Zootaxa 5255 (1): 101–112
https://www.mapress.com/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5255.1.14
http://zoobank.org/urn:lsid:zoobank.org:pub:3955F173-2E65-46FC-8A40-200536DAABE8

Pheretimoid earthworms (Clitellata: Megascolecidae) cultivated in a vermifacility in Los Baños, Laguna, Philippines, with description of a new species

NONILLON M. ASPE[1*] & MARIE CHRISTINE M. OBUSAN[2]
[1]*College of Marine and Allied Sciences, Mindanao State University at Naawan, Naawan 9023, Misamis Oriental, Philippines.*
[2]*Institute of Biology, College of Science, University of the Philippines, Diliman, Quezon City, Philippines;*
mmobusan@up.edu.ph; https://orcid.org/0000-0002-5453-1881
**corresponding author. nonillon_aspe@yahoo.com; https://orcid.org/0000-0003-0025-6017*

ABSTRACT

We report two pheretimoid earthworm species cultivated in Folia Tropica vermifacility in Los Baños, Laguna, Philippines. One species is *Pheretima losbanosensis* **sp. nov.**, which belongs to *P. dubia* group in Sims & Easton 1972, characterized by having three pairs of spermathecal pores in 6/7–8/9. It has an adult size of 220–228 mm x 8–9 mm; and has 26–36 and 56–66 setae on vii and xx, respectively. The other species is *Metaphire bahli* Gates, 1945, characterized by having three pairs of spermathecal pores in 6/7–8/9 and post-clitellar genital markings at 17/18 and 18/19. The vermicasts of the two species are commercially produced and are harvested as organic fertilizers. We promote the utilization of native species for vermiculture and vermicomposting rather than using the introduced vermicomposting species African nightcrawler *Eudrilus eugeniae*.

Key words: Pheretimoids, new species, vermiculture, Laguna, Philippines

INTRODUCTION

Vermiculture is a technology that cultures earthworms for various purposes, mainly on vermicomposting and the utilization of their vermicasts as organic fertilizers. In bigger vermifacilities, the earthworms are used to expand a vermicomposting operation or are sold to customers who use them for the same or other purposes. Many countries in Europe, the Americas, Australia, and Asia have been practicing vermiculture and vermicomposting (Edwards & Arancon 2006; Edwards & Arancon 2022). The species that is popularly cultured anywhere in the Philippines is *Eudrilus eugeniae*, also known as the African nightcrawler, a species introduced to the Philippine soils from Africa around 1980s (Guerrero 2005; Blakemore 2016a). Their growth and reproductive rates, as well as the efficacy of their vermicasts as alternative to synthetic fertilizers have been well established (Edwards & Arancon 2006; Edwards & Arancon 2022). However, introduced species have the potential to become invasive if not regulated and may have detrimental effects to 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). A mismanaged and abandoned vermifacility in Bukidnon Province that used to cultivate African nightcrawlers caused the species to spread across the area. Consequently, we observed a lot of African nightcrawlers in the open but very little or no native species. A vermifacility in Los Baños called Folia Tropica has been successfully culturing local earthworm species and commercializing their vermicasts as organic fertilizers. They are sustainably distributing their vermi-products in well-known commercial centers in the country for already a decade now. The proprietor, Dr. Raymundo Lucero, observed that the local species grow and reproduce faster and has better vermicast yields than the African nightcrawler, thus, he promotes the utilization of local species rather than the introduced ones. As the species cultivated in Folia Tropica were previously unknown to science, this paper reports the taxonomic identification of the local species cultured in the vermifacility.

The introduction and utilization of the African nightcrawler to the Philippine soils was a result of the lack of knowledge on the indigenous resources in the country. However, as a result of active taxonomic work on earthworm

Accepted by A. Nguyen: 17 Jun. 2022; published: 15 Mar. 2023

species in the Philippines, more scientific information has been made available in recent years, from around 10 species recorded prior to 2004, to more than 200 species (e.g. Aspe & James 2014; 2016; Aspe *et al*. 2021). The Philippine native species are collectively referred as pheretimoids, originally belonging to the largest genus of family Megascolecidae, *Pheretima*, which were reallocated into 12 genera (e.g. Sims & Easton 1972; Easton 1979; Blakemore 2007). This article is part of the series of reports of the earthworm fauna of the Philippines (Aspe & James 2014; 2015; 2016; 2017; Aspe *et al*. 2021; Hong 2018; Hong & James 2004; 2008a; 2008b; 2008c; 2009; 2010; 2011a; 2011b; 2021; James 2004; 2005; 2006; James *et al*. 2004). Molecular phylogenetic analyses (James 2005; Aspe *et al*. 2016; Aspe & James 2018) have also been conducted to elucidate the earthworm species' diversification mechanism and distribution in the archipelago. In the case of Los Baños, Laguna, Hong & James (2008b) previously described three species from Mt. Makiling. Here, we report *Pheretima losbanosensis* **n. sp.** and *Metaphire bahli*, adding to the record of species in Laguna Province. In addition, the study presents the record the first native species in the Philippines being utilized for vermicomposting.

MATERIAL AND METHODS

The Study Area

Los Baños is a city in Laguna Province located 63 km southeast of Metro Manila, Philippines. It lies on the northern slopes of the long dormant volcano Mt. Makiling and is popular among tourists for its hot spring resorts. Los Baños hosts the University of the Philippines Los Baños (UPLB) along with other local and international research centers such as the International Rice Research Institute (IRRI), the ASEAN Center for Biodiversity, the Southeast Asian Ministers of Education Organization-Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEAMEO-SEARCA), the Philippine Rice Research Institute (PhilRice), and the Philippine Carabao Center. In 2000, Los Baños was declared as a Special Science and Nature City of the Philippines through Presidential Proclamation No. 349.

Sampling and Morphological Examination

A Gratuitous Permit was secured from the Biodiversity Management Bureau of the Department of Environment and Natural Resources (BMB-DENR) before the collection of specimens. Sampling was conducted in Los Baños Folia Tropica, Pleasant Village, Los Baños, Laguna on 2 July, 2019. The collected specimens were rinsed and killed with 5% ethanol prior to fixing them in 95% ethanol. All descriptions are based on external examination and on dorsal dissection under a stereomicroscope, following the terminology and conventions of Easton (1979). Descriptions of body color are based on living specimens. Body dimensions refer to fixed material. Measurements are in millimetres. The generic diagnoses and assignment to species groups follow Sims & Easton (1972). For convenience in species comparison, the new species were initially compared with the other members of the same species group by their sizes and the distance between spermathecal pores and between the male pores. Types are deposited in the Annelids Section of the National Museum of the Philippines (NMA) and the University of the Philippines-Los Baños Museum of Natural History (UPLBMNHLS).

DNA Extraction, Sequencing, and Analysis

Total genomic DNA was extracted from muscle tissues of the earthworm samples using the Wizard® Genomic DNA Purification Kit (Promega, USA). DNA samples were submitted to the Philippine Genome Center (PGC), University of the Philippines, Diliman for cytochrome c oxidase subunit I (COI) gene amplification and capillary sequencing. The PCR mixture contained genomic DNA, COI-F_N (TTTGAGCCGGAATAATTGG) and COI-R_N COI (TCGAAGAATGATGTATTTAGGTTTCG) primers (Aspe *et al*. 2016), Taq buffer, DNA polymerase, and dNTP mix. The cycling profile was as follows: denaturation for 1 min at 95 °C, annealing for 45 s at 52 °C, and extension for 1 min at 70 °C for 30 cycles with initial denaturation for 5 min at 95 °C and final extension for 10 min at 72 °C. PCR amplification of RBCL gene with ~1500bp size was confirmed by electrophoresis in 1.2% agarose gel. Capillary sequencing reactions were performed with ABI BigDye® Terminator v3.1 Cycle Sequencing Kit. The cycling profile was as follows: denaturation for 10 s at 96 °C, annealing for 5 s at 50 °C, and extension for 4 min at 62 °C for 25 cycles with initial denaturation for 1 min at 96 °C.

COI sequences of *Pheretima* species from Aspe & James (2018) and *M. bahli* from Blakemore (2016b) and Prasankok *et al.* (2013) were retrieved from NCBI for phylogenetic analysis with the sequence of the species from this study. The sequences were aligned using MUSCLE (Edgar 2004). The genetic distance was calculated using the Kimura 2 parameter (K2P) model performed in MEGA 7.0 with bootstrap of 1,000 (Kumar *et al*. 2016). Phylogenetic reconstruction was performed with Maximum Likelihood (ML) method using the model GTR + G + I. The tree was rooted using *Pontodrilus litoralis* (Megascolecidae) as outgroup.

RESULTS

Systematics

Family Megascolecidae Rosa, 1891

Genus *Pheretima* (*Pheretima*) Kinberg, 1867

Type species *Pheretima montana* Kinberg, 1867

Generic diagnosis. Body circular in cross section, with numerous setae regularly arranged equatorially around each segment; setae absent on first and last segments. Male pores paired within copulatory bursae opening on segment xviii; one or more pairs of spermathecal pores in intersegmental furrows between 4/5 and 8/9. Clitellum annular, covering three segments (xiv–xvi). Single midventral female pore on xiv. Genital markings usually absent. Internally, oesophageal gizzard usually originating in viii; a pair of caeca originating in xxvii, extending forward. Ovaries and funnels free in xiii. Male sexual system holandric, with paired testes and funnels enclosed in sacs in x and xi, and seminal vesicles in xi and xii. Spermathecae one pair, multiple pairs, sometimes single and located midventrally, or sometimes lacking. Nephridia present on spermathecal duct(s). One pair of prostate glands, racemose. Copulatory bursae present; secretory diverticula lacking on coelomic surface of copulatory bursae.

***Pheretima losbanosensis* n. sp.**
(Figure 1)

Material examined. Holotype: adult (PNM 4649), Pleasant Village, Los Banos, Laguna (14°09'29.9"N, 121°15'17.4"E), 24 m asl., Luzon Island, Philippines, coll. N. Aspe, M.R.F. Mapile 2 July, 2019. Paratypes: 3 adults (PNM 4650); 2 adults (UPLBMNHLS (EW) 158), same collection data as for holotype.

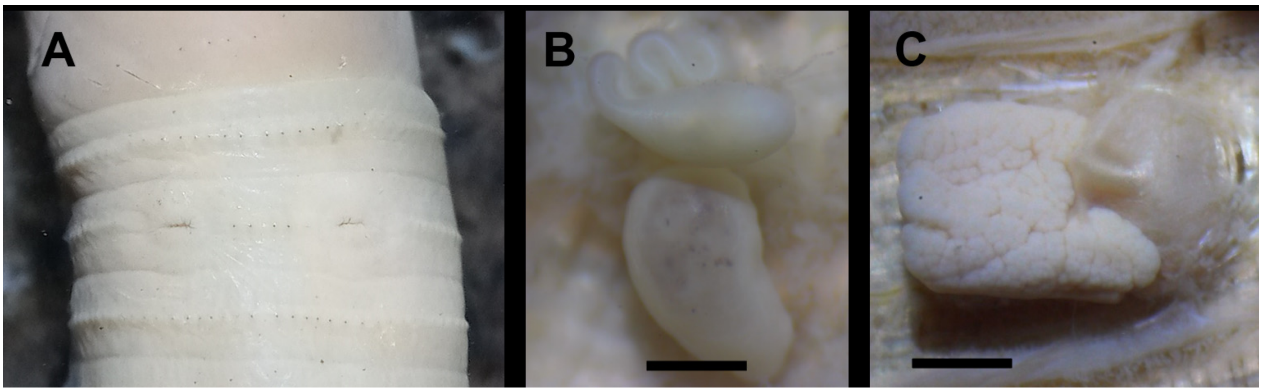

FIGURE 1. *Pheretima losbanosensis* **n. sp.** (A) Male pores area. (B) Spermatheca. (C) Prostate gland. Scale bar: 1mm.

Etymology. The species is named after Los Baños City in Laguna Province, the type locality.

Diagnosis. Large brown worms with adult size of 220–228 mm x 8–9 mm; equators pigmented; three pairs of spermathecal pores at 6/7–8/9; distance between spermathecal pores and between male pores 0.18 and 0.15 circumference apart ventrally, respectively; setae on vii and xx 26–36 and 56–66, respectively. Spermathecae paired in vii, viii, and ix, with nephridia on ducts; each spermatheca small with ovate ampulla, stout muscular duct, stalked diverticulum attached to duct near ampulla, terminating in ovate receptacles, stalks long and thin, some with convolutions; prostates small in xvii to xix ; penis present.

Description. In live animals, dorsal brown, ventral pale, equators pigmented; Length 220–228 mm (n=6 adults); diameter 8–9 mm at x, 8 mm at xx; body cylindrical in cross-section, tail blunt; 117–128 segments. First dorsal pore at 12/13; spermathecal pores three pairs at 6/7–8/9, inconspicuous, distance between spermathecal pores 5 mm (0.18–0.20 circumference apart ventrally). Female pore single in xiv. Openings of copulatory bursae paired in xviii, distance between openings 3.5–4 mm (0.15–0.16 circumference apart ventrally), 4–6 setae between openings. Clitellum annular, from xiv to xvi. Setae evenly distributed around segmental equators; 26–36 setae on vii, 56–66 setae on xx, dorsal setal gaps present, ventral setal gaps lacking. Genital marking lacking.

Septa 5/6–8/9 and 10/11–13/14 thin, 9/10 lacking. 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 originating in xxvii, extending forward to xxiii; Hearts in x to xiii, esophageal; commissural vessels in vi, vii, and ix, lateral; those in viii extend to gizzard; supra-esophageal vessel extends from x to xiii; extra-esophageal vessel joins ventral esophageal wall in xi, receives efferent parieto-esophageal vessel in xiii.

Ovaries and funnels free in xiii. Spermathecae paired in vii, viii, and ix, with nephridia on ducts; each spermatheca small with ovate ampulla, stout muscular duct, stalked diverticulum attached to duct near ampulla, terminating in ovate receptacles, stalks long and thin, some spermathecae with convoluted stalks. 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; vas deferens slender, free from body wall to ental end of prostatic ducts; prostates small in xvii to xix, each a single, dense, racemose mass; short muscular duct entering center of copulatory bursa; paired small copulatory bursae xviii; secretory diverticula lacking; tapering penis present.

Remarks. *Pheretima losbanosensis* **n. sp.** belongs to *P. dubia* group in Sims & Easton (1972), characterized by having three pairs of spermathecal pores in 6/7–8/9. Including the new species, *P. dubia* group is now composed of 12 species: *P. philippina* Rosa, 1981 from Cebu and Mindoro; *P. callosa* Gates, 1937 from Benguet Province; *P. balbalanensis* Hong & James, 2010; *P. banaoi* Hong & James, 2010 from Kalinga Province; *P. lamaganensis* Hong & James, 2011a; *P. julkai* Hong & James, 2011a from Mountain Province; *P. globosa* Hong & James, 2011b in Kalbaryo, Pagudpud, Ilocos Norte; *P. vungtauensis* Nguyen *et al*. 2018 from Vietnam; and *P. dubia* Horst, 1893; *P. korinchiana* Cognetti, 1922; and *P. poiana* Michaelsen, 1913 from Indonesia (Table 1). Among the members of *P. dubia* group, the new species is most similar to *P. philippina* in length (180–240 mm). However, the latter has slate gray pigmentation (vs. brown in *P. losbanosensis* **n. sp.**), has more setae in vii and xx (44 and >70, respectively vs. 26–36 and 55–60 in *P. losbanosensis* **n. sp.**), has its first dorsal pore in 11/12 (vs. 12/13 in *P. losbanosensis* **n. sp.**), and has no dorsal setal gaps. The caeca in *P. philippina* extends from xxvi to xxiii and its prostate gland is confined only in xviii while the caeca in *P. losbanosensis* **n. sp.** extends from xxvii to xxiii and its prostate gland extends from xvii to xix. Also, the spermathecal ampulla of *P. philippina* is pear-shaped, the stalked diverticulum terminating in small, round receptacle, and the stalk is shorter than the ampulla. On the other hand, the spermathecal ampulla and the receptacle of the new species are ovate, and the stalk is longer than the ampulla. *Pheretima poiana* (290 mm) and *P. callosa* (330 mm) are larger than the new species (220–228 mm) while *P. korinchiana* (83–180 mm), *P. dubia* (80–140 mm), *P. vungtauensis* (132–169), *P. julkai* (49–69 mm), *P. banaoi* (53–60 mm), *P. lamaganensis* (46–55 mm), *P. balbalanensis* (42–50 mm) and *P. globosa* (35–42 mm) are significantly smaller. *Pheretima losbanosensis* **n. sp.** has ovate spermathecal diverticulum and short prostatic duct while *P. poiana* has sausage-shaped spermathecal diverticulum and the prostatic duct is in an S-curve. *Pheretima poiana* also has distinctively long tubular dorsal lobes of the seminal vesicles, which are absent in *P. losbanosensis* **n. sp.** The distances between spermathecal pores and between male pores are wider in *P. poiana* (0.4 and 0.29, respectively), *P. vungtauensis* (0.4 and 0.35, respectively), *P. korinchiana* (0.19–0.22 and 0.19–0.23, respectively), *P. dubia* (0.38 and 0.17, respectively), *P. lamaganensis* (0.27–0.28 and 0.21–0.22, respectively), *P. julkai* (0.20–0.23 and 0.18–0.20, respectively), *P. balbalanensis* (0.28–0.32 and 0.22, respectively), *P. banaoi* (0.19–0.22 distance between male pores only), and *P. globosa* (0.32 and 0.22, respectively) compared with the new species (0.18 and 0.15, respectively). There are more setae on vi and xx in *P. callosa* (71 and 114, respectively) and more setae on vii in *P. lamaganensis* (43–44) while there are fewer setae on xx in *P. balbalanensis* (41–42) and in *P. banaoi* (46–47) compared with the new species (26–36 and 55–66, respectively). Also, there are more setae between male pores in *P. korinchiara* (12), *P. vungtauensis* (11–17),

TABLE. 1. Comparison among members of the *P. dubia* group of Sims & Easton (1972). The species are listed in order of size from largest to smallest.

Characters	*callosa* Gates 1937	*poiana* Michaelsen 1913	*philippina* Rosa 1981	*losbanosensis* **n. sp.**	*korinchiana* Cognetti 1922	*vungtauensis* Nguyen *et al*. 2018	*dubia* Horst 1893	*julkai* Hong & James 2011a	*banaoi* Hong & James 2010	*lamaganensis* Hong & James 2011a	*balbalanensis* Hong & James 2010	*globosa* Hong & James 2011b
Length (mm)	330	290	180–240	220–228	83–180	132–169	80–140	49–69	53–60	46–55	42–50	35–42
Width (mm)	16	?	7	8–9	?	4.1–6.1	?	3.5–4	3–3.6	2.5–3.2	2.2–2.7	2.3–2.7
Pigmentation	?	gray	gray	brown	brown	grayish brown	brown	light purple-brown	yellowish-brown	brown	reddish-brown	light brown
No. of segments	?	>110	115–125	117–128	?	91–125	?	90–95	89–97	84–93	72–85	71–80
First dorsal pore	?	?	11/12	12/13	?	11/12 or 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	29–31, 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	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	0.2–0.23	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	0.18–0.2	0.22	0.21–0.22	0.22	0.22
Caeca	?	?	xxvi–xxiii	xxvii–xxiii	?	xxvii–xxiv or xxiii	?	xxvii–xxv	xxvii–xxiv	xxvii–xxv	xxvii–xxiv	xxvii–xxvi
Prostate glands	?	?	xviii	xvii–xix	?	xvi–xviii	?	xxvii–xxviii	xvi–xviii	xvii–xviii	xvii–xviii	xvii–xviii

P. dubia (10), *P. lamaganensis* (7–10), *P. julkai* (6–10), *P. balbalanensis* (6–8), *P. banaoi* (6–12) and *P. globosa* (9). In addition, the new species differs from the other species in the relative length of caeca (xxvii–xxiii in *P. losbanosensis* n. sp. vs. xxvii–xxv in *P. julkai* and *P. lamagensis*, xxvii–xxiv in *P. banaoi* and *P. balbalanensis*, and xxvii–xxvi in *P. globosa*) and size of prostate glands (xvii–xix in *P. losbanosensis* **n. sp.** vs. xvi–xviii in *P. vungtauensis* and *P. banaoi* and xxvii-xxviii in *P. julkai*, *P. banaoi*, *P. lamagensis*, *P. balbalanensis* and *P. globosa*).

Genus *Metaphire* Sims & Easton, 1972

Type species *Rhodopis javanica* Kinberg, 1867

Generic diagnosis. Body cylindrical. Setae numerous, regularly arranged around each segment. Clitellum annular, xiv–xvi. Male pores paired within copulatory pouches on xviii, rarely xix or xx. Female pores single, rarely paired. Spermathecal pores usually large transverse slits, seldom small; paired, occasionally single or multiple, between 4/5 and 9/10. Intestinal caeca present, originating in or near xxvii. Testes holandric, rarely proandric or metandric. Prostatic glands racemose. Copulatory pouches present, often with stalked glands; secretory diverticula absent. Ovaries paired, xiii. Spermathecae paired, rarely single or numerous. Nephridia on spermathecal ducts lacking.

***Metaphire bahli* Gates, 1945**
(Figure 2)

Material examined. 3 adults (PNM 4651); 2 adults (UPLBMNHLS (EW) 159), Pleasant Village, Los Baños, Laguna (14°09'29.9"N, 121°15'17.4"E), 24 m asl., Luzon Island, Philippines, coll. N. Aspe, M.R.F. Mapile 2 July, 2019.

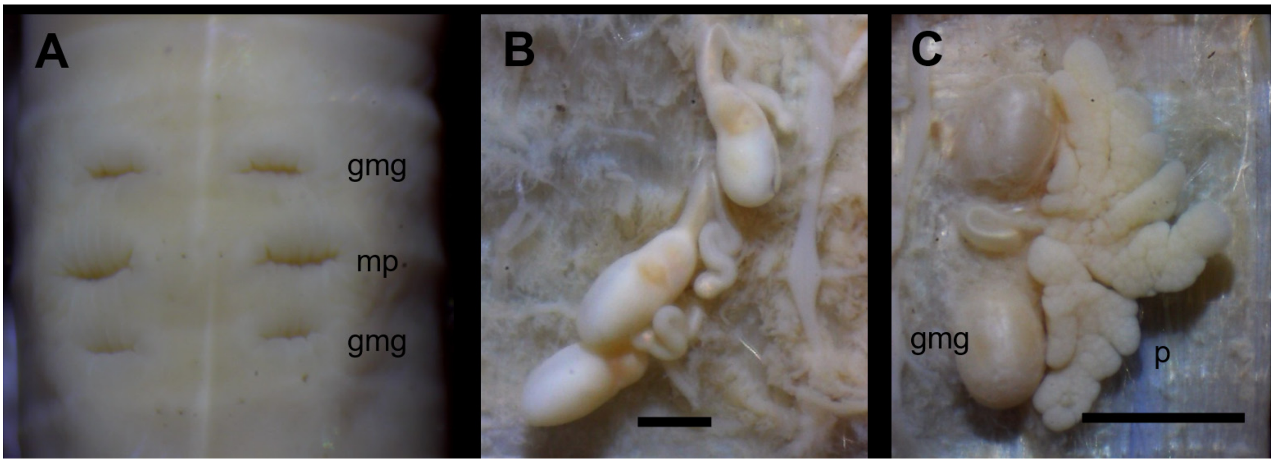

FIGURE 2. *Metaphire bahli*. (A) Male pores (mp) area showing the genital markings (gmg). (B) Spermathecae. (C) Prostate gland (p) showing the genital marking glands (gmg). Scale bar: 1mm.

Diagnosis. Brown worms with adult size of 58–145 mm x 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 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.

Remarks. *Metaphire bahli* belongs to *M. peguana* group in Sims & Easton (1972), characterized by having three pairs of spermathecal pores in 6/7–8/9 and post-clitellar genital markings at 17/18 and 18/19. The species group includes: *M. peguana* Rosa, 1890; *M. saigonensis* Omodeo, 1957; *M. kiengiangensis* Nguyen et al. 2015; *M. dorsomultitheca* Nguyen et al. 2015; *M. nhuongi* Nguyen, 2016; *M. doiphamon* Bantaowong & Panha, 2016 in Bantaowong *et al*. 2016; *M. haui* Nguyen *et al*. 2020; and *M. narraensis* Aspe *et al*. 2021. First recorded in Sri Lanka, *M. bahli* has also been recorded in 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 2016b). In the Philippines, *M. bahli* had been recorded in Batangas, Manila, Angeles City, Pangasinan, Panay, Negros and Sibuyan Island (Thai & Samphon 1989; Blakemore 2016b). The morphological features of *M. bahli* in Los Baños, Vietnam and India in general coincide (Table 2). However, there are discordances in some characters. For example, the distance between spermathecal pores and between male pores in *M. bahli* in Los Baños is 0.16 and 0.16 circumference apart ventrally, respectively. On the other hand, Nguyen *et al*. (2017) reported that the distance between spermathecal pores and between male pores in *M. bahli* in Vietnam are 0.25 and 0.20 circumference apart ventrally, respectively. Narayanan *et al*. (2019) did not provide information on the distance between spermathecal pores and between male pores in *M. bahli* in India. The pre- and postclitellar setae in *M. bahli* in Los Baños and in Vietnam more or less belong to the same range (39–49 in the preclitellar segments and 40–72 in the postclitellar segments). However, this is in contrast to Narayanan *et al*.'s (2019) description of *M. bahli* in India wherein the number of setae in the preclitellar segments reaches 73+ while it reaches 91+ setae in the postclitellar segments.

TABLE 2. Comparison of some morphological characters among *Metaphire bahli* collected from India, Vietnam and the Philippines.

Characters	India Narayanan *et al*. 2019	Vietnam Nguyen *et al.* 2017	Philippines present study
Length	76–121	58–100	118–145
Diameter	4–5	3.3–4.4	5.5
Segments	79–119	53–114	90–116
Pre-clitellar setae	73+	39–47 (viii)	43–49 (vii)
Post-clitellar setae	91+	40–72 (xxx)	58–63 (xx)
Dist. bet. sper. pores	?	0.25	0.16
Dist. bet. male pores	?	0.20	0.16
Setae bet. male pores	?	4–5	3–8
Caeca	xxvii–xxiv	?	xxiv–xxiv
Prostate glands	xvii–xx	?	xvi–xx

Genetic distance and phylogenetic analysis

A COI sequence was successfully obtained from *Pheretima losbanosensis* **n. sp**. However, despite several attempts, including the designing of new primers, no COI sequencewas obtained from *M. bahli* collected in Folia Tropica. The K2P distance between *Pheretima losbanosensis* **n. sp.** and the other pheretimoid species based on COI sequences varies between 15% to 20.9% (Table 3). This range is in concordance with the results of Nguyen *et al*. (2018; 2020) on the analysis on the interspecific genetic distance of other pheretimoid species. In particular, the K2P distance of *Pheretima losbanosensis* **n. sp.** with the other species of the *Pheretima dubia* group ranges between 15% and 18.4%. The phylogenetic analysis based on COI sequences generated a tree with weakly supported branches and therefore does not give a conclusive result (Figure 3). *Pheretima losbanosensis* **n. sp.** falls in a weakly supported clade with other species of the *Pheretima dubia* group (with 3 pairs of spermathecae). The phylogenetic relationship among members of the *P. dubia* group and the *P. darnleiensis* group (with 4 pairs of spermathecae) in Aspe & James (2018) has also not been resolved even with the use of multiple gene markers that reached a length of more than 2,600 bp. More robust data such as the use of complete mitochondrial genome may be needed to resolve the phylogenetic relationship among various earthworm groups (e.g. Hong *et al*. 2017; Shekhovtsov *et al*. 2020; Liu *et al*. 2021).

TABLE 3. K2P distance of the COI gene calculated by MEGA 7.0.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Pontodrilus litoralis LC125897																	
Metaphire bahli KC404844	0.210																
Metaphire bahli KT626581	0.213	0.008															
Metaphire bahli KT626589	0.213	0.004	0.012														
Metaphire bahli KT626598	0.207	0.012	0.004	0.017													
Pheretima vergrandis LC127233	0.194	0.220	0.225	0.223	0.225												
Pheretima malindangensis LC127234	0.208	0.238	0.241	0.238	0.235	0.177											
Pheretima boniaoi LC127235	0.204	0.206	0.212	0.206	0.209	0.197	0.169										
Pheretima camiguinensis LC127236	0.220	0.206	0.208	0.208	0.211	0.185	0.180	0.172									
Pheretima sp. NVizcaya3 LC268858	0.249	0.203	0.203	0.206	0.201	0.220	0.213	0.194	0.170								
Pheretima sp. Poro LC268868	0.188	0.217	0.217	0.217	0.214	0.172	0.160	0.181	0.142	0.179							
Pheretima sp. Negros2 LC268872	0.209	0.192	0.200	0.197	0.197	0.181	0.172	0.170	0.101	0.193	0.144						
Pheretima sp. Negros4 LC268873	0.222	0.228	0.228	0.228	0.228	0.181	0.195	0.209	0.160	0.199	0.153	0.200					
Pheretima sp. Panay2 LC268878	0.228	0.197	0.189	0.200	0.187	0.188	0.187	0.216	0.167	0.157	0.144	0.177	0.188				
Pheretima sp. Panay3 LC268879	0.238	0.214	0.217	0.217	0.217	0.198	0.206	0.214	0.192	0.188	0.180	0.173	0.199	0.150			
Pheretima sp. Panay4 LC268882	0.203	0.206	0.203	0.212	0.201	0.206	0.247	0.228	0.187	0.174	0.171	0.192	0.213	0.143	0.164		
Pheretima sp. Siquijor LC268886	0.215	0.187	0.189	0.189	0.187	0.214	0.179	0.185	0.128	0.185	0.157	0.130	0.172	0.171	0.180	0.194	
Pheretima losbanosensis **n. sp.** LC706862	0.209	0.190	0.195	0.190	0.195	0.205	0.179	0.190	0.183	0.189	0.158	0.167	0.185	0.155	0.150	0.184	0.159

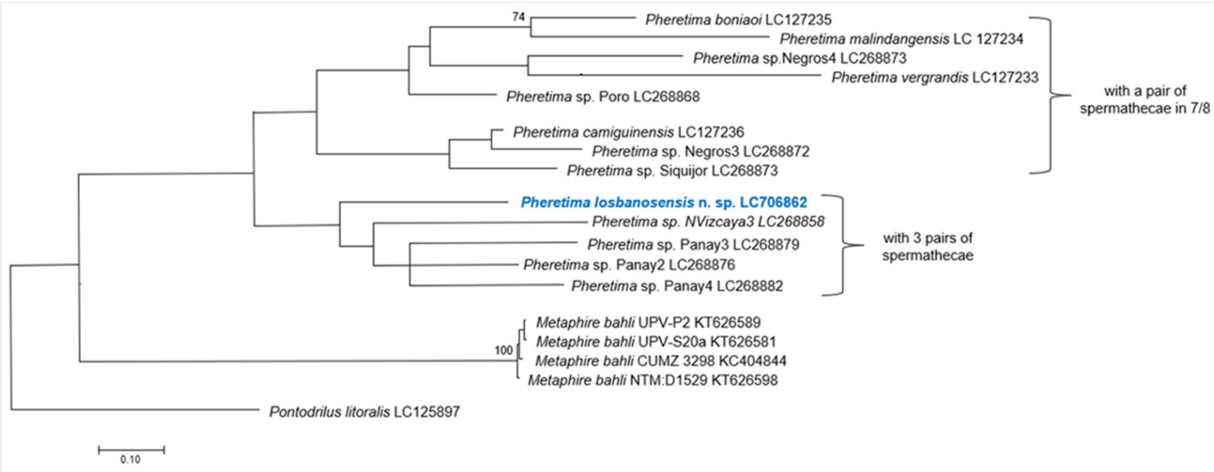

FIGURE 3. Maximum Likelihood tree using COI constructed following the GTR + G + I model showing the relationship of *P. losbanosensis* n. sp. with other *Pheretima* species and *Metaphire bahli*. The accession nos. are reflected with the taxa. Bootstrap values lower than 70% indicate weak support and are not reflected.

DISCUSSION

For already a decade of operation of Folia Tropica vermifacility, this is the first time that the earthworms they are culturing have been taxonomically identified. Although Dr. Lucero, the proprietor of Folia Tropica, observed that *P. losbanosensis* **n. sp.** and *M. bahli* grow and reproduce faster and have better vermicast yields than that of the African nightcrawler, biological tests of the earthworms and physico-chemical analyses of the vermicasts need to be conducted to quantify and verify these observations. We have an ongoing study investigating the composition and diversity of gut and cast microbiota of *P. losbanosensis* **n. sp.** and *M. bahli*. Based on our preliminary data, their mineralization activities may be associated with their microbial interactions. In pot experiments conducted at the International Rice Research Institute (unpublished), the presence of *M. bahli* has been observed to increase N content and growth of upland rice and maize in sandy (unpublished).

Other pheretimoid species have been studied for their benefits in agriculture. Waqar *et al*. (2019) reported the contribution of *Amynthas gracilis* (Kinberg 1867) in decomposition and soil nitrogen mineralization. Fresh casts of *Pheretima* sp. and *M. peguana* were reported to have higher pH, porosity, moisture, organic matter, and exchangeable cations including calcium, magnesium, potassium, and sodium (Boonchamni *et al*. 2019). Vermicomposts processed by some *Metaphire* species have also offered beneficial effects to soil microbial properties. For example, soils amended with vermicomposts treated with *M. posthuma* demonstrated higher enzymatic activities, bacterial and catabolic diversity, and nitrogen content (Doan *et al*. 2013). Soil aggregate stability and plant growth were also positively affected by earthworm activities such as burrowing, mixing, and casting as in the case of *M. guillelmi* (Yu *et al*. 2008). In addition, *M. posthuma* was also reported to bioprocess toxic compounds (Das *et al*. 2015) and its extracts and casts exhibited antimicrobial activity (Kumar *et al*. 2012; Bansal *et al*. 2015). Casts of *M. tschiliensis tschiliensis* were also characterized to have higher nutrient content associated with higher gut microbial composition (Teng *et al*. 2012).

Both *P. losbanosensis* **n. sp.** and *M. bahli* are characterized to have epigeic living habit, being found to be abundant in the large mounds of composts feeding on decaying organic matters. Epigeic earthworms can promote the decomposition of organic matter and improve the availability of nutrients in the soil (Ravindran *et al*. 2014). Epigeic earthworms can also effectively promote the microbial secretion of enzymes into the soil, thereby improving enzyme activity at the soil surface (Caravaca & Roldán 2003). The high adaptability of *M. bahli* as reflected by its wide geographic distribution is a factor why it is suitable for cultivation. As for *P. losbanosensis* **n. sp.**, more surveys need to be conducted to verify its geographic distribution. In vermiculture and vermicomposting, aside from the vermicasts products being sold, earthworms are also being sold and brought to other areas. Although we promote the cultivation of native species rather than using exotic species such as the African nighcrawler, we discourage the introduction of local species by bringing to other areas.

ACKNOWLEDGEMENTS

We express our gratitude to Dr. Raymundo Lucero of Folia Tropica for the kind accommodation and assistance during sampling, to the local government of Los Baños for granting Prior Informed Consent to collect specimens, and to the Biodiversity Management Bureau of the Department of Environment and Natural Resources (BMB-DENR) for granting Gratuitous Permit (GP No. 268). To Mr. Mark Fellizar of UPLB for his kind assistance in the field; to Mr. Aroin B. Verdera, Ms. Jamaica Ann A. Caras and Ms. Maria Reynalen F. Mapile for the technical assistance. We also thank Dr. Jessica Simbahan of UP Diliman for the support. We express our thanks to our respective universities, the University of the Philippines Diliman and Mindanao State University at Naawan for their support. N. Aspe is grateful to Nagao Natural Environment Foundation and the MSU-Naawan Foundation for Science and Technology Development, Inc. for partly funding this work.

REFERENCES

Aspe, N.M. & James, S.W. (2014) New species of *Pheretima* (Oligochaeta: Megascolecidae) from the Mt. Malindang range, Mindanao Island, Philippines. *Zootaxa*, 3881 (5), 401–439.
https://doi.org/10.11646/zootaxa.3881.5.1

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

Aspe, N.M. & James, S.W. (2016) New species of *Pheretima, Amynthas, Polypheretima*, and *Pithemera* (Clitellata: Megascolecidae) from Mindanao and associated islands, Philippines. *Zoological Studies*, 55 (8), 1–33.

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

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

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

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

Bansal, N., Gupta, R.K., Singh, D. & Shashank, S. (2015) Comparative study of antibacterial activity of two different earthworm species, *Perionyx excavatus* and *Pheretima posthuma* against pathogenic bacteria. *Journal of Applied and Natural Science*, 7 (2), 666–671.

Bantaowong, U., Chanabun, R., James, S.W. & Panha, S. (2016) Seven new species of the earthworm genus *Metaphire* Sims & Easton, 1972 from Thailand (Clitellata: Megascolecidae). *Zootaxa*, 4117 (1), 63–84.
https://doi.org/10.11646/zootaxa.4117.1.3

Blakemore, R.J. (2007) Updated checklist of pheretimoid (Oligochaeta: Megascolecidae: Pheretima auct.) taxa, 109 pp. http://www.annelida.net/earthworm/Pheretimoids.pdf

Blakemore, R.J. (2016a) Eco-taxonomic profile of an iconic vermicomposter– the 'African nightcrawler' earthworm, *Eudrilus eugeniae* (Kinberg, 1867). *African Invertebrates*, 56 (3), 527–548.

Blakemore, R.J. (2016b) Darwin's earthworms (Annelida, Oligochaeta, Megadrilacea) with review of cosmopolitan *Metaphire peguana*–species group from Philippines. *Opuscula Zoologica (Budapest)*, 47 (1), 09–30.

Bohlen, P.J., Scheu, S., Hale, C.S., McLean, M.A., Migge, S., Groffman, P.M. & Parkinson, D. (2004) Non-native invasive earthworms as agents of change in northern temperate forests. *Fronters in Ecology and Environment*, 2, 427–435.

Boonchamni, C., Iwai, C.B. & Ta-oun, M. (2019) Physical-chemical properties of earthworm casts in different earthworm species. *International Journal of Environmental and Rural Development*, 10 (1), 1–5.

Caravaca, F., Roldán, A. (2003) Effect of *Eisenia foetida* earthworms on mineralization kinetics, microbial biomass, enzyme activities, respiration and labile c fractions of three soils treated with a composted organic residue. *Biology and Fertility of Soils,* 38, 45–51.
https://doi.org/10.1007/s00374-003-0612-7

Cognetti, D.M.L. (1922) Descrizione di tre nuovi megascolecini. *Bollettino dei Musei di Zoologia et Anatomia comparata della Reale Universita di Torino*, 37, 1–6.

Das, S., Bora, J., Goswami, L., Bhattacharyya, P., Raul, P., Kumar, M. & Bhattacharya, S.S. (2015) Vermiremediation of water treatment plant sludge employing *Metaphire posthuma*: a soil quality and metal solubility prediction approach. *Ecological*

Engineering, 81, 200–206.
Doan, T.T., Jusselme, D.M., Lata, J.C., Van Nguyen, B. & Jouquet, P. (2013) The earthworm species *Metaphire posthuma* modulates the effect of organic amendments (compost vs. vermicompost from buffalo manure) on soil microbial properties. A laboratory experiment. *European Journal of Soil Biology*, 59, 15–21.
Easton, E.G. (1979) A revision of the 'acaecate' earthworms of the *Pheretima* group (Megascolecidae: Oligochaeta): *Archipheretima, Metapheretima, Planapheretima, Pleionogaster* and *Polypheretima*. *Bulletin of the British Museum (Natural History)*, 35, 1–126.
Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32, 1792e1797.
Edwards, C.A. & Arancon, N.Q. (2006) The science of vermiculture: The use of earthworms in organic waste management. In: Guerrero, R.D III and del Castillo, M.R.G. (eds.) Vermiculture for Developing Countries. Proceedings of the International Symposium Workshop on Vermi Technologies for Developing Countries (ISWVT 2005). Philippine Fisheries Association, Inc. Philippines.
Edwards, C.A. & Arancon, N.Q. (2022) The use of earthworms in organic waste management and vermiculture. In: Biology and Ecology of Earthworms. Springer, New York, NY.
https://doi.org/10.1007/978-0-387-74943-3_14.
Gates, G.E. (1937) Notes on some species of *Drawida* and *Pheretima* with descriptions of three new species of *Pheretima*. *Bulletin of the Museum of Comparative Zoology at Harvard College*, 80, 305–335.
Gates, G.E. (1945) On some earthworms from Ceylon II. *Spolia Zeylanica*, 24, 69–90.
Guerrero, R.D.III. (2005) Vermicomposting gets high marks in the tropics (Philippines). *BioCycle*, 46 (8), 60. Available from: https://www.biocycle.net/vermicomposting-gets-high-marks-in-the-tropics-philippines/ (Accessed 12 Mar. 2023)
Holdsworth, A.R., Frelich, L.E. & Reich, P.B. (2007) Effects of earthworm invasion on plant species richness in northern hardwood forests. *Conservation Biology*, 21, 997–1008.
Hong, Y. (2018) Two new earthworm species of genus *Pithemera* Sims and Easton, 1972 (Clitellata: Megascolecidae) from Mt. Tapulao, Luzon Island, Philippines. *Journal of Asia-Pacific Biodiversity*.
https://doi.org/10.1016/j.japb.2018.02.002
Hong, Y. & James, S.W. (2004) New species of *Amynthas* Kinberg, 1867 from the Philippines (Oligochaeta: Megascolecidae). *Revue Suisse de Zoologie*, 111, 729–741.
Hong, Y. & James, S.W. (2008a) Nine new species of earthworms (Oligochaeta: Megascolecidae) of the Banaue rice terraces, Philippines. *Revue Suisse de Zoologie*, 115, 341–354.
Hong, Y. & James, S.W. (2008b) Three new earthworms of the genus *Pheretima* (Oligochaeta: Megascolecidae) from Mt. Makiling, Luzon Island, Philippines. *Zootaxa*, 1695, 45–52.
https://doi.org/10.11646/zootaxa.1695.1.2
Hong, Y. & James, S.W. (2008c) Two new earthworms of the genus *Pheretima* (Oligochaeta: Megascolecidae) from Mt. Isarog, Luzon Island, Philippines. *Journal of Natural History*, 42, 1565–1571.
https://doi.org/10.1080/00222930802000398.
Hong, Y. & James, S.W. (2009) New earthworms of the *Pheretima urceolata* species group (Oligochaeta: Megascolecidae) from southern Luzon, Philippines. *Zootaxa*, 2059, 33–45.
Hong, Y. & James, S.W. (2010) Six new earthworms of the genus *Pheretima* (Oligochaeta: Megascolecidae) from Balbalan-Balbalasang, Kalinga Province, the Philippines. *Zoological Studies*, 49, 523–533.
Hong, Y. & James, S.W. (2011a) New earthworm species of the genus *Pheretima* (Clitellata: Megascolecidae) from Mountain Province, Philippines. *Journal of Natural History*, 45, 1769–1788.
https://doi.org/10.1080/00222933.2011.560726
Hong, Y. & James, S.W. (2011b) New species of *Pheretima, Pithemera*, and *Polypheretima* (Clitellata: Megascolecidae) from Kalbaryo, Luzon Island, Philippines. *Raffles Bulletin of Zoology*, 59, 19–28.
Hong, Y. & James, S.W. (2021) Three new earthworm species of *Pheretima* Kinberg, 1867 (Clitellata: Megascolecidae) from Mt. Tapulao, Luzon Island, Philippines. *Journal of Natural History*, 55 (19-20), 1213–1225.
https://doi.org/10.1080/00222933.2021.1940336.
Hong, Y., Kim, M.J., Wang, A.R. & Kim, I. (2017) Complete mitochondrial genome of the earthworm, *Amynthas jiriensis* (Clitellata: Megascolecidae). *Mitochondrial DNA Part A: DNA Mapping, Sequencing, and Analysis*, 28 (2),163–164.
Horst, R. (1893) Descriptions of earthworms. No. 7. On Malayan earthworms. *Nores Leyden Museum*, 15, 316–329.
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 (2), 289–313.
James, S.W. (2005) New genera and new species of earthworms (Clitellata: Megascolecidae) from southern Luzon, Philippines. *Systematics and Biodiversity*, 2 (3), 271–279.
James, S.W. (2006) The earthworm genus *Pleionogaster* in southern Luzon, Philippines. *Organisms, Diversity and Evolution*, 6, 1–20. Electronic Supplement 8.
James, S.W., Hong, Y. & Kim, T.H. (2004) New earthworms of *Pheretima* and *Pithemera* (Oligochaeta: Megascolecidae) from Mt. Arayat, Luzon Island, Philippines. *Revue Suisse de Zoologie*, 111 (1), 3–10.
James, S.W. (2005) Preliminary molecular phylogeny in the *Pheretima* group of genera (Crassiclitellata: Megascolecidae) using Bayesian analysis, *in*: V.V. Pop, A.A. Pop (Eds.), Advances in Earthworm Taxonomy II (Annelida: Oligochaeta). Cluj University Press, Cluj-Napoca, Romania, pp. 129–142.
Kinberg, J.G.H. (1867) *Annulata nova.* Ofvers K. Vetensk Academy, Ford Stockholm, 23, 97–103.

Kumar, S., Stecher. G. & Tamura, K. (2016) MEGA7: Molecular Evolution Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33, 1870–1874.

Kumar, V., Bharti, A., Negi, Y.K., Gusain, O., Pandey, P. & Bisht, G.S. (2012) Screening of actinomycetes from earthworm castings for their antimicrobial activity and industrial enzymes. *Brazilian Journal of Microbiology*, 43 (1), 205–214.

Liu, H., Zhang, Y., Xu, W., Fang, Y. & Ruan, H. (2021) Characterization of five new earthworm mitogenomes (Oligochaeta: Lumbricidae): Mitochondrial phylogeny of Lumbricidae. *Diversity*, 13, 580.
https://doi.org/10.3390/d13110580.

Michaelsen, W. (1913) Die Oligochaten von Neu-Caledonien und den benachbarten Inselgruppen. *Nova Caledonia*, 1, 173–280.

Narayanan, S.P., Sathrumithra, S., Anuja, R., Christopher, G., Thomas, A.P. & Julka, J.M. (2019) First record of the exotic earthworm *Metaphire bahli* (Gates, 1945) (Oligochaeta: Megascolecidae) from India. *Opuscula Zoologica (Budapest)*, 50 (1), 99–103.

Nguyen, T.T., Trinh, B.K.T., Nguyen, H.T. & Nguyen, A.D. (2015) On the polythecate earthworms of the genus *Metaphire* (Oligochaeta: Megascolecidae) from Vietnam, with descriptions of three new species. *Raffles Bulletin of Zoology*, 63, 461–470.

Nguyen, T.T., Nguyen, A.D., Binh, T.T. & Blakemore, R.J. (2016) A comprehensive checklist of earthworm species and subspecies from Vietnam (Annelida: Clitellata: Oligochaeta: Almidae, Eudrilidae, Glossoscolecidae, Lumbricidae, Megascolecidae, Moniligastridae, Ocnerodrilidae, Octochaetidae). *Zootaxa*, 4140 (1), 1–92.

Nguyen, T.T., Trinh, B.K.T., Nguyen, H.T. & Nguyen, A.D. (2017) Earthworms (Annelida: Oligochaeta) from islands of Kien Hai District, Kien Giang Province, Vietnam, with descriptions of two new species and one subspecies. *Journal of Natural History*, 51 (15–16), 883–915.
https://doi.org/10.1080/00222933.2017.1294213.

Nguyen, T.T., Nguyen, N.Q. & Nguyen, A.D. (2018). First record of the earthworm genus *Pheretima* Kinberg, 1867 sensu stricto in Vietnam, with description of a new species (Annelida: Clitellata: Megascolecidae). *Zootaxa*, 4496 (1), 251–258.

Nguyen, T.T., Nguyen, N.Q., Lam, D.H. & Nguyen, A.D. (2020) Six new species of the genus *Metaphire* Sims & Easton, 1972 (Annelida: Oligochaeta: Megascolecidae) from southeastern Vietnam. *Raffles Bulletin of Zoology*, 68, 220–236.

Nguyen, T.T. (2016) Two new earthworm species (Oligochaeta, Megascolecidae) from Vietnam. *Tap chi Sinh hoc (Academy Journal of Biology)*, 38(4), 442-448.
https://doi.org/10.15625/0866-7160/v38n4.8829.

Omodeo, P. (1957) Oligocheti dell'Indochina e del Mediterraneo Orientale. *Memorie del Museo Civico di Storia Naturale di Verona*, 5, 321–336.

Prasankok, P., Bantaowong, U., James, S.W. & Panha, S. (2013) Low heterogeneity in populations of the terrestrial earthworm, *Metaphire peguana* (Rosa, 1890), in Thailand, as revealed by analysis of mitochondrial DNA COI sequences and nuclear allozymes. *Biochemical Systematics and Ecology*, 51, 8–15.
https://doi.org/10.1016/j.bse.2013.07.001

Ravindran, B., Contreras-Ramos, S.M., Wong, J.W.C., Selvam, A. & Sekaran, G. (2014) Nutrient and enzymatic changes of hydrolysed tannery solid waste treated with epigeic earthworm *Eudrilus eugeniae* and phytotoxicity assessment on selected commercial crops. *Environmental Science and Pollution Research.* 21, 641–651.
https://doi.org/10.1007/s11356-013-1897-1

Rosa, D. (1890) Viaggio di Leonardo Fea in Birmanica e regioni vicine, XXVI. *Preichetidi. Annali del Museo Civico di Storia Naturale, Giacomo Doria*, 10, 107–122.

Rosa, D. (1891) Die exotischen Terricolen des k. k. naturhistorischen Hofmuseums. *Annalen des K. K. Naturhistorischen Hofmuseums*, 6, 379–406.

Shekhovtsov, S.V., Golovanova, E.V., Ershov, N.I., Poluboyarova, T.V., Berman, D.I., Bulakhova, N.A., Szederjesi, T. & Peltek S.E. (2020) Phylogeny of the *Eisenia nordenskioldi* complex based on mitochondrial genomes. *European Journal of Soil Biology*, 96, 103137.
https://doi.org/10.1016/j.ejsobi.2019.103137.

Sims, R. & Easton, E. (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.
https://doi.org/10.1111/j.1095-8312.1972.tb00694.x

Teng, S.K., Aziz, N.A.A., Mustafa, M., Aziz, S.A. & Yan, Y.W. (2012) Evaluation on physical, chemical and biological properties of casts of geophagous earthworm, *Metaphire tschiliensis tschiliensis*. *Science Research and Essays*, 7 (10), 1169–1174.

Thai, T.B. & Samphon, K. (1989) Initial remarks on the fauna of earthworms in Laos. *Thong Bao Khoa Hoc (Hanoi), 1989, 61–75. [Vietnamese:* Thái TB, Samphon K *(1989)* Nhận xét býớc đầu về khu hệ giun đất Lào (từ cao nguyên Mýờng Phuôn đến cao nguyên Bua La Vên). *Thông báo khoa học ĐHSPHN*, 1, 61–75].

Waqar, A., Shah, G.M., Bakhat, H.F., Shahid, M., Aslam, M., Ashraf, M.R., Hafeez, R., Murtaza, B. & Rashid, M.I. (2019) The earthworm species *Pheretima hawayana* influences organic wastes decomposition, nitrogen mineralization and maize N recovery. *European Journal of Soil Biology*, 90, 1–8.

Yu, J., Hu, F., Li, H. & Mi, C. (2008) Earthworm (*Metaphire guillelmi*) effects on rice photosynthates distribution in the plant–soil system. *Biology and Fertility of Soils*, 44 (4), 641–647.