



AENSI Journals

Journal of Applied Science and Agriculture

ISSN 1816-9112

Journal home page: www.aensiweb.com/JASA



Phenotypic Plasticity in a Local Population of Earthworms from Upper Nilo Tigbao, Zamboanga Del Sur, Philippines

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ARTICLE INFO

Article history:

Received 25 June 2014

Received in revised form

8 July 2014

Accepted 10 August May 2014

Available online 30 August 2014

Keywords:

Earthworms Morphology

Plasticity

ABSTRACT

This study was conducted to determine morphological plasticity among selected populations of earthworms from Upper Nilo, Tigbao, Zamboanga del Sur, Philippines. There were 60 phenotypic characters that were used for the comparison. Three species were identified and were compared between populations. These were *Pheretima* sp. *Pontoscolex corethrurus*, and *Eudrilus euginae*. Results show variability within and among populations especially in their internal and external morphological characters such as the clitellum cover, location of gizzard, last heart location, nephridia, pigmentation, intestinal caeca, clitellum origin, clitellum shape, prostomium type, location of seminal vesicles, and male pore location. This study have shown that variability within and among populations of earthworms can be considered a common occurrence and this can be attributed to the species response to the different conditions of the locations where they inhabit.

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To Cite This Article: Ma. Theresa C. Pareño, Mark Anthony J. Torres, Muhmin Michael E. Mantingand Cesar G. Demayo, Phenotypic Plasticity in a Local Population of Earthworms from Upper Nilo Tigbao, Zamboanga Del Sur, Philippines. *J. Appl. Sci. & Agric.*, 9(11): 245-256, 2014

INTRODUCTION

Earthworms are best known for contributing soil fertility and flow of water in the soil. They could also be an indicator if the soil is healthy or not. In agriculture, determining species variability and their plasticity is necessary since phenotypically plastic organisms display alternative phenotypes in different environments. It is widely appreciated that possessing alternative phenotypes can affect fitness especially in today's soil ecological situation, uncontrolled deforestation in watershed areas resulting to unabated soil erosion can affect species status and survival especially the earthworms. Likewise, the study is necessary since there are limited information on this group of organisms considered very important in soil ecology and agriculture. The Philippines lack more studies about the taxonomic classifications of earthworms down to the species level. While there are reported new species of earthworms in the genus *Pleionogaster* Michaelsen, 1892, this has long been known from a few isolated collections made in the Philippines during the last two centuries (Easton, 1979; James, 2004) and its range and diversity has remained unknown. Since phenotypic plasticity can arise as an adaptation to variable environments (Hunges *et al.*, 2003), for it to be adaptive within different kinds of environments, we should try to understand that there must be a level of regulation over the point and the right timing of the expression of plasticity across generations for the cause of maintaining the response in the genetic mechanism (Rollwagen, 1996). Many soil ecosystem engineers (Lavelle *et al.*, Santra and Bhowmik, 2001) argue that poor agricultural practices may deplete earthworms' species diversity as a valuable resource within an individual's lifetime. Changes in land use patterns are directly affecting their composition and population structure different agro-climatic regions (Banchart and Julka, 1997). Since there are limited studies of this group of organisms here in the Philippines, this study was therefore conducted. Most of the studies did not consider within species and population variability aside from the absence of comprehensive documentations. It was therefore important that classification of species will be done not only by qualitatively describing the external morphological characteristics of the sample but should be extended to variations between individuals within and among populations of the identified species. This study was limited to a selected number of populations in a mountain agricultural village of Upper Nilo, Tigbao, Zamboanga del Sur, Philippines.

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Methodology:

A total of 57 earthworms were collected in the sampling site located in Upper Nilo, Tigbao, Zamboanga del Sur, Philippines (123°14'00" to 123°16'45" Latitude and 7°44'45" to 8°49'10" Longitude) through opportunistic sampling. The earthworms were collected by digging at least 2 feet in the soil, photographed alive then fixed in 5% ethanol solution for 1-3 minutes and preserved in 10% formalin solution

In the laboratory, the earthworms were rinsed with water to remove the formalin, mounted and the external and internal morphology of each sample examined. A total of 66 characters were used in the examination and described (Table 1). Sixty of these characters were based on a published study on selected species of earthworms (Apuan *et al.*, 2010) and additional 6 characters from a study on new species of *amynthas*, *pheretima* and *pleionogaster* (clitellata: megascolecidae) of the Mt. Kitanglad range Mindanao island, Philippines" (James, 2004).

Table 1: Character states and corresponding codes.

CODE	CHARACTER STATES
	Prostatic gland (0) Absent (1) Present
	Prostatic gland opening: (0) prostatic pores in xvii and xix, separate from male pores in xvii (acanthodriline); (1) a pair of prostates, tubular or racemose, opened to the exterior in xviii together with the sperm ducts (megascoleceine); (2) absence of prostates.
	Prostatic gland shape: (0) glandular portion has central lumen on bisection, either long or short cylinder-like structure (tubular); (1) bunch like or lobular with characteristic branching that is unrecognizable macroscopically (racemose); (2) absence of prostates.
	Male pore orientation: (0) anterior to segment xvi; (1) posterior to segment xvi; (2) male pore is not developed or not visible.
	Male pore location: (0) male pore located on segment xvii; (1) male pore located on segment xviii; (2) male pore is not developed or not visible.
	(3) male pore on segment xiv (4) male pore on segment xv (5) male pore on segment xvi
	Last heart location: (0) last heart posterior to segment xi; (1) last heart exactly on segment xi. (2) last heart location on segment ix (3) last heart location on segment xii (4) last heart location on segment xiv (5) last heart location on segment xv (6) last heart location on segment xvi (7) last heart location on segment xviii (8) last heart location on segment x (9) last heart location on less than v (10) last heart location on 1/2x
	Nephridia: (0) single pair of nephridia in each segment, usually large in size (holoic); (1) small and plenty that are either scattered or conglomerated along intestine, esophagous or near spermathecae (meroic). (2) No visible nephridia are construed as meroic.
	Setal arrangement: (0) 8 setae per segment usually in 4 pairs (lumbricine); (1) setae numerous and arranged equally around each segment (perichaetine); (2) setal counts vary in different parts of the body increasing from 8 segments anteriorly to > 8 per segment posteriorly.
	Pre-testicular spermathecae: (0) present; (1) absent
	Location of gizzard: (0) gizzard on seg v; (1) gizzard on seg viii; (2) gizzard on seg ix; (3) gizzard on seg xi; (5) gizzard on seg vii; (6) gizzard on seg vi; (7) gizzard on seg xx; (8) gizzard on seg xxv; (9) gizzard on seg xxvii.
	Testes: (0) testes occupying segments x and xi (holandric); (1) testes occupying only segment xi (metandric); (2) testes occupying only segment x
	Gizzard: (0) absent; (1) present
	Intestinal caeca: (0) present; (1) absent.
	Copulatory pouches: (0) absent; (1) present.
	Setae between male pores: (0) absent; (1) present
	Dorsal pore: (0) absent; (1) present
	Spermathecae: (0) absent; (1) present
	Posteriormost spermathecal pore: (0) paired; (1) unpaired; (2) absent.
	Number of spermathecal pairs: (0) 2 pairs on opposite line; (1) not visible; (2) 1 pair on opposite line; (3) 3 pairs on opposite line; (4) 4 pairs on opposite line; (5) unpaired.
	Clitellum origin: (0) origin on segment xii; (1) origin on segment xiii; (2) origin on segment xiv; (3) origin on segment xvii; (4) origin on segment xviii; (5) not developed (6) origin on segment 1/2xiii
	Clitellum cover: (0) 4 segments covered; (1) 10 segments covered; (2) 9 segments covered; (3) 13 segments covered; (4) 2 segments covered; (5) not developed; (6) 3 segments covered; (7) 5 segments covered; (8) 6 segments covered.
	Clitellum shape: (0) annular; (1) saddle type; (2) not developed.
	Genital markings: (0) absent; (1) present.
	Most posterior genital markings: (0) absent; (1) unpaired; (2) paired
	Genital markings anterior to clitellum: (0) absent; (1) unpaired; (2) paired
	Number of unpaired genital markings (GM): (0) not visible; (1) paired; (2) single genital marking; (3) 4 unpaired GM; (4) 5 unpaired GM; (5) 2 unpaired GM; (6) 3 unpaired GM.
	Number of paired genital markings (GM): (0) no visible genital markings; (1) 2 pairs GM; (2) 3 pairs GM; (3) 4 pairs GM; (4) 5 pairs GM; (5) 6 pairs GM; (6) 8 pairs GM; (7) 10 pairs GM; (8) unpaired
	Female gonopore: (0) absent; (1) present
	Number of female gonopore: (0) not visible; (1) single; (2) paired
	Body pigmentation: (0) pink; (1) dark brown; (2) reddish purple; (3) pink (4) dark gray (5) unpigmented (6) brown
	Prostomium type: (0) epilobic; (1) tanylobic; (2) zygalobic.
	Spermathecal pores on intersegment 4/5: (0) absent; (1) present
	Spermathecal pores on intersegment 5/6: (0) absent; (1) present.
	Spermathecal pores on intersegment 6/7: (0) absent; (1) present
	Spermathecal pores on intersegment 7/8: (0) absent; (1) present
	Spermathecal pores on intersegment 8/9: (0) absent; (1) present
	Genital markings on 8: (0) none; (1) paired; (2) unpaired
	Genital markings on 9: (0) none; (1) paired; (2) unpaired
	Genital markings on 10: (0) none; (1) paired; (2) unpaired
	Genital markings on 11: (0) none; (1) paired; (2) unpaired.
	Genital markings on 12: (0) none; (1) paired; (2) unpaired

Genital markings on 13: (0) none; (1) paired; (2) unpaired
 Genital markings on 14: (0) none; (1) paired; (2) unpaired.
 Genital markings on 15: (0) none; (1) paired; (2) unpaired.
 Genital markings on 16: (0) none; (1) paired; (2) unpaired
 Genital markings on 17: (0) none; (1) paired; (2) unpaired.
 Genital markings on 17/18 (0) none (1) paired (2) unpaired
 Genital markings on 18/19 (0) none (1) paired (2) unpaired
 Genital markings on 19/20 (0) none (1) paired (2) unpaired
 Genital markings on 2/3 of 21 (0) none (1) paired (2) unpaired
 Genital markings on 18: (0) none; (1) paired; (2) unpaired
 Genital markings on 19: (0) none; (1) paired; (2) unpaired
 Genital markings on 20: (0) none; (1) paired; (2) unpaired
 Genital markings on 21: (0) none; (1) paired; (2) unpaired
 Genital markings on 22: (0) none; (1) paired; (2) unpaired
 Genital markings on 23: (0) none; (1) paired; (2) unpaired.
 Genital markings on 24: (0) none; (1) paired; (2) unpaired
 Genital markings on 25: (0) none; (1) paired; (2) unpaired
 Genital markings on 26: (0) none; (1) paired; (2) unpaired
 Genital markings on 27: (0) none; (1) paired; (2) unpaired
 Genital markings on 28: (0) none; (1) paired; (2) unpaired
 Genital markings on 29: (0) none; (1) paired; (2) unpaired
 First dorsal pore location: (0) not visible; (1) intersegment 3/4; (2) intersegment 4/5; (3) intersegment 5/6; (4) intersegment 10/11; (5) intersegment 11/12; (6) intersegment 12/13; (7) intersegment 13/14.
 Origin of intestine: (0) seg 14; (1) seg 15; (2) seg 16; (3) seg 17; (4) seg 18; (5) seg 19.
 Seminal vesicles (0)absent (1) present
 Location of seminal vesicles (0) seminal vesicles \leq iv (1) seminal vesicle on segment xiv (2) seminal vesicles on segment xv (3) absent (4) seminal vesicles on segment xi (5) seminal vesicles on segment xiii (6) seminal vesicles on segment xiv

The paleontological statistics software (PAST) developed by Hammer *et al* (2001) was used for constructing, editing, and storing the codes for the description of the phenotypic characters. Support for the branches was calculated by bootstrapping technique using 1000 replication.

RESULTS AND DISCUSSIONS

The results of the study revealed 13 groups of earthworms based on the 66 characters which were used in the descriptions. These are presented as follows:

Group 1 (Fig. 1). These earthworms are pink in color. Prostomium type is prolobous, prostatic gland is absent, no visible male pore orientation, last heart location segment VIII or exactly on segment XI, nephridia are small and plenty that are either scattered or conglomerated along intestine, esophagous or near spermathecae (merioic), setae is lumbricine which means that there are 8 setae per segment usually in 4 pairs. Spermathecae is absent, gizzard is present located on segment XI or segment VI. Testes is holandric which means it occupied the segment X and XI. There is no intestinal caeca, copulatory pouch, and dorsal pores. Origin of clitellum is on segment XIV or XVIII. Clitellum is ix segments covered and saddle type. There are no genital markings and no female gonopore. Origin of intestine is on segment XV. No seminal vesicles. The earthworms are living in a clay type of soil beside a river 2-3 inches deep.



Fig. 1: External and Internal photograph of group 1.

Group2 (Fig. 2). These earthworms are reddish brown in color, prostatic gland is present. Prostatic gland opening is on XII or XIII, prostatic gland shape tubular. Male pore location is on XVII. Last heart location located exactly on segment XI. Nephridia is meroic but on some species is not visible. Setal arrangement is on 8

setae per segment usually in 4 pairs or setae numerous and arranged equally around each segment. Gizzard is located on segment 6, testes is holandric. Intestinal caeca is absent, copulatory pouch is absent, setae between male pores is absent, no dorsal pore visible, no female gonopore, no seminal vesicles, clitellum origin is on segment xii, clitellum cover is iv segments and no genital markings. These earthworms are living besides the rice field in the decayed hay area 1-2 ft. deep the soil type is loamy.



Fig. 2: External and Internal photograph of group 2.

Group 3 (Fig. 3). These earthworms are reddish purple in color. Prostatic gland is present, prostatic pores in xvii and xix (acanthodriline) and they have a tubular prostatic gland shape. Male pore is located at segments XVII and XVIII. Last heart location is on segment IX. Nephridia is meroic, setal arrangement varies from 8 setae per segment usually in 4 pairs (lumbricine). No pretesticular spermathecae. Location of gizzard ranges from V, VIII, and XI. Testes is holandric which means it is occupying segment X and XI. Copulatory pouch is not visible on most specimens. Intestinal caeca is not visible. Setae between male pores are visible on some of the specimens. Dorsal pore is not visible, paired spermathecae is present on some specimens. Clitellum shape is annular, origin of clitellum ranges from segment XII, XIII, and XIV. Clitellum cover varies from segments II and IV. There are no genital markings. Prostomium type of some specimens is tanybolic. No visible dorsal pore. Origin of intestine is from segment XV. Location of seminal vesicles ranges is on segment X. These earthworms were collected beside the rice field in the decayed hay area 1-2 ft. deep and the soil type is loamy.



Fig. 3: External and Internal photograph of group 3.

Group 4 (Fig. 4). These earthworms are reddish purple and pink on coloration, prostatic gland may be present or absent, its prostatic gland opening pores on XVII and XIX, separate from the male pores in XVIII. Prostatic gland shape is tubular, male pore located on segment XV, last heart location either on XI, XIV, XV, XVI. Some species has no visible nephridia which are construed as meroic, other species may have small and plenty on that are scattered or conglomerated along intestine, esophagous or near spermathecae. Setal arrangements on some species are lumbricine, perichaetine, and some species varies on setal counts in different parts of the body. Pretesticular spermathecae is absent. Gizzard located on segment viii. Testes is holandric

which means it occupied the segments X and XI. Intestinal caeca is absent, copulatory pouch is present in some species. There are no setae between male pores. In some species dorsal pore is present, spermathecae is absent. Clitellum originated on segment XII or XIII. On some species the clitellum is covered from ix segments and 2 segments. Clitellum shape is saddle type or annular. No genital markings, some species have female gonopore. Prostomium type is tanybolic or prolobous. Some species have a dorsal pore located on segment 4/5. Origin of intestine varies on segments XI, XXI and XVIII. Location of seminal vesicles on some species is on segment X, XII. These earthworms are living in a loamy soil 1-2 ft. deep.



Fig. 4: External and Internal photograph of group 4.

Group 5 (Fig. 5). The color of these earthworms is reddish purple. Prostatic pores in xvii and xix, separate from the male pores in XVII (acanthodroline) or a pair of prostates, tubular or racemose, opened to the exterior in XVIII together with the sperm ducts (megascolicine). Male pore location ranges on segment XIV, XVI. Last heart location ranges from VIII, XI, XII. Nephridia is meroic, setal arrangement varies from 8 setae per segment usually in 4 pairs (lumbricine) or setae are arranged equally around each segment (perichaetine). Pretesticular spermathecae is not visible on most specimens. Gizzard is located on segment VIII. Testes are holandric. Intestinal caeca is not visible on some specimens. Copulatory pouch is absent. Setae between male pores are not visible on some specimens. Dorsal pore is present. Spermathecae is paired on some specimen 1 pair spermathecae on opposite line. Clitellum is originated on segments XII, XIII, XVII. Clitellum is II to III segments covered and clitellum shape is annular. No genital markings present. Female gonopore is absent. Prostomium type varies from epilobic and tanybolic. Spermathecal pores on intersegment 6/7. Origin of intestine varies from XIV, XVI. Seminal vesicles located on segment XI, XII, XIII. These earthworms were collected in the decayed hay location near the rice field and the soil type is loamy.



Fig. 5: External and internal photograph of group 5.

Group 6 (Fig. 6). The body color of these earthworms varies from reddish purple to dark brown. Prostatic gland opening is a pair of protates tubular or racemose, open to the exterior in xviii together with the sperm duct. Prostatic gland shape is bunch like or lobular with characteristic branching that is unrecognizable macroscopically (racemose). Male pore location is on segment XVII. Last heart location on segment XI, XII. Nephridia is meroic, setae numerous and arrange equally on around each segment (perichaetine). Pretesticular spermathecae is not visible. Location of gizzard is on VI, VIII. Testes is holandric, intestinal caeca is absent, copulatory pouch is present on some specimen, dorsal pore is not absent, spermathecae is visible on some specimen. Spermathecae is is paired on posterior most spermathecal pore. Number of spermathecal pairs is one pair on the opposite line. Clitellum origin is on segment XIV, XVII, XVIII. There are no genital markings. Prostomium type is tanybolic and epilobic. Female gonopore is single. Spermathecal pores on intersegment 4/5, 6/7. Origin of intestine is on segment XIV, XV. Seminal vesicles are located on segment XII. These earthworms were located under the decayed woods and the soil type is peaty.



Fig. 6: External and internal photograph of group 6.

Group 7 (Fig. 7). The colors of these earthworms is pink. There is an absence of prostates, male pore is not developed or not visible. Last heart varies from segment XI, VIII, XV, XVI, and XIII. Nephridia is small and plenty that are either scattered or conglomerated along intestine, esophagus or near spermathecae (meroic). Setal arrangement is lumbricine. No pretesticular spermathecae. Gizzard located on segment VI on some specimens on segment XI. Testes are occupying segments X and XI (holandric). Intestinal caeca is absent. No copulatory pouches. No setae between male pores. No dorsal pore. Spermathecae is absent. Clitellum origin varies from segments XII, XIII, XIV, and XVII. Clitellum is covered by vii to viii segments. Clitellum shape is saddle type. No genital markings. No female gonopore. Prostomium type is proloubous. Origin of intestine varies on segment XIV, XV, XVIII and XIX. Seminal vesicles is absent except for other specimens which are located on 10, 14. These earthworms are found on the clay type of soil.



Fig. 7: External and Internal photograph of group 7.

Group 8 (Fig. 8). The colors of these earthworms is pink. There is an absence of prostates, male pore is not developed or not visible. Last heart location varies on each specimen on segment viii, xi, xiv, and xiii. Nephridia is small and plenty that are either scattered or conglomerated along intestine, esophagous or near spermathecae (meroic). There are 8 setae per segment usually in 4 pairs (lumbricine). There are no pretesticular spermathecae, gizzard on segment VI. Testes occupying on segment X and XI (holandric). Intestinal caeca is absent. A copulatory pouch is absent. Setae between male pores are absent. No dorsal pore. Spermathecae is absent. Clitellum origin varies from segments XII, XIII, XIV, and XVIII. Clitellum cover varies from vii to viii segments. Clitellum shape is saddle type. No genital markings. Female gonopore is absent. Prostomium type is prololobous. Origin of intestine varies from segment XIV, XV, XVII, and XIX. Seminal vesicle is absent except for the some specimens which has seminal vesicles on segment X and XIV. These earthworms are found near the fishpond which has a peaty types of soil.



Fig. 8: External and Internal photograph of group 8.

Group 9 (Fig. 9). These earthworms are dark brown in color. Prostatic gland opening is megascolicine and prostatic gland shape is racemose. Male pore location is on XV to XVII. Last heart is located on segment xiii. Nephridia is meroic. Setal arrangement is numerous and arranged equally around each segment. Pretesticular spermathecae is present or absent on some specimens. Gizzard located on segment VI. Testes occupying only segment XI (metandric). Intestinal caeca is present. Copulatory pouch is present. Setae between male pores are present. Dorsal pore is absent. Spermathecae is present. Posterior most spermathecal pores are paired. Clitellum originated on segment XIII, clitellum is iii segments covered. Clitellum shape is annular. There are no genital markings. Single female gonopore on some specimen may be present. Prostomium type is epilobic. Spermathecal pores on intersegment 4/5, 5/6, 6/7, 8/9 varies on different species. Origin of intestine is XIV. Location of seminal vesicles varies on segment XI to XII. These earthworms were collected in the decayed hay in a loamy soil.



Fig. 9: External and Internal photograph of group 9.

Group 10 (Fig. 10). These earthworms are reddish purple in color. Prostatic pores in XVII and XIX, separate from male pores in XVII (acanthodriline) but in some specimens there is a pair of prostates tubular or racemose opened to the exterior in XVIII together with the sperm ducts (megascolicine). Prostatic gland shape has glandular portion has central lumen on bisection either long or short cylinder like structure (tubular) on few specimens the shape is bunch like or lobular with characteristic branching that is unrecognizable macroscopically (racemose). Male pore location is on segment XV, XVI, XVII, and XVIII. Last heart location is on some species is anterior to segment V, other specimens on segment X, XIII, X. Nephridia is merioic on few specimens are small and plenty that are either scattered or conglomerated along intestine, esophagous or near spermathecae (merioic). Setal arrangement is 8 setae per segment usually in 4 pairs (lumbricine). Prestesticular spermathecae is absent. Gizzard location is on segments V, X, VIII, XI. Testes occupying segments X and XI (holandric) on other specimens it only occupies only on segment XI (metandric). Intestinal caeca is visible on most specimens. No copulatory pouch but visible on some specimens. Setae between male pores are absent but are visible on some specimens. Dorsal pore is not visible on some specimens. No spermathecae. Clitellum origin is on segment XII, XIII, XIV, and XVIII. Clitellum cover on some specimens are II, III, and IV segment covered. Clitellum shape is annular. No genital markings except on 1 specimen. The 1 specimen has paired genital markings. Some specimens has female gonopore, other specimens have paired and single female gonopore. Prostomium type of most specimens is tanybolic and the other one is zygotobic. No spermathecal pores. The other one has genital markings on XV-XVIII. On some specimen the dorsal pore is on intersegment 11/12 and 13/14. Origin of intestine is on segments, XIV, XV, XVI, XVII. Location of seminal vesicles on some species varies from IV, X, XIII, XIV, XXVI. These earthworms are found on the elevated loamy soil



Fig. 10: External and Internal photograph of group 10.



Fig. 11: External and Internal photograph of group 11.

Group 11 (Fig. 11). The color of these earthworms varies from reddish purple to dark brown. There's a pair of prostates, prostatic gland opening is acanthodrilin some is megascolicine, and prostatic gland shape is tubular. Male pore location is on segment XV, XVII. Last heart location is posterior to segment V, on segment XI, and XII. Nephridia is meroic. Setae is lumbricine. Pretesticular spermathecae is absent. Location of gizzard varies on segment VI - XI. Testes are holandric. Intestinal caeca is present and absent in some specimen. Copulatory pouch is absent, setae between male pores is present, dorsal pore is absent, paired spermathecal pore on some specimen but absent on some species. Clitellum origin varies on segment XII, XIV, and XVII, clitellum cover varies on II, III, IV segments covered. Clitellum shape is annular. No genital markings present. Single female gonopore is present most some specimens. Prostomium type is epilobic and tanylobic, spermathecal pores on intersegment 6/7. Origin of intestine is on segment XIV. Location of seminal vesicles varies on IV to X. First dorsal pore location is on intersegment 13/14 and 10/11.

Group 12 (Fig. 12). The color of these earthworms varies on reddish purple to dark brown. Prostatic pore on XVII and XIX separate from the male pore in xvii on some specimens or megascolicine. Male pore location varies on segment XIV to XVIII. Last heart location varies from segment IX to XI. Nephridia is small and plenty that are either scattered or conglomerated along intestine, esophagus or near spermathecae (meroic). There are 8 setae per segment usually in 4 pairs in (lumbricine). Pretesticular spermathecae is absent. Gizzard location varies on segments V to VIII. Testes is holandric which is occupying the segment X and XI. Intestinal caeca is absent but visible on some specimen, copulatory pouch is present on some specimens, setae between male pores is absent but present on some, and dorsal pore is visible. Spermathecae is absent but present on some. Number of spermathecal pairs is one pair in opposite line on some specimens. Spermathecal pores on intersegment 6/7. Clitellum origin is varies from XIII, XIV, and XVII. Most Clitellum is covered by II to III segments. Clitellum is annular. No genital markings. Prostomium type is tanylobic and epilobic. Female gonopore is present. Origin of the intestine varies from segments XV, XVII, and XIX. Dorsal pore on intersegment 12/13, 13/14 Seminal vesicles is present on some specimens and is located on segment X, XIV. Intestinal origin varies from XIX to XIV. Seminal vesicles is located on segments X and XI.



Fig. 12: External and Internal photograph of group 12.

Group 13 (Fig. 13). The color of these earthworms varies from reddish purple to dark brown. Prostatic gland is acanthodrilin on some species and megascolicine. Prostatic gland shape is tubular or racemose on some species. Male pore location varies from segments XV - XVII. Last heart location is on segment XIII. Nephridia is meroic. Setae numerous and arrange equally around each segment (perichaetine). Pretesticular spermathecae may be present on some species. Gizzard location on segment VI. Testes are metandric or testes occupying only segment XI. Intestinal caeca is present. Setae between male pores are present. No dorsal pore. Spermathecae is present. Posterior most spermathecal pair is paired. Clitellum originated on segment XIII. Clitellum is 3 segments covered. Clitellum shape is annular. No genital markings. Single female gonopore may

be visible. Prostomium type is epilobic. Spermathecal pores on intersegment 4/5, 5/6, 6/7, 8/9 varies on different specimens. Origin of intestine is on segment XIV. Location of seminal vesicles on varies on segment XI to XII.

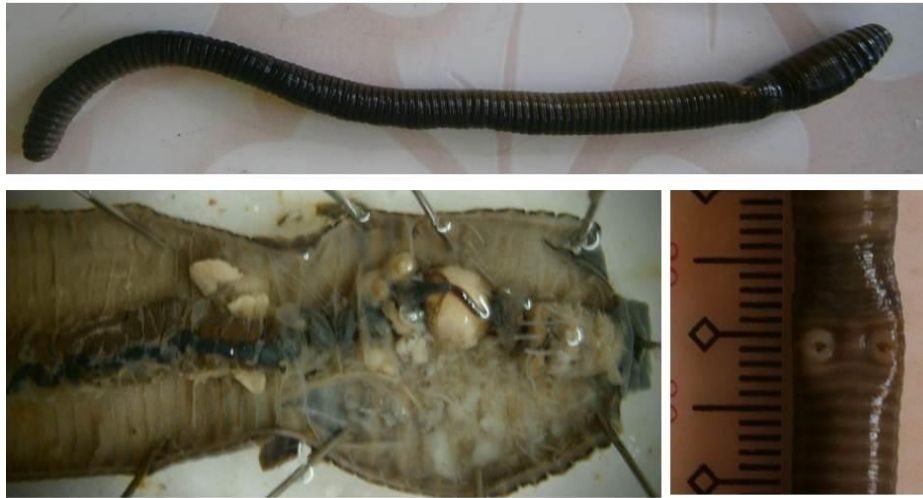


Fig. 13: External and Internal photograph of group 13.

Results of the phenetic analysis show three groups were found to belong to *Pheretima sp.* (G5, G11 and G13), 8 groups to *Eudriluseuginae* (G2, G3, G4, G5, G6, G9, G10, G12) and 4 groups to *Pontoscolex* (G1, G7, G8). Based on observations from the all outgroups used, most of the specimens collected were grouped and belong to the species *Eudrilus euginae* (Fig. 14) and the specimens only vary on the location of heart and seminal vesicles. High variability were observed in the genus *Eudrilus* based on these structures. Studies show this is a common occurrence in the species. Based on ecological considerations of the earthworms used in vermiculture, according to Blakemore (1997) seminal vesicles were located on segment XI and XII but a study of earthworm species in Guyana, according to the study Abdullah A.A and Saywack P. (2011) it shows that the seminal vesicles of these earthworms were located on segment XIX – XXV, in segments XI to XII for little-known earthworm species from the Ivory Coast (Oligochaeta: Acanthodrilidae: Benhamiinae and Eudrilidae) (Csuzdi *et al.*, 2009) and in four Economically Important Species of Earthworm in Thailand (Loongyai *et al.*, 2011). Results in these studies clearly indicate that phenotypic plasticity is expected in earthworms because of its highly variable seminal vesicles. Such phenomenon was reported also in the seminal vesicles of *Pontoscolex corethrurus* (1856) which found to be paired and located on segment xiii (Muller, 1856) and also in segments XV-XVII (Shen, 2005). It is therefore not a wonder that the majority of the specimens collected *E. euginae* will also vary on the distribution and location of seminal vesicles (Fig. 14).

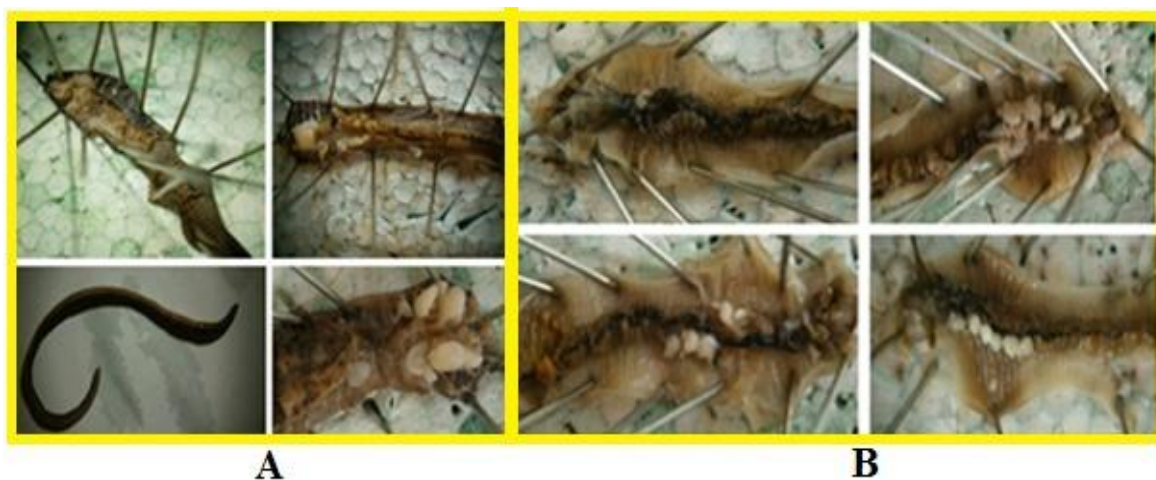


Fig. 16: A. External and Internal photograph of *E. euginae* B. Variability of the seminal vesicles of *P. Corethrurus*.

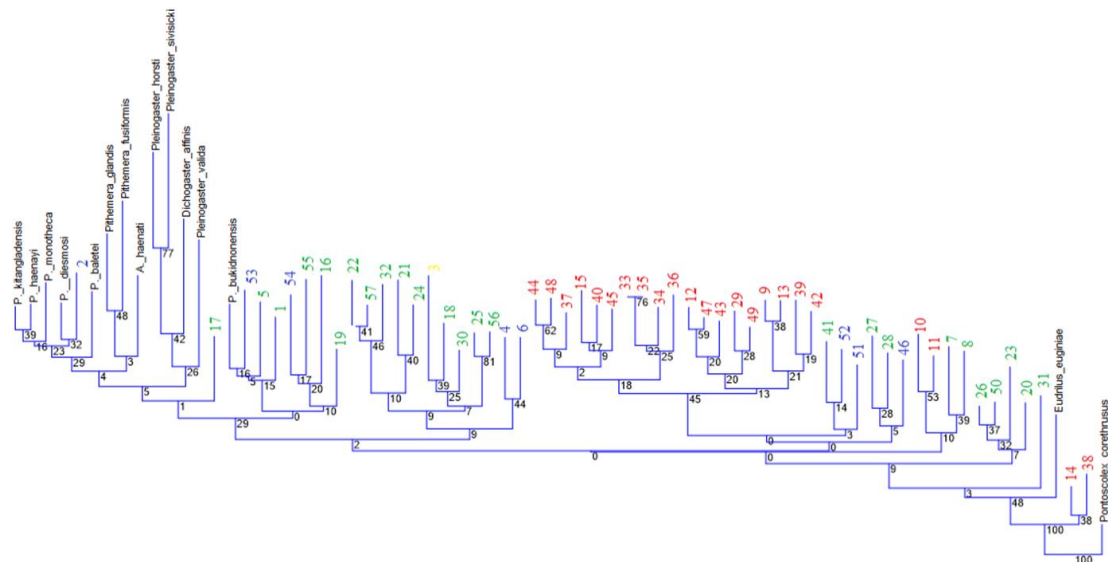


Fig. 1: Neighbor joining clustering of individuals in relation to the various identified species of earthworms.

Conclusion:

This study showed variability within the collected species of earthworms since there are differences with the groups especially on the characters listed: clitellum, cover, location of gizzard, last heart location, nephridia, pigmentation, intestinal caeca, clitellum origin, clitellum shape, prostomium type, location of seminal vesicles, and male pore orientation. There is therefore a need to describe using more important tools to be able to understand the nature of diversity within, between, and among individuals, populations of a suspected new species of earthworms.

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