DIVERSITY OF EARTHWORM SPECIES WITHIN A RELATIVELY DISTURBED FOREST OVER LIMESTONE IN MT. GUIMBA LOBOC, BOHOL, PHILIPPINES

College of Agriculture and Natural Resources
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A Thesis
Presented to the Faculty of the
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In Partial fulfillment
Of the Requirements for the Degree
in Bachelor of Science in Environmental Science

Jea Marie J. Simbajon

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APPROVAL SHEET

This thesis entitled **"DIVERSITY OF EARTHWORM SPECIES WITHIN A RELATIVELY DISTURBED FOREST OVER LIMESTONE IN MT. GUIMBA LOBOC BOHOL, PHILIPPINES"** prepared and submitted by Jea Marie J. Simbajon, in partial fulfillment of the requirements for the degree BACHELOR OF SCIENCE IN ENVIRONMENTAL SCIENCE has been examined and recommended for acceptance and approval for oral defense.

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ABSTRACT

Earthworms are present in almost all terrestrial ecosystems and represent one of the most important components of the soil invertebrate macrofauna. This study was conducted to look into the diversity of earthworm species within a relatively disturbed forest over limestone in Mt. Guimba, Loboc, Bohol. This study employed three randomly established 10m x 10m plots that were surveyed for earthworm species. The result showed that there were three identified genera found in the site. These genera include: *Polypheretima, Amynthas* and *Pheretima*. Among these genera, *Polypheretima* was found to be the most abundant in the area. The total diversity index showed that the area was diverse in earthworm species. Environmental factors such as soil moisture, pH and elevation were found to be associated to the diversity of earthworm species such as *Polypheretima* sp. 2. These findings suggest that conservation and sustainable management of the habitat of this species is important.

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THE PROBLEM AND ITS SCOPE

Rationale

Karst landscapes encompass 15% of the Earth's surface (White, 2021). Karst landscape is type of landscape where the dissolving of bedrock consist of soluble rock types like limestone, marble, and gypsum creates sinkholes, sinking streams, cavern, springs and characteristic features (National Park Service, 2021). Due to its unique carbonates bedrock, karst soil development is slow, taking between 250,000 and 850,000 years and 630,000 to 7,800,000 years to reach 10 cm soil under favorable conditions that stimulate weathering and pedogenesis (Yuan and Cai, 1988; Yuan and Cai, 1988; Cao *et al.*, 2004 as cited by Wang *et al.*, 2019). As a result, the majority of karst soils have direct contact with bedrock, making them vulnerable to severe soil erosion as a result of extreme weather events (Cao, *et al.*, 2004, as cited by Wang *et al.*, 2019).

Karst areas are a significant hub for diversification and outstanding biodiversity, making this area a subject for ecological and evolutionary studies (Cai *et al.*, 2015, Clements *et al.*, 2016, *Oliver et al.*, 2017, as cited by Yang *et al*, 2021). Rare plants that need calcium-rich nutrition, cave invertebrates, salamanders, frogs, bats and potentially considerable microbiological biodiversity all depend on the diverse ecological niches and microclimatic conditions supplied by the karst terrain, both on the surface and underneath. (Discovery Islands Ecosystem Mapping, n.d.)

Earthworms, also known as angleworms, are among the 1,800 species of terrestrial worms in the class Oligochaeta (phylum Annelida), with members of the genus *Lumbricus* thriving in the karst landscape (Britannica, 2020). In addition, earthworms can survive in every soil type around the world if there is enough moisture and organic matter available. Earthworm activity is important because it improves nutrient cycling by incorporating detritus into mineral soils quickly, as well as mucus production related with water excretion in earthworm guts, which boosts the activity of other beneficial soil organisms, which leads to organic production (Bhadauria and Saxena, 2010).

In the Philippines, there are around 200 species with eight genera of native earthworms that was recorded and documented (Aspe & James 2014, 2015). According to the species described and identified, the Philippines has high diversity and local endemicity of earthworms. There are 18 species of earthworms described from Mt. Kitanglad by James (2004) and 22 species from Mt. Malindang by Aspe and James (2014, 2015). Also, there are 17 new earthworm species in the genera *Pheretima (Pheretima), Pheretima (Parapheretima), Amynthas, Polypheretima*, and *Pheretima*, from various localities on Mindanao, Camiguin, and Dinagat Island described by Aspe and James (2016). In Bohol, there are three recorded genera that are being identified on previous study; these are *Pheretima, Polypheretima and Pleinogaster* (Flores, 2009). On the recent study being done, *Amynthas* sp. were included on the species that can be found in Bohol (Jose et al, 2021).

This study would serve as the baseline study of earthworm in Mt. Guimba which aimed to explain the ecology and biology of earthworm species on the area.

Literature Background

The following related reading served as the legal basis of the study:

Republic Act 9147 or the Wildlife Resource Conservation and Protection Act which upholds the protection of biological diversity, and so as conserving their vital and critical habitats for their perpetual existence. This act also promotes and support scientific studies on the conservation of the country's biological diversity.

Chapter 1, Section 2. The Declaration of Policy. States that is shall be the policy of the state to conserve the country's wildlife resources and their habitats for sustainability. In the pursuit of this policy, it pinpoints an objective which promotes biodiversity research. Its objective is to initiate or support scientific studies on biological diversity.

Furthermore, Chapter 2, Section 14. Bio prospecting. Bio prospecting would be permitted only if any proponents signs an agreements pledging to follow and adhere to the secretary's affairs, terms and conditions for the protection of biological diversity.

Before issuing the required pursuit, the Secretary or his designated representative shall require the applicant to obtain prior informed consent from the concerned indigenous cultural communities, local communities, and

management board through Republic Act 7586, or private individual or entity in consultation with the concerned agencies. The applicant must clearly disclose the purpose and nature of bio prospecting operation in a language and procedure that the general public understands. Indigenous people's prior informed consent must be sought in compliance with applicable laws. Concerned bodies must act on the bio prospecting proposal within a fair time frame.

Moreover, Chapter 2, Section 7. Collection of Wildlife. Gathering of wildlife samples or subjects were allowed in relation to Section 6 of this Act. Provided, in gathering, correct and ethical methods of wildlife collection with least or no harmful effect to the population of the existing wildlife species and so as their habitats shall be required. In collection of wildlife done by indigenous people, the collection of sample was allowed for traditional use and not for trade. Further, the collection and use for the mentioned purpose of an individual shall no include the threatened species.

Chapter 2, Section 15. Scientific Researches on Wildlife. The gathering and the use of biological resources use for scientific research and not for commercial utilization shall be permitted upon the implementation of an undertaking/agreement with and issuance of a gratuitous permit by the secretary or authorized representative; provided, however, that prior clearance from concerned bodies must be obtained prior to the issuance of the gratuitous permit: Provided, further that the last paragraph of Section 14 applies.

The following related literatures provided the background information of this research:

Earthworms are detritivores organisms that mediated the circulation of organic matter in nature. They increase the nutrients bioavailability for plants and aids in soil aeration, bioturbation, and humidity matrix (Industrial and Municipal Sludge, 2019). Earthworm plays substantially on these ecological processes such as water, carbon, and nutrient circulation which is present in ecosystem. Earthworms were also a significant participant on food chain since they constitute as diet for some predators (Bouche, 1977 as cited in Rorat and Vandenbulcke, 2019). Earthworms thrive in different terrestrial ecosystems around the world and their burrowing act on soil have substantial effect on the soil structure and soil fertility (Lavelle and Lepage, 1997 as cited in Bothe & Drake, 2007). And thus, they are known as ecosystems engineers (Lavelle et al., 1998 as cited in Coleman and Wall, 2015).

Earthworms are classified into three distinct groups which are based on their localization in soil, feeding behavior, and borrowing. The Epigeic, thrives, eats, and lives above soil surface, Anecic, live in semi-permanent burrows that are more or less vertical and open to the soil surface, and the Endogeic generally reside on the surface for about 10-15 cm, consume dirt, and dig enormous systems of tunnels to seek for food, which promptly replace with their cast (Karaca, 2011 as cited Lamande et al., 2003: Lee, 1959: Bouche, 1977). Their burrows were typically sub horizontal orientation (Karaca, 2011).

Bohol is among the islands in the Central Philippines that has karstic landscape. Bohol's main karst covers the southern half of Bohol. Karstic terrains cover sink holes, springs, estaralles, caves, poljes, and rich clay soils which

principal component material was limestone (Urich, 1989). However, karstic landscape has negative downside, the ecosystem is characterized by shallow soil, low water holding capacity, and the rainfall leads to the loss of top soil which consist or organic matter, destruction of soil fertility which subsequently leads to degraded and barren soil (Zhao and Hou, 2019).

The following related studies justified the conduct of this study:

A study conducted by Lugo *et. al.* (2006), on two sites which was a karst forest (Pugando, Juan Nieves, and Ollas y Calderas) and alluvial forest, it revealed that no species of earthworm were observed in karstic forests compared to alluvial forest. However, both sites were observed with earthworm cast. They argued that the absence of earthworm on karstic forest were remnants of previous sampling/study conducted in an area and the depth of their soil. They also added that karst forest was characterized by rocky relief and abrupt water infiltration which makes the earthworm prevails on deeper soil profile or close where under rocks or clumped distribution.

Li et al., (2018) studied on the interaction of Earthworm and Arbuscular Mycorrhizal fungi in increasing nutrient availability and plant growth in karst soil on the 0-15 cm layer of desertified soil in the karst land in Xindian Experimental Station, Kumming, South Western-China. They found that earthworms and Arbuscular mycorrhizal have substantial effect on plant growth in nutrient deficient soils in karst desertification land than in other ecosystem. Their study had concluded that earthworm and fungi (AM) can uphold the growth of the plant and root colonization by complementary but collaborative mechanism by

reorganizing nutrient availability for plants most specially on nutrient-limited soils. Thus, their presence were vital in karst desertification ecosystem where foliage ad soil were complex to revitalize compared to other land-use types.

On the study conducted by Modra, *et al.*, (2017) on the Maravian karst, a karst system in Czech Republic, they found that the relevance of earthworm on detecting mycobacteria through their cast was high. They also added that on the Bull Rock Cave (Mavarian Karst) 200 and 300 earthworms per 1 m^2 were observed (Karel, Kavarik, personal communication).

A compilation made by Phillips *et al.*, (2019) on the Global Distribution of Earthworm Diversity notes that in Asian sites, including Brazil in South America which falls under tropical region, it was mentioned that these sites were dominated by giant earthworms which occurs at low species richness and low densities which they have expected to have stupendous/ high biomass but low abundance. But these elucidated patterns were not proven due to the small number of sites or sample points for biomass model compared to diversity.

A study was conducted on the population of earthworm on the effect long-term crop sequences and bio-covers under no tillage in 2 study sites: the MTREC (Middle Tennessee Research and Education Center) - Spring Hill Tennessee and the MLRA (Major Land Resources Area). Both sited were under the karst region. Their study revealed three identified species of earthworm on each site: *D. coroliniana, L., rubellus*, and *Amynthas* spp.

As the study done by Aspe and Samuel (2018) on the molecular phylogenetic and biographic analysis of the earthworms of the Philippines

showed that a tree of taxonomy of earthworm species of *Amynthas, Pithemera, Polypheretima*, and *Pheretima* and *Pheretima* lineage requires revision and changes due to monophyly. Analysis showed that in *Pheretima* lineages undergone abrupt diversification with indicated as contributors. They also added that the four representative islands show that during the Pleistocene periods there shows an evidence of multiple colonization. However, during the times when the water had risen, the four major islands has been divided into smaller pieces and thus the movement and colonization were reduced.

A biodiversity survey conducted by Flores (2007) on the distribution of earthworm in Central Visayas showed that in Bohol and in Cebu, *Pleionoglaster* appears to be diverse. The overall genera found in Bohol were; *Pheretima* spp., *Pleionogaster* spp., *Polypheretima* spp., and *Pontoscolex corethrurus.*

THE PROBLEM

Statement of the Problem

The main objective of this study was to identify the diversity of earthworm species within a relatively disturbed forest over limestone in Mt. Guimba, Loboc, Bohol, Philippines.

Specifically, this research aimed to answer the following objectives:

1. What are the species account?

2. What are the abundance of earthworm species?

3. What are the species diversity of earthworms?

4. What are the factors that can shape species diversity of earthworms?

Significance of the Study

The result of the study would be beneficial to the community because the researcher can share his expertise on earthworm on how to identify them and acquire them. This would also help future researchers to support their future studies related on this field of research specialization.

Conservationist. Earthworms were affected by various anthropogenic and non-anthropogenic interventions. To combat this issue, the researcher would assess the diversity of earthworm in the local. By this, this would help them create a plan for conserving these ecologically significant species.

Environmentalist. Earthworms were keystone species that modifies the environment to make it a better place. The researcher would lend himself to understand its nature and ecology which aids the environmentalist to incorporate them of their environmental protection measures.

Extension Workers. The output of this study would give information to the extension workers. This would help them analyze and decide prospect earthworm studies and earthworm quantification.

Students. The study would be useful to the students as it would serves as their reference or guideline in conducting another study related to earthworm.

RESEARCH METHODOLOGY

Design

This diversity survey was laid out using site selection that lies within Mt. Guimba specifically in relatively disturbed forest.

Environment

The study was conducted on 8-12 December 2021 at Mt. Guimba within Barangay Oy. It is situated in the North Western part of the municipality with the coordinates of 9.651633 and 124.015230 and 173 m above sea level. The mountain has a total area of 185 hectares (Figure 1). The bioprospecting occurred on the relatively disturbed forest of Mt. Guimba. The area chosen was slightly covered with ferns and small trees and the ground was covered with dried leaves, twigs and small rocks.

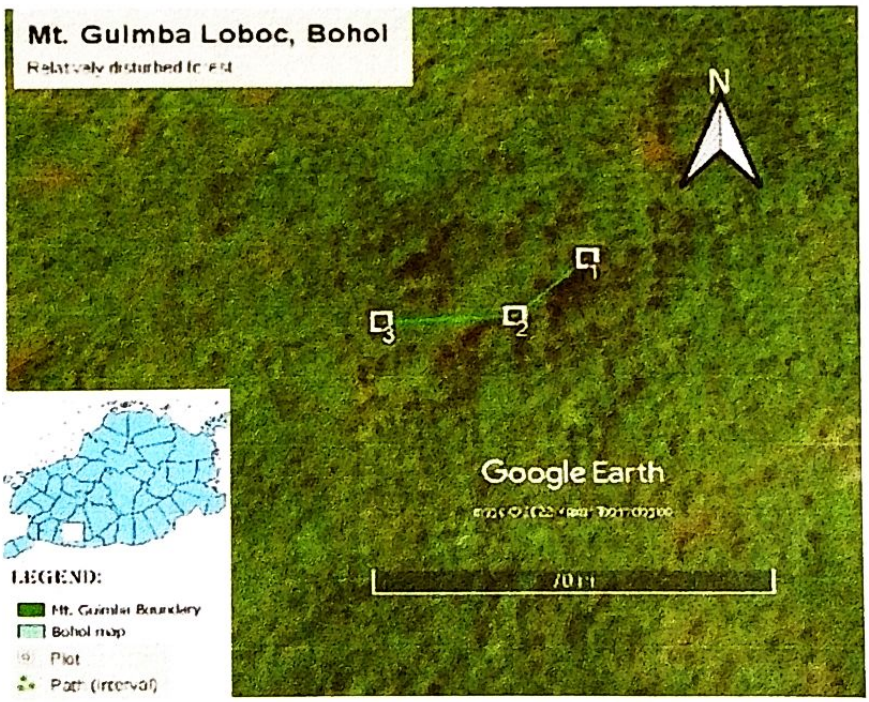

Figure 1. Location of the study site

Material and Equipment

The material and equipment used were the following: glass jars, formaldehyde, crowbar, shovel, machete, gloves, camera, references, record, ball pen, and pencil.

Procedure

Permits from the locality were gathered prior to the sampling. Sampling were conducted following the methods in Aspe et. al (2009), the site were determined based on the vegetation covering, floor covering such as wood debris, rocks and leaf litters, and so with the stiffness of the site were also considered. In the relatively disturbed forested area of Mt. Guimba three replicates of one row 10m x 10m quadrat was established with an average horizontal distance of 20m between plots. Within the plots, shovel, trowels, and hoes was used to dig 10 holes of 0.5m (length) x 0.5m (width) x 0.3m (depth). The surrounding habitat where the bioprospecting took place was observed and the soil moisture, elevation and soil pH was gathered as well. Earthworms were gathered and cleaned in tap water before killed in 10% ethanol and stored in a closed container with 10% formalin three times the volume of the samples to guarantee that all of the specimens in the bag were well preserved. After two days, the formalin was removed and replaced with 80% ethanol. The earthworm specimens were brought to Bohol Island State University- Bilar Campus for taxonomic identification. Taxonomic identification was based on morphometrics and meristic characteristics of the earthworm (Figure 2). This method was taken from the study of Easton (1979) as cited in Jose et al. (2021). In addtion, the aid of reliable field guide and expert verification were also considered in this study.

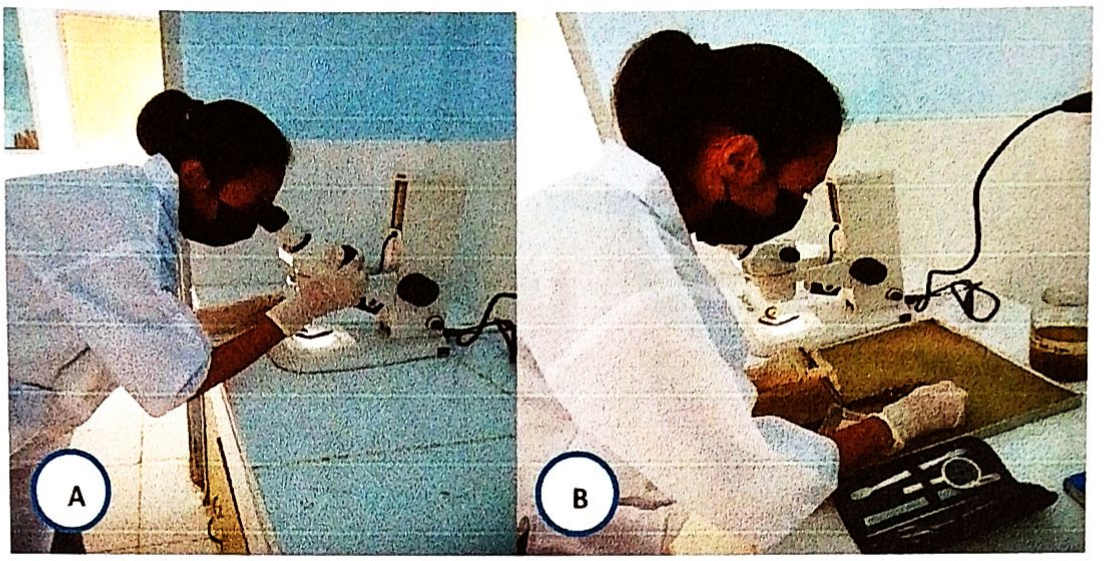

Figure 2. Examining the external feature of earthworm (A); Dissecting of earthworm specimen (B)

Data Analysis

Relative abundance is the percent composition of an organism of a particular kind relative to the total number of organisms in the area. The abundance of a species is divided by the total abundance of all species combined (Heaney, 1997; Kunz, 1982; Hodgkison, 2004).

$$\text{Relative Abundance (\%)} = \frac{\text{No. of individual species}}{\text{Total no. of individual species}} \times 100$$

The Shannon-Wiener diversity index was used to assess the diversity of earthworms. The proportion of species 1 relative to the total number of species (Pi) was calculated and multiplied by the natural log of this proportion (lnPi) and the resulting product was summed across species (Magurran 1984).

$$H = -SUM [(p_i) x \ln (p_i)]$$

Where, H = Index of species diversity

$$P_i = \frac{\text{Number of individuals of one species}}{\text{Total number of individuals in the samples}}$$

Moreover, the Canonical Correspondence Analysis was used to determine the factors that shapes the species diversity of earthworms. The analysis was conducted using PAST Software.

DEFINITION OF TERMS

Abundance. An ecological concept referring to the relative representation of a species in a particular ecosystem. It is usually measured as the number of individuals found per sample.

Composition. A part or element of a larger whole

Disturbed Area. An area where vegetation, topsoil or overburden is removed

Diversity. Range of different things.

Earthworm. A terrestrial invertebrate that belongs to the phylum Annelida and are commonly found in soil, eating a wide variety of organic matter.

Karst. A topography formed from the dissolution of soluble rocks such as limestone, dolomite, and gypsum.

Karst land. An area of land made up of limestone.

Lime. Also called burnt lime, calcium oxide, caustic lime, calx, quicklime.

Morphology. The study of the forms of things.

Site. A piece of ground that is used for a particular purpose or where a particular thing happens.

Soil Parameters. Indicate the state of soil ecosystem characteristics, which especially reflect production, buffering, filter and other soil functions.

Species Diversity. Is simply the number and relative abundance of species found in a given biological organization (population, ecosystem, Earth).

CHAPTER 2

PRESENTATION, ANALYSIS AND INTERPRETATION OF DATA

This chapter deals with the presentation, analysis and interpretation of the study. To achieve the purpose of this study, the researcher made use of the numbers of collected genus captured in the selected site of Mt. Guimba specifically in the relatively disturbed forest. Data on earthworms are presented and analyzed.

A. Species Account of Earthworms

The genera were identified based on the field guide with its morphometric description. All specimens were verified by Dr. Nonillon M. Aspe, an earthworm taxonomist of the Philippines.

A total of three known genera were found to be in the site. These genera includes *Polypheretima*, *Amynthas* and *Pheretima* under the family Megascolicidae. Atleast five putatively new species of earthworms were identified.

Family Megascolecidae

*Amynthas (*Kinberg 1867*)*

Generic Diagnosis. Body circular in cross section, with numerous setae regularly arranged equatorially around each segment; setae absent on first and last segments. Male pores paired and superficial, opening on xviii. Spermathecal pores usually paired, positioned either, intersegmental or intrasegmental

between 4/5 and 8/9. Clitellum annular, covering three segments, from xiv to xvi. Single female pore midventrally on xiv. Genital markings present or absent. Ovaries and funnels free in xiii. Male sexual system holandric, or proandric. Spermathecae a single pair, multiple pairs, sometimes single and located midventrally, or sometimes lacking. Nephridia on spermathecal duct lacking. One pair of prostate glands, racemose. Copulatory bursae lacking.

Remarks. *Amynthas* is highly diverse and is dominant in China (Aspe 2016). There are currently 19 known species of *Amynthas* in the Philippines (Aspe & James 2017). This does not include yet the species described below.

Amynthas sp. (Figure 3)

Amynthas sp. is a dark-brown worm; equator unpigmented; length is 140-165 cm; diameter is 4-6.5 mm. Body circular in cross section; tail tapered; with 72-108 segments; first dorsal pore at 12/13; two pairs of spermathecal pores extending from segment vii/viii; female pore at segment xiv; male pores on xviii. Clitellum at segment xiv-xvi. Setae unevenly distributed around the equator in some segments; dorsal and ventral setal gaps lacking; nephridia on spermathecal duct is lacking. Gizzard is prominent; three pairs of heart at xii-xiii; a pair of prostate gland at xvii-xix; caeca at xviii; copulatory bursae lacking.

Remarks. So far, no known species is morphologically similar with *Amynthas* sp. and is putatively a new species. Further morphological examination is required to verify the taxonomic identification of the species.

Figure 3. *Amynthas* sp.

Pheretima sp. (Kinberg 1867)

Generic diagnosis. Body circular in cross section, with numerous setae regularly arranged equatorially around each segment; setae absent on first and last segments. Male pores paired within copulatory bursae opening on segment xviii; one or more pairs of spermathecal pores in intersegmental furrows between 4/5 and 8/9. Clitellum annular, covering three segments from xiv to xvi. Single midventral female pore on xiv. Genital marking usually absent. Internally, esophageal gizzard usually originating in xxvii, extending forward. Ovaries and funnels free in xiii. Male sexual system holandric, with paired testes and funnels enclosed in sacs in x and xi, and seminal vesicles in xi and xii. Spermathecae one pair, multiple pairs, sometimes single and located midventrally, or sometimes lacking. Nephridia present on spermathecal duct (s); one pair of prostate glands,

racemose. Copulatory bursae present; secretory diverticula lacking on coelemic surface of copulatory bursae.

Remarks. *Pheretima* occupies the Southeast Asian archipelagos and towards the Pacific, with high species richness in the Philippines, followed by the Indonesian archipelago and New Guinea (Aspe 2016). Currently, there are 99 species of *Pheretima* in the Philippines, suggesting that the archipelago may be the center of species radiation for this genus (Aspe & James 2017).

Pheretima sp. (Figure 4)

Pheretima sp. is a light brown worm with thread-like body; segmental equator pigmented; length is 103-150 mm; diameter is 5-8 mm. Body circular in cross section; tail tapered; with 65-114 segments; first dorsal pore at 12/13; Paired spermathecal pores extending from segment 5/6; female pore at xiv; male pores at xviii. Clitellum at segment xiv-xvi. Setae unevenly distributed around the equator in some segments; dorsal and ventral setal gaps lacking. Nephridia on spermathecal duct present. Gizzard is prominent; a pair of heart at ix-x; a pair of prostate gland at xiv-xv; caeca at segment xxvii; copulatory bursae present.

Remarks. So far, no known species is morphologically similar with *Pheretima* sp. and is putatively a new species. Further morphological examination is required to verify the taxonomic identification of the species.

Figure 4. *Pheretima* sp.

Polypheretima (Michaelsen 1934)

Generic diagnosis. Body cylindrical; setal arrangement perichaetine; annular clitellum covering segments xiv-xvi; pair of male pores in xviii on circular porophores that may be within copulatory bursae; ventral genital markings present or absent; esophageal gizzard in viii; intestine begins in xv or xvi; nephridia on spermathecal ducts lacking; caeca lacking; male sexual system usually holandric, with testes and funnels enclosed in paired sacs in x and xi; seminal vesicles in xi and xii; spermathecal pores small, spermathecal diverticula simple and usually ectal in origin; prostates racemose; copulatory bursae may or may not be present; ovaries free in xiii; oviducts lead to single or closely paired opening (Easton 1979).

Remarks. *Polypheretima* is diverse in the Indonesian archipelago, in New Guinea, in the Myanmar-Laos-Thailand-Vietnam area, and in the Philippines (Ape 2016). Currently, there are 18 species of *Polypheretima* species known in the Philippines. This does not include yet the species described below.

Polypheretima sp. 1 (Figure 5A)

Polypheretima sp. 1 is a brown worm with thread-like body; segmental equator unpigmented; length is 115-165 mm; diameter is 4 mm at segment xiv and 4.5 mm at xxv. Body circular in cross section; tail tapered; with 315- 424 segments; first dorsal pore at 13/14; two pairs of spermathecal pores at segment 6/7-7/8; female pore at xiv; male pores at xviii. Clitellum at xiv-xviii. Setae unevenly distributed around the equator in some segments; dorsal and ventral setal gaps lacking. Genital markings lacking. Gizzard is prominent; nephridia on spermathecal duct lacking; caeca lacking; prostate at xvii; copulatory bursae lacking.

Remarks. So far, no known species is morphologically similar with *Polypheretima* sp. 1 and is putatively a new species. Further morphological examination is required to verify the taxonomic identification of the species.

Figure 5. *Polypheretima* sp. 1

Polypheretima sp. 2 is a light pink worm with thread-like body; equator unpigmented. Length is 119-150 mm; diameter is 4.5 mm at segment viii and 5 mm on segment xv. Body circular in cross section; tail tapered; first dorsal pore at segment 12/13; Two pairs of spermathecal pore at segment 6/7-7/8; female pore at xiv; male pores at xviii. Clitellum at xiv-xvi. Setae unevenly distributed around the equator in some segments; dorsal and ventral setal gaps lacking. Genital markings lacking. Gizzard is prominent. Nephridia on spermathecal duct lacking; caeca lacking; prostate at xviii; copulatory bursae lacking.

Remarks. So far, no known species is morphologically similar with *Polypheretima* sp. 2 and is putatively a new species. Further morphological examination is required to verify the taxonomic identification of the species.

Figure 6. *Polypheretima* sp. 2

Polypheretima sp. 3 is a pinkish white worm with a thread-like body; segmental equator unpigmented. Length is 120-140 cm; diameter is 4-5 mm. Body circular in cross section; tail blunt; first dorsal pore at segment 12/13. Three pairs of spermathecal pore at 6/7-7/8; female pore at xiv; male pores at xviii. Clitellum at xiv-xvi. Setae unevenly distributed around the equator in some segments; dorsal and ventral setal gaps lacking. Genital markings lacking. Gizzard is prominent. Nephridia on spermathecal duct absent; caeca lacking; prostate at xviii-xx; copulatory bursae lacking.

Remarks. So far, no known species is morphologically similar with *Polypheretima* sp. 3 and is putatively a new species. Further morphological examination is required to verify the taxonomic identification of the species.

Figure 7. *Polypheretima* sp. 3

B. Relative Abundance of Earthworms

Table 1 shows the relative abundance of the collected species in the sampling plots. *Polypheretima* sp. 2 *and Polypheretima* sp. 3 had the highest abundance value of 28%, followed by *Polypheretima* sp.1 (26%), *Pheretima* sp. (10%) and *Amynthas* sp. with 8%.

In terms of occurrence, three known genera were captured during the period and these were: *Amynthas, Pheretima* and *Polypheretima.* The genus *Polypheretima* was the most abundant because it can be found in all plots and it has the highest total number of individuals collected. According to Fragoso (1999), the genus *Polypheretima* are continuous breeders with high fecundity and characterized by its high number of species and individuals. Together with *Polypheretima, Amynthas and Pheretima* can also be seen in the relatively disturbed forest.

Table 1. Relative abundance of earthworms within relatively disturbed forest

Family	Genus	Species	Coordinates	Abundance	Percentage
Megascolecidae	*Amynthas*	*Amynthas* sp.	09°38'40.6"N, 124°01'12.7"E	0.08	8%
	Pheretima	*Pheretima* sp.	09°38'40.8"N, 124°01'12.8"E	0.1	10%
	Polypheretima	*Polypheretima* sp.1	09°38'40.1"N, 124°01'12.0"E	0.26	26%
		Polypheretima sp.2	09°38'40.1"N, 124°01'11.9"E	0.28	28%
		Polypheretima sp.3	09°38'39.9"N, 124°01'11.1"E	0.28	28%

C. Earthworm diversity

Figure 6 shows the species diversity of earthworms in sampling plots. Plot 3 had the highest H'=1.728, followed by Plot 2 (H'=1.575) and Plot 1 with H'=1.517. The combined plots had an H' index of 1.535 which shows that the area was diverse. This is expected since the area has adequate soil moisture and has mild alkalinity which are the preference of most earthworms (Edwards,1998).

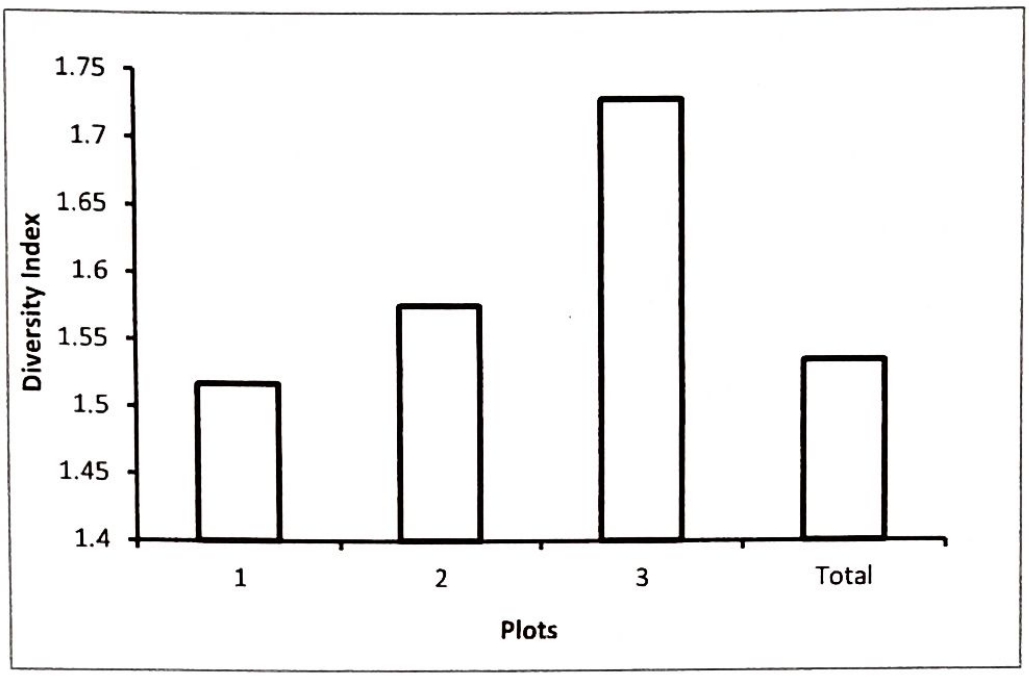

Figure 8. Species Diversity of Earthworms in Sampling Plots

In terms of species accumulation, it found out that there was a gradual increase in the number of species collected in each day. This increase signifies that the area was diverse in earthworm species. Moreover, if the sampling period was done longer, there could be more species collected because the curve has not yet stabilized.

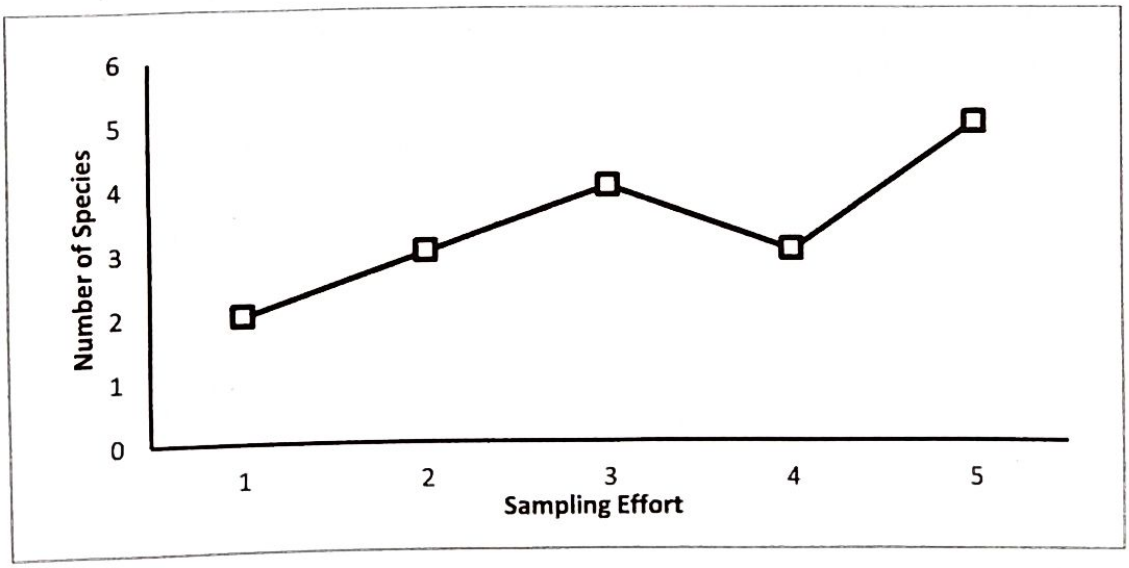

Figure 9. Species Accumulation Curve

D. Factors that influence species diversity of earthworms

In order to analyze the factors which shapes the diversity of earthworms, three environmental factors (soil moisture, pH and elevation) were analyzed using the Canonical Correspondence Analysis. The analysis revealed that *Polypheretima* sp. 2 was mostly dependent on soil moisture, pH and elevation as it accumulates closest to the environmental factors in the y-axis. This is in accordance to the study conducted by Kale et al., (2009) which states that the activity and population of earthworms are mostly influence by soil moisture because water constitutes 75-90 percent of their body weight. Moreover, according to Bantaowong et al. (2011), alkaline soil with pH ranging from 7.1-8 were found to be relevant to *Polypheretima* sp. 2, this is because these kind of soils facilitate the morphology of earthworms. He also added that as the elevation gets higher, the diversity also increases.

On the other hand, *Amynthas* sp., *Polypheretima* sp. 1, *Polypheretima* sp. 3 and *Pheretima* sp. were found to be negatively affected to soil pH, moisture and elevation. This implies that these species of earthworms thrives in any habitat requirements and season.

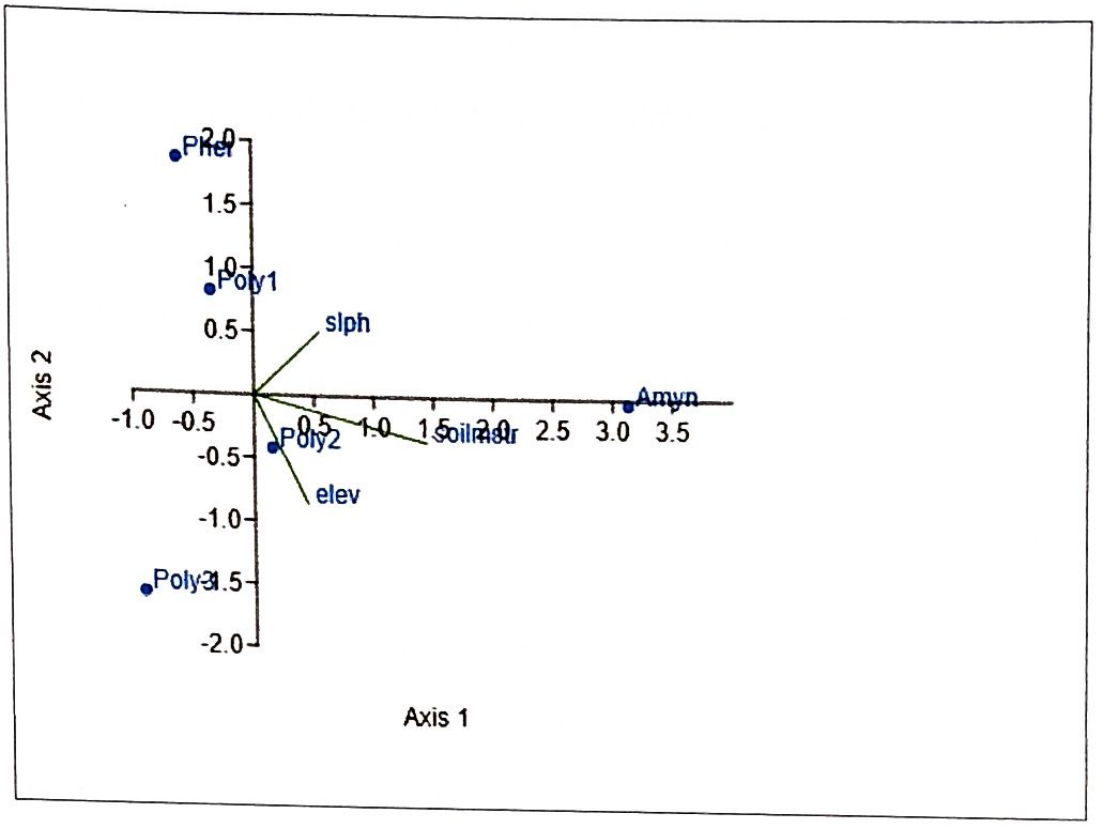

Figure 10. Canonical correspondence analysis of species and environmental factors association. The eigenvalue of axis 1 (horizontally) and axis 2 was 91.52% and 8.48%. Environmental factors were labeled as (slph = soil pH, soilmstr = soil moisture, elev = elevation, Pher = *Pheretima* sp., Amyn = *Amynthas* sp., Poly 1 = *Polypheretima* sp. 1, Poly 2 = *Polypheretima* sp. 2, Poly 3 = *Polypheretima* sp. 3)

CHAPTER 3

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

This chapter presents the summary of findings derived from statistical analysis, conclusions derived from findings and recommendations derived from the conclusions.

Summary of Findings

There were three identified genera found within the relatively disturbed forest over limestone in Mt. Guimba and these include: *Polypheretima, Amynthas* and *Pheretima*. Out of three genera present, the most abundant genus is *Polypheretima* spp. with the highest total value of 82%. This genus was found on every plot and is characterized by its high number of individuals. The second high value of abundance was *Pheretima* sp. (10%), followed by *Amynthas* sp. (8%).

In terms of species diversity on each plot, Plot 3 had the highest diversity value of 1.728. The high diversity value of the plot could be attributed to the habitat that is suitable to earthworm communities. On the other hand, Plot 3 had the lowest diversity value of 1.575, this could be possibly because of the collected species that is low in the area. In terms of the H' index across plots, the value is 1.535 which is an implication that the area is highly diverse in earthworm species.

In terms of CCA, *Polypheretima* sp. 2 was found to be most relevant to soil moisture, pH and elevation. However, *Amynthas* sp., *Polypheretima* sp. 1, *Polypheretima* sp. 3 and *Pheretima* sp. were found to be not associated to soil moisture, pH and elevation. This signifies that these species thrive regardless of any habitat requirements and season.

Conclusions

Based on the findings of the study, the researcher comes up with the following conclusions:

There were three identified genera by the researcher using the field guide of Dr. Nonillon Aspe. These genera include: *Polypheretima, Amynthas* and *Pheretima*. Among these genera, the most abundant genus was *Polypheretima* with the highest total value of 82%. It is due to the high number of individuals and species collected across plots. With regards to the diversity index on each plot, Plot 3 had the highest diversity value of 1.728 because of the suitable habitat for earthworm communities, followed by Plot 2 with diversity value of 1.575 and Plot 1 with diversity value of 1.517. Overall, the area was diverse with an H' index of 1.535. In terms of the factors that shapes the diversity of earthworm species, *Polypheretima* sp. 2 was found to be associated to soil moisture, pH and elevation, while *Amynthas, Polypheretima* sp.1, *Polypheretima* sp. 3 and *Pheretima* sp. were found to be not associated.

Considering the result, relatively disturbed forest area in general had high diversity. The result found that earthworms could survive in disturbed ecosystems, particularly in moist and alkaline areas. In addition, earthworm species differ in their susceptibility to adapt in a certain area due to differences in their ecology, feeding habits and physiological characteristics as well.

Recommendations

1. Conduct further studies on the diversity of earthworms in the same site for a longer period of time in order to identify more species

2. Conduct further studies of earthworm species on different sites to identify the factors that influence the diversity of these species

3. Increase the sampling days for more collection of species

4. Submit the unknown species on the expert for verification

5. Raise awareness for the public on the benefits and harmful effects of earthworms for better understanding and conservation purposes

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APPENDICES

APPENDIX A

Republic of the Philippines
BOHOL ISLAND STATE UNIVERSITY – Bilar Campus
Zamora, Bilar, Bohol

04 December 2021

HON. LEON A. CALIPUSAN
Municipal Mayor
Loboc, Bohol, Philippines

Dear Sir,

Good Day!

I am Jea Marie J. Simbajon, a fourth year Bachelor of Science in Environmental Science Student of Bohol Island State University – Bilar Campus.

I am currently working on my undergraduate thesis entitled "DIVERSITY OF EARTHWORM SPECIES OVER THE KARST LANDSCAPE IN RELATIVELY DISTURBED FORESTED AREA OF MT. GUIMBA LOBOC BOHOL, PHILIPPINES". The place is still under your Jurisdiction hence this letter. In line with this, I would like to ask for your approval to allow me to conduct a study starting December 2021- March 2022 every weekdays, 8am – 5pm in Mt. Guimba.

Hoping for a positive response with regards to this matter. Thank you and God bless.

Respectfully yours,

JEA MARIE J. SIMBAJON (Sgd)
Researcher

Noted:

REIZL P. JOSE, PhD (Sgd)
Research Adviser

Recommending Approval:

NOEL T. LOMOSBOG, PhD (Sgd)
Dean, CANR

Approved:

HON. LEON A. CALIPUSAN (Sgd)
Municipal Mayor

Republic of the Philippines
BOHOL ISLAND STATE UNIVERSITY – Bilar Campus
Zamora, Bilar, Bohol

04 December 2021

HON. MARCELO C. ORACION
Barangay Captain
Oy, Loboc, Bohol

Dear Sir,

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Recommending Approval:

NOEL T. LOMOSBOG PhD (Sgd)
Dean, CANR

Approved:

HON. MARCELO C. ORACION (Sgd)
Barangay Captain

Republic of the Philippines
BOHOL ISLAND STATE UNIVERSITY – Bilar Campus
Zamora, Bilar, Bohol

04 December 2021

HON. TIRSO P. ARAT
Barangay Captain
Tigbao, Loboc, Bohol

Dear Sir,

Good Day!

I am Jea Marie J. Simbajon, a fourth year Bachelor of Science in Environmental Science Student of Bohol Island State University – Bilar Campus.

I am currently working on my undergraduate thesis entitled "DIVERSITY OF EARTHWORM SPECIES OVER THE KARST LANDSCAPE IN RELATIVELY DISTURBED FORESTED AREA OF MT. GUIMBA LOBOC BOHOL, PHILIPPINES". The place is still under your Jurisdiction hence this letter. In line with this, I would like to ask for your approval to allow me to conduct a study starting December 2021- March 2022 every weekdays, 8am – 5pm in Mt. Guimba.

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NOEL T. LOMOSBOG, PhD (Sgd)
Dean, CANR

Approved:

HON. TIRSO P. ARAT (Sgd)
Barangay Captain

Republic of the Philippines
BOHOL ISLAND STATE UNIVERSITY – Bilar Campus
Zamora, Bilar, Bohol

04 December 2021

HON. VIRGILIO SARIGUMBA
Barangay Captain
Jimilian, Loboc, Bohol

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Research Adviser

Recommending Approval:

NOEL T. LOMOSBOG, PhD (Sgd)
Dean, CANR

Approved:

HON. VIRGILIO SARIGUMBA (Sgd)
Barangay Captain

Republic of the Philippines
BOHOL ISLAND STATE UNIVERSITY – Bilar Campus
Zamora, Bilar, Bohol

04 December 2021

Ssg. RENATO PALOMARES
Patrol base Commander
Quinoguitan, Loboc, Bohol

Dear Sir,

Good Day!

I am Jea Marie J. Simbajon, a fourth year Environmental Science Student of Bohol Island State University – Bilar Campus.

I am currently working on my undergraduate thesis entitled "DIVERSITY OF EARTHWORM SPECIES OVER THE KARST LANDSCAPE IN RELATIVELY DISTURBED FORESTED AREA OF MT. GUIMBA LOBOC BOHOL, PHILIPPINES". The place is still under your Jurisdiction hence this letter. In line with this, I would like to ask for your approval to allow me to conduct a study starting December 2021- March 2022 every weekdays, 8am – 5pm in Mt. Guimba.

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JEA MARIE J. SIMBAJON (Sgd)
Researcher

Noted:

REIZL P. JOSE, PhD (Sgd)
Research Adviser

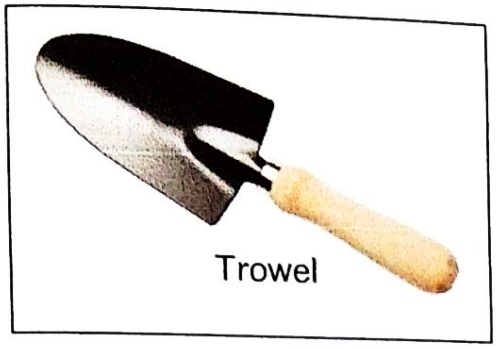
Recommending Approval:

NOEL T. LOMOSBOG, PhD (Sgd)
Dean, CANR

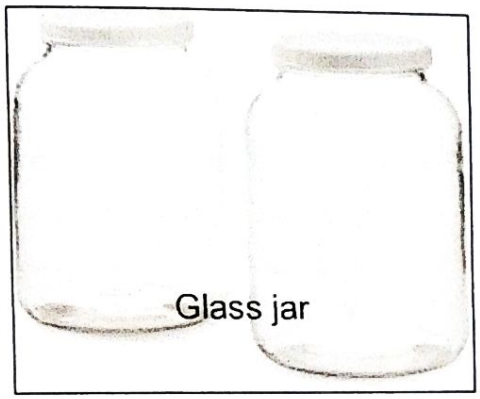
Approved:

Ssg. RENATO PALOMARES (Sgd)
Patrol base Commander

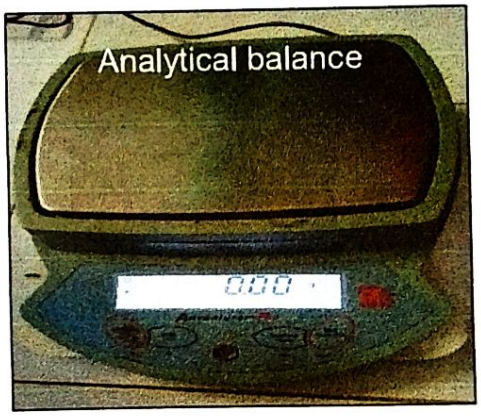
APPENDIX B


Trowel


Axe


Glass jar


Shovel


Analytical balance


Soil pH meter


Oven

APPENDIX C

Total number of individual species per plot

Species	Plot 1	Plot 2	Plot 3
Amynthas sp.	1	2	1
Pheretima sp.	1	2	2
Polypheretima sp. 1	3	7	3
Polypheretima sp. 2	5	6	3
Polypheretima sp. 3	6	6	2
Total	16	23	11

Diversity index on each plot

Species	Plot 1	Plot 2	Plot 3	Individuals
Amynthas sp.	1	2	1	4
Pheretima sp.	1	2	2	5
Polypheretima sp. 1	3	7	3	13
Polypheretima sp. 2	5	6	3	14
Polypheretima sp. 3	6	6	2	14
Total number of species	5	5	5	
Total number of earthworms	16	23	11	
Diversity Index	1.517	1.575	1.535	
Standard Error	1.019804	1.07703	0.3741657	

Raw data for Canonical Correspondence Analysis

Plot	slph	soilmstr	elev	*Poly1*	*Poly2*	*Poly3*	*Pher*	*Amyn*
1	7.5	67.06503	101	0	0	0	1	0
1	7.5	73.49702	101	1	2	0	0	0
1	7.4	74.92519	101	0	1	0	0	0
1	7.5	83.13625	101	1	1	0	0	0
1	7.5	88.82257	101	0	0	0	0	1
1	7.4	78.9753	101	0	0	1	0	0
1	7.3	55.80731	102	0	1	0	0	0
1	7.3	67.39384	102	1	0	1	0	0
1	7.3	56.15595	102	0	1	1	0	0
1	7.4	67.44388	102	0	1	0	0	0
2	7.3	93.63543	97	0	0	0	0	1
2	7.5	66.47558	88	0	1	1	0	0
2	7.5	65.48964	88	2	0	0	0	0
2	7.5	66.21516	88	0	0	0	1	0
2	7.5	69.07061	88	1	2	0	0	0
2	7.3	64.75638	88	1	0	0	0	0
2	7.4	74.87145	89	0	1	0	0	0
2	7.5	65.28516	89	0	2	1	0	1
2	7.4	75.54642	89	0	0	0	1	0
2	7.5	57.26965	89	1	0	1	0	0
3	7.5	55.97228	90	2	0	1	0	0
3	7.4	77.73651	90	1	1	0	0	0
3	7.5	56.04367	90	1	1	0	0	0
3	7.7	65.05129	89	0	0	0	0	1
3	7.4	54.18776	88	0	0	0	1	0
3	7.5	67.36428	86	1	2	0	0	0
3	7.6	71.9882	88	0	1	0	0	0
3	7.6	65.52681	85	0	0	0	1	0
3	7.5	67.21416	84	0	1	0	0	0
3	7.4	73.24341	86	1	0	1	0	0

APPENDIX D

Measuring the soil pH of soil samples (A); Reading the coordinates of the collected earthworm specimen (B); Preserving of earthworm specimen (C); Measuring the length of earthworm (D)