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DIVERSITY, DISTRIBUTION, AND ABUNDANCE OF ANNELIDA IN UiTM NEGERI SEMBILAN FOREST RESERVE

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Abstract

Annelida is a universal, abundant, and varied collection of creatures that can be discovered on terrestrial, freshwater as well as in marine. Their biological importance relies not only on the comparatively high number of species but also on their often-high abundance. However, knowledge of Annelida in UiTM Negeri Sembilan Forest Reserve is poorly known and documented resulted due to a lack of awareness of their role in the ecosystem. Hence, this research was done to identify the species and investigate the diversity and abundance of Annelida in three different areas in UiTM Negeri Sembilan Forest Reserve. This research was advantageous in helping to manage, preserve, protect, and conserve Annelida's habitat for ecological sustainability and may contribute to society's knowledge and information to other researchers about Annelida's diversity. A sampling collection was conducted seven times in April 2022 at Site A, Site B and Site C. The specimens were hand-sorted and placed in a plastic pail. Then, the specimens were brought to the laboratory for the identification process. A total of 134 individual Annelida samples were collected from Site A (N = 85), Site B (N = 11) and Site C (N = 38). From detailed identifications and observations based on morphological characteristics, they were classified into two species which are *Pontoscolex corethrurus* (N = 110) and *Haemadipsa zeylanica* (N = 24). The statistical analysis shows the richness (R) = 0.20, diversity (H) = 0.47, evenness (E) = 0.68. The Analysis of Variance (ANOVA) of this study shows both length and weight were significant to distinguish between *P. corethrurus* and *H. zeylanica* species. Based on this research, the Annelida diversity in the UiTM Forest Reserve is relatively low. However, further detailed research is needed to discover more about the variety of annelids in this area.

Keywords: Annelida, Diversity, *Haemadipsa zeylanica*, *Pontoscolex corethrurus*, UiTM Forest Reserve

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Introduction

Annelida is a phylum of segmented worms classified as part of the Kingdom Animalia. They are bilaterally symmetrical invertebrates and their segmented bodies differentiate them from all other organisms. According to Capa and Hutchings (2021), Annelida is a universal, abundant, and varied collection of creatures that can be discovered on terrestrial, freshwater, as well as in the marine. Most of the phylum Annelida is found in forests, especially in the deadwood regions.

This invertebrate phylum has about 9000 species grouped into three classes, which are sea worms underclasses of Polychaeta, earthworms underclasses of Oligochaeta and leeches that underclasses of Hirudinea (Molnár *et al.*, 2021). According to Bora *et al.*, (2021), the phylum Annelida plays a major role in a variety of pedological activities such as soil health improvement, organic matter decomposition, soil microbial activity, and soil reclamation. Therefore, this phylum improves the ecosystem, especially in the UiTM Forest Reserve.

Annelida plays a significant part in the decomposition of organic materials. Unfortunately, knowledge of the Annelida at UiTM Forest Reserve is poorly known. Lack of information on the variety and

distribution of Annelida, as well as a lack of awareness about their importance in environments and undiscovered taxonomic diversity, are all reasons why Annelida has frequently overlooked components of the ecosystem. Research conducted by Hamid (2017) found that only a few data are obtained from their study titled the study of Earthworms Diversity which is conducted at UiTM Kuala Pilah. Besides that, a study by Noormi *et al.,* (2018) also mentioned there are few earthworms are found in their research about Species Composition and Biodiversity. Hence, this study is carried out to document the diversity and distribution of Annelida in UiTM Negeri Sembilan Forest Reserve. The objectives of this study are to identify the species of Annelida located in UiTM Forest Reserve, Negeri Sembilan and to determine the diversity and abundance of Annelida in three different areas of UiTM Forest Reserve, Negeri Sembilan. Hence, this study will be useful for those who want to discover more about the UiTM Negeri Sembilan Forest Reserve, as it will provide preliminary research for conservation and preservation efforts on ecosystem health and forest resources, which are required for sustainable development.

Methods

Study Area

The sampling was conducted in the forest reserve which is located in UiTM Kuala Pilah with the geocoordinates of 2.7890° N, 102.2180° E (Figure 1). The site is surrounded by hills and forests. UiTM Forest Reserve is classified as a tropical rainforest because it has a humid subtropical climate and a high possibility for earthworms to live (Hamid, 2017). There is numerous forest litter found in the terrestrial, especially in the forest reserve. The study areas were divided into three sites that are labelled as A, B and C. Site A is a forest area (2° 47’ 42.745" N 102° 13’ 6.55" E), Site B is a forest margin (2° 47’ 40.0" N 102° 13’00.0" E) and lastly is Site C, which is the water tank area (2° 47' 42.9" N and 102° 13' 13.9" E) (Figure 2). These three sites were randomly chosen.

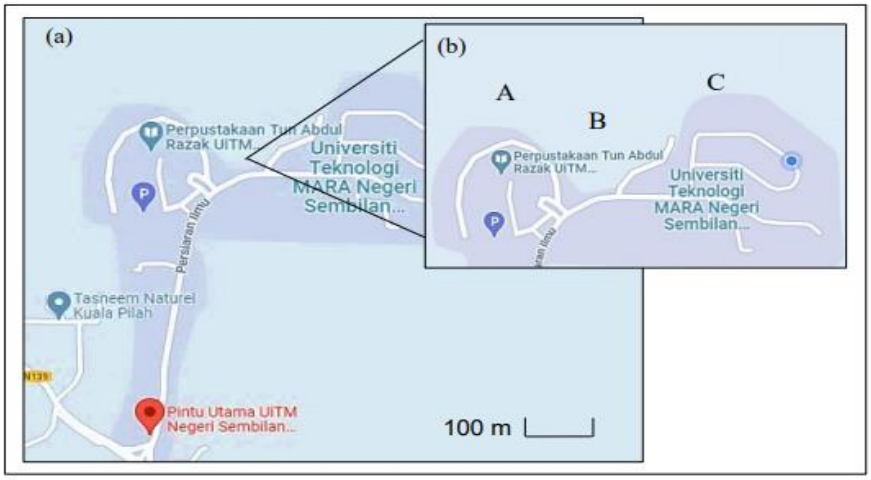

Figure 1. (a) UiTM Kuala Pilah Campus, (b) 3 plots of study sites, which is labelled as A, B and C (Google Map, 2022)

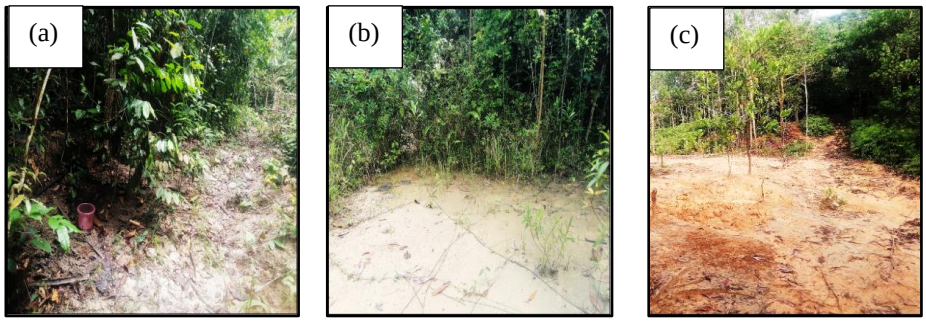

Figure 2. (a) Site A: Forest Area, (b) Site B: Forest Margin, (c) Site C: Water Tank

Apparatus and materials

The apparatus that used in this study are analytical balance, stereoscopic microscope, white plastic sheet, magnifying glass, white tile, plastic pail, zip lock bag, meter ruler, tissue paper, small container and metal tray. The chemical materials used are 70-80% ethyl alcohol and distilled water.

Methods

Sample was collected using hand-sorting methods (Gutiérrez-López *et al.*, 2016). The samplings were conducted seven times in April 2022 and 10m x 10m sampling at each site randomly chosen. A spade used to extract a 20-centimeter-deep soil core in three distinct locations. The samples were collected in three different locations which are sites A, B and C. The images of the samples were captured using Huawei Y9's phone camera. Their morphological characteristics were recorded. After that, they were placed in the plastic pail and brought them to the laboratory. In the laboratory, the samples were bathed in distilled water and dried with tissues. The morphometric characteristics such as length and weight were observed. At the same time, they were kept in laboratory condition. A stereoscopic microscope and magnifying glass were used to identify the species. Besides that, the sample identification was observed by referring to identification keys from articles, journals and official websites. The samples were preserved in 70-80% ethyl alcohol. Next, data analysis such as the Margalef Richness Index (R), Shannon Weiner's Diversity Index (H), Evenness Index (E) and Analysis of Variance (ANOVA) were calculated. The species richness in this study was determined by the Margalef Richness Index (R). This valuable tool is used for the interpretation of time series data from changing environments and for the conservation of the natural environment (Iglesias-Rios and Mazzoni, 2014) while Shannon Weiner's Diversity Index (H) is used to measure the number of individuals in each growth step or genus in a habitat community based on the circumstances of the organism's population (Ulfah *et al.,* 2019). The Evenness Index (E) was also used in this research to represent the number of individuals in a community between species. The lower the evenness index, the lower the population uniformity (Ulfah *et al.,* 2019). Lastly, ANOVA is a statistical method that splits the observed variance data to determine whether the observed results are significant (Karthik and Rama Mohan Rao, 2021). The ANOVA statistical test was done using SPSS Version 20.

Result and Discussion

Collection of Annelida samples

After five days of sampling, there were 134 individual samples from Site A, Site B, and Site C were collected and identified. Based on the characteristics of the sample that was collected, there were two families of Annelida, which described two different genera and represented two species. From the research, the abundant species in the sampling areas was *Pontoscolex corethrurus* which had 110 individuals, followed by *Haemadipsa zeylanica* which had 24 individual samples. The highest number of Annelida found was at Site A which 85 individuals followed by Site C and Site B with 38 and 11 samples (Table 1). Site A is a forest region covered with high trees and moist soil, while Site B is a forest margin and Site C is a water tank area. These two sites are near the building of the campus. The total number of Annelida's samples collected for each family and genera is shown in Table 1.

Table 1. Family and Genera species found in each sampling sites

Species	Family	Genera	Site A	Site B	Site C	No. of individuals
Pontoscolex corethrurus	Glossoscolecidae	Pontoscolex	61	11	38	110
Haemadipsa zeylanica	Haemadipsidae	Haemadipsa	24	0	0	24
					Total	**134**

Collection of Species in the UiTM Forest Reserve

Based on results in this study, *P. corethrurus* is the most dominant species in the UiTM Forest Reserve compared to *H. zeylanica,* which the species was most abundant in Site A compared to Site B and Site C. The image of Annelida samplings is shown in Figure 1. Soil temperature and moisture are the abiotic factors that have a bigger influence on the habitat and ecosystem of Annelida species like earthworms and leeches. Because earthworms and other soil animals have diverse responses to environmental conditions, soil usually represents an interactive set of components in which their distribution may differ (Khan *et al.*, 2021).

They also stated that pH, organic matter content, percent nitrogen, potassium and phosphorous content affect diversity and abundance in different locations. Besides that, research conducted by Sankar and Patnaik (2018) stated that habitat conditions have an important influence on soil physico-chemical characteristics and the number of earthworm populations.

The other species found is *H. zeylanica*. This species is the type of leeches and brown in colour and only found in Site A. According to Phillips *et al.* (2020), leeches can be found in freshwater, estuarine, and marine aquatic habitats, as well as wet terrestrial ecosystems, on all continents and seas except terrestrial Antarctica. A study conducted by Adamiak-Brud *et al.,* (2018) reported that the main significant factors influencing the distribution of leech species are waterbody and sampling type and also land use. A terrestrial leech species including *H. zeylanica*, which has multiple subspecies or variants, has not been thoroughly investigated (Chen et *al.,* 2011). According to Schnell *et al.* (2015), to properly organise and perform a successful leech collection, it is necessary to know the factors that determine leech abundance.

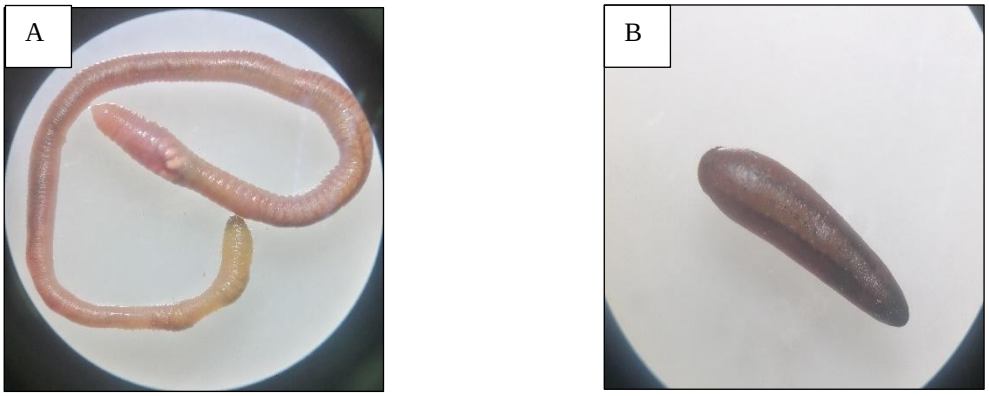

Figure 3. Annelida species captured under stereoscopic microscope (A) *Pontoscolex corethrurus*, and (B) *Haemadipsa zeylanica*)

Families of Annelida Collection in the UiTM Forest Reserve

In this research, the families that were discovered are the Glossoscolecidae and Haemadipsidae. The family of Glossoscolecidae is abundantly dispersed and distributed throughout the world, especially in terrestrial and forest habitats. According to Ansari and Saywack (2010), Glossoscolecidae is one of the major families of earthworms besides Lumbricidae, Megascolecidae, Moniligastridae and Eudrilidae. In South America only, there are about 400 species in the family of Glossoscolecids (Christoffersen, 2007).

For the Haemadipsidae, this family is found all throughout the Indo-Pacific, which includes almost all of the world's biodiversity areas (Fahmy *et al.*, 2019). In addition, one of the families that are exclusively terrestrial is Haemadipsidae (Phillips *et al.*, 2020). They also stated Haemopidae are predatory worms that live on land and in water, and they are distributed throughout Europe and North America. A study conducted by Borda and Siddall (2011) found that the typical family of Haemadipsidae involves duognathous which have two-jawed endemics from continental and volcanic

islands such as Australia, Indonesia, Madagascar, Papua New Guinea, Philippines, Seychelles and South Pacific islands. They also added that trignathous have three-jawed, which are known as Tritetrabdella species, and the species Haemadipsa are restricted to the Indian subcontinent and range into east and south-east Asia.

Annelida species richness and diversity

Table 2. Diversity index

Title	Value
Number of species	2
Number of individuals	134
Margalef Richness Index (R)	0.20
Shannon Weiner's Index (H)	0.47
Evenness Index (E)	0.68

Margalef Richness Index (R)

This index was used to measure the total number of species in the community. Based on Table 2, the value of the Margalef Richness Index (R) in this study is 0.20 which is in a low range compared to the study of Annelida communities in Merambong Shoal (R= 3.26). This is because 18 species of Annelida was discovered (Shi et *al.*, 2014). They also stated that the higher richness of Annelida species is because the Merambong Shoal was located near the open sea and had an environment that was more stable with minor salinity changes and a large dissolved oxygen amount. The low range in the UiTM Forest Reserve is low because only two species were discovered. According to Gamito (2010), since it attempts to control for sampling effects, the Margalef index is highly sensitive to sample size. Thus, the more abundant species in the sampling location means the site is rich with species.

Shannon Weiner's Index (H)

This study gained a value of 0.47 in Table 2. This shows that UiTM Kuala Forest Reserve displayed low diversity. Besides that, the value of the Shannon-Weiner Index in 10 stations at Pekan-Dungun, Kuala Terengganu and Kudat-Balambangan Island is 3.88 which is in the high range. This is due to 368 species were found (Rosli *et al.*, 2016). According to Singh *et al.*, (2020), the number of earthworms in various ecosystems such as yards, meadows, woodlands, leaf litter, and agricultural fields differed greatly.

Evenness Index (E)

From Table 2, this research obtained an Evenness Index (E) value of 0.68 which is in moderate range. However, this study has higher index than a study of Earthworms diversity at UiTM Forest Reserve in the year 2017 that obtained a value of 0.25 **(**Hamid, 2017). This is because the species are present in similar proportions regularly. In addition, Shi *et al.*, (2014) found that the evenness index at Merambong Shoal in Johor is 0.89, which is in the high range. They also stated that the polychaete population in that region was still in an equilibrium range. Thus, the evenness of a species can range from zero to one, with zero indicating no evenness and one indicating total evenness.

Analysis of Variance (ANOVA)

ANOVA was used to evaluate the significance of the difference between morphological characters between individuals. In this study, the morphological characters are the length and weight of *P. corethrurus* and *H. zeylanica* species. From Table 3, the P-value for length is 0.000 and the P-value for weight is 0.048. Thus, the data is statistically significant since the data is less than the P-value, which is 0.05. Both length and weight are significant to distinguish between *P. corethrurus* and *H. zeylanica* species. This is due to the great environmental conditions in the UiTM Kuala Pilah forest reserve for this habitat's species (Marfur *et al.*, 2022).

Table 3. ANOVA result for morphometric measurements of *P. corethrurus* and *H. zeylanica* species

Morphometric characteristic	Between species	
	F-value	**P-value**
Length	22. 421	**0.000***
Weight	3.995	0.048

Malaysia, including UiTM Kuala Pilah, has a tropical rainforest environment with annual rainfall ranging from 2000 to 2500 mm (Hazir *et al.*, 2020). The rainy season may be a good time to collect leeches and earthworms. Even in a moist tropical rainforest, Annelida samples may be less easily present for sampling under dryer conditions (Abrams *et al.*, 2019). They also stated season, weather, and microhabitat factors could all affect Annelida collection results. According to Schnell *et al.* (2015), to properly organize and perform a successful leech collection, it is necessary to know the factors that determine leech abundance. Even though this study gets both significant values, length is more accurate than weight for distinguishing the species. This is because the length value is less than the weight value.

Allentoft-Larsen *et al.*, (2021) stated in another study that the morphological studies on these annelids would provide a diverse lineage for comparison, increase knowledge of the significant Annelida adaptations, and assist in explaining the adaptive nature of annelids.

Conclusion

In a nutshell, the total number of samples of Annelida that were successfully analyzed and collected in this research was 134, which were classified into two species which are *P. corethrurus* (114) and *H. zeylanica* (20). The diversity index shows the UiTM Forest Reserve has quite a low diversity of Annelida. The low diversity and abundance of annelid species found in this study were affected by the annelids that were collected. This might occur due to the sampling period's short duration. According to the findings obtained, it cannot be concluded that the diversity, distribution, and abundance of Annelida in the UiTM Negeri Sembilan Forest Reserve were affected by threats such as anthropogenic activity and pollution. Thus, increasing the collection duration would result in higher collected specimens and the discovery of more Annelida species. Besides that, the sampling area of the site must be bigger to find more species of Annelida. Hence, it is suggested that additional research into the diversity of Annelida in the UiTM Forest Reserve be conducted in order to have advanced observations and findings in this region for the time being.

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Author Contribution

Baniran, S. K. - collecting data, data processing and analysis, manuscript writing; Rahim, N. A. - supervision, manuscript writing, review and editing.

Conflict of Interest

Author declares no conflict of interest.

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