

ABUNDANCE AND DIVERSITY OF SOIL MITES BENEATH RABBIT CARCASSES ACROSS VARIOUS HABITATS IN PENINSULAR MALAYSIA

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ABSTRACT

Studies related to understanding the abundance and diversity of mites from beneath carrion soil is still limited, especially in Malaysia. This study aims to explore mite diversity and abundance in soil beneath rabbit carcasses throughout decomposition stages across various locations. In this study, 12 rabbit carcasses were placed at four different habitats in Malaysia namely forest reserve, highland, rural area, and oil palm plantation. The soil beneath rabbit carcasses were collected at the interval of 3-days (duration of observation was 34-days) and examined for the presence of Acari. The mites were identified to the family level morphologically and their relative abundance (RA), and diversity (richness, Simpson's Diversity Index, Shannon-Wiener's Index and Evenness) were calculated. Generally, mites were found to be highly abundant during the advanced decay stage of decomposition in all the four locations. The highest relative abundance of mites observed at advanced decay stage were macrochelids in forest (31.85%), histiostomatids in highland (37.06%), acarids in rural area (35.26%) and oil palm plantation (50.77%). Mite diversity varied across habitats and decomposition stages. During the early stages, the oil palm plantation showed the highest diversity, whereas the forest exhibited greater family richness. In the later stages of decomposition, rural and highland habitats demonstrated higher diversity and evenness, indicating habitat-related differences in mite community structure during carrion decomposition. In summary, the mites retrieved from the soil beneath the decomposing rabbit carcasses present significant potential as indicators for

detecting temporary disturbances in various habitats, based on their dynamic patterns of abundance and diversity.

Keywords: Soil mites; forensic acarology; Macrochelidae; Histiostomatidae; Acaridae; ecology

ABSTRAK

Kajian berkaitan pemahaman kelimpahan dan kepelbagaian hama daripada tanah di bawah bangkai masih terhad, khususnya di Malaysia. Kajian ini bertujuan untuk meneroka kepelbagaian dan kelimpahan hama dalam tanah di bawah bangkai arnab sepanjang peringkat pereputan di pelbagai lokasi. Dalam kajian ini, sebanyak 12 bangkai arnab ditempatkan di empat habitat berbeza di Malaysia, iaitu hutan simpan, tanah tinggi, kawasan luar bandar dan ladang kelapa sawit. Tanah di bawah bangkai arnab dikutip pada selang masa setiap tiga hari (tempoh pemerhatian selama 34 hari) dan diperiksa untuk kehadiran Acari. Hama dikenal pasti secara morfologi sehingga ke peringkat famili dan kelimpahan relatif (RA) serta kepelbagaian (kekayaan spesies, Indeks Kepelbagaian Simpson, Indeks Shannon-Wiener dan keseragaman) dikira. Secara umum, hama didapati sangat melimpah pada peringkat pereputan lanjut di keempat-empat lokasi. Kelimpahan relatif tertinggi yang diperhatikan pada peringkat ini ialah Macrochelidae di hutan (31.85%), Histiostomatidae di tanah tinggi (37.06%), serta Acaridae di kawasan luar bandar (35.26%) dan ladang kelapa sawit (50.77%). Kepelbagaian hama berbeza mengikut habitat dan peringkat pereputan. Pada peringkat awal, ladang kelapa sawit menunjukkan kepelbagaian tertinggi, manakala hutan mempamerkan kekayaan famili yang lebih tinggi. Pada peringkat akhir pereputan, habitat luar bandar dan tanah tinggi menunjukkan kepelbagaian dan keseragaman yang lebih tinggi, sekali gus menunjukkan perbezaan struktur komuniti hama yang berkait dengan habitat semasa proses pereputan bangkai. Secara keseluruhannya, hama yang diperolehi daripada tanah di bawah bangkai arnab yang mereput menunjukkan potensi yang signifikan sebagai penunjuk untuk mengesan gangguan sementara dalam pelbagai habitat, berdasarkan corak dinamik kelimpahan dan kepelbagaian mereka.

Kata kunci: Hama tanah; akarologi forensik; Macrochelidae; Histiostomatidae; Acaridae; ekologi.

INTRODUCTION

The decomposition of carcasses leads to the emergence of temporary ‘cadaver decomposition islands (CDIs)’ resulting from the variable nutrients that accumulate beneath them throughout the breakdown of organs and tissues (Carter et al. 2007; Miles et al. 2025). The process of decomposition is initiated by microbial activity, leading to the emission of carbon-based compounds such as putrescine, as well as sulfur-containing substances like dimethyl sulfide into the surrounding environment, which are particularly appealing to insects including flies and beetles (Debruyn et al. 2025; Kalinová et al. 2009). These insects often display a phoretic relationship with mites, and their association with decomposing remains has been shown to be advantageous in forensic investigations (Perotti & Braig 2009).

Currently, various uses of mites in facilitating forensic investigations involve determining the minimum post-mortem interval (mPMI), interpreting death circumstances, and connecting suspects to the crime scene, among other elements (Kamaruzaman et al. 2018; Prichard et al. 1986; Szelecz et al. 2018). The utilization of Acari in forensic science can be traced back to the 18th century when the forensic entomologist, Mégnin, employed the mite

species *Tyrophagus longior* (Astigmata: Acaridae) to determine the mPMI of a mummified female infant (Mégnin 1894; Perotti 2009). This evidence suggests that the abundance of Acari, specifically the mites when thoroughly evaluated for their dynamics, could function as a significant tool in case investigations.

Thus, it is important to study the abundance and diversity of mite communities across various habitats. The habitat types in the current study were chosen based on the findings of Sea and Beauregard (2018), which identified common body dumping sites used by criminals, such as forests and highlands. In Malaysia, research on carcass decomposition from different habitats, especially involving mites, remains scarce. Silahuddin et al. (2015) briefly noted the presence of mites from the orders Mesostigmata, Prostigmata, Astigmata, and Oribatida throughout the decomposition process in forests, rural areas, and highlands, suggesting their forensic potential; however, the study did not account for decomposition stages. The current study, therefore, intends to address this limitation by analyzing the abundance, diversity, and richness of some mite families throughout the different stages of decomposition, as well as across a spectrum of habitats including forest, highland, rural settings, and oil palm plantations.

MATERIALS AND METHODS

Carcass Preparation and Placement

Male white rabbits (*Oryctolagus cuniculus* Linnaeus) weighed between 1.75 and 2.0 kg were used as carcass models. Three rabbits ($n = 3$) were allocated for each location as biological replicates and observed for 34 days to complete decomposition. Three replicates per site were used to capture natural variability, reduce random error, and improve the reliability of the results while remaining logistically feasible. The rabbits were purchased from a local farm and kept in the Laboratory Animal Care Unit (LACU), Faculty of Medicine, Universiti Teknologi MARA (UiTM), Selangor, Malaysia prior to the study. Prior inspection of the rabbits was performed to ensure they were mite-free before recruited. On the day of the placement, the rabbits were euthanized with pentobarbital overdose at 150 mg/kg (200 mg/ml, 2.25 ml) via intracardiac injection. The rabbit carcasses were immediately sealed in clinical waste disposal bags and transported to the study sites within 2 to 3 hours to prevent insect colonization. On the study site, each carcass was assigned in an anti-scavenging cage measuring approximately 100 cm (W) 100 cm (L) x 50 cm (H). The use of anti-scavenger cages was necessary to prevent the carcasses from being eaten or removed; however, the cages may have altered the microclimate by modifying temperature, humidity, and airflow, and may have limited insect access, potentially affecting the decomposition rate and insect colonization patterns. The distance between each carcass was between 10 - 20 m. These study sites were chosen because they mimicked the corpse dumping sites. For each rabbit carcass, the stages of decomposition as defined by Payne (1965) and DeBruyn et al. (2025) were recorded according to days.

Study Sites

The four habitats selected for this research were forest, highland, rural area, and oil palm plantation. The first study site was in Bukit Lagong Forest Reserve, Selangor state, Malaysia (3.25°N 101.62°E, 368 m above sea level (a.s.l)). The area was covered with tiny plants and tall trees such as *Shorea platyclados*, *S. ovata*, and *Palaquium burckii*. The rabbit carcasses were placed on small quadrats directly on the topsoil to ease the process of soil collection.

The second study location was within the compounds of the Malaysian Agricultural Research and Development Institute (MARDI) in Cameron Highlands, Pahang, Malaysia (4.47°N 101.38°E, 1450 m a.s.l.). Two of the carcasses were surrounded by strawberry guava

(*Psidium cattleianum*) and morning glory (*Ipomoea purpurea*) while the last replicate was located near a banana tree (*Musa acuminata*) and an uncultivated rice field (*Oryza sativa*).

The third study spot was in a rural area of Negeri Sembilan state, Malaysia (2.73°N, 102.59°E, 38 m a.s.l.). The estimated size of the rural area was 1,465,400 m² with 534 village residents. The study was conducted among the scattered houses where the distance of the rabbit carcasses was between 10 to 20 m from each other. Some of the trees surrounding the carcasses include mango trees (*Mangifera indica*), pineapple trees (*Ananas comosus*), pudding plants (*Codiaeum variegatum*), banana trees (*Banana caturra*), shrubs of pandan (*Pandanus amaryllifolius*), lime (*Citrofortunella microcarpa*), and coconuts (*Cocos nucifera*) trees. The fourth study site was an oil palm plantation, located about 20 km from the previous rural area. It has an area of 21,513,900 m². All three cages were surrounded by palm trees (*Elaeis guineensis*).

Soil Sampling

The soil samples (100 g per sample) were randomly collected from beneath the rabbit carcasses, with ten subsamples taken from each carcass and combined. Soil samples were collected from the top 3 cm to target the layer closest to the decomposing carcass, where surface-associated mites are most likely to occur, and to standardize sampling across sites at the interval of 3 days for a total of 34 days of decomposition at each location. The initial soil samples were collected approximately 1 hour after the carcasses were placed. The time taken to complete the study was eight months as work at each site was conducted sequentially. The sampling schedule was kept within the interval at each habitat and not daily to minimize the disruption to the population of mites in the soil beneath the carcasses. The soil samples were placed separately in labelled zip-lock bags according to the designated carcasses. Direct sampling of mites from the carcasses was not performed as the objective of this was to focus on the mites thriving in the soil beneath the rabbit carcasses.

Mite Extraction

The soil samples were placed on modified Berlese-Tullgren funnels for two consecutive days (i.e., 48 hours) after soil collection in the field to extract the soil-dwelling mites (Southwood 1978). Then, the extracted mites were transferred into vials containing 70% ethanol for long-term preservation before the mounting process.

Morphological Identification of Mite

The preserved mites were placed in lactophenol for the clearing process (Nadchatram & Dohany 1974). Larger and heavily sclerotized mite specimens were punctured using a fine needle to allow entrance of lactophenol. After 4 to 7 days, the cleared specimens were mounted on glass slides in Hoyer's medium and covered with thin round coverslips. The slides were subsequently placed in an oven (Memmert, Germany) at 40°C for a week for the dehydration process. The specimens were then identified using taxonomic keys up to the family level (Krantz & Walter 2009).

Climatological Data

The temperature (°C) and the amount of precipitation (mm) during the study period were documented daily using a temperature data logger (Elitech, China) and a rain gauge. The fieldwork was conducted at different time points due to man-power limitations. However, there was no overt seasonal variation in Malaysia at different times; hence there were no noticeable changes in temperature other than due to land elevation.

Data Analysis

The identified mites were then counted for their abundance, richness, and diversity indices (i.e., Simpson's Diversity Index (1-D), Simpson's Dominance Index, Shannon-Wiener Diversity Index (H') and evenness (E), according to family and during carcass' stages of decomposition, with means and standard deviations calculated from three replicates in each ecoregion. Two-way ANOVA and Tukey's test were employed to compare between the locations. The definitions and formulas for relative abundance and diversity indices were summarized in Table 1, following Heo et al. (2015). The relative abundance data (%) was arcsine square root transformed, and two-way Analysis of Variance (ANOVA) and Tukey's test were performed to compare between the mite families found in the four locations. The relationship between mite abundance and environmental variables (temperature and rainfall) at different decomposition stages was assessed using simple linear regression analysis. The strength of the association was expressed using the coefficient of determination (r^2), and statistical significance was determined at $P < 0.05$. The statistical analyses and graphs were generated by Graphpad Prism 8 (GraphPad Software Inc., USA) and Microsoft Excel (Microsoft, USA).

Table 1. The definitions and formulas for relative abundance (RA) and diversity indices

Formula	Definition
Relative abundance $RA (\%) = \frac{TF}{\sum TG}$	Relative abundance is obtained by dividing the number of family from one group by the sum number of species from all groups. RA (%) is relative abundance, TF is the total number of one family/group and TG is the total number from all groups/families.
Family Richness (S)	The number of different families found in a particular ecoregion.
Simpson's Diversity Index (D) $1 - D = \sum_{i=1}^s P_i^2$	The probability that two randomly selected individuals in the community belong to the same family. D is Simpson's Index; S is family richness and P_i is the proportions of the families.
Simpson's Dominance Index $D = \sum_{i=1}^s P_i^2$	
Shannon-Wiener's Index (H) $H = - \sum_{i=0}^n P_i (\ln P_i)$	A measure of order (or disorder) observed within a particular ecoregion and is characterized by the number of individuals observed for each species in each ecoregion. H is Shannon-Wiener's Index, n is the number of collected individuals and P_i is the proportions of the families.
Evenness (E) $E = \frac{H}{\ln S}$	Evenness (E) is the similarity in the abundance of different families. E is Evenness, H is Shannon-Wiener's Index and $\ln S$ is natural log of species richness

RESULTS

Carcass Decomposition and Relative Abundance

Five stages of decomposition (fresh, bloated, active decay, advanced decay, and skeletonized) were observed on rabbit carcasses assigned to each habitat (Figure 1). A total of 1,709 individual mites were collected across all decomposition stages and study locations, with average individual mites of 156 ± 55.1 from the forest, 154 ± 24.6 from the highland, 50 ± 22.6 from the rural area, and 101 ± 45.9 from the oil palm plantation. During the early stages of decomposition (fresh, bloated, and active decay), oribatids had the highest relative abundance (Figure 2). However, at the later stages of decomposition (advance decay and skeletonized), their relative abundance decreased. During advanced decay stage in the forest, Macrochelidae showed the highest relative abundance (31.85%), followed by cohort Oribatida (31.08%) and Histiostomatidae (26.39 %). Meanwhile, in the highland, the most prevalent family was Histiostomatidae (37.06%), followed by oribatids (32.62%) and Acaridae (25.07%). In rural areas and oil palm plantations, acarids were most prevalent at 35.26 % and 50.77%, respectively. During the skeletonized stage, mites of the family Histiostomatidae (43.54%) and Acaridae (39.23%) continued to be the highest in abundance in the highland and rural areas, respectively. Meanwhile, oribatids dominated the forest (44.08%) and oil palm plantation (47.78%).



Ecoregions studied	Forest reserve	Highland	Oil palm plantation	Rural
Fresh stage	 Day 1 - 2	 Day 1 - 3	 Day 1 - 2	 Day 1 - 2
Bloated stage	 Day 2 - 3	 Day 4 - 5	 Day 2 - 3	 Day 2 - 3
Active Decay	 Day 4 - 6	 Day 6 - 10	 Day 4 - 9	 Day 4 - 6
Advance Decay	 Day 7 - 27	 Day 10 - 30	 Day 10 - 17	 Day 7 - 12
Skeletonized	 Day 28 - 40	 Day 31 - 40	 Day 18 - 40	 Day 13 - 40

Figure 1. The decay activities of rabbit carcasses during stages of decomposition, duration in days, and ecoregions in Peninsular Malaysia over the study period

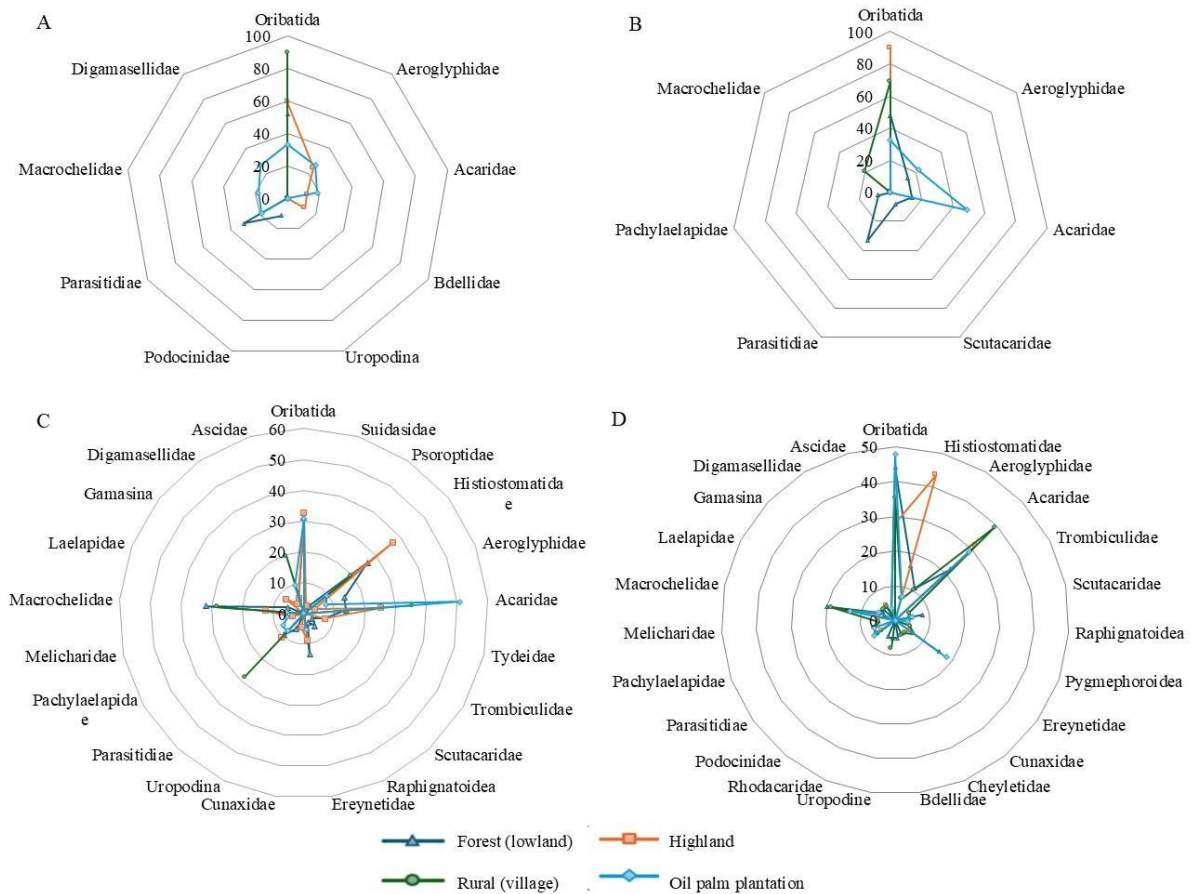


Figure 2. Relative abundance (%) of mites in four ecoregions of Malaysia at different stages of decomposition: A. Fresh and bloat stage; B. Active Decay; C. Advance Decay; D. Skeletonized

Figure 3 to 6 illustrated the temporal pattern of mite abundance across the different stages of decomposition at different habitats. In the forest reserve (Figure 3), only a few mite families and cohorts, including Oribatida, Uropodina, and mesostigmatids such as Parasitidae and Podocinidae, were observed during the early stages of decomposition. During the advanced decay stage, Histiostomatidae and Macrochelidae were highly abundant, although their numbers declined slightly during the skeletonization stage. In the highland site (Figure 4), a small number of mites from the cohorts and suborder Oribatida, Astigmata (Aeroglyphidae, Acaridae), Prostigmata (Bdellidae), Mesostigmata (Macrochelidae), and Uropodina were observed during the fresh, bloated, and active decay stages. In contrast, during the advanced decay stage, mite families such as Histiostomatidae, Acaridae, Parasitidae, and Macrochelidae became more abundant and remained present until the skeletonization stage. As shown in Figure 5, only a small number of mites were observed from the fresh to the advanced decay stages, including members of the cohorts and suborders Oribatida, Astigmata (Histiostomatidae and Acaridae), and Mesostigmata (Ascidae, Macrochelidae, and Parasitidae). During the skeletonized stage, the abundance of mites increased markedly, particularly for the families Acaridae and Macrochelidae. In Figure 6 (oil palm plantation), a small number of mites were observed during the fresh, bloated, and advanced decay stages, including families such as Aeroglyphidae, Acaridae, Cunaxidae, Melicharidae, and Macrochelidae. During the advanced decay stage, Acaridae increased in abundance and remained consistent until the skeletonized stage. The photographs of the most abundant mites during advanced decay stage are reported

in Figure 7. Statistical analysis showed several significant differences ($P < 0.05$) between the families of mites in in all four habitats at different decomposition stages (Table 2 & Table 3)

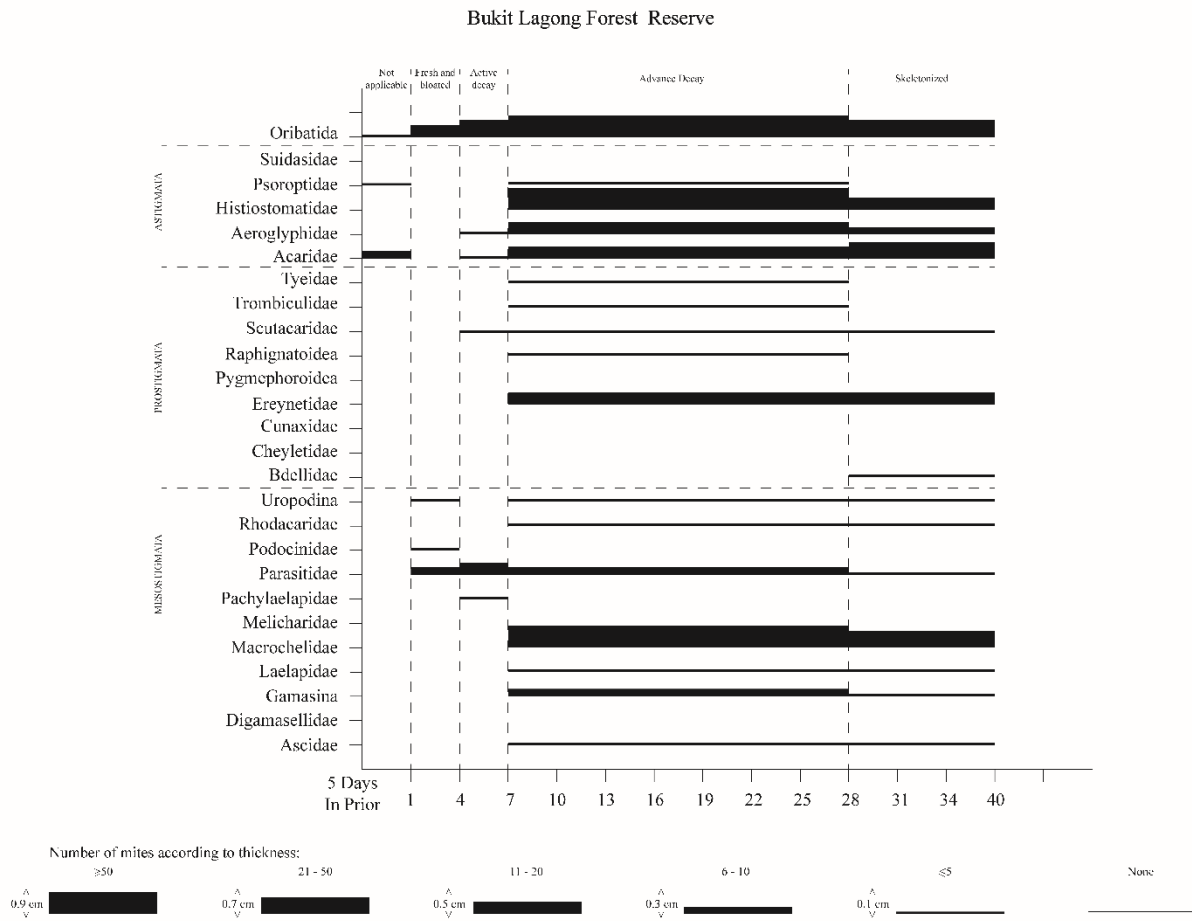


Figure 3. The abundance of mites according to families and cohort at different stages of decomposition in forest reserve

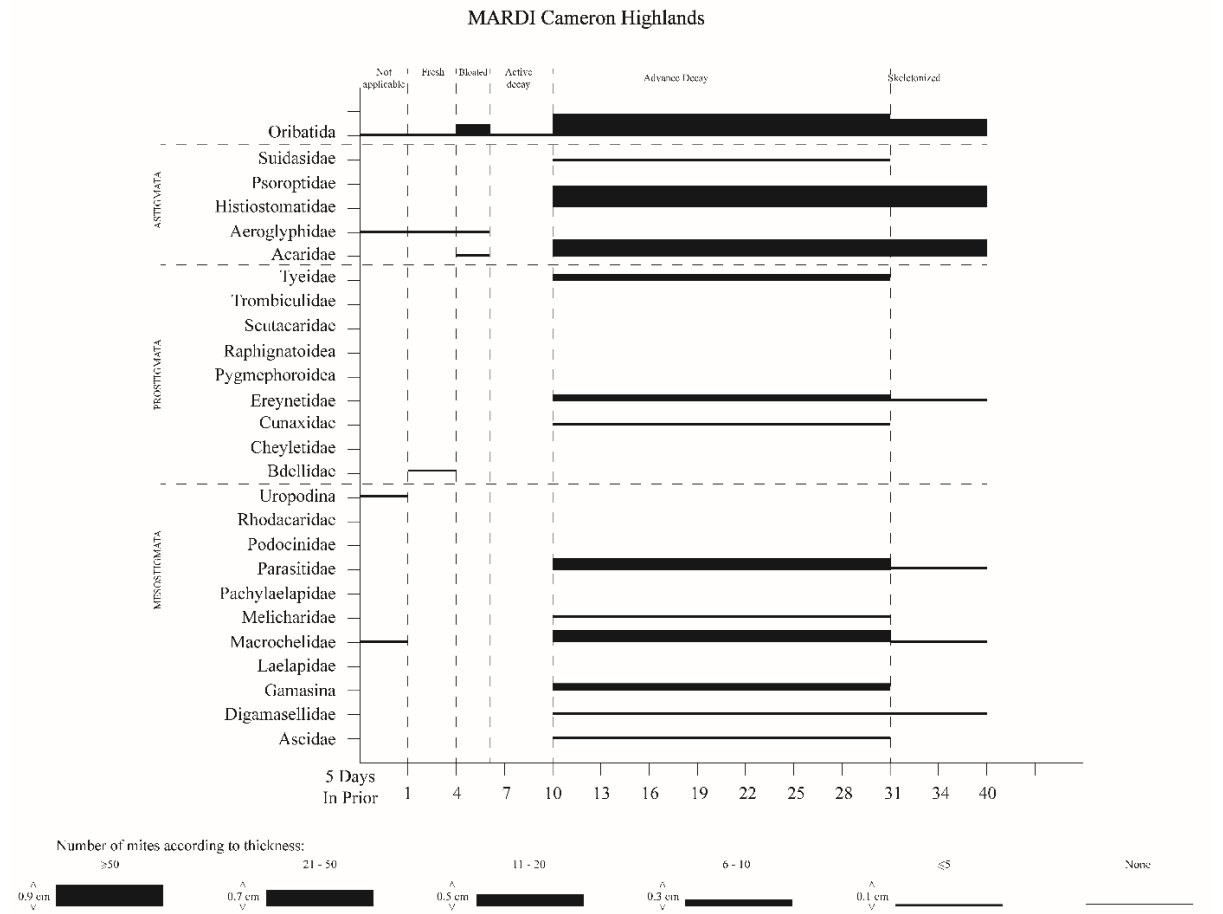


Figure 4. Abundance of mites according to families and cohort at different stages of decomposition in highland

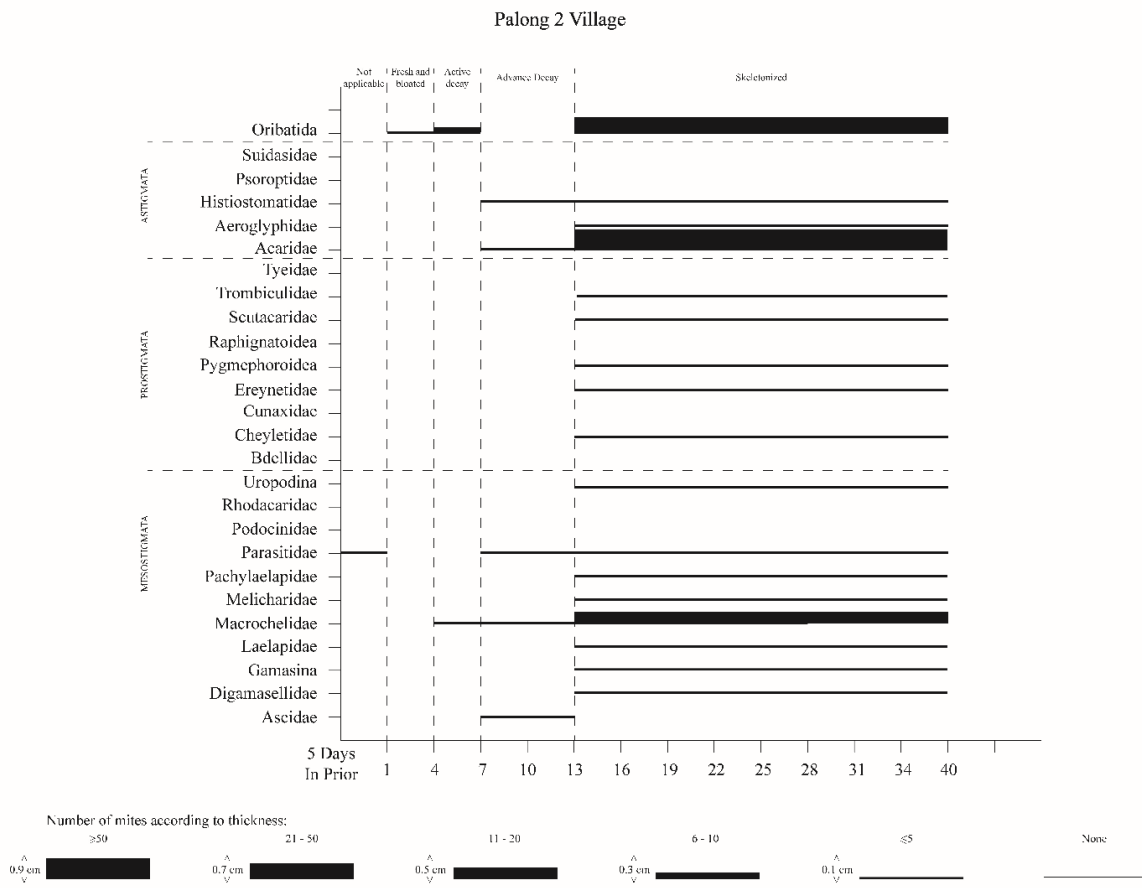


Figure 5. Abundance of mites according to families and cohort at different stages of decomposition in village

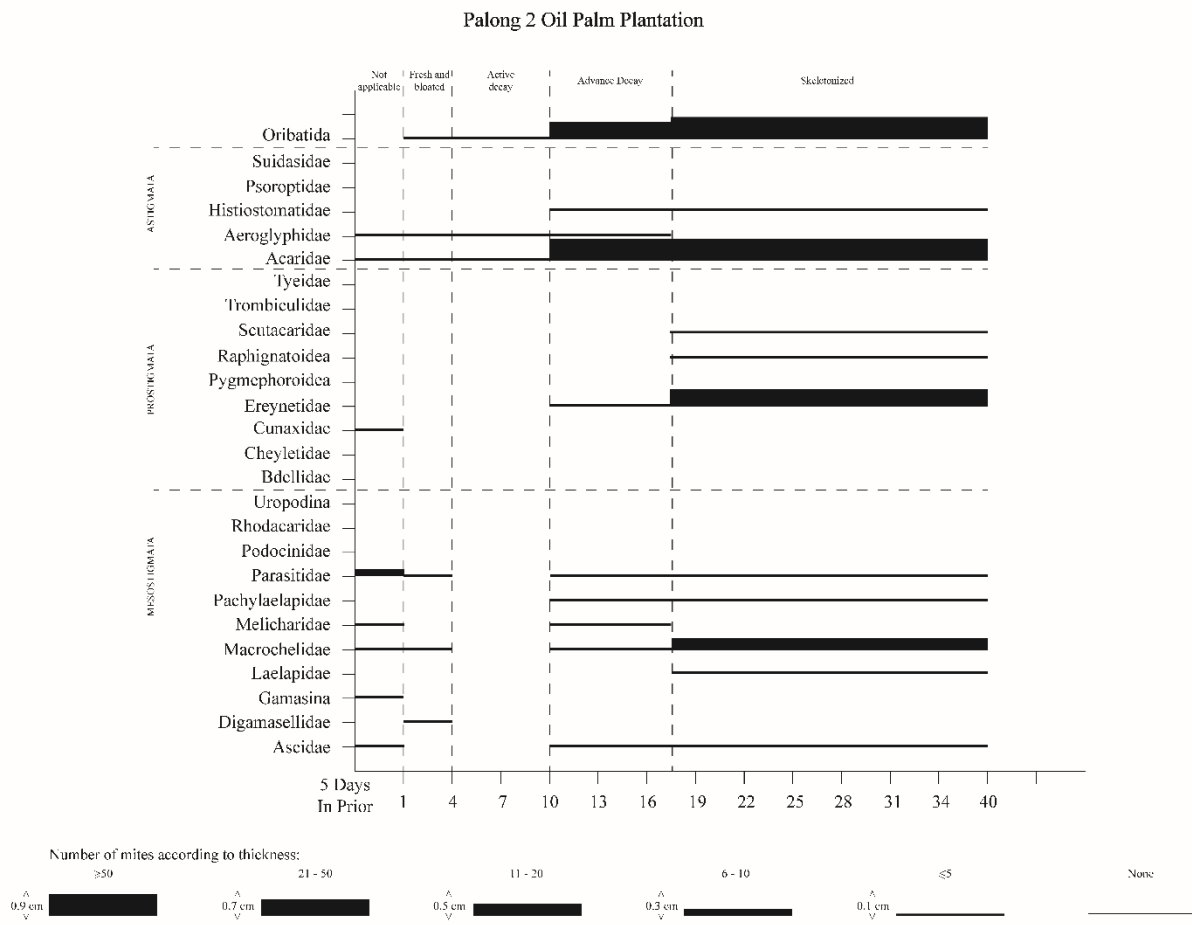


Figure 6. Abundance of mites according to families and cohort at different stages of decomposition in oil palm plantation



Figure 7. Images of the most abundant mite family collected at each study site in Peninsular Malaysia: A. Macrochelidae (Acari: Mesostigmata) from forest reserve. B. Histiostomatidae (Acari: Astigmata) from highland. C. Acaridae (Acari: Astigmata) from rural. D. Acaridae (Acari: Astigmata) from the oil palm plantation

Table 2. Two-Way ANOVA data that highlights the comparison between the relative abundance of mites at four different ecoregions in Malaysia and at different stages of decomposition

Stages of Decomposition	Source of Variance	Sum of Squares (SS)	Degree of Freedom (DF)	MS	F Value (DFn, DFd)	P Value
Fresh and bloat stage	Row Factor	96.67	8	12.08	F (8, 16) = 3.591	P=0.0141
	Column Factor	14.44	3	4.815	F (3, 6) = 1.042	P=0.4393
	Interaction: Row Factor x Column Factor	72.22	24	3.009	F (24, 48) = 1.239	P=0.2588
Active Decay	Row Factor	105.3	6	17.55	F (6, 12) = 5.074	P=0.0082
	Column Factor	70.29	3	23.43	F (3, 6) = 2.151	P=0.1951
	Interaction: Row Factor x Column Factor	164	18	9.114	F (18, 36) = 2.274	P=0.0177
Advance Decay	Row Factor	7961	20	398	F (20, 40) = 18.34	<i>P<0.0001</i>
	Column Factor	1361	3	453.6	F (3, 6) = 10.10	P=0.0092
	Interaction: Row Factor x Column Factor	7291	60	121.5	F (60, 120) = 3.601	<i>P<0.0001</i>
Skeletonized	Row Factor	11145	22	506.6	F (22, 44) = 13.05	<i>P<0.0001</i>
	Column Factor	149.6	3	49.87	F (3, 6) = 0.9508	P=0.4736
	Interaction: Row Factor x Column Factor	3667	66	55.56	F (66, 132) = 1.663	P=0.0069

Table 3. The multiple comparison analysis (Tukey’s Post Hoc Test) of different mite families according to stages of decompositions and ecoregions

Stages of Decomposition	Comparison	Mean Difference	Standard Error	95%CI of Diff.	P Value	
Fresh and bloat stage	Oribatida					
	Forest (lowland) vs. Rural (village)	4.667	1.273	1.280 to 8.054	0.0033	
	Forest (lowland) vs. Oil palm plantation	4.333	1.273	0.9464 to 7.720	0.0071	
	Highland vs. Rural (village)	5	1.273	1.613 to 8.387	0.0015	
	Highland vs. Oil palm plantation	4.667	1.273	1.280 to 8.054	0.0033	
Active Decay	Oribatida					
	Forest (lowland) vs. Highland	9	1.635	4.597 to 13.40	<0.0001	
	Forest (lowland) vs. Rural (village)	7.333	1.635	2.931 to 11.74	0.0004	
	Forest (lowland) vs. Oil palm plantation	9	1.635	4.597 to 13.40	<0.0001	
	Parasitidae					
	Forest (lowland) vs. Highland	5.333	1.635	0.9306 to 9.736	0.0124	
	Forest (lowland) vs. Rural (village)	5.333	1.635	0.9306 to 9.736	0.0124	
	Forest (lowland) vs. Oil palm plantation	5	1.635	0.5973 to 9.403	0.0208	
	Advance Decay	Oribatida				
		Forest (lowland) vs. Rural (village)	31.67	4.743	19.31 to 44.02	<0.0001
Forest (lowland) vs. Oil palm plantation		22.33	4.743	9.975 to 34.69	<0.0001	
Highland vs. Rural (village)		43.67	4.743	31.31 to 56.02	<0.0001	
Highland vs. Oil palm plantation		34.33	4.743	21.98 to 46.69	<0.0001	
Histiostomatidae						
Forest (lowland) vs. Highland		-16.33	4.743	-28.69 to -3.975	0.0043	
Forest (lowland) vs. Rural (village)		20.33	4.743	7.975 to 32.69	0.0002	
Forest (lowland) vs. Oil palm plantation		19.67	4.743	7.308 to 32.02	0.0004	
Highland vs. Rural (village)		36.67	4.743	24.31 to 49.02	<0.0001	
Highland vs. Oil palm plantation		36	4.743	23.64 to 48.36	<0.0001	
Acaridae						
Forest (lowland) vs. Oil palm plantation		-15.33	4.743	-27.69 to -2.975	0.0085	

Stages of Decomposition	Comparison	Mean Difference	Standard Error	95%CI of Diff.	P Value
	Highland vs. Rural (village)	14	4.743	1.642 to 26.36	0.0196
	Rural (village) vs. Oil palm plantation	-20.67	4.743	-33.02 to -8.308	0.0002
	Macrochelidae				
	Forest (lowland) vs. Highland	18.67	4.719	6.308 to 31.02	0.0008
	Forest (lowland) vs. Rural (village)	24.67	4.719	12.31 to 37.02	<0.0001
	Forest (lowland) vs. Oil palm plantation	25.33	4.719	12.98 to 37.69	<0.0001
Skeletonized	Oribatida				
	Forest (lowland) vs. Highland	30.67	4.719	18.39 to 42.95	<0.0001
	Forest (lowland) vs. Rural (village)	23.33	4.719	11.05 to 35.61	<0.0001
	Highland vs. Oil palm plantation	-32.33	4.719	-44.61 to -20.05	<0.0001
	Rural (village) vs. Oil palm plantation	-25	4.719	-37.28 to -12.72	<0.0001
	Histiostomatidae				
	Forest (lowland) vs. Highland	-15	4.719	-27.28 to -2.721	0.0099
	Highland vs. Rural (village)	21	4.719	8.721 to 33.28	0.0001
	Highland vs. Oil palm plantation	20.33	4.719	8.054 to 32.61	0.0002

Diversity Indices

The violin plot (Figure 8) presents the distribution of Simpson's Diversity Index, Shannon-Wiener Index, Evenness, and Richness across different locations at each stage of decomposition. Overall, considerable variation was observed among replicates within all habitats, especially in the early stages (fresh, bloat, and active decay stages) where statistical analysis showed no significant difference between the habitats except for highland and oil palm plantation ($P < 0.02$) for Simpson's Diversity Index. During the fresh and bloat stages, the oil palm plantation exhibited the highest diversity, with Simpson's Diversity Index of 0.93 ± 0.38 , corresponding to the highest Shannon's Diversity Index (1.38 ± 0.26) and relatively high evenness (0.73 ± 0.33). However, the highest family richness during these stages was recorded in the forest habitat (2.5 ± 2.12). At the active decay stage, the rural area showed the highest diversity, with Simpson's Diversity Index of 0.89 ± 0.19 and Shannon's Diversity Index of 0.82 ± 0.26 , while the forest displayed the highest evenness (0.76 ± 0.03) and family richness (2.67 ± 1.53). Diversity patterns became more consistent during the later stages of decomposition (advanced decay and skeletonized stages).

During the advanced decay stage, the rural area recorded the highest Simpson's Diversity Index (0.82 ± 0.14), followed by the highland (0.76 ± 0.06). In contrast, the highest Shannon's Diversity Index was observed in the highland (1.67 ± 0.25), followed by the rural area (1.55 ± 0.05). Both habitats differed significantly from the forest (highland: $P < 0.02$; rural: $P < 0.04$). The highest evenness was observed in the rural area (0.95 ± 0.03), and comparison analysis (Figure 8) indicated significantly higher evenness among habitats ($P < 0.001$). However, the forest recorded the greatest family richness at this stage (13.0 ± 1.73). At the skeletonized stage, the rural area again showed the highest diversity, with Simpson's Diversity Index of 0.76 ± 0.06 , Shannon's Diversity Index of 1.64 ± 0.20 , evenness of 0.75 ± 0.08 , and family richness of 9.0 ± 1.0 . Family richness in the highland differed significantly from both the rural area and forest ($P < 0.01$).

Furthermore, Simpson's Dominance Index values (Table 4) indicated low to moderate dominance across all habitats, including forest, highland, oil palm plantation, and rural areas. The relatively low dominance values suggest that mite communities were not strongly dominated by a single family, indicating a more balanced distribution of taxa within the assemblages.

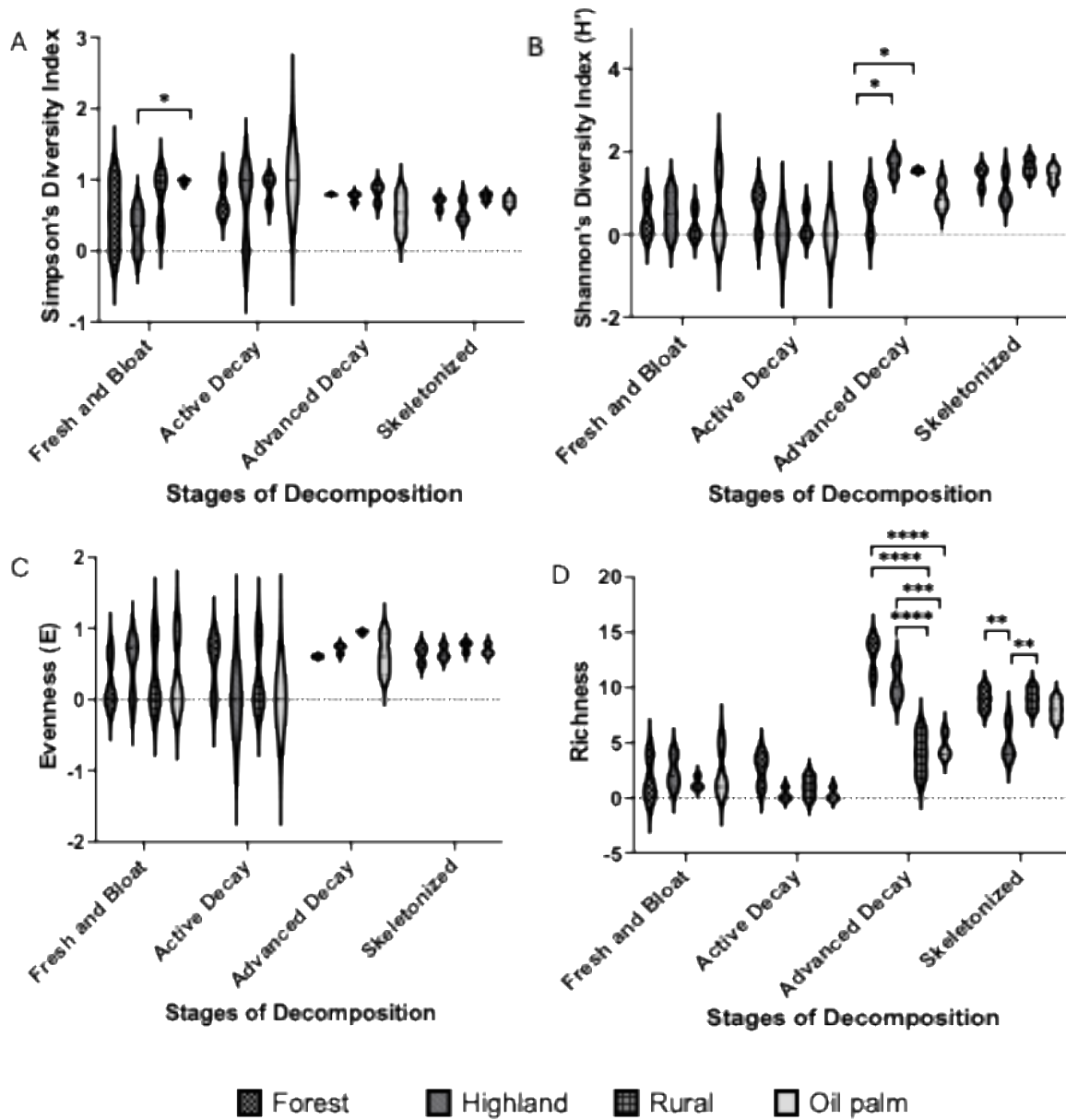


Figure 8. The diversity indices of soil mites beneath rabbit carcasses at different stages of decomposition in Peninsular Malaysia. A: Simpson's Diversity Index; B: Shannon's Diversity Index; C: Evenness; and D: Richness

Table 4. Simpson's Dominance Index (D) of mite families across different habitats and decomposition stages

Stages of Decomposition	Habitats							
	Forest	Interpretation	Highland	Interpretation	Rural	Interpretation	Oil palm plantation	
Fresh and Bloat	0.26	Low	0.52	Moderate	0.22	Low	0.07	Low
Active Decay	0.29	Low	0	Low	0.11	Low	0	Low
Advanced Decay	0.21	Low	0.24	Low	0.18	Low	0.46	Low
Skeletonized	0.32	Low	0.46	Low	0.24	Low	0.3	Low

Climatological Data

The mean temperature and precipitation for the forest were $24.4 \pm 1.43^\circ\text{C}$, and $2.72 \pm 8.19\text{mm}$, while in the highland, they were $21.2 \pm 2.13^\circ\text{C}$, and $0.77 \pm 1.08\text{mm}$, respectively. The average temperature and precipitation in rural areas were $28.2 \pm 1.20^\circ\text{C}$ and $3.86 \pm 4.26\text{mm}$, while in the oil palm plantation, they were $31.4 \pm 1.20^\circ\text{C}$ and $0.28 \pm 0.45\text{mm}$, respectively (Figure 9 and Figure 10). Simple linear regression analysis was conducted to examine the relationship between mite abundance and environmental variables (temperature and rainfall) across the different habitats (Figure 11). For temperature, moderate relationships were observed in the forest ($r^2 = 0.53$) and highland ($r^2 = 0.50$), while weaker relationships were detected in the oil palm plantation ($r^2 = 0.39$) and rural area ($r^2 = 0.01$). However, these relationships were not statistically significant, with P-values of 0.2754 for both forest and highland, 0.3745 for oil palm plantation, and 0.8992 for the rural area. Similarly, regression analysis for rainfall showed relatively strong relationships in the forest ($r^2 = 0.74$) and oil palm plantation ($r^2 = 0.62$), but weaker relationships in the highland ($r^2 = 0.29$) and rural area ($r^2 \approx 0.00$). Nevertheless, none of the correlations were statistically significant, as indicated by P-values of 0.1410 (forest), 0.2121 (oil palm plantation), 0.4587 (highland), and 0.9965 (rural area).

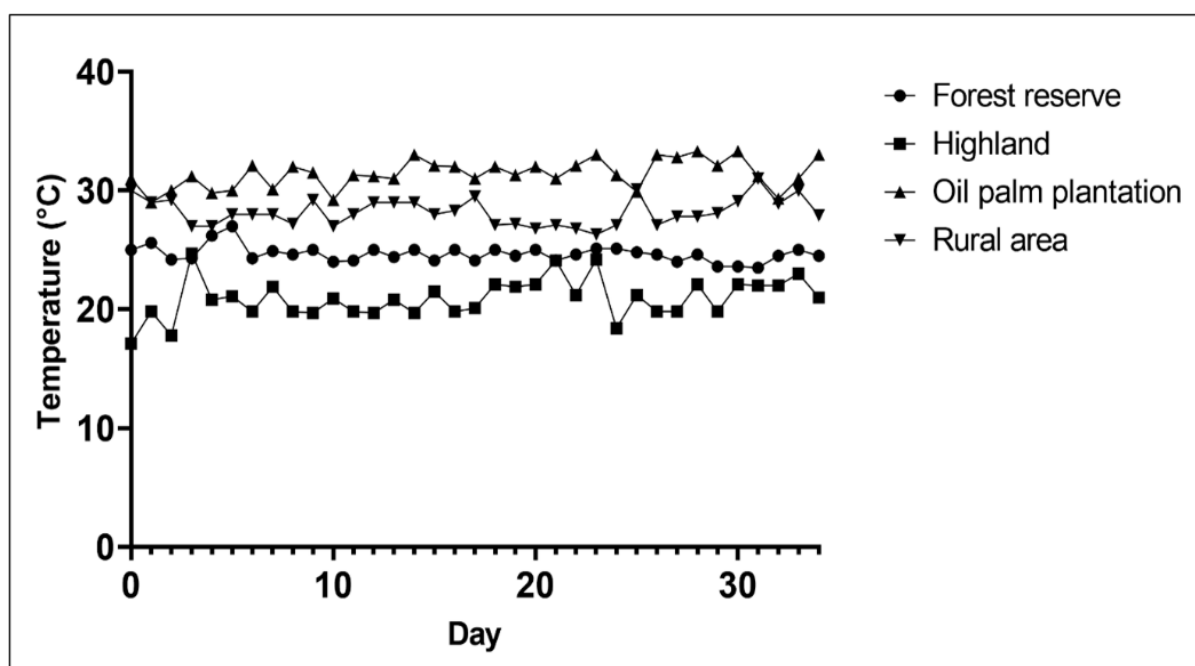


Figure 9. Daily environmental temperature ($^\circ\text{C}$) of the four ecoregions: forest, highland, village (rural), and oil palm plantation in Peninsular Malaysia over the study period

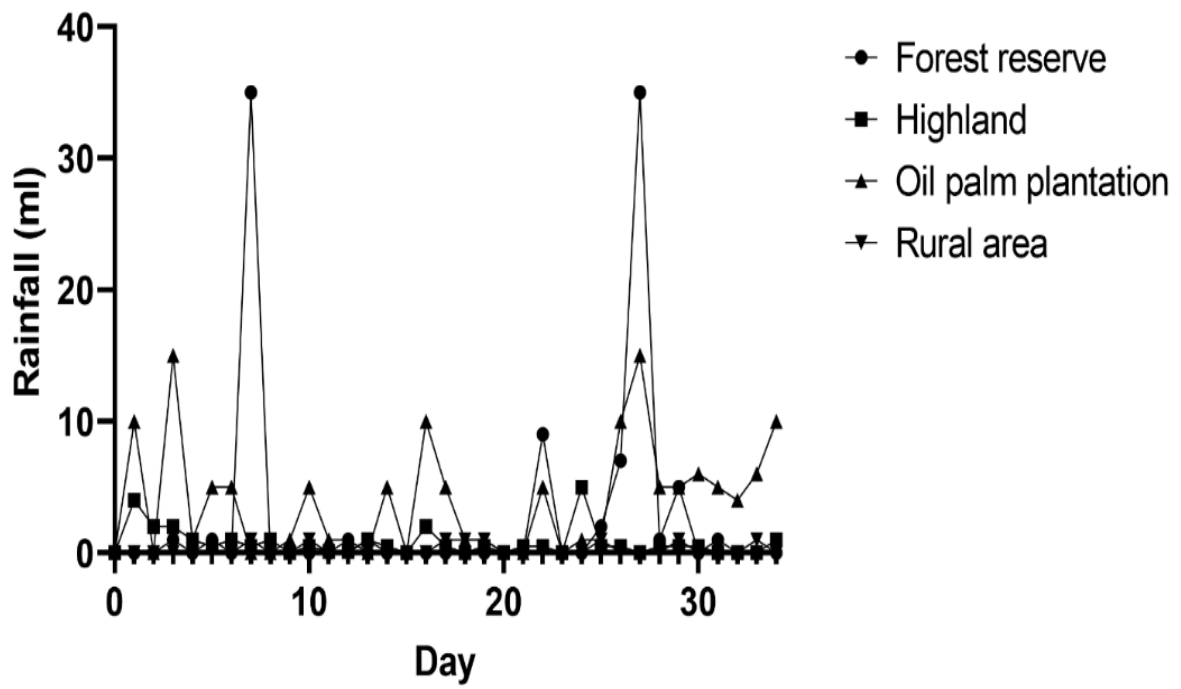


Figure 10. Daily amount of rainfall (mm) of the four ecoregions: forest, highland, village (rural), and oil palm plantation in Peninsular Malaysia over the study period

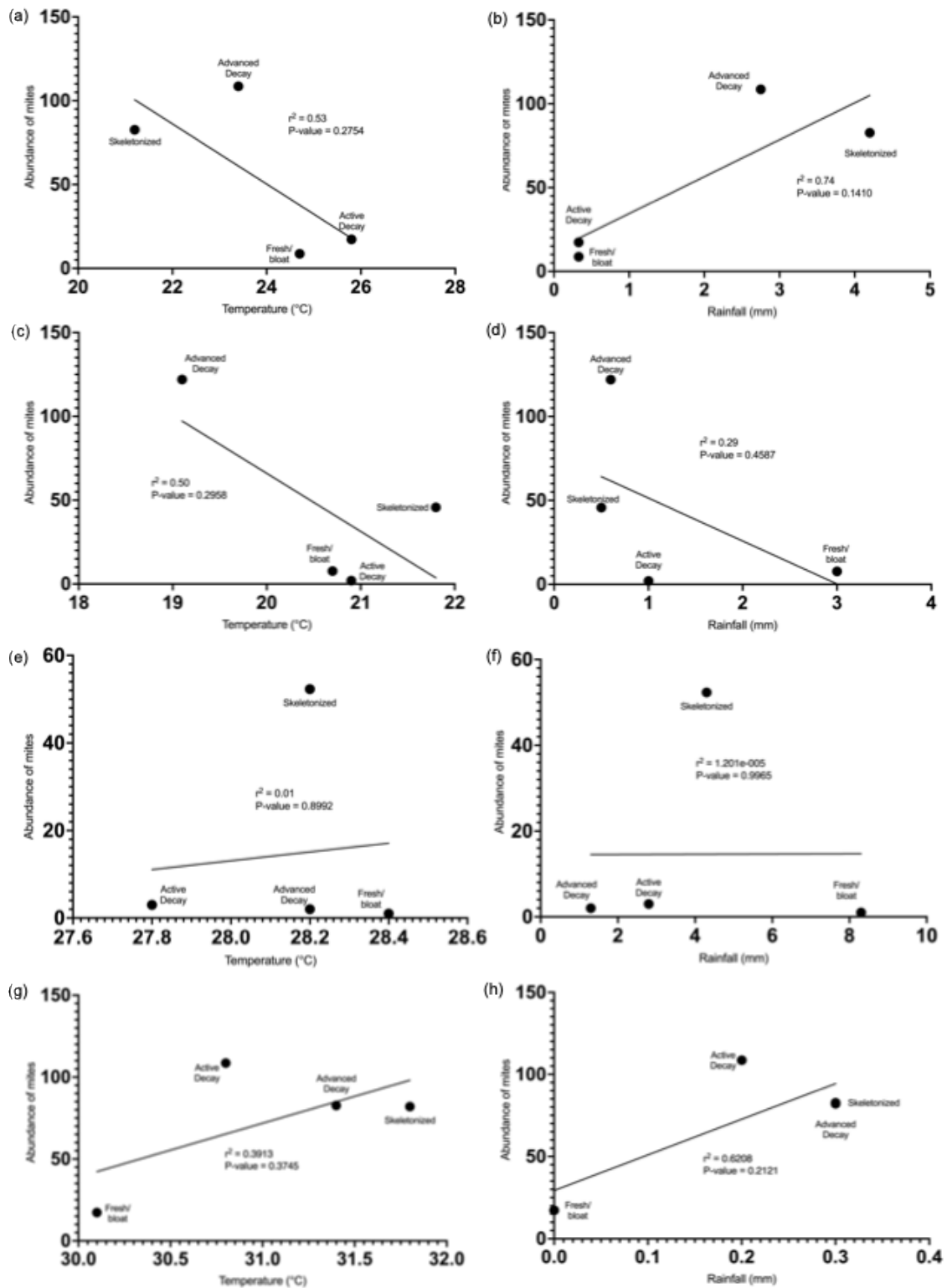


Figure 11. Simple linear regression analysis showing the relationship between mite abundance and environmental variables (temperature and rainfall): (a-b) forest; (c-d) highland; (e-f) rural area; (g-h) oil palm plantation. Regression results indicate the strength of association expressed as r^2 and corresponding P-values

DISCUSSION

Mites play a role in the decomposition of animal carcasses and human bodies throughout every stage, from the early fresh stage to the final skeletal phase of dry decomposition (Braig & Perotti 2009). We observed similar findings in our study, where the patterns of several mites, such as oribatids, histiostomatids, macrochelids, and acarids showed dynamics in abundance and diversity over the period of decomposition of rabbit carcasses. In this study, the most abundant family in the forest reserve, Macrochelidae, likely outcompeted oribatids for resources, as this family has been reported to be a predatory Mesostigmata (Krantz 1998). Another study on pig decomposition study also reported the order Mesostigmata to be dominant compared to the Oribatida (Heo et al. 2021). Over time, when the resources were nearly depleted, the macrochelids and acarids decreased in number, possibly indicating that some of them were phoretic on newly emerged flies or beetles in search of other potential resources (Seeman & Walter 2023).

However, this pattern of mite abundance does not apply to highland and rural areas (village and oil palm plantation). This was likely due to the environments in these locations being conducive for the mites to thrive (providing enough food and space). Histiostomatids were reported to feed on fungal materials (Mohamed 2018). The highland study location was an agricultural land surrounded by flower and compost materials, which could explain why histiostomatids continued to thrive there. Acarids are synanthropic mites commonly recognized as storage pests and they live together with house dust mites (Chmielewski 2009; Ho & Nadchatram 1984). The abundance of Acaridae found there was likely enhanced by the presence of several human settlements, providing their access to more resources, particularly food and living space. This is because acarids require moist and very humid environments to reproduce actively (Mullen et al. 2019).

To apply the dynamics of soil mite abundance in forensic investigations, one must consider the surrounding environment of the carcasses as these mites are specific to their microhabitats. From this study, we proposed the use of oribatids to detect the early stages of carcass decomposition in soil. Previously, oribatids have been used as bioindicators for soil health as their biodiversity changes upon soil disturbance (Eeva & Penttinen 2009). As for advanced decay stage, the abundance of mites such as macrochelids (in the forest), histiostomatids (in the highland), and acarids (in rural areas and oil palm plantations) can be useful especially for cases related to dead body removal or detection of clandestine graves. Besides, they also have the potential to be used as forensic geo-indicators for different types of localities as their abundance varies according to ecoregions. However, to utilize mites for this purpose, more studies are necessary in similar ecoregions to confirm the consistency of the mite abundance patterns in the soil beneath.

The diversity trends of soil mite communities across different ecoregions and decomposition stages demonstrated spatial and temporal differences. In this study, one factor that differed was that carcasses were placed in locations with different land-use types. The observed differences in diversity indices among habitats may reflect how each environment responds to this carcass-derived disturbance. Habitats with more complex vegetation and substrate structure, such as forests, may support greater family richness due to higher microhabitat heterogeneity (Brockerhoff et al. 2017), whereas more open habitats such as oil palm plantation creates specific ecological conditions that can support high densities of certain mite species (Foster et al. 2011) and may facilitate rapid colonization and shifts in community composition. These findings highlight the influence of habitat characteristics and carcass-

associated disturbance in shaping mite assemblages during decomposition. Differences in diversity, evenness, and family richness observed across habitats may also be associated with the disturbance created by the carcass itself. This suggests that the observed changes in mite diversity may be due to the temporary disturbance caused by the carcasses themselves. Mites are known to be sensitive and to alter their community composition in response to environmental changes (Gormsen et al. 2006). The introduction of carrion into an environment represents a localized resource pulse that alters the surrounding microhabitat through the release of nutrients, decomposition fluids, and increased microbial and arthropod activity (Crippen et al. 2015). This temporary disturbance can modify soil conditions and microclimatic factors, creating favorable conditions for the colonization and proliferation of various mite taxa.

The absence of noticeable seasonal variations in the study was attributed to Malaysia's consistent climate year-round and its proximity to the equator. Despite this, the rate of rabbit decomposition in the forest was found to be quicker compared to other habitats. One potential contributing factor could be the rich insect diversity (Barton & Evans 2017), as well as the impact of heavy rainfall during the active decay and skeletonized phases. Prior studies have shown that heavy rainfall can accelerate the disappearance of elephant carcasses by washing away the remains along with the surrounding soil (Douglas-Hamilton & Burrill 1991). The analysis of environmental factors indicated that both temperature and rainfall had a limited influence on mite abundance across the studied habitats. Although the forest showed some relationship with both variables, these were not statistically significant, suggesting that environmental conditions alone do not strongly determine mite abundance. Weaker relationships were observed in the highland, oil palm plantation, and rural areas, highlighting that other factor, such as microhabitat conditions, moisture, vegetation structure (Gong et al. 2023) and the localized disturbance caused by carrion, likely play a more important role in shaping mite communities (Rai et al. 2021). These findings highlight the complex interplay of environmental and ecological factors in driving mite assemblages during decomposition. To provide a better understanding, the use of larger carcasses or extending the observation period could help capture the full temporal dynamics of mite colonization and succession during decomposition as previous studies suggested that carcass mass affected decomposition rate (Oh et al. 2024). One limitation of the current study is that the small carcass size may have shortened the observation period and restricted the detection of longer-term community changes (Komar & Beattie 1998). Another limitation of this study is that soil samples were collected only at a depth of 3 cm, which may not fully represent the vertical distribution of mite communities within the soil profile. Some mite taxa may occur at deeper soil layers, particularly during later stages of decomposition when nutrients and decomposition fluids penetrate further into the substrate (Rai et al. 2021). Future studies incorporating greater sampling depths could provide a more comprehensive understanding of the vertical distribution and diversity of mites associated with carrion decomposition. Despite these limitations, the study provides valuable insights into the patterns of mite diversity and abundance across habitats, emphasizing the importance of habitat characteristics and carcass-associated disturbance in shaping arthropod succession during decomposition.

CONCLUSION

The abundance of mites collected during carcass decomposition can be used as habitat marker. Mites of three families, namely Macrochelidae, Histiogastromatidae, and Acaridae, which were found in large numbers in this study, could serve as indicators for a forested areas, highlands, and disturbed soil ecosystems, such as human settlements (e.g., rural) or agricultural land (e.g.,

oil palm plantation), respectively. However, there was no discernible difference in the mite diversity between the four locations, likely due to the smaller size of rabbit carcasses that contributed to a faster decomposition rate. Therefore, a follow-up study is required to confirm these findings using larger carcasses, such as pigs or, if feasible, human cadavers. The application of forensic acarology in Malaysia is still in its infancy stage. Further studies on taxonomy and morphological identification of mites are needed to provide a suitable identification key for the mites of forensic importance in this region. In addition, species identification remains challenging due to taxonomic complexity and limitations in both morphological and molecular approaches, highlighting the need for integrated methods to improve accuracy (Jones et al. 2025). As we learn more about the succession of mites on carcasses, producing a checklist of forensically important mites is recommended. Tremendous amount of work is required to catalog the geographical distribution of mites in Malaysia, especially those associated with decomposing bodies. More forensic case reports, especially those with findings of mite species, should be published and recorded, including the use of mites in various investigations.

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AUTHORS DECLARATIONS

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Conflict of Interest

The authors declared no conflict of interests.

Ethical Approval

Approval from Animal Ethic Committee, the Faculty of Medicine, UiTM Selangor (Reference: 600-FF(PS.17/2/1)) was obtained prior to the commencement of this study.

Data Availability Statements

No data was used for the research described in the article

Author's Contributions

Nurul Azmiera was responsible for investigation, writing of the original draft, and reviewing and editing the manuscript. Mariana Ahamad contributed to the methodology and was involved in reviewing and editing the manuscript. Chong Chin Heo was responsible for conceptualization, supervision, and reviewing and editing the manuscript.

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