

Highland Stream Chironomidae of Malaysia: Distribution and Environmental Assessment Potential

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Abstract Most chironomids ecological studies has focuses on lowland area and related to the pollution issues. This study examined the diversity of chironomids (Diptera: Chironomidae) in ten highland streams of Pahang, Malaysia, spanning elevations from 436 to 1,575 m. Fifty stations were sampled using a Surber net, with water quality parameters analyzed following APHA protocols. Water quality assessment based on the Malaysian Water Quality Index (WQI) showed all streams were clean (WQI 88–97; Class I–II). Although total suspended solids (TSS) varied significantly ($p < 0.05$), values remained within good water quality thresholds. Substrate composition, canopy cover, and water velocity also differed significantly between streams, reflecting distinct habitat conditions. A total of 4,859 chironomid larvae were collected, representing three subfamilies: Chironominae (49%), Orthocladiinae (44%), and Tanypodinae (7%). Twenty-nine genera were identified from Chironominae and Orthocladiinae, while Tanypodinae was identified only to the subfamily level. Chironominae, dominated by *Polypedilum* and *Rheotanytarsus* were more abundant at lower elevations, whereas Orthocladiinae, dominated by *Tvetenia*, *Cricotopus*, and *Rheocricotopus*, prevailed at higher altitudes. Canonical correspondence analysis (CCA) revealed altitude, substrate type, and TSS as key factors influencing the distribution. These findings provide the first comprehensive account of highland chironomids in Malaysia and highlight their potential as bioindicators for assessing tropical montane streams.

Keywords: Biological indicator, Ecological indices, Water quality, Biological monitoring.

Introduction

The Chironomidae is one of the largest families within the order Diptera, with more than 5,000 species recorded worldwide [14],[20] and constitutes more than 50% of the benthic macroinvertebrate community structure in freshwater ecosystems [10]. Chironomids are reported to have the widest distribution globally, occurring across all continents, with records ranging from an altitude of 5,600 m above sea level in the Himalayan Range, Nepal, to a depth of 1,642 m below sea level in Lake Baikal, Russia [18]. In addition, chironomids exhibit high diversity and abundance, inhabiting all types of aquatic habitats [12]. These characteristics have drawn the attention of researchers to highlight the potential of chironomids as biological indicators, providing valuable information for ecosystem assessment and river water quality monitoring.

Studies on the use of chironomids in biological monitoring programs of ecosystems have been widely conducted in European countries, the United States, and several Asian countries such as Japan and China. This represents a direct measurement of changes in water quality caused by certain factors, based on the presence or absence of a particular chironomid species or genus in a specific habitat. Baseline databases on chironomid diversity, taxonomy, and ecology have been developed for the

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Holarctic region [6], the United Kingdom [11], and Australia [13], providing essential information for evaluating the potential of chironomids as biological indicators in biological monitoring programs. Aquatic ecosystem assessments have been introduced alongside conventional methods because they can enhance the effectiveness of biological monitoring programs. Issues such as high operational costs can be addressed because the method does not require complex or expensive equipment and is highly cost-effective in the long term. In addition, this method is environmentally friendly as it does not require the use of chemicals that could disrupt the ecosystem of the study area.

The use of chironomids as biological indicators is less popular in the Asian region [22], although they have been widely employed as biological monitoring tools in North America and Europe [19],[26]. The greatest limitation in using chironomids as biological indicators in Malaysia is the lack of taxonomic keys for identification to the lowest taxonomic level, as well as insufficient information on the diversity and autecology of most chironomid species.

Several brief studies on chironomids have been conducted in Malaysia, such as a toxicity studies of heavy metals [30], and [15] on certain chironomid species. Research on the ecotoxicological monitoring of *Chironomus* spp. using a genotoxicological approach was conducted by [5]. Their studies focused only on the application of chironomids and not on taxonomy and diversity, especially in high altitude streams. Studies on chironomid diversity are very scarce, such as in paddy field areas and polluted ecosystems [4],[3] as well as in pristine ecosystems [1],[2]. This study was undertaken to estimate chironomid diversity and distribution within Malaysian highland ecosystems and bioassessment potential using chironomids.

Materials and Methods

The sampling areas in the highland region are located in the Titiwangsa Range, namely Janda Baik, Fraser Hill, and Cameron Highlands. The study was undertaken between 10th. September 2023. All three areas are well-known as highland resort destinations in Pahang State, Peninsular Malaysia. A total of 10 streams were selected, with six located in Janda Baik (03° 18' 29.3" - 03° 19' 49.7"N: 101° 49' 46.5" - 101° 53' 15.2"E), three in Cameron Highlands (04° 28' 45.8" - 04° 31' 10.7"N : 101° 22' 58.0" - 101° 24' 22.4"E), and one stream in Fraser Hill (03° 43' 38.5"-03° 43' 40.9"N:101° 42' 41.4" - 101° 42' 45.4"E). The geographical coordinates of each study area are presented in Table 1. A total of five sampling points were selected for each river, at an estimated 100–200m interval. Three replicates were collected for water and chironomids samples at each sampling point, representing both sides of the riverbank and midstream zone.

Stream water quality was measured in-situ for dissolved oxygen, temperature, conductivity, pH and TDS using a pre-calibrated YSI Pro meter and dissolved oxygen compensation was made based on atmospheric pressure (mmHg). A water sample was collected for BOD₅, COD, NH₄-N and orthophosphate analysis following HACH methods. A Surber net with a mesh size of 250 µm and a quadrat opening measuring 0.3 m × 0.3 m was used for chironomid sampling. The observational method was employed to assess physical and habitat characteristics such as river depth and width, the percentage and type of substrate composition, canopy shading, and riparian vegetation cover along the riverbanks [31],[17]. Environmental information was recorded in a habitat assessment form to support the water quality and biological data obtained.

The Malaysian Water Quality Index (WQI) was applied to evaluate the cleanliness level and to classify the water quality status of the studied streams. The integration of the six sub-indices of dissolved oxygen (siDO), biochemical oxygen demand (siBOD), chemical oxygen demand (siCOD), ammoniacal nitrogen (siAN), total suspended solids (siTSS), and pH (siPH) produced a single WQI value as shown below:

$$WQI = 0.22 (siDO) + 0.19 (siBOD) + 0.16 (siCOD) + 0.15 (siAN) + 0.16 (siTSS) + 0.12 (siPH)$$

where si represents the sub-index for each respective parameter

A one-way ANOVA statistical comparison test was conducted for comparison purposes using SPSS ver 21 software, and a multivariate CCA analysis was used to exhibit environmental factors on the chironomid presence using CANOCO ver 4.5.

Results and Discussion

Water Quality

The ten studied streams from Janda Baik, Fraser Hill, and Cameron Highlands exhibited distinct physical characteristics reflective of their respective altitudinal levels and surrounding land cover (Table 1). Streambed substrate composition varied considerably among sites, with cobble, pebble, and sand proportions (c:p:s) ranging from sand-dominated beds (e.g. Sg. Sum Sum, 0:10:90), coarse substrate (eg. Sg. Luruh (10:40:50) and Sg. Pauh (10:50:40) to intermediate substrates (e.g. Sg. Benus and Sg. Chemperoh, 30:30:40).

Water Temperature and pH

Water temperature showed a clear altitudinal gradient (Table 2). The lowest temperature was in Cameron Highlands (e.g. 17.9 °C at Sg. Pauh) and Fraser Hill (e.g. 18.4 °C at Sg. Bertam), whereas Janda Baik streams were warmer (up to 23.6 °C at Sg. Sum Sum). This pattern is expected, as higher elevations have cooler climates. In contrast, pH varied more with local conditions than altitude: Janda Baik tributaries tended to be slightly acidic (pH: 6.2–6.3 at Sg. Ceringin, Tampik, Sum Sum), whereas Fraser Hill and Cameron Highland sites were near-neutral (pH; 7.2–7.5). A few Janda Baik sites were just below the class I lower bound, although still within broader class II limits (6–9). In general the pH values indicate mostly unpolluted, natural conditions, with only mild acidity at some headwater sites.

Dissolved Oxygen

The DO concentrations were high throughout the network (6.9–8.7 mg/L), reflecting cold, clear-flowing water with excellent re-aeration. All but one site met the class I criterion (>7 mg/L). Sg. Bettam (Bukit Fraser) had a slightly lower DO (6.94 mg/L), placing it in class II (5–7 mg/L). In terms of saturation, most sites were well-oxygenated (>80%). A few mid-elevation streams (Sg. Bertam 73%, Sg. Pauh/Palas 76–77%) and within class III. The DO data indicates very good water quality and nearly all sites satisfy National Water Quality Standard (class I) for DO concentration.

BOD₅ and COD

Both biological and chemical oxygen demand were uniformly low across all sites. BOD₅ ranged only 0.3–0.8 mg/L, well within the class I limit of 1 mg/L. Likewise, COD was typically 2–6 mg/L far below the class I cut-off of 10 mg/L. These low values indicate minimal organic in these forest streams. In other words, the water has a very low organic load, consistent with pristine conditions. There is no evidence of elevated organic stress and all sites clearly meet class I quality.

Table 1. Physical characteristics of studied streams

Physical Charac.	Janda Baik						Fraser Hill	Cameron Highlands		
	Sg. Benus	Sg. Chemperoh	Sg. Luruh	Sg. Ceringin	Sg. Tampik	Sg. Sum Sum	Sg. Jeriau	Sg. Bertam	Sg. Pauh	Sg. Palas
Substrate *c:p:s	30:30:40	30:30:40	10:40:50	20:50:30	10:50:40	10:40:50	10:30:60	0:10:90	10:50:40	20:40:40
Canopy (%)	<30	<50	>70	30-40	60-70	<70	>70	<20	>80	0
Depth (m)	0.2-0.5	0.1-0.4	0.1-0.4	0.2-0.5	0.1-0.4	0.2-0.4	0.2-0.4	0.1-0.3	0.1-0.2	0.2-0.4
Width (m)	7-9	2-5	4-7	3-5	2-3	2-5	2-6	1-1.5	1.5-4	1-1.5
Flow (m/s)	0.51 ± 0.08	0.65 ± 0.11	0.47 ± 0.33	0.27 ± 0.03	0.23 ± 0.02	0.26 ± 0.05	0.4 ± 0.07	0.07 ± 0.15	0.4 ± 0.07	0.34 ± 0.17
Altitude (m)	436-445	475-482	445-482	452-465	447-459	440-457	988-1002	1490-1536	1454-1484	1562-1575

Table 2. Water quality across ten studied highland streams

District	Stations	Water temp. (°C)	pH (mg/L)	DO (mg/L)	BOD ₅ (mg/L)	COD (mg/L)	TSS (mg/L)	NH ₃ -N (mg/L)
Janda Baik	Sg. Chemperoh	22.4 ± 0.01	6.85 ± 0.08	8.11 ± 0.12	0.43 ± 0.22	4.94 ± 0.27	29.53 ± 7.64	0.19 ± 0.09
	Sg. Benus	22.9 ± 0.04	7.02 ± 0.05	7.25 ± 0.09	0.78 ± 0.36	5.18 ± 0.30	28.15 ± 9.78	0.18 ± 0.02
	Sg. Luruh	21.9 ± 0.04	6.98 ± 0.10	8.21 ± 0.27	0.47 ± 0.21	4.83 ± 0.39	18.29 ± 6.41	0.21 ± 0.02
	Sg. Cheringin	22.5 ± 0.06	6.26 ± 0.01	7.49 ± 0.21	0.39 ± 0.06	1.88 ± 0.62	4.05 ± 1.04	0.03 ± 0.01
	Sg. Tampik	22.5 ± 0.1	6.26 ± 0.01	7.34 ± 0.07	0.32 ± 0.07	2.93 ± 0.68	10.45 ± 3.99	0.01 ± 0.01
Fraser Hill	Sg. Sum Sum	23.6 ± 0.14	6.25 ± 0.01	7.71 ± 0.15	0.36 ± 0.03	2.21 ± 0.78	20.65 ± 10.34	0.11 ± 0.02
	Sg. Jeriau	21.9 ± 0.18	7.49 ± 0.11	7.39 ± 0.24	0.61 ± 0.29	4.28 ± 2.21	37.37 ± 13.73	0.21 ± 0.08
Cameron Highland	Sg. Bertam	18.4 ± 0.05	7.19 ± 0.11	6.9 ± 0.23	0.49 ± 0.44	5.92 ± 4.53	29.41 ± 17.46	0.23 ± 0.16
	Sg. Pauh	17.9 ± 0.05	7.24 ± 0.03	7.23 ± 0.05	0.41 ± 0.06	6.01 ± 1.14	5.14 ± 2.25	0.09 ± 0.04
	Sg. Palas	19.2 ± 0.09	7.24 ± 0.06	7.13 ± 0.10	0.64 ± 0.06	5.37 ± 1.26	3.09 ± 0.85	0.10 ± 0.02

TSS and Ammonia Nitrogen

TSS and ammonia showed the greatest variability and indicated the only substantive water-quality concerns. Most sites had very low TSS (<20 mg/L), but three exceeded the class I threshold (<25 mg/L). Notably, Sg. Jeriau (Fraser Hill) had TSS 43 mg/L (class II), and two Janda Baik streams were 28–30 mg/L (class II)(Table 2). These spikes suggest local sediment inputs, perhaps from bank erosion, trail runoff or nearby agriculture. In general, however, most streams had TSS in the class I range, indicating good water quality. Ammonia-nitrogen was also generally low. Most sites had ammonia nitrogen <0.1 mg/L (class I). A few streams were slightly higher (Sg. Luruh, Sg. Chemperoh, Sg. Benus 0.18–0.21 mg/L; Sg. Jeriau 0.21; Sg. Bertam 0.23 mg/L), placing them in class II (0.1–0.3 mg/L). Overall, the ammonia nitrogen levels are low compared to polluted rivers, but any value >0.1 mg/L indicates a slight departure from the most pristine class I standard.

Overall, all the studied streams can be classified as clean and free from significant pollution elements. Although the one-way ANOVA indicated significant differences for certain parameters among the studied streams, the observed values still remain below the threshold for a clean aquatic ecosystem. The findings show that the studied highland streams have a water quality index (WQI) within class I to II, indicating good and clean water quality, free from significant pollution elements, and capable of supporting sensitive aquatic life. In particular, DO, BOD, and COD are uniformly class I at every station. Only few sites with elevated TSS or NH₃-N. Only dissolved oxygen in Sg. Bertam’s exhibits the lowest quality. Overall, all studied highland streams are mostly pristine and only signed by slightly low quality of turbidity and ammonia in a few locations.

Chironomidae Distributions and Composition

A total of 4,859 chironomid individuals were successfully sampled from the 10 study streams (Table 3). Three Chironomidae subfamilies consisted of Chironominae, Orthoclaadiinae, and Tanypodinae dominated studied streams (Figure 1). The subfamily Chironominae was found to dominate streams located in lower altitude areas, namely the Janda Baik. In contrast, the subfamily Orthoclaadiinae dominated streams in higher altitude areas, Fraser Hill and Cameron Highlands. Chironominae represent 49% of total collected, followed by Orthoclaadiinae (44%), and Tanypodinae (7%). A total of 14 genera from the subfamily Chironominae and 15 genera from the subfamily Orthoclaadiinae were successfully identified. However, 315 individuals from the subfamily Tanypodinae could not be identified to the genus level due to the lack of taxonomic references for the larval stage.

A total of 2,017 individuals from the tribe Chironomini (Subfamily: Chironominae) and 374 individuals from the tribe Tanytarsini (Subfamily: Chironominae) were recorded across the study sites. Within

Chironomini, ten genera were identified, and four of which belonged to the non-Harnischia complex, namely *Polypedilum*, *Chironomus*, *Glyptotendipes*, and *Microtendipes*. *Polypedilum* was the most dominant genus in this group, accounting for 1,927 individuals (98% of the non-Harnischia complex) (Figure 2).

In contrast, the Harnischia complex was dominated by *Cryptochironomus* (20 individuals; 43% of the Harnischia complex) and *Harnischia* (17 individuals; 30%). For the tribe Tanytarsini, *Rheotanytarsus* was the overwhelmingly dominant genus, comprising 351 individuals (94% of the total Tanytarsini composition). The remaining 6% consisted of *Cladotanytarsus* (14 individuals), *Tanytarsus* (8 individuals), and *Virgatanytarsus* (1 individual) (Figure 2).

The subfamily Chironominae was the most dominant and abundant chironomid group in the Janda Baik streams, which are located at relatively lower altitudes. A total of 1,768 Chironominae individuals were recorded, accounting for 63% of all chironomids collected in the area. Within this subfamily, *Polypedilum* was most prevalent in Sungai Benus, with 796 individuals recorded, representing 41% of the total *Polypedilum* collected. Second abundant represented by *Cricotopus* (Subfamily:Orthoclaadiinae) dominated in Sungai Cheringin and Sungai Tampik (S5) with 427 individuals, comprising 69% of the total *Cricotopus* abundance. The subfamily Tanypodinae was less common, with the highest number (113 individuals, 36% of total Tanypodinae) recorded in Sungai Cheringin .

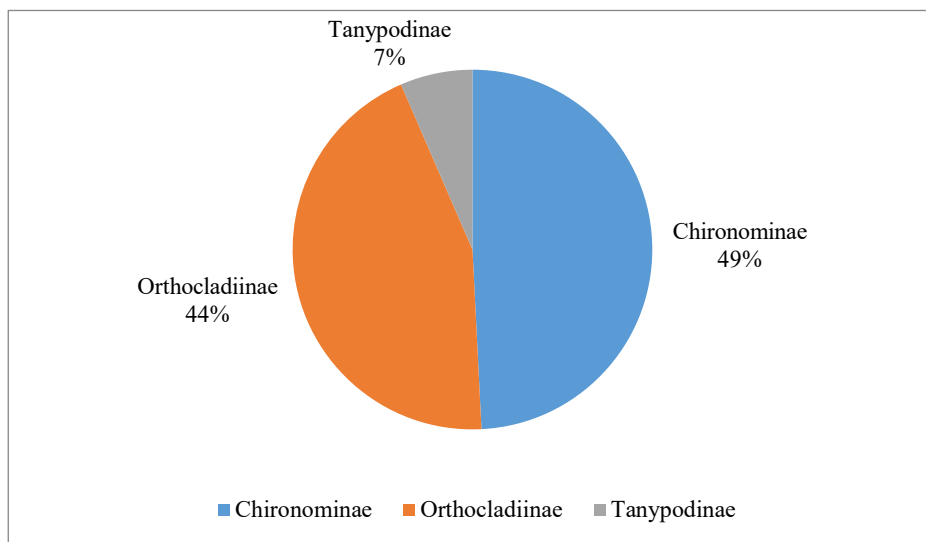


Figure 1. Subfamily composition dominating highland ecosystems

Overall, Chironomidae distribution patterns were uneven across streams, largely due to the dominance of specific genera such as *Polypedilum* and *Cricotopus*. Taxa richness comparisons revealed that Sungai Benus exhibited the highest richness (14 taxa), followed by Sungai Tampik, Sungai Cheringin, Sungai Luruh, Sungai Sum Sum, and Sungai Chemperoh. Similarly, Sungai Benus recorded the highest overall abundance of Chironomidae (952 individuals), followed by Sungai Cheringin, Sungai Sum Sum, Sungai Luruh, Sungai Tampik, and Sungai Chemperoh.

Sungai Jeriau, located in Fraser Hill, has a higher altitude than Janda Baik and exhibits an almost uniform distribution pattern along the stream. The numbers of the subfamilies Chironominae, Orthoclaadiinae, and Tanypodinae obtained were not significantly different, however, Orthoclaadiinae subfamily dominated the study area with a composition of 55% replacing Chironominae subfamily. *Rheocricotopus* was the most dominant genus with 18 individuals, followed by the subfamily Tanypodinae with 17 individuals, and the genus *Polypedilum* with 13 individuals. Although Sungai Jeriau showed uniform distribution of Chironomidae, it also recorded the lowest taxa richness and abundance, with eight taxa and a total of 82 individuals.

The subfamily Orthoclaadiinae was the predominant and most widely distributed chironomid group in the high altitude streams within the Cameron Highlands, except for the Bertam River, which was dominated by *Polypedilum* (Figure 3). Previous study has reported that *Polypedilum* is highly tolerant to organic matter content [8],[21]. The Sg. Bertam has relatively high ammonia nitrogen and TSS values, as well

as the lowest dissolved oxygen value among all the studied streams, due to its slow current velocity and anthropogenic potential from agricultural activities. Therefore, the composition of *Polypedilum* is relatively high compared to Orthocladiinae genera. In total, 1,318 Orthocladiinae individuals were recorded from Cameron Highlands rivers, accounting for 67% of the total chironomid specimens collected from this area. Orthocladiinae are known to be well-adapted to low temperatures (0–25 °C) and are typically associated with highland environments [7].

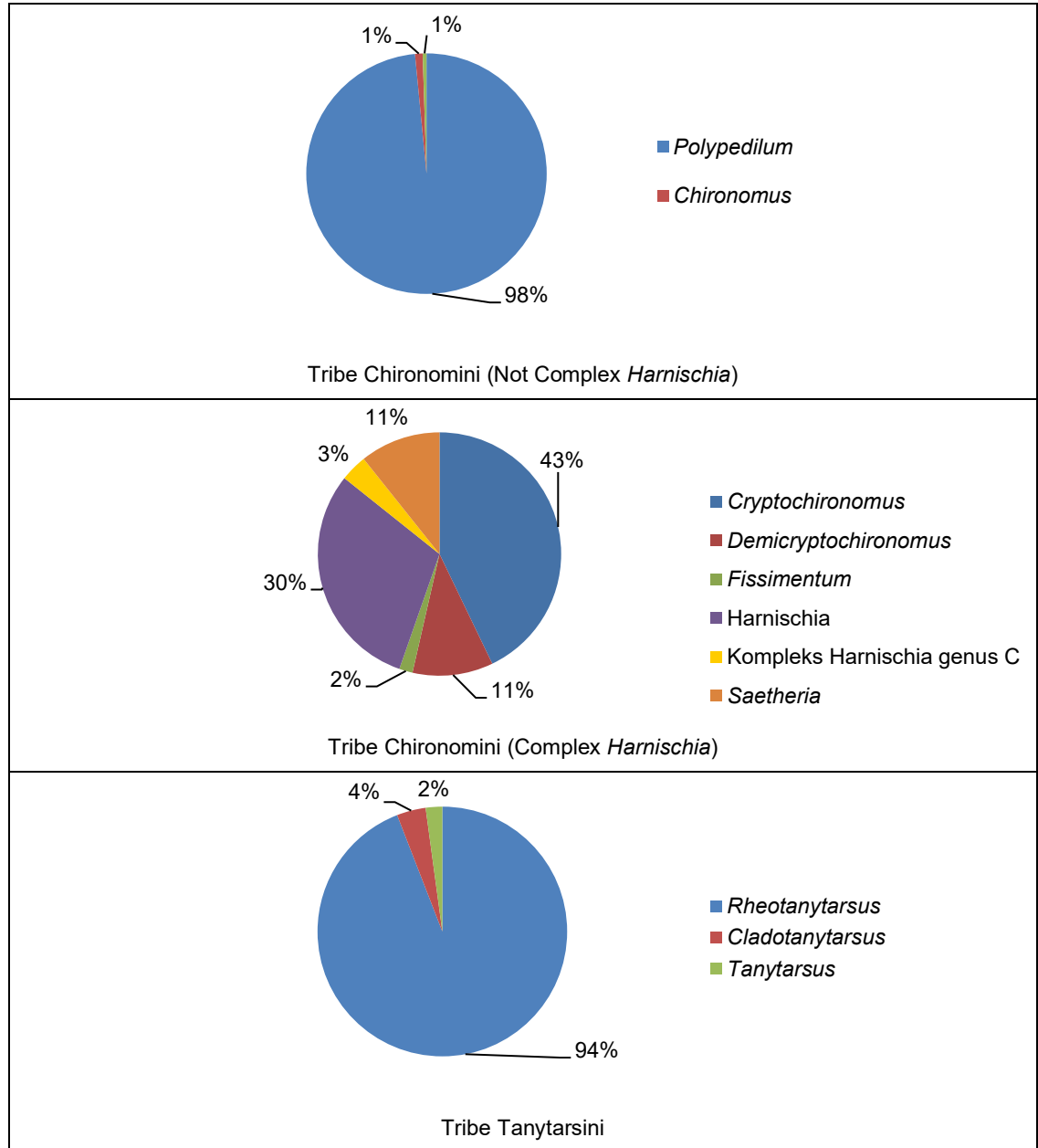


Figure 2. Chironominae subfamilies proportions

Table 3. Distribution and abundance of Chironomidae across highland ecosystems

	Janda Baik					Fraser Hill	Cameron Highland			Total	
	Sg. Benus	Sg. Chemperoh	Sg. Luruh	Sg. Cheringin	Sg. Tampik	Sg. Sum Sum	Sg. Jeriau	Sg. Bertam	Sg. Pauh		Sg. Palas
Subfamili : Chironominae											
Tribe : Chironomini											
Genus :											
<i>Polypedilum</i>	796	126	254	167	36	211	13	142	48	134	1927
<i>Cryptochironomus</i>	3	0	1	15	0	4	0	0	1	0	24
<i>Chironomus</i>	2	0	1	0	0	0	0	1	16	0	20
<i>Demicryptochironomus</i>	0	0	0	0	0	0	0	0	5	1	6
<i>Fissimentum</i>	0	0	0	0	1	0	0	0	0	0	1
<i>Glyptotendipes</i>	0	0	0	2	1	1	0	0	0	0	4
<i>Harnischia</i>	0	0	0	2	2	0	1	0	2	10	17
Kompleks <i>Harnischia</i> genus C	1	0	0	0	0	0	0	0	1	0	2
<i>Microtendipes</i>	2	1	0	5	1	0	0	0	1	0	10
<i>Saetheria</i>	5	0	0	0	0	1	0	0	0	0	6
Tribe : Tanytarsini											
Genus :											
<i>Rheotanytarsus</i>	28	5	23	11	22	20	5	19	164	54	351
<i>Cladotanytarsus</i>	2	1	0	7	4	0	0	0	0	0	14
<i>Tanytarsus</i>	0	0	0	1	1	1	1	0	4	0	8
<i>Virgatanytarsus</i>	0	0	0	0	1	0	0	0	0	0	1
Subfamili : Orthoclaadiinae											
Genus :											
<i>Cricotopus</i>	3	2	11	375	54	13	2	24	4	138	626
<i>Rheocricotopus</i>	66	14	13	19	43	124	18	0	129	9	435
<i>Tvetenia</i>	0	0	0	0	0	0	8	1	348	435	792
<i>Eukiefferiella</i>	13	0	16	1	0	1	7	5	5	70	118
<i>Bryophaenocladus</i>	0	0	1	0	0	0	0	0	0	0	1
<i>Cardiocladius</i>	2	0	1	4	1	0	0	0	0	23	31
<i>Heleniella</i>	0	0	1	0	0	0	0	0	5	0	6
<i>Limnophyes</i>	0	0	0	0	0	0	0	0	0	2	2
<i>Paracricotopus</i>	0	0	0	0	0	0	0	4	28	56	88
<i>Parametriocnemus</i>	6	1	0	0	0	1	3	0	9	3	23
<i>Paratrissocladus</i>	0	0	0	0	0	0	0	0	8	0	8
<i>Pseudorthocladus</i>	0	0	1	0	0	0	1	0	0	0	2
<i>Onconeura</i>	0	0	0	0	0	0	0	0	1	0	1
<i>Synorthocladus</i>	0	0	0	0	0	0	0	0	3	0	3
<i>Thienemanniella</i>	0	1	0	0	0	0	6	1	0	3	11
Orthoclaadiinae genus 1	1	0	0	0	0	0	0	0	0	0	1
Orthoclaadiinae genus 2	0	0	0	0	0	1	0	0	0	0	1
Orthoclaadiinae genus 3	0	0	0	0	0	0	0	0	0	4	4
Subfamili : Tanypodinae											
Total Individu	952	194	338	722	194	414	82	197	822	944	4859
Number of Taxa	14	8	11	12	12	11	11	8	19	14	

The genus *Tvetenia* was particularly dominant in the Pauh River and Palas River, with 783 individuals representing 99% of the total *Tvetenia* composition. Other Orthoclaadiinae genera recorded exclusively from Cameron Highlands streams, including *Limnophyes*, *Paracricotopus*, *Paratrissocladius*, *Onconeura*, and *Synorthocladus*. The restricted distribution of these genera may be attributable to a combination of environmental and ecological factors unique to the highland streams. Chironomids patterns of distribution within Cameron Highlands streams were generally uneven due to the marked dominance of *Tvetenia* and *Polypedilum*. Taxa richness was highest in the Pauh River, with 18 recorded taxa, followed by the Palas River and the Bertam River. In terms of abundance, the Palas River supported the largest number of individuals (944), followed by the Pauh River and the Bertam River. *Tvetenia* is consistently associated with cold, well-oxygenated, rocky headwater streams rich in epilithic algae. In Mediterranean montane streams, [24] found that *Tvetenia* species (*T. discoloripes* and *T. bavarica-calvescens*) were indicator taxa of siliceous headwaters characterized by low temperatures and torrential flow. Likewise, they also reported that *Tvetenia* larvae (*T. calvescens/bavarica*) predominated in stable non-glacial alpine reaches (with coarse substrates and abundant periphyton) rather than in extreme glacial zones.

More broadly, *Tvetenia* larvae are typical orthoclad grazers of stream biofilms. Orthoclaadiine midges like *Tvetenia* dwell on stones and vegetation in flowing water and feed by scraping epilithic algae. In agreement with this, [23] emphasize that *Orthocladus/Cricotopus* (close relatives of *Tvetenia*) dominate the benthos as periphyton scrapers. In practical terms, *Tvetenia* and allied orthoclads often dominate the insect community in pristine high-elevation riffles, but their abundances decline in warmer or nutrient-enriched conditions.

Previous research has highlighted the strong influence of environmental factors on the distribution and composition of chironomid assemblages. Factors such as altitude and water temperature [16], substrate type [27], canopy cover [25], and the presence of aquatic vegetation and mosses [9],[28] have all been shown to shape chironomid communities.

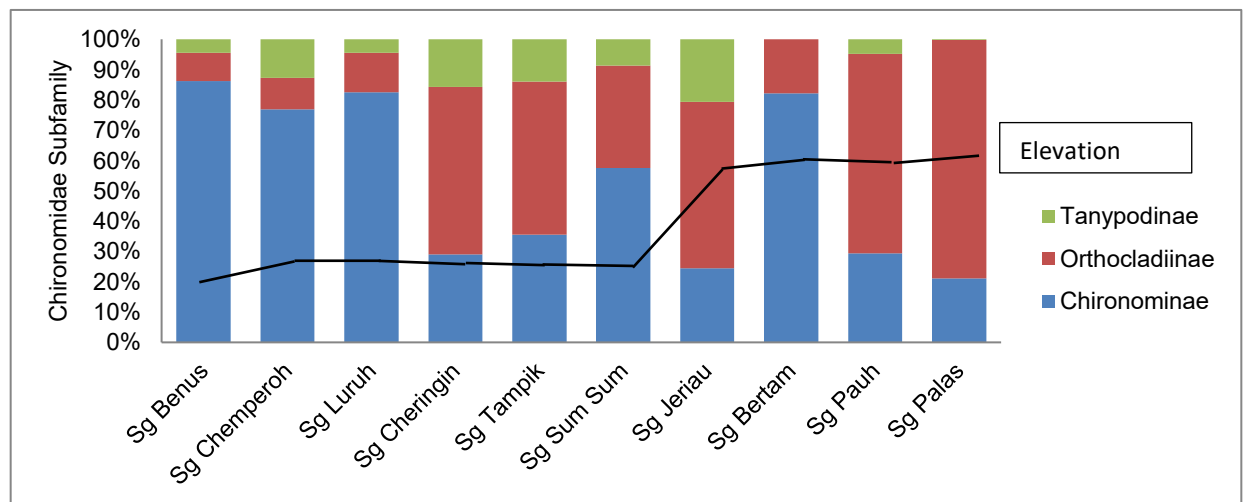


Figure 3. Chironomidae subfamilies distributions along the gradient

In the present study, water quality among the surveyed rivers appeared relatively homogeneous, with no significant differences detected except for total suspended solids (TSS). However, one-way ANOVA results revealed significant variation ($p < 0.05$, $\alpha = 0.05$) in physical habitat features, including substrate composition, canopy cover, and flow velocity. These findings suggest that while water chemistry (pristine quality) may not be a major differentiating factor, habitat heterogeneity plays a key role in structuring benthic assemblages across highland streams. The Canonical Correspondence Analysis (CCA) result demonstrated that environmental variables exerted strong influences on the distribution of chironomid groups across the study sites. Two distinct grouping patterns were observed, one primarily associated with altitude, and another influenced by nutrient concentrations in the water column (Figure 4). Among these factors, altitude emerged as the most dominant driver, influencing nearly half of the chironomid recorded. This strong effect is attributable to the markedly different elevations among the three study streams. Specifically, ten out of fifteen genera belonging to the subfamily Orthoclaadiinae (*Tvetenia*, *Paracricotopus*, *Eukiefferiella*, *Thienemanniella*, *Cardiocladus*, *Limnophyes*, *Onconeura*, *Synorthocladus*, *Paratrissocladius*, and *Heleniella*) were significantly structured by altitude. These

findings corroborate the observations by [32], who reported increased Orthoclaadiinae diversity with rising elevation. The present study further demonstrates that altitude is a key determinant shaping the distribution of specific Orthoclaadiinae taxa in highland ecosystems.

Chironomids exhibit a broad spectrum of environmental tolerance, from pristine to heavily disturbed ecosystems [29]. Owing to this ecological versatility, chironomids have gained significant attention from researchers worldwide as reliable biological indicators of environmental conditions. They are now widely incorporated into biological monitoring programmes globally, with their use as bioindicators being actively promoted in developed countries [25]. In the context of Malaysian highland streams, *Polypedilum* is an excellent bioindicator for lower altitude streams (Janda Baik and Fraser Hill) considering it consistently present across all studied streams. However, *Cricotopus* and *Tvetenia* (Subfamily:Orthoclaadiinae) appear to have strong potential as indicators of higher altitude stream (Cameron Highland) as both were exhibits domination in few stations. Nevertheless, it is recommended that *Polypedilum* be identified to the species level, as different species within this genus may exhibit varying levels of tolerance to water quality, reflecting its broad ecological distribution. By contrast, *Tvetenia* may serve as a more specific indicator of high altitude, unpolluted rivers with cooler water temperatures, given its restricted occurrence in elevated environments such as Fraser's Hill and Cameron Highlands.

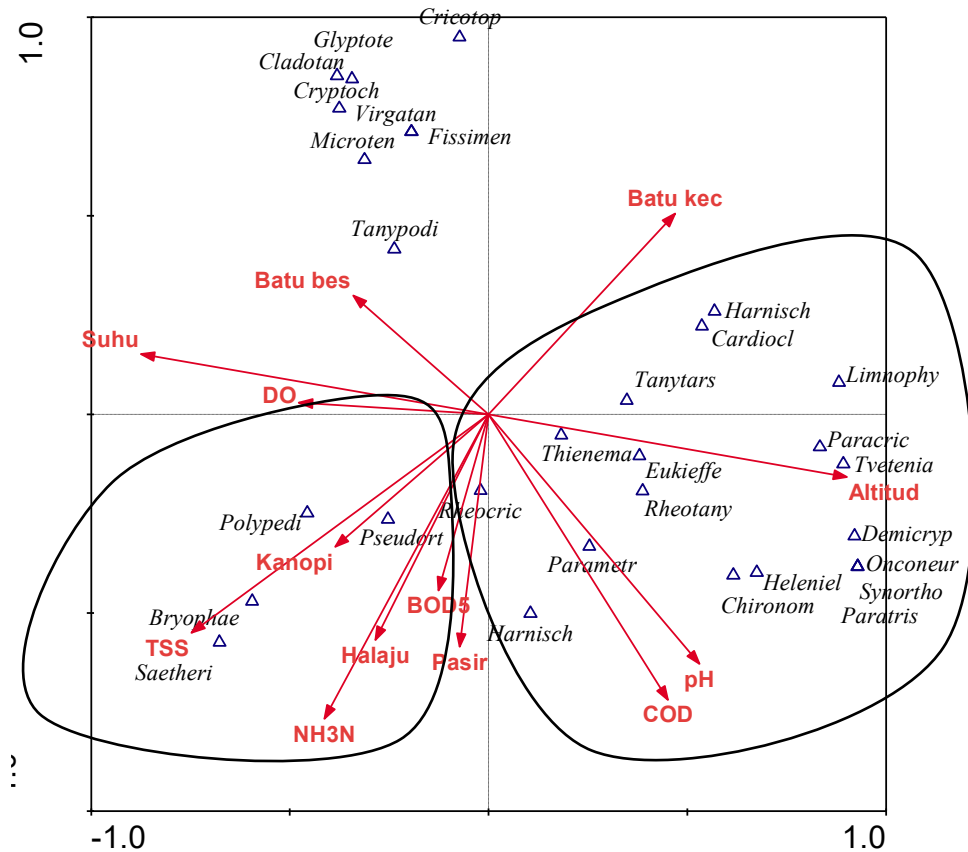


Figure 4. Environmental component factors shaping chironomids presents

Conclusion

This study successfully achieved its objectives by demonstrating the applicability of chironomids, for highland stream assessment in Malaysia. All ten streams surveyed across three highland regions of differing altitudes were classified as having good water quality based on the Malaysian water quality standards. Community composition varied with altitude, with Orthocladiinae dominating higher-altitude sites such as Fraser Hill and Cameron Highlands, whereas Chironominae dominated the lower altitude site of Janda Baik. Multivariate CCA analysis highlighted the environmental drivers of dominant taxa were altitude, sandy substrates, and total suspended solids (TSS). These findings emphasize the strong potential of family and genus of chironomid assemblages as reliable bioindicators for monitoring and managing highland stream ecosystems in Malaysia. Ultimately, this research underlines the importance of *Polypedium* (Subfamily: Chironominae) and *Tvetenia* (Subfamily: Orthocladiinae) as biological indicators for highland ecosystem and ecologically meaningful tool for sustaining biodiversity and guiding conservation strategies in fragile highland environments.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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