

## Comparisons of earthworm community structure between different ecotypes



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### ABSTRACT

The Philippines is considered to be a biodiversity “hotspot” of high species richness and endemism but the increased in human population and the destruction of the environment triggered the decline of biodiversity. Habitat complexity and the specialized niches available to animals are lost due to deforestation and anthropogenic activities. As good bioindicators and soil ecosystem engineers, earthworms play an important role for the decomposition, remediation of the soil in the form of their casts and food source for the other animals. This study was conducted to determine the biodiversity of earthworms from 3 different ecotypes namely forest, riverbank, and agricultural area. To determine on to what genus they belong, there were a total of 64 morphological characters that were used. There are a total of 4 genera that were identified from the 3 families namely, *Eudrilidae*, *Megascolecidae*, and *Glossoscolecidae*. The 4 genus were namely, *Eudrilus eugeniae*, *P. corethrurus*, and 2 *pheretima sp.* This study shows that these species were actually invasive that even in the forest they are still present. But results show the forest has the most number of species compared to the other ecotypes like river and agricultural area.

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### 1. Introduction

The Philippine ecosystem is characterized by a high degree of biological diversity. However, population pressure, poverty and survival needs have resulted in rapid destruction of the environment. It is thought that about 90% of Philippine land area was once forested. But due to massive forest exploitation, excessive annual cuts and weak reforestation efforts it dropped to less than 20%. Based on the analysis almost 9.8 million hectares of forests were lost. If the current rate of deforestation is maintained, it is projected that there will be no forest covers within the next decade (Suarez and Sajise, 2010). Earthworm populations have been recognized as bio-indicators of soil fertility and health, because earthworm improves soil properties by playing an active role in the decomposition of organic materials, nutrient cycling, soil formation, and the improvement of soil structure by channeling and bioturbation (Fragoso and Lavelle, 1992; Liu and Zou, 2002).

The differences in habitat preferences may affect the community structure of earthworms in sites altered by land-use changes. In Banaue rice terraces, there's a major threat because of the earthworms. These are called the giant earthworms, known as the species under *Polypheretima elongata* and another unknown species under the genus *Pheretima*. They cause major threat because they burrow holes in the landscape resulting a landslide or the destruction of the landscape where the rice is usually grown. They made it to the rice terraces because of the destruction of their habitats. In recent years, the increasing global trade, travel, and transport had rapidly increased the rate of introduction and diversity of alien species (Paoletti, 1999). There are several species of earthworms that are invasive non-native ones and to mention a few are as follows: *Pontoscolex corethrurus* (origin Brazil), *Polypheretima elongata* (origin SE Asia, probably Indonesian region), *Amyntas corticis* (origin mainland E Asia), *Dichogaster saliens* (origin Africa), *Nematogonia occidentalis* (origin unclear), *Perionyx excavates* (origin India).

The need to conduct such surveys on earthworms is accentuated by the rapid destruction of forest communities particularly in the tropics (Rickart et al., 1991). Assessment surveys of earthworm communities are increasingly used to determine

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conservation priorities. Quantifying the Earthworm communities has gained increasing importance in environment impact assessments, planning of conservation, and also ecological research. This study determines to examine the influence of environmental changes on earthworm community structure.

## 2. Materials and methods

### 2.1. Collection of samples

Samples were collected through opportunistic sampling from the different ecotypes such as river, forest and agricultural area one to two feet below the ground. Collected earthworms were then washed with water and fixed with ethyl alcohol for preservation. Earthworms were preserved using 80% of ethanol and put in the screw capped test tube for the morphological examination.

### 2.2. Morphological analysis

Segmentation of earthworms support diversified functions of body parts and tissues. Fig. 1 represents the anatomy of the earthworms and their basic body parts. There are a total of 64 characters represented by codes were used in the examination. The characters were based on the study of Apuan et al. (2010), 6 additional characters from the study of James (2004) and 2 additional characters by Gates (1972).

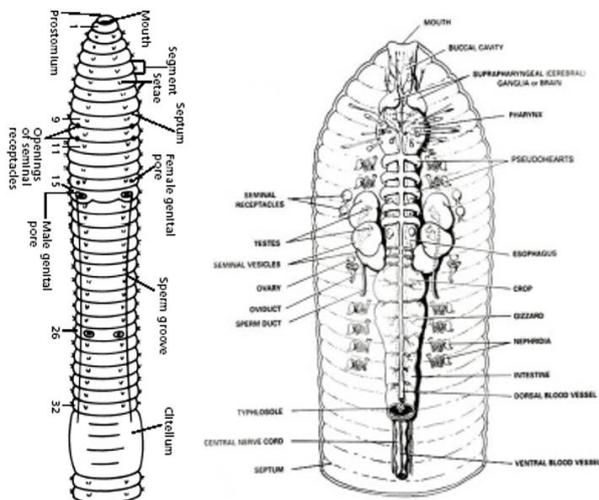


Fig. 1: External and internal morphology of Earthworms

## 3. Results

There are a total of 5 species of earthworms under the family Eudrilidae, Megascolecidae, Glossoscolecidae were identified (Figs. 2-5).

*P. corethrurus* (Fig. 2) is characterized by the obvious external feature of clitellum that is saddle shaped and is covered by 7 to 9 segments from the anterior, the setae is arranged 8 setae per segment and is single pointed. The pigmentation of this species is unpigmented to slightly pink.

*Eudrilus eugeniae* has an obvious external feature like the purple sheen and the posterior segments are evenly tapered to a point (Blackburn, 1989). They have sizes that ranges from about 10cm in length to huge specimens of over 12cm and size may depend on habitat (Segun, 1998). They are known to contribute to soil processes through faecal excretion in form of casts, burrows, feeding and digestion (Tian et al., 1995). These earthworms are of great importance especially in vermicomposting, bait, food for fish, birds, etc.



Fig. 2: External feature of *P. corethrurus*



Fig. 3: External feature of *Eudrilus eugeniae*

The genus *Amyntas* is one of the invasive and dominant terrestrial earthworm genera that occur throughout Thailand and nearby countries.



Fig. 4: External feature of *Amyntas* sp

The *Pheretima* group of species is the largest group of earthworms in the world (Sims and Easton, 1972) from the family Megascolecidae. This species is not applicable in the biological species concept because they have different sizes and anatomically not compatible (Aspe et al., 2009). Members of Family Megascolecidae have annular clitellum. The male pores are a single pair, usually on segment 18. The female pores are either paired or there is only a single median pore, nearly always on segment 14. Most of the *Pheretima* species were darkly pigmented. In the study conducted by Gates (1972).

Two of these species belongs to the family Megascolecidae, one in Glossoscolecidae and one in the family Eudrilidae. There are a lot of samples from the family Glossoscolecidae from each of the ecotype dominating the other species. One species was identified as *P. corethrurus* of Family

Glossoscolecidae, an exotic species that originated from Brazil (Table 1).



Fig. 5: External features of A. *Pheretima sp.1* and B. *Pheretima sp. 2*

Table 1: Species distribution of earthworms from different ecotypes

Species	Ecotype		
	Forest	Riverbank	Agriculture
<i>Eudrilus eugeniae</i>	-	+	+
<i>Pheretima sp. 1</i>	+	+	-
<i>P. corethrurus</i>	+	+	+
<i>Amyntas sp.</i>	+	+	+
<i>Pheretima sp 2</i>	+	-	-

Table 2 shows that between the 3 different ecotypes forest has the most number of species of earthworms and is the most diverse. This is due to the less anthropogenic activities happening in the area compared to the river and agricultural area. Results also show that there are 3 invasive species that are present in each ecotype. Namely *P. corethrurus*, *Eudrilus eugeniae*, and *Amyntas sp.* Endogeic worms were present in all the different ecotypes and each of the ecotypes were dominated by *P. corethrurus*. *P. corethrurus* are most abundant in the river side and agricultural area.

### 3.1. Forest

The result of the neighbor joining clustering of earthworm populations above shows two groups from the forest. The result of the study revealed 2 groups of earthworms based on the 63 phenotypic characters used. They are presented as follows:

- Group 1: Clitellum cover is from segments 2-8. Clitellum origin is from segment 12-15. Other individuals don't have a male pore and the other earthworms have their male pore located at segment 13-19. Setal arrangement of this group is very variable that they vary from numerous and arranged equally around each segment (Perichaetine) and setae counts vary in different parts of the body from more than 8 setae per segment to less than 8 in the posterior. Dorsal pores are present in some individuals and are located in segment 12/13. Clitellum shape is annular and also saddle type. There are no genital markings present. Female gonopore is present on

some individual. Their pigmentation is brown and pink, some individuals are unpigmented. Prostomium type is tanylobic and epilobic. There are no spermathecal pores present. Last heart location varies from segments 11-17. There are no pre-testicular present. Gizzard locations vary from segments 6-9; 17-25. Testes are absent. Intestinal caeca and copulatory pouches are absent. Spermathecae is located from segment 6-9. Origin of intestine is located on segment 12, 14, 15, 17, 21, 25, 26. Seminal vesicles are located on segment 9, 10, 13-15 and 17.

- Group 2: Clitellum is not developed. Male pore is not developed. Setae counts vary in different parts of the body from 8 segments to less than 8 segments in the posterior. There are no spermathecal pores present. There are no setae between male pores. There are no dorsal pores present. There are no genital markings present. There are no female gonopores present. The pigmentation of this group is pink. Prostomium type is tanybolic. Prostates are not present. Last heart location is located on segments 15 and 16. Nephridia are small and plenty that either scattered (meraic). There are no pretesticular spermathecae present. Gizzard is located on segment 12. There are no testes present. There are also no intestinal caeca and copulatory pouches. Origin of intestine is located on 14-15, and 18. Location of seminal vesicles is located on segment 12.

Of the 2 groups, the variations between the 3 species groups were described in Table 2.

### 3.2. Agricultural area

Neighbor joining clustering of individuals in relation to the various identified species of earthworms from the agricultural area revealed 2 groups based on the use of 63 phenotypic characters. The two groups have the following characters:

- Group 1: Clitellum cover is from segments 2-4, 6, 8, and 9. Clitellum origin is from segments 11-14. Male pore location is located on segment 15-18. Dorsal pore is not present. Dorsal pore is absent. Setae of this group vary from numerous and arranged equally around each segment (Perichaetine), setae count vary in different parts of the body from greater than 8 segments to less than 8 segments in the posterior, setae numerous and arrange equally but setae are closer (near). Clitellum shape is annular and saddle type. There are no genital markings. Some individuals have female gonopore present. The pigmentation is pink and brown to dark brown. The prostomium type is epilobic and tanybolic. There are no spermathecal pores present. Some individuals have prostatic gland and are tubular and racemose. Last heart location is on segments 9-14. Location of gizzard is on segments from 12, 14, 17 and 23. Testes are

absent. Intestinal caeca and copulatory pouches are absent. Spermathecae are located on segments 6 and 8. Origin of intestine 15, 17-19, and 21. Location of seminal vesicles 9-12.

- Group 2: Clitellum covers vary from segments 6, 8, and 9. Clitellum origin is from segments 12-14. There are no male pores present. Setae counts vary in different parts of the body from greater than 8 segments in the anterior and less than 8 segments in the posterior. There are no dorsal pores present. Clitellum shape is saddle type. There are no genital markings present. There are no female gonopores

present. Prostomium type is tanybolic. This group of earthworms is unpigmented. Some individuals have spermathecae on segments 5-9. Prostatic glands are absent. Nephridia are meroc. There are no testes present. There are no intestinal caeca and copulatory pouches present. Location of gizzard is on segments 12, 14, and 23. Origin of intestine is on segments 19, 21, and 25. Seminal vesicles are located on segment 11, 12, 14, and 15. Morphological variations of three selected species groups of earthworm populations found in the agricultural area are shown in Table 3.

**Table 2:** Morphological variations of the different earthworm populations found in the forest area

Characters	<i>P. corethrurus</i>	<i>Pheretima</i> sp.	<i>Amyntas</i> sp.
Clitellum origin	13, 14, 15	13,12, 14	Absent
Clitellum cover	6, 7, 8	3, 2, 5	Absent
Male pore location	Absent	18, 17	Absent
Setal arrangement	setae count vary in different parts of the body from 8>8 posterior	setae numerous and arranged equally around each segment (Perichaetine)	setae count vary in different parts of the body from 8>8 posterior
Dorsal pore	Absent	Present	Absent
Clitellum shape	Saddle type	Annular	absent
Genital markings	Absent	18-22	Absent
Female gonopore	Absent	Paired	Absent
Pigmentation	Unpigmented	brown	Pink
Spermathecal pore	Absent	Absent	Absent
Prostomium type	tanybolic	Epilobic	tanybolic
Prostatic gland	Absent	present	Absent
Prostatic gland location	Absent	17-18	Absent
Prostatic gland Shape	Absent	racemose	Absent
Last heart location	9-15, 17	10, 11, 14,16	42721
Location of gizzard	6, 7, 9, 17,23	6-8, 9, 10, 25	42687
Origin of intestine	14,16,17,19,21	12, 15, 21,25	11,12,14,17,18,19,
location of seminal vesicles	10, 11, 12,15	9, 10, 12, 14, 15	Absent

**Table 3:** Morphological variations of the 3 earthworm populations found in the agricultural area

Characters	<i>E. Eugeniae</i>	<i>P. corethrurus</i>	<i>Amyntas</i> sp.
Genital markings	none	absent	absent
Prostomium type	epilobic	Tanybolic	Tanybolic
Prostatic gland shape	tubular	absent	absent
Last heart location	9, 10, 11	10,11,12,14,16	9, 10, 11, 12,14
Location of gizzard	14, 15	14, 15	14, 15,6,23
Testes	absent	absent	absent
Intestinal caeca	absent	absent	absent
Copulatory pouches	absent	absent	absent
Spermathecae	absent	absent	Absent
Location of spermathecae	absent	absent	absent
Origin of intestine	15, 16, 18, 19, 25	18, 19, 21, 25	15,19,21,25
location of seminal vesicles	10, 11, 12	11,12,13, 14,15	11
Clitellum cover	4, 10	9, 8, 10	Absent
Clitellum origin	12, 13	12,13,14	absent
Male pore location	16, 17	Absent	absent
Setal arrangement	setae numerous and arranged equally around each segment	setae count vary in different parts of the body	setae numerous and arrange equally but setae are closer(near)
Setae between MP	present	Absent	absent
Dorsal pore	absent	absent	absent
Clitellum shape	Saddle type	Saddle type	absent

### 3.3. Riverbank

Neighbor joining clustering of individuals in relation to the various identified species of earthworms from the riverbank based on morphological variations reveals 2 groups of earthworms from the riverbank area. Using the 63 phenotypic characters the two groups has the following characters:

- Group 1: Clitellum cover is from segments 2-4, 6-8, and some individuals don't have clitellum at all. Those individuals who have clitellum have their clitellum originated from segments 11-15, and 17. Male pores are located on 13 and 16-18. Setal arrangements vary from setae numerous and arranged equally around each segment (Perichaetine) and setae counts vary in different parts of the body from greater than 8 setae per segment and less than 8 setae per segment in the posterior. Dorsal pores are absent. Clitellum shape varies from annular and saddle type. Some individuals have genital markings present on segments 10-12, 19-22, and 18-22. Spermathecal pores are absent. Prostatic glands are present and vary in shape from tubular and racemose. Some individuals have pre-testicular spermatheca present. Location of gizzard varies on segments 7,

12, 14, 17, 23, and 25. Some individuals in this group have their testes located on segments 6 and 7. Intestinal caeca and copulatory pouches are absent. Those individuals who have their spermatheca present are located on segments 6-8. Origin of intestine varies on segments 14, 15, 19, 21, and 25. Location of the seminal vesicles are located on segment 9, and 11- 13 (Table 4).

- Group 2: Clitellum cover is from segments 6-9. Clitellum origin is 12 and 13. Male pore is not present. Setae counts vary in different parts of the body from more than 8 setae per segment to less than 8 setae in the posterior. Dorsal pore is not present. There are no female gonopores. This group is unpigmented. Prostomium type is tanybolic. There are no spermathecal pores present. Prostatic glands are absent. Last heart location is on segments 9, 11, 12 and 15. Nephridia are single pair or holoic. There are no pre-testicular spermatheca present. Location of gizzard is on segments 14, 17, and 20. Testes are absent. Intestinal caeca and copulatory pouches are absent. Origin of intestine is on segments 21 and 25. Seminal vesicles are absent. Morphological variations of selected earthworm populations found in the riverbank area are shown in Table 4.

**Table 4:** Morphological variations of the different earthworm populations found in the riverbank area

Characters	<i>E. Eugeniae</i>	<i>P. corethrurus</i>	<i>Pheretima sp.</i>	<i>Amyntas sp.</i>
Clitellum cover	3,4,5	9, 8, 10	3,2,3	absent
Clitellum origin	9, 13, 14, 7	12-15	12-15	absent
Male pore location	13, 16, 17, 18	Absent	17, 18	
Setal arrangement	setae numerous and arranged equally around each segment	setae count vary in different parts of the body	setae numerous and arranged equally around each segment	setae numerous and arrange equally but setae are closer(near)
Setae between MP	present	Absent	present	absent
Dorsal pore	absent	absent	Present 12-13	absent
Dorsal pore	absent	absent	Present 12-13	absent
Clitellum shape	Saddle type	Saddle type	annular	absent
Genital markings	none	absent	18-22	absent
Clitellum shape	Saddle type	Saddle type	annular	absent
Last heart location	9, 10, 12	9-13, 16	11-14, 17	10-14/8/2/2016
Location of gizzard	7, 14, 17, 23	13-15	13-15	11, 13, 14, 15
Testes	absent	absent	Present 5, 7, 11	absent
Intestinal caeca	absent	absent	absent	absent
Copulatory pouches	absent	absent	Absent	absent
Spermathecae	absent	absent	Present	Absent
Location of spermathecae	absent	absent	6-8	absent
Origin of intestine	17, 19, 21, 25	14, 17, 18, 19,25	14, 16, 17, 28, 21	12, 14, 18, 19, 25, 26
location of seminal vesicles	9,11, 15	9-14	9-13, 17	12, 13, 14

Results of this study have shown the wide distribution of the different earthworm groups. Earthworms play an ecological significance in the part of the biodiversity since there are many fauna and flora that depends on them. While there are few information about interactions between native and

non-native species, they are however critical to understanding the potential consequences of ongoing biological invasions. The existence of biological invasions in soil is largely overlooked because soil is an opaque medium, where the biota is inconspicuous (Hendrix and Bohlen, 2002; Ehrenfeld

and Scott, 2001). In the study conducted by Zou et al. (2006) the conversion of the natural environment to industrialized environment will lead the anecic earthworms mostly the native ones to disappear because it decreases the soil surface litter due to disturbances that leads to the dominance of the endogeic species *P. corethrusus*. *P. corethrusus* can borrow deep into mineral soil layers and regenerate rapidly even in nutrient poor soils (Lavelle et al., 1999) and this explains why this species has a high density in agricultural area.

The destruction of the natural habitat causing alteration in the chemical and physical properties of the soil most likely hampered the development and activity of the native earthworms; thus, alter the earthworm community structures in these areas (Aspe et al., 2009). The destruction of the natural habitat by anthropogenic activities, which drastically affected the soil environment and most likely favored the establishment of the widespread introduced species *P. corethrusus* (Aspe et al., 2009). *P. corethrusus* has been said to be "the most widely distributed earthworm" though that honor also has been claimed for several lumbricid species (Gates, 1972).

The occurrence of the exotic species in the farmlands, grasslands and the disturbed forests is most likely associated with anthropogenic activities. When the forest was converted to industrialized lands, their natural habitat has been destroyed resulting in the loss of the native species as the cultivated lands became favorable for the propagation of the exotic species. This group of species is also abundant in the agricultural area because they are able to thrive in the harsh and stressful environment in the farmlands due to the use of pesticides where the native species cannot (Aspe et al., 2009). Human immigration and sustained anthropogenic activities favored the establishment of *P. corethrusus*, which also is responsible for the disappearance of the native species in the area from the study conducted by Lapiéd and Lavelle (2003). *P. corethrusus* do not necessarily require a mate for them to reproduce and that they have the ability to start a new population by only one individual. That may explain its rapid population growth, which is a big advantage over the other species (Aspe et al., 2009).

Introduced species like *Pheretima* also dominate the earthworm fauna in most soils. A general feature is that native earthworm species are found in relatively undisturbed soils whereas introduced species are more likely to be encountered in disturbed soils (Hendrix, 1995; Kalisz and Dotson, 1989). A study conducted by Callahan et al. (2002) shows there are greater than 75 % of the total number of earthworms collected were exotic species and only at least 20 % consisted of juvenile lumbricids that may have been native or exotic species (the family Lumbricidae is represented by both native and introduced species in the samples studied in this study).

#### 4. Conclusion

The overwhelming abundance of exotic species in the site where there is less or non-anthropogenic activities are quite alarming. This study shows that even the undisturbed sites are also dominated by exotic species like *P. corethrusus*.

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