SHORT COMMUNICATION | *Philippine Journal of Systematic Biology*
DOI 10.26757/pjsb2022a16007

Earthworms Species Diversity and Populations in Initao-Libertad Protected Landscape and Seascape, Misamis Oriental, Philippines

John Edward P. Sacay[1] and Nonillon M. Aspe[2*]

Abstract

Earthworm diversity and populations in the seaward and landward portions of Initao-Libertad Protected Landscape and Seascape (ILPLS) were assessed. Soil physico-chemical properties in the area were also assessed to explain such occurrence. *Pheretima* cf. *lantapanensis*, *Amynthas* sp. and *Polypheretima* sp., all belonging to family Megascolecidae, were detected in the area. ILPLS has a very low diversity of earthworms and the soil physico-chemical properties had no significant relationship with earthworm species diversity. The soil physico-chemical properties between the seaward and landward portions of the protected area were not significantly different. This study was undertaken to provide baseline information on earthworm species diversity in ILPLS, which will serve to help strengthen the policy on the conservation and protection of biodiversity in the protected area.

Keywords: *Amynthas*, Annelida, Megascolecidae, *Pheretima*, *Polypheretima*

Introduction

Earthworms are an extremely important component of terrestrial fauna and are vital for soil health and primary productivity (Deleporte 2001; Hendrix & Bohlen 2002). This is attributed to their ability to ingest organic material and facilitate the redistribution of organic matter throughout the soil profile (Zhang & Hendrix 1995; Kooch *et al*. 2007). The burrowing activity of earthworms in the soil creates channels and pores that will facilitate gas exchange and favors the flow of water and solutes (Rombke *et al*. 2005; Sautter *et al*. 2006). Hence, earthworms are important in maintaining the ecological balance of any forest ecosystem.

Subsequently, several studies on earthworm diversity in the Philippines have been conducted. At present, there are around 200 species of earthworms recorded in the Philippines, which have mostly been collected from protected areas (e.g. James *et al*. 2004; James 2004; Hong & James 2004; 2008; Aspe & James 2014; 2015, Aspe *et al*. 2021). Molecular phylogenetic analyses on Philippine earthworms have also been conducted to shed light on the species' mechanism of diversification and distribution across the archipelago (James 2005; Aspe *et al*. 2016; Aspe & James 2018). Of the ~200 known species in the country, >60 species of earthworms were discovered in Mindanao Island (James 2004; Aspe & James 2014; 2015; 2016; 2017). However, not all protected areas in the Philippines or in Mindanao have been surveyed yet.

Initao-Libertad Protected Landscape and Seascape (ILPLS) is a protected area of limestone forest located between the municipalities of Initao and Libertad in Misamis Oriental, Philippines (Figure 1). Although ILPLS is a protected area, its seaward portion is a tourism zone where there are a lot of infrastructure developments such as concrete roads, offices, canteen, hostel and concrete cottages. These anthropogenic activities result to the disturbance of the natural environment. Although there were also some infrastructure developments in the landward portion of ILPLS, it is now regarded as a protection zone where strictly no one can enter inside except the workers of ILPLS and the personnel of the Department of Environment and Natural Resources (DENR). These two portions are divided by a widened national highway, thus separating populations of species between the two portions.

Here, species diversity and relative abundance of the earthworm species in ILPLS were assessed. The soil physico-chemical properties of the seaward and landward portions of ILPLS were also assessed to determine their impact on the species diversity and abundance of earthworm species in the area. This study provides the first record of earthworm species in ILPLS, which will serve to help strengthen the policy on the conservation and protection of biodiversity in the area.

Materials and Methods

Sampling and identification of earthworms

Prior to the conduct of sampling activity, a permit was secured from the Protected Area Management Board of the ILPLS. Sampling was done in the seaward and landward portions of ILPLS from January to March 2021 during a long dry season. The method of collection followed that of Aspe *et al*. (2009) using 10 m x 10 m plots to assess earthworm abundance. Three plots, which serve as replicates, were set each for the seaward and landward portions of ILPLS. Within the plots, 12 holes of 0.5 m x 0.5 m x 0.3 m (depth) were dug to collect earthworms which were sorted based on their size, shape and color. Opportunistic sampling was also conducted to assess species richness, including collecting earthworms in the soils outside the plots and above ground such as the barks of trees, vines, stone, and logs. The collected specimens were cleaned with tap water, killed by 10% ethanol and put in sealable plastic bags filled with a volume of 10% formalin that was at least three times the total volume of the

[1]Community Environment and Natural Resources Office, Department of Environment and Natural Resources, Initao, 9022 Misamis Oriental, Philippines
[2]College of Marine and Allied Sciences, Mindanao State University at Naawan, Naawan, 9023 Misamis Oriental, Philippines

*Corresponding author: nonillon.aspe@msunaawan.edu.ph

Date Submitted: 09 June 2022
Date Accepted: 06 May 2023

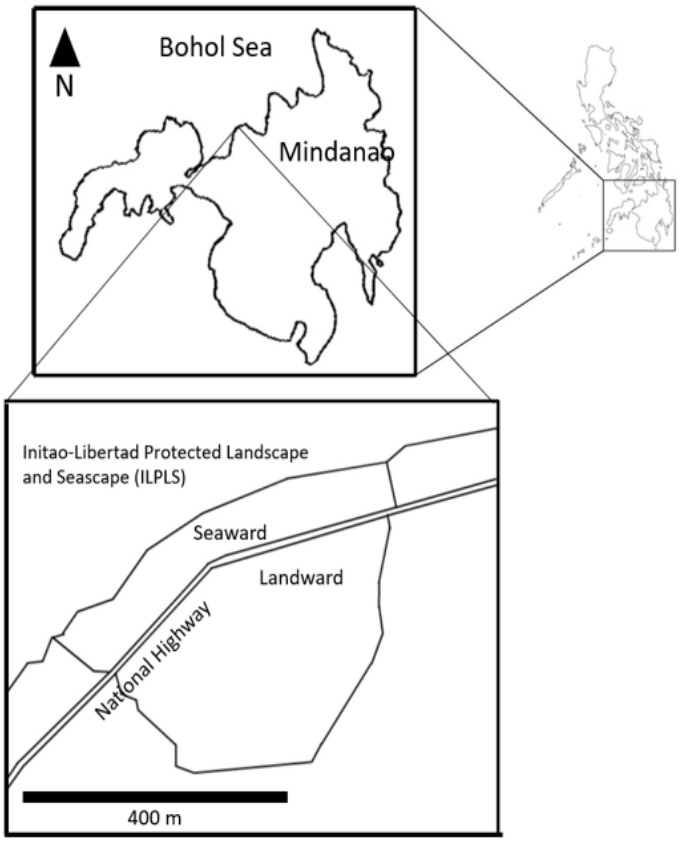

Figure 1. Map of the Philippines and Mindanao showing the Initao-Libertad Protected Landscape and Seascape (ILPLS) in Misamis Oriental.

earthworms for proper preservation. After two days, formalin was replaced with 95% ethanol and the specimens prepared for morphological examination.

The external and internal parts of the adult specimens were examined for taxonomic identification following the method of Aspe *et al*. (2009). The specimens were then morphologically compared with closely related species (e.g. James 2004; Aspe & James 2014; 2015; 2016; 2017).

Assessment of soil physico-chemical properties

Data on physical and chemical properties, which include soil temperature, moisture content, soil pH, organic matter (OM) composition, and the proportions of sand, silt and clay, were collected in both landward and seaward portion of ILPLS and compared. The soil physico-chemical properties were also analyzed to see if these have significant effects on the distribution of earthworm species and in shaping the species composition in the sampling sites.

Data analysis

Relative abundance and Shannon's diversity index were calculated. The data were analyzed using unpaired T-test. Correlation between species diversity and individual physico-chemical parameter was also done to test for significant relationship between variables.

Results and Discussion

Species accounts

Three species, namely *Pheretima* cf. *lantapanensis* Aspe & James, 2016, *Amynthas* sp. and *Polypheretima* sp., of family Megascolecidae were found in Initao-Libertad Protected Landscape and Seascape. All the species are native to the Philippine soils. The comparison among the species in ILPLS are shown in the Supplementary Table (S1).

Pheretima cf. lantapanensis Aspe & James, 2016 (Figure 2A*)*
Diagnosis. Small, brown worm, length ranges from 74–77 mm (n=3) with a diameter of 2–3 mm at segment x while 3–4 mm at segment xx, body is circular form, segments ranges from 97–100. First dorsal pore at intersegment 11/12, spermathecal pores paired at intersegment 5/6, copulatory bursae openings paired in xviii, 0.2 mm male pore distance with setae ranging from 5-8 between openings, Clitellum annular from segments xiv to xvi, setae in segment vii ranging from 34–43, setae in segment xx ranging from 47–50. Caeca originate in segment xxvi to segment xxiii. Pair of spermathecae at segment vi. Prostate glands at segments xvii–xix. Round copulatory bursae at segment xviii.

Remarks. *Pheretima* cf. *lantapanensis*, which belongs to the *Pheretima urceolata* species group of Sims & Easton (1972), characterized by having spermathecal pores opening only on intersegment 5/6, was first recorded from Brgy. Songco of the municipality of Lantapan, Bukidnon Province, approximately 80 km from ILPLS. The specimens of *P. lantapanensis* from Bukidnon were amputee, thus, there was no information on their actual length and the number of segments. However, the other morphological characters match except in the distance between male pores (0.20 vs. 0.23), the number of setae in xx (47–50 vs. 31–36) and the length of caeca (xxvi–xxiii vs. xxvii–xxiii). Further morphological examination and even molecular characterization are required to verify the species identification.

***Amynthas* sp.** (Figure 2B)
Diagnosis. Brown worm, adult length 140 mm (n=1, adult), diameter of 8 mm at segment x and 10 mm at segment xx; body, circular form; body has 120 segments. First dorsal pore at intersegment 12/13. Spermathecal pores absent. Distance between male pores 3 mm with 6 setae in between. Clitellum from segments xiv to xxvi, in segment vii 41 setae while 52 setae in segment xx. Caeca originating in segment xxvii and extending to segment xxiv. Spermathecae lacking, copulatory bursae lacking, prostate glands at segments xvii–xix.

Remarks. Among the species of *Amynthas*, *Amynthas* sp. is morphologically most smilar to *Amynthas dinagatensis* Aspe & James, 2016 in size, the first dorsal pore in 12/13, gizzard in xiii–x, intestinal origin in xiii and in having no spermathecae. However, *A. dinagatensis* has more body segments (163–167 vs. 120 in *Amynthas* sp.), more setae between male pores (20 vs. 6 setae in *Amynthas* sp.) and have longer caeca (covering seven segments from xvii–xxi vs. covering four segments from xxvii–xxiv) than *Amynthas* sp. No other *Amynthas* species in the Philippines lack spermathecae. Also, other *Amynthas* species in the Philippines are much smaller than *Amynthas* sp.

Figure 2. (A) *Pheretima* cf. *lantapanensis* (B). *Amynthas* sp. (C) *Polypheretima* sp. of Initao-Libertad Protected Landscape and Seascape, Misamis Oriental.

No other known *Amynthas* species is morphologically similar with *Amynthas* sp., which is putatively a new species. As there was only one specimen collected from ILPLS, further sampling is required to better describe the morphological features of *Amynthas* sp. Likewise, further morphological examination is required to verify the taxonomic identification of the species.

***Polypheretima* sp. (Figure 2C)**

Diagnosis. Unpigmented worm, adult length ranges from 69–79 mm (n= 3), 3 mm at segment x while 4 mm in segment xx; body, circular formation; body segments after clitellum was very close, segments ranges from 123 to 137; 34–42 setae in segment vii; 50–54 setae in segment xx. First dorsal pore at intersegment 12/13. Spermathecal pores lacking. Distance between male pores ranging from 0.2 to 0.23 mm with 5 to 8 setae between openings, clitellum from segments xiv to xxvi, genital markings paired in segments xix–xxi and in line with the male pores. Caeca lacking. Spermathecae lacking, copulatory bursae shallow, prostate glands at segments xvii–xix.

Remarks. *Polypheretima* sp. belongs to the *Polypheretima elongata* group, characterized by having a pair of genital markings on xix and successive segments in line with the male pores, paired batteries of up to 28 spermathecae in vi and/or vii, and shallow copulatory bursae with no stalked glands. Among the members of *Po. elongata* group, *Polypheretima* sp. is similar to *Po. mindanaoensis* Aspe & James 2015, *Po. jenniferae* Aspe et al. 2021 and *Po. sahlani* Fahri et al. 2017 in having no spermathecae and in having the first dorsal pore in 12/13. However, *Polypheretima* sp. markedly differs from these species in size (74–77 mm × 3–4 mm vs. 90–118 mm × 5.1–7 mm in *Po. mindanaoensis*, 161–198 × 3.2–5.5 in *Po. jenniferae* and 152–195 mm × 4.5–6 mm in *Po. sahlani*), in the number of segments covered by the genital markings (xix–xxi vs. xix–xxv in *Po. mindanaoensis* and xix–xxiii in *Po. jenniferae* and *Po. sahlani*), and in the number of setae on vii (34–43 vs. 41–53 in *Po. mindanaoensis*, 43–64 in *Po. jenniferae* and 78–89 in *Po. sahlani*). No other known *Polypheretima* species is morphologically similar with *Polypheretima* sp., which is putatively a new species. Further morphological examination is required to verify the taxonomic identification of the species.

Species diversity and relative abundance of earthworms

Among the species, *Polypheretima* sp. has the highest number of individuals collected with a total of 68 individuals. This is followed by *Pheretima* cf. *lantapanensis* with 34 individuals. Only one individual of *Amynthas* sp. was collected in the area. There is no significant difference in the abundance among the species between the landward and seaward portions of ILPLS (Supplementary Table S2). Shannon's index (H) for species diversity of earthworms in ILPLS showed to be very low, with only three species in the landward portion (H= 0.702686) and only two species in the seaward portion (H= 0.646724). Sampling during the dry season, wherein the soil has very low moisture content, is seen as a factor for this occurrence. Various climatic factors like temperature, precipitation, soil moisture, as well as extreme climate events like drought and flood have shown to alter the composition and functioning of communities in the soil (Singh *et al*. 2019). Activity of most earthworms is interrupted during dry periods or under high temperatures. To overcome the adverse period, they usually move into the deeper soil layers and may undergo 'diapause' or transform into a quiescent stage. During this period the worm stops feeding and constructs a spherical chamber lined with mucus within which it usually rolls into a tight ball or a loose knot (Jairajpuri 1993).

There is an average of two individuals/m^2 in the landward portion of ILPLS while there is an average of 1.43 individuals/m^2 in the seaward portion. Similarly, Jose *et al*. (2021) reported an average of two individuals/m^2 of indigenous species in Rajah Sikatuna Protected Landscape in Bohol wherein six species were detected. Meanwhile, Aspe and James (2014) reported an average of <1 individual/m^2 in Mt. Malindang in Zamboanga Peninsula. However, there is significantly higher number of exotic species, particularly *Pontoscolex corethrurus* Muller, 1856, in Mt. Malindang with an average of 45 individuals/m^2. No exotic species was observed in ILPLS.

Soil physico-chemical properties of the sampling sites

The sampling activity was conducted during the long dry season, thus, there was relatively high soil temperature, low organic matter content and low moisture content (Supplementary Table S3). There was no significant difference in the values of the physico-chemical properties between the seaward and landward portions of ILPLS. Silt composition was also observed to be higher (average of 53.33%) with very low sand composition (average of 4.83) for both the seaward and landward portions of ILPLS. Soil temperature influences soil moisture and organic matter. Increase in soil temperature increases the organic matter decomposition by increasing the movement of soluble substrates in the soil (Fierrer *et al*. 2005) and stimulate microbial activities (Fang *et al*. 2005). Increase in soil temperature also leads to heat-induced cracks in the sand-sized particles that consequently leads to breakdown and reduction of the amount of sand-sized particles and increase in the silt content of the soil (Pardini *et al*. 2004; Inbar *et al*. 2004). Lee (1985) reported that such soil features do not usually support a large number of earthworms. Lee (1985) and Jairajpuri (1993) stated that soil temperature and moisture are two important factors that influence earthworm seasonality and distribution. In sub-tropical climate like that of India, earthworms are active and abundant mainly during summer rains and prolonged drought decreases their numbers significantly. However, although most earthworms favor good quality soil rather than poor ones, they can still survive in many different soils provided that there is sufficient food and moisture (Kubiena 1955).

Conclusion and Recommendations

Sampling of earthworms was conducted in the seaward and landward portions of ILPLS. Three species of earthworms were identified, namely *Pheretima* cf. *lantapanensis*, *Amynthas* sp. and *Polypheretima* sp., all of which are native, belonging to the family Megascolecidae and are putatively new species. The soil physico-chemical properties in the seaward and landward portions of ILPLS showed no significant differences. Also, no single soil physico-chemical properties was shown to have significant relationship with earthworm species diversity and abundance. We here recommend a thorough morphological examination to verify the taxonomic identification of the species. We also recommend that thorough ecological studies and monitoring of these organisms, including sampling during the wet season to conserve earthworm diversity and populations. In addition, we recommend that studies on the biology of these species and their potential benefits in agriculture, soil bioremediation and discovery of natural products from these species be undertaken.

Acknowledgements

We would like to thank the Protected Area Management Board in allowing us to conduct sampling activity in ILPLS. Special thanks to Mr. Edgar Castañares and Mr. Eric John Florida for their contribution in the analyses of data.

Literature Cited

Aspe, N.M. & S.W. James, 2014. New species of *Pheretima* (Oligochaeta: Megascolecidae) from the Mt. Malindang Range, Mindanao Island, Philippines. *Zootaxa*, 3881: 401-439. doi:10.11646/zootaxa.3881.5.1.

Aspe, N.M. & S.W. James, 2015. New *Polypheretima* and *Pithemera* (Oligochaeta: Megascolecidae) species from the Mt. Malindang Range, Mindanao Island, Philippines. *Journal of Natural History*, 42: 1-23. doi:10.1080/00222933.2015.1021875.

Aspe, N.M. & S.W. James, 2016. New species of *Pheretima, Amynthas, Polypheretima*, and *Pithemera* (Clitellata: Megascolecidae) from Mindanao and associated islands, Philippines. *Zoological Studies*, 55: 1-33. doi:10.6620/ZS.2016.55-08.

Aspe, N.M. & S.W. James, 2017. Pheretimoid earthworms (Clitellata: Megascolecidae) from Mt. Apo, Mindanao Island, Philippines with description of eight new species. *Raffles Bulletin of Zoology*, 65: 357-372.

Aspe, N.M. & S.W. James, 2018. Molecular phylogeny and biogeographic distribution of pheretimoid earthworms (Clitellata: Megascolecidae) of the Philippine archipelago. *European Journal of Soil Biology*, 85: 89-97. doi:10.1016/j.ejsobi.2018.02.001.

Aspe, N.M, H. Kajihara & S.W. James, 2016. A molecular phylogenetic study of pheretimoid species (Megascolecidae) in Mindanao and associated Islands, Philippines. *European Journal of Soil Biology*, 73: 119-125. doi:10.1016/j.ejsobi.2016.02.006.

Aspe, N.M., R.E. Manasan, A.B. Manlavi, M.L.E. Patiluna, M.A.B. Sebido, M.C.M. Obusan, J.F. Simbahan & S.W. James, 2021. The earthworm fauna of Palawan, Philippines with description of nineteen new pheretimoid species (Clitellata: Megascolecidae). *Journal of Natural History*, 55: 733-797. doi:10.1080/00222933.2021.1923849.

Aspe, N.M., O.M. Nuñeza & M.A. Torres, 2009. Diversity and distribution of earthworms in Mt. Malindang, Philippines. *Journal of Nature Studies*, 8: 59-67.

Deleporte, S., 2001. Changes in the earthworm community of an acidophilus lowland beech forest during a stand rotation. *Soil Biology*, 37: 1-7.

Fahri, R. Amaliah, Annawaty & A.D. Nguyen, 2017. The earthworm genus *Polypheretima* Michaelsen, 1934 (Annelida: Clitellata: Megascolecidae) from Sulawesi, Indonesia, with descriptions of four new species. *Raffles Bulletin of Zoology*, 65: 559-573.

Fang, C.M., P. Smith, J.B. Monorieff & J.U. Smith, 2005. Similar response of cabile and resistant soil organic matter pools to changes in temperature. *Nature*, 436: 881-883.

Fierrer, N., J.M. Gruine, K. McLauchlan & J.P. Schimel, 2005. Litter quality and the temperature sensitivity of decomposition. *Ecology*, 86: 320-326.

Hendrix, P.F. & P.G. Bohlen, P.G. 2002. Exotic earthworm invasions in North America: Ecological and policy implications. *Bioscience*, 9: 1-11.

Hong, Y. & S.W. James, S.W. 2004. New species of *Amynthas* Kinberg, 1867 from the Philippines (Oligochaeta: Megascolecidae). *Revue Suisse de Zoologie*, 111: 729-741.

Hong, Y. & S.W. James, 2008. Nine new species of earthworms (Oligochaeta: Megascolecidae) of the Banaue rice terraces, Philippines. *Revue Suisse de Zoologie*, 115:

341-354.

*I*nbar, A., M. Lado, M. Sternberg, H. Tenau & M. Beh-Hur, 2004. Forest fire effects on soil chemical and physiochemical properties, infiltration, runoff and erosion in a semi–arid Mediterranean region. *Geoderma*, 221: 131-138.

Jairajpuri, M.S., 1993. Earthworms and vermiculture: An introduction. In: Director, Zoological Survey of India, editor. Earthworm Resources and Vermiculture. Calcutta: Zoological Survey of India, pp. 1-5.

James, S.W., 2004. New species of *Amynthas, Pheretima* and *Pleionogaster* (Oligochaeta: Megascolecidae) of the Mt. Kitanglad Range, Mindanao Island, Philippines. *Raffles Bulletin of Zoology*, 52: 289-313.

James, S.W., 2005. Preliminary molecular phylogeny in the *Pheretima* group of genera (Crassiclitellata: Megascolecidae) using Bayesian analysis. In: Pop VV, Pop AA, editors. Advances in Earthworm Taxonomy II (Annelida: Oligochaeta). Cluj-Napoca (Romania): Cluj University Press, pp. 129–142.

James, S.W. Y. Hong & T.H. Kim, 2004. New earthworms of *Pheretima* and *Pithemera* (Oligochaeta: Megascolecidae) from Mt. Arayat, Luzon Island, Philippines. *Revue Suisse de Zoologie*, 111: 3-10.

Jose, R.P., N.M. Aspe, W.A. Aureo, R.Y. Parba, C.D. Capunhag & C.I. Narido, 2021. Earthworm diversity and populations in different habitats of Rajah Sikatuna Protected Landscape, Bohol, Philippines. *Philippine Journal of Systematic Biology*, 15(1): 1-11. doi:10.26757/pjsb2021a15001.

Kooch, Y., H. Jalilvand, M.A. Bahmanyar, M.R. Pormajidian & M.S. Gilkalayee, 2007. The effective soil factors on distribution of earthworms in forest ecosystem units. Proceeding of the 10th Congress of Soil Sciences in Iran, 221-223.

Kubiena, W.L., 1955. Animal activities in soils as a decisive factor in establishment of humus forms. In: Kevan, D.K.M., editor. Soil Zoology: A Symposium. London: Butterworths, pp. 73-82.

Lee, K.E., 1985. Earthworms: Their Ecology and Relationships with Soils and Land Use. Sydney: Academic Press.

Pardini, G., M. Gispert & G. Dunjo, 2004. Relative influence of wildlife in properties and erosion processes in different Mediterranean environments in NE Spain. *Science of the Total Environment*, 328: 237-246.

Rombke, J., S. Jansch & W. Diden, 2005. The use of earthworms in ecological soil classification and assessment concepts. *Forest Ecology and Management*, 41: 42-53.

Sautter, K.D., G.G. Brown, S.W. James, D.H. Pasini, N. Nunes & P. Benito, 2006. Present knowledge on earthworm biodiversity in the state of Parana. Brazil. *European Journal of Soil Biology*, 42: S296-S300.

Sims, R. & E. Easton, 1972. A numerical revision of the earthworm genus *Pheretima* with the recognition of new genera and an appendix on the earthworms collected by the Royal Society North Borneo Expedition. *Biological Journal of the Linnean Society*, 4: 169-268. doi:10.1111/j.1095-8312.1972.tb00694.x.

Singh, J., M. Schädler, W. Demetrio, G.G. Brown & N. Eisenhauer, 2019. Climate change effects on earthworms––a review. *Soil Organisms*, 91: 114-138.

Zhang, Q.L. & P.F. Hendrix, 1995. Earthworm (*Lumbricus rubellus* and *Aporrectodea caliginosa*) effects on carbon flux in soil. *Soil Science Society of America Journal*, 59: 816-823.