

SPATIAL DISTRIBUTION OF *Rattus* SPECIES (RODENTIA: MURIDAE) IN OIL PALM PLANTATIONS OF PENINSULAR MALAYSIA WITH SPECIES VERIFICATION USING CYTOCHROME OXIDASE I (COI) GENE

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ABSTRACT

Rats have caused severe problems in oil palm production in Malaysia. Rattus species is the majority group of Muridae found in this area, and it is crucial to know about the species distribution in oil palm plantations (OPP) in Peninsular Malaysia. DNA barcoding method using Cytochrome Oxidase I (COI) gene was performed to identify all rat species captured in OPP aside from morphological identification. The data were then used to estimate the species diversity based on palm tree age. A total of 341 rats were captured and identified as Rattus tiomanicus, R. argentiventer, R. rattus diardii, R. exulans and R. tanezumi. Among these species, R. tiomanicus dominated the plantation with the highest diversity index ($H' = 1.31$), followed by R. argentiventer and R. rattus diardii. Most species of rats were commonly dispersed in the mature oil palm area. The annual precipitation showed a negative correlation (-0.258 , $p < 0.05$) with the species abundance, indicating that rats were more abundant during the dry season. In conclusion, the identification of rat species using molecular tools conforms to the morphological identification to determine the rats' distribution in the OPP. This can be associated with the oil palm age stage and abiotic factors of seasonal change.

Keywords: molecular identification, oil palm age, rat pest, species diversity.

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INTRODUCTION

The oil palm is one of the most rapidly increasing crops in the tropics (Kushairi *et al.*, 2017; Shevade and Loboda, 2019; Xu *et al.*, 2020). In 2019, Malaysia was the second-biggest share (20.7%) globally, behind Indonesia (33.9%) in producing palm oil and palm kernel oil market (MPOC, 2020). Since 1990, oil palm has remained the largest crop in Malaysia, contributing to Malaysia's third-largest external trade in 2019 (MPOC and MPOB, 2020). More than 5.9 million hectares of land have been converted from other crops such as rubber plants into oil palm plantations (OPP) to meet the market demand in 2019 (MPOC, 2020). However, this establishment has invited rats as notorious pests in the OPP (Hood *et al.*, 2019). Rats can cause damage up to 24% of fresh fruit bunch (FFB) in OPP (Chung, 2012; Rizuan and Noor Hisham, 2015).

Management of rat infestations in OPP has been dealt with using physical, biological and chemical methods. Physical methods such as shooting, dog hunting, and trapping were successful against individual rats but did not guarantee complete control of the rat populations (Lupo, 2011; Saravanan *et al.*, 2020). The barn owl, *Tyto alba javanica* is a classic example of a biological control approach to control rats in oil palm after its discovery in a Johor, Malaysia OPP in the late 1960s (Kamarudin *et al.*, 2019; Labuschagne *et al.*, 2016; Zainal Abidin *et al.*, 2021). On the other hand, the chemical approach is often considered the most favourable, cost-effective, humane, and gives the fastest result (Chia, 2005). Anticoagulant rodenticides (ARs) are chronic rodenticide that is probably the most effective and commonly used to manage rodents (van den Brink *et al.*, 2018). The plantation in Malaysia has started using the ARs by mixing them with a wide range of materials for field acceptability and practicality since the mid-1960s (Wood, 1976; Wood and Fee, 2003).

The genus *Rattus* has been identified as the primary rat pest infesting OPP (Ikhsan *et al.*, 2020), known to be distributed widely in Southeast Asia and comprises 561 species in 126 genera (Musser and Carleton, 2005). Rats are small mammals that quickly adapt in oil palm habitat due to easy access, breed and safety from predators (Puan *et al.*, 2011b; Tri Kwatrina *et al.*, 2018). Three main *Rattus* species were recorded in an oil palm plantation in Malaysia: *R. rattus diardii*, *R. argentiventer* and *R. tiomanicus* (Hafidzi and Saayon, 2001; Puan *et al.*, 2011a; 2011b). *Rattus rattus diardii* and *R. tiomanicus* dominate the matured palm area, while *R. argentiventer* was dominant in younger tree areas (Chia, 2005; Hafidzi and Saayon, 2001). This is because of the large body size of the *R. argentiventer* that limits its ability to climb off the older palm tree, which is high for them (Corley and Tinker,

2003; Liao *et al.*, 1991; Puan *et al.*, 2011a; Wood and Fee, 2003).

Morphological characteristics and skull measurements have been helpful tools in identifying rat species in the field. However, misidentification due to similarities and vast external features can happen during identification. Therefore, the molecular analysis will support the confirmation of rats' identification from the field study. Thus, this study aimed to investigate the rat species present in the OPP using genetic information and determine the species diversity of rats based on the age of the palm trees and seasonal factors.

MATERIALS AND METHODS

Study Site

Rats were collected from five OPP located in three states of Peninsular Malaysia from December 2018 until December 2019. The study sites located in Johor were: 1) Universiti Malaya (UM) Oil Palm Research Plantation at Kota Tinggi (N2.02916, E103.87076), 2) Paya Lang Plantation (N2.61125, E102.69362) and 3) Tambang Plantation (N2.63018, E102.70772) at Segamat, while in Pahang; 4) Pukin Plantation at Rompin, (N2.73600, E103.03200) and in Perak; 5) Tumbuh Hangat Plantation (N4.313903, E100.929009) at Perak Tengah (Figure 1). These study sites represent two age stages of OPP. Paya Lang estate and Tambang estate were young oil palm age (3-7 planting years), while UM plantation and Tumbuh Hangat plantation were matured oil palm age (10-15 planting years). Pukin estate contained both types of oil palm growth phase. The monthly rainfall data between December 2018 to December 2019 for each trapping location were provided by the plantation management office.

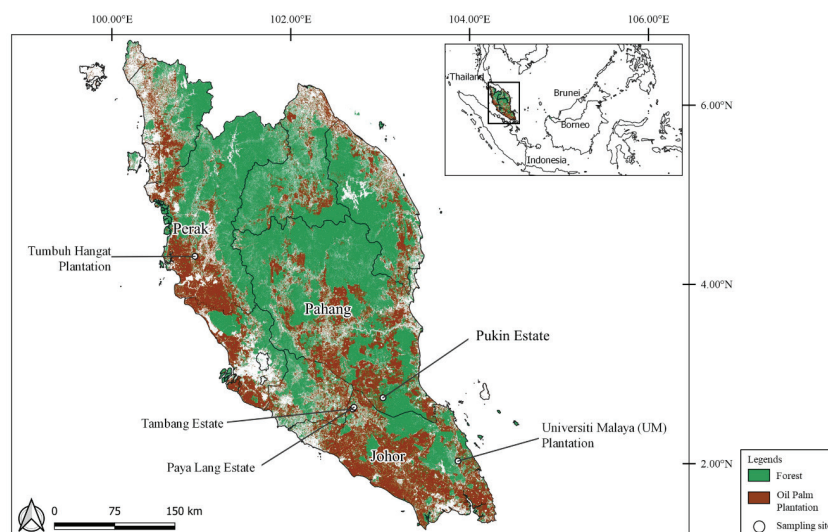


Figure 1. Sampling site selected at oil palm plantation in three states of Peninsular Malaysia.

Sampling Technique

A total of 150 rat trap cages, 28 cm x 15 cm x 12 cm, were placed in every plantation and set up randomly between frond heaps at the inter-planting rows of the selected plots in the plantations. Fresh oil palm fruit, salted fish and fresh corn pieces were used as bait in each trap (Mohd-Taib and Ishak, 2021; Saarani *et al.*, 2021). The traps were checked twice per day, during early morning and late afternoon, for five consecutive days at each plantation.

Rat's Handling and Research Approval

All captured rats were euthanised using Zoletil® through intramuscular injection (>50 mg kg⁻¹) (Ferrari *et al.*, 2005; Mohd-Taib and Ishak, 2021). Liver samples were extracted and stored at -20°C. In contrast, the body and skull of the sample were kept in 70% ethanol as wet specimens (Tingga *et al.*, 2012) and then were deposited at the Museum of Zoology, Universiti Malaya, Malaysia. Five linear measurements were measured: head to body length (HB), tail length (TL), hindfoot length (HF), ear length (E) and weight following Herbreteau *et al.* (2011). This study received approval from the Institutional Animal Care and Use Committee, Universiti Malaya, Malaysia (UM IACUC) (S/18042019/26112018-04/R) and the Department of Wildlife and National Parks [JPHL&TN(IP): 100-34/1.24 Jld. 14(02)].

Species Identification

Morphological and deoxyribonucleic acid (DNA) sequences were used for species identification. Morphological identification was based on five body measurements which include body length (BL), TL, HF, E and weight (Aplin *et al.*, 2003; Francis, 2008). In addition, all individuals were identified based on morphological features such as the colour of the dorsal fur, belly fur, mid-belly line, and tail, the texture of the dorsal fur and guard hairs, the length of the snout, and the number and position of the mammae (Aplin *et al.*, 2003; Lim, 2015; Paramasvaran *et al.*, 2013).

DNA Extraction

A total of 341 samples of genomic DNA of *Rattus* species were extracted from liver tissues using the GF-1 Tissue DNA Extraction kit (Vivantis®) following a protocol provided by the manufacturer. A total of 250 µL of Tissue Lysis Buffer (Buffer TL) was added to the liver tissue together with 20 µL of Proteinase K and 12 µL of Lysis Enhancer solution. The mixture was mixed immediately and incubated in the water bath at 65°C for 1 hr. After that, 560 µL of Tissue Genomic DNA Binding Buffer

(Buffer TB) were added and mixed thoroughly by pulsed-vertexing and incubated for 10 min in a 65°C water bath. The column then underwent washing steps where the column was loaded with 650 µL of wash buffer and centrifuged at 5000 x g for 1 min, and the solution that flowed through was discarded, and the same step was repeated. 50 µL of preheated Elution Buffer was added directly onto the column membrane and allowed to stand at room temperature for 2 min. The DNA obtained was stored at -20°C. NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific, USA) was used to quantify the isolated genomic DNA's concentration and purity.

PCR Amplification and DNA Barcoding

Molecular identification was carried out by amplifying a partial mitochondrial cytochrome oxidase I (COI) gene. The polymerase chain reaction (PCR) was employed to amplify the targeted locus using Mastercycler® Nexus (Eppendorf North America, Inc.). PCR was conducted by using the PCR reagent, which were included 12.5 µL of 2X Power Taq MasterMix (BioTeke, Beijing), 0.5 µL of each 10 micromolar (µM) of forward and reverse primer, 1.0 µL of genomic DNA template and 10.5 µL of ultrapure water added to make the final volume of 25 µL, as per suggested by the manufacturer. PCR was performed by using a set of established primer corresponding to the (mtDNA) COI marker, forward Batl. 5310 primer 5 'CCTACTCRGCCATTTTACCTATG' 3 and reverse R6036R primer 5 'ACTTCTGGGTGT CCAAAGAATCA' 3 (Robins *et al.*, 2007). The PCR condition was as follows; initial denaturation for 4 min at 94°C, continued with 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 48°C, an extension for 1 min at 72°C and a final extension stage for 10 min at 72°C, before holding-up to 10°C after the reaction was completed (Ikbal *et al.*, 2019a; 2019b).

The amplicons were purified and sent out for sequencing to the third-party company (1st BASE DNA Sequencing Division). Sequencing was done using the Applied Biosystems 3730XL Genetic Analyser via the Sanger Sequencing method. Both aligned forward and reversed sequences were trimmed using Molecular Evolutionary Genetics Analysis Version X (MEGA X) (Kumar *et al.*, 2018). All assembled sequences were searched in the National Centre for Biotechnology Information (NCBI) database using BLAST (<http://BLAST.ncbi.nlm.nih.gov/BLAST.cgi>) and the MEGA X software for a first sequence quality assessment and preliminary species identification. As standards for successful conspecific identification, we chose the top-matching hit with the greatest (>98%) maximum percent identity score. We

reconstructed the species identification for all of our sequences using phylogenetic tree analysis as was demonstrated in Pulgarín-R *et al.* (2021).

$$R = \frac{\sum_{i=1}^k (i-1)Y_i}{T} \quad (2)$$

Phylogenetic Analysis

All sequenced DNA was analysed by building phylogenetic trees to visualise the species speciation together with representatives of each *Rattus* species sequence from GenBank; EF186528 *Rattus* sp. ABTC, HM217499 *Rattus* sp. ABTC, HM217498 *Rattus* sp. R3, FR775826 *R. tiomanicus*, JX533999 *R. tanezumi*, KC010285 *Rattus* sp. R3, KC010291 *R. tiomanicus* and KC617850 *R. exulans*. The phylogenetic trees were built using the Neighbour Joining (NJ) by MEGA X software. The Kimura-2-Parameter (K2P) algorithm model was used with a bootstrap value of 1000. JF459655 *Leopoldamys sabanus* (Muridae; Murinae) was included as the outgroup for this analysis.

Data Analyses for Species Diversity

Data assessed for normality by the Shapiro-Wilk test ($p=0.581$, $p>0.05$) were normally distributed. The Minitab 17 software was fully utilised for the analysis of variance (ANOVA) test for all variables used in this study. The significance of differences between males and females were investigated using a t-test for every external measurement. General trends of the external measurement were studied via principal component analysis (PCA). Collective data of the individuals captured from different localities were used to compute Shannon diversity index (H') by using PAST 3.26 (PAleontological STatistics) computer software (Hammer *et al.*, 2001). Cluster analysis was used to distinguish the species composition's similarity in the five locations, using the Euclidean (Pythagorean) distance measure. The Pc-Ord Version 5 programme (Grandin, 2006) was used for cluster analysis. Regression analysis was used to estimate the relationships between species abundance and annual rainfall using Minitab version 19.0.

To calculate the estimated population of rats in the oil palm plantation, we used the non-removal trapping system using the Zippin method (Zippin, 1958). Estimating the rats' population was based on the first, second and third catches, where the rats trapped would be counted and not rereleased. The total catch (T) was calculated based on the Equation (1);

$$T = \sum_{i=1}^k Y_i \quad (1)$$

where k is the number repeat of sampling and Y_i is caught on the i th captured. The ratio (R) of the total catch was calculated based on the Equation (2);

After the R was determined, the captured individuals' probability was estimated using a $(1-q^k)$ probability graph (Figure 2). The estimated population size (N_o) was calculated using the Equation (3);

$$N_o = \frac{T}{(1-q^k)} \quad (3)$$

The standard deviation (SD) of N_o is given by Equation (4);

$$SD(N_o) = \sqrt{\frac{N_o(N_o-T)T}{T^2 - N_o(N_o - T) \left(\frac{k \cdot p^2}{[1-p]}\right)}} \quad (4)$$

RESULTS

Species Identification

A total of 341 rats were collected throughout 9120 trap nights in five locations of OPP in Peninsular Malaysia. A total of five species (*i.e.*, *Rattus tiomanicus*, *Rattus rattus diardii*, *Rattus argentiventer*, *Rattus exulans* and *Rattus tanezumi*) were identified in the field based on morphological characteristics (Figure 3). *Rattus tiomanicus* was identified based on pure-white belly fur with dark midline strike, smooth olive-brown dorsal fur and 10 mammary tits. While *R. argentiventer* belly fur appears silvery grey with no midline middle streak, uniformly dark tail and dorsal fur of orange-brown colour intermixed with black. *Rattus rattus diardii* has greyish dorsal fur and whitish or grey-based belly fur with cream to buff tips on a combination of 2 (pectoral pairs) + 3 (inguinal pairs) mammae. The dorsal fur of *R. tanezumi* has a shade of brown, while the belly fur is whitish or grey based fur with pale yellow or orange tips. The only species with eight pairs of mammary tits was identified as *R. exulans*, the smallest rats among the *Rattus* genus with spiny reddish-brown dorsal fur, grey belly fur with a white-tip fur. The characteristics were referred from previous studies and guidebooks (Aplin *et al.*, 2003; Francis, 2008; Lim, 2015; Paramasvaran *et al.*, 2013).

All the COI sequences were aligned with no stop codon, insertion and deletion observed, a total of 708 bp of COI gene were retrieved, including 499 conserved sites, 209 variable sites, 163 Parsimony informative and 46 Singleton. All species except

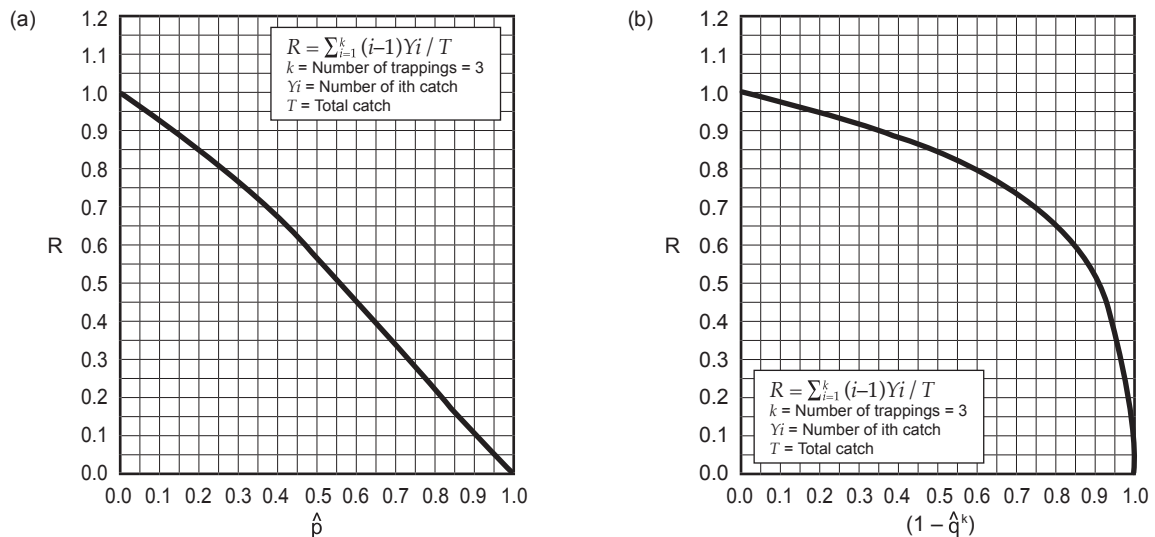


Figure 2. (a) Graphs for estimation from ratio R in removal trapping (b) Graph to estimate the p-value.

R. rattus diardii were confirmed by the DNA barcoding method of the COI gene marker with a high (>99%) match score. A total of 117 unclassified *Rattus* species were identified based on COI gene marker, including 88.9% *Rattus* sp. R3 and 11.1% *Rattus* sp. ABTC. In this study, all these unclassified *Rattus* species were referred to as *R. rattus diardii* based on their morphological field identification.

The phylogenetic relationship of the five *Rattus* species recorded in this study was reconstructed in NJ trees prior to the BLAST result (Figure 4). The sequences analyses revealed that *Rattus* COI falls into five well-supported clades categorised into *R. rattus diardii*, *R. tiomanicus*, *R. argentiventer*, *R. tanezumi*, and *R. exulans* with high bootstrap value ($96 \leq Bp \leq 100$). *Rattus tanezumi* is clustered with 86% bootstrap values with a clade of *R. rattus* originating (100%) as a sister taxon. Three mitochondrial lineages are associated with the house rat, which is *Rattus* sp. R3, *R. rattus diardii* and *R. rattus* (RrC) lineage IV form a single clade. The *R. rattus diardii* clade represents a cryptic species because molecular data was not accessible in the GenBank database, yet these lineages were clustered together in the same clade. This study included a sequence of *R. norvegicus* to show a special relationship between *R. rattus diardii* and *R. rattus* species.

Principal component (PC) 1 shows 63% of the variation, whereas PC2 shows 27% variation in morphological measurements. The highest PCA loadings from PC1 were total length, followed by HB and weight, indicating a high contribution of these morphological measurements in distinguishing species (Table 1). While weight (0.082) has the largest PCA loading in PC2, followed by E, the hindfoot was negatively correlated with the other variables (-0.623). The PCA plots the morphological measurement of all species produced by two contrasting groups (Figure 5). The first group

comprised of *R. tiomanicus* and *R. argentiventer*, with only a few *R. rattus diardii* individuals spotted. The second group included *R. rattus diardii*, *R. exulans* and *R. tanezumi*. However, some *R. tiomanicus* and *R. argentiventer* overlapped with the other species in group 2. These plots revealed that species from the same group closely resembled each other for specific morphological measurements, as mentioned earlier. One way ANOVA analysis of the morphological measurements between males and females showed no significant difference with a *p*-value of 0.245 ($p > 0.05$).

The ANOVA analysis of the *Rattus* species' external measurements showed no significant difference ($p = 0.273$, $p > 0.05$). However, *post hoc* analysis using Tukey's honestly significant difference test showed that this genus head-to-body measurement could be divided into two groups with a significant difference of $p < 0.05$ separated into alphabetical groups in Table 2. *Rattus tiomanicus* and *R. exulans* have smaller HB (159-389 mm) and weight (10-200 g). In contrast, *R. argentiventer* and *R. rattus diardii* have a larger body size (176-462 mm) and weight (21-332 g). In this study, the TL appeared shorter than the HB for all species captured. *Rattus tiomanicus* and *R. exulans* BL showed a significant difference between *R. argentiventer* and *R. rattus diardii*. Simultaneously, the mean TL for every species was significantly different from each other, where *R. exulans* has the shortest tail among other *Rattus* species. For hindfoot measurement, only *R. exulans* was significantly different ($p < 0.05$) than the other species, with an average of 25.06 ± 1.56 mm. While for E measurement, *R. tiomanicus* was significantly different ($p < 0.05$) than the other species with an average length of 28.07 ± 0.54 mm. The number of female rats trapped was higher (51.73%) than male (48.27%) (Table 2).

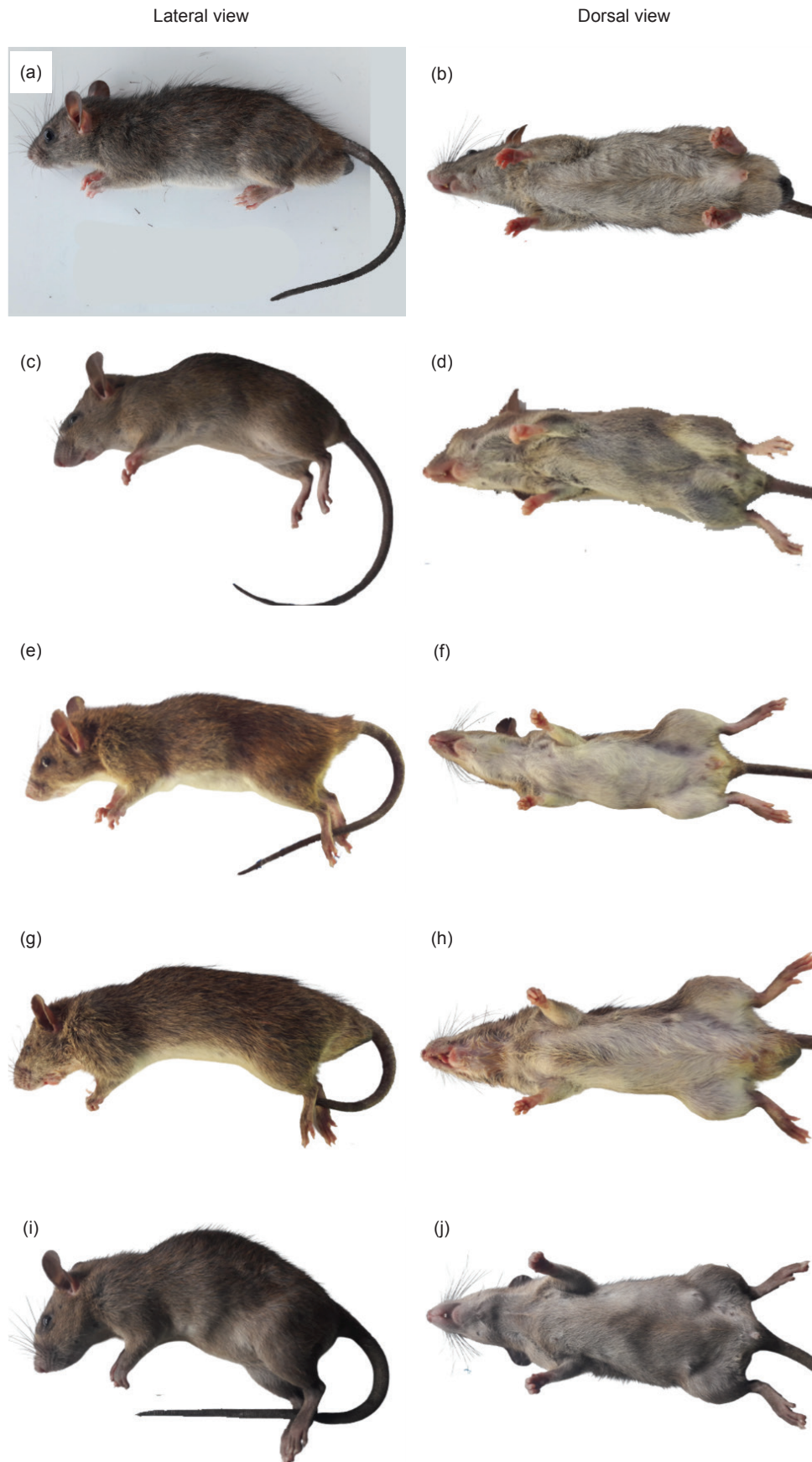


Figure 3. *Rattus* species captured in the oil palm plantation. (a-b) *Rattus rattus diardii* (c-d) *Rattus exulans* (e-f) *Rattus tiomanicus* (g-h) *Rattus argentiventer* (i-j) *Rattus tanezumi*.

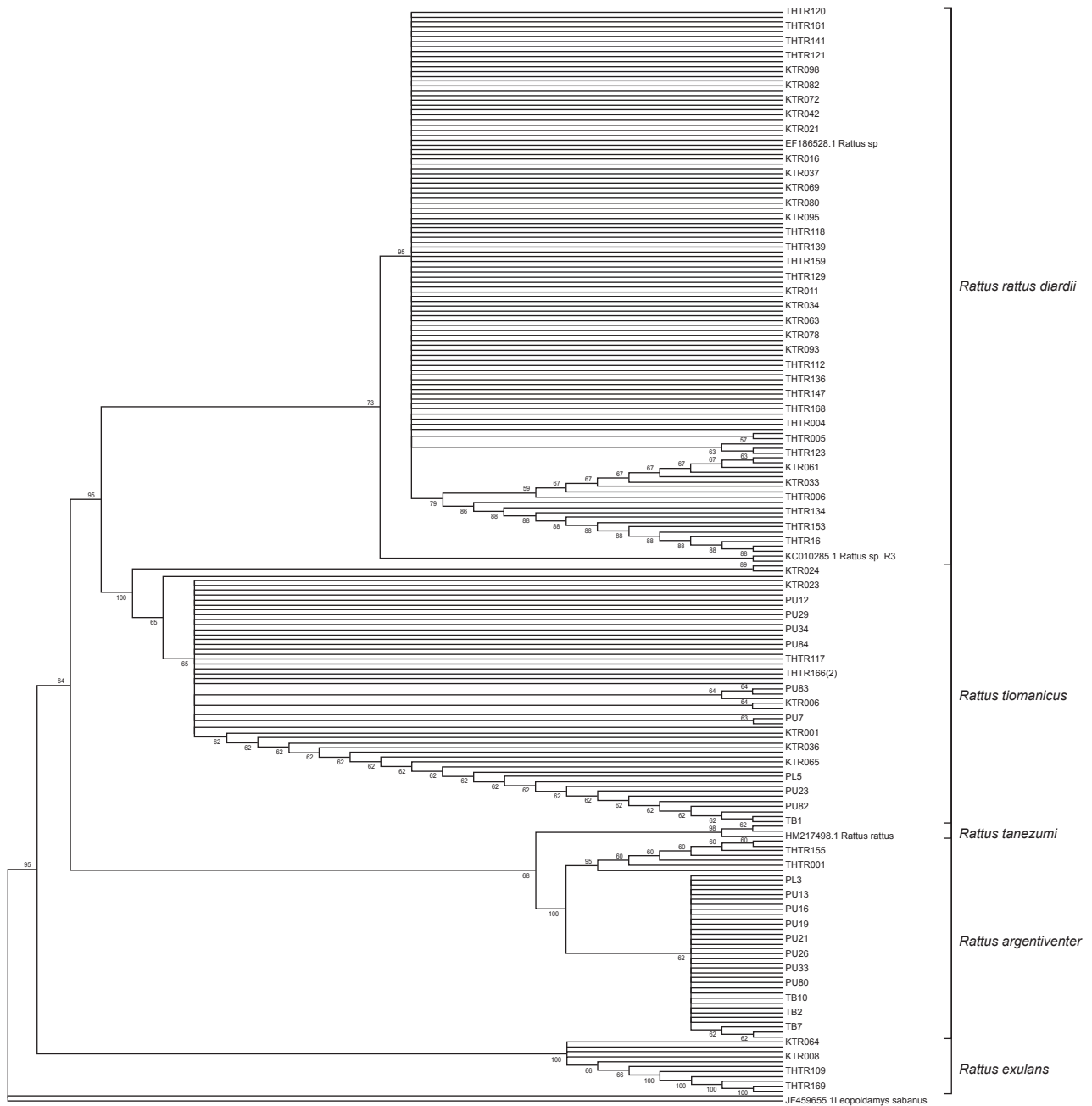


Figure 4. The Neighbour-Joining (NJ) phylogenetic tree (NJ/MP=89/76) illustrates the relationships between the Rattus species based on the COI genes using the Kimura-2-parameter algorithm and 1000 bootstrap replication. The optimal tree sum of branch = 0.5739 is shown for the NJ tree.

TABLE 1. FACTOR LOADINGS FOR THE PRINCIPAL COMPONENT ANALYSIS (PCA) BASED ON SIX EXTERNAL CHARACTERISTICS

Variable	PC1	PC2	t-test analysis against gender		
			df	p-value	p-variance
Head to body length	0.481	0.047	339	0.0816	0.8756
Tail length	0.455	0.113	339	0.7089	0.3272
Total length	0.505	0.086	339	0.2449	0.7600
Ear	0.029	0.763	339	0.7403	0.2754
Hind foot	0.286	-0.623	339	0.8591	0.8789
Weight	0.473	0.082	339	0.5089	0.7249
Eigenvalue	3.797	1.6357	-	-	-
% total variance	63.1712	27.2895	-	-	-

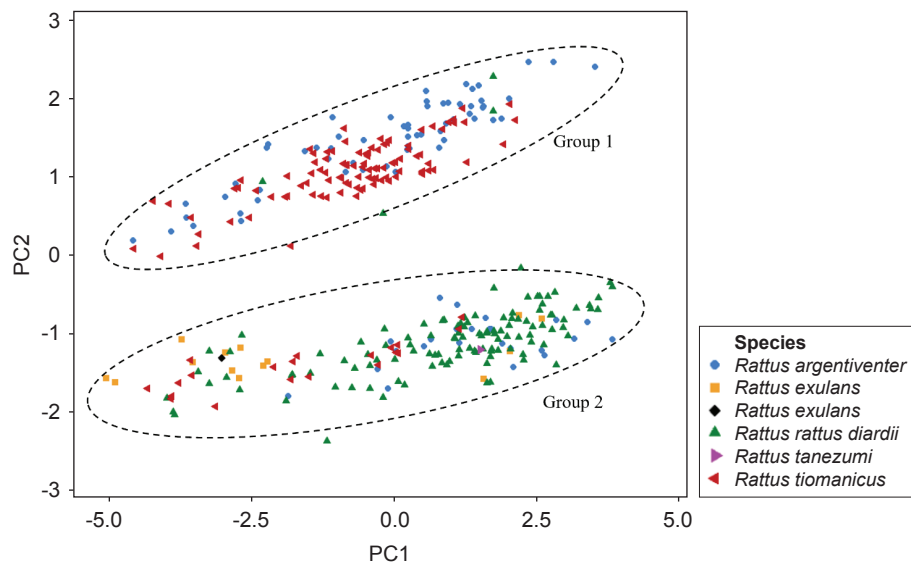


Figure 5. PC1 and PC2 of the PCA for six external measurements of the *Rattus* species.

TABLE 2. LIST OF SPECIES AND MEAN MEASUREMENT ± STANDARD ERROR (maximum and minimum ranges)

Species	Sex		Measurement (mm)				Weight (g)
	M	F	HB	TL	HF	E	
<i>Rattus tiomanicus</i>	66	59	148.00 ± 2.38 ^b (85-200)	141.69 ± 2.49 ^c (90-189)	19.76 ± 0.38 ^a (17-35)	28.07 ± 0.54 ^b (14-37)	88.07 ± 3.42 ^b (19-118)
<i>Rattus argentiventer</i>	34	51	162.60 ± 3.19 ^a (84-260)	156.20 ± 2.45 ^b (92-202)	23.05 ± 0.87 ^a (10-40)	29.63 ± 0.73 ^a (16-40)	129.46 ± 5.95 ^a (30-239)
<i>Rattus rattus diardi</i>	59	56	173.95 ± 2.58 ^a (98-220)	167.75 ± 2.41 ^a (94-220)	32.45 ± 0.44 ^a (16-39)	20.31 ± 0.29 ^a (14-35)	131.61 ± 5.29 ^a (21-332)
<i>Rattus exulans</i>	9	6	129.82 ± 9.17 ^b (80-205)	122.06 ± 7.26 ^c (79-180)	25.06 ± 1.56 ^b (17-39)	16.71 ± 0.72 ^a (12-22)	66.60 ± 15.8 ^b (10-200)
<i>Rattus tanezumi</i>	0	1	184 ^{ab}	163 ^{abc}	35 ^{ab}	20 ^{ab}	143 ^{ab}

Note: *Alphabets indicate the significant level ($p < 0.05$).

N - total individuals; HB - head to body length; TL - tail length; HF - hind foot; E - ear.

Diversity and Abundance

The three most abundant species collected in this study were *R. tiomanicus* (36.6%, 125 individuals), *R. rattus diardi* (33.7%, 115 individuals), and *R. argentiventer* (24.93%, 85 individuals) (Figure 6). Only 15 individuals of *R. exulans* and one individual *R. tanezumi* were captured throughout this sampling. The value of the H' strongly supports that the *R. tiomanicus* ($H' = 1.31$, $E = 0.77$, $D_{Mg} = 0.83$) was the most dominant rat in OPP (Figure 7). *Rattus argentiventer* has the second-highest value of H' compared to *R. rattus diardi*, although the number of individuals for this species was lower than *R. rattus diardi* with Shannon diversity index ($H' = 1.19$, $E = 0.82$, $D_{Mg} = 0.68$) and ($H' = 0.73$, $E = 0.70$, $D_{Mg} = 0.42$), respectively. The *R. rattus diardi* was only captured in UM plantation and Tumbuh Hangat plantation but not in the other three plantations. Aside from *R. rattus diardi*, *R. exulans*

has the lowest diversity index and richness score among the other species, with $H' = 0.50$ and D_{Mg} of 0.37. *Rattus tanezumi* scored 0 for H' and D_{Mg} indicating the lowest evenness index between other species with only one individual captured in all OPP sites.

The species composition in all locations showed a significant difference ($H' = 1.24$; $df = 6$, $p < 0.05$) (Table 3). Tumbuh Hangat plantation has the highest diversity of species ($N = 111$; $H' = 1.18$), while Paya Lang has the lowest diversity score ($N = 47$, $H' = 0.46$). Pukin plantation ($N = 97$, $H' = 0.74$) with both young and mature palm trees scored a higher diversity index than UM plantation ($N = 67$, $H' = 0.64$) due to differences in the species number. The estimated population size for each plantation is shown in Table 3. In line with the highest rat abundance in Tumbuh Hangat plantation, the estimated population size scored the highest with 314 ± 62 individuals per hectare, followed by

Pukin plantation (124 ± 28 /ha) and UM plantation (103 ± 17 /ha). The younger palm trees (3-7 planting years) area has a lower recorded population of rats, fewer than 50 individuals per hectare. It showed that the rat abundance was concentrated in the matured palm area (10-15 planting years) than in the young palm.

Rattus species captured in the UM and Tumbuh Hangat plantations showed a 100% similarity index, while Tambang and Paya Lang plantations shared 89% of the clustering analysis (Figure 8). Pukin plantation was separated from Paya Lang and Tambang plantations by a similarity index of less than 75%. Based on the clustering analysis, the five *Rattus* species were grouped into three with an overlapping value of >95%. Group 1 consisted of *R. tiomanicus* and *R. argentiventer*, where these species were concentrated in Paya Lang plantation, Tambang plantation, and Pukin plantation with a similarity score of 90%. *Rattus rattus diardii* and *R. exulans* were grouped into group 2 captured from UM and Tumbuh Hangat plantations, while *R. tanezumi* was categorised in Group 3 (100%) alone and can only be found in Tumbuh Hangat plantation.

Association of Rats Species with Rainfall and Oil Palm Age

The monthly data collection of *Rattus* with the mean rainfall dataset of all locations was recorded from December 2018 to December 2019 (Figure 9a). Four changes in the mean rainfall have been recorded throughout the sampling periods between four quartiles of the year. The average rainfall data throughout the sampling period in all locations was 192.73 mm. The first drop of the mean rainfall was recorded between December 2018 to February 2019, with an average rainfall of 125.75 mm. During this period, a significantly high number of rats were trapped, with 20 to 63 individuals that included *R. argentiventer*, *R. tiomanicus*, *R. rattus diardii* and *R. exulans*. The highest captured species were *R. argentiventer* (N=63), while *R. exulans* (N=9) was the lowest. The second quartile of the year recorded an increased rainfall was between April 2019 to June 2019 (189.41 mm). The total trapped individuals reduced when the rainfall increased, especially in May 2018 (N=10, 198.70 mm), and the number increased in June

TABLE 3. SPECIES DIVERSITY OF RATS CAPTURED IN FIVE DIFFERENT OPP

Plantation	Ha	Captured individuals	Shannon diversity index (H')	Estimated population size (N_e /Ha)
Tambang Plantation	134	19	0.68	18 ± 16
Paya Lang Plantation	141	47	0.46	48 ± 13
UM Plantation	136	67	0.64	103 ± 17
Pukin Plantation	142	97	0.74	124 ± 28
Tumbuh Hangat Plantation	142	111	1.18	314 ± 62
Total	695	341	1.24	920 ± 90

Note: H' - shannon diversity index, N_e /Ha - estimated population size.

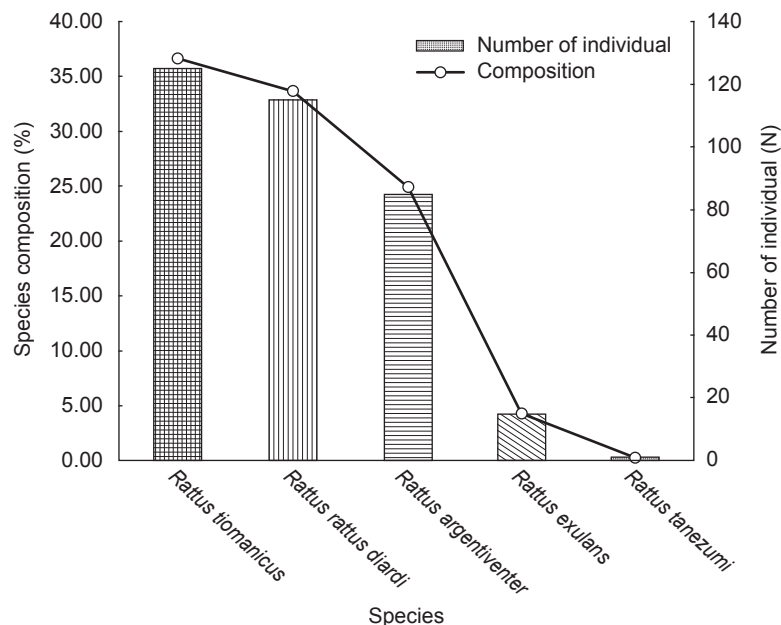


Figure 6. Species composition and the number of individuals captured of the *Rattus* species collected in OPP of Peninsular Malaysia.

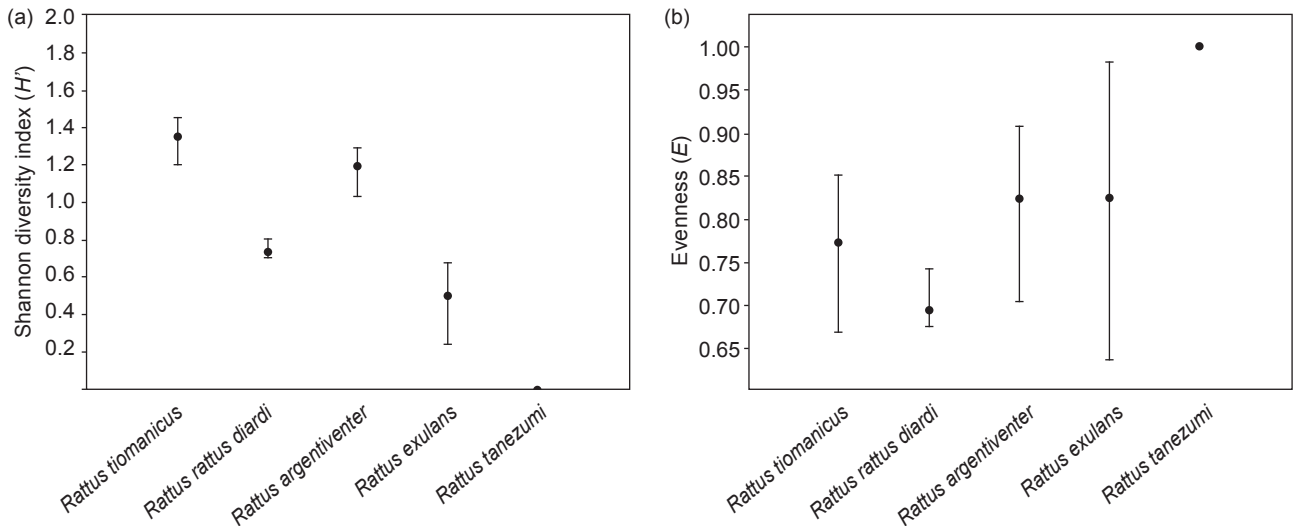


Figure 7. Mean box and whisker plot for each rat species captured with (a) Shannon diversity index (H'), (b) Evenness (E).

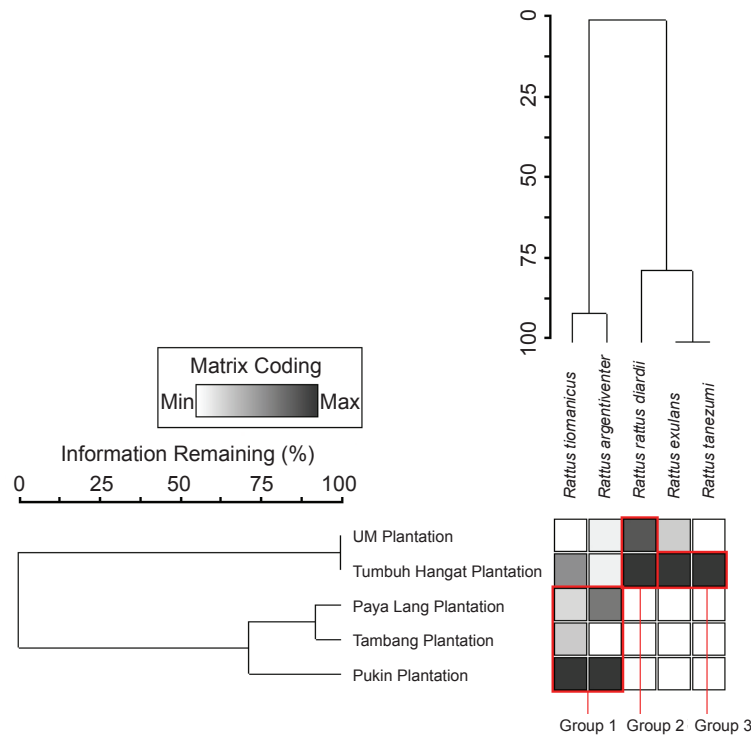


Figure 8. Clustering analysis between the composition of *Rattus* species in the OPP.

2019 (N=51, 173.10 mm) with the decreasing rainfall. With the drop of rainfall in July 2019 (134.10 mm), the number of trapped rats rose to the highest (N=77) compared to the other sampling months. The number of trapped rats dropped from September 2019 to December 2019, when the wet season started with an average rainfall of 286.44 mm. Overall, the trapped rats showed a significant negative correlation ($r=-0.258$, $p=0.038$) with the average rainfall. The number of trapped rats decreased with the increase of mean rainfall (Figure 9b; $R^2=6.68\%$, $F=4.51$, $DF=64$, $p<0.05$). We observed

a non-significant correlation ($r=0.305$, $p=0.618$) between the two palm age groups based on the captured rats species in this study ($R^2=9.28\%$, $F=0.31$, $p=0.618$).

DISCUSSION

Reference DNA sequences under *R. rattus diardii* (Jentink, 1880) were unavailable in the GenBank, which could explain why our sequences were matched with *Rattus* sp. R3 and *Rattus* sp. ABTC

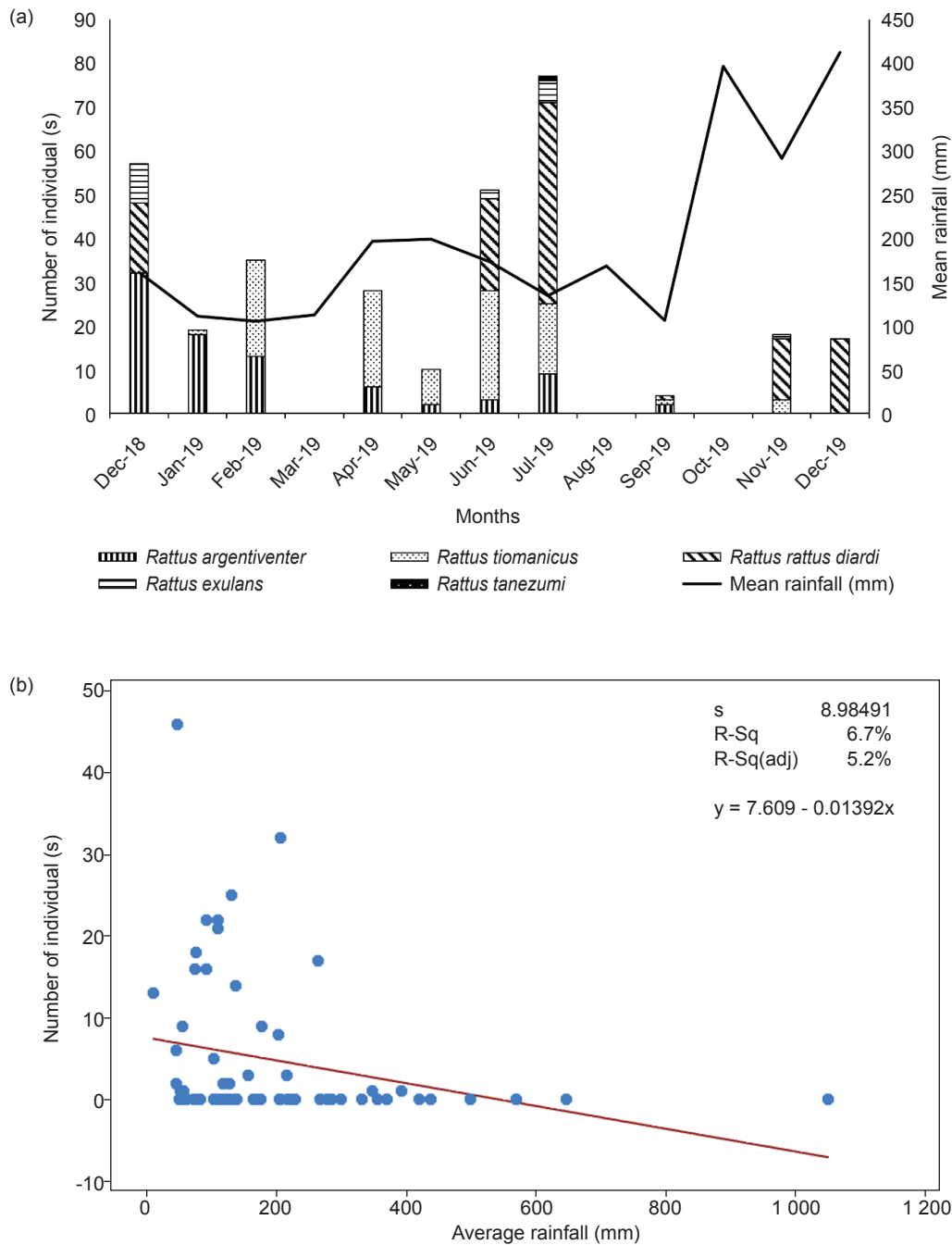


Figure 9. (a) Comparing individuals collected with the mean monthly rainfall data (mm). (b) Species abundance decreases across a regional gradient of mean rainfall (mm).

of unknown *Rattus* species. This was supported by the phylogenetic tree that shows the R3 lineage was grouped with our *R. rattus diardi* sequences. However, based on previous studies of unrecognised sequences of original data, both *Rattus* sp. R3 and *Rattus* sp. ABTC were initially identified as *R. rattus diardi* by field identification based on the physical appearance of this species. However, in another study conducted by Robins *et al.* (2007), sequences of *R. rattus diardi* collected from Kuala Lumpur were deposited as *Rattus* sp.

ABTC according to their voucher name instead of the original species name. While the *Rattus* sp. R3 was proposed by Pagès *et al.* (2010) when the author used a DNA sequence of unidentified *Rattus* sp. from Thailand and then later classified them under the R3 lineage based on the phylogenetic analysis. In Indonesia, this species was also referred to as *R. rattus diardi* by local taxonomists as the species responsible for the infestation and damage of the oil palm tree (Andru *et al.*, 2013; Ikhsan *et al.*, 2020).

Rattus rattus diardii clade was well separated from other species in this study, including *R. rattus* and *R. norvegicus* retrieved from GenBank data sources. Thus, this study emphasises a suitable terminology for all individuals in this clade, such as *R. rattus diardii* (Malayan House Rat) (Jentink, 1880). The other *Rattus* species recorded in this study were well separated in their well-supported clade. DNA-based identification has proven to be helpful to distinguish cryptic species with the development of genetic lineage divergence to eliminate the taxonomic uncertainty introduced by morphological identification (Hebert *et al.*, 2004). However, morphology identification is still needed to distinguish rat species better since it does not change as rapidly as the genetic lineage (Hebert *et al.*, 2004; Matisoo-Smith and Robins, 2009; Wada *et al.*, 2003).

Rattus rattus diardii came second as the most abundant species captured in this study but came third in the diversity index score. *Rattus rattus diardii* was a typical household and urban pest (Lim, 2015). In Borneo, Zainal Abidin *et al.* (2021), live trapping revealed that *R. rattus diardii* was the predominant species in the oil palm plantation. The feeding preferences of *R. rattus diardii* are not limited to fruit bunches but also any leftover food present surrounding the worker settlements area within the plantation, as we observed their existence in two plantations (UM and Tumbuh Hangat plantation). In contrast to the other plantation, which was remote from human settlements, the two plantations were surrounded by human settlements, such as the aborigine's village, other crop fields, and the surrounding residential area.

This study observed *R. tiomanicus* as the most dominant species, especially in the matured oil palm plantation. *Rattus tiomanicus* has adapted well to the oil palm plantation due to its agility and arboreal habits that make it difficult to control. Small body size gives this species the advantage of becoming a great climber with their well-developed slight ridges pattern on their broad footpads. Furthermore, a longer tail than the BL gives advantages to the arboreal rats to keep their balance while climbing (Hori *et al.*, 2011). *Rattus tiomanicus* spend most of their time on trees but was often found on the ground (Francis, 2008). Other species such as *R. rattus diardii*, *R. tanezumi* and *M. hellwaldii* share the same characteristic of having a longer tail than HB, but the climbing ability is not as good as *R. tiomanicus* (Ikhsan *et al.*, 2020). In this study, we have observed that *R. rattus diardii* and *R. exulans* shared the same characteristic of the TL as *R. tiomanicus* to maintain their stability and balance. While in comparison, the tail of the terrestrial rat, such as *R. argentiventer* is usually shorter than the HB (Carrizo *et al.*, 2014).

In Peninsular Malaysia, *R. tiomanicus* was reported as the main rat pest infesting the oil palm plantation along with *R. rattus diardii* and *R. argentiventer* (Hafidzi and Saayon, 2001; Nursyazana *et al.*, 2013; Puan *et al.*, 2011a; 2011b; Rizuan and Noor Hisham, 2015). However, the situation in Borneo OPP was different, where the *R. tiomanicus* was less recorded and predominated with *R. rattus diardii*, *Maxomys whiteheadi*, *R. argentiventer* and *R. exulans* (Abidin *et al.*, 2014; Buckle *et al.*, 1997; Phua *et al.*, 2018; Stryjek *et al.*, 2018). In Indonesia, the population of *R. tiomanicus* was reported growing and expanding after converting the secondary forest to OPP (Nugroho and Santosa, 2018; Santosa and Rejeki, 2019).

The next most dominant rat species in OPP was *R. argentiventer*, and even the abundance was relatively low compared to *R. rattus diardi*. This species was more likely to invade the young palms (Corley and Tinker, 2003; Wood, 1976) as they were terrestrial rats and the limitation to climb as the palm grows higher (Yasuma and Andau, 2000). However, the correlation between the palm age and the abundance of *R. argentiventer* was negative in Puan *et al.* (2011b) studies, where this species was widely found in OPP with matured palms. The same observation was recorded in this study, where many rats were reported in mature palm planting sites such as Tumbuh Hangat plantation and Pukin plantation. There was a possibility that this species has migrated from the surrounding paddy field at Tumbuh Hangat plantation that may have caused the high rate of capture as they were a common species of grassy field and paddy field (Tristiani and Murakami, 2003; Tristiani *et al.*, 2003).

Rattus argentiventer was initially an important small mammal pest of the rice field in Southeast Asia (Buckle *et al.*, 1982; Tristiani *et al.*, 2000). However, an increasing number of these species has been recorded in OPP, leading to a potential threat for this crop. Their presence in OPP has been reported since 1976 (Payne *et al.*, 1985; Wood, 1976), and their growing population has caused great damage to FFB in oil palm (Hafidzi and Saayon, 2001; Zainal Abidin *et al.*, 2021). With an abundance of oil palm fruit available in OPP, this species can survive and adapt to its new environment moving away from its natural habitat in the rice field.

Only a small number of the Polynesian rat, *R. exulans* were captured in this study. This species was concentrated only in the Tumbuh Hangat plantation, surrounded by forest, human settlements, and other agriculture crops. *Rattus exulans*, like *R. rattus diardii*, is a rodent that is commonly found in households and lives in forest and agricultural environments (Paramasvaran *et al.*, 2013). Smaller rats, such as *R. exulans* and *M. whiteheadi* were often recorded in immature palm areas (Rizuan and Noor Hisham, 2015).

However, in Tumbuh Hangat plantation, the oil palm trees were in a mature stage, and there was a possibility that this site was in their foraging pathway during the trapping session.

The connection between the activity of rats and ground materials can be species-specific and can differ depending on the palms' age (Puan *et al.*, 2011c). As indicated in this study, the captured rats' population size has a higher density in the matured planted palm tree area of Pukin plantation, UM plantation and Tumbuh Hangat plantation. The clustering analysis also supports the rat distribution according to the palm planted age where the *R. tiomanicus*, *R. rattus diardii* and *R. exulans* concentrated on the higher and older palms from the five species identified. The older palm areas also have a higher diversity of rats relative to the young palm area. Radio-tagged movements of *R. tiomanicus* in the oil palms in Malaysia have shown a preference for the use of ground vegetation areas, and the frond heaps were another vital habitat structure for rats, especially as shelters (Buckle *et al.*, 1997). It was suggested that frond heaps could benefit rats by improving foraging or hiding spots from predators (Lambert *et al.*, 2008). Denser and grassy crop environment was preferred by *R. argentiventer* for foraging, nesting, and protection (Brown and Singleton, 2001). This species was positively associated with high ground vegetation cover of small shrubs in the plantation with young palm trees.

More female individuals were captured than male rats (Table 2). Female rats were easily caught as they foraged their young individually around their nest, while the male rats have a more extended home range but are limited to their territory (Priyambodo, 2003; Takahashi and Miczek, 2013). Although female rats' foraging ranges are different throughout their life expectancy, their nutritional requirement is high, especially during gestation and lactation stages (Sadleir *et al.*, 1973; Tristiani *et al.*, 2003). The home range of rats is dispersed individually between males and females during the pre-mating season. However, their home range completely overlapped before the breeding season, where the males are clustered around the females (Tristiani *et al.*, 2000). In theory, males should be easily captured in the field due to their larger home range than females. However, females were captured more frequently in this study due to the female-biased ratio. Like most mammals, rodents produce roughly equal males and females (Labov *et al.*, 1986; Rosenfeld *et al.*, 2003; Rugh, 1968), but litters with significant sex imbalances can emerge spontaneously. However, due to various external factors, including stress, competition and a shortage of food in nature, the resulting litters are biased toward females (Pratt and Lisk, 1989; Wolff, 2007).

The absence of sexual dimorphism was also

obviously recognised. In this study, the sizes of male *Rattus* showed no differences compared to the females. Several studies on the morphometric variation of *R. rattus* using conventional morphometrics confirmed the absence of sexual dimorphism and age variation (Faleh *et al.*, 2012; Ventura and Fuster, 2000). However, *Aethomys ineptus* *Calomys musculus*, *Akodon azarae* and *Oxymycterus rufus* population showed a strong sexual dimorphism between males and females (Abdel-Rahman *et al.*, 2009; Martínez *et al.*, 2014). Based on the PCA plots, the PC1 scores were positive, indicating that the first PC score increases with all the external variables. This implies that the high PC1 score is associated with larger measurements of the variables.

The trapped rats' distribution in the oil palm plantation showed a significant difference between the rainy and dry seasons. The strong influence of precipitation on the rat's abundance in the regression analysis could be due to food availability changes with the rainfall pattern (Taylor and Green, 1976). In some studies, high annual rainfall triggered the outbreak of *R. villosissimus* in central Australia because this species is not physiologically well adapted to dry conditions (Greenville *et al.*, 2013). There was an important link between high rainfall (>600 mm) and rodent abundance, especially in tropical and arid zones with annual rainfall patterns to determine the species' abundance (Madsen and Shine, 1999; Singleton, 2008). Seasonal samplings can be continued in the future to confirm the association between precipitation and the distribution and abundance of rats in the study areas.

CONCLUSION

In conclusion, molecular identification using COI gene marker was proven to be helpful to verify the correct ambiguity of the morphological identification of rat pests in OPP. Molecular tools can provide consistent data and reduce the variations for species identification. *R. tiomanicus*, *R. rattus diardii* and *R. argentiventer* are the three dominant pest species observed infesting the oil palm plantation with a significant population coverage per hectare. More data that include biotic and abiotic factors such as intraspecific and interspecific interaction, nutrient flows, or weather should be considered to estimate this pest's population dynamics in the oil palm plantation.

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