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Three new earthworm species of the genus *Polypheretima* Michaelsen, 1934 (Oligochaeta: Megascolecidae) from Vietnam

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Abstract

The paper provides descriptions of three new species of the earthworm genus Polypheretima Michaelsen, 1934 from Dong Nai Province, South Vietnam. They are named Po. cattienensis sp. nov., Po. militium sp. nov., and Po. cordata sp. nov.. All three species are characterized by spermathecal pores in 5/6/7 and the absence of genital markings. Po. cattienensis sp. nov. is distinguished by paired spermathecal pores and seven spermathecae per porus. Po. militium sp. nov. is diagnosed by paired spermathecal pores and a variable number of spermathecae, 21-40 altogether, with 7-17 in 5/6 and 11-23in 6/7. Po. cordata sp. nov. is recognized by one pair of spermathecal pores in 5/6 and two in 6/7, by only one spermatheca per porus, and by a heart-shaped spermathecal ampulla.

Key words: Clitellata, Megascolecidae, Polypheretima, earthworms, new species, Dong Nai, Vietnam

Introduction

Polypheretima Michaelsen, 1934 was firstly established as a subgenus of the genus Pheretima (Michaelsen 1934) and later elevated to genus rank (Easton 1979). Polypheretima is distinguished from other genera within the family Megascolecidae by the absence of intestinal caeca, intestinal gizzards and a creeping sole; it has a cylindrical body, male pores on circular porophores, no crescentic genital markings, small spermathecal pores and simple spermathecal diverticula (Easton 1979).

To date, fourty-three Polypheretima species have been described, but only two of them, Polypheretima elongata (Perrier, 1872) and P. taprobanae (Beddard, 1892), are common species (Blakemore 2007). All other species are narrowly and strictly found in New Guinea, Indonesia, Philippines and Vietnam (Easton 1979; Blakemore 2007).

In Vietnam, the genus Polypheretima has been recorded with ten species, Polypheretima annamensis (Stephenson, 1931), Po. colonensis (Thai, 1996), Po. elongata (Perrier, 1872), Po. grandisetosa (Thai, 1996), Po. kyhaensis (Thai, 1996), Po. mekongmontis Nguyen, Tran & Nguyen, 2014, Po. parataprobanae (Thai & Nguyen, 1993), Po. spiridonovi (Thai, 1996), Po. taprobanae (Beddard, 1892), and Po. touranensis (Michaelsen, 1934) (Stephenson 1931; Michaelsen 1934b; Nguyen 1993; Thai 1996, 2000; Nguyen et al. 2014). The number of species is expected to be higher due to Vietnam's location in the tropical region and a lack of intensive surveys in this country. This paper contributes to the better understanding of the earthworm fauna of Vietnam with descriptions of three new species.

Methods and materials

All material was collected during 10-11/2013 in Dong Nai Province, Vietnam. Examined material was preserved in formalin 4%. Taxonomic terminology and system follow Easton (1979). Holotypes and paratypes are being kept in the Laboratory of Zoology, Department of Biology, Can Tho University (CTU). Some paratypes are deposited at the Zoological Museum, Faculty of Biology, Hanoi University of Education (HNUE).

Body length, diameter and weight including gut content were measured after fixation and cleaning of the body surface with tissue paper.

Abbreviations: C = Clitellate specimen/specimens (e.g., "5C" = 5 clitellate specimens).

Taxonomic part

Genus Polypheretima Michaelsen, 1934

Pheretima (*Polypheretima*) Michaelsen, 1934a: 15. *Metapheretima* Michaelsen (part.)—Sims & Easton 1972: 205, 233. *Polypheretima* Michaelsen —Easton 1979: 28.

Type species. Perichaeta stelleri Michaelsen, 1892, by original designation.

Distribution. Philippines, Indonesian archipelago, Papua New Guinea, Malaya peninsula and Vietnam (Easton 1979).

Remarks. Michaelsen (1934a) proposed a subgenus *Polypheretima* for a species group characterized by multiple spermathecae arranged in pairs of transverse groups, with two or more than two per group, and absence of intestinal caeca. However, Sims & Easton (1972) did not support the concept of the subgenus *Polypheretima* because of variations among individuals in terms of number of spermathecae, for example in *Pheretima elongata*. They placed all *Polypheretima* species in *Metapheretima* and suggested to wait for more polythecal conditions to separate *Polypheretima* into a genus. Soon after, Easton (1979) revised the systematics of the 'acaecate' *Pheretima* group and raised *Polypheretima* to full generic rank.

Polypheretima cattienensis sp. nov.

(Fig. 1, Table 2)

Examined material. *Holotype*: 1C (CTU-EW040-h01) natural forest (11°25'30.9N; 107°25'42.2E), 122 m asl, Cat Tien National Park, Tan Phu District, Dong Nai Province, Vietnam, 13/10/2013, coll. Le Van Nhan. *Paratypes*: 4C (CTU-EW040-p01) and 2C (HNUE-B240. CT1. Par.), same data as for holotype.

Diagnosis. Male pores deeply located inside copulatory pouches; copulatory pores crescentic. Genital markings absent in male and spermathecal regions. Spermathecae arranged in two pairs of pores in intersegments 5/6/7, seven spermathecae per porus. Ampulla slightly expanded distad; distal and basal parts of ampulla not clearly differentiated. Diverticula shorter than ampulla, attached to ampulla near base.

Etymology. Named after the Cat Tien National Park, a biosphere reserve in South Vietnam, where the type material was found.

Description. *External characters:* Body generally cylindrical, but slightly bigger in segments iv–vii. Large size, length 115–147 mm, diameter 6.41–8.63 mm, body weight 2.82–4.28 gr, 132–189 segments. Segments i-vii darkish green, other segments paler, rather uniformly colored. Prostomium epilobous (2/3). First dorsal pore in 12/13. A darkish blue line present mid-dorsally from post-clitellum to the end. Setae perichaetine, never excessively crowded ventrally, regularly arranged around each segment, 90–114 in viii, 95–129 in xxx, 3–8 between male porophores in xviii; setae distance aa=2ab, zz=2zy. Clitellum annular, xiv–xvi, smooth without setae and dorsal pores. Female pore single, mid-ventral in xiv. Spermathecal pores large, in two pairs, lateroventral in intersegments 5/6/7. Male porophores highly elevated; male pores located deeply inside copulatory pouches in xviii; copulatory pores crescentic. Ventral distance between male porophores about 0.25x body circumference. Genital markings absent in male and spermathecal regions.



FIGURE 1. *Polypheretima cattienensis* **sp. nov.**, paratype. **A-B.** Male pore region, ventral view (**A**), lateral view (**B**) (mp = male pore). **C.** Spermathecal region, lateral view (sp = spermathecal pore). **D.** Spermathecae in left pore in 5/6 (amp = ampulla, dv = diverticula). **E.** Prostate gland. **F.** Copulatory pouch, transverse body section. Scales = 1mm.

Internal characters: Septa 4/5/6/7/8 thickened, 8/9/10 absent, 11/12/13 thinned. Oesophageal gizzard large, but short within ix–x. Intestinal origin at xv; caeca absent. Last hearts in xiii. Pharyngeal micronephridia poorly developed on septum 4/5, and more developed on septum 5/6. Lymph glands absent or poorly present only in xv–xx. Spermathecae arranged in two pairs of pores in 5/6/7; seven spermathecae per porus. Ampulla slightly expanded distally, distal and basal parts of ampulla not clearly differentiated. Diverticula shorter than ampulla, attached to ampulla near base. Accessory glands absent. Typhlosole simple, lamelliform. Holandric. Testis sacs not separated. Seminal vesicles well developed within xi–xii. Ovaries paired, poorly developed on septum 12/13. Oviduct on septum 12/13 ventrally. Prostate glands racemose, paired in xviii, two main branches extending from xvii–xix; prostatic ducts C-shaped, ending at elevated, large chambers in coelom. No accessory glands.

Remarks. The species belongs to the *Po. polytheca* species-group Easton, 1979 characterized by multiple spermathecae per pore, but differs from other members in having two pairs of pores in intersegments 5/6/7, seven spermathecae per pore, and copulatory pouches. The species is somewhat similar to *Polypheretima parataprobanae* (Thai & Nguyen, 1993 in Nguyen 1993) in the morphology of the male region, first dorsal pore in 12/13 and in the presence of a copulatory pouch. However, *Po. parataprobanae* differs from the new species in smaller body size (length 60–71 mm, diameter 4.5–6 mm), spermathecal pores paired lateroventrally in only 7/8, genital. The new species is also similar to *Po. aringeana* (Beddard, 1990) from Malaya in first dorsal pore in 12/13, spermathecal pores paired in 5/6/7, and multiple spermathecae per porus. *Po. aringanea* differs from the new species in the presence of genital markings in both male and spermathecal regions, 7–10 spermathecae per porus in vi and vii, and shallow copulatory pouches.

Locality and habitat. Cat Tien National Park is located in Dong Nai province, about 170km north-east from Ho Chi Minh City. It is known as a biosphere reserve and the best conserved forest in Vietnam, with high diversity of animals, plants, and habitats. The species was found a depths of 5–10 cm under the soil surface in closed evergreen forests. Material was collected in October during dry season in southern Vietnam.

Polypheretima militium sp. nov.

(Fig. 2, Tables 1, 2)

Examined material. *Holotype:* 1C (CTU. EW041–h01) garden (11°08'33.4N; 107°00'72.9E), 45 m asl, village No.2, Ma Da commune, Vinh Cuu District, Dong Nai province, 10/10/2013, coll. Duong Chi Trong. *Paratypes:* 4C (CTU.EW041–p01) and 2C (HNUE–B241. CT1. Par.), same data as for holotype.

Diagnosis. Male pores located inside copulatory pouches; copulatory pores O-shaped. Genital markings absent in male region and spermathecal regions. Spermathecal pores arranged in two pairs of pores in intersegments 5/6/7; spermathecae small, about 21–40 altogether, 7–17 per segment in 5/6 and 11–23 per segment in 6/7. Ampulla oval; duct 1/3 as long as ampulla. Diverticula cylindrical, not twisted, slightly expanded distad, much longer than ampulla, attached to ampulla at base.

Etymology. "militium" = "of the soldiers". Named in memory of Vietnamese soldiers in the Vinh Cuu District during the Vietnam War.

Description. *External characters:* Small to medium size, slightly bigger in xvi–xxx; body length 58–97 mm, diameter 2.58–3.57 mm, weight 0.36–1.56 gr, 137–164 segments. Body uniformly light-grey. Clitellum whitishbrown. Prostomium epilobous (1/3). First dorsal pore in 12/13 or 13/14. Setae perichaetine; preclitellar setae stouter and thicker than postclitellar setae, 67–90 in viii, 36–62 in xxx, 7–11 between male porophores in xviii; setae distance aa=2ab, zz=2zy. Clitellum annular, xiv–3/4xvi, without setae and dorsal pores. Female pore single, mid-ventral in xiv. Spermathecal pores two pairs, lateroventral in intersegments 5/6/7. Male porophores highly elevated; male pores located inside copulatory pouches in xviii; copulatory pores O-shaped. Ventral distance between male porophores about 0.35x body circumference. Genital markings absent in spermathecal and male regions.



FIGURE 2. *Polypheretima militium* **sp. nov.**, paratype. **A-B.** Male pore region, ventral view (**A**), lateral view (**B**) (mp = male pore). **C.** Spermathecal region, lateral view (sp = spermathecal pore). **D.** Spermathecae in left pore in 5/6 (amp = ampulla, dv = diverticula). **E.** Prostate gland. **F.** Copulatory pouch, transverse body section. Scales = 1mm.

Material examined	Position	Left	Right	Total
Holotype	5/6	4	3	7
	6/7	7	6	13
Paratype 1	5/6	6	6	12
	6/7	7	7	14
Paratype 2	5/6	6	6	12
	6/7	10	10	20
Paratype 3	5/6	5	5	10
	6/7	7	9	16
Paratype 4	5/6	8	7	15
	6/7	11	12	23
Paratype 5	5/6	4	5	9
	6/7	5	6	11
Paratype 6	5/6	8	9	17
	6/7	10	9	19

TABLE 1. Number of spermathecae in the type series of Polypheretima militium sp. nov.

Internal characters: Septa 4/5/6/7/8 thickened, 8/9/10 absent, 11/12/13 thinned. Oesophageal gizzard large within ix–x. Intestinal origin at xv; caeca absent. Last hearts in xiii. Pharyngeal micronephridia well-developed on septum 4/5, poorly developed on septum 5/6. Lymph glands absent. Spermathecae small, about 21–40 altogether, 7–17 per segment in 5/6 and 11–23 per segment in 6/7. The number of spermathecae variable in specimens (Table 1). Ampulla oval; duct about 1/3 as long as ampulla. Diverticula cylindrical, not twisted, slightly expanded distad, much longer than ampulla, attached to ampulla at base. Typhlosole simple, lamelliform. No accessory glands. Holandric. Testis sacs separated. Seminal vesicles well developed in xi–xii. Oviduct on septum 12/13 ventrally. Ovaries absent. Prostate glands racemose, paired in xviii, two main branches extending from xvii–xix; prostatic ducts getting bigger distally, ending at xviii. No accessory glands.

Remarks. *Polypheretima militium* **sp. nov.** belongs to the *Po. polytheca* species-group Easton, 1979 characterized by multiple spermathecae per pore, but differs from other members in having two pairs of pores in intersegments 5/6/7, about 21–40 small spermathecae altogether (7–17 per segment in 5/6 and 11–23 per segment in 6/7), and copulatory pouches. It is rather close to *P. colonensis* (Thai, 1996) in the following characters: body size, setae distance (aa=2ab, zz=2zy), spermathecal pores paired in 5/6/7, no genital markings in spermathecal and male regions, septa 8/9/10 absent, last hearts in xiii. However, *Po. colonensis* differs from the new species in being metandric and bithecal, first dorsal pore in 11/12, and intestinal origin at xvii. The species is also similar to *Po. cattienensis* **sp. nov.** (see above), but distinguished by the body colour and the shape of the copulatory pouches (comp. Figs 1A, 2A). *Po. militium* is more variable than *Po. cattienensis* regarding the location of the first dorsal pore (in 12/13 or 13/14) and the number of spermathecae per porus and per segment.

Locality and habitat. Vinh Cuu Natural Reserve is located in Dong Nai province, about 100 km north-east from Ho Chi Minh city. It is considered as the best-conserved area with high biodiversity values including animals, plants and habitats. The species was found a depths of 5–10 cm under the soil surface in a garden in which long-term trees were planted. Material was collected in October during dry season in southern Vietnam.

Polypheretima cordata sp. nov.

(Fig. 3, Table 2)

Examined material. *Holotype:* 1C (CTU. EW042–h01) natural forest (11°06'89.5N; 107°03'10.3E), about 100 m asl, Ma Da commune, Vinh Cuu District, Dong Nai Province, 13/10/2013; coll. Duong Chi Trong. *Paratypes:* 5C (CTU. EW042–p01) and 2C (HNUE–B242. CT1. Par.), same data as for holotype.

Further material. 4C (CTU. EW042–k01) natural forest (11°25'42.0N; 107°25'38.1E), 122 m asl, Cat Tien National Park, Tan Phu District, Dong Nai Province, Vietnam, 13/10/2013, coll. Le Van Nhan.

Diagnosis. Male pores located inside copulatory pouches; copulatory pores O-shaped. Genital markings absent in spermathecal and male regions. Three pairs of spermathecal pores, lateroventral in intersegments 5/6/7: one in 5/ 6 and two in 6/7; one spermatheca per pore. Ampulla characteristically heart-shaped; ducts as long as ampulla. Diverticula shorter than ampulla, more or less waved in the middle, attached to ampulla at base.

Etymology. The new species is named after the specific shape of the ampulla.

Description. *External characters:* Medium size, body cylindrical, length 98–168 mm, diameter 3.17–3.99 mm, weight 0.76–1.63 gr, 110–154 segments. Body uniformly whitish-brown. Clitellum darkish-brown. Prostomium prolobous. First dorsal pore in 12/13. Setae perichaetine, regularly arranged around segment, 61–90 in viii, 60–84 in xxx, 7–9 between porophores in xviii; setae distance aa=ab, zz=zy. Clitellum annular, xiv–3/4xvi, without setae and dorsal pores. Female pore single, mid-ventral in xiv. Three pairs of spermathecal pores lateroventral in intersegments 5/6/7: one pair in 5/6 and two in 6/7. Male porophores elevated; male pores located inside copulatory pouches in xviii; copulatory pores O-shaped. Ventral distance between male porophores about 0.3x body circumference. Genital markings absent in spermathecal and male regions.



FIGURE 3. *Polypheretima cordata* **sp. nov.**, paratype. **A-B.** Male pore region, ventral view (**A**), lateral view (**B**) (mp = male pore). **C.** Spermathecal region, lateral view (sp = spermathecal pore). **D.** Spermathecae in left pore in 5/6/7 (amp = ampulla, dv = diverticula, ag = accessory glands). **E.** Prostate gland. **F.** Copulatory pouch, transverse body section. Scales = 1mm.

Internal characters: Septa 4/5/6/7 thickened, 8/9/10 absent, 10/11/12 thinned. Oesophageal gizzard within viii–x. Intestinal origin at xv; caeca absent. Last hearts in xiii. Pharyngeal micronephridia well developed on septa 4/5/6. Lymph glands absent. A pair of spermathecae in 5/6 and two pairs of spermathecae in 6/7, one spermathecae per pore. Ampulla characteristically heart-shaped; ducts as long as ampulla. Diverticula shorter than ampulla, more or less waved in the middle, attached to ampulla at base. Two accessory glands before spermathecae in 5/6/7. Typhlosole lamelliform, but poorly developed. Holandric. Testis sacs separated. Seminal vesicles well developed in

xi–xii. Oviduct well developed in 12/13. A pair of ovaries developed in xiii. Prostatic glands racemose in xvii–xix, rarely extending to xx; prostatic ducts small, slightly getting bigger distally. Accessory glands absent.

Remarks. *Polypheretima cordata* **sp. nov.** belongs to the *Po. polytheca* species-group Easton, 1979 characterized by multiple spermathecae per pore or per segment, but differs from other members in having three pairs of spermathecal pores in intersegments 5/6/7 (one in 5/6 and two in 6/7), and copulatory pouches. The species is similar to *Po. grandisetosa* (Thai, 1996) in the following characters: prostomium prolobous; genital markings absent in spermathecal and male regions; spermathecal pores in 5/6/7; last hearts in xiii; septa 8/9/10 absent; diverticula shorter than ampulla in length; a pair of ovaries in xiii. However, *Po. grandisetosa* differs from the new species in being metandric, lacks of copulatory pouches, spermathecal pores two pairs in 5/6/7 and having 4-5 macrosetae dorsally on vi.

Locality and habitats. The species was found a depths of 5–10 cm under the soil surface in closed evergreen forests in Vinh Cuu Natural Reserve and Cat Tien National Park. Material was collected in October during dry season in southern Vietnam.

Discussion

Blakemore (2007) listed nine *Polypheretima* species in Vietnam including three valid species, *Po. annamensis* (Stephenson, 1931), *Po. elongata* (Perrier, 1872), *Po. taprobanae* (Beddard, 1892), and other six species marked as "*species inquirenda*". Nguyen *et al.* (2014) have confirmed their status as valid species and described another new species from the Mekong delta. To date, there are 13 *Polypheretima* species recorded in Vietnam including three new species described in this paper. Those are:

- Po. annamensis (Stephenson, 1931), from Lam Dong province, southern Vietnam.
- Po. colonensis (Thai, 1996), from Con Lon Island, Ba Ria-Vung Tau province, southern Vietnam.
- Po. grandisetosa (Thai, 1996), from Con Lon Island, Ba Ria-Vung Tau province, southern Vietnam.
- Po. elongata (Perrier, 1872), widely distributed in Vietnam.
- Po. kyhaensis (Thai, 1996), from Quang Nam province, southern Vietnam.
- Po. parataprobanae (Thai & Nguyen, 1993), from Thua Thien Hue province, central Vietnam.
- Po. spiridonovi (Thai, 1996), from Khanh Hoa province, southern Vietnam.
- Po. taprobanae (Beddard, 1892), widely distributed in Vietnam.
- Po. touranensis (Michaelsen, 1934), from Da Nang province, southern Vietnam.
- Po. mekongmontis Nguyen, Tran & Nguyen, 2014, from Kien Giang province, southern Vietnam.
- *Po. cattienensis* **sp. nov.**, from Dong Nai province, southern Vietnam.
- *Po. militium* **sp. nov.**, from Dong Nai province, southern Vietnam.
- Po. cordata sp. nov., from Dong Nai province, southern Vietnam.

Interestingly, almost all Vietnamese *Polypheretima* species have a restricted distribution in southern Vietnam, and they are rarely found north of the Haivan pass, which is a natural barrier between southern and northern Vietnam. Especially two species appear to have an extremely restricted distribution: *Po. colonensis* and *Po. grandisetosa* have been recorded only from a small island in southernmost Vietnam. Both species are metandric, a rare condition in *Polypheretima*, known so far only in species from Papua and Less Sunda regions (Easton 1979).

The Vietnamese *Polypheretima* species can be divided into two groups (Table 2). The first group "bithecal", characterised by one pair of spermathecae per segment, includes *Po. annamensis*, *Po. colonensis*, *Po. grandisetosa*, *Po. kyhaensis*, *Po. parataprobanae*, *Po. taprobanae*, and *Po. touranensis*. The other group "polythecal" is characterized by many pairs of spermathecae per segment, and consists of *Po. spiridonovi*, *mekongmontis*, *Po. cattienensis*, *Po. militium*, and *Po. elongata*. *Po. cordata* occupies an intermediate position, being bithecal in one segment and polythecal in the other. Furthermore, the spermathecae in the polythecal segment are not arranged in groups as in the other species of the polythecal species group.

Located in the tropical region and one of the biodiversity centers in the world, Vietnam is known to have rich fauna and flora (Sterling *et al.* 2006). The current number of *Polypheretima* species has not yet reflected the high diversity of the genus in Vietnam. It is believed that more intensive surveys, especially in southern Vietnam, could reveal more new species to be discovered.

Species	First dorsal pore	Sperma- thecal pores	Sperma- thecae type	GMM	GMS	Male organ system	Copu- latory pouches	Intestinal origin	Last hearts
Po. annamensis	12/13	6/7	Bithecal	+	-	Holandric	+	XV	xiii
Po. colonensis	11/12	5/6/7	Bithecal	-	-	Metandric	-	xvii	xiii
Po. grandisetosa	11/12	5/6/7	Bithecal	-	-	Metandric	-	xvii	xiii
Po. kyhaensis	11/12	5/6/7/8/9	Bithecal	+	+	Holandric	-	XV	xii
Po. parataprobanae	12/13	7/8	Bithecal	-	+	Holandric	-	xvi	?
Po. taprobanae	12/13	7/8	Bithecal	+	+	Holandric	-	XV	xii
Po. touranensis	12/13	7/8/9	Bithecal	+	-	Holandric	-	?	?
Po. spiridonovi	12/13	4/5/6/7	Polythecal	-	-	Holandric	-	XV	xii
Po. elongata	12/13	5/6/7	Polythecal	+	-	Holandric	+	XV	xiii
Po. mekongmontis	12/13	4/5/6/7	Polythecal	+	-	Holandric	+	xv or xvi	xii
Po. cattienensis	12/13	5/6/7	Polythecal	-	-	Holandric	+	XV	xiii
Po. militium	12/13	5/6/7	Polythecal	-	-	Holandric	+	XV	xiii
Po. cordata	12/13	5/6/7	Intermediate	-	-	Holandric	+	XV	xiii

TABLE 2. Marker characters for Vietnamese *Polypheretima* species (GMM=genital markings in male region; GMS=genital markings in spermathecal region; + = present; - = absent)

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