Chironomidae (Order: Diptera) diversity in relation to water quality of highland rivers at Cameron Highlands, Malaysia

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Abstract

Aim: This study aims to determine Chironomidae diversity and distribution in three small rivers at Cameron Highlands in relation to their response to water quality conditions.

Methodology: Fifteen sites from three rivers (Palas River, Pauh River and Bertam River) were chosen for water quality measurements and Chironomidae sampling. Water quality was analysed following APHA standard methods and chironomid was identified to genus level. The rivers were classified based on Malaysian's Water Quality Index (WQI) and macroinvertebrates diversity and distribution was analysed using diversity indices. Comparison between sites and river was undertaken using one-way ANOVA and chironomid appearance in relation to water quality was evaluated using Pearson correlation test.

Results: Chironomidae (Order: Diptera) distribution at high altitude rivers in Malaysia is not well documented although this group of insect has a wide range of distribution in tropical region. Based on Malaysian water quality index (WQI), result shows all rivers were in Class I. Although still in Class I, Bertam River has slight lower water quality due to higher ammoniacal nitrogen and total suspended solids. The diversity indices also demonstrate that Bertam Rivers was in stress conditions, and other two were in moderate conditions. Three subfamilies (Chironominae, Orthocladiinae and Tanypodinae) were recorded, which comprises of 1963 individuals. *Tvetenia* (Subfamily: Orthocladiinae) was the most dominant but only dominated Palas River and Pauh River. Another genus from Orthocladiinae (*Crocotopus* and *Rheocrocotopus*) also recorded dominant only in Palas River and Pauh River, but lesser in Bertam River. Only *Polypedilum* (Subfamily: Chironominae) which was second highest recorded, dominated in all rivers, even highest in Bertam River. Amongst dominant genus, only *Polypedilum* was found dominant in all rivers, others were only dominating Palas River and Pauh River only.

Interpretation: This findings exhibit wide range of adaptations and potential use of *Polypedilum* as biological indicator. Due to availability of incomplete keys, identification to species level cannot be undertaken. However, this study reveals thirteen different sub-group of *Polypedilum* based mouthpart organs, which represent different species for tropical ecosystems.

Key words: Biological indicator, Chironomids, Diversity indices, Freshwater, *Polypedilum*

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Introduction

Chironomids has very high diversity and composition in freshwater ecosystems and more than 15000 species been recorded (Beermann *et al.*, 2018). Chironomids exhibit wide range of adaptations to various types of habitat and water quality differences, therefore been recorded in most types of freshwater ecosystems (Serra *et al.*, 2017). Chironomids compositions reported to represent one-third of world insect and most abundant in Holartic region.

Information of chironomids in Neotropical and Oriental regions was limited. However, several intensive studies on chironomids distribution in China, Japan and India were conducted, yet lack of taxonomic keys. Malaysia is one of the countries located within Oriental region and chironomids distribution was very scarce due to heterogeneity of the habitat types. There are four chironomids subfamilies reported in Malaysia namely Chironominae, Orthoclaadiinae, Tanypodinae dan Diamesinae. First Chironomidae recorded in Malaysia was *Pentaneura batuensis* (Tanypodinae:subfamily), *Eusmittia cavernae* (Orthoclaadiinae), *Paratendipes inarmatus* (Chironominae:subfamily) by Freeman, (1962) on his research at Batu Cave Selangor. Sub-family Diamensinae known to adapt to cooler region and Willassen (1988) reported *Diamesa cranstoni* at Kinabalu Mountain ecosystem at 3000m asl. This finding proved possibility of discovering another species in Malaysia within highland ecosystems.

In Malaysia, studies on chironomids concentrated on the midges as bio-indicator to estimate the quality of the freshwater ecosystem (Prabhakaran *et al.*, 2017; Azmi *et al.*, 2016, Ahmad and Hanisah, 2018; Norhafizah-Azwa *et al.*, 2018; Ahmad and Siti Hafizah 2017). Only few studies were carried out to estimate the chironomids diversity such as Al-Shami *et al.* (2010a, 2010b, 2011) in paddy field, Ahmad *et al.* (2008, 2014) and Appalasamy *et al.*, 2018) in pristine rivers. Meanwhile, Zalela (2014) undertook a comprehensive study on chironomids diversity and distributions within upstream of Langat River in Selangor.

Chironomids distribution in highland ecosystems in Malaysia is not well documented. Malaysia has few aesthetic highland ecosystems such Genting Highland, Frasier Hill and Cameron Highlands. For instance, Cameron Highlands is assertively developed for tourism attractions and at the same times practiced agronomic practices for tea plantations and cash crops plants such as strawberry and vegetables. This study was conducted to fulfil information gap regarding chironomids diversity within highland ecosystems. Cameron Highlands have approximately 123 tributaries that flow water into three main river namely Telom River, Bertam River and Lemoi River. Those streams and rivers provide sufficient habitat for aquatic macroinvertebrates.

Materials and Methods

Study sites: This study was conducted at two tributaries namely Pauh River and Palas River and Bertam River, which is one of

main river in in Cameron Highlands. Even though only upstream of Bertam River was covered in this study, the study area is affected by many land use activities. Bertam River flows through various types of land use such agriculture (strawberry, flower, cash crops), residential and tourism activities. which within order 2 and 3 river size. Pauh River (order 2) is flowing through protected forest that managed by Forestry Department. This river receives minimum anthropogenic impact compared to Bertam River and being used as recreational river. Since located within protected forest, the river has high canopy cover (app. 80%). Palas River is an order 2 tributary flows downstream through tea plantation before confluent to Telom River. This river located within tea plantation area and therefore has lack of canopy cover. Geographical coordination and general physical characteristics of those rivers are presents in Table 1.

Water quality: Water quality has direct influence on chironomids present and distributions. *In situ* water quality measurements were undertaken for dissolved oxygen, pH and water temperature using pre-calibrated YSI Professional Series made in USA. Water samples were collected for chemical oxygen demand (COD), suspended solids (TSS) and ammoniacal-nitrogen analysis. A PVC coated BOD bottle was used to collect river water for biochemical oxygen demand (BOD₅) analysis. All water quality analyses were undertaken following standard method APHA (2012). The Malaysian water quality index proposed by department of environment the water quality was used to determine rivers water quality.

Chironomids: Surber net (dimension 0.3 m x 0.3 m, pore size 250 µm) was used to collect chironomids in all stations due to shallow water. Collected materials were rinsed few times and preserved with ethanol (70%) in sampling plastic bags. Five sampling stations were selected from each river and three replicates were collected from each station. In laboratory, samples were rinsed with tap water to remove preservative agent and chironomids samples were manually pickup using soft fine forceps and kept in 90% ethanol. Chironomids samples were soak in KOH solution for 20 min and then neutralised with acetate acid. All samples were then prepared into permanent slide following Andersen *et al.* (2013). Chironomid samples were identified to the lowest taxonomic possible using Andersen *et al.* (2013), Cranston (2004;2013) and Epler (2010; 2014).

Data analysis: Malaysian water quality index (WQI) was calculated using six selected formula and water quality comparison between rivers were examined using one-way ANOVA. The WQI formula is as below.

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

si = sub-index

Table 1 : Geographical coordination and physical characteristics of studied rivers

Rivers	Bertam river	Pauh river	Palas river
Substrates *c:p:s	0:10:90	10:50:40	20:40:40
Canopy (%)	<20	>80	0
Water Depth (m)	0.1–0.3	0.1–0.2	0.2–0.4
River Width (m)	1–1.5	1.5–4	1–1.0
Velocity (m s ⁻¹)	0.7 ± 0.15	0.4 ± 0.07	0.34 ± 0.17
Altitude (m)	1490–1536	1454–1484	1562–1575
Geography Coordination	101°23'09.3" E04°28'43.4" N	101°253'87.6" E04°28'89.0" N	101°25'22.5" E04°31'69.0" N

*Note: c ('cobble') = large stone, p = pebble, s = sand

The Shannon diversity index was calculated to estimate chironomid richness and composition in highland rivers. The Pielou Evenness Index and Margalef Richness Index were also calculated to exhibits domination and evenness within studied rivers. The similarity and differences of indices between rivers were tested using Pearson correlation and one-way ANOVA after confirmation of normal distributions respectively.

Results and Discussion

Water quality: In average, the water temperature for all rivers was $17.9 \pm 0.05^\circ\text{C}$. highest water temperature was detected at Palas River that located in tea plantation. With small water body and least canopy cover make highest water temperature in Palas River. Similar result exhibits by Bertam River as second highest in river water temperature (Table 2). Water temperature at highland river obviously much lower than lowland rivers, which normally ranges between 25°C to 30°C . Altitude with canopy cover and water depth determine river water temperature. For example, water temperature of lowland river was ranging between 26°C and 27°C (Fawaz et al., 2013 and Ahmed et al., 2020). Similar pattern was obtained for organic substances parameters such as biochemical oxygen demand (BOD_5) and COD concentrations. Based on Malaysian National Water Quality Standard, both parameters classify all rivers into Class I. The total suspended solids (TSS) in all rivers also low and classified all rivers in class I. However, even though still in class I, Bertam River exhibits slightly lower quality compared to others especially on TSS. As main tributary, this river receives water from smaller order tributaries which carry high TSS concentrations due to assertive agriculture activities. The one-way ANOVA test indicates insignificant different each parameter between rivers ($p > 0.05$). Ammoniacal nitrogen was detected highest in Bertam River which expected due to agriculture wastes.

The Malaysian Water Quality Index (WQI) was calculated based on six selected parameters and results were presented in Fig. 1. Pauh River and Palas River were classified as in Class I based on Malaysian Water Quality Standard (NWQS), but Bertam River was in Class II due to high TSS concentration in water body. However, WQI index value (87.5), indicates all studied rivers are in clean condition includes Bertam River even though contains TSS concentrations. Uncontrolled land opening for agriculture predicted to cause high sedimentation in Bertam River especially after rain.

Chironomidae composition and distribution: A total of 1963 chironomid individual were recorded which consists of three sub-families and 29 genus. Chironomidae represented by three subfamilies namely Chironominae, Orthocladiinae and Tanypodinae. Subfamily Chironominae characterized by two tribes Chironomini and Tanytarsini with 325 and 241 individuals respectively. Subfamily Orthocladiinae represented by 15 families (1315 ind) and subfamily Tanypodinae was not identified to lower taxa due to insufficient key available and 42 individuals

Fig. 1 : WQI comparison between three rivers**Table 2:** Water quality for three studied rivers in Cameron Highlands

River	Temp. ($^\circ\text{C}$)	pH (mg l^{-1})	DO (mg l^{-1})	BOD_5 (mg l^{-1})	COD (mg l^{-1})	TSS (mg l^{-1})	$\text{NH}_3\text{-N}$ (mg l^{-1})
Bertam River	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
Pauh River	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
Palas River	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

were recorded. Orthoclaadiinae and Chironominae exhibit quite similar taxa richness between rivers, and few genus from each subfamily showed domination (Table 3).

Chironomids density between three rivers were tested using one-way ANOVA and result indicates homogeneity of composition between rivers ($p=0.299$, $\alpha=0.05$). Palas River and Pauh River have approximately even chironomids density, and both were higher than Bertam River. Genus composition from all subfamilies are presented in Fig. 2. All dominant genus were present in all rivers, but with lower composition in Bertam River except *Polypedilum*.

Tvetenia (subfamily: Orthoclaadiinae) was the most dominant genus recorded but only one individual was recorded in Bertam River. *Polypedilum* is second dominant genus and found highest in Bertam River. *Rheotanytarsus*, *Eukiefferiella*, *Paracricotopus* and *Cricotopus* were well distributed between rivers but lowest in Bertam River.

Diversity indices based on chironomids data for all rivers

are presented in Table 4. Shannon diversity was lowest at Bertam River (0.971) and 1.788 and 1.715 for Pauh River and Palas River respectively. Bertam River also exhibits lowest for Pielou evenness and Margalef Richness index. This simply explains lower quality of Bertam River ecosystem based on chironomid populations.

High altitude rivers reported to have higher Orthoclaadiinae taxa and similar result was recorded in Palas River and Pauh River. Hamerlik et al. (2018) in his study at high altitude streams also found Orthoclaadiinae as dominant subfamily and lack of Chironominae, which might be largely related to the preference for tropical and subtropical lowlands that exhibited by most members of this subfamily. Ahmad and Hanisah (2018) in their study at high altitude river in tropical region found Chironominae as most dominant subfamily followed by Tanypodinae and Orthoclaadiinae.

This study found Bertam River was dominated by *Polypedilum* (subfamily: Chironominae). As regards to NWQS and WQI indices, Bertam River has the lowest as compared to

Table 3 : Checklist of Chironomidae in three studied rivers

	River			
	Bertam river	Pauh river	Palas river	Total
Subfamily: Chironominae				
Tribe: Chironomini				
<i>Polypedilum</i>	142	48	134	324
<i>Cryptochironomus</i>	0	1	0	1
<i>Chironomus</i>	1	16	0	17
<i>Demicryptochironomus</i>	0	5	1	6
<i>Harnischia</i>	0	2	10	12
Kompleks <i>Harnischia</i> genus C	0	1	0	1
<i>Microtendipes</i>	0	1	0	1
Tribe: Tanytarsini				
<i>Rheotanytarsus</i>	19	164	54	237
<i>Tanytarsus</i>	0	4	0	4
Subfamily: Orthoclaadiinae				
<i>Cricotopus</i>	24	4	138	166
<i>Rheocricotopus</i>	0	129	9	138
<i>Tvetenia</i>	1	348	435	784
<i>Eukiefferiella</i>	5	5	70	80
<i>Cardiocladius</i>	0	0	23	23
<i>Heleniella</i>	0	5	0	5
<i>Limnophyes</i>	0	0	2	2
<i>Paracricotopus</i>	4	28	56	88
<i>Parametriocnemus</i>	0	9	3	12
<i>Paratrisocladius</i>	0	8	0	8
<i>Onconeura</i>	0	1	0	1
<i>Synorthocladius</i>	0	3	0	3
<i>Thienemanniella</i>	1	0	3	4
Orthoclaadiinae genus 1	0	0	4	4
Subfamily: Tanypodinae	0	40	2	42
Total individual	197	822	944	1963
No of Taxa	14	8	11

Fig. 2 : Taxa composition for three studied rivers: (a) Taxa composition more than 50 individuals and (b) Taxa composition less than 50 individuals.

Palas River and Pauh River. Bertam River contains high suspended solids due to aggressive land opening. However, *Polypedilum* was recorded most dominant in this river. The genus reported to have high environmental gradient adaptations (Sriariyanuwath et al., 2015) and has many feeding behaviours (Beermann et al, 2018; Leite-Rossi et al., 2019). Previous studies indicate this genus is tolerable to organic concentrations (Bazzanti, 2000; Garcia and Laville, 2000; Leszczyńska et al., 2019). Instead of having turbid water, Bertam River also found to have higher ammoniacal. This study indicates good adaptation of this genus to turbid water and high ammoniacal nitrogen concentrations. It's important to identify the genus to species level to confirm biological indicator species. However, this study unable to identify the genus to lower taxonomic level due to incomplete keys available for species identification. However,

based on morphological differences, this study has discovered thirteen groups of *Polypedilum*. Scarcity in tropical region taxonomic key limits the study to propose a specific biological indicator species.

Chironominae and Orthoclaadiinae diversity and composition vary with altitude and water temperature change (Helson et al., 2006; Garay et al., 2020). Instead of those factors, anthropogenic activities also affect those subfamilies. Cranston (2004) reported that *Polypedilum* is very diverse in tropical region. Bertam River which affected by agriculture activities and has higher organic substances was dominated by *Polypedilum* and this genus was reported tolerant to organic contaminations (Bazzanti, 2000; Kranzfelder and Ferrington, 2018; Montes et al., 2020).

Note : drawing not to scale

Fig. 3 : Variety of *Polypedilum* spp. head capsule discovered in this study.

Taxonomic from subtropical and tropical region is still scarce with only few species were well elaborated (Cranston *et al.*, 2013). This genus reported abundant in sandy substrates due to its feeding habit (collectors). Bertam River substrates dominated by sand (90%) and therefore preferred by *Polypedilum* group. This study has recorded 324 individuals and almost half were in Bertam River. Ventromental plate morphology was the easiest way to differentiate from another genus (Fig. 3). *Polypedilum* also recognised by having five segmented antennae, mandible with dark dorsal teeth and two inner teeth. This study reveals thirteen groups of *Polypedilum* which indicates

possibility to establish new species from *Polypedilum* genus. There were few different names were misused for same organism and existing sub-genus is not suitable to be used for certain larval.

Chironomids are important agent for biological monitoring worldwide due to their wide range of tolerant and this group has been explored as biological indicator for water quality in develop countries (Lencioni *et al.*, 2012; Czechowski *et al.*, 2020). This study reveals the potential of *Polypedilum* as biological indicator at high altitude ecosystems. At genus level, *Polypedilum* exhibits large range of tolerant and therefore it is

Table 4: Diversity indices for three studied rivers

Indices	Bertam River	Pauh River	Palas River
Shannon diversity (H)	0.971	1.788	1.715
Pielou evenness (E)	0.47	0.59	0.633
Margalef richness (D_{Mg})	1.32	2.83	2.04

important to identify to species level for river quality assessment purpose. Similar result was reported by Zalela (2014) on her study at low altitude rivers, where *Polypedilum* also dominated low altitude rivers (Chertoprud et al., 2018). Only at species level, a true biological indicator could be confirmed because within a single genus, species may respond in a distinct manner to environmental changes (Lenat and Resh, 2001; Beermann et al, 2018).

In conclusion, the study on hydrology at Cameron Highland demonstrates that upstream streams were not affected significantly by anthropogenic activities as compared to middle stretch or downstream area. Even though Bertam River exhibits lower water quality compared to Pallas and Pauh River, studied area (Upstream of Palas River, Pauh River and Bertam River) are still within class I water quality. Similar results were observed for chironomid composition and diversity. *Tvetenia* (Subfamily: Orthocladiinae) exhibits domination at highland and good water quality. Only *Polypedilum* exhibits good tolerant to lower water quality river (Bertam River). This study reveals that genus *Polypedilum* at studied rivers has thirteen different groups below genus level that makes large range water quality tolerant. Therefore, species confirmation is very crucial for identifications of biological indicator at species level especially for *Polypedilum* spp.

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Add-on Information

Authors' contribution: A.K. Ahmad: Main author and supervisor of the project; A. Hafizah: Identification of chironomids; S.O. Sharifah Aisyah: Statistical analysis and co-researcher.

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