

Taxonomy of Earthworm Species Collected from East Coast of Guyana

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AA and SJ designed the study, wrote the protocol and interpreted the data. Author GPP carried out the research work and performed preliminary data analysis. Authors GPP and SJ managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

Earthworms contribute significantly towards the proper functioning of our ecosystem and are environmentally and economically beneficial to the well-being of other organisms. These soil dwelling organisms serve to enhance vegetation growth in an eco-friendly manner among other benefits such as alternative source of proteins that are vital for the existence of life on earth. Earthworm diversity varies across different parts of the globe and species identification is a key to each of the specific regions such as Guyana. The present study was conducted during the year 2014-15 at University of Guyana, Georgetown during the rainy season period. The primary objective of this investigation was to successfully collect, identify and classify local Earthworm species. Earthworm samples were collected from a local organic kitchen garden in Cane Grove at GPS location 218° SW (6° 37'27" N 57° 55'8" W) by utilizing the hand sorting method. Cane Grove is a rural agricultural area in the Demerara- Mahaica Region of Guyana located on the coastal plain along Mahaica River. The area from which earthworms were collected was generally moist and showed rich organic composition. Hand fork was used for digging the soil and samples of earthworms collected were placed into a vermi-container. The collected specimens were taken to the lab where taxonomic studies were conducted. The external morphology, internal anatomy and

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the ecological features were carefully examined. Based on this investigation, the species was grouped into the family Megascolecidae and identified as *Perionyx excavates* (Perr.). This study has showed that *Perionyx excavatus* (Perr.) is an important epigeic species in Guyana.

Keywords: Morphology; anatomy; *Perionyx excavates*.

1. INTRODUCTION

In this investigation taxonomical studies were conducted to confirm *Perionyx excavates* (Perr.). The life cycle and the potential of this species for breaking down organic wastes have been documented by various authors under controlled conditions. It is a necessity that suitable organic materials are required in sufficient amounts for optimum growth and reproduction. *Perionyx excavates* (Perr.) exist mostly in organic wastes and high moisture contents [1-5]. They use a wide variety of organic material for food and even in unfavorable conditions they extract adequate nourishment from the soil for survival. The quality and quantity of food available influences not only their population size but also the rate of growth and fecundity [6].

There are over 1800 species of earthworms that occur globally, most of which have been introduced to new areas by the activities of man. Moreover, they hardly ever exist in deserted areas, in areas under continuous snow and ice, mountain ranges and in areas with deficient vegetative growth. These factors act as natural barriers against the spread or migration of earthworm species. Earthworms do not exist in seas and in oceanic soils as well because they cannot tolerate the high levels of salinity even for short periods [7]. They live in just about any type of soil with the precise quantity of moisture and organic constituent. Their color ranges from brownish-black to purple with the exception of some being green. Those that are reddish brown in appearance are due to the presence of the pigment hemoglobin in their blood [8].

The taxonomic complexity when identifying earthworms is their morphological characteristics, both external and internal, which often show high variability between individuals. However in earthworm systematics, reproductive organs are believed to be less affected by environmental influences through time and are evolutionarily more conservative [9]. In Guyana limited studies were conducted on earthworm taxonomy hence in this investigation locally available earthworms were collected and correctly identified using taxonomic tools to confirm *Perionyx excavates* (Perr.).

2. METHODOLOGY

Vermiculture units were constructed at GPS location 325° NW (6° 37'27" N 57° 55'8" W) on the East Coast of Guyana approximately 35km away from the capital city Georgetown (Fig. 1).

Laboratory experiments were conducted at University of Guyana, Georgetown. Local earthworms were collected by the hand sorting technique [10,11]. Three large plastic buckets were filled with a layer of sand and stone, soil, cow manure and leaf litter as vermiculture units. The earthworms were kept in vermiculture units at 22°C and the moisture content was maintained at 75 to 80% by sprinkling it with tap water whenever required. The compost was checked on a regular basis so as to make sure that the earthworms were alive and not escaping from the buckets. Cow manure and leaf litter were replaced at monthly intervals so as to avoid scarcity of food [12].

For the purpose of examining the external morphologies samples were carefully washed with tap water and preserved in 70% alcohol solution for observation. Digital pictures of the species were taken and the external features were recorded [12].

On the other hand dissection was done so as to view the internal anatomy. In doing so earthworms were washed and placed into Petri dish with cotton wool soaked in 119.38 g/mol of chloroform. The Petri dish was covered until there were no signs of movement. Each of the specimens was then placed into a dissecting tray containing water with its ventral side facing up. Water was used as a mean of carefully removing the unwanted dirt found in the intestinal tract. The two ends of sample were then pinned down. With the use of a razor blade and dissecting scissors each of the specimens was carefully cut right down the middle portion on the ventral region. It is important not to apply much pressure since this can cause damages to the internal organs. Pins and tweezers were further used to carefully spread open the earthworm for examination. The internal anatomy of the specimen was then observed using a magnifying hand lens and dissection microscope [12].

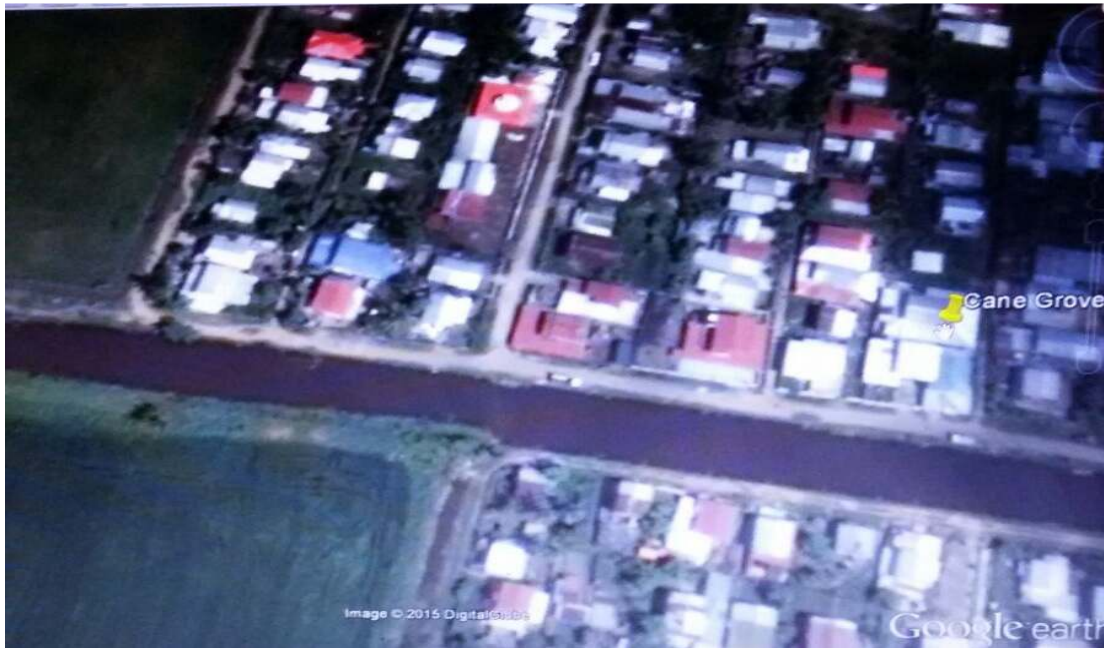


Fig. 1. Aerial view of study area (picture taken from Google earth)

A small portion about the size of one centimeter square on the ventral region was carefully removed to investigate the body setae. The detached portion was washed to remove unwanted dirt and tissues. It was then placed into 5% of caustic potash (KOH) solution and boiled for 10 minutes until the integument had dissolved to produce a yellowish solution. This was poured into a large watch glass and allowed to cool. Water was added; allowing it to stand for a few minutes until the setae dropped at the bottom. The setae were collected and placed onto a microscope slide. Excess water was carefully removed and the sample was viewed under a compound light microscope at X10 magnification to see if the setae were isolated and visible. The shape and arrangement of the setae were noted and microscopic pictures were taken [13]. The length of the body setae was also calculated. A drop of DPX mount was placed on the specimen to make it a permanent mount. A cover slip was then placed over it carefully so as to avoid air bubbles. The prepared slide was then labeled and stored for future reference [12]. The dissected specimens were kept in vials containing 70% alcohol for future reference. A total amount of five samples were examined using the same procedure.

3. RESULTS AND DISCUSSION

Perionyx excavatus (Perr.) are diagnosed by numerous setae. Its gizzard is absent or reduced

in segments five or six. Its digestive system lacks caeca, calciferous glands and typhlosole but have they calciferous tissues in some portion of the oesophagus. Latero-oesophageal hearts are found in segments ten to twelve while prostates and male pores are in segment eighteen. Clitellum is annular and setae are retained. They also have holonephridia with pre-septal funnels [14].

On the subject of taxonomy, the identified earthworm is *Perionyx excavatus* (Perr.) from the class Clitellata, order Haplotaxida and family Megascolecidae. The data presented in Table 1 is supported by previous studies conducted by [14-17].

In earthworm taxonomy, the external morphological features as well as the internal anatomy and the ecological features are critical for species identification and classification [12]. The size of the earthworm as well as the number and arrangement of the segments are also tools that are used for classification [18]. This investigation have revealed that *P. excavatus* (Perr.) ranges from 70 mm to 190 mm in length with body segment ranging from 155-294 (see Fig. 1 in appendix). This is in disparity with research conducted by [14] who found that the *P. excavatus* (Perr.) consist of up to 155 segments with body length of up to 180 mm. The morphology of the clitellum is also another equally important taxonomic tool for species classification. Usually the number of segments

Table 1. Morphological features and internal anatomy of earthworm studied (Figs 2-7)

Taxonomic characters	Sample A	Sample B	Sample C
Body shape	Elongated	Elongated	Elongated
Color	Purplish dorsally and reddish brown ventrally	Purplish dorsally and reddish brown ventrally	Purplish dorsally and reddish brown ventrally
Body length	190 mm	70 mm	100 mm
Number of segments	294	263	155
Shape of Prostomium	Epilobous	Epilobous	Epilobous
Location of setae	Ventral region	Ventral region	Ventral region
Shape of setae	Slightly f-shaped	Slightly f-shaped	Slightly f-shaped
Arrangement of body setae	Ring- shaped (Lumbricine)	Ring- shaped (Lumbricine)	Ring- shaped (Lumbricine)
Length of body setae	0.457 mm	0.457 mm	0.357 mm
Shape of clitellum	Cylindrical	Cylindrical	Cylindrical
Number of segments from head to clitellum	13 segments	13 segments	14 segments
Length of clitellum	9 mm	5 mm	5 mm
Diameter of clitellum	5 mm	3 mm	3 mm
Number of segment on clitellum	6	5	5
Position of the male pore	19 th segment	18 th segment	18 th segment
Position of septa	7-8	7-8	8-9
Position of female pore	14 th segment	13 th segment	13 th Segment
Nephridia	Holonephric	Holonephric	Holonephric
Number of seminal vesicle	4 (2pairs)	4 (2pairs)	4(2pairs)
Position of seminal vesicle	11 th segment	12 th segment	12 th segment
Number of spermatheca	2 pairs	2 pairs	2 pairs
Position of spermatheca	8-9 segment	7-8 segment	7-8 segment
Ovaries	Paired in 13 th segment	Paired in 13 th segment	Paired in 13 th segment
Position of nephridiopores	5 th Segment	5 th Segment	5 th Segment
Position of heart	13 th segment	12 th segment	13 th segment
Position of gizzard	6 th segment	6 th segment	5 th segment
Esophagus	Swollen and in segment 10-12 and in 13 it is swollen to form lateral pouches.	Swollen and in segment 10-12 and in 13 it is swollen to form lateral pouches.	Swollen in segment 10-12 and in 13 it is swollen to form lateral pouches.
Dorsal blood vessel	On pharynx	On pharynx	On pharynx

forming the clitellum varies with species (Ismail, 1997). In *P. excavates* (Perr.), the clitellum is recognized as a dark cylindrical structure composed of 5- 6 segments with a diameter of 3- 5 mm. This annular but indistinct aspect of the earthworm varies from 5 mm to 9 mm in length with a total of 13 to 14 segments forming the

region from the head to clitellum (see Fig. 2 in appendix). Its Prostomium is epilobous in shape with slightly ventral cleft on the Peristomium (see Fig. 3 in appendix). Numerous body setae are located on the ventral region with lumbricine arrangement and are slightly f-shaped when viewed under a compound light microscope. The

length of the body setae when calibrated varies from 0.357 mm to 0.457 mm (see Fig. 4 in appendix). Subsequently, the shape of Prostomium and arrangement of body setae are also taxonomic tools [14-17].

Considering the fact that *P. excavatus* is a hermaphroditic earthworm, the location of the male and female genital pore is vital. This investigation has disclosed that the male genital pore is located on segments 18 to 19 while the female genital pore is located on segments 13 to 14. However the male pores are in close approximation with deep, wedge-shaped clefts in a common depressed but tumid field. No setae are present in the tumid region between the male genital pores. Complementary to this, there are circles of 5 to 6 black penial setae in each cleft. The ovaries are paired on segment 13. This is supported by research conducted earlier [14-17].

Further, internal examination has revealed that two pairs of seminal vesicles are on segments 11 to 12. On the other hand, two pairs of spermatheca are present on segments 7 to 8 and 8 to 9. The Nephridiopores is positioned on segments 5 while the heart is within segments 12 to 13 and gizzard on segments 5 to 6. In segments 10 to 12 the esophagus is swollen and vascularised, but more importantly it is swollen on segments 13 to form lateral pouches with ridged internal surfaces. Blood vessels continuously lie dorsally on the pharynx. Additionally the spermathecal pores form two pairs on 7 to 8 and 8 to 9 segments. Large, open pores are present with intervening furrows obliterated, same width apart as male pores. The septa are found on segments 7 to 8 and 8 to 9. The nephridia are holonephric with convoluted tubules in the clitellar region. These worms moves rapidly to escape handling and has a lashing response. The gut content is composed mainly of organic material (see Fig. 5 in appendix for internal anatomy). This is also supported by previous studies conducted [14-17,19].

Another essential point about *Perionyx excavates* (Perr.) is that they release small, lemon-shaped cocoons after mating which hatches in approximately 21 days (see Fig. 6 in appendix). Their cocoons are rough and spindle shaped with bristles at the pointed end [20]. *Perionyx excavates* (Perr.) have luminescent appearance with blue-violet sheen on its skin which is visible only when exposed to bright light. These worms are highly active and like other species of earthworm they have an opening at

each of its end; with the anterior opening being the mouth and the posterior opening being the anus. Sexual maturity is met in approximately 35 days and is recognized by the development of clitellum. Their growth stages ranges from cocoon to immature worm that further develop into an adult [20].

Moreover, research have shown that *P. excavates* (Perr.) are widely distributed in the Australasian region (Australia, New Zealand); India; Asia and Oceania; North and Central America [21] and is now a pioneer species to Guyana. They have a significant role for effectively managing organic wastes. It is a valuable source of feed for poultry and has the ability to transform many types of complex organic matters such as animal manure, rice straw etc into stabilized vermicompost that contains higher nutrients and make them available in an exchangeable forms that can be easily and readily absorbed by plants. Experiment in broiler implied that *Perionyx excavates* (Perr.) is a very nutritious feed for poultry [22].

4. CONCLUSION

The taxonomic features noted from earthworms that were collected locally have confirmed to be *Perionyx excavates* (Perr.). The study also reveals that *Perionyx excavates* (Perr.) is an important epigeic species in Guyana.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX



Fig. 2. The number of segments and length of *P. excavatus*



Fig. 3. Morphology of clitellum and number of segments from head to clitellum



Fig. 4. Morphology of prostomium and peristomium

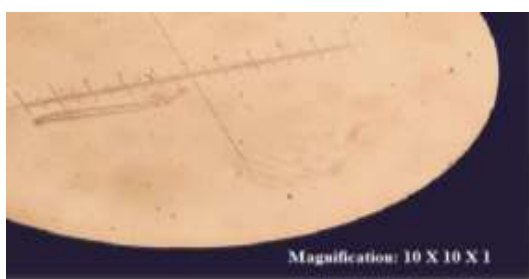


Fig. 5. View of the setae through Microscope

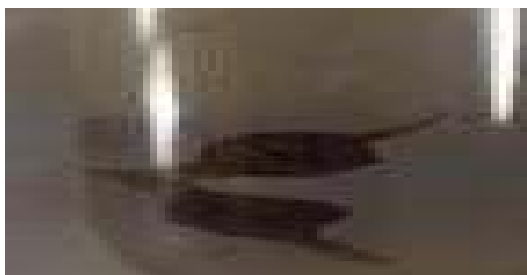


Fig. 6. Lemon-shaped cocoons



Fig. 7. Internal anatomy

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