https://doi.org/10.17576/serangga-2026-3102-03

CHIRONOMIDAE (DIPTERA) ASSEMBLAGES IN TROPICAL RIVERS OF PENINSULAR MALAYSIA: ADAPTATION TOWARD DIFFERENT ALTITUDES

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Received: 16 September 2025; Accepted: 22 July 2026; Published: 23 August 2026

ABSTRACT

Chironomidae (Order: Diptera) known to have wide distribution worldwide. However, their distribution in Malaysian ecosystem is not well studied, especially at different altitude. A preliminary survey on chironomid diversity between highland and lowland was undertaken. Chironomids from both ecosystems were recorded and their adaptation to water quality were examined. Seven streams from Janda Baik Pahang and four streams from Cameron Highland represent highland streams and nine streams from Hulu Langat Selangor represent lowland streams. Sampling was undertaken using Surber net and chironomids were identified to lowest taxonomy level possible. The Malaysian water quality index (WQI) was measured for both catchmens. A total of 7361 chironomids consists of three sub-families and 38 genera were recorded from both study areas. Highland ecosystem exhibits higher chironomid density, but low in diversity compared to the lowland ecosystem. However, the turnover rate of the midges (β-diversity) was higher in lowland ecosystem. Sixteen genera found to inhabitants in both ecosystems. Even most of water quality parameters were not differ significantly except water temperature, the genus still exhibits habitat preferences and twenty-two genus were not sharing the ecosystems. *Tvetenia*, *Cricotopus* and *Rheocricotopus* (subfamily: Orthocladiinae) dominating highland ecosystem, and in contra *Polypedilum*, *Cryptochironomus*, and *Chironomus* (Subfamily:Chironominae) dominating lowland ecosystem. The species indicator analysis identified one genus that was significant indicator species for the lowland ecosystem, *Nanocladius* (subfamily: Orthocladiinae). This study significantly important in providing a checklist for chironomids in tropical rivers of Peninsular Malaysia.

Keywords: Macroinvertebrates; freshwater diversity; water quality; biological indicator

ABSTRAK

Chironomidae (Order: Diptera) diketahui mempunyai taburan yang meluas di seluruh dunia. Walau bagaimanapun, taburan kumpulan ini dalam ekosistem di Malaysia, khususnya pada altitud yang berbeza, masih kurang dikaji. Satu tinjauan awal mengenai kepelbagaian Chironomidae antara ekosistem tanah tinggi dan tanah rendah telah dijalankan. Chironomidae daripada kedua-dua ekosistem direkodkan, dan hubungannya dengan kualiti air turut dikaji. Sebanyak tujuh sungai di Janda Baik, Pahang dan empat sungai di Cameron Highlands mewakili ekosistem tanah tinggi, manakala sembilan sungai di Hulu Langat, Selangor mewakili ekosistem tanah rendah. Persampelan dijalankan menggunakan jaring Surber dan spesimen Chironomidae dikenal pasti sehingga ke aras taksonomi terendah yang dapat ditentukan. Indeks Kualiti Air (WQI) Malaysia turut diukur bagi kedua-dua kawasan tadahan. Sebanyak 7,361 individu Chironomidae yang terdiri daripada tiga subfamili dan 38 genus telah direkodkan daripada kedua-dua kawasan kajian. Ekosistem tanah tinggi menunjukkan kepadatan Chironomidae yang lebih tinggi tetapi kepelbagaian yang lebih rendah berbanding ekosistem tanah rendah. Walau bagaimanapun, kadar pergantian genus (β-kepelbagaian) adalah lebih tinggi di ekosistem tanah rendah. Sebanyak 16 genus didapati mendiami kedua-dua ekosistem. Walaupun kebanyakan parameter kualiti air tidak menunjukkan perbezaan yang signifikan, kecuali suhu air, genus Chironomidae masih memperlihatkan keutamaan habitat yang berbeza, dengan 22 genus hanya ditemui dalam salah satu ekosistem. *Tvetenia*, *Cricotopus* dan *Rheocricotopus* (Subfamili: Orthocladiinae) mendominasi ekosistem tanah tinggi, manakala *Polypedilum*, *Cryptochironomus* dan *Chironomus* (Subfamili: Chironominae) mendominasi ekosistem tanah rendah. Analisis spesies penunjuk mengenal pasti satu genus, iaitu *Nanocladius* (Subfamili: Orthocladiinae), sebagai spesies penunjuk yang signifikan bagi ekosistem tanah rendah. Kajian ini penting dalam menyediakan senarai semak Chironomidae bagi sungai-sungai tropika di Semenanjung Malaysia.

Kata kunci: Makroinvertebrata; kepelbagaian air tawar; kualiti air; penunjuk biologi

INTRODUCTION

The Chironomidae (Order: Diptera), is the most diversified and abundant of all aquatic insect families (Helson et al. 2006). According to Armitage et al. (1995) and Pape et al. (2011), there were at least 15000 species of Chironomidae globally. More recent study by Prat et al. (2014), reported that more than 20000 chironomid species were described, which putting them as the largest family among the aquatic insects. According to Ferrington Jr (2008), these midges appeared to be richer in the northern hemisphere in comparison the southern hemisphere, but the greatest richness was in northern mid-latitudes. In temperate regions as of Europe, chironomids midges consist of 1262 species (Sæther & Spies 2013). However, chironomids taxonomy information from tropical region is poorly recovered due to the conflict between the need for a basic understanding of insect biodiversity including taxonomic research (Helson et al. 2006; Jackson & Sweeney 1995). Taxonomic information is crucial specially to assess the impacts of the environment to which the organism was exposed such as the relationship of chironomids midges and the water pollution (Al-Shami et al. 2010; Ahmad et al. 2014).

Despite of they are the most diversify family among the insects, chironomid midges also have a wide range of environmental gradients adaptation (Ahmad et al. 2014; Ferrington Jr 2008). These midges are considered as a very speciose group as they can be found in a variety of habitats. They were found in various different habitat such as the pristine aquatic ecosystem (Ahmad et al. 2014), polluted paddy field (Al-Shami et al. 2010), brackish water (Parma &

Krebs 1977), sedimented riverbed (Bervoets et al. 1998) and sewage (Seldén et al. 2013). This ubiquitous distribution contributes to the suitability of the midges as water quality indicator (Nicacio & Juen 2015). However, in some part of the world such as in the oriental area, not many studies on chironomids distribution and diversity been carried out (Ahmad et al. 2014). As a result, the paucity of chironomids taxonomic keys of tropical area led to the lack of the midges' checklists which contributed less guidance for freshwater entomologists and ecologists to determine the condition of the rivers as their accurately.

Chironomidae taxonomic studies in Malaysia were considerably scarce and the information on their distribution was limited despite of high number of species. Ferrington Jr (2008) reported that, approximately 105 genera and 359 species of chironomids was recorded in oriental area. However, most of the Chironomidae identification was based on the keys from another region such as Australasian region (Cranston 1982) and Holarctic region (Wiederholm 1983). In this region, most of the taxonomic studies focused on the identification of single species such as from genus *Microtendipes* (Qi et al. 2012), *Nilothauma* (Qi et al. 2016) and *Shangomyia* (Cranston 2003) and *Sublettea*, recorded from Sulawesi, Indonesia (Ashe & O'Connor 1995).

In Malaysia, few studies were conducted on chironomids taxonomy such as Ahmad et al. (2012) in clean rivers and Al-Shami et al. (2006) from polluted paddy field. Other studies, such as Ahmad et al. (2008), Al-Shami et al. (2009), Vedamanikam & Shazilli (2008) and Warrin et al. (2008) on the Malaysian chironomids, found Chironominae, Orthocladinae and Tanypodinae were common and abundant subfamilies. Amongst these, Chironominae was the most dominant subfamily in tropical ecosystems. This limited information however, contributed greatly to studies of diversity, density and the roles of chironomids in water quality assessment at this region (Al-Shami et al. 2010; Shuhaimi-Othman et al. 2011). Spatial and temporal variation in Chironomidae assemblages is important to be studied in order to further understand the biological adaptations with the environment conditions. Chironomidae exhibited vast distribution between lowland and highland ecosystems. Ahmad et al (2021) reported that similar Chironomidae family reported in lowland were found in highland ecosystems. Amongst important variables that influence Chironomidae distributions is water temperature (Maiolini & Lenchioni 2001) and different between cold stenothermal and warm euthermal species (Cranston 1995). Malaysia has many esthetic highland and lowland river systems, which widely utilized as recreational area. However, very limited information on chironomids distributions between these ecosystems is produced as many studies focused on contamination assessments. The limited comparison between these two ecosystems has resulted in insufficient information on the variation and similarity of chironomid taxa, thereby constraining their application as reliable biological indicators. This study was conducted to compare chironomids assemblages at different altitude rivers system and to understand their adaptation towards water quality differences.

MATERIAL AND METHOD

Study Sites

The study was conducted at two different states namely Pahang and Selangor. Geographically, Pahang represents east coast of Malaysia Peninsular and Selangor is at the west. Streams from Pahang state represent high altitude streams and low altitude streams were sampled from Selangor state. High altitude streams located between 436 m to 1575 m from sea level and low altitude streams are between 65 m to 150 m. High altitude streams located in Janda Baik and Cameron Highland districts in Pahang and low altitude streams are located in Hulu Langat district in Selangor). A total of 23 stations were sampled, which seven rivers were from Janda Baik and four rivers were from Cameron Highland and twelve sites were sampled from Langat River and tributaries. High altitude streams are flow through dense cash crops and tea plantations (Cameron Highland) and villagers (Janda Baik), whereas low altitude streams are within recreation and small residential areas (Hulu Langat). As sampling was undertaken at upstream site of the streams/rivers, all sites are shallow and dominated by cobble sand. The physical characteristics of streams from both localities are simplified in Table 1. Habitat criteria such as stream width and depth, percentage and type of substrates, canopy cover and riparian vegetation were estimated as been suggested by Barbour et al. (1999).

The average ambient air temperature at highland sites (Janda Baik dan Cameron Highland) is 18°C±0.2°C whereas 31ºC±0.4°C at lowland (Hulu Langat, Selangor). Studied catchments are affected by typical tropical monsoon climate with high humidity (from 70% to 98%) and vary annual rainfall. The average rainfall in Peninsular Malaysia is 650 mm from December to May 2023, meanwhile the highest precipitation was recorded in east coast with maximum rainfall is 1000 mm (June – November 2023). The physical characteristics of the study streams were measured in the field. Altitude was determined using a Garmin eTrex 10 handheld GPS. Canopy cover and substrate composition were assessed through visual estimation. Stream depth and width were measured manually using a rope and weighted plumb line, whereas water velocity was measured using a Flowatch Handheld Flow Meter (ENVCO model). Result for rivers characteristic for both ecosystems are presented in Table 1.

Table 1. A physical characteristics river from highland rivers (Janda Baik and Cameron Highland) and lowland (Hulu Langat, Selangor)

	Lowland	**Highland**
Altitude (m) asl	65-150	436-1575
Substrates (c:p:s) *	70:40:70	30:50:90
Canopy cover (%)	10-80	<10–80
Depth (m)	<1	0.1–0.5
Width (m)	3–11	1–9
Water flow (m/s)	0.26–1.23	0.07–0.65

*c:p:s = cobble: pebble: sand

Chironomids Larvae Sampling

Chironomids larvae were collected using a Surber's net (40 cm wide x 40cm long and 40 cm high with 0.16 m^2 sampling area and net mesh size of 0.3 mm) which is suitable for shallow water (Merritt et al. 2008). At each sampling station, three replicates sample were collected. The net was firmly embedded into the river substrate with opening is facing the flow. Substrate within the quadrate was vigorously disturbed and any large materials were hand-lifted and washed to dislocate the aquatic macroinvertebrates. Collected samples were cleaned, labeled precisely and preserved with 70% ethanol.

Prior to identification, each chironomid sample was prepared for permanent slide by treated with 10% potassium hydroxide and neutralized using the glacial acetic acid. Each chironomid sample was then mounted on slide upside down and sealed with formaldehyde resin. The slide was inspected using stereo microscope (model Olympus SZX9) and identification was undertaken following Cranston (1982), Epler (2001), Wiederholm (1983) and Andersen et al. (2013).

Water Quality Measurement and Analysis

Dissolved oxygen (DO), pH, water velocity and water temperature was were measured *in situ* using a YSI Professional Plus multi-probe. A velocity meter (CDM 9000 Digitizer) was used to measure the streams velocity. Water samples were collected using polyethylene (PE) bottles (1000 mL) for chemical oxygen demand (COD), ammoniacal-nitrogen (NH_3-N) and total suspended solid (TSS) analyses. The biochemical oxygen demand (BOD_5) water sample was collected using polyethylene coated bottle (300 mL). Water sampling was undertaken following the Standard Methods for Examination of Water and Wastewater (APHA 1995). The COD, NH_3-N, BOD_5 and TSS were analyzed following HACH DR/2500 procedures.

Statistical Analysis

The analysis was computed using the IBM SPSS statistical program version 22.0. Biodiversity patterns of the chironomids, alpha, beta and gamma diversity were calculated following Whittaker (1960) and Heino et al. (2015). In this study, we calculated the average number of taxa at each river ($\alpha_{average}$), while beta diversity, $\beta = \gamma / \alpha_{average}$, being the means of each catchment according to Baselga (2010), Boyero et al. (2012) and Che Salmah et al. (2014). According to Sepkoski (1988), alpha diversity ($\alpha_{average}$) is used to measure the compacting of taxa within a habitat which reflects division of ecological resources, while β indicates the amount of turnover in species composition along environmental. In addition, gamma (γ) was simply the total number of chironomid taxa detected in all studied rivers of each of the catchment. Diversity of chironomids in all catchments was calculated using pattern of distribution Shannon (*H*'). For richness index, Menhinick's (*R*2) was estimated, and evenness was analyzed using Pielou evenness index (*J*'). All ecological indices were calculated using the paleontological statistics software package for education and data analysis (PAST 3 version 3.16). Canonical Correspondence Analysis (CCA) was computed to estimate the impacts of physico-chemical parameters on Chironomidae taxa. The significance of the generated canonical axes was measured using the Monte Carlo test ($P<0.05$) with 500 iterations. The CCA was performed using CANOCO (version 4.5) and the biplot ordination diagrams were drawn with CanoDraw for Windows 4.1. Significant indicator taxa of Chironomidae discriminating between both catchments were identified using indicator species analysis. This analysis was computed using PC-ORD software package (version 6.0, MjM Software). The analysis was based on the presence or absence data (Al-Shami et al. 2017), and as recommended by Heino et al. (2015). According to Dufrene & Legendre (1997), the good indicator taxa are those that occur at nearly all sites in each group and only rarely in any other groups. The indicator value ranges from 0 to 100 that pertains to perfect indication. The significance of the indicator value for each taxon was tested by Monte Carlo tests with 4999 iterations at significance level of 0.05.

RESULTS AND DISCUSSION

According to Ferrington Jr (2008), the oriental riverine habitats support an exceptionally high richness and abundance of Chironomidae. In the present study, remarkably rich chironomids larvae were recorded from two different altitude catchments in Malaysian river systems. A total of 7361 individual was recorded and higher altitude streams contain more individual and taxa richness (Table 2). Chironomids density at higher altitude streams is 359.9 ind./m^2 compared to lower altitude streams 220.6 ind./m^2 regardless to uneven sampling size. Three subfamilies were recorded in both catchments, which are Chironominae, Orthocladiinae and Tanypodinae. (Figure 2). Rosa et al. (2011, 2013) reported that allochthonous input from riparian provides food for midges (Figure 1). This could be the most logical reason to explain higher abundance of chironomids in highland catchment compared to lowland catchment. Ahmad et al. (2014)

added that habitat for chironomids is ubiquitous and they can be found in various type of environments. Similarly, Coffman & Ferrington (1984) mentioned that chironomids were frequently occurring in high density in all kinds of ecosystems, but scrapper feeder likes chironomids are abundant at allochthonous ecosystems.

Chironominae subfamily was represented by two tribes, Tanytarsini and Chironomini. Only *Polypedillum* from Chironomini tribe recorded dominating both catchments, whereby *Cladotanytarsus* and *Rheotanytarsus* that are from Tanytarsini tribe were dominating lower altitude and higher altitude ecosystems respectively. Two genera from Ortholcadiinae subfamily also exhibited domination in both catchments that are *Rheocricotopus* and *Cricotopus, whereas Tvetenia and Eukiefferiella* found dominating highland catchment.

These subfamilies are common in the tropical rivers and they inhabited in large variant of habitats from the clean to the polluted water (Ahmad et al. 2008; Al-Shami et al. 2011; Ahmad et al. 2012). The biodiversity measurements expressed as alpha, beta and gamma are shown in Table 3. Specifically, local (measured as $\alpha_{average}$) and regional (γ-diversity) diversities (measures of taxa richness) were higher in lowland catchment. The highland catchment has wide range of alpha diversity, which from 8 (lowest diversity, (α_{min}) and 20 (highest diversity, (α_{max}). In addition, the γ-diversity was 40 taxa for lowland catchment and only 33 taxa in highland. However, the turnover rate of chiromonids was higher in highland catchment (3.59) probably due to more habitats variability. The diversity and richness indices (*H*' and *R*2) values indicated that chironomids were more diversify in lowland catchment in comparison to rivers of highland. These midges were almost evenly distributed in rivers of both catchments according to Pielou index (*J*'), although more genera of Chironomidae were collected from lowland river.

The water quality between streams from same catchment showed an insignificant variation (Table 4). However, BOD_5, COD and water temperature between lowland catchment and highland catchment were found significantly different (independent *t*-test, $P<0.05$, α=0.05). Water temperature variation between elevations could be assumed to play major role in determining chironomids distribution. The suspended solid in streams from both catchments is very low and was not differ significantly among streams in each catchment (paired *t*-test, $P>0.05$). Only water temperature was found significantly different between highland and lowland streams (independent t-test, $P<0.05$, α=0.05) and might significantly influence chironomids population. In average, based on the Malaysian WQI, all streams in both catchments are classified in clean class. Study was undertaken at upstream site of the catchments and therefore good water quality was gathered.

Table 2. A comparison of taxa, abundant and diversity of Chironomidae between lowland and highland streams

Subfamili	Tribe	Genus	Lower Altitude	Higher Altitude
Chironominae	Tanytarsini	*Cladotanytarsus*	120	14
		Rheotanytarsus	71	351
		Tanytarsus	18	8
		Sublettea	2	
		Virgatanytarsus		1
		Tanytarsini genus 1	3	

Subfamili	Tribe	Genus	Lower Altitude	Higher Altitude
		Tanytarsini genus 2	1	
	Chironomini	*Polypedilum*	971	1938
		Microtendipes	151	10
		Chironomus	87	20
		Stictochironomus	4	
		Stenochironomus	1	
		Glypotendipes	1	4
		Shangomyia	1	
		Cryptochironomus	148	24
		Saetheria	17	6
		Demicryptochironomus	7	6
		Harnischia	7	19
		Robackia	1	
		Paracladopelma	1	
		Chironomini genus 1	1	
		Chironomini genus 2	1	
		Chironomini genus 3	1	
		Chironomini genus 4	1	
		Chironomini genus 5	2	
		Chironomini genus 6	1	
Orthocladiinae		*Rheocricotopus*	419	435
		Cricotopus	128	626
		Parametriocnemus	22	23
		Eukiefferiella	15	118
		Thienemanniella	14	
		Nanocladius	12	
		Tvetenia	13	792
		Parakiefferiella	1	
		Xylotopus	1	
		Corynoneura	1	
		Bryophaenocladius		1
		Cardiocladius		31
		Heleniella		6
		Limnophyes		2
		Paracricotopus		88
		Paratrissocladius		8
		Pseudorthocladius		2
		Onconeura		1
		Synorthocladius		3
		Thienemanniella		11
		Orthocladiinae genus 1	1	1
		Orthocladiinae genus 2	1	1

Subfamili	Tribe	Genus	Lower Altitude	Higher Altitude
		Orthocladiinae genus 3	1	4
Tanypodinae			254	315
		Total individual	2502	4859
		Density (individual/m^2)	220.6	359.9
		Shannon diversity (*H'*)	2.092	1.925
		Menhinick richness (R2)	0.799	0.473
		Pielou evenness (J')	0.567	0.550

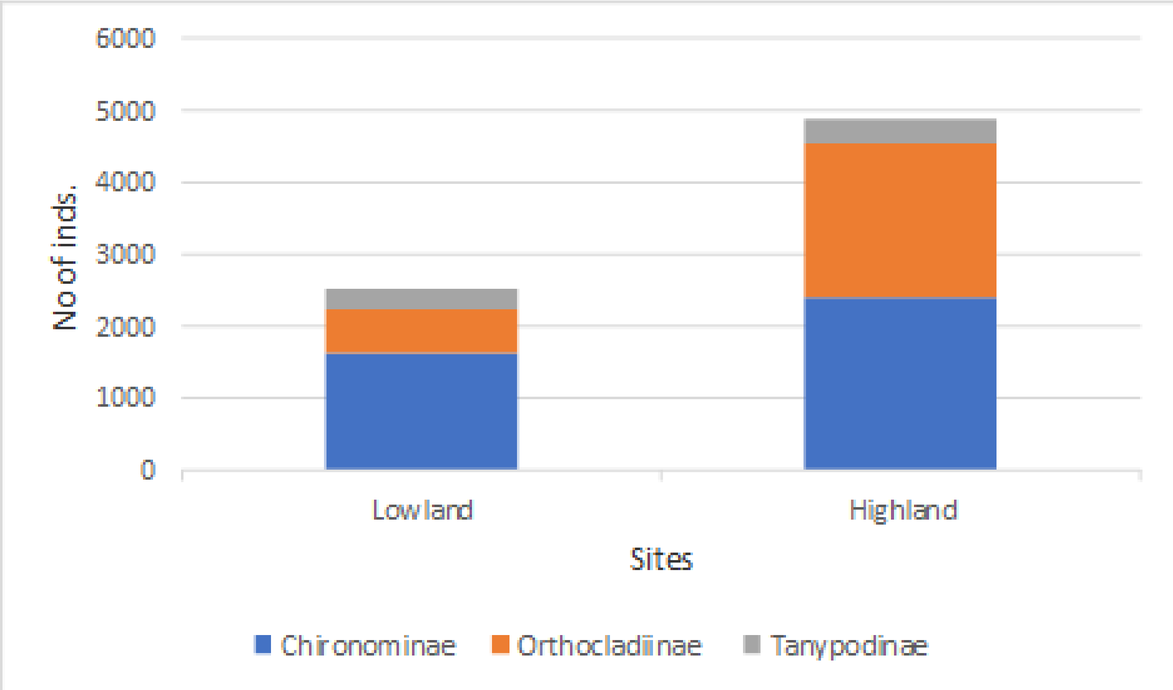

Figure 1. Subfamilies composition between lowland and highland catchments

Table 3. Diversity indices of Chironomidae (Order: Diptera) between lowland catchment and highland catchment

Biodiversity Indices	Lower Altitude	High Altitude
α_{max}	21	20
α_{min}	11	8
$\alpha_{average}$	15	9
β-diversity ($\gamma/\alpha_{average}$)	2.67	3.59
γ-diversity	40	33
Shannon-Wiener (*H'*)	2.092	1.925
Menhinick (R2)	0.799	0.473
Pielou (*J'*)	0.567	0.550

The multivariate CCA was applied to infer water quality influence on Chironomidae population. In this study, the canonical correlation model exhibited a significant of water quality on chironomids taxa (Table 5). For lowland catchment, the first two canonical axes in the ordination plot displayed negative associations with escalating amounts of COD, BOD_5, water temperature and TSS but positive association with DO. Meanwhile, the chironomids showed positive trend with increasing of pH and COD in axes one and ammoniacal nitrogen, pH, COD and TSS in highland catchment (Figure 2).

Polypedilum and *Rheocricotopus,* the dominant genus in both catchments showed a positive association with DO and TSS in highland catchment. At lowland catchment, only *Polypedilum* associated with DO and *Rheocricotopus* has negative association with elevated TSS. The most abundance genus in Orthocladiinae subfamily that is *Tvetenia* does not has any significant association with any water quality parameters. Members of Tanypodinae subfamily preferred to inhabit rivers with higher temperature as they positively associated with water temperature in both catchments.

Table 4. Physico-chemical parameters and WQI scores of two catchments (Mean±SD)

Water Parameters	**Lower Altitude**	**Higher Altitude**
Temperature (°C)	25.7±1.72	21.3±0.08
DO (mg/L)	7.20±0.60	7.48±0.06
NH_3-N (mg/L)	0.10±0.08	0.14±0.01
pH	7.02±0.35	6.88±0.06
BOD_5 (mg/L)	0.67±0.65	0.49±0.03
COD (mg/L)	4.46±5.50	4.35±0.30
TSS (mg/L)	10.01±0.01	18.61±2.04
WQI	96.05	92.62

The indicator analysis was used to identify indicator species of the catchments. Resultant indicator values (IV) were tested for significance using Monte Carlo test (4999 permutations). All taxa from both catchments were tested and only one significant indicator species was identified at lowland catchment (Table 6). *Nanocladius* (sub-family: Orthocladiinae) was identified as an indicator for lowland catchment with IV value of 57.1 ($P<0.05$). Similarly, *Sublettea*, *Tanytarsus*, *Harnischia* and *Glypotendipes* (sub-family: Chironominae) are also good indicator species for both catchments (Table 6). Other genus from Orthocladiinae such as *Thienimanniella* is not significant as an indicator for both catchments but is good indicator species based on its indicator value.

Table 5. Features of Canonical Correspondence Analysis (CCA) of six WQI parameters and chironomids abundance in two catchments at 500 iterations. Monte Carlo test significant at *$P<0.05$*

		Lower Catchment				**Higher Catchment**			
		Axis 1	**Axis 2**	**Axis 3**	**Axis 4**	**Axis 1**	**Axis 2**	**Axis 3**	**Axis 4**
Constrained correlation coefficients for all variables	Temperature	-0.8039	-0.0474	0.407	0.1884	-0.9086	-0.0904	-0.1898	0.1767
	DO	**0.6598**	0.3004	-0.5424	-0.2599	-0.5448	0.1584	0.0794	0.2414
	NH_3-N	0.0239	-0.1049	**0.8415**	0.3794	-0.0765	**0.903**	-0.2032	0.2239
	pH	0.3202	-0.6487	0.3644	-0.4719	**0.7282**	**0.5912**	-0.0467	0.1815
	BOD_5	-0.3132	**0.8843**	-0.2723	0.0578	0.1785	0.4806	-0.5319	-0.0625
	COD	-0.1836	**0.8604**	-0.0593	0.1911	**0.6733**	**0.6305**	0.0923	-0.1122
	TSS	-0.5041	-0.4096	-0.4141	**0.6374**	-0.521	**0.6124**	-0.1005	0.4287
	WQI	0.2977	-0.4821	-0.5533	-0.3964	-0.2767	-0.7283	0.16	-0.204
Eigenvalues		0.272	0.212	0.19	0.167	0.317	0.142	0.131	0.08
Cumulative percentage variance of species-environment relation		26.6	47.4	66	82.3	38.6	56	72	81.8

Note: Any factor whose eigenvalue is less than 1.0 is in most cases not going to be retained for interpretation. Unless it is very close or has a readily understood and interesting meaning.

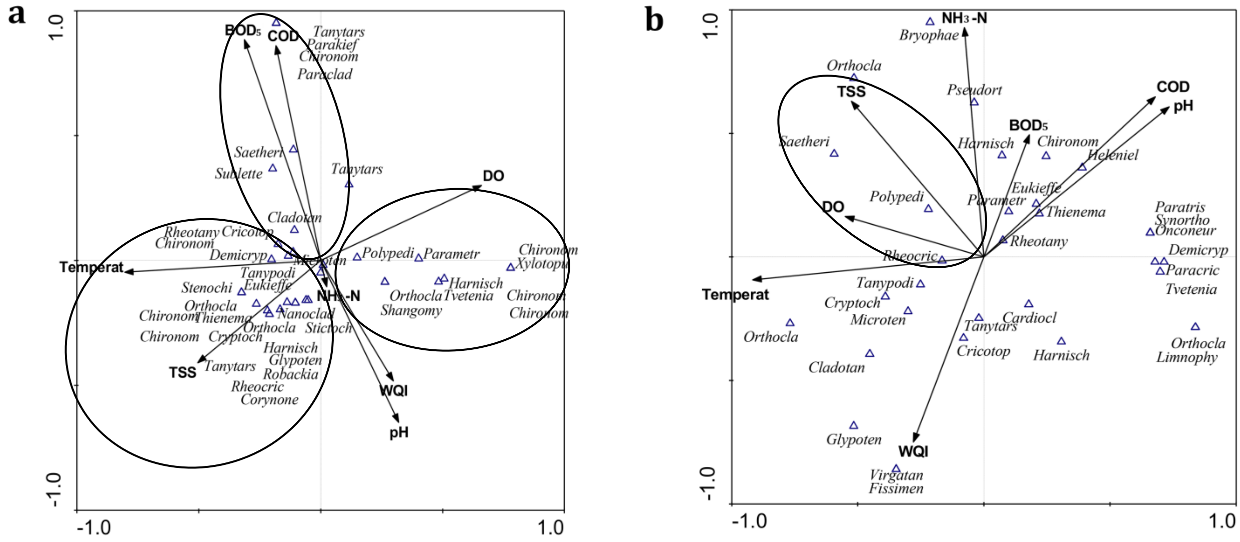

Figure 2. The ordination plot of Canonical Correspondence Analysis (CCA) for the first two canonical axes of the Chironomidae taxa and physico-chemical parameters in two different catchments, a) lowland Catchment (LC) and b) Highland Catchment (HC). Monte Carlo test significant at $P<0.05$

Table 6. The indication value (IV) of the Chironomidae taxa from lowland and highland catchments (Monte Carlo test, 4999 permutations, significance when $P<0.05$)

Genus (Sub-Family)	Catchments	Indicator Value	*P-Value*
Nanocladius (Orthocladiinae)	LC	57.1	0.015
Sublettea (Chironominae)	LC	28.6	0.150
Tanytarsus (Chironominae)	LC	28.6	0.162
Thienemanniella (Orthocladiinae)	LC	28.6	0.159
Harnischia (Chironominae)	LC	23.2	0.665
Thienemanniella (Orthocladiinae)	HC	40.0	0.185
Glypotendipes (Chironominae)	HC	22.1	0.518
Heleniella (Orthocladiinae)	HC	20.0	0.494
Pseudorthocladius (Orthocladiinae)	HC	20.0	0.486

Note: LC = Lower catchment, HC = Higher catchment

DISCUSSION

Biodiversity Pattern of Chironomidae in Two Different Catchments of Tropical Rivers

In the present study, remarkably rich chironomids larvae were recorded from two different catchments in Malaysian river systems. However, highland catchment supports higher chironomids density. Many factors explain this finding such as substrate type, temperature regime, food resource and contaminations. Highland catchments like Janda Baik and Cameron Highland are established as tourism destination and have pristine ekosistems. Both area is good water quality and well manage catchment and therefore have cooler and constant temperature. Many chironomids are cold-adapted and attain high densities in cooler streams (Hannah et al. 2023). Thus, highland sites can host chironomid assemblages that are numerically dominant because they are well adapted to the prevailing temperature regime (Eggermont & Heiri 2012). Most streams/ rivers in highland catchments flow beneath dense forest canopies, which provide better canopy and allochthonous inputs. Rosa et al. (2011, 2013) mentioned that the input from allochthonous provides food for midges. This could be the most logical reason to explain the high abundance of chironomids in highland catchment compared to lowland catchment which is warmer. Some highland reaches possess a complex mosaic of flow velocities and microhabitats (riffles, glide margins, pools) that create refugia and permit rapid recolonisation of chironomids. Fu et al. (2019) reported that stable cobble and mixed substrates, common in upland streams, favour higher chironomid abundance. This result is in agreement with river continuum concept proposed by Vannote et al. (1980), where high diversity of aquatic insects including the chironomids in low order stream that flows within the dense vegetation that provide sufficient amount of allochthonous inputs into water body. In addition, according to Rosa et al. (2013), Chironomidae richness in rivers surrounded by the forest reserve was related to abundant of leaf litter that retained the food particles for the midges. Many chironomid taxa in these reaches act as *collector–gatherers* (deposit feeders) that ingest FPOM and microbially-conditioned detritus or exploit biofilms on organic fragments.

Lowland reaches system typically contain a mosaic of habitat types (slow-flowing channels, pools, backwaters, marshes, ponds, detrital accumulations, macrophyte beds and soft sediments). This cause more chironomid taxa to coexist and increasing local (α) and among-site (β) richness. Empirical work on stream invertebrates and chironomids supports a strong role for local habitat heterogeneity in driving taxon richness and β-diversity. Therefore, all calculated diversity indices pointed toward higher diversity and richness of Chironomidae in lowland catchment although the abundance of the midges was two-folds higher in highland catchment. The number of genera recorded in lowland catchment is 40 compared to 33 in the highland catchment. In this study, three subfamilies of Chironomidae were recorded from both catchments, Chironominae, Orthocladiinae and Tanypodinae. According to Ahmad et al. (2008), these subfamilies was common in the tropical rivers of Malaysia and they inhabited different type of habitat from the clean upper stream, to the polluted water of the downstream (Al-Shami et al. 2011; Ahmad et al. 2014). Not only in Malaysian rivers, Chironomidae subfamilies also common in other tropical rivers in different regions. For example, Sriariyanuwath et al. (2015) found that *Polypedilum* sp. was presence in high abundance in the tropical rivers of Thailand. *Polypedilum* and *Rheotanytarsus* were the most dominant genus recorded in this study. Restello et al. (2014) recorded highest *Polypedilum* and *Rheotanytarsus* and these two genera represent 40% of the total Chironomidae in tropical rivers of Brazil. These two genera can tolerate to wide range of disturbance including from the agricultural wastes. Nevertheless, *Polypedilum* and *Rheotanytarsus* were often indicated as pollutants tolerant taxa, including the pollution caused by the agricultural activities (Gresens et al. 2007; Macdonald & Taylor 2006). However, *Polypedilum* was recorded dominating clean ecosystems. Therefore, identification at the species level is crucial to distinguish intolerant species from tolerant species.

The most abundance genus from Orthocladiinae subfamily were *Tvetenia, Cricotopus*, *Rheocricotopus* and *Eukieferiella. Cricotopus*, *Rheocricotopus* were dominant in both catchments, whereas *Tvetenia* and *Eukieferiella* were only dominant in highland catchment. Syrovátka et al. (2009) reported in their study that the *Tvetenia* sp. and *Cricotopus* sp. as well as *Rheocricotopus* sp. dominated the rivers with higher velocity rivers. Meanwhile, *Cricotopus* sp. was common in tropical rivers (Rosa et al. 2013; Sriariyanuwath et al. 2015). Habitats in highland areas are typically characterized by fast-flowing streams, low temperatures, and coarse substrates and similar genera were recorded in this highland catchment.

In this study, Tanypodinae subfamily was unable to be identified at genus level due to insufficient taxonomic keys. Unlikely, the number of individuals of this subfamily was relatively high in both catchments. In the neighbour country of Thailand, the abundance of Tanyponidae was reported by Sriariyanuwath et al. (2015). However, this study found that among all the subfamilies, Tanyponidae was considered as less diversify compared to subfamilies Chironominae and Orthocladiinae and similar results were reported by Prat et al. (2014) and Matthew-Bird et al. (2016). With high density in both ecosystems, species-level identification is highly valuable for recognizing bioindicator species.

Physico-Chemical Parameters Influenced the Chironomidae Distribution

The water quality of streams in lowland and highland catchments are in excellent state and categorized in class 1 based on Water Quality Index (WQI). The WQI was not significantly differ between the two catchments except water temperature. Based on CCA analysis, water temperature in association with TSS exhibit significant influence on at lowland, but minimum effects at highland catchment chironomids (Figure 2). *Polypedilum* which abundant in both catchments may be represented by different species at different catchments due to different temperature preferences. *Polypedilum* at highland exhibited good relation with TSS and DO, but insignificant relationship with TSS at lowland catchment. In contrast, *Polypedilum* distribution in the lower altitude catchment was driven by the amount of dissolved oxygen. However, this pattern is common since this genus inhabited various conditions of the rivers and very adaptive to any change of the water physico-chemical parameters (Gresen et al. 2007). In this study, the positive association of *Rheotanytarsus* with pH and COD in high altitude catchment is inline with the findings by Gresen et al. (2007) and Restello et al. (2014) which foundt this genus occurs frequently in forested or agricultural rivers, that pH and COD are the important predictors in the river systems.

The PC-ORD analysis revealed distinct patterns in chironomid community composition between lowland and highland streams. Ordination using Non-metric Multidimensional Scaling (NMDS) produced clear separation between the two altitude groups, indicating that altitude is a major structuring factor for chironomid assemblages. Stress values from NMDS were within an acceptable range (<0.20), suggesting a reliable representation of community dissimilarities.At lowland catchment, Chironomini tribe, particularly *Polypedilum*, *Microtendipes* and *Chironomus* clustered strongly in the ordination space. These genera are often associated with higher organic enrichment and warmer water conditions, reflecting the environmental setting of lower streams. In contrast, highland catchment assemblages were characterized by abundance of Orthocladiinae subfamily that were *Tvetenia*, *Cricotopus* and *Rheocricotopus,* which known cooler, oxygen-rich, and fast-flowing water preference, and they contributed significantly to the ordination separation. Indicator Species Analysis (ISA) further supported these patterns. *Polypedilum*, *Cryptochironomus*, and *Chironomus* were identified as significant indicator taxa for low-altitude sites, whereas *Tvetenia*, *Cricotopus*, and *Eukiefferiella* emerged as strong indicators for high-altitude streams.

Overall PC-ORD analysis demonstrates that altitude significantly separating chironomid community structure, with Chironomini associated with lowland conditions and Orthocladiinae characterizing upland sites. These findings highlight the potential use of chironomid assemblages as potential bioindicators of environmental gradients in highland stream ecosystems. Diversity indices verified the ordination results. Although total density was higher at higher altitudes, the Shannon diversity index was slightly lower. This suggests that higher altitude communities were numerically dominated by a few cold-water specialists (e.g., *Tvetenia*), while lower altitude communities exhibited greater taxonomic balance among genera.

CONCLUSION

This study clearly demonstrates that altitude serves as a distinct determinant in the distribution and dominance of Chironomidae larvae. Streams in highland catchments are more strongly associated with the subfamily Orthocladiinae compared to Chironominae. Temperature was found to be a significant factor distinguishing between these two subfamilies. *Tvetenia*, *Cricotopus*, and *Eukiefferiella* serve as reliable biological indicators for Janda Baik and Cameron Highland ecosystems, whereas *Polypedilum*, *Cryptochironomus*, and *Chironomus* are better indicators for lowland catchments. The subfamily Tanypodinae exhibited comparable adaptability across both ecosystems, and identification at a lower taxonomic resolution is essential before definitive conclusions can be drawn. The same applies to the genus *Polypedilum*.This study proved that at the family level Chironomidae exhibit a broad tolerance range across both highland and lowland freshwater ecosystems. Therefore, taxonomic identification at the genus and species levels provides greater resolution for distinguishing differences in ecological tolerance associated with altitudinal gradients. Therefore, identification to species level is crucial to reveal a good biological indicator.

ACKNOWLEDGEMENTS

We express our heartfelt gratitude to various people involved in this study for their tireless help during the fieldwork. We are grateful to Department of Earth Science and Environment, Faculty Science and Technology, Universiti Kebangsaan Malaysia for providing laboratory facilities to conduct this research. We are also express our gratitude to Dr. Pete Cranston, Adjunct Professor of ANU, Australia and Emeritus Professor of University of California Davis, US for his great advice and guidance in chironomids identification.

AUTHORS DECLARATIONS

Funding Statement
This research received no external funding.

Conflict of Interest
The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

Ethic Declarations
Formal ethical approval was not required according to the institutional guidelines.

Data Availability Statements
The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Authors Contributions
Ahmad Abas Kutty served as the main author and was responsible for data analysis and preparation of the original manuscript. Siti Hafizah Ahmad was responsible for benthos sample collection. Wan Mohd Hafezul Wan Abdul Ghani contributed to data analysis. Zalela Abdul Aziz was responsible for benthos identification. All authors reviewed and approved the final manuscript.

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