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Three new species and a new record of pheretimoid earthworms (Crassiclitellata, Megascolecidae) from Misamis Oriental, Philippines, with data from the mitochondrial genome

Nonillon M. Aspe[1], Huifeng Zhao[2,3], Maria Reynalen F. Mapile[4], Maria Christine M. Obusan[4], Yufeng Zhang[2], Edgar Castañares[5], Eric John D. Florida[5,6], Donghui Wu[3,7]

1 *College of Fisheries and Marine Sciences, Mindanao State University at Naawan, Naawan 9023, Misamis Oriental, Philippines*
2 *Hebei Key Laboratory of Animal Diversity, College of Life Science, Langfang Normal University, Langfang 065000, China*
3 *Key Laboratory of Wetland Ecology and Environment, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun 130102, China*
4 *Institute of Biology, College of Science, University of the Philippines, Diliman, Quezon City 1101, Philippines*
5 *College of Environment and Life Sciences, Mindanao State University at Naawan, Naawan 9023, Misamis Oriental, Philippine*
6 *Institute of Soil Research, Department of Forest and Soil Sciences, University of Natural Resources and Life Sciences, Vienna, Austria*
7 *State Environmental Protection Key Laboratory of Wetland Ecology and Vegetation Restoration, School of Environment, Northeast Normal University, Changchun 130117, China*

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Corresponding authors: Nonillon M. Aspe (nonillon.aspe@msunaawan.edu.ph); Donghui Wu (wudonghui@iga.ac.cn)

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Abstract

We describe three new species of pheretimoid earthworms from Misamis Oriental, Mindanao Island, Philippines. One species is *Polypheretima andresi* **sp. nov.**, belonging to the *Polypheretima elongata* group of Easton (1979), characterized by having a pair of genital markings in xix, successive segments in line with the male pores, paired batteries of up to 28 spermathecae in vi and/or vii, and shallow copulatory bursae. Two species belong to *Pheretima*, namely *Pheretima elnorroae* **sp. nov.** of the *Pheretima sangirensis* species group of Sims and Easton (1972), characterized by having a pair of spermathecal pores in the intersegmental furrow of 7/8, and *Pheretima naawanensis* sp. nov. of the *Pheretima dubia* species group of Sims and Easton (1972), characterized by having three pairs of spermathecal pores in 6/7–8/9. Furthermore, for the first time, *Metaphire bahli* has been recorded on Mindanao Island. The mitochondrial genomes of the four species are provided, which included 13 protein-coding genes. A phylogenetic analysis is performed, and the taxonomic status of the four species is supported. The newly described species confirms the diverse assemblage of earthworms in the Philippines and highlights the need for extensive surveys of earthworms and investigating them for potential uses and applications.

Key Words

Mitochondrial genome, new species, pheretimoid earthworms, Philippines

Introduction

Highly regarded as "ecosystem engineers," earthworms are a critical aspect of terrestrial fauna that plays a significant role in maintaining soil health and primary productivity (Guerrero 2009; Shipitalo and Korucu 2017). Their ability to consume organic material and distribute it throughout the soil profile is key to this process (Zhang and Hendrix 1995; Kooch et al. 2007). Additionally, the burrowing activities of earthworms help lessen soil erosion and create essential channels and pores in the soil, enabling gas exchange, water movement, and the transport of solutes (Feller et al., 2003; Fierer 2019). Ultimately, the presence of earthworms is vital for preserving the ecological balance in natural ecosystems. Evidently, earthworms are vital in terrestrial ecosystems, but surprisingly, only a few species have been studied thoroughly, and many more are awaiting description and further exploration (Shipitalo and Korucu 2017; Fierer 2019).

Published reports on the discovery and taxonomic description of indigenous species affirm that the Philippines is a vital geographical resource for earthworms. Countless taxonomic studies have already been conducted around the archipelago, resulting in the discovery of more than 200 indigenous species belonging to family Megascolecidae (Aspe and James 2014; 2015; 2016; 2017; 2018; Aspe and Obusan 2023; Aspe et al. 2016; 2021; Hong 2018; Hong and James 2004; 2008a; 2008b; 2008c; 2009; 2010; 2011a; 2011b; 2021; James 2004; 2005a; 2005b; 2006; 2009; James et al. 2004; Sacay and Aspe 2022). In the province of Misamis Oriental in Mindanao Island, there are no published studies on the description of new species yet, but there is one study conducted by Sacay and Aspe (2022) wherein *Pheretima* cf. *lantapanensis* Aspe & James, 2016, *Amynthas* sp., and *Polypheretima* sp. were detected in Initao-Libertad Protected Landscape and Seascape, a protected area of limestone forest.

With the advent of molecular techniques, earthworm taxonomy and systematics have significantly improved in the last 17 years (Marchán et al. 2022). Previous phylogenetic analyses of earthworms use multiple genes to resolve evolutionary relationships among different earthworm groups (e.g., Chang et al. 2008; Zhao et al. 2015; Aspe et al. 2016; Aspe and James 2018). However, relationships among many earthworm taxa still remain unresolved, especially since most earthworm phylogenetic studies often constitute isolated and unconnected attempts to answer very specific evolutionary questions (Zhao et al. 2015; Aspe and James 2018; Marchán et al. 2022). The use of mitogenomes represents an interesting alternative to multigene phylogenetic analysis due to its significantly higher number of gene markers covered (Zhao et al. 2022; Sato et al. 2023). It has proved to be useful in resolving formerly troublesome phylogenies, clarifying the relationships within phylogenetically difficult groups where high divergence rates made other markers ineffective (Ramesh et al. 2012; Kern et al. 2020). In this paper, we describe three new earthworm species and a new record in Misamis Oriental, providing the first-ever mitogenomic data from the earthworm species of the Philippines.

Materials and methods

Collections sites and sampling

The province of Misamis Oriental, located in the Northern Mindanao region at 8.5046°N, 124.6220°E, borders the Bohol Sea to the north, Bukidnon to the south, Lanao del Norte to the west, and Agusan del Norte to the east. With a land area of 3,131.52 km^2, the coastal province is surrounded by Macajalar Bay, Gingoog Bay, Bohol Sea, and Iligan Bay. The province is rich in flora and fauna species (Canencia and Daba 2015; Sanguila et al. 2016; Guadalquiver et al. 2019; Ramos et al. 2020; Daso et al. 2021).

Indigenous earthworms were collected in Brgy. Mapulog (8°25'06"N, 124°21'37"E) and Brgy. Poblacion (8°26'24.5"N, 124°17'22.4"E and 8°25'24.4"N, 124°17'21.3"E) in Naawan and Brgy. Paniangan (8°23'11.6"N, 124°18'11.3"E) in Manticao, Misamis Oriental, Philippines. A gratuitous permit was secured from the Department of Environment and Natural Resources (DENR) Region 10 Community Environment and Natural Resources Office (CENRO) Oriental before specimen collection. Sampling was conducted from January 2020 to September 2022. Adult earthworms, characterized by the presence of clitellum, were collected from the above-mentioned sites by digging and hand sorting methods. Upon collection in the field, earthworms were sorted to putative species based on identifying characters, including body size, coloration, and number and location of spermathecal pores. The collected earthworms were rinsed with water and killed with 10% ethanol prior to preservation with 95% ethanol.

Morphological examination of earthworm species

All descriptions were based on the examination of external and internal characters using a stereomicroscope, following the terminology and conventions of Easton (1979). Live samples were used for the description of body color, while fixed samples were used for the measurement of body dimensions in millimeters (mm). The generic diagnoses and assignment to species groups follow Sims and Easton (1972). The new species were initially compared with the other members of the same species group in terms of size, distance between the male pores, and distance between spermathecal pores for convenience. Holotypes were deposited in the Philippine National Museum-Annelid Collection (PNM), Manila, Philippines, while paratypes were deposited in the Mindanao State University at Naawan Biological Specimens Repository (MSUN-A), Misamis Oriental, Philippines.

DNA extraction, sequencing, and analysis

Total genomic DNA was extracted from muscle tissues of earthworms using the TIANamp Genomic DNA Kit (Tiangen Biotech Co., Beijing, China) following the manufacturer's instructions. Regions of the COX1 were amplified through the polymerase chain reaction (PCR) using a PCR mixture containing the genomic DNA, forward COI-F_N (5-TTTGAGCCGGAATAATTGG-3) and reverse COI-R_N COI (5-TCGAAGAATGATGTATTTAGGTTTCG-3) primers (Aspe et al. 2016), DNA polymerase, dNTP mix, and Taq buffer. The cycling profile was as follows: denaturation for 30 s at 95 °C, annealing for 45 s at 51 °C, and extension for 1 min at 72 °C for 35 cycles with an initial denaturation for 5 min at 95 °C and a final extension step for 5 min at 72 °C. PCR amplification was confirmed through electrophoresis in 1% agarose gel. Positive amplifications were sequenced using Sanger sequencing by ABI 3730 automated sequencer in Tianyihuiyuan Biotech Co., Ltd. (Beijing, China).

For obtaining the data of the mitochondrial genome of the earthworm species, next-generation sequencing (NGS) was done with the BGISEQ500 platform (BGI Genomics Co., Ltd., Wuhan, China). Before NGS, the genomic DNA was fragmented using a shortgun strategy. After repairing the blunt ends, adenylating 3' ends, and ligating adapters, the fragmented DNA was amplified by PCR. More than two gigabytes of raw data of each species was sequenced; for the quality control of raw data, see Zhao et al. (2022). The clean data was assembled by MitoZ v2.4 (Meng et al. 2019), and then the nearly completed mitogenomes that contain 13 protein-coding genes (CDS), 22 transfer RNAs (tRNAs), 2 ribosomal RNAs (rRNAs), and a control region are obtained. The mitogenomes were submitted to GenBank (accession number is PP266604–PP266607) after gene checking. To determine the interspecific genetic divergence with other closely related species, K2P genetic distance was calculated using COX1 data in MEGA5 (Tamura et al. 2011).

Thirteen PCGs of mitogenomes were chosen for phylogenetic analyses. The sequences were aligned using MAFFT v7.0 (Katoh and Standley 2013) and concatenated by the Perl script FASconCAT-G_v1.04.pl (Kück and Longo 2014). Phylogenetic trees were reconstructed with the maximum likelihood method and Bayesian inference. A maximum likelihood tree was performed by IQ-TREE v2.1.2 (Nguyen et al. 2015) with ultrafast 5,000 bootstraps. Bayesian tree was performed by MrBayes V3.2 (Ronquist et al. 2012). The tree was rooted using *Perionyx excavatus* (EF494507) as an outgroup.

Mitogenomic data from Sato et al. (2023) were included for the phylogenetic analyses. However, many of the taxa in their analyses were found to have been misidentified and may have used invalid species names or synonyms. Therefore, the taxa that were included in their analyses were carefully screened and corrected using materials such as Blakemore (2007) before including them in the analyses while the other mitogenomic data were excluded. The following are some of their misidentified taxa that were corrected or excluded in the analyses: "*Pheretima okutamaensis*" is a synonym of *Metaphire vesiculata*; "*Pheretima iizukai*" and "*Pheretima lactea*" are synonyms of *Amynthas fuscatus*; "*Pheretima silvatica*" is a synonym of *Amynthas tappensis*; "*Amynthas divergens*" is a synonym of *Amynthas corticis*; "*Amynthas masatakae*" is a synonym of *Amynthas robustus*; "*Metaphire agrestis*" is a synonym of *Amynthas agrestis*.

Results and discussion

Morphological data

Polypheretima andresi **sp. nov.**

https://zoobank.org/06AF235C-D159-447F-AD52-972D628682E0

Fig. 1

Material examined. ***Holotype*** • adult (PNM 4680), Brgy. Paniangan, Manticao, Misamis Oriental, near the quarry site of the Republic Cement Iligan, Inc. (8°23'11.6"N, 124°18'11.3"E), 410 m asl., Mindanao Island, Philippines, collectors: N. Aspe, E. Castañares, E.J. Florida, G. Marapao, 20 September 2022. ***Paratypes*** • adults (MSUN-A-0001), same collection data as for holotype.

Etymology. The species is named in honor of Andres Obusan, the son and inspiration of one of the authors of this work.

Diagnosis. Brown worm with adult length 92–178 mm, diameter 4.5–5.9 mm; equators pigmented; 145–248 segments; first dorsal pore at 12/13; 8–9 setae between male pores; male openings 0.25 circumference apart ventrally; paired genital markings in xix to xxiii in line with male pores; spermathecae absent; prostates large in xv to xx.

Description. In live animals, dorsal brown, ventral pale, equators pigmented; Length 92–178 mm (n = 4 adults); diameter 4.5–5.9 mm at x, 4.5–5.0 mm at xx; body cylindrical in cross-section, tail blunt; 145–248 segments. First dorsal pore at 12/13; spermathecal pore absent. Female pore single in xiv, distance between openings 4 mm (0.25 circumference apart ventrally), 8–9 setae between openings. Clitellum annular, from xiv to xvi. Setae evenly distributed around segmental equators; 60–64 setae on vii, 66–70 setae on xx, dorsal and ventral setal gaps absent. Genital marking present in segments xix to xxiii; in one individual, the right genital marking on xxiii is lacking.

Septa 4/5–8/9 muscular, and 9/10–10/11 lacking, 11/12–13/14 muscular. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body near septum/body wall junction. Large gizzard extending from ix to x, esophagus with low vertical lamellae x–xiii, intestinal origin xiv, no caeca; hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix, lateral.

Ovaries and funnels free in xiii. Spermathecae absent. Male sexual system holandric, testes and funnels enclosed in paired sacs in x, xi; seminal vesicles xi, xii, each with digitate dorsal lobe; vesicles of xi enclosed in testes sac; vasa deferentia slender, free from body wall to ental end of prostatic ducts; prostates large in xv to xx, each a single, dense, racemose mass; short muscular duct entering inconspicuous copulatory bursa.

Remarks. *Polypheretima andresi* sp. nov., belongs to the *Po. elongata* group of Sims and Easton (1972), characterized by having a pair of genital markings on xix, successive segments in line with the male pores, and shallow copulatory bursae. Members of the group include *Po. elongata* Perrier , 1872, *Po. stelleri* Michaelsen, 1892,

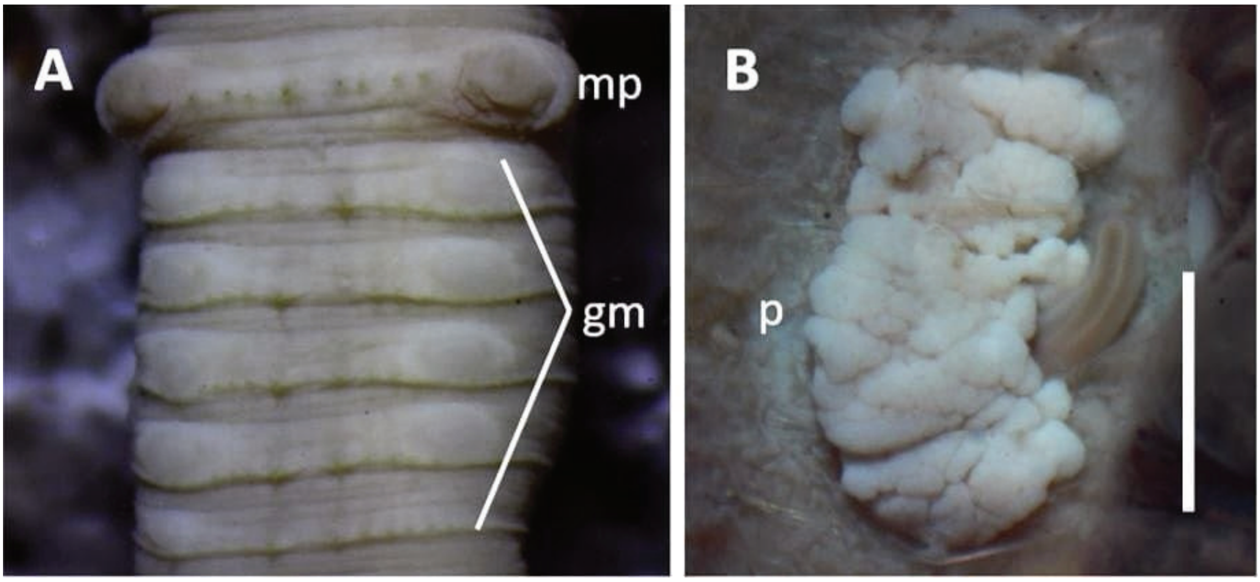

Figure 1. *Polypheretima andresi* sp. nov. **A.** Male pores (mp) and genital markings (gm); **B.** Prostate gland (p). Scale bar: 1mm.

Table 1. Comparison of *Polypheretima andresi* sp. nov. with closely related members of the *Po. elongata* group.

Character	*Po. jenniferae* Aspe et al., 2021	*Po. sahlani* Fahri et al., 2017	*Po. cokelat* Fahri et al., 2017	*Po. andresi* sp. nov.	*Po. irawanensis* Aspe et al., 2021	*Po. kalimpaaensis* Fahri et al., 2017	*Po. kinabaluensis* Beddard & Fedarb, 1895	*Po. fleischmani* Aspe et al., 2021	*Po. bukidnonensis* Aspe & James, 2015	*Po. mantalingahanensis* Aspe et al., 2021	*Po. mindanaoensis* Aspe & James, 2016
Length	161–198	152–195	54–185	92–178	174	124–156	~150	99–149	131	91–124	90–118
Width	3.2–5	4.5–6	4.5–6	4.5–5.9	6.5–7	3–5	?	4–5.2	5–6.5	3–4	5.1–7
No. of segments	160–218	113–281	169–214	145–248	178	150–223	~200	157–168	147	104–115	140–141
First dorsal pore	12/13	12/13	12/13	12/13	11/12	12/13	?	12/13	12/13	12/13	12/13
Setae on vii, post-clitellar setae	43–64, 53–74	78–89, 59–68	52–63, 46–62	60–64, 66–70	94, 79	42–74, 48–52	<40 in vii, ?	50–53, 59–65	39–45, 46–58	38–43, 42–49	41–53, 44–46
Male pores spacing	0.27	0.5	0.4	0.25	0.22	0.35	?	0.25–0.29	0.22	0.23–0.25	0.23
Setae bet. male pores	7–14	8–10	8–10	8–9	11	8–10	?	14–16	6–7	11–14	10
Genital markings	xix–xxiii	xix–xxiii/xxiv	xix–xxii/xxiii	xix–xxiii	xix–xxii	xix–xx/xxi		xix–xxii	xix–xxi	xix–xxi	xix–xxv/xxvi
No. of spermathecae	0	0–3 in vi, 0 in vii	3–5 per battery in vi, 2–5 per battery in vii	0	6 per battery in vi, 12 per battery in vii	3–7 per battery in vi and vii	6–12 per battery in vi and vii	4 per battery in vi, 6 per battery in vii	8–11 per battery in vi, 7–11 per battery in vii	4 per battery in vi and vii	0–5 per battery in vi and vii
Prostate glands	xvi–xxi	xvi–xix	xvii–xix	xv–xx	xvii–xviii	xvi–xx	?	xvi–xix	xvii–xix	xvi–xix	xvi–xxi

Po. everetti Beddard & Fedarb, 1895, *Po. kinabaluensis* Beddard & Fedarb, 1895, *Po. phacellotheca* Michaelsen, 1899, *Po. bukidnonensis* Aspe & James, 2015, *Po. mindanaoensis* Aspe & James, 2016, *Po. cokelat* Fahri et al., 2017, *Po. elongatoides* Fahri et al., 2017, *Po. kalimpaaensis* Fahri et al., 2017, *Po. sahlani* Fahri et al., 2017, *Po. fleischmani* Aspe et al., 2021, *Po. irawanensis* Aspe et al., 2021, *Po. jenniferae* Aspe et al., 2021, *Po. mantalingahanensis* Aspe et al., 2021, *Po. puertoprincesaensis* Aspe et al., 2021, *Po. victoriaensis* Aspe et al., 2021, and *Po. andresi* sp. nov. Among the species in the *Po. elongata* group, *Po. andresi* sp. nov. is relatively similar to *Po. jenniferae*, *Po. sahlani*, *Po. cokelat*, *Po. irawanensis*, *Po. kalimpaanensis*, *Po. kinabaluensis*, *Po. fleischmani*, *Po. bukidnonensis*, *Po. mantalingahanensis* and *Po. mindanaoensis* in size (Table 1). However, the new species (4.5–5.9 mm) has a thinner body than that of *Po. irawanensis* (6.5–7 mm) but has a body thicker than that of *Po. mantalingahanensis* (3–4 mm). *Polypheretima andresi* sp. nov. has a wider space between male pores (0.25) than that of *Po. irawanensis* (0.22), *Po. bukidnonensis* (0.22), and *Po. mindanaoensis* (0.23) but narrower than that of *Po. jenniferae* (0.27), *Po. sahlani* (0.5), *Po. cokelat* (0.4), and *Po. kalimpaanensis* (0.25). The new species has fewer setae on vii (60–64) than that of *Po. sahlani* (78–89) and *Po. irawanensis* (94) but has more setae on the same segment than that of *Po. kinabaluensis* (<40), *Po. fleischmani* (50–53), *Po. bukidnonensis* (39–45), *Po. mantalingahanensis* (38–43), and *Po. mindanaoensis* (41–53). The new species also has fewer post-clitellar setae (66–70) than that of *Po. irawanensis* (79) but more in the same area compared with *Po. cokelat* (46–62), *Po. kalimpaanensis* (48–52), *Po. fleischmani* (59–65), *Po. bukidnonensis* (46–58), *Po. mantalingahanensis* (42–49), and *Po. bukidnonensis* (44–46). Notably, the new species is also similar to *Po. jenniferae* and some individuals of *Po. sahlani* and *Po. mindanaoensis* in having no spermathecae but differs from these species in the position of the prostate glands (xv–xx vs. xvi–xxi, xvi–xix). Gates (1972) suggested that the absence of spermathecae in species or adult individuals may result in parthenogenetic reproduction.

In a separate study, *Po. andresi* sp. nov., which showed to have a potential for vermiculture for the purpose of various applications, has been assessed for its gut microbiota profile. Initial results show that there is a significantly higher diversity of gut microorganisms in *Po. andresi* sp. nov., which have potential benefits in agriculture, bioremediation, and in medicine compared to the gut microbiota in the African nightcrawler *Eudrilus eugeniae*, which is a popular vermiculture commodity in the Philippines (Mapile et al. unpublished).

Pheretima elnorroae **sp. nov.**

https://zoobank.org/5653B500-B16F-485B-BA5E-B2BF882E427E

Fig. 2

Material examined. ***Holotype*** • adult (PNM 4682), Brgy. Mapulog, Naawan, Misamis Oriental (8°25'06"N, 124°21'37"E), 845 m asl., Mindanao Island, Philippines, collectors: N. Aspe, E. Castañares, E.J. Florida, G. Marapao, 28 January 2020. ***Paratypes*** • adults (MSUN-A-0002), same collection data as for holotype.

Etymology. The species is named in honor of Dr. Elnor C. Roa, the incumbent Chancellor of the Mindanao State University at Naawan, who inspires the primary author of her visionary leadership.

Diagnosis. Brown worm with adult length 95–122 mm, diameter 5–10 mm; equators pigmented; 117–128 segments; 7–9 setae between male pores; spermathecal pores 0.18 circumference apart ventrally; male openings 0.13–0.18 circumference apart ventrally. Spermathecae paired in viii, large with ovate ampulla, stalked diverticulum terminating in sausage-shaped receptacles; paired small copulatory bursae in xvii–xxi; genital markings lacking; caeca simple and long, originating in xxvii, extending forward to xviii; prostates large in xvi to xxii, penis present.

Description. In live animals, dorsal brown, ventral pale, equators pigmented; Length 95–122 mm (n = 4 adults); diameter 5–7 mm at x, 7–10 mm at xx; body cylindrical in cross-section, tail blunt; 117–128 segments. First dorsal pore at 11/12; spermathecal pore one pair at 7/8, inconspicuous, distance between spermathecal pores 4 mm (0.18 circumference apart ventrally). Female pore single in xiv, openings of copulatory bursae paired in xviii, extending to xxi, distance between openings 4 mm (0.13–0.18 circumference apart ventrally), 7–9 setae between openings. Clitellum annular, from xiv to xvi. Setae evenly distributed around segmental equators; 43–71 setae on vii, 45–79 setae on xx, dorsal setal gaps absent, ventral setal gaps present. Genital marking lacking.

Septa 4/5–8/9 muscular, and 9/10–10/11 thin, 11/12–13/14 muscular. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body near septum/body wall junction. Large gizzard extending from ix to x, esophagus with low vertical lamellae x–xiii, intestinal origin xv, caeca simple and long, originating in xxvii, extending forward to xviii; Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix, lateral.

Ovaries and funnels free in xiii. One pair of spermathecae in viii, with nephridia on ducts; each spermatheca large with ovate ampulla, short muscular duct, stalked diverticulum attached to duct near ampulla, terminating in irregular sausage-shaped receptacle, stalk thin. Male sexual system holandric, testes and funnels enclosed in paired sacs in x, xi; seminal vesicles xi, xii, each with digitate dorsal lobe; vesicles of xi enclosed in testes sac; vasa deferentia slender, free from body wall to ental end of prostatic ducts; prostates large in xvi to xxii, each a single, dense, racemose mass; short muscular duct entering on posterior margin of copulatory bursa; paired small copulatory bursae xvii–xxi; tapering penis present.

Remarks. *Pheretima elnorroae* sp. nov., belongs to the *Pheretima sangirensis* group of Sims and Easton (1972), characterized by having a pair of spermathecal pores in the intersegmental furrow of 7/8. Members of the group include *Ph. sangirensis* Michaelsen, 1891, *Ph. apoensis* Aspe & James, 2016, *Ph. camiguinensis* Aspe & James, 2016, *Ph. sibucalensis* Aspe & James, 2016, *Ph. timpoongensis* Aspe & James, 2016, *Ph. alba* James, 2004, *Ph. asurgo* James, 2004, *Ph. baungonensis* James, 2004, *Ph. ceramensis* James, 2004, *Ph. diesmosi* James, 2004, *Ph. lagunasensis* James, 2004, *Ph. mariae* James, 2004, *Ph. monoporata* James, 2004, *Ph. paucisetosa* James, 2004, *Ph. quincunxia* James, 2004, *Ph. rubida* James, 2004, *Ph. rugosa* James, 2004, *Ph. vicinipora* James, 2004, *Ph. virgata* James, 2004, *Ph. immanis* Aspe & James, 2014, *Ph. boniaoi* Aspe & James, 2014, *Ph. lago* Aspe & James, 2014, *Ph. longigula* Aspe & James, 2014, *Ph. longiprosta* Aspe & James, 2014, *Ph. maculodorsalis* Aspe & James, 2014, *Ph. malidangensis* Aspe & James, 2014, *Ph. misamisensis* Aspe & James, 2014, *Ph. nolani*

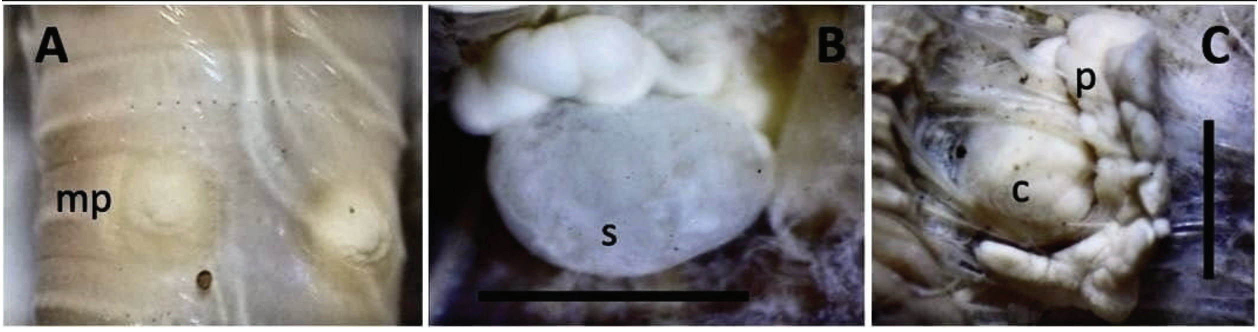

Figure 2. *Pheretima elnorroae* sp. nov. **A.** Male pores (mp); **B.** Spermatheca (s); **C.** Prostate gland (p) and copulatory bursa (c). Scale bar: 1mm.

Table 2. Comparison of *Pheretima elnorroae* sp. nov. with the closely related members of the *Ph. sangirensis* group.

Characters	***Ph. boniaoi*** **Aspe & James, 2014**	***Ph. diesmosi*** **James, 2004**	***Ph. elnorroae*** **sp. nov.**	***Ph. camiguinensis*** **Aspe & James, 2016**	***Ph. nunezae*** **Aspe & James, 2014**	***Ph. paucisetosa*** **James, 2004**	***Ph. baungonensis*** **James, 2004**	***Ph. riparianensis*** **Hong & James, 2008**	***Ph. baracatanensis*** **Aspe & James, 2017**	***Ph. nolani*** **Aspe & James, 2014**
Length	101–133	128	95–122	63–122	>116	115	107	75–105	100	89–97
Width x, xx	5–5.5, 5.5–6	3.4, 3.4	5–7, 7–10	3.5–4.8, 3.5–5.2	8.5, 9	5.5, 6.0	4.5, 4.2	?	4–5, 4.8–6.5	4.5–5
Dorsal pigmentation	purplish brown dorsal stripes	brown	brown	reddish brown	dark gray-brown	?	brown	violet brown	brown	purplish brown
Segments	98–108	102	117–128	87–103	?	89	?	100–102	94	111
1st dorsal pore	?	12/13	11/12	?	12/13	11/12	13/14	?	12/13	12/13
Setal gaps D, V	+, -	+, -	-, +	?	+, -	+, -	-, -	+, +	+, -	+, -
Sperm. pore distance	0.14	0.23	0.18	0.17	0.28	0.16	0.38	0.07	0.32	0.14
Male pore distance	0.03	0.15	0.13–0.18	0.15–0.18	0.22	0.13	0.19	0.11	0.20–0.22	0.12
Setae between openings	0	0	7–9	1–4	9	2	8	5–7	4–5	2
Setae vii, xx	59–78, 62–68	28, 38	43–71, 45–79	32–34, 39–44	46, 51	22,28	38,50	37–43, 46–58	43–46, 44–51	33–48, 42
Gizzard	viii-ix	viii	ix-x	?	ix	?	viii	viii-x	viii-x	viii
Intestinal origin	xvii	xvii	xv	xv	xv	xvi–xxv	xxvii–xv	xvi	xv	xv
Caeca	xxvii–xxiv	xxvii to xxv	xxvii–xviii	xxvii–xxiv/ xxiii/ xxii	xxvii–xxiv	xvii–xxv	xxvii–xxv	xxvii–xxiii	xxvii–xxiv	xxvii–xxii
Prostate	xvi–xxi	?	xvi–xxii	xvii–xx/ xxi	xvii–xix	?	?	?	xvi–xix	xv–xx
Copulatory bursae	xvii–xx	xviii	xvii–xxi	xvii–xix	viii	xviii	xviii	?	xvii–xix	xvii–xix
Penes	-	-	+	+	+	+	-	+	-	+

Aspe & James, 2014, *Ph. nunezae* Aspe & James, 2014, *Ph. tigris* Aspe & James, 2014, *Ph. wati* Aspe & James, 2014, *Ph. baracatanensis* Aspe & James, 2017, *Ph. floresi* Aspe & James, 2017, *Ph. solisi* Aspe & James, 2017, *Ph. bontocensis* Hong & James, 2021, *Ph. riparianensis* Hong & James, 2021, and *Pheretima elnorroae* sp. nov. Among these, the new species is relatively similar to *Ph. boniaoi*, *Ph. diesmosi*, *Ph. camiguinensis*, *Ph. nunezae*, *Ph. paucisetosa*, *Ph. baungonensis*, *Ph. riparianensis*, *Ph. baracatanensis*, and *Ph. nolani* in size (Table 2). But *Ph. elnorroae* sp. nov., has the greatest number of body segments among the closely related species (117–128 vs. 87–111). The new species has a thicker body (5–10 mm) than that of *Ph. diesmosi* (3.4 mm), *Ph. camiguinensis*

(3.5–5.2 mm), *Ph. baungonensis* (4.2–4.5 mm), and *Ph. nolani* (4.5–5 mm). It has a wider distance between spermathecal pores (0.18) than that of *Ph. boniaoi* (0.14), *Ph. riparianensis* (0.07), and *Ph. nolani* (0.14) but has a narrower distance between spermathecal pores than that of *Ph. diesmosi* (0.23), *Ph. nunezae* (0.28), *Ph. baungonensis* (0.38), and *Ph. baracatanensis* (0.32). It also has a wider distance between male pores than that of *Ph. boniao* (0.03) and *Ph. riparianensis* (0.11) but has a narrower distance than that of *Ph. nunezae* (0.22) and *Ph. baracatanensis* (0.20–0.22). The new species also has more pre- and postclitellar setae (43–71, 45–79) than that of *Ph. diesmosi* (28, 38), *Ph. camiguinensis* (32–34, 39–44), and *Ph. paucisetosa* (22, 28); it has more preclitellar setae than that of *Ph. baungonensis* (38) and *Ph. riparianensis* (37–43) and has more postclitellar setae than that of *Ph. nolani* (42). Notably, the new species has the longest caeca (xxvii–xviii) among its closely related species. The new species also has penes, while *Ph. boniao*, *Ph. diesmosi*, *Ph. baungonensis*, and *Ph. baracatanensis* have none. In addition, the new species differs in the position of the prostate gland (xvi–xxii) from that of *Ph. boniaoi* (xvi–xxi), *Ph. camiguinensis* (xvii–xx/xxi), *Ph. nunezae* (xvii–xix), *Ph. baracatanensis* (xvi–xix), and *Ph. nolani* (xv–xx).

***Pheretima naawanensis* sp. nov.**

https://zoobank.org/C3CA5886-4B25-4F43-BCE7-D83B4831B8DA

Fig. 3

Material examined. ***Holotype*** • adult (PNM 4683), Brgy. Poblacion, Naawan, Misamis Oriental (8°26'24.5"N, 124°17'22.4"E), 164 m asl., Mindanao Island, Philippines, collectors: E. Castañares, 10 February 2020. ***Paratypes*** • adults (MSUN-A-0003), same collection data as for holotype.

Etymology. The species is named after the municipality of Naawan, Misamis Oriental, where it was collected.

Diagnosis. Brown worm with adult length 82–100 mm, diameter 6–9 mm; equators pigmented; 67–120 segments; three pairs of spermathecal pores at 6/7/8/9. Spermathecae large with ovate ampulla, stalked diverticulum terminating in sausage-shaped receptacles; paired small copulatory bursae in xvii–xviii; genital markings lacking; caeca simple originating in xxvii, extending forward to xxiii; prostates large in xvii to xix; penis present.

Description. In live animals, dorsal brown, ventral pale, equators pigmented; Length 82–100 mm (n = 2 adults); diameter 6–9 mm at x, 7–8 mm at xx; body cylindrical in cross-section, tail blunt; 67–120 segments. First dorsal pore at 13/14; spermathecal pore three pairs at 6/7/8/9, inconspicuous, distance between spermathecal pores 5.2 mm (0.18 circumference apart ventrally). Female pore single in xiv, openings of copulatory bursae paired in xviii, extending to xxi, distance between openings 4.2 mm (0.17 circumference apart ventrally), 4–5 setae between openings. Clitellum annular, from xiv to xvi. Setae evenly distributed around segmental equators; 29–33 setae on vii, 69–71 setae on xx, dorsal and ventral setal gaps absent. Genital marking lacking.

Septa 4/5–8/9 muscular, and 9/10–10/11 thin, 11/12–13/14 muscular. Dense tufts of nephridia on anterior faces of 5/6 and 6/7; nephridia of intestinal segments located mainly on body near septum/body wall junction. Large gizzard extending from ix to xi, esophagus with low vertical lamellae x–xiii, intestinal origin xv, caeca simple originating in xxvii, extending forward to xxiii; Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix, lateral.

Ovaries and funnels free in xiii. Three pairs of spermathecae in vii, viii, ix with nephridia on ducts; each spermatheca large with ovate ampulla, short muscular duct, stalked diverticulum attached to duct near ampulla, terminating in sausage-shaped receptacle, stalk thin. Male sexual system holandric, testes and funnels enclosed in paired sacs in x, xi; seminal vesicles xi, xii, each with digitate dorsal lobe; vesicles of xi enclosed in testes sac; vasa deferentia slender, free from body wall to ental end of prostatic ducts; prostates large in xvii to xix, each a single, dense, racemose mass; short muscular duct entering on lateral margin of copulatory bursa; copulatory bursae small in xvii–xviii; tapering penis present.

Remarks. *Pheretima naawanensis* sp. nov., belongs to the *Ph. dubia* group of Sims and Easton (1972), characterized by having three pairs of spermathecal pores in 6/7–8/9. Members of the group include *Ph. callosa* Gates, 1937, *Ph. poiana* Michaelsen, 1913, *Ph. philippina* Rosa, 1981, *Ph. losbanosensis* Aspe & Obusan, 2023, *Ph. korinchiana* Cognetti, 1922, *Ph. vungtauensis* Nguyen et al., 2018, *Ph. dubia* Horst, 1893, *Ph. julkai* Hong & James, 2011, *Ph. banaoi* Hong & James, 2010, *Ph. lamaganensis* Hong & James, 2011, *Ph. balbalanensis* Hong & James, 2010, *Ph. globosa* Hong & James, 2011, and *Ph. naawanensis* sp. nov. In terms of the distance between spermathecal pores, the new species is similar to *Ph. losbanosensis*, a significantly larger worm, while the other members of the species group have a wider distance between male pores (0.19–0.4) (Table 3). Similar to *Ph. dubia* (0.17), the new species has a wider distance between male pores than that of *Ph. losbanosensis* (0.15) but has a narrower distance between male pores than that of the other members of the species group (0.18–0.29). In terms of length, *Ph. naawanensis* sp. nov. is relatively similar to *Ph. korinchiana* Cognetti, 1922, and *Ph. dubia* Horst, 1893. However, the new species has fewer preclitellar setae (29–33) and more postclitellar setae than the two species (38, 43, and 52, respectively). It also has fewer setae between male pores (4–5) than the two species (12 and 10, respectively). In addition, the new species has a narrower distance between spermathecal pores and between male pores (0.18 and 0.17, respectively) than *Ph. korinchiana* (0.19–0.22) and has a significantly narrower distance between spermathecal pores than *Ph. dubia* (0.38). *Pheretima naawanensis* sp. nov., is so far the only species belonging to the *Ph. dubia* group to be recorded from Mindanao Island, geographically isolated from any member of the species group.

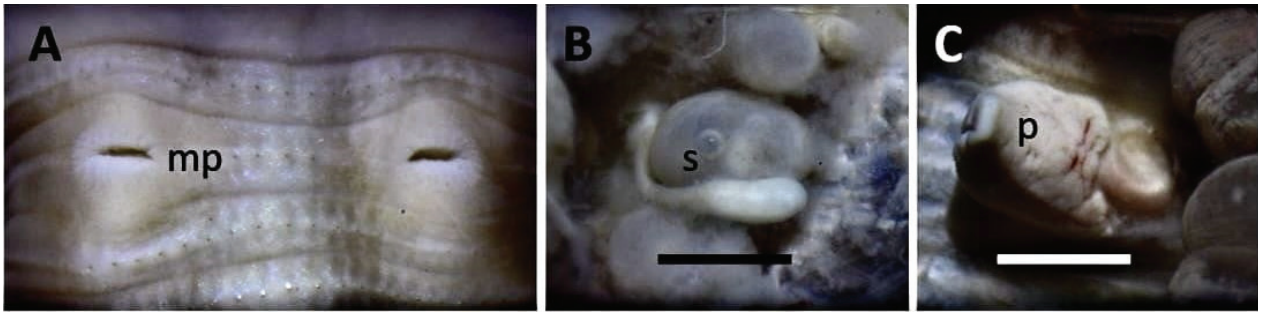

Figure 3. *Pheretima naawanensis* sp. nov. **A.** Male pores (mp); **B.** Spermathecae (s); **C.** Prostate gland (p). Scale bar: 1mm.

Table 3. Comparison among members of the *Ph. dubia* group of Sims and Easton (1972). The species are listed in order of size from largest to smallest.

Characters	*Ph. callosa* Gates, 1937	*Ph. poiana* Michaelsen, 1913	*Ph. philippina* Rosa, 1981	*Ph. losbanosensis* Aspe & Obusan, 2023	*Ph. korinchiana* Cognetti, 1922	*Ph. vungtauensis* Nguyen et al., 2018	*Ph. dubia* Horst, 1893	*Ph. naawanensis* sp. nov.	*Ph. julkai* Hong & James, 2011	*Ph. banaoi* Hong & James, 2010	*Ph. lamaganensis* Hong & James, 2011	*Ph. balbalanensis* Hong & James, 2010	*Ph. globosa* Hong & James, 2011
Length (mm)	330	290	180–240	220–228	83–180	132–169	80–140	82–100	49–69	53–60	46–55	42–50	35–42
Width (mm)	16	?	7	8–9	?	4.1–6.1	?	6–9	3.5–4	3–3.6	2.5–3.2	2.2–2.7	2.3–2.7
Pigmentation	?	gray	gray	brown	brown	grayinsh brown	brown	brown	light purple-brown	yellowish-brown	brown	reddish-brown	light brown
No. of segments	?	>110	115–125	117–128	?	91–125	?	99–237	90–95	89–97	84–93	72–85	71–80
First dorsal pore	?	?	11/12	12/13	?	11/12 or 12/13	?	12/13	10/11	12/13	11/12	11/12	11/12
Setae on vii, xx	71, 114	50 (v); 52 (xix)	44, >70	26–36, 55–60	38, 43	31–49 (viii), 57–82 (xxv)	38, 52	29–33, 69–71	29–31, 51–54	25–35, 46–47	43–44, 23–30	29–31, 41–42	29, 52
Setae bet. male pores	?	?	?	4–6	12	11–17	10	4–5	6–10	6–12	7–10	6–8	9
Dist. bet. sper. pores	?	0.4	5th–6th setal lines	0.18	0.19–0.22	0.4	0.38	0.18	0.2–0.23	0.19	0.27–0.28	0.28–0.32	0.32
Dist. bet. male pores	?	0.29	7th–8th setal lines	0.15	0.19–0.23	0.35	0.17	0.17	0.18–0.2	0.22	0.21–0.22	0.22	0.22
Caeca	?	?	xxvi–xxiii	xxvii–xxiii	?	xxvii–xxiv or xxiii	?	xxvii–xxiii	xxvii–xxv	xxvii–xxiv	xxvii–xxv	xxvii–xxiv	xxvii–xxvi
Prostate glands	?	?	xviii	xvii–xix	?	xvi–xviii	?	xvii–xix	xxvii–xxviii	xvi–xviii	xvii–xviii	xvii–xviii	xvii–xviii

Similar to *Po. andresi* sp. nov., *Pheretima naawanensis* sp. nov. has shown to have potential for vermiculture and has been explored in the College of Agriculture, Forestry, and Environmental Science of the Mindanao State University at Naawan for various applications such as in agriculture and aquaculture.

Metaphire bahli Gates, 1945

Fig. 4

Material examined. ***Holotype*** • adult (PNM 4681), Poblacion, Naawan, Misamis Oriental, just adjacent to the building of the College of Agriculture, Forestry, and Environmental Science of the Mindanao State University at Naawan (8°25'24.4"N, 124°17'21.3"E), 154 m asl., Mindanao Island, Philippines, collectors: E.J. Florida, J.C. Cajes, 17 February 2020. ***Paratypes*** • adults (MSUN-A-0004), same collection data as for holotype.

Diagnosis. Brown worm with adult size of 58–145 mm × 3.3–6 mm; equators pigmented; three pairs of spermathecal pores at 6/7–8/9; distance between spermathecal pores 0.16–0.25 circumference apart ventrally; distance between male pores 0.14–0.20 circumference apart ventrally, 3–8 setae between openings; setae on vii and xx 39–49 and 40–72, respectively; genital markings in 17/18 and 18/19 in line with male pores; male region strongly concave to form an ellipsoid-shaped or rounded area. Spermathecae

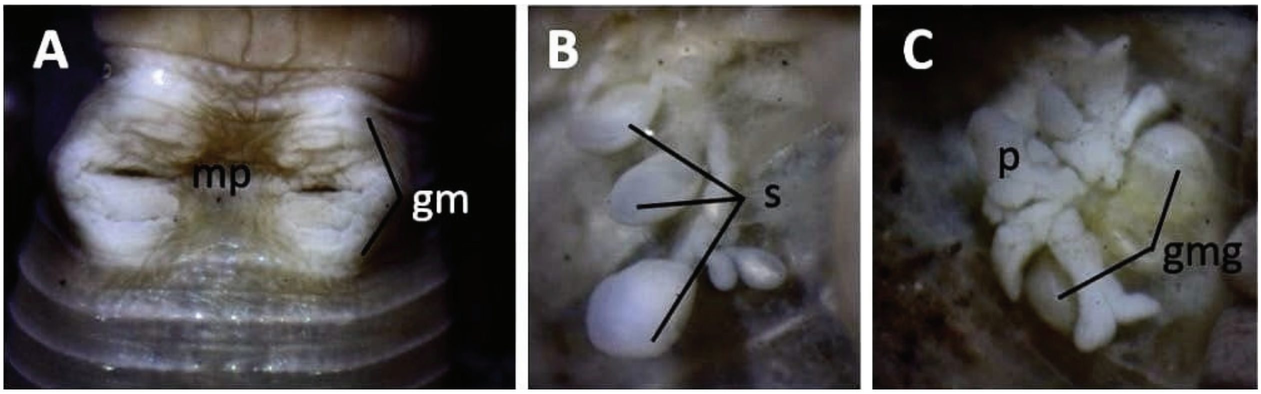

Figure 4. *Metaphire bahli*. **A.** Male pores (mp) with genital markings (gm); **B.** Spermathecae (s); **C.** Prostate gland (p) with genital marking glands (gmg). Scale bar: 1mm.

paired, postseptal in vii, viii, and ix, no nephridia on ducts; each spermatheca large with pyriform ampulla, stout muscular duct, stalked diverticulum attached to duct near the base of the duct, terminating in ovate receptacles, stalks long, convoluted; male sexual system holandric; copulatory bursae not prominent; round genital marking glands present in xvii and xix; intestinal origin xv, caeca simple originating in xxvii, extending forward to xxiv; prostates large in xvi to xx, each a single, dense, racemose mass; copulatory bursae not prominent; round genital marking glands present in xvii and xix (Aspe and Obusan 2023).

Remarks. This is the first record of *Metaphire bahli* on Mindanao Island. *Metaphire bahli* had been recorded in Batangas, Manila, Angeles City, Pangasinan, Panay, Negros, and Sibuyan Island (Thai and Samphon 1989; Blakemore 2016) and previously documented being cultured for its vermicast in a vermifacility in Los Baños City, Laguna (Aspe and Obusan 2023). Outside of the Philippines, *M. bahli* had also been recorded in Sri Lanka, Vietnam (Nguyen et al. 2016, 2017, 2020), India (Narayanan et al. 2019), Thailand (Prasankok et al. 2013), Laos and Cambodia (Gates 1945), as well as in Darwin, Australia (Blakemore 2016). *Metaphire bahli* has been explored in the College of Agriculture, Forestry, and Environmental Science of the Mindanao State University at Naawan for various applications such as in agriculture and aquaculture.

Molecular analyses

The interspecific genetic distance of closely related species based on COX1 is shown in Table 4. The whole dataset ranges from 11.6% to 29.8%, and the K2P distance of the three new species ranges from 17.6% to 27.5%, supporting the new species status from a genetic divergence view. In other studies, the interspecific distance in different earthworm groups ranges between 15% and 28% (Admassu et al. 2006; Huang et al. 2007; Chang et al. 2008; Novo et al. 2009; Dong et al. 2019), which are in agreement with our results. The K2P analysis also confirmed the genetic identity of *M. bahli* collected in Naawan with another sequence of *M. bahli* obtained from GenBank.

The mitogenomes of *Polypheretima andresi* sp. nov., *Pheretima elnorroae* sp. nov., *Pheretima naawanensis* sp. nov., and *Metaphire bahli* are the first mitogenomes that were obtained from the earthworm species from the Philippines. The previous phylogenetic analyses of earthworms from the Philippines used specific genes that were concatenated (Aspe et al. 2016; Aspe and James 2018). The mitogenomic structures of the four species are shown in Fig. 5, and gene order and location in double strands are identical with all published mitogenomes of other earthworms (e.g., Zhao et al. 2022; Koo and Hong 2023). The four mitogenomes are nearly complete, and only the control region is not assembled completely due to the large amount of short AT repeats that are difficult to assemble in NGS reads. The details of the mitochondrial genomes are provided in Suppl. material 1.

The results of the phylogenetic analyses are shown in Figs 6, 7. The results support the new species status of the three pheretimoid species from the phylogenetic perspective. The trees generated from Bayesian Inference and maximum likelihood more or less reflect the same results, wherein the new Philippine species are placed at the basal clades, supporting the hypothesis in Aspe and James (2016) that the Philippines may be the center of species radiation for these groups in eastern Asia. Further studies involving more taxa belonging to these groups have to be conducted to verify the results. *Polypheretima andresi* sp. nov. forms as a sister taxon with *Polypheretima elongata*, while *Pheretima elnorroae* sp. nov. forms as a sister taxon with *Pheretima naawanensis* sp. nov. In Aspe and James (2018), *Polypheretima* showed to be non-monophyletic while the taxonomic grouping in *Pheretima* is still not very clear. Further phylogenetic studies using mitogenomic data need to be conducted to verify the taxonomic grouping of these two genera. Meanwhile, the trees depict a monophyletic clade for *Metaphire* and *Amynthas*, which agrees with the results of Sato et al. (2023). However, *Metaphire* and *Amynthas*, respectively, showed to be non-monophyletic groups, which is also consistent with the results of Sato et al. (2023) on the mitogenomic phylogenetics of the earthworms in Japan and with other phylogenetic studies using multiple gene markers (e.g., Chang et al. 2008; Zhao et al. 2015; Aspe et

Table 4. K2P distance of some *Metaphire*, *Pheretima*, and *Polypheretima* species based on COX1.

	Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	*Pheretima_vergrandis*_LC127233																	
2	*Pheretima_malindangensis*_LC127234	0.209																
3	*Pheretima_boniaoi*_LC127235	0.222	0.191															
4	*Pheretima_camiguinensis*_LC127236	0.212	0.200	0.201														
5	*Pheretima_timpoongensis*_LC127237	0.226	0.187	0.227	0.116													
6	*Pheretima*_sp_LC268858	0.259	0.238	0.223	0.193	0.218												
7	*Pheretima*_sp_LC268867	0.248	0.223	0.191	0.213	0.200	0.176											
8	*Pheretima*_sp_LC268878	0.212	0.216	0.249	0.191	0.196	0.174	0.194										
9	*Pheretima*_sp_LC268879	0.228	0.234	0.234	0.222	0.250	0.202	0.218	0.171									
10	*Pheretima*_sp_LC268882	0.242	0.298	0.264	0.226	0.237	0.192	0.217	0.166	0.172								
11	*Pheretima*_sp_LC268883	0.258	0.223	0.225	0.211	0.189	0.239	0.227	0.235	0.255	0.233							
12	*Pheretima_naawanensis* sp. nov.	0.250	0.212	0.230	0.221	0.228	0.226	0.199	0.177	0.176	0.220	0.239						
13	*Pheretima_elnorroae* sp. nov.	0.217	0.211	0.211	0.154	0.173	0.229	0.223	0.226	0.259	0.230	0.216	0.232					
14	*Metaphire_bahli*	0.262	0.275	0.242	0.234	0.231	0.217	0.205	0.203	0.237	0.230	0.198	0.223	0.262				
15	*Metaphire_bahli*_KT626581	0.262	0.275	0.242	0.234	0.231	0.217	0.205	0.203	0.237	0.230	0.198	0.223	0.262	0.000			
16	*Polypheretima_andresi* sp. nov.	0.190	0.246	0.220	0.193	0.193	0.222	0.239	0.209	0.221	0.216	0.222	0.243	0.196	0.230	0.230		
17	*Polypheretima*_sp_LC268874	0.226	0.212	0.230	0.215	0.225	0.213	0.193	0.215	0.225	0.236	0.211	0.202	0.210	0.220	0.220	0.216	
18	*Pontodrilus_litoralis*_LC125897	0.210	0.208	0.205	0.220	0.210	0.248	0.197	0.242	0.240	0.236	0.192	0.217	0.238	0.210	0.210	0.193	0.254

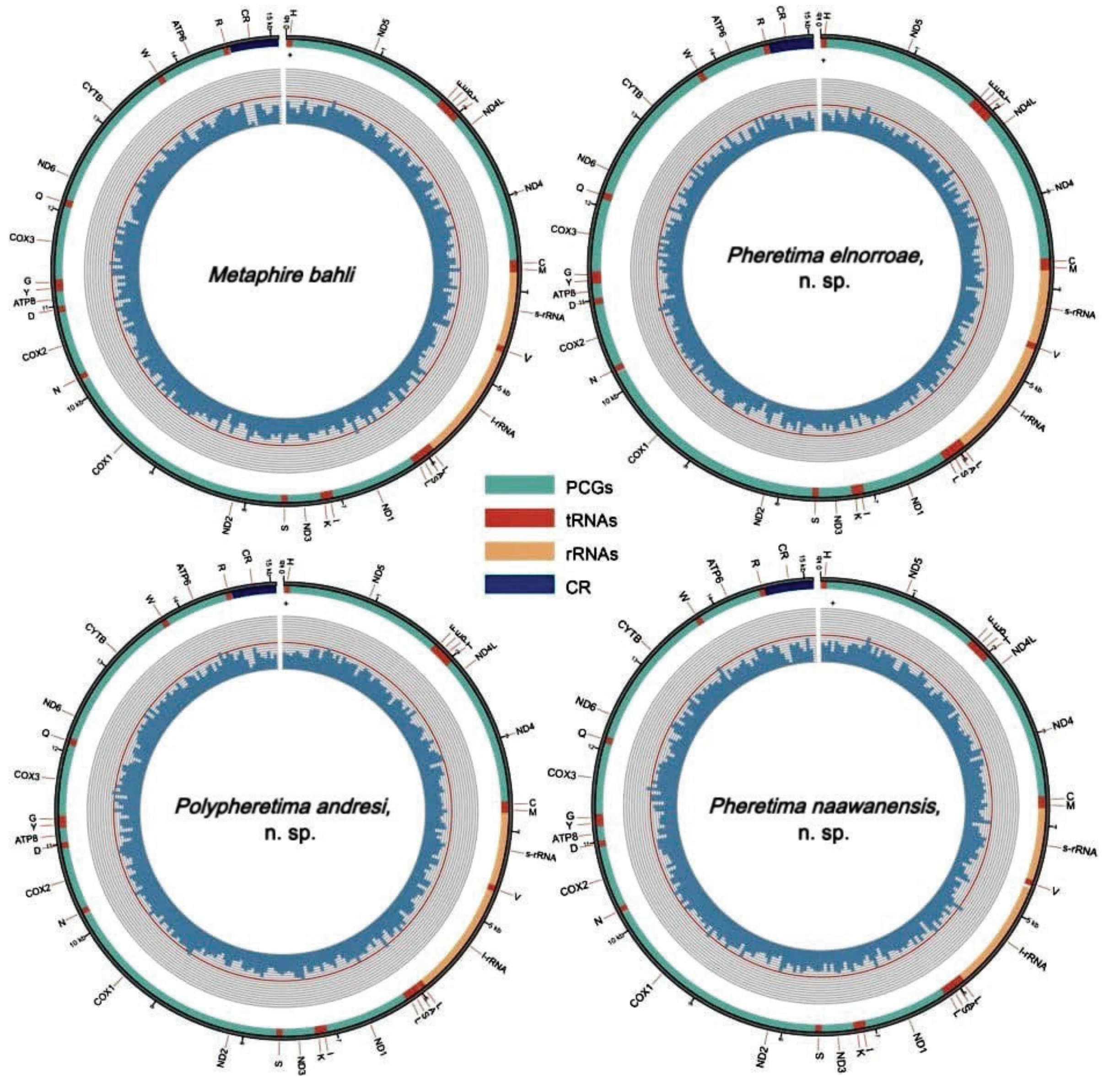

Figure 5. Mitogenomic structure of the four new species. The inner circles indicate the GC content in every 50-site window, and the outer circle shows the arrangement of the genes; *trnH* was set as the start of the mitogenome. All genes are coded on the majority strand.

al. 2016; Aspe and James 2018). Aspe and James (2018) stated that the non-monophyly of these genera suggests that the characters used by Sims and Easton (1972), particularly the presence of copulatory bursae in *Metaphire* and the absence of the same in *Amynthas* to delineate each genus, are homoplasious and do not reflect phylogeny. Though Zhao et al. (2015) proposed that *Metaphire* and *Amynthas* be reconsidered as one genus, a way to re-assess the taxonomic grouping of these two genera may be through carefully re-examining the male pores and the copulatory bursae of the specimens by using histological analysis and supporting it with molecular data as done in the more recent papers on the earthworms of Vietnam (e.g., Nguyen et al. 2020, 2022).

Vermiculture, a technology that cultures earthworms for various applications, has been practiced in many countries in Europe, the Americas, Australia, and Asia (Edwards and Arancon 2006; Edwards and Arancon 2022). In the Philippines, the species that has been popularly cultured is the African nightcrawler, *Eudrilus eugeniae*, which was introduced to the Philippine soils from Africa around the 1980s (Guerrero 2005; Blakemore 2016) to be used as feeds for tilapia. During that time, there was still a lack of knowledge on the diversity of indigenous earthworm species in the country. However, the introduction of species from one geographically isolated area to another can potentially be an ecological threat due to species invasion if the population growth of the introduced species becomes unregulated. This will have detrimental effects on the natural environment, such as alteration of nutrient storage and availability in the soil and displacement of indigenous species (Bohlen et al. 2004; Holdsworth et al. 2007; Aspe et al. 2009). Aspe and Obusan (2023) promoted the cultivation of indigenous earthworm species rather than using exotic ones and dis-

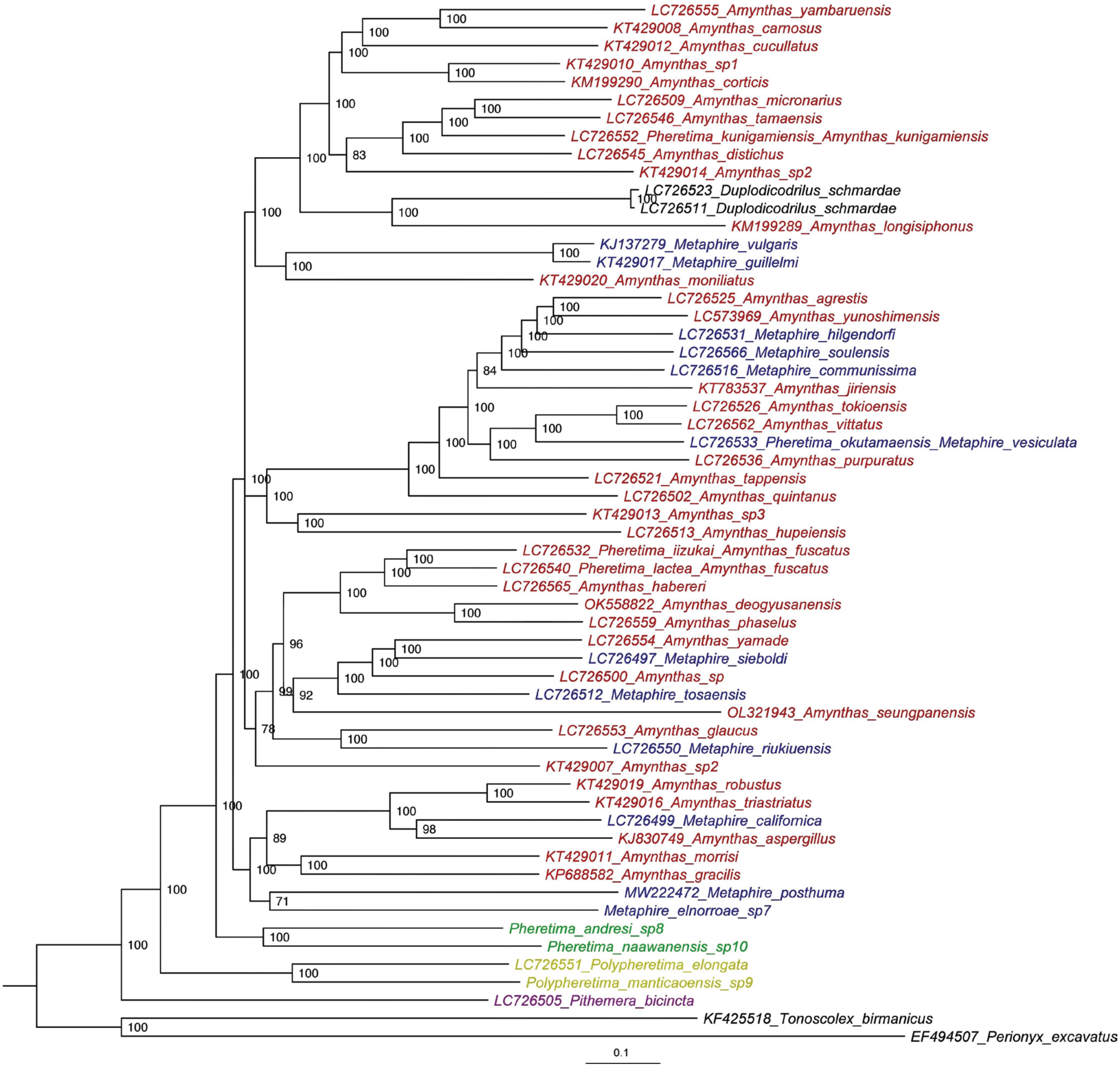

Figure 6. The Bayesian phylogenetic tree based on 13 PCGs of mitogenomes of Megascolecidae. The four new species are highlighted. Yellow refers to *Polypheretima*, green refers to *Pheretima*, blue refers to *Metaphire*, and red refers to *Amynthas*.

couraged the introduction of local species to other areas. Recently, cultivation of indigenous species has started to become known in the Philippines. For example, *Pheretima losbanosensis* and *Metaphire bahli*, two indigenous earthworm species in Los Baños, Laguna, have successfully been cultured and commercialized for their vermicasts as organic fertilizers (Aspe and Obusan 2023). Likewise, *Polypheretima jenniferae* from Palawan Island (Aspe et al. 2021) and the new species reported herein are also being explored for various applications. Mapile et al. (2023; unpublished) analyzed the microbiota in the gut and the vermicasts of *Pheretima losbanosensis*, *Polypheretima jenniferae*, and *Polypheretima andresi* sp. nov., for potential application in agriculture and found out that there is significantly higher diversity of microorganisms in the indigenous species compared to that of the introduced species *Eudrilus eugeniae*. As more and more indigenous species are discovered in the country, their potential applications should be harnessed for the benefit of mankind. Thus, taxonomic and systematic works are essential scientific steps that would avoid taxonomic misidentification of potential natural resources that can be utilized for the benefit of society.

Conclusion

In this work, three new pheretimoid earthworm species, namely *Polypheretima andresi* sp. nov., *Pheretima elnorroae* sp. nov., and *Pheretima naawanensis* sp. nov., were described from Misamis Oriental, Philippines, adding to the rich earthworm diversity of Mindanao Island and of the Philippine archipelago as a whole. In addition, this work presents the first record of *Metaphire bahli* on Mindanao Island. The mitogenomes obtained in this study, which are the first mitogenomic data obtained from the earthworm

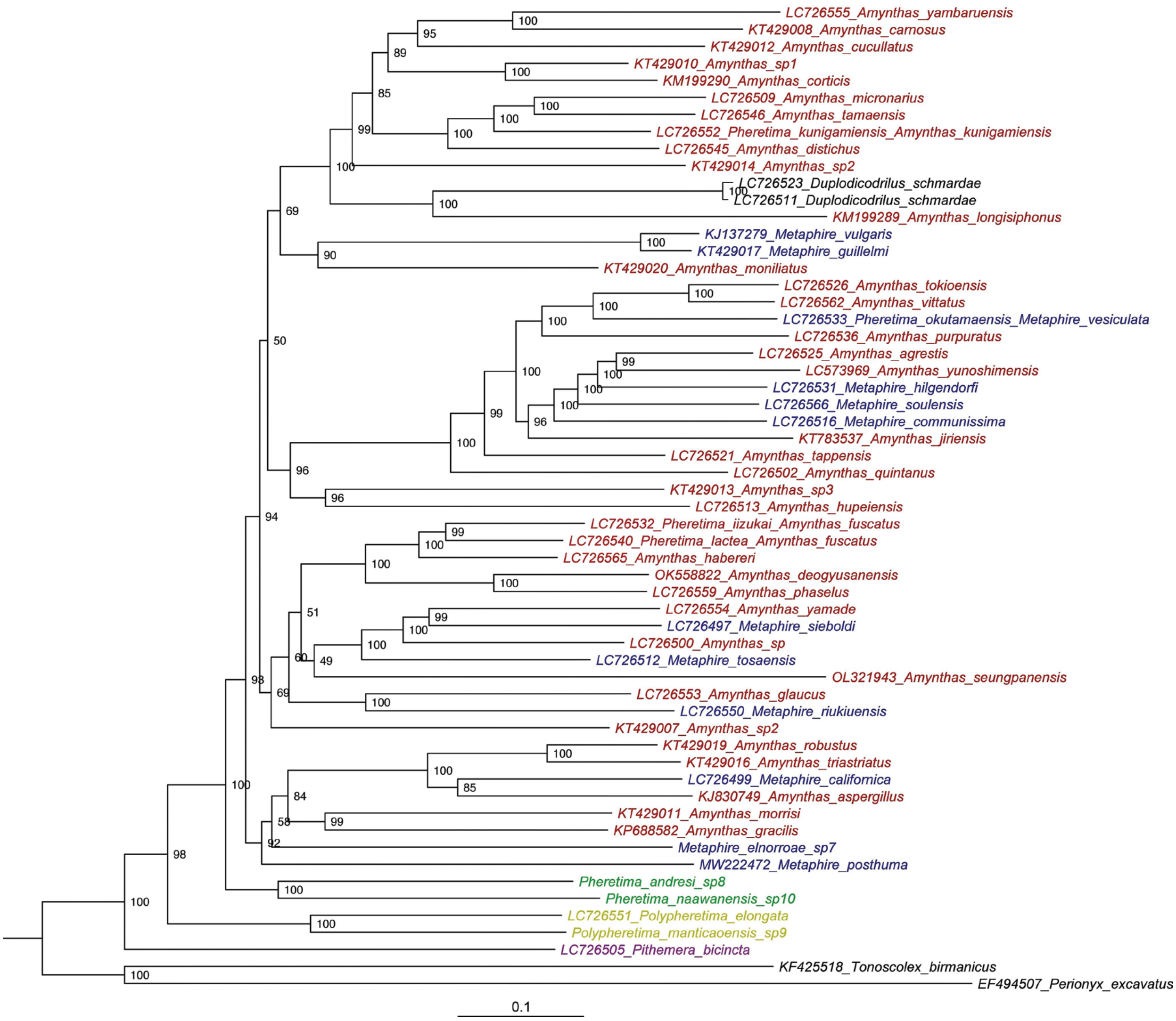

Figure 7. The maximum likelihood phylogenetic tree based on 13 PCGs of mitogenomes available from GenBank using IQTREE. The four new species are highlighted. Yellow refers to *Polypheretima*, green refers to *Pheretima*, blue refers to *Metaphire*, and red refers to *Amynthas*.

species of the Philippines, support the new species status of the three species. Phylogenetic analyses showed non-monophyly in *Metaphire* and *Amynthas*, supporting previous studies using multiple gene markers. On the other hand, further mitogenomic analyses are required to shed light on the taxonomic groupings of *Polypheretima* and *Pheretima* by including more samples belonging to these genera. The species described in this paper are just the initial findings on the diversity of earthworms in the province of Misamis Oriental. More earthworm species await to be discovered, as many of the sites across the province and across Mindanao Island are yet to be explored.

Acknowledgments

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Supplementary material 1

Supplementary data

Authors: Nonillon M. Aspe, Huifeng Zhao, Maria Reynalen F. Mapile, Maria Christine M. Obusan, Yufeng Zhang, Edgar Castañares, Eric John D. Florida, Donghui Wu

Data type: docx

Explanation note: **table S1.** Information of mitogenome of *Metaphire bahli*, total length 15082 bp.; **table S2.** Information of mitogenome of *Pheretima elnorroae* sp. nov., total length 15048 bp.; **table S3.** Information of mitogenome of *Polypheretima andresi* sp. nov., total length 15065 bp.; **table S4.** Information of mitogenome of *Pheretima naawanensis* sp. nov., total length 15099 bp.

Link: https://doi.org/10.3897/zse.101.135702.suppl1