the citrus genome using biotinylated oligonucleotide sequences bound to streptavidin-coated magnetic particles. *Biotechniques* 16:656-62 (1994).

Kirton LG, Cheng S. Ring-barking and root debarking of dipterocarp seedlings by termites in an enrichment planting site in Malaysia. *Journal of Tropical Forest Science* 19:67-72 (2007).

Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, Wilson AC. Dynamics of mitochondrial evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA* 86:6196-200 (1989).

Levinson G, Gutman GA. High frequencies of short frameshifts in poly-CA/TG tandem repeats borne by bacteriophage M13 in *Escherichia coli* K12. *Nucleic Acids Res.* 15:5323-38 (1987).

Lewis PO, Zaykin D. Genetic data analysis: computer program for the analysis of allelic data, 2001, <http://lewis.eeb.uconn.edu/lewishome/software.html> Accessed 2011 April 1.

Li W, Godzik A. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics Applications Note* 22 (13):1658-59 (2006).

Litt M, Luty JA. A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Am. J. Hum. Genet.* 44:397-401 (1989).

Liu H, Beckenbach A. Evolution of the mitochondrial cytochrome oxidase II gene among 10 orders of insects. *Mol Phylogenetic Evol* 1:41-52 (1992).

Liu K, Muse SV. Powermarker: Integrated analysis environment for genetic marker data. *Bioinformatics* 21 (9):2128-29 (2005).

Mace GM, Gittleman JL, Purvis A. Preserving the tree of life. *Science* 300:1707-9 (2003).

Mariette S, Le Corre V, Austerlitz F, Kremer A. Sampling within the genome for measuring within-population diversity: trade-offs between markers. *Mol. Ecol.* 11:1145-56 (2002).

Marini M, Mantovani B. Molecular relationships among European samples of *Reticulitermes* (Isoptera, Rhinotermitidae). *Molecular Phylogenetics and Evolution* 22 (3):454-59 (2002).

Mayden R L. A hierarchy of species concepts: the denouement in the sage of the species problem. In Claridge MF, Dawah HA, Wilson MR, editors. *Species: The Units of Biodiversity*. London: Chapman & Hall; 1997. p. 381-424.

Mayr E. Systematics and the origin of species. New York: Columbia University Press; 1942. p. 151.

Mayr E. Populations, Species, and Evolution. Cambridge, MA: Harvard Univ. Press, Cambridge, MA. 1970.

Meglécz E. MICROFAMILY: A computer program for detecting flanking region similarities among different microsatellite loci. *Mol. Ecol. Notes* 7:18-20 (2007).

Meglécz E, Anderson SJ, Bourguet D, Butcher R, Caldas A, Cassel-Lundhagen A, d'Acier AC, Dawson DA, Faure N, Fauvelot C, Franck P, Harper G, Keyghobadi N, Kluetsch C, Muthulakshmi N, Nagaraju J, Patt A, Péténian F,

Silvain J-F, Wilcock HR. Microsatellite flanking region similarities among different loci within insect species. *Insect Molecular Biology* 16: 175-185 (2007).

Mirol PM, Mascheretti S, Searle JB. Multiple nuclear pseudogenes of mitochondrial cytochrome b in *Ctenomys* (Caviomorpha, Rodentia) with either great similarity to or high divergence from the true mitochondrial sequence. *Heredity* 84:538-47 (2000).

Mitchell JD. Termites as pests of crops, forestry, rangeland and structures in Southern Africa and their control. *Sociobiology* 40:47-69 (2002).

Miura T, Roisin Y, Matsumoto T. Molecular phylogeny of the nasute termite genus *Nasutitermes* (Isoptera: Termitidae) in the Pacific tropics. *Molecular Phylogenetics and Evolution* 17 (1):1-10 (2000).

Moritz C, Dowling TE, Brown WM. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Ann. Rev. Ecol. Syst.* 18:269-92 (1987).

Nei M, Tahima F, Tateno Y. Accuracy of estimated phylogenetic trees from molecular data. *J. Mol. Evol.* 19:153-70 (1983).

Noirot C, Darling JPEC. Termite nests: architecture, regulation and defence. In: Abe T, Bignell DE, Higashi M, editors. *Termites: Evolution, Sociality, Symbioses, Ecology*. Dordrecht: Kluwer Academic Publishers; 2000. p. 121-39.

Ostrander EA, Jong PM, Rine J, Duyk G. Construction of small-insert genomic DNA libraries highly enriched for microsatellite repeat sequences. *Proceedings of the National Academy of Sciences of the USA* 89:3419-23 (1992).

Paetkau D. Microsatellites obtained using strand extension: An enrichment protocol. *Biotechniques* 26:690-97 (1999).

Paterson H. The recognition concept of species. In Vrba E, ed. *Species and speciation*. Pretoria: Transvaal Museum. 1985. p. 21-29

Pfenninger M, Schwenk K. Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evol Biol* 7:121-26 (2007).

Pinheiro F, Palma-Silva C, Barros FD and Cozzolino S. Cross-amplification and characterization of microsatellite loci for the Neotropical orchid genus *Epidendrum*. *Genet. Mol. Biol.* 32 (2):337-39 (2009).

Polizzi JM, Forschler BT. Factors affecting aggression among the worker caste of *Reticulitermes* spp. subterranean termites (Isoptera: Rhinotermitidae). *Journal of Insect Behaviour* 12:133-46 (1998).

Posada D. Collapse: describing haplotypes from sequence alignments, 2006, <http://darwin.uvigo.es/soft-ware/collapse.html> Accessed 2009 October 3.

Poulton EB. What is a species? *Proceedings of the Entomological Society of London* 1903:Ixxvii–cxvi (1904).

Primmer CR, Painter JN, Koskinen MT, Palo JU, Merilä J. Factors affecting avian cross-species microsatellite amplification. *J Avin Biol* 36:348-60 (2005).

Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics* 155:945-59 (2000).

Quicke DLJ, Belshaw R, Lopez-Vaamonde C. Preservation of hymenopteran specimens for subsequent molecular and morphological study. *Zoologica Scripta* 28:261-67 (1999).

Rassman K, Schlotterer C, Tautz D. Isolation of simple-sequence loci for use in polymerase chain-reaction-based DNA fingerprinting. *Electrophoresis* 12:113-18 (1991).

Roisin Y, Leponce M. Characterizing termite assemblages in fragmented forests: a test case in the Argentinian Chaco. *Austral Ecology* 29:637-46 (2004).

Roonwal ML, Chhotani OB. Wing microsculpturing in termite genera *Odontotermes*, *Hypotermes* and *Microtermes* (Termitidae: Macrotermitinae) and its taxonomic value. *Zool Anz* 178:236-62 (1986).

Rouland-Lefèvre C. Symbiosis with Fungi. In: Abe T, Bignell DE, Higashi M, editors. *Termites: Evolution, Sociality, Symbioses, Ecology*. Dordrecht: Kluwer Academic Publishers; 2000. p. 289-306.

Roy V, Demanche C, Livet A, Harry M. Genetic differentiation in the soil-feeding termite *Cubitermes* sp. *affinis subarquatus*: occurrence of cryptic species revealed by nuclear and mitochondrial markers. *BMV Evolutionary Biology* 6:102 (2006).

Salunke BK, Salunkhe RC, Dhotre DP, Khandagale AB, Walujkar SA, Kirwale GS, Ghate HV, Patole MS, Shouche YS. Diversity of *Wolbachia* in *Odontotermes* spp. (Termitidae) and *Coptotermes heimi* (Rhinotermitidae) using the multigene approach. *FEMS Microbiol Lett* 307 (1):55-64 (2010).

Schlötterer C, Tautz D. Slippage synthesis of simple sequence DNA. *Nucleic Acids Research* 20:211-15 (1992).

Schlötterer C. Evolutionary dynamics of microsatellite DNA. Chromosoma 109:365-71 (2000).

Schmidt AM, Trindl A, Korb J. Isolation and characterization of 10 microsatellite loci in the magnetic termite, *Amitermes meridionalis* (Isoptera: Termitidae). Mol. Ecol. Notes (2007).

Shay JW, Werbin H. New evidence for the insertion of mitochondrial DNA into the human genome: significance for cancer and aging. Mutat. Res. 275:227-35 (1992).

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann Ent Soc Am 87:651-701 (1994).

Simpson GG. Principles of animal taxonomy. New York: Columbia University Press; 1961. 247 p.

Snyder TE. Catalog of the termites (Isoptera) of the world. Smithsonian Miscellaneous Collections 112(3953):1-490 (1949).

Sørensen LL, Coddington JA, Scharff N. Inventorying and estimating subcanopy spider diversity Using semiquantitative sampling methods in an Afromontane forest. Environ. Entomol. 31 (2):319-30 (2002).

Stamatakis A. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688-90 (2006).

Strand M, Prolla TA, Liskay RM, Petes TD. Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. *Nature* 365:274-76 (1993).

Swofford DL. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts. (2003).

Tautz D. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research* 17:6463-71 (1989).

Templeton A. The meaning of species and speciation: A genetic perspective. In: Otte D, Endler J, eds. *Speciation and its consequences*. Sunderland, MA: Sinauer; 1989. p. 3-27.

Thapa RS. Termites of Sabah. *Sabah Forest Record No. 12*. Sabah Forest Department, Sabah; 1981. 374 p.

Thiel T, Michalek W, Varshney RK, Graner A. Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). *Theor. Appl. Genet.* 106 (4):411-22 (2003).

Tho YP. Termites of Peninsular Malaysia. Kirton LG, editor. *Malayan Forest Records No. 36*. Kuala Lumpur: Forest Research Institute Malaysia; 1991. 224 p.

Thorsness PE, Fox TD. Nuclear mutations in *Saccharomyces cerevisiae* that affect the escape of DNA from mitochondria to the nucleus. *Genetics* 134:21-28 (1993).

Tsutsui ND, Case TJ. Population genetics and colony structure of the argentine ant (*Linepithema humile*) in its native and introduced ranges. *Evolution* 55:976-85 (2001).

Turelli M, Hoffmann AA. Rapid spread of an inherited incompatibility factor in California *Drosophila*. *Nature* 353:440-42 (1991).

Turelli M, Hoffmann AA, McKechnie SW. Dynamics of cytoplasmic incompatibility and mtDNA variation in natural *Drosophila simulans* populations. *Genetics* 132: 713-23 (1992).

Uva P, Clement J-L, Austin JW, Aubert J, Zaffagnini V, Quintana A, Bagnères A-G. Origin of a new *Reticulitermes* termite (Isoptera, Rhinotermitidae) inferred from mitochondrial and nuclear DNA data. *Molecular Phylogenetics and Evolution* 30:344-53 (2004).

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* 4:535-38 (2004).

Van Valen L. Ecological species, multispecies, and oaks. *Taxon* 25:233-39 (1976).

Vargo E. L. Hierarchical analysis of colony and population genetic structure of the eastern subterranean termite *Reticulitermes flavipes*, using two classes of molecular markers. *Evolution* 57:2805-18 (2003).

Vargo EL, Husseneder C, Grace K. Colony and population genetic structure of the Formosan subterranean termite, *Coptotermes formosanus*, in Japan. *Mol. Ecol.* 12:2599-608 (2003).

Vargo EL, Husseneder C. Genetic structure of termite colonies and populations. In: Bignell DE, Roisin Y, Lo N, editors. *Biology of Termites: A Modern Synthesis*. Springer. p. 321-348.

Weber JL, May PE. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am. J. Hum. Genet.* 44:388-96 (1989).

Werren JH, Windsor DM. *Wolbachia* infection frequencies in insects: evidence of a global equilibrium? *Proc. R. Soc. Lond. B Biol. Sci.* 267:1277-85 (2000).

Wright S. The genetical structure of populations. *Annals of Eugenics* 15:323-354 (1951)

Wu K, Jones R, Danneberger L, Scolnik PA. Detection of microsatellite polymorphisms without cloning. *Nucleic Acids Research* 22:3257-58 (1994).

Zane J, Bargelloni L, Patarnello T. Strategies for microsatellite isolation: a review. *Mol. Ecol.* 11:1-16 (2002).

Zhang D-X, Hewitt GM. Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. *Mol. Ecol.* 12 (3):563-84 (2003).