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Diversity of Earthworm Species within a Relatively Undisturbed Forest over Limestone in Mt. Guimba, Loboc, Bohol, Philippines

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ABSTRACT

Earthworms are keystone species that shape the terrestrial ecosystem into a conducive and productive system. However, the data supporting their ecology and biology were scarce, specifically in the karst areas of Mt. Guimba, Bohol. Hence, this investigation was carried out. Three randomly established 10m x 10m plots were utilized for the assessment. The findings revealed that the area is very low in earthworm diversity species; however, the detected earthworms are putatively new species. *Polypheretima* spp. was found to be abundant.

Polypheretima sp. 1, *Polypheretima* sp. 2, and *Pheretima* sp. 1 were associated with soil pH, soil moisture, and elevation. *Polypheretima* sp. 2 was sensitive to soil pH, while P*olypheretima* sp. 3 was closely associated elevation. Ultimately, these findings are not only for baseline information but also for conserving and protecting their natural habitat.

***Keywords**:* Biodiversity, *Pheretima, Polypheretima*, terrestrial ecosystem

INTRODUCTION

Earth's surface is covered by 15% karst landscape (White, 2021). Karst is a type of landscape where the bedrock consists of soluble rock types such as limestone, marble, and gypsum (National Park Service, 2022). Karst areas are significant hubs for diversification and outstanding biodiversity, making them subjects for ecological and evolutionary studies (Yang et al., 2021).

Earthworms are organisms that can thrive in specialized landscapes such as karst. They can live in any soil type across the world, provided the soil conditions are retained (Bhadauria & Saxena, 2009). Earthworms are major soil invertebrates that regulate ecosystem processes (Lavelle et al., 1997). They are perhaps considered the most important soil organisms influencing soil organic matter breakdown, soil structure development, and nutrient cycling (Aspe et al., 2009). In a study of earthworms in red pine forests in a limestone area in Korea, earthworms were observed to be abundant and have altered the soil texture and increased nutrient availability through the production of soil casts (Mun & Kim, 1991). This also aligns with the study of Robinson et al., (1992), who stated that the presence of lime or inherent to the soil can increase the abundance of earthworms in the soil. Hence, studying earthworms in the karst landscape would be interesting as new species of earthworm may be detected in the area.

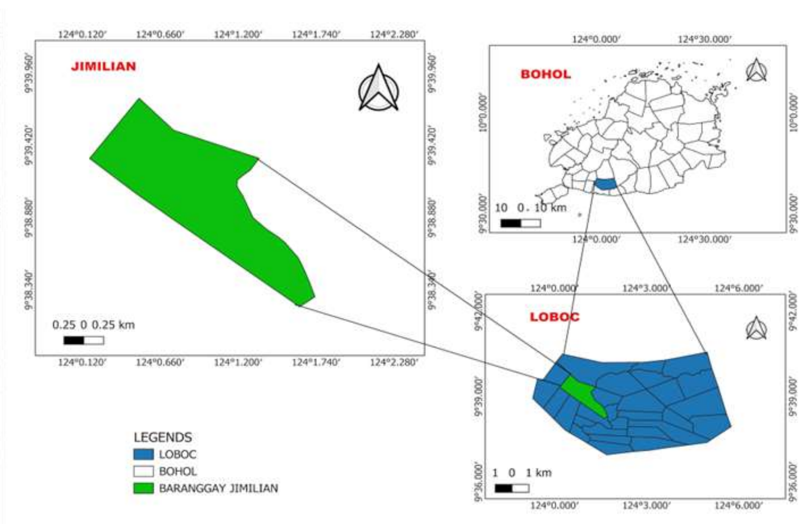
In the Philippines, the knowledge on earthworms was very limited prior to the year 2004. Decades of taxonomic studies being done in the Philippines have resulted in the detection of around 200 species of native earthworms representing nine pheretimoid genera that exhibit high endemicity (e.g., Aspe and James, 2016; Aspe et al., 2021). In Bohol, three genera were identified in a previous study by Flores (2007), including *Pheretima, Polypheretima,* and *Pleionogaster*. A more recent study conducted in Rajah Sikatuna Protected Landscape, Bohol, identified the genera *Pheretima, Polypheretima,* and *Amynthas,* which were putatively new species (Jose et al., 2021). As only a few areas have been surveyed in Bohol, this study provides additional data on earthworms that explain the ecology and biology of earthworm species in the karst landscape of Mt. Guimba on Bohol Island.

MATERIALS AND METHODS

The study was conducted at Mt. Guimba within Barangay Jimilian, Loboc. It is situated in the northwestern part of the municipality with coordinates of 9.651633 and 124.015230 (Figure 1). The mountain encompasses a total area of 185 hectares. Bioprospecting specifically occurred in the relatively undisturbed forest area of Mt. Guimba, which is under the limestone formation in Bohol (Urich, 1989). The area is surrounded by lush vegetation, including Mahogany trees, native trees, shrubs, and boulders. The forest floor is covered by decayed leaves, twigs, tree trunks, and rocks as well.

Figure 1

Location of the study site in Mt. Guimba, Brgy. Jimilian, Loboc, Bohol, Philippines



Sampling

Before initiating the study, permits for the conduct and collection of earthworm specimens were obtained from the local government and the Department of Environment and Natural Resources issued a Wildlife Gratuitous

Permit. The sampling followed the methods of Aspe et al. (2009). In the relatively undisturbed forest area of Mt. Guimba, three replicates of a 10m x 10m quadrat were randomly established in one row, with an average horizontal distance of 20m between plots (Figure 1). Within the plots, shovels, trowels, and hoes were used to dig ten holes measuring 0.5m (length) x 0.5m (width) x 0.3m (depth). Ten composite soil samples were collected within the bioprospecting site for soil moisture and soil pH analysis, and elevation data were gathered as well. Earthworms were collected, sorted by color, labeled accordingly with coordinates, and cleaned in tap water before being preserved in 70% ethanol and stored in a closed container with 10% formalin, ensuring three times the volume of the samples to guarantee the proper preservation of all specimens in the glass jar.

Morphological examination of specimens

The collected samples were transported to the Biology Laboratory of Bohol Island State University-Bilar, where they underwent thorough examinations, both externally and internally, utilizing a stereoscopic microscope. The examination was based on the observable characteristics of the specimen that are presented in earthworm taxonomic literature (e.g., Aspe & James, 2014; 2016; 2017; Aspe et al., 2021).

Data analysis

Relative abundance was employed to identify the abundance of various species present at the site of investigation. The calculation is expressed by the formula:

Relative Abundance= (Number of individual species/Total number of individual species) x100

Shannon-Weiner Index was utilized to assess the diversity of earthworms. The proportion of species relative to the total number of species (Pi) was calculated, and this proportion was then multiplied by the natural log of Pi (InPi). The resulting products were summed across species (Magurran et al., 2005).

$$H' = -\sum_{i=1} (Pi * LNPi)$$

Where:

H'= Shannon diversity index
∑= Sum from the species to species S
Pi= Fraction of the entire population made up of species i
S= Number of the species encountered

Furthermore, the **Canonical Correspondence Analysis** (CCA) was used to determine the association between species and environmental factors (Hammer and Harper, 2001). This analysis was conducted using PAST version 4. Edaphic data were collected through composite soil extraction, assessing 10 soil samples for pH and moisture content (Singh et al., 2016). Elevations were determined using a Global Positioning System (GPS).

RESULTS AND DISCUSSIONS

A total of five putatively new species were identified during the survey under the genera of Polypheretima and Pheretima, belonging to the family Megascolecidae.

Species accounts

Family Megascolecidae

Pheretima (Kinberg, 1866)

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 markings are usually absent. Internally, esophageal gizzard usually originates in viii, a pair of caeca originating in xxvii, extending forward. Ovaries and funnels are 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 is present on the spermathecal duct (s). One pair of prostate glands, racemose. Copulatory bursae present; secretory diverticula lacking on the coelomic surface of copulatory bursae.

***Pheretima* sp. 1 (Figure 2A)**

Pheretima sp. 1 is a dark brown worm with thread-like body, length 130-140 mm and diameter 8 mm to 9 mm on segment x. Body circular in cross section. Equator pigmented covering segment 11/12/13. Externally, Three pairs of spermathecal pores are is present from 6/7-8/9 anterior to clitellum, clitellum annular; female pores are present on xiv, posterior to clitellum; a pair of male pores are present on xviii, number of setae is less than 100 and distributed unevenly on every segments. Sixty-seven segments extend from prostomium to periproct. Internally, three pairs of spermathecae on vii to ix, nephridia are present on the spermathecal duct, gizzard is prominent, posterior to gizzard, four hearts present on segment xi to xiv, copulatory bursa is present, caeca is present at segment xxvii-xxiv, and prostate gland on segment xv.

Remarks: *Pheretima* sp. 1 belongs to the *Pheretima* dubia group of species, characterized by three pairs of spermathecae from 6/7 to 8/9. *Pheretima* sp. 1 is similar to *Pheretima korinchiana* Cognetti, 1922, *Pheretima* vungtauensis Nguyen et al., 2018, and *Pheretima* dubia Horst, 1893 in size. However, the three latter species are not recorded in the Philippines and are geographically distant from Bohol. No member of the *Pheretima dubia* group has the same features as *Pheretima* sp. 1, which may be a new species. A thotough morphological examination will be done to describe the morphological features and taxonomically identify the specimens at the species level.

Pheretima sp. 2 (Figure 2B)

Pheretima sp. 2 is a light brown worm with a thread-like body. 80 mm-90 mm in length and diameter 5-6 mm. Body circular in cross-section. Clitellum annular and unpigmented, number of setae per segment is less than 100 and distributed unevenly, gizzard is prominent, a pair of spermathecae are present on segment 5/6, nephridia in the spermathecal duct are present, copulatory bursae is absent, caecum originates in xxvii.-xii.

Remarks: *Pheretima* sp. 2 belongs to the *Pheretima urceolata* group of species, characterized by a pair of spermathecae in 5/6. The other *Pheretima* species detected in Bohol belong (Jose et al., 2021) to the *Pheretima sangirensis* group, characterized by a pair of spermathecae in 7/8. Although there are members of the *Pheretima urceolata* group in other parts of the Philippines that have similar features to that of *Pheretima* sp. 2, they are geographically distant from Bohol; thus, Pheretima sp. 2 may putatively be a new species. A thorough morphological examination will be done to taxonomically identify the the specimens at the species level.

Figure 2

A. Pheretima sp. 1. B. Pheretima sp. 2

Polypheretima (Michealsen, 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 a single or closely paired opening (Easton, 1979).

***Polypheretima* sp. 1 (Figure 3A)**

Polypheretima sp. 1 is a light-brown worm-pigmented segmental equator; diameter is 5 to 6 mm located in segment viii; adult length is 140 mm-150 mm; first dorsal pores at 13/14; clitellum annular from segment xiv to xvi; setae unevenly distributed around the segmental equator; a pair of spermathecal pores present at segment 5/6. Genital markings are lacking. Nephridia is absent on the spermathecal duct, the female single pore is unnoticeable, and caeca lacks pair of prostate glands in xvii-xx.

Remarks: *Polypheretima* sp. 1 is similar to *Polypheretima fruticosa* Hong and James, 2008 from Luzon Island, the only *Polypheretima* species documented in the Philippines with a pair of spermathecae in 5/6. However, the latter is much smaller (39-61 mm) and has genital markings unlike Polypheretima sp. 1. There is no similar *Polypheretima* species recorded in Jose et al. (2021) with *Polypheretima* sp. 1, which may putatively be a new species. Further morphological examinations will be conducted to establish the taxonomic identification of the species.

***Polypheretima* sp. 2 (Figure 3B)**

Polypheretima sp. 2 is an unpigmented worm with a length of 120-160 mm, 4-5 mm in diameter on segment v, clitellum unpigmented. A pair of spermathecal pores is present at segment 5/6. Setae per segment is less than 100 and distributed unevenly; genital markings are absent. Nephridia on the spermathecal duct is absent; gizzard is prominent at segment x; copulatory bursae is inconspicuous; caeca is lacking.

Remarks: P*olypheretima* sp. 2 is another *Polypheretima* species in Bohol with a pair of spermathecae in 5/6. Although *Polypheretima* sp. 2 and *Polypheretima* sp. 1 overlap in size, the former is unpigmented, while the latter has light brown pigmentation and may putatively be a new species. Further morphological examination will be done to taxonomically identify the specimens at the species level.

***Polypheretima* sp. 3 (Figure 3C)**

Polypheretima sp. 3 is a dark brown worm with a length of 80-90 mm and a width of 3-4 mm. Clitellum was unpigmented. Two pairs of spermathecal pores are present at segments 6 and 6/7. Setae per segment is less than 100 and distributed unevenly. Nephridia on the spermathecal duct is absent; gizzard is prominent at segment x; copulatory bursae is lacking

Remarks: *Polypheretima* sp. 3 is similar to *Polypheretima perlucidula* Hong and James, 2008 and *Polypheretima pagudpudensis* Hong and James, 2011 from Luzon Island and *Polypheretima zamboangensis* Aspe and James, 2016 from Mindanao Island in having two pairs of spermathecae in 5/6 and 6/7. However, *Polypheretima perlicidula* and *Polypheretima pagudpudensis* are shorter (38-63 mm), while *Polypheretima zamboangensis* is significantly longer (223-306 mm). In addition, *Polypheretima pagudpudensis* and *Polypheretima zamboangensis* have genital markings, while *Polypheretima* sp. has none. No other *Polypheretima* species is morphologically similar to *Polypheretima* sp. 3, which is putatively a new species. Further morphological examination will be conducted to taxonomically identify the specimens at the species level. Table 1 shows the comparison of the general features of the species in Mt. Guimba, Loboc, Bohol.

Table 1

Comparison of the earthworm species in Mt. Guimba, Loboc, Bohol, Philippines.

Character	*Polypheretima* sp. 1	*Polypheretima* sp. 2	*Polypheretima* sp. 3	*Pheretima* sp. 1	*Pheretima* sp. 2
Body pigmentation	Light brown	Unpigmented	Dark brown	Dark brown	Light brown
Size (mm)	140-150 x 5-6	120-160 x 4-5	80-90 x 3-4	130-140 x 8-9	80-90 x 5-6
Spermathecae	1 pair in 5/6	1 pair in 5/6	2 pairs in 5/6/7	3 pairs in 6/7-8/9	1 pair in 5/6
Nephridia on spermathecal ducts	Absent	Absent	Absent	Present	Present
Copulatory bursae	Inconspicuous	Inconspicuous	Absent	Conspicuous	Conspicuous
Size of prostate gland	xvii-xx	?	?	xv-xvii	?
Length of caeca	Absent	Absent	Absent	xxvii-xxiv	xxv-xvvii

Figure 3

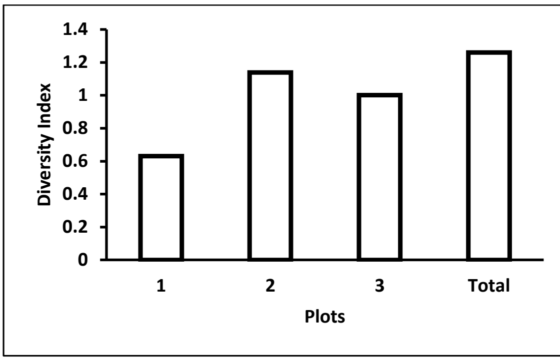
Genus Polypheretima. A. Polypheretima sp. 1. B. Polypheretima sp. 2. C. Polypheretima sp. 3.

Species diversity of earthworms

The relatively undisturbed forest of Mt. Guimba exhibits a diversity index of 1.26, indicating a very low diversity of earthworm species at the site (Figure 4). Low species diversity of earthworms was also observed in the forest over limestone in Initao-Libertad Protected Landscape and Seascape, Misamis Oriental (Sacay & Aspe, 2022), where species of *Pheretima, Polypheretima,* and *Amynthas* were observed. Temperature, precipitation, soil moisture, as well as extreme climate events like drought and flood are some environmental factors that may alter the composition and functioning of communities in the soil (Singh et al., 2019). The activity of most earthworms is interrupted during dry periods, and they usually move into the deeper soil layers. They may undergo 'diapause', wherein they stop feeding, to overcome the adverse period (Jairajpuri, 1993). On the other hand, the low diversity of earthworms in the area may also be attributed to inadequate sampling efforts that could create bias in the results. Thus, a more exhaustive sampling needs to be conducted to enhance the representation of earthworm diversity in the area.

Figure 4

Species diversity on each plot



Relative abundance

Table 2 displays the relative abundance of earthworms recorded in three sampling plots within a relatively undisturbed forest. The genus *Polypheretima* spp. was found to be abundant, whereas *Pheretima* spp. revealed to be the least abundant on the site. In terms of occurrence, five (5) species were most frequently captured during the sampling period, and these were the following; *Polypheretima* sp. 1 (46%), *Polypheretima* sp. 2 (25%), *Polypheretima* sp. 3 (25%), *Pheretima* sp. 1(2%), and *Pheretima* sp. 3 (2%). *Polypheretima*, the most abundant genus across plots, can be found in various regions, including the Philippines, Indonesian Archipelago, Papua New Guinea, and Malaya Peninsula (Aspe, 2016; Nguyen et al., 2015). In the Philippines, 18 known Polypheretima species exist (Jose et al., 2021), although the species described in this study still need to be included. Alongside *Polypheretima*, *Pheretima* species are also present in Southeast Asia and the Pacific region (Aspe, 2016; Aspe & James, 2014). Currently, in the Philippines, which may have been thought to be the center of species radiation of *Pheretima*, 99 known species are documented on the country (Aspe & James, 2017). However, the species described in this study still need to be included.

Table 2

Relative abundance of earthworms in a relatively undisturbed forest

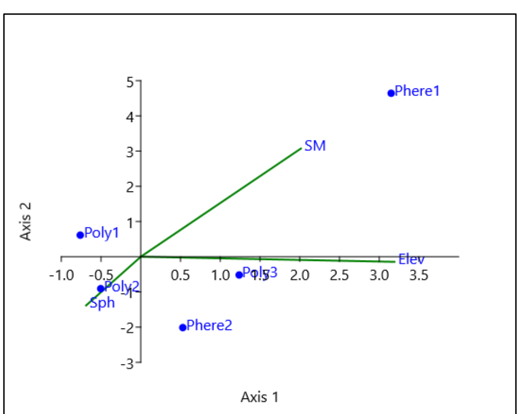
Family	Genus	Species	N	Relative Abundance (%)
Megascolecidae	*Polypheretima*	*Polypheretima* sp. 1	20	46%
		Polypheretima sp. 2	11	25%
		Polypheretima sp. 3	11	25%
	Pheretima	*Pheretima* sp. 1	1	2%
		Pheretima sp. 2	1	2%
		Total	**44**	**100%**

Factors that influence species diversity of earthworms

Figure 5 presents the canonical correspondence analysis of species and their association with environmental factors. The analysis revealed various species closely associated with the prevailing factors. *Polypheretima* sp. 1, *Polypheretima* sp. 2, *Polypheretima* sp. 3, and *Pheretima* sp. 1 were closely associated with soil pH (7.3- 7.7), soil moisture (58.3%- 97.28%), and accumulated near the centroid where environmental factors lie. *Polypheretima* sp. 2 was highly sensitive to soil pH, while *Polypheretima* sp. 3 was associated with elevation (258-261 m). On the other hand, *Pheretima* sp. 1 was found to be a species that could thrive neither with nor without the prevailing factors included in this study.

Figure 5

Canonical correspondence analysis of species and environmental factors association. The eigenvalue of axis 1 (horizontally) and axis 2 (vertically) was 59.13% and 40.86 %. Environmental factors were labeled as: Sph= Soil ph; SM= Soil Moisture; and Elev= Elevation. Species were labeled as: Poly1= Polypheretima sp. 1; Poly2= Polypheretima sp. 2; Poly3= Polypheretima sp. 3; Phere1= Pheretima sp. 1; and Phere2= Pheretima sp. 2.



The analysis implies that the association of various earthworm species with environmental factors (*Polypheretima* sp. 1, *Polypheretima* sp. 2, *Polypheretima* sp. 3, and *Pheretima* sp. 1.) was explained by Bothe and Drake (2007) and Bhadauria and Saxena (2009). They suggested that earthworms thrive in any terrestrial ecosystem when soil conditions, such as sufficient soil moisture, soil pH, and elevation, are met. Although *Polypheretima* sp. 2 was found to be highly sensitive to soil pH, a study by Aspe et al. (2009) revealed that soil pH was insignificant for earthworms, as they can thrive in a wide range of soil pH. In their study, *Pithemera* sp., *Polypheretima* sp., and some *Pheretima* species thrived and reproduced in soil with a pH as low as 3.7. While the *Polypheretima* sp. 3 was found to be closely associated with elevation, this could be due to isolation, making this species thrive at high elevations. Earthworms are considered generalists, thriving in any terrestrial ecosystem as long as soil conditions are met (Bothe & Drake, 2007; Bhadauria & Saxena, 2009), aligning with the characteristics of *Pheretima* sp. 1, which was found to be a generalist species.

Given that Bohol is under the domain of karst landscape wherein the soil is rich in Calcium carbonate or lime and has varied geographical relief (Urich, 1989). These distinct characteristics of Bohol usher the earthworms to thrive, given that earthworms prefer soils that are high in pH (alkaline), which contributes to their abundance (Robinson et al., 1992). The results show that the area exhibited very low diversity, which may be due to insufficient sampling effort. However, this does not imply that the perpetuation of different species was impeded. The karst landscape in Bohol provides a unique environment that supports earthworm diversity, along with the presence of soil moisture. In addition, the varied gradient of elevation in relation to the variation of species richness could perhaps be attributed to the biotic and abiotic factors prevailing in the area (González et al., 2007). Moreover, the predictability of the earthworm preferences plays a pivotal role in its existence.

These results signify that earthworms, as a keystone species, are significant in terms of land use management and conservation. Their association with different environmental conditions underscores the need for sustainable practices or absolute conservation measures in both the karst areas and their surrounding environment to ensure the continuation of the direct and regulating services performed by earthworms, which are vital for ecosystem health. By implementing research-based management strategies, the quality of ecosystems can be strengthened, supporting the existence and proliferation of earthworms and leading to sustainable and inclusive development. Conservation efforts should prioritize preserving the unique environmental conditions of karst landscapes, such as soil pH, and moisture, through sustainable land use practices and absolute conservation measures. Understanding the factors influencing earthworm distribution and diversity in these landscapes is crucial for developing effective conservation strategies that safeguard these ecosystems and the essential ecological roles played by earthworms.

CONCLUSIONS

Mt. Guimba, a relatively undisturbed forest, revealed a very low diversity of earthworm species. This result was influenced by a short sampling effort, which affected the species captured during the sampling period. However, Bohol, as an island that belongs to the karst domain, would be a significant hub for earthworm survey since the inherent lime content of the soil would contribute to the abundance and diversity of earthworm species. Additionally, the putativeness and uniqueness of the earthworm species and Bohol, respectively, would be a baseline data for efficient soil management strategies and conservation practices to be done.

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