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Diversity and spatial distribution of Order Diptera from Kuala Keniam National Park, Pahang, Malaysia

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Abstract. *Amin IN, Fathiah KN, 'Aliyaa NN, Badrina MNN, Nadiah MYN, Hasnah KS, Norhafizah MZ, Khairiyah MHS. 2023. Diversity and spatial distribution of Order Diptera from Kuala Keniam National Park, Pahang, Malaysia. Biodiversitas 24: 4668-4674.* A study on the diversity and distribution of order Diptera was conducted in different Kuala Keniam National Park, Malaysia landscapes. Three malaise traps were placed in three landscapes: the forest fringe, middle forest, and inner forest. A total of 1920 Diptera were recorded from 19 families and 109 morphospecies of order Diptera. Anthomyiidae, Calliphoridae, Pipunculidae, Scenopinidae, Ulidiidae, Tephritidae, Ceratopogonidae, Stratiomyidae, Asilidae, Syrphidae, Tipulidae, Ephydridae, Cecidomyiidae, Dolichopodidae, Phoridae, Culicidae, Muscidae, Sciaridae, and Drosophilidae were the identified families. The family with the highest number of individuals collected was Phoridae, with 89 individuals (5 morphospecies) collected from the forest fringe, followed by 271 individuals (6 morphospecies) from the middle forest and 697 individuals from the inner forest (7 morphospecies). Pipunculidae had the least collected individuals, with only one in the inner forest. The forest fringe recorded the highest Shannon-Wiener Diversity Index (2.13), followed by the middle forest (1.68) and the inner forest (1.04). The forest fringe also recorded the highest Evenness Index and Margalef Richness Index with 0.44 and 3.16, respectively. The distribution of Diptera differed significantly along the environment gradients from the forest fringe to the inner forest ($p<0.05$), and the pairwise comparison showed that the difference comes from Trap 2 and Trap 3 ($p<0.001$). The forest fringe was more diversified than the middle and the inner forest, even though the number of individuals was low. Vegetation types and sunlight might be the reasons behind this discovery. The results from this study can be used as baseline data for future reference on the diversity of Diptera from the forest reserve.

Keywords: Abundance, biodiversity, environmental gradients, fly, mosquito

INTRODUCTION

Insects are the most diverse group of organisms in the 3-billion-year history of life and are the most ecologically dominant animals on land (Grimaldi and Engel 2006). These six-legged creatures live in all habitats, which is rare in aquatic environments (Campbell et al. 2014). Insects are small and mostly can fly, enabling them to exploit small and scattered resources (Price et al. 2011). These invertebrates are the center of food webs, consumed by birds, bats, small mammals, reptiles, amphibians, spiders, and other insects (Goulson 2019). Significant roles of insects can be observed in the world and humankind, from being a source of food to a source of inspiration for art, design, and literature (Price et al. 2011). Commonly grouped into 27 to 32 orders, among them are Diptera, which are the major order of insects, with about 150,000 described species and expected to comprise more than a quarter of a million species from 150 families (Cranston and Gullan 2009; Rocha-Ortega et al. 2021). Except for the open sea and glaciers, Diptera populate every habitat and continent (Courtney et al. 2017). Species in order Diptera are plenteous, and they settle in specific microhabitats or breeding sites, therefore giving a highly needed assessment for habitat quality and conservation planning (Foottit and Adler 2017). Diptera is divided into two suborders, Nematocera and Brachycera (Hall and Gerhardt 2002). They hold significant roles as pollinators, decomposers, parasitoids, and vectors for diseases (Resh and Cardé 2003; Wajnberg and Colazza 2013; Coetzee 2014; Sarwar 2020).

Kuala Keniam National Park is under the jurisdiction of the Department of Wildlife and National Parks Malaysia and is 25 km north away from the gateway of Pahang National Park, Kuala Tahan. It has a tropical climate with an annual rainfall of approximately 2660 mm and is rich in forest vegetation with trees, climbers, epiphytes, and palms (Zani and Suratman 2011). For research purposes regarding biodiversity, ecology, sustainability, and climate change, a research station has been developed by Universiti Teknologi MARA (UiTM) in Kuala Keniam (Hamid and Suratman 2010).

No study has been conducted on the distribution and diversity of Diptera at Kuala Keniam National Park. What dipteran species are present and whether their distribution differs between environmental gradients are unknown. Nowadays, human activities have threatened the diversity

of flora and fauna, including Diptera, which can lead to species extinction. These threats include habitat loss, forest degradation, forest fragmentation, polluting and harmful substances, the spread of invasive species, global climate change, direct overexploitation, and co-extinction of species dependent on other species (Cardoso et al. 2020). Hence, learning and studying insect diversity is crucial for developing action plans to prevent dipteran extinction.

From this research, we sought to understand the diversity and abundance of Diptera at different selected sampling sites in Kuala Keniam National Park and the relationship between the distribution of Diptera and the environmental gradient from the forest fringe to the inner forest. The questions addressed in this research are: (i) What Dipteran families are present in Kuala Keniam National Park?; (ii) Does the distribution of order Diptera differ in different landscapes from the forest fringe to the inner forest?; (iii) What is the extent of anthropogenic impact on the insect distribution in Kuala Keniam National Park?. This study will be helpful to understand better the diversity and abundance of dipterans in Kuala Keniam National Park as it will provide information on the distribution, classification, family composition, roles, and importance of order Diptera in Kuala Keniam National Park. This study can also serve as baseline data for further research.

MATERIALS AND METHODS

Study site

In 1939, the National Park, or Taman Negara, was declared for conservation as a protected area, encompassing the states of Pahang, Terengganu, and Kelantan (Ibrahim and Hassan 2011). The National Park was established to protect wildlife, conserve nature and the ecological system, and for recreation and ecotourism activities (Hayati et al. 2010). The National Park in Pahang has a total area of 2477 km from the 4343 km total of all National Parks in Malaysia (UNESCO World Heritage Centre 2014). Pahang National Park has permanent high temperatures, ranging from 20°C at night to 35°C during the day with adequately high humidity (Suratman 2012). Kuala Keniam National Park (4º31'07.17" N, 102º28'31.26" E) is one of the protected forests within Pahang National Park and is located 120 to 250 m above sea level (Suratman et al. 2010). Kuala Keniam National Park Forest is characterized by a lowland Dipterocarp forest (Asari and Suratman 2010).

Sampling methods

Malaise traps were used in this study for collecting the insects (Figure 1). Malaise traps with collecting jars half-filled with 70% alcohol were set up at three different landscapes of Kuala Keniam National Park. Three traps were set up, separated by 250 m (Figure 2). Trap 1 was set up at the forest fringe, Trap 2 at the middle forest, and Trap 3 at the inner forest. Forest fringe is the more open area with less tree canopy and higher exposure to sunlight. Along the transect line, as it was set up through the middle and inner forest, the area was more covered with canopies and large trees. Malaise trap is commonly used to assess diverse terrestrial arthropod communities because it can collect many samples with modest effort (Steinke et al. 2021). The traps were left unattended for three days before the specimens were collected and brought to the laboratories. The samples were then sorted and identified according to their respective families.

Data analysis

Three diversity indices, namely the Shannon-Wiener diversity index, Evenness Index, and Margalef Richness Index, were calculated using PAST (Paleontological Statistics), and the Kruskal-Wallis test was performed to observe the differences in the distribution of Diptera across the three different landscapes.

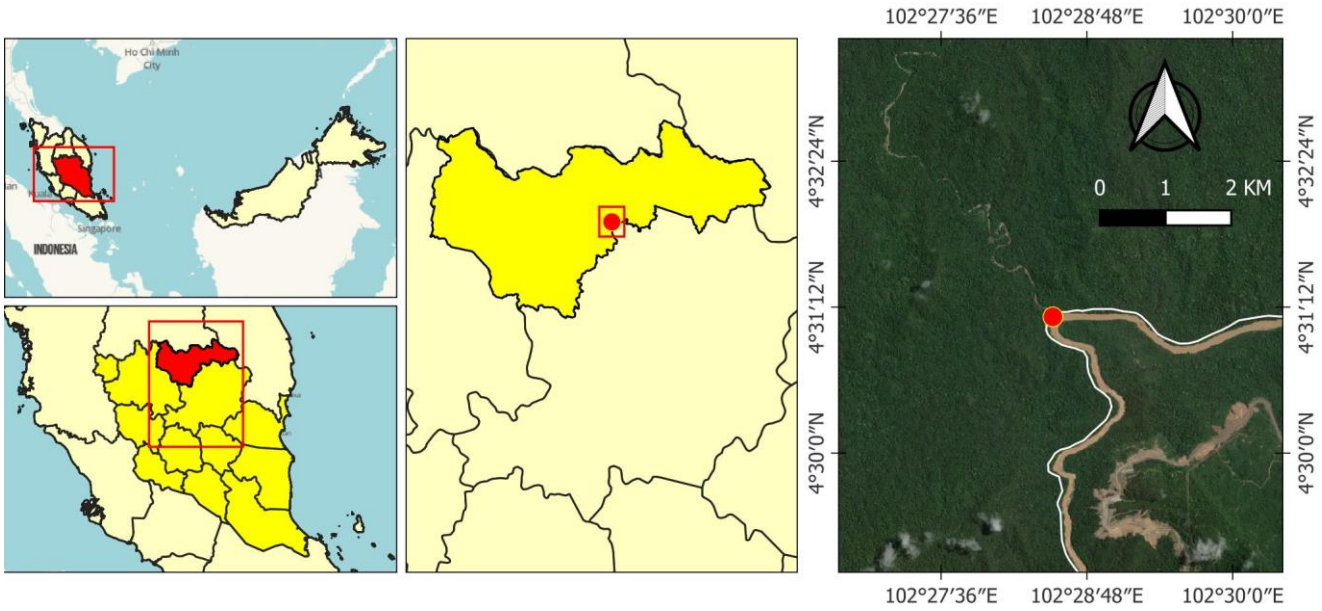

Figure 1. Map of study area in lowland forest of Kuala Keniam, Taman Negara Pahang, Pahang, Malaysia

Figure 2. Malaise trap at Kuala Keniam National Park, Pahang, Malaysia

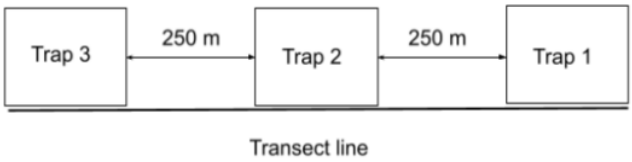

Figure 3. Arrangement of Malaise traps

Calculations on the Shannon-Wiener diversity index that has been used to show diversity in the study area were based on the formula:

$$H' = \sum_{i=1}^{S} pi\ log\ e\ pi$$

Where,
H': the value of the Shannon-Wiener Diversity Index
pi: the proportion of species
log e: the natural logarithm of Pi
S: the number of species in the community

Calculations on the Evenness Index analysis were conducted to show evenness and dominance among families under the order of Diptera based on the formula:

$$(E) = H' / ln\ S$$

Where,
H': Shannon Wiener Diversity Index
S: number of species

Calculations on the Margalef's Richness Index were used to state the function of this index based on the formula:

$$D_{Mg} = \frac{(S-1)}{In\ N}$$

Where,
S: number of species
N: the number of individuals

RESULTS AND DISCUSSION

The overall population of Diptera at Kuala Keniam National Park, Pahang

In this study, 19 Diptera families were identified, comprising 109 morphospecies with 1920 individuals. Families identified in Kuala Keniam National Park were Anthomyiidae, Calliphoridae, Pipunculidae, Scenopinidae, Ulidiidae, Tephritidae, Ceratopogonidae, Stratiomyidae, Asilidae, Syrphidae, Tipulidae, Ephydridae, Cecidomyiidae, Dolichopodidae, Phoridae, Culicidae, Muscidae, Sciaridae, and Drosophilidae (Figure 3).

Phoridae was the most abundant family of Diptera from the samples collected, covering 55.1% of the samples, including 1057 individuals. This high number of individuals is supported by Brown (2001), who stated that Phoridae are the most diverse family among Brachycera. Phoridae are also abundant in human habitats (Wang et al. 2020). The most apparent characteristic of Phoridae is their hunched back those points outwards (Namaki-Khameneh et al. 2021). Many Diptera species were fed on decomposing organic matter, while others prey on slug eggs and parasitize insects (Merritt et al. 2009). Phoridae was also known from many studies to have having high reproduction rate and adaptability, causing the high abundance. Phorid flies can boost their development from eggs to adults in high temperatures (Chen and Porter 2020). They can also adapt to the environment to reproduce their eggs. Phoridae exhibited parasitic behavior in producing their eggs by laying their eggs near or directly to the hosts, as the larvae could feed on the hosts' tissues (Core et al. 2012). This adaptation behavior is advantageous when suitable spots for laying eggs are unavailable or during dry seasons. The short life cycle of Phoridae also causes an increase in their population size as they reproduce more rapidly (Alcaine-Colet et al. 2015).

The second most dominant family was Dolichopodidae, with 281 individuals recorded (14.64%). Members of this family are commonly known for their long legs and metallic body color. They consume fungi, flies, and mites as food (Cicero et al. 2017). Dolichopodidae adults are known to stay in high-humidity habitats and forests (Kechev 2021). Dolichopodidae rely on high agricultural intensification and are the most diverse in high vegetation areas (Kautz and Gardiner 2019). This explains the high diversity and abundance of Dolichopodidae found in Kuala Keniam National Park, Pahang.

Another family with abundant individuals collected was Sciaridae, with 274 total individuals, comprising 14.28% of individuals identified. Sciaridae are mostly known by the name of "fungus gnats." Their adults usually do not bring any concerns, but their larvae are pests and will damage plants (Hartley 1992). Sciaridae adults favor places with high moisture (Gerhardt and Hribar 2019).

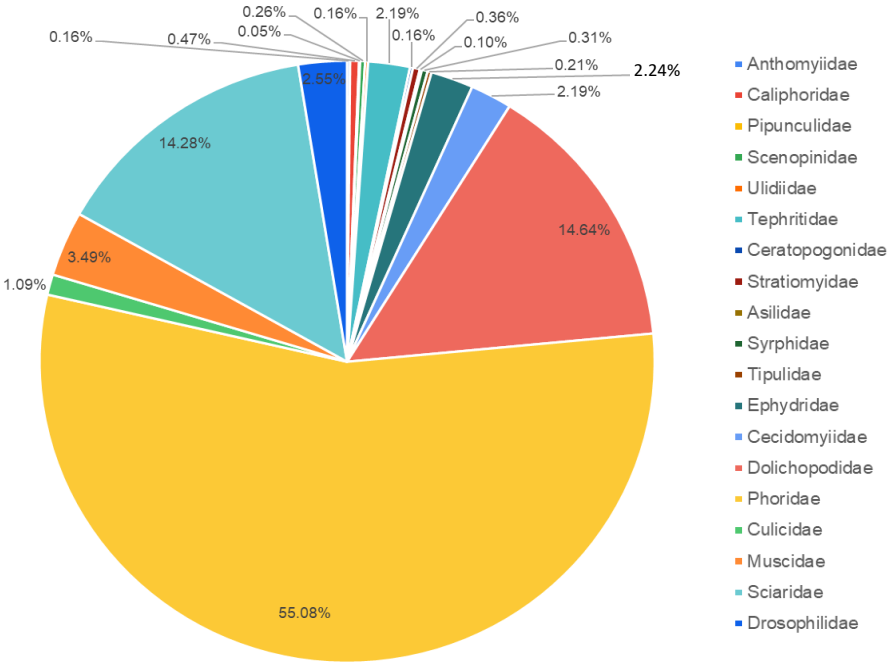

Figure 4. Percentage of Diptera families collected in Kuala Keniam National Park, Pahang, Malaysia

Kuala Keniam National Park is humid like any other rainforest in Malaysia and thus is favorable for them. Fungus gnats also thrive well where the surrounding organic matter is wet (Gibb 2015). The rest of the families recorded less than 100 individuals each, with Pipunculidae having the lowest percentage, only 0.05% of the total number of individuals (Figure 3).

The abundance of insects in each trap

Trap 3 (from the inner forest) recorded the highest number of total individuals of Diptera with 1032 individuals, followed by Trap 2 (from the middle forest) with 589 individuals, and lastly, Trap 1 (from the forest fringe) with 299 total individuals of Diptera (Table 1). The highest number of insects, with 54% of total individuals, was recorded from Trap 3, while Trap 2 recorded the second highest number of insects with 31%. Trap 1 had the least number of insects, covering only 15% of the total individuals collected in Kuala Keniam National Park, Pahang.

Overall, 109 morphospecies of Diptera were recorded from Kuala Keniam National Park. Trap 1 had the most diverse Diptera with 72 morphospecies (41%), followed by Trap 2 with 61, which comprised 35% of the total morphospecies. The least diverse of Diptera, with 41 morphospecies, represented by 24% of morphospecies, came from Trap 3.

From the information gathered, it can be concluded that Trap 1 had the most diversity of Diptera due to the highest number of morphospecies found despite having the least number of individuals collected. Kuala Keniam forest area has a uniform distribution of tree species and a stable tree population (Suratman et al. 2010). The outer area has less vegetation richness than the middle and inner areas, which might contribute to the lower abundance of Diptera individuals due to a lack of food sources. Trap 3, set up in the inner forest, recorded the highest number of individuals that the Phoridae family majorly contributed. Research has shown a higher population density of *Pseudacteon* species (Diptera: Phoridae) near rivers and forests (Calcaterra et al. 2005).

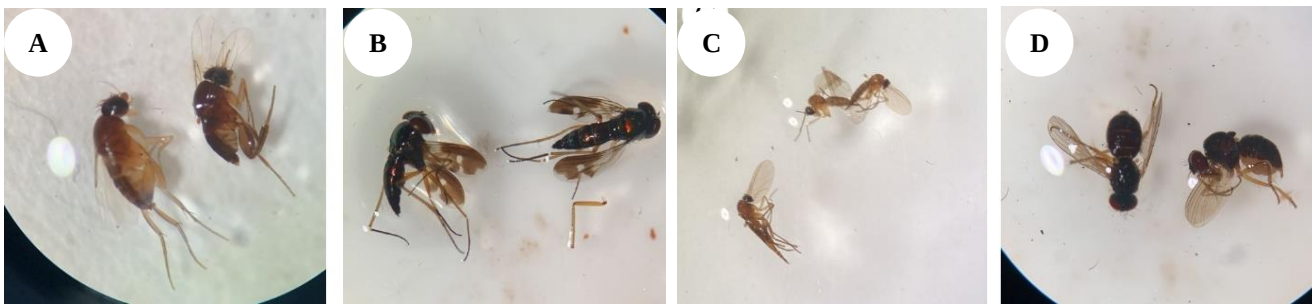

Figure 5. Some samples were sorted by families, including A. Phoridae; B. Dolichopodidae; C. Sciaridae; D. Muscidae

Table 1. The abundance and diversity of Diptera from the different traps

Order	Morphospecies	Trap			Total Individual
		1	2	3	
Anthomyiidae	2	2 (1)	1 (1)	0	3
Calliphoridae	7	1 (1)	8 (6)	1 (1)	10
Pipunculidae	1	1 (1)	0	0	1
Scenopinidae	3	3 (1)	2 (2)	0	5
Ulidiidae	2	2 (2)	0	1 (1)	3
Tephritidae	3	9 (3)	6 (2)	27 (3)	42
Ceratopogonidae	3	1 (1)	0	2 (2)	3
Stratiomyidae	4	5 (3)	0	2 (1)	7
Asilidae	2	1 (1)	1 (1)	0	2
Syrphidae	3	6 (3)	0	0	6
Tipulidae	3	3 (3)	1 (1)	0	4
Ephydridae	7	13 (4)	27 (5)	3 (2)	43
Cecidomyiidae	5	14 (2)	20 (3)	8 (1)	42
Dolichopodidae	11	22 (8)	58 (5)	201 (4)	281
Phoridae	7	89 (5)	271 (6)	697 (7)	1057
Culicidae	8	5 (4)	16 (4)	0	21
Muscidae	15	38 (7)	19 (7)	10 (8)	67
Sciaridae	10	70 (9)	132 (8)	72 (7)	274
Drosophilidae	13	14 (7)	27 (10)	8 (4)	49
Total Individual	109	299	589	1032	1920
Percentage		15%	31%	54%	
Total Family	19	19	14	12	
Total Morphospecies	109	66	61	41	

Note: *The value in the bracket is the total number of morphospecies

Moreover, Banafshi et al. (2013) found that *Anopheles superpictus* (Diptera: Culicidae) larvae were mostly found along river edges with high sunlight exposure. Similarly, the available water bodies in the inner forest may serve as spots for laying eggs, which explains the high number of individuals for Phoridae and other families recorded there.

The overall results indicate that the diversity and abundance of the order Diptera are high in Kuala Keniam National Park, Pahang. The abundance of Diptera collected might be due to the rainy season during the sample collection. A previous study has proven that wet seasons contribute to the high abundance of insects collected (Abdul-Hamid et al. 2014). Srisuka et al. (2021) also found that blackflies were the most abundant throughout the monsoon while at its lowest in the dry season. The anthropogenic impact in the study sites seems to affect only the forest fringe area. The abundance of Diptera collected in the forest fringe was lower compared to others, possibly due to the tourism activities in the area, as the area is more exposed and accessible to visitors from the nearby campsites. The diversity of species is also high during low-temperature seasons. Rooke et al. (2020) stated that *Drosophila* (Family: Drosophilidae) acts differently regarding social and collective behavior when alone and in a group. Phoridae were the most abundant in the inner forest, possibly caused by their social interaction to move together in large groups to the area. The number of samples collected generally might also be due to the period spent collecting the samples, where enough insects were collected to get this result. However, the number could increase if the Malaise traps were left longer.

Species diversity, evenness, and richness of Diptera

The diversity of Diptera collected from the three traps - the forest fringe (Trap 1), the middle forest (Trap 2), and the inner forest (Trap 3) - was analyzed using PAST version 4 software. The obtained values for the Shannon-Wiener Diversity Index (H′), Evenness Index (E′), and Margalef Richness Index (R′) are tabulated in Table 2.

The Shannon-Wiener Diversity Index indicates that the diversity was at a peak in Trap 1 (H′: 2.13) than in Trap 2 (H′: 1.68) and Trap 3 (H′: 1.04). Despite having the lowest number of individuals collected, Trap 1 was also the most diverse compared to Trap 2 and Trap 3. In most ecological studies, the range of H′ is between 1.5 and 4.5, and it rarely exceeds 5 (Mwakalukwa et al. 2014). All three traps from the forest fringe towards the inner forest recorded a value of H′ from 1.0 to 2.5. The low diversity at all three traps may be because the samples were collected during the rainy season. During this wet period, insects will seek safety by limiting foraging and seeking shelter as rainfall causes immediate mortality of larvae and eggs and shifts insect development (Skendžić et al. 2021).

For the Evenness Index, Trap 1 recorded the highest value (E′: 0.44), followed by Trap 2 (E′: 0.39) and Trap 3 (E′: 0.24). The Evenness Index ranges from 0 to 1, where 1 means complete evenness in the area. This further proves that Trap 1 showed the least dominance of species compared to the other two traps. The E′ value will decrease as the number of Diptera individuals is higher than that of the other orders.

Table 2. Shannon-Wiener Diversity Index (H′), Evenness Index (E′), and Margalef Richness Index (R′)

Trap	H′	E′	R′
1	2.13	0.44	3.16
2	1.68	0.39	2.04
3	1.04	0.24	1.69

Table 3. Pairwise comparisons of traps

Traps	*P*-value
Trap 1-Trap 2	0.197
Trap 2-Trap 3	<0.001
Trap 1-Trap 3	0.099

The high diversity value from this trap led to a higher evenness as no species dominated this trap. The huge variation between the highest and the lowest number of orders collected in each trap led to the low range of the Evenness Index. If no families dominated the traps, a high E′ value would be obtained (Smith and Wilson 1996). However, in this study, all traps had an E′ value of less than 0.5, indicating that some orders dominated the landscapes.

The Margalef Richness Index value for Trap 1 was 3.16, the highest compared to other traps. The R′ values for Trap 2 and Trap 3 were 2.04 and 1.69, respectively. The highest R′ value for Trap 1 means it had the highest species richness. Trap 1 caught the highest number of morphospecies, even though fewer individuals were collected in this trap than others. The Margalef Richness Index has no limit and varies according to the number of species. Because of its sensitivity to sample size, it only considers one aspect of diversity (species richness) when comparing the sites (Kocatas and Bilecik 1992).

Kruskal-Wallis test was used to determine the significant difference in distribution for all dipteran families between the three traps. There was a considerable variation in the Diptera distribution among the traps at various sites comprehensively ($p<0.05$), and pairwise comparison showed that the difference came from the Trap 2-Trap 3 pair (Table 3).

In conclusion, overall, 1920 individuals of Diptera from different families were successfully collected from the selected sites in Kuala Keniam National Park. Comparatively, most individuals were caught in Trap 3 (the inner forest), followed by Trap 2 (the middle forest) and Trap 1 (the forest fringe). The Shannon-Wiener Diversity Index (H′) indicated that the three traps had high insect diversity, with Trap 1 having the highest H′ value, followed by Traps 2 and 3. Additionally, the data was not normally distributed, and there was a great disparity in insect order between traps with a *p*-value less than 0.05. Trap 1 also had the greatest values for the Evenness Index (E′) and Margalef Richness Index (R′). This might be because Trap 1 was located in a region with lower temperatures and more humidity. This study can also serve as a baseline for future entomology research in Kuala Keniam and raise conservation awareness.

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