

**GENETIC DISTANCE AND PHYLOGENETIC RELATIONSHIPS OF
Tetraponera rufonigra Jerdon (HYMENOPTERA: FORMICIDAE) POPULATIONS
IN PENANG, MALAYSIA**

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ABSTRACT

Tetraponera rufonigra, commonly known as the arboreal bicolored ant has gained its notoriety as one of the medically-important pest insects in Malaysia. *Tetraponera rufonigra* is recognizable by its excruciating sting, causing a severe anaphylaxis especially to those with the history of allergy. The aim of this study was to assess the phylogenetic relationships among the populations of *T. rufonigra* inferred from 503bp of the mitochondrial DNA Cytochrome Oxidase I (COI) gene. The data obtained were analysed using the T-coffee, ClustalX, MEGA 6.0 and PAUP software. A major monophyletic clade was formed consists of haplotype 1 and haplotype 2. Haplotype 1 consists of samples mostly from industrial areas, differing from the other populations studied by one singleton base. This study will enhance the knowledge about the population's relationship of *T. rufonigra* as well as its control and role in fragile ecosystem of Penang, Malaysia.

Keywords: *Tetraponera rufonigra*, arboreal bicolored ant, phylogenetic, cytochrome oxidase subunit 1(CO1).

ABSTRAK

Tetraponera rufonigra, biasa dikenali sebagai semut arboreal dwi-warna telah mendapat perhatiannya sebagai salah satu daripada serangga perosak berkepentingan-perubatan di Malaysia. *T. rufonigra* dikenali dengan sengatan yang menyakitkan, menyebabkan anafilaksis yang teruk terutama kepada mereka yang mengalami alergi. Tujuan kajian ini adalah untuk menilai hubungan filogenetik di kalangan populasi *T. rufonigra* yang disimpulkan daripada 503 bp DNA Sitokrom Oksidase I (COI) mitokondria. Data yang diperoleh dianalisis menggunakan perisian T-Coffee, ClustalX, MEGA 6.0 dan PAUP. Satu klad monofiletik utama terbentuk terdiri daripada haplotype 1 dan haplotip 2. Haplotip 1 terdiri daripada sampel yang kebanyakannya dari kawasan perindustrian, berbeza dari populasi lain melalui satupangkalan tunggal. Kajian ini akan meningkatkan pengetahuan tentang hubungan taburan populasi *T. rufonigra* serta kawalannya di ekosistem yang rapuh di Pulau Pinang, Malaysia.

Kata kunci: *Tetraponera rufonigra*, semut arboreal dwi-warna, phylogenetik, subunit sitokrom oksida 1 (CO1).

INTRODUCTION

Tetraponera rufonigra Jordan (Hymenoptera: Formicidae) which belongs to the subfamily Pseudomyrmecinae is one of the more distinctive groups inhabiting arboreal stratum in tropical and sub-tropical regions (Ward 2001). There are three genera of Pseudomyrmecinae: *Myrcidis* Ward (two species), *Pseudomyrmex* Lund (~200 species) and *Tetraponera* F. Smith (~100 species). The genus *Tetraponera* from The Old World, pseudomyrmecines are distributed throughout Paleotropics, with Eocene/Oligocene fossils known from Europe (Bolton 1994). Although less attention is given to this genus, a taxonomic revision and phylogenetic study of the Indo-Australian species of *Tetraponera* had classified the genus into four species groups i.e. *allaborans*, *nigra*, *pilosa* and *rufonigra*. The genus was believed to be originated from Africa and dispersed on numerous occasions into Asia (Ward 2001). Based on research by Ward and Downie (2005), *Tetraponera* is possibly paraphyletic which related to the New World pseudomyrmecines comprising of genera *Pseudomyrmex* + *Myrcidris*. Besides, estimated date of species divergence between *Pseudomyrmex* and *Tetraponera* (represented by *T. rufonigra*) was probably 54 Mya (Ward & Brady 2003).

Tetraponera rufonigra (locally known as ‘Semut Selangor’) is a large bicolored ant species with abroad dark head and gaster and light orange-brown body. This species is well-distributed around the Indian subcontinent as well as Southeast Asia (Davidson et al. 2003). They appear to be invasive, probably due to human-mediated introduction at these respective regions in the past. Adding to this, the nesting habits of *Tetraponera* species are known to be generalist inhabitants, generally in dead hollow twigs and stems. In general, studies on *T. rufonigra* are extremely limited in Malaysia. The first study on the genus *Tetraponera* was on nucleotide content of the cytochrome b region in the mitochondrial DNA of *T. rufoniger* (Jermin & Crozier 1994). More than a decade after, Chong and Lee (2010) studied inter- and intraspecific aggression in the invasive long-legged ant (*A. gracilipes*) and *T. rufonigra*. They stated that *T. rufonigra* could effectively compete against *A. gracilipes* during group interactions. The most recent study was conducted by Norasmah et al. (2012) concerning diurnal foraging activity and nutrient preferences of *T. rufonigra* in Penang Island, Malaysia.

Mitochondrial DNA (mtDNA) had been widely used in phylogenetic studies of insects due to its relatively high substitution rate suitable for constructing a phylogenetic tree (Galtier et al. 2009). Furthermore, mtDNA evolves more rapidly in differentiation between the closely related species. In this study, a partial cytochrome oxidase subunit 1 (COI) was chosen as a genetic marker for *T. rufonigra*. This marker has been used to determine phylogenetic relationships in relatively lower categorical levels such as in families, genera, species or populations (Hwang & Kim 1999).

To date, genetic diversity and phylogenetic relationship of *T. rufonigra* from Malaysia and other countries are unknown. Therefore, in this study, genetic diversity and phylogenetic relationships of *T. rufonigra* in Penang Island were determined using partial COI gene sequence. Sequence comparison of COI gene among *T. rufonigra* collected from different locations were also reported.

MATERIALS AND METHODS

Sampling of *Tetraponera rufonigra*

Samples of *T. rufonigra* workers were collected from several locations within eastern part of Penang Island. These locations were mapped based on collection locality data using ArcGIS 10.3 Software (ESRI 2011) (Figure 1). Workers of *T. rufonigra* in Penang Island were collected using a glass jar containing a bait. The tuna (protein) approximately weight 0.2-0.3 grams were used as a food source (bait) in each trap and placed near the infested trees especially in the foraging area. The traps were collected after 30 minutes and brought back to the laboratory for sorting and identification purpose. All specimens were labelled and kept according to the locations. The specimens were preserved in 95% ethanol for DNA extraction, COI amplification and sequencing. A total of 28 specimens (including one samples of outgroup, *Tetraponera hespera* with an accession number of DQ176236.1) where five workers' head from each site were used in this study (Table 1).

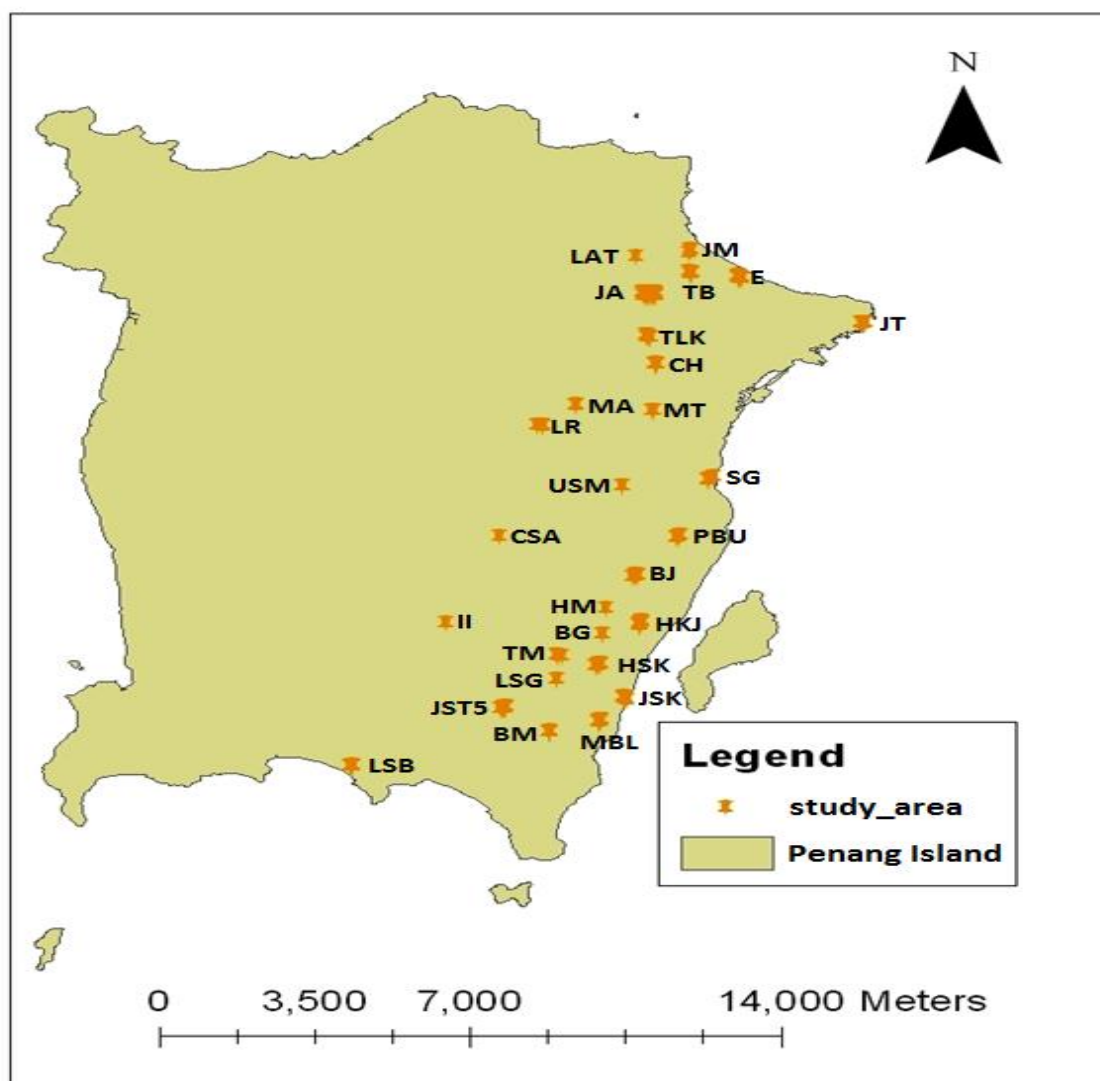


Figure 1. Map of Penang Island showing study locations of *Tetraponera rufonigra* collected for this study. Abbreviations are listed in Table 1.

Table 1. Collection sites at Penang Island, Malaysia specimens' voucher, haplotype and accession number of each *Tetraponera rufonigra* specimens in this study.

Specimen_voucher	Location code	Collection sites	Collection_date	Country	Isolation source	Isolate	Lat_Lon	Haplotype	Accession number
USM: PBU: FS001	PBU	Persiaran BatuUban	4-Dec-15	Malaysia	<i>Acacia mangium</i>	FS001	05° 21.090'N 100° 18.308'E	Haplo 1	KX398205
USM: LSG: FS002	LSG	Lorong Sg. Tiram	5-Dec-15	Malaysia	<i>Cerbera odollam</i>	FS002	05° 18.572'N 100° 16.853'E	Haplo 1	KX398206
USM: HSK: FS003	HSK	Hilir Sg. Kluang	11-Dec-15	Malaysia	<i>Tabebuia rosea</i>	FS003	05° 18.243'N 100° 17.699'E	Haplo 1	KX398207
USM: MBL: FS004	MBL	Medan Bayan Lepas	12-Dec-15	Malaysia	<i>Tabebuia rosea</i>	FS004	05° 17.874'N 100° 17.389'E	Haplo 1	KX398208
USM: JST5: FS005	JST5	Jln. Sg. Tiram 5	6-Dec-15	Malaysia	<i>Cassia fistula</i>	FS005	05° 18.087'N 100° 16.226'E	Haplo 1	KX398209
USM: MA: FS006	MA	Medan Angsana	20-Dec-15	Malaysia	<i>Cassia javanica</i> spp. <i>nodosa</i>	FS006	05° 23.421'N 100° 17.070'E	Haplo 1	KX398210
USM: TLK: FS007	TLK	Taman LumbaKuda	26-Dec-15	Malaysia	<i>Tabebuia</i> sp.	FS007	05° 24.609'N 100° 17.959'E	Haplo 1	KX398211
USM: LSB: FS008	LSB	Lintang Sg. Batu	27-Dec-15	Malaysia	Dead tree	FS008	05° 17.053'N 100° 14.362'E	Haplo 1	KX398212
USM: USM: FS009	USM	Universiti Sains Malaysia	20-Nov-15	Malaysia	<i>Mimosop selengi</i>	FS009	05° 21.615'N 100° 18.180'E	Haplo 2	KX398213
USM: CH: FS010	CH	Cheeseman	10-Nov-15	Malaysia	<i>Pongamia pinnata</i>	FS010	05° 24.151'N 100° 18.033'E	Haplo 1	KX398214
USM: II: FS011	II	Isle of Island	25-Nov-15	Malaysia	Shrubs	FS011	05° 18.315'N 100° 15.594'E	Haplo 2	KX398215
USM: LR: FS012	LR	Lebuh Rambai	19-Dec-15	Malaysia	<i>Swietenia macrophylla</i>	FS012	05° 23.038'N 100° 16.669'E	Haplo 2	KX398216
USM: SG: FS013	SG	Sungai Gelugor	5-Nov-15	Malaysia	<i>Mangifera indica</i>	FS013	05° 22.126'N 100° 18.716'E	Haplo 1	KX398217
USM: BJ: FS014	BJ	Bukit Jambul	8-Nov-15	Malaysia	<i>Swietenia macrophylla</i>	FS014	05° 19.893'N 100° 17.480'E	Haplo 1	KX398218
USM: BG: FS015	BG	Bukit Gedung	15-Nov-15	Malaysia	<i>Azadirachta indica</i>	FS015	05° 18.995'N 100° 16.880'E	Haplo 2	KX398219
USM: TM: FS016	TM	Taman Mahsuri	12-Nov-15	Malaysia	<i>Ficus</i> sp.	FS016	05° 19.671'N 100° 17.283'E	Haplo 1	KX398220
USM: MT: FS017	MT	Medan Tembaga	10-Jan-16	Malaysia	<i>Cerbera odollam</i>	FS017	05° 23.332'N 100° 18.014'E	Haplo 1	KX398221

(Table 1. continued)

Specimen voucher	Location code	Collection sites	Collection date	Country	Isolation source	Isolate	Lat Lon	Haplotype	Accession number
USM: BM: FS018	BM	BatuMaung	16-Jan-16	Malaysia	<i>Casuarina equisetifloa</i>	FS018	05° 17.655'N 100° 16.770'E	Haplo 1	KX398222
USM: CSA: FS019	CSA	Cangkat Sg. Ara	31-Jan-16	Malaysia	<i>Mangifera indica</i>	FS019	05° 19.929'N 100° 15.923'E	Haplo 2	KX398223
USM: HKJ: FS020	HKJ	Halaman Kg. Jawa	5-Feb-16	Malaysia	<i>Azadirachta indica</i>	FS020	05° 19.585'N 100° 17.875'E	Haplo 1	KX398224
USM: JA: FS021	JA	Jesselton Avenue	12-Feb-16	Malaysia	<i>Azadirachta indica</i>	FS021	05° 25.424'N 100° 17.887'E	Haplo 1	KX398225
USM: E: FS022	E	Edgecumbe	6-Feb-16	Malaysia	<i>Cerbera odollam</i>	FS022	05° 25.665'N 100° 19.070'E	Haplo 1	KX398226
USM: JSK: FS023	JSK	Jln. Sg. Keluang	5-Feb-16	Malaysia	<i>Delonix regia</i>	FS023	05° 18.863'N 100° 17.383'E	Haplo 1	KX398227
USM: TB: FS024	TB	Taman Berjaya	6-Feb-16	Malaysia	<i>Plumeriarubra cultivar</i>	FS024	05° 25.744'N 100° 18.457'E	Haplo 1	KX398228
USM: LAT: FS025	LAT	Lorong Air Terjun	12-Feb-16	Malaysia	<i>Swietenia macrophylla</i>	FS025	05° 26.031'N 100° 17.778'E	Haplo 1	KX398229
USM: JM: FS026	JM	Jln. Midland	13-Feb-16	Malaysia	<i>Azadirachta indica</i>	FS026	05° 26.145'N 100° 18.457'E	Haplo 1	KX398230
USM: HM: FS027	HM	Halaman Macalister	14-Feb-16	Malaysia	<i>Cerbera odollam</i>	FS027	05° 25.170'N 100° 18.904'E	Haplo 1	KX398231

Morphological Characterization and Species Identification

Tetraponera rufonigra were identified by using keys to species of genus *Tetraponera* based on worker caste (Ward 2001; Xu & Chai 2004). Identifications were based on 10 specimens from each collection and enumerated with mean lengths (mm) of antennae and antennal club formed with apical and pre-apical segments, and two-segmented petiole connecting gaster and thorax. Measurements were denoted in mm at 250x magnification, using a micrometer in the ocular lens of a dissection microscope. Other morphological characters supplementing identification were body colour, head shape, length of head and body and prominence of eyes. Morphology was further illustrated by the following measurements, namely Head length (HL) midline length of head proper, from posterior margin to the anterior extremity of clypeus (just before mandibles), Head width (HW) maximum width of eyes, including the eyes, profemur length (FL) length of the profemur, profemur width (PW) maximum measurable width of profemur, petiole length (PL) length of the petiole measured in lateral view from the lateral flanges of the anterior to posterior margin of the petiole, petiole height (PH) maximum height of the petiole measured in lateral view and dorsal petiole width (DPW) maximum width of the petiole measured in dorsal view (Figures 2 - 3). The indices used in further illustrations were calculated as follows: a) cephalic index (CI), HW/ HL; b) petiole width index (PWI), DPW/PL. The measurements were recorded as a number of units on the micro meter in the left ocular and were converted to mm (1 unit=0.4 mm) using a calibration scale. For example, a number of units recorded at 4.68 units are equivalent to 1.87 mm.

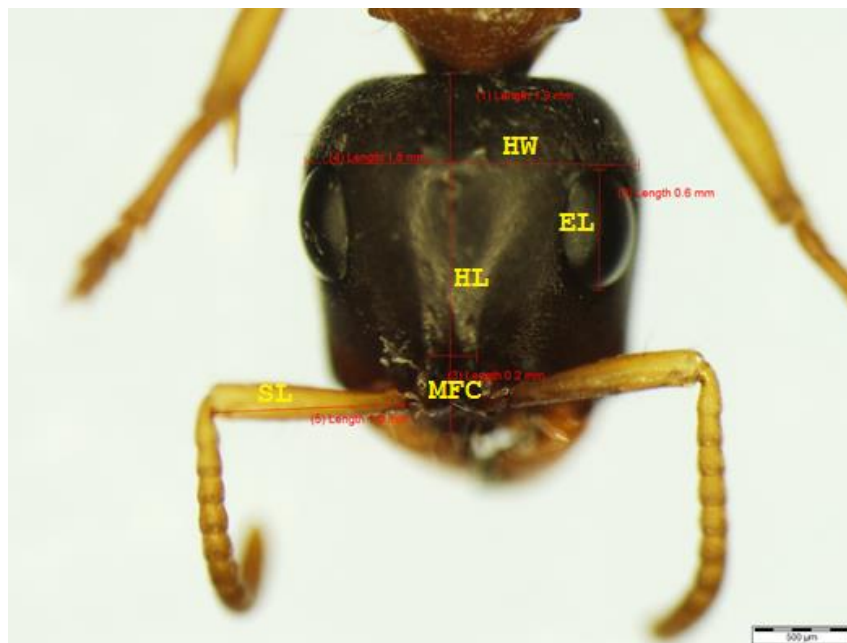


Figure 2. Morphological characters and various measurements of *Tetraponera rufonigra* in frontal view.



Figure 3. Morphological characters and various measurements of *Tetraponera rufonigra* petiole dorsal view.

DNA Extraction

DNA of *T. rufonigra* was extracted using DNeasy Blood & Tissue Kit, Lot Number 151027140 (Qiagen, Valencia, CA) with a slight modification. Prior to DNA extraction, specimens were sterilized using 100% ethanol, rinsed with sterile distilled water and air dried for few seconds. The specimens were homogenized in the cell lysis buffer with Proteinase K at 4°C, and then incubated for an hour at 60°C. The lysates were then subjected to protein precipitation, and ethanol wash, followed by the elution step using 50 μL buffer AE. The elution step was repeated twice to give a final volume of 100 μL. The DNA concentration purity and quality were quantitated using Nanodrop 2000c (Thermoscientific, USA).

Polymerase Chain Reaction (PCR) Amplification and DNA Sequencing

A 579 bp fragment of the cytochrome oxidase I (COI) gene was amplified using standard forward and reverse primers from previous sample of *T. rufonigra* deposited at Genebank with an accession number FJ436846 (Heil et al. 2009; Kautz et al. 2009) (F'. '5-TCCTGATATGGCCTACCCCC-3' and R'. '5-AATCCGATGGCTAATATTGCGT-3'). PCR was performed in a total volume of 50 μL, containing 25 μL Master Mix (Qiagen, Valencia, CA), 2.5 μL of each primer, 5 μL DNA template, and 15 μL ddH₂O (Table 3). The PCR amplification was carried out using a 'touchdown' procedure. The PCR profile was set following Ab Majid and Kee (2015); Seri Masran and Ab Majid (2017); Sabtu and Ab Majid (2017) at initial denaturation at 94°C, 1 cycle for 4 minutes and followed by a touchdown phase of thirty cycles with denaturation at 94°C for 30 seconds, and hybridization at 60°C for 30 seconds (for first cycle) decreasing 0.5°C per cycle to 45.5°C in the last cycle and extension at a temperature of 72°C for 1 minute. Another thirty cycles of PCR were done with denaturation at 94°C for 30 seconds, annealing at 45°C for 30 seconds and extension at 72°C for 1 minute. Final extension was set at 72°C for 10 minutes before storage at 4°C.

Single band of PCR products were visualized on 0.8% agarose gel stained with ethidium bromide. Approximately 45μL of PCR product were subsequently purified using QIAquick PCR Purification Kit, Lot number 148022416 (Qiagen, Valencia, CA) (Ward &

Downie 2005) and sequenced by 1st BASE DNA Sequencing Services Company (1st BASE Laboratories, Serdang, Malaysia). All sequences were deposited in GeneBank under accession number KX398205 to KX398231 (Table 1).

Phylogenetic Analysis

Nucleotide sequences were aligned by using multiple alignment programmes, T-Coffee (Notredame et al. 2000) and ClustalX (Thompson et al. 1997). Phylogenetic relationship was examined and constructed by using Molecular Evolutionary Genetics Analysis (MEGA), version 6.0 (Tamura et al. 2013; Kheng et al. 2019). The Maximum likelihood (ML) tree was constructed with HKY model due to lowest Akaike's Information Score (AIC) score from best fits models. AIC is a goodness of fit test that compares the amount of information lost in a statistical model when that model is compared to the data (Nei & Kumar 2000). However, the Neighbor-joining (NJ) analyses were done based on substitution model using PAUP4.0b10 (Swofford 2002) and NJ tree was constructed based on the genetic distance determined by Kimura 2-parameter with 1000 replication. The *Tetraoponera hespera* (accession number of DQ176236.1) was used as the outgroup to root the tree.

RESULTS

Morphological Characterization

A total of 270 individuals of *T. rufonigra* representing 10 individuals from every 27 populations were identified using morphological characters. Individuals were mostly broad-headed 1.7–2.0 (CI>0.85). The head and gaster were dark brown to brownish-black, mesosoma and petiole were light orange to brown, while, mandibles, antennae, protibial and tarsi were medium-brown to yellowish-brown. All individuals showed 92-96% similarity with *T. rufonigra* sequences available in the NCBI Genbank. Values of Scape Index (SI) of several individuals were below the range as measured by Ward (2001).

Phylogenetic Analysis

A total of 170 individuals from 27 populations of *T. rufonigra* in Penang Island, Malaysia were successfully genotyped and scored for COI markers. The average fragment size for COI gene of *T. rufonigra* was approximately 579 bp. BLAST analysis of COI gene sequences against the NCBI GenBank database showed percentage similarities ranging from 92% to 95%.

A 379 bp of the partial COI gene of 27 sequences of *T. rufonigra* and the sequence of outgroup, *T. hespera* were successfully obtained after the sequence alignment using T-Coffee Molecular Software (Notredame et al. 2000) and ClustalX (Thompson et al. 1997). The average values of nucleotide frequencies were as follows: Adenine (A=28.4 %), Thymine (T=35.5 %), Cytosine (C=20.9 %), and Guanine (G=15.2%), consistent with the findings of Jermin and Crozier (1994). Of the 379 bp of the partial COI gene, 275 were conserved sites, 104 sites were variables and 103 were a singleton. The high number of conserved sites indicated that this genetic marker was highly conserved.

Genetic relationships between the populations as shown in the NJ with two major groups were formed (Figure 4). Among 27 populations sequenced, two haplotypes were identified. Most of the populations were in Haplotype 1, except for Taman Mahsuri (TM), Cangkat Sg. Ara (CSA), Sungai Gelugor (SG), Lebu Rambai (LR) and Cheeseman (CH) populations (Haplotype 2). Phylogenetic trees of NJ revealed the monophyly of *T. rufonigra* supported by 50% of bootstrap value. One major monophyletic group consisted of all

Haplotype 1 populations (Figure 4). Meanwhile, the remaining populations were in Haplotype 2, appeared to be closely related to the major group populations.

Genetic distances between the populations from 27 different localities were low. Population within the same haplotype shows 0.00 genetic distance, while between different haplotype harbour 0.003 values. This was due to minor nucleotide variations in the same species.

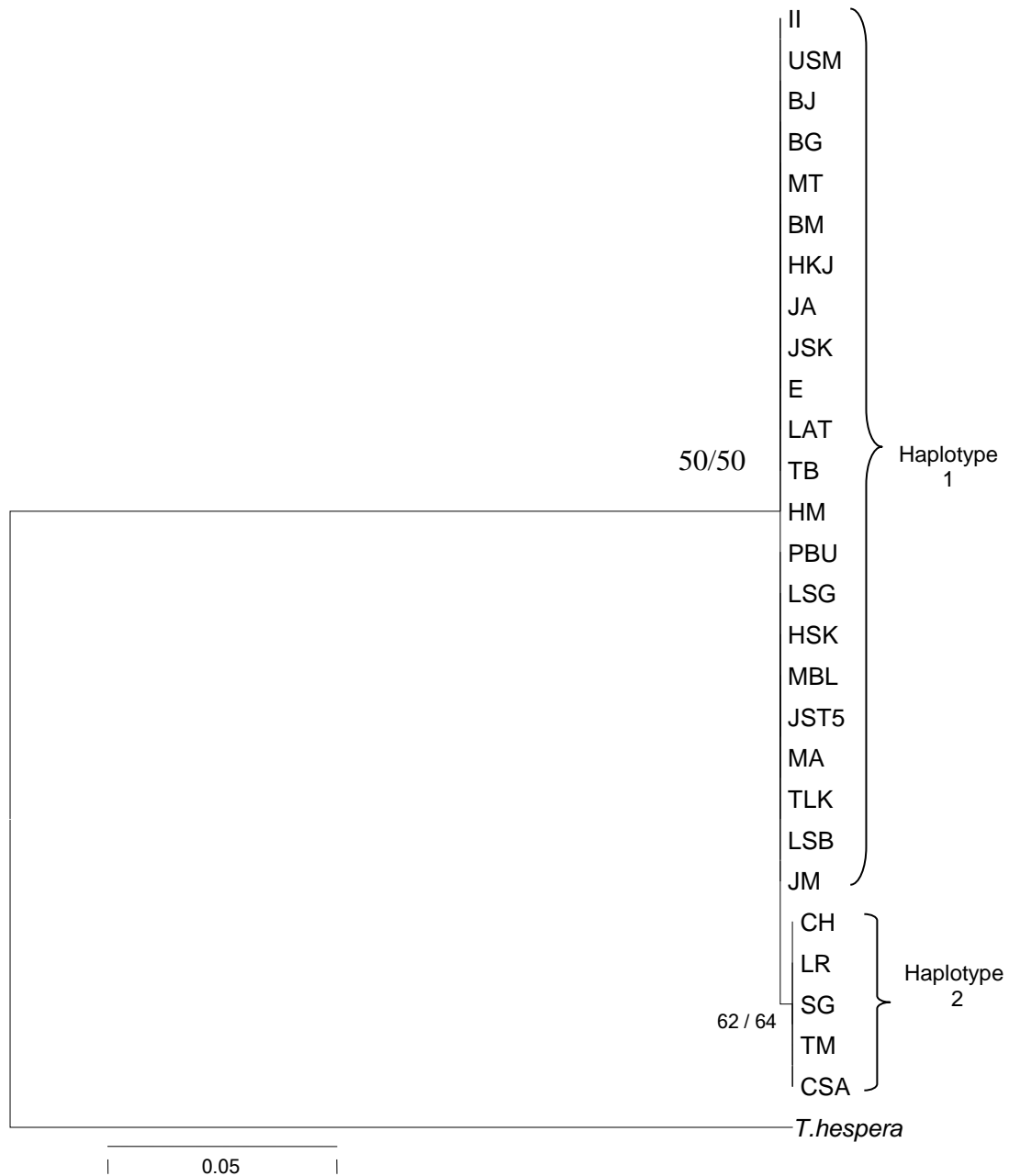


Figure 4. Neighbour-Joining tree representing COI gene sequences all *Tetraponera rufonigra* populations in 27 different infested locations against the outgroup (*T. hespera*, DQ176236.1) downloaded from NCBI GeneBank database. The tree was constructed based on pairwise distances (p-distance) and 1000 bootstrap replications. Abbreviation for each of the study site in Table 1

DISCUSSION

Genetic distance between the individuals obtained from 27 different localities was low, indicating a close relationship between the populations. A close relationship between populations was also due to the low gene flow and genetic dispersal. *T. rufonigra* exhibited a monogyne colony, where the queens have long-range nuptial flights and could form a new colony independently without the help of workers (Ward 2001). Although this species has a long-distance dispersal ability, habitat specialization, however, may have limited the range expansion of this species (Kumar & Mishra 2008). Other species known to have smaller range expansion is *T. binghami*, which is restricted to wet forests.

According to Gathorne-Hardy et al. (2002), the distribution of plants and animals' taxa in Wallace's line is influenced by the role of habitat and climatic tolerances. *Tetraponera rufonigra* collected from this study probably experienced a panmictic population due to the low level of genetic variation among the populations. Almost all individuals in the populations were genetically identical to each other. This finding was supported by Davidson (2003) where they stated *T. rufonigra* was a monotypic species group, forming a single population with no sub-species. Moreover, low genetic variation among the populations in the present study might result from recent colonization, supported by Yumoto and Maruhashi (1999), who suggested populations with low genetic variability were possibly resulted from recent colonization due to invasion by other species of ants occupying the same area.

Two haplotypes were identified i.e. Haplotype 1 and Haplotype 2. Haplotype 1 was mostly the populations from industrial areas surrounded by young and newly cultivated trees, while, populations in Haplotype 2 were from residential areas with many tall trees. Members of *T. rufonigra* are arboreal ants and are always found foraging on tall trees and can travel up to the apex of trees (Gaume et al. 2005). However, adaptation may have occurred to populations of *T. rufonigra* in Haplotype 2 as these ants foraged for foods on the ground instead of on the trees. Moreover, the residential areas experienced fewer disturbances in terms of human activities and development compared to industrial areas in Haplotype 1. Numerous ant species are highly sensitive to microclimate fluctuations and habitat structures; therefore, they immediately respond to any environmental changes (Gunawardene 2008; Klimes et al. 2015; Rizali et al. 2008; Patkar & Chavan 2014).

ML and NJ trees analyses revealed the monophyly of *T. rufonigra* supported by more than 50% bootstrap. The major group consisted of all Haplotype 1 populations and a minor group consisted of Haplotype 2 populations. The tree was also supported by mean pair-wise genetic distance (0.003), suggesting all *T. rufonigra* populations were clustered together showing no significant difference between different localities. All populations had low nucleotide variations, and might, for this reason, all populations were clustered in one monophyletic group. Locations might also affect the result as sampling sites selected in this study were somewhat close to each other. In this study, the distribution of *T. rufonigra* in Penang Island was not restricted to any particular plant species where they were found on ornamental trees, fruit trees and shrubs. According to Ward and Downie (2005), *T. rufonigra* is labeled as an ant-plant generalist with generalized twig nesting habits and is not tied to any particular plant species. Furthermore, populations of *T. rufonigra* were high in areas with leaf litter or left-over foods scattered around the trees, implying that these ant populations preferred disturbed conditions. A similar observation was reported by Abe et al. (2012) where

T. rufonigra was found at Anak Krakatau, Indonesia that had been frequently disturbed by volcanic activity and dominated by poor vegetation.

CONCLUSION

There was a low genetic distance among populations based on pairwise genetic distances. Based on phylogenetic tree constructed, populations from Taman Mahsuri (TM), Cangkat Sungai Ara (CSA), Sungai Gelugor (SG), Lebu Rambai (LR) and Cheeseman (CH) shared the same clade, while, other 22 localities were in another clade. This indicated that *T. rufonigra* from sites in the same shared clade were closely related to each other. Low genetic variation between the populations were also observed. Further analysis such as population genetic structure of *T. rufonigra* should be executed to better understand the genetic diversity and phylogenetic relationship of this species

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REFERENCES

- Abe, T., Yamane, S. & Onoyama, K. 2012. Ants collected on the Krakatau Islands 100 years after the great eruptions. *Biogeography* 14: 65–75.
- Ab Majid, A. H. & Kee, K. L. 2015. Genotyping of tropical bed bugs, *Cimex hemipterus* F. from selected urban area in Malaysia inferred from microsatellite marker (Hemiptera: Cimicidae). *Entomology, Ornithology & Herpetology: Current Research* 04(3): 3–7.
- Bolton, B. 1994. Book review identification guide to the ant genera of the world. *Psyche: A Journal of Entomology* 101(3-4):203–208.
- Chong, K. F. & Lee, C. Y. 2010. Inter- and intraspecific aggression in the invasive long-legged ant (Hymenoptera: Formicidae). *Journal of Economic Entomology* 103(5): 1775–1783.
- Davidson, D. W. 2003. Explaining the abundance of ants in lowland tropical rainforest canopies. *Science* 300: 969–972.
- ESRI. 2011. ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute.
- Galtier, N., Nabholz, B., Glémin, S. & Hurst, G. D. D. 2009. Mitochondrial DNA as a marker of molecular diversity: A reappraisal. *Molecular Ecology* 18(22): 4541–4550.
- Gathorne-Hardy, F. J., Syaukani, Davies, R. G., Eggleton, P. & Jones, D. T. 2002. Quarternary rainforest refugia in south-east Asia: Using termites as indicators. *Biological Journal of the Linnean Society* 75(4): 453–466.
- Gaume, L., Zacharias, M. & Borges, R. M. 2005. Ant-plant conflicts and a novel case of castration parasitism in a myrmecophyte. *Evolutionary Ecology Research* 7(3): 435–452.
- Gunawardene, N. R. 2008. Assessing factors influencing the spatial distribution of species diversity in ground dwelling ant assemblages in lowland wet forest of southwest Sri Lanka. PhD. Dissertation. Curtin University of Technology, Australia.
- Heil, M., Teuber, M. G., Clement, L. W., Kautz, S., Verhaagh, M. & Bueno, J. C. S. 2009. Divergent investment strategies of *Acacia* myrmecophytes and the coexistence of mutualists and exploiters. *Proceedings of the National Academy of Sciences* 106: 18091-18096.
- Hwang, U. W. & Kim, W. 1999. General properties and phylogenetic utilities of nuclear ribosomal DNA and mitochondrial DNA commonly used in molecular systematics. *Korean Journal of Parasitology* 37(4): 215–228.

- Jermiin, L. S. & Crozier, R. H. 1994. The Cytochrome-B region in the mitochondrial-DNA of the ant *Tetraponera rufoniger* – sequence divergence in Hymenoptera may be associated with nucleotide content. *Journal of Molecular Evolution* 38: 282–294.
- Kautz, S., Lumbsch, H. T., Ward, P. S. & Heil, M. 2009. How to prevent cheating: A digestive specialization ties mutualistic plant-ants to their ant-plant partners. *Evolution* 63: 839–853.
- Kheng, H. P., Hung, P. K. J., Huat, O. K., Fah, B. C., Joseph, J. & Mahadi, N. M. M. 2019. laccases repertoire of a subterranean termite *Coptotermes curvignathus* Holmgren (Blattodea: Rhinotermitidae). *Serangga* 24(2):169-197.
- Klimes, P., Fibich, P., Idigel, C. & Rimandai, M. 2015. Disentangling the diversity of arboreal ant communities in tropical forest trees. *PLoS ONE* 10: 1–24.
- Kumar, D. & Mishra, A. 2008. Ant community variation in urban and agricultural ecosystems in Vadodara District (Gujarat State), western India. *Asian Myrmecology* 2: 85–93.
- Nei, M. & Kumar, S. 2000. *Molecular Evolution and Phylogenetics*. New York: Oxford University Press.
- Norasamah, B., Chin, Y. J. & Abu Hassan, A. 2012. A preliminary study on the diurnal foraging activity and nutrient preferences of *Tetraponera rufonigra* (Hymenoptera: Formicidae) in Pulau Pinang, Malaysia. *Malaysian Applied Biology* 41: 51–54.
- Notredame, C., Higgins, D. G. & Heringa, J. 2000. T-Coffee: a novel method for fast and accurate multiple sequence alignment. *Journal Molecular Biology* 302: 205-217.
- Patkar, N. B. & Chavan, R. J. 2014. Diversity of ants (Hymenoptera: Formicidae) from undisturbed and disturbed habitats of Great Indian Bustard Wildlife Sanctuary, (MS), India. *International Journal of Science Research* 3: 398–401.
- Rizali, A., Bos, M. M., Buchori, D., Yamane, S. & Schulze, C. H. 2008. Ants in tropical urban habitats: the myrmecofauna in a densely populated area of Bogor, West Java, Indonesia. *Journal of Biosciences* 15: 77–84.
- Sabtu, F. S. & Ab Majid, A. H. 2017. Genetic variation and population structure of the arboreal bicolored ant *Tetraponera rufonigra* Jerdon from selected urban locations in eastern Penang Island, Malaysia. *Journal of Asia-Pacific Entomology* 20(4): 1350–1357.
- Seri Masran, S. N. A. & Ab Majid, A. H. 2017. Genetic Diversity and Phylogenetic relationships of Cytochrome C Oxidase Subunit I in *Cimex hemipterus* (Hemiptera: Cimicidae) populations in Malaysia. *Journal of Medical Entomology* 54(4): 974-979.
- Swofford, D. L. 2002. PAUP* version 4.0 b10. Phylogenetic analysis using parsimony (*and other methods). Sunderland, MA, USA: Sinauer Associates.

- Tamura, K., Stecher, G., Peterson, D., FilipSKI, A. & Kumar, S. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology Evolution* 30: 2725-2729.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. 1997. The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 24: 4876-4882.
- Ward, P. S. 2001. Taxonomy, phylogeny and biogeography of the ant genus *Tetraopenera* (Hymenoptera: Formicidae) in the Oriental and Australian regions. *Invertebrate Taxonomy* 15(5): 589–665.
- Ward, P. S. & Brady, S. G. 2003. Phylogeny and biogeography of the ant subfamily Prionomyrmecinae (Hymenoptera: Formicidae). *Invertebrate Systematics* 17: 361–386.
- Ward, P. S. 2001. Taxonomy, phylogeny and biogeography of the ant genus *Tetraopenera* (Hymenoptera: Formicidae) in the Oriental and Australian regions. *Invertebrate Taxonomy* 15(5): 589–665.
- Ward, P. S. & Downie, D. A. 2005. The ant subfamily Pseudomyrmecinae (Hymenoptera: Formicidae): Phylogeny and evolution of big-eyed arboreal ants. *Systematic Entomology* 30: 310–335.
- Xu, Z. H. & Chai, Z. Q. 2004. Systematic study on the ant genus *Tetraopenera* F. Smith (Hymenoptera: Formicidae) of China. [Chinese abstract]. *Acta Zootaxon* 29: 63–76.
- Yumoto, T. & Maruhashi, T. 1999. Pruning behavior and intercolony competition of *Tetraopenera* (*Pachysima*) *aethiops* (Pseudomyrmecinae, Hymenoptera) in *Barteria fistulosa* in a tropical forest, Democratic Republic of Congo. *Ecological Research* 14(4): 393–404.