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THREE NEW EARTHWORM SPECIES OF THE GENUS *Metaphire* Sims & Easton, 1972 (Oligochaeta, Megascolecidae) FROM DONG NAI PROVINCE, VIETNAM

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ABSTRACT: Three new earthworm species are described from Dong Nai Province, Vietnam, namely *Metaphire malayanoides* Nguyen & Lam sp. n., *Metaphire grandiverticulata* Nguyen & Lam sp. n., and *Metaphire xuanlocensis* Nguyen & Lam sp. n. Both *M. malayanoides* sp. n. and *M. grandiverticulata* sp. n. have four pairs of spermathecal pores in ventrolateral 5/6/7/8/9. However, *M. malayanoides* is distinguished by having two to eight pairs of genital markings located in intersegmental furrows, starting from 19/20; *M. grandiverticulata* sp. n. is characterized by having only one pair of genital markings between male porophores in xviii, and very large tube-shaped diverticulum. *M. xuanlocensis* sp. n. has one pair of spermathecal pores inside the spermathecal chambers in 7/8, accessory glands stalked and attached to the enlarged base of prostatic duct, and the presence of penial setae.

Keywords: Megascolecidae, *Metaphire*, earthworms, new species, Dong Nai, Vietnam.

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INTRODUCTION

Dong Nai province is located in the South Eastern part of Vietnam, with an area of 5903.4 km^2 (10°30' to 11°35'N and 106°45' to 107°35'E). This region has typical trophic climate with two distinct seasons: the rainy season (from April to November), and the dry season (from December to next March); the average temperature is from 23.9 to 29°C. Moreover, Dong Nai is located in the transition zone between the Central Highlands and Southern Plains of Vietnam. It, thus, harbors a very rich and diverse fauna (Le et al., 2010).

To date, 220 species and subspecies were reported from Vietnam (Nguyen, 2016; Nguyen et al., 2016a, b, 2017). Almost all species belong to the *Pheretimoid* group (198 species) with two most diverse genera, *Amynthas* Kinberg, 1867 (113 species), and *Metaphire* Sim & Easton, 1972 (55 species).

Currently, 17 species have been known from Dong Nai, mostly in the genus *Metaphire* Sim & Easton, 1972 (10 species) (Nguyen, 2016). The number of recorded species is far from reflecting the diversity of this area. Therefore, our work contributes to the knowledge on the earthworm fauna of this region by descriptions of three new species.

MATERIALS AND METHODS

Earthworms were collected in Dong Nai province in rainy season (September and October) from 2012 to 2014 (fig. 1). They were searched by digging to the soil and hand sorting leaf litters. Worms were killed with 2% formalin, transferred to 4% for fixation in 12 hours, and then preserved in new 4% formalin.

Line drawings were made using a Motic DM143 FBGG C stereoscopic light microscope. Colour images were taken using camera attached directly to the microscope. Drawings and images were grouped using Adobe Illustrator CS6.

Holotypes and paratypes are deposited in the Laboratory of Zoology, Department of

Biology, Can Tho University (CTU), Can Tho city, Vietnam.

Worms diameter were measured in pre-clitellar region (segment viii), clitellum and post-clitellar (segment xxx). Setae ratios aa/ab and zz/zy were determined on segments viii and xxx. T% was measured in segment xxxv.

Abbreviations: mp = opening of copulatory pouch; gm = genital marking; sp = spermathecal pores; amp = ampulla; dv = diverticulum; ag = accessory gland; prd = prostatic duct; ps = penial setae; sd = spermathecal duct.

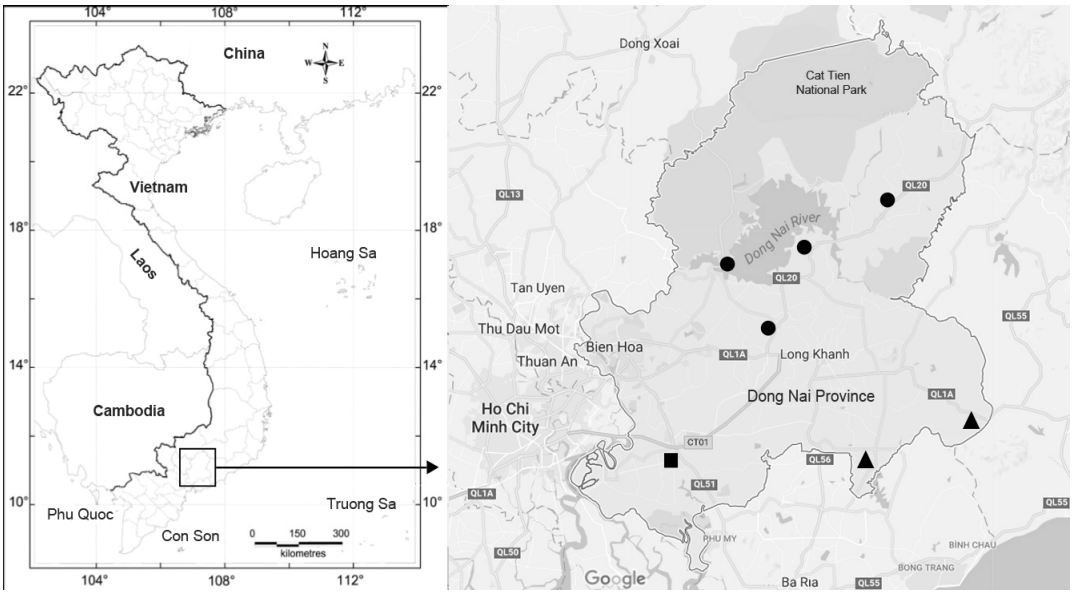

Figure 1. Collection sites of 3 new earthworms

●*: Metaphire malayanoides*; ■*: Metaphire grandiverticulata*; ▲*: Metaphire xuanlocensis*

RESULTS AND DISCUSSION

***Metaphire malayanoides* Nguyen & Lam, sp. n.** (Figs. 2-3)

Examined material: *Holotype*: 1 mature (CTU-EW.084.h01), rubber plantation (11°08'34.3''N; 107°13'32.6''E), La Nga commune, Dinh Quan district, Dong Nai province, 14/10/2013, coll. Le Van Nhan.

Paratypes: 8 matures (CTU-EW.084.p02), same data as for holotype; 5 matures (CTU-EW.084.p03), long-term tree garden (10°59'40.9''N; 107°09'08.0''E), Quang Trung commune, Thong Nhat district, Dong Nai province, 15/10/2013, coll. Le Van Nhan.

Further materials: 16 matures (CTU-EW.084.04), same data as holotype; 31 matures, 2 juveniles (CTU-EW.084.05), same data as sample CTU-EW.084.p03; 14 matures (CTU-EW.084.06), annual crop (11°06'40.9''N; 107°03'12.2''E), Ma Da commune, Vinh Cuu district, Dong Nai province, 18/10/2012, coll. Duong Chi Trong; 34 matures, 1 juvenile (CTU-EW.084.07), long-term tree garden (11°13'53.7''N; 107°22'55.1''E), Phu Vinh commune, Dinh Quan district, Dong Nai province, 14/10/2013, coll. Le Van Nhan.

Diagnosis: Medium size worm, length 63-155 mm (111.8±24.6 mm), diameter ca. 4.0-5.9 mm (4.9±0.7 mm). First dorsal pore in 12/13. Clitellum with setae and dorsal pores. Spermathecal pores lateroventrally paired in intersegmental furrows 5/6/7/8/9. Holandric. Genital marking ventrally paired in 19/20-23/24 and also present inside copulatory pouches but invisible from outside. Intestinal caeca simple. Septa 8/9/10 absent.

Etymology: Named after the particular similarity to *Metaphire malayana* (Beddard, 1900).

Vietnamese name: Giun cận mã lai

Description: *External characters*: Body cylindrical, medium size; length 111.8±24.6 mm (N=14), diameter ca. 4.9±0.7 mm, weight 1.75±0.66 g, 103±28 segments. Dorsum brownish grey, but ventrum paler. Prostomium 1/2 epilobous. First dorsal pore in 12/13. Setae perichaetine; pre-clitellar setae stouter and slightly sparser than post-clitellar setae, 43-73 in viii, 50-79 in xxx, 12-17 between male porophores in xviii. Setal distance aa = 1.2-1.5ab, zz = 1.2-1.5zy. Clitellum annular, xiv-xvi, blackish brown, smooth, with presence of setae and dorsal pores. Female pore single, mid-ventral on xiv.

Spermathecal pores four pairs in 5/6/7/8/9. No genital makings in the spermathecal region. Male pores located inside copulatory pouches which opening ventrally on setal ring in xviii; ventral distance between openings of copulatory pouches about 0.33x body circumference. Two to eight pairs of small, round genital markings from 19/20 to 26/27, in line with male pores; genital markings also present inside the copulatory pouches, but invisible from outside.

Internal characters: Septa 5/6/7/8 thickened, 8/9/10 absent, 10/11/12/13 thin. Oesophageal gizzard large, within viii-x. Intestine origin at xv; caeca simple, originating at xxvii and extending anteriorly to xxiv. Last hearts in xiii. Pharyngeal micronephridia well developed in 5/6/7. Lymph glands absent. Typhlosole simple, lamelliform; T% = 21%.

Spermathecae four pairs in vi-ix. Ampulla large, main part usually folded, and enlarged at distal part; duct muscular, stout, and about two-third of ampulla length. Diverticulum slender, shorter than ampulla, attached to the base of ampulla duct; seminal chamber large, opalescent. Accessory glands absent from the spermathecal region.

Holandric, testes sacs ventrally paired in x and xi, and separated. Seminal vesicles whitish, paired in xi and xii. Ovaries well developed, paired in 12/13. Ovisacs paired in xiii. Prostate glands racemose, deeply lobuled, paired within xvi-xx or xvi-xxi; prostatic ducts short, U-shaped. Accessory glands unstalked, flowed into copulartory pouches (fig. 3k), but in small coelomic and muscular chambers if opening out in intersegmental furrows (fig. 3l).

Locality and habitat: Worms were mostly found from 5-10 cm deep in to the soil in rubber plantations, and long-term tree gardens in red basaltic soils.

Remarks: Genital markings of the new species are variable, from two to eight pairs in intersegmental furrows (from 19/20 to 26/27). However, the most common type has five pairs (39/109 matures) (fig. 2).

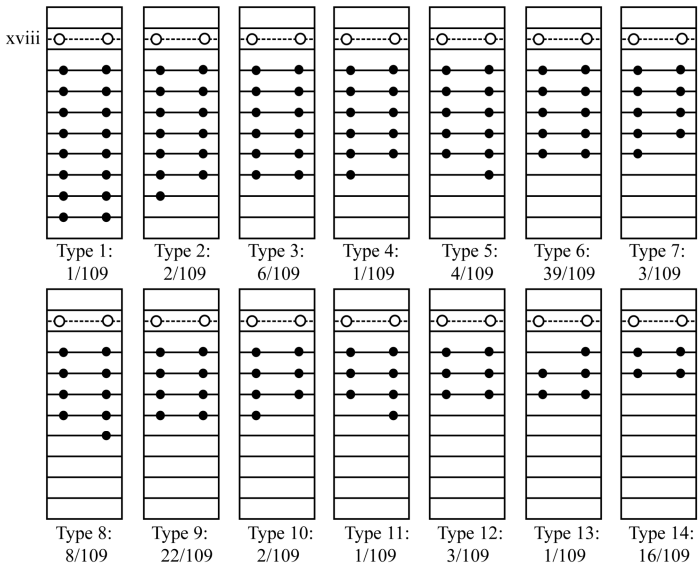

Figure 2. Variation of genital markings in the male region of *M. malayanoides* sp. n.

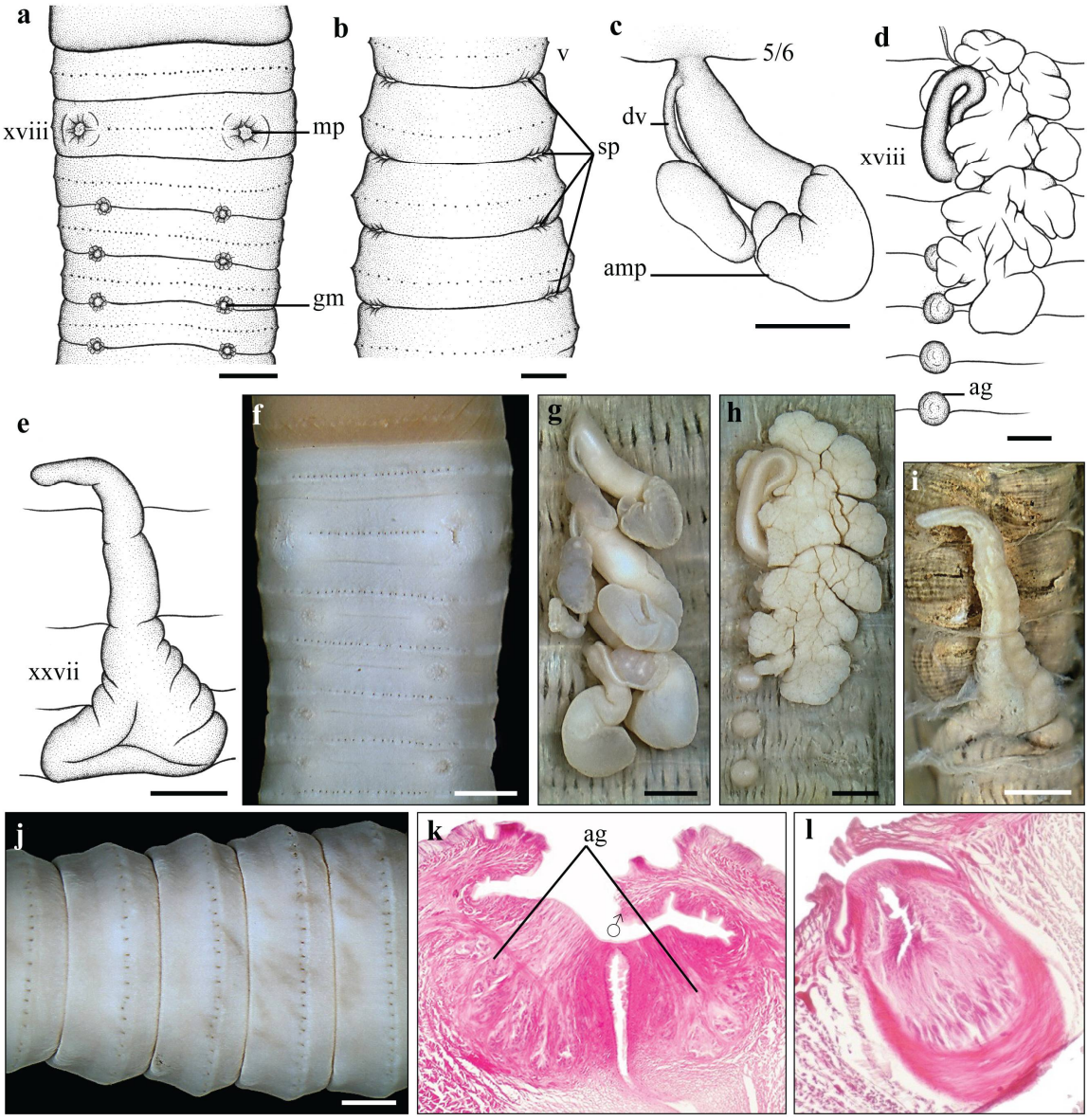

Figure 3. Metaphire malayanoides Nguyen & Lam, sp. n.

a, f: Male pore region; b, j: Spermathecal region, ventral view; c, g: Spermathecae, right side; d, h: Prostate glands, right side; e, i: Intestinal caecum; k: Copulatory pouch, transverse body section; l: Accessory gland, transverse body section. Scale bar = 1 mm.

The new species is similar to *M. dipapillata* (Thai & Tran, 1986), *M. neoexilis* (Thai & Samphon, 1988), *M. posthuma* (Vaillant, 1868) (redescribed by Bantaowong et al. (2011)), *M. nhuongi* Nguyen, 2016, *M. varellana* (Michaelsen, 1934), *M. pulauensis* (Beddard, 1900), and *M. malayana* (Beddard, 1900) by having four pairs of spermathecal pores in 5/6/7/8/9, post-clitellar genital markings present, holandric, intestinal caeca simple. However, *M. malayanoides* has first dorsal pore in 12/13, bithecal, genital markings paired in intersegmental furrows (from 19/20 to 26/27), ovisacs only in 12/13, intestinal origin at xv, micronephridia in 5/6/7/8 while *M. dipapillata* has first dorsal pore in 11/12, polythecal, one pair of genital markings in 17/18, ovisacs in 12/13 and 13/14, intestinal origin at xvi and

micronephidia in 5/6/7. The new species has 2-8 pairs of genital markings present in intersegmental furrows (from 19/20 to 26/27), accessory glands chamber shaped, lymph glands absent while those species have 2 pairs of genital markings in xvii and xix, accessory glands massed in *M. posthuma* but invisible in *M. nhuongi*; Moreover, the new species is smaller than *M. nhuongi* but equal to with *M. posthuma* in size. The new species is also distinguished from *M. neoexilis* and *M. varellana* by having genital markings paired in intersegmental (from 19/20 to 26/27), testis sacs separated, ovisacs paired in 12/13, intestinal origin at xv, lymph glands absent while those species have genital markings paired only in xvii, testes sacs connected, ovisacs invisible, intestinal origin at xvi and lymph glands present from 27/28.

Especially, *M. malayanoides* sp. n. is particularly similar to *M. malayana* (Beddard, 1900) in having genital markings paired in the male region but absent in xvii. However, the new species has the first dorsal pore in 12/13, no genital markings in the spermathecal region, two to eight pairs of intersegmental genital markings from 19/20, testes sacs separated. In contrast, *M. malayana* (Beddard, 1900) from Malaysia, Myanmar, and Thailand has the first dorsal pore in 11/12, various genital markings in vi-ix (the spermathecal region), four to eight pairs of genital markings in xvii-xxiv, testes sacs connected (Beddard, 1900; Gates, 1949, 1972).

***Metaphire grandiverticulata* Nguyen & Lam, sp. n.** (Fig. 4, Table 1)

Examined material: *Holotype*: 1 mature (**CTU-EW.089.h01**), long-term tree garden (10°44'29.1''N; 106°58'31.0''E), Long Phuoc commune, Long Thanh district, Dong Nai province, 21/10/2014, coll. Le Van Nhan.

Paratypes: 9 matures (CTU-EW.089.p02), same data as for holotype.

Further material: 13 matures, 1 juvenile (CTU-EW.089.03), same data as for holotype.

Diagnosis: Small worm, length 69-92 mm (75.3±9.2 mm), diameter ca. 2.3-2.7 mm (2.9±0.9 mm). First dorsal pore in 12/13. Spermathecal pores lateroventrally paired in intersegmental furrows 5/6/7/8/9. Genital markings ventrally paired in xviii. Spermathecal diverticulum extremely large. Holandric. Intestinal caeca simple. Septa 8/9/10 absent.

Vietnamese name: Giun diverticulum lớn.

Table 1: Character comparison among *Metaphire grandiverticulata* sp. n., *M. bucculenta* (Gates, 1935), *M. neoexilis* (Thai & Samphon, 1988), and *M. varellana* (Michaelsen, 1934)

No.	Characters	*M. grandiverticulata*	*M. bucculenta* (1)	*M. neoexilis* (2)	*M. varellana* (3)
1	Length (mm)	2.9±0.9	135	52	105
2	Diameter ca. (mm)	0.35±0.16	5	2-2.5	2-4.5
3	Prostomium	Epi	Epi	Pro	?
4	First dorsal pore	12/13	11/12	12/13	11/12
5	GM in male region	1 pair, xviii	Absent	1 pair, xvii	1 pair, xvii
6	Testes sacs	Separated	Connected	Connected	Connected
7	Intestinal origin	xv	xvi	xvi	?
8	Prostate glands	Poorly lobuled	Deeply lobuled	Deeply lobuled	Deeply lobuled

GM: Genital markings; 1: Gates (1935) and Chen (1936); 2: Thai & Samphon (1988); 3: Michaelsen (1934).

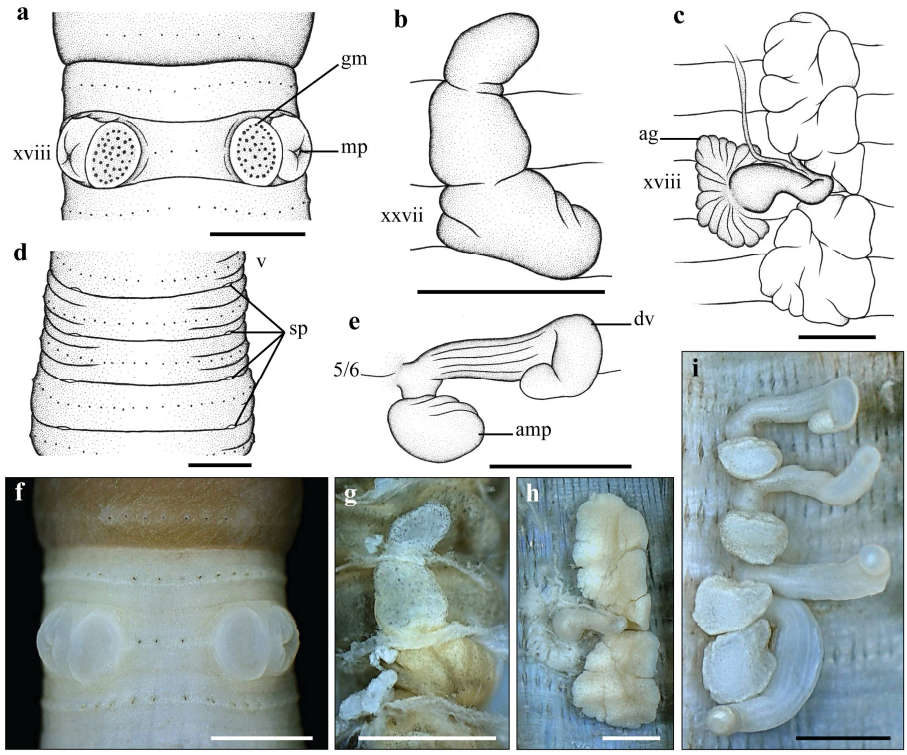

Figure 4. Metaphire grandiverticulata Nguyen & Lam, sp. n.

a, f: Male pore region; b, g: Intestinal caecum; c, h: Prostate glands, right side; d: Spermathecal region, ventral view; e, i: Spermathecae, right side. Scale bar = 1 mm.

Etymology: *"grandiverticulata"* is an adjective derived from Latin "*grandis*" and "*diverticula*" to emphasize the large spermathecal diverticulum.

Description: *External characters*: Body cylindrical, small. Length 75.3±9.2 mm (N=10), diameter ca. 2.9±0.9 mm, weight 0.35±0.16 g, 106±19 segments. Dorsum brownish grey, ventrum paler. Prostomium 1/2 epilobous. First dorsal pore in 12/13. Pre-clitellar setae stouter and thicker than post-clitellar, 45-65 in viii, 35-49 in xxx, 2-7 between male porophores in xviii; setal distance aa = 1.2-2ab, zz = 1.2-2zy. Clitellum annular, xiv-xvi, blackish brown, smooth; dorsal pores absent, setae small at ventrum. Female pore single, mid-ventral on xiv.

Spermathecal pores four pairs, in lateroventral 5/6/7/8/9. Genital markings absent in the spermathecal region. Male pores located inside copulatory pouches which opening ventrally on setal ring in xviii; ventral distance between openings of copulatory pouches about 0.35x body circumference. A pair of elipsoidal genital markings in xviii, located next to and as large as male porophores.

Internal characters: Septa 5/6/7/8 thickened, 8/9/10 absent, 10/11/12/13 thin. Oesophageal gizzard large, within viii-x. Intestine origin at xv; caeca simple, within xxvii-xxv. Last heart in xiii. Pharynegal micronephridia well developed in 5/6/7. Lymph glands sac-shaped, present from 27/28. Typhlosole simple, lamelliform; T% = 16%.

Spermathecae four pairs in vi-ix. Ampulla small, heart-shaped; ducts muscular, extremely short. Diverticulum longer than ampulla, stouter than ampulla ducts, sometimes enlarged at the middle, and attached to the base of ampulla duct; seminal chamber small, bullet-shaped. Accessory glands absent in the spermathecal region.

Holandric, testes sacs in x and xi, horseshoe-shaped, and separated. Seminal vesicles well developed in xi and xii. Ovaries

paired in 12/13. Ovisacs invisiable. Prostate glands racemose, poorly lobuled, paired within xvi-xxi; prostatic ducts short, enlarged at the base like a small bulb. Accessory glands sessile, located next to prostatic ducts.

Remarks: The new species is somewhat similar to *M. bucculenta* (Gates, 1935), *M. neoexilis* (Thai & Samphon, 1988), and *M. varellana* (Michaelsen, 1934), by having four pairs of spermathecal pores in 5/6/7/8/9, genital markings in the male region, intestinal caeca simple. However, the new species is different from those species in having genital markings one pair in xviii while that character is absent in *M. bucculenta*, one pair in xvii in *M. neoexilis* and *M. varellana*. Moreover *M. grandiverticulata* is also distinguished by having separated testes sacs while others have connected testes sacs. In addition, the new species also have some differences from those species for each one in prostomium, first dorsal pore, and prostate glands (table 1). *M. grandiverticulata* sp. n. has a pair of genital markings on setal line in xviii, separated testes sacs while *M. bucculenta* has genital markings paired and be pre-setal on xviii, ventrally connected testes sacs.

***Metaphire xuanlocensis* Nguyen & Lam, sp. n.** (Fig. 5, Table 2)

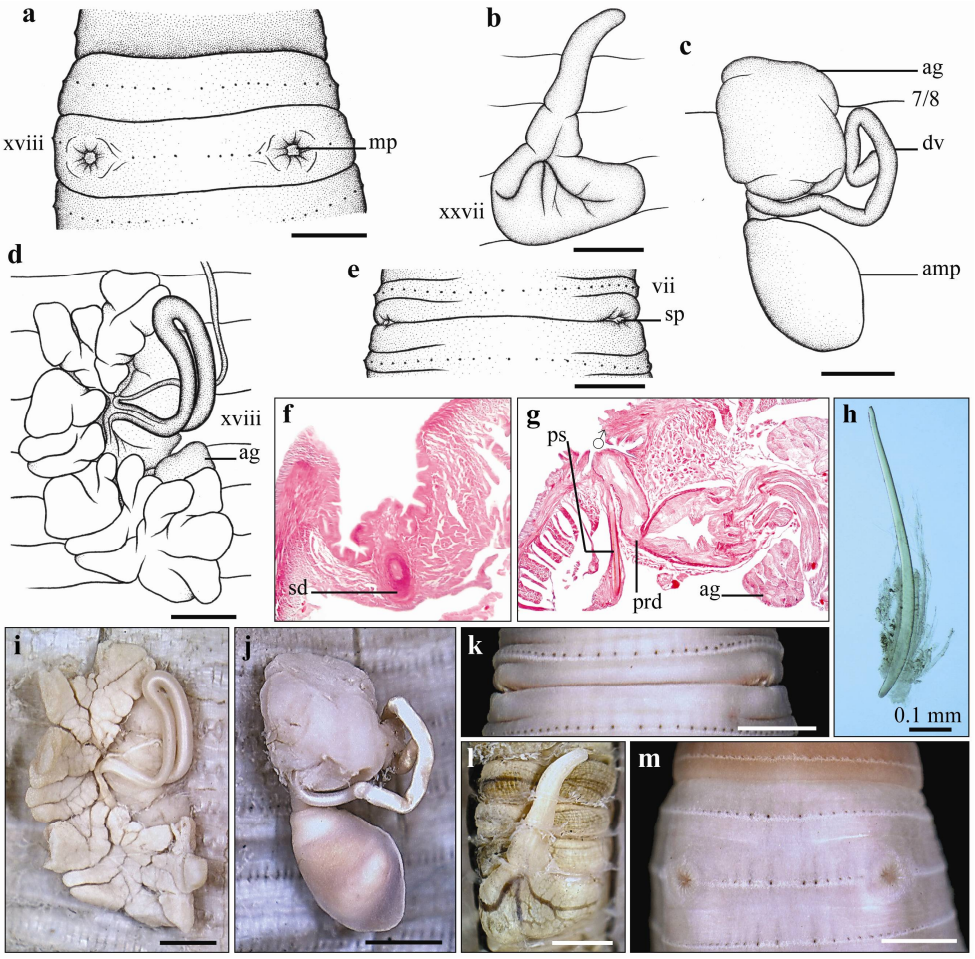

Figure 5. Metaphire xuanlocensis Nguyen & Lam, sp. n.

a, m: Male pore region, ventral view; b, l: Intestinal caecum; c, j: Spermathecae, left side; d, i: Prostate glands, left side; e, k: Spermathecal region, ventral view; f: Spermathecal chamber, transverse body section; g: Copulatory pouch, transverse body section; h: Penial setae. Scale bar = 1 mm.

Table 2: Character comparison among *Metaphire xuanlocensis* sp. n., *M. phaluongana* (Do & Huynh, 1992), *M. dacnomontis* (Thai & Huynh, 1992), and *M. arrobustoides* (Thai, 1984)

STT	Đặc điểm	*M. xuanlocensis*	*M. phaluongana* (1)	*M. dacnomontis* (1)	*M. arrobustoides* (2)
1	Length (mm)	85.4±14	52-90	180	>90
2	Diameter (mm)	3.41±0.56	3-4	6.5-7	4.5-5
3	Spermathecal pores	7/8	7/8	6/7	7/8/9
4	Spermathecal chamber	Present	Absent	Absent	Absent
5	First dorsal pore	11/12 or 10/11	11/12	12/13	11/12
6	Penial setae	Present	Absent	Present	Present
7	GM in male region	Absent	Absent	Absent	1 pair, xviii
8	GM in spermathecal region	1 pair, 7/8	Absent	Absent	Absent
9	Testes sacs	Separated	Connected	Connected	Connected
10	Origin of diverticulum	Middle of ampulla duct	Base of ampulla duct	Base of ampulla duct	Base of ampulla duct
11	Intestinal caeca	Simple	Simple	Simple	Separated

GM: Genital markings; 1: Thai et al. (1992); 2: Thai (1984).

Examined material: *Holotype*: 1 mature (CTU-EW.086.h01), mango garden (10°48’57.9’’N; 107°32’32.9’’E), Xuan Hoa commune, Xuan Loc district, Dong Nai province, 11/09/2012, coll. Nguyen Van Thang.

Paratypes: 9 matures (CTU-EW.086.p02), same data as for holotype.

Further materials: 17 matures (CTU-EW.086.03), plantations (10°42’45.2’’N; 107°19’29.0’’E), Lam Son commune, Cam My district, Dong Nai Province, 22/10/2013, coll. Le Van Nhan.

Diagnosis: Medium worm, length 81-122 mm (85.4±14 mm), diameter ca. 3.56-4.18 mm (3.41±0.56 mm). Body uniformly brownish. One pair of spermathecal pores in lateroventral 7/8. Genital markings invisible. Penial setae present. Accessory glands attached to the enlarged base of prostatic duct. Holandric. Intestinal caeca simple. Septa 8/9/10 absent.

Etymology: Named after the type locality, Xuan Loc District.

Vietnamese name: Giun xuân lộc.

Description: *External characters*: Body cylindrical, medium. Length 85.4±14mm (N=15), diameter ca. 3.41±0.56 mm, weight 0.8±0.3 g, 110±8 segments. Body uniformly, light brown. Prostomium 1/2 epilobous. First dorsal pore in 11/12, sometimes in 10/11(3 matures). Pre-clitellar and post-clitellar setae not different,61-82 in viii, 58-76 in xxx, 8-13 between male porophores; setal distance aa = 1.2-1.5ab, zz = 1.2-2zy. Clitellum annular, xiv-xvi, reddish brown; setae and dorsal pores absent. Female pore single, mid-ventral on xiv.

Spermathecal pores one pair, inside spermathecal chambers (fig. 5f) which opening lateroventrally in 7/8. Only one pair of small genital markings located next to spermathecal pores in 7/8. Male pores located inside copulatory pouches which opening ventrally on setal ring in xviii; ventral distance between openings of copulatory pouches about 0.35x body circumference. No genital markings in the male region.

Internal characters: Septa 5/6/7/8 thickened, 8/9/10 absent, 10/11/12/13 thin.

Oesophageal gizzard large, within viii-x. Intestinal origin at xv; caeca simple, within xxvii-xxiv or xxvii-xxv. Last hearts in xiii. Pharynegal micronephridia well developed in 5/6/7. Lymph glands lobuled, present from 27/28. Typhlosole simple, lamelliform; T% = 16%.

Spermathecae one pair in viii. Ampulla large, pear-shaped; duct muscular, about a half of ampulla length. Diverticulum slender, attached to the base of ampulla duct; seminal chamber tube-shaped, about a half of diverticulum length, and rolled at distal part. Accessory glands massed, stalked, and covered the base of ampulla and diverticulum.

Holandric, testes sacs paired in x and xi, ventrally, separated. Seminal vesicles well developed, paired in xi and xii. Ovaries poorly developed in xiii. Ovisacs invisible. Prostate glands racemose, deeply lobuled, paired within xvi-xx or xvi-xxi; prostatic ducts long, hairpin-shaped, enlarged at the base. Penial setae J shaped, located next to the prostatic ducts, about 0.7 mm long and 0.025 mm wide with slightly hooked tip which contained a shallow trench at the top (fig. 5h). Accessory glands stalked and attached to the enlarged part of prostatic ducts (fig. 5g).

Remarks:

The new species is similar to *M. phaluongana* (Do et Huynh, 1992) in having one pair of spermathecal pores in 7/8, first dorsal pore present in 11/12, the absence of genital markings in the spermathecal region, intestinal origin at xv, last hearts in xiii, holandric, simple intestinal caeca, and more or less same size. However, the new species differs from *M. phaluongana* in having a pair of genital markings in 7/8, separated testes sacs, presence of penial setae. *M. phaluongana* has no genital markings in the spermathecal region, connected testes sacs and absence of penial setae.

In addition, penial setae are known to be present in *M. dacnomontis* and *M. arrobustoides* (Thai, 1984), but the new species is clearly distinguished from those in some important characters. *M. xuanlocensis* sp. n. has a pair of spermathecal pores in 7/8, a pair of genital markings in the spermathecal region, ventrally separated testes sacs, diverticulum attached to middle of ampulla duct; while *M. dacnomontis* has spermathecal pores in 6/7, no genital markings in the spermathecal region, connected testes sacs, diverticulum attached to base of ampulla duct (table 2). The new species is also distinguished from *M. arrobustoides* in having one pair of spermathecal pores in 7/8, one pair of genital markings in 7/8, separated testes sacs, and simple intestinal caeca; whereas *M. arrobustoides* has two pairs of spermathecal pores in 7/8/9, no genital markings in the spermathecal region but one pair in xviii, connected testes sacs, and serrated intestinal caeca.

CONCLUSION

The recent papers showed the structure of copulatory pouches and the type of accessory glands by some of the body cross section slides (Nguyen, 2016; Nguyen et al., 2017); the receptacles chamber and the penial setae were mentioned in previous papers even though these structure were rarely found in Pheretimoids. Those structure can be useful to explain the value of the taxonomical characters for further classifying of Pheretimoids.

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