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## THE FIRST RECORD OF *Metaphire birmanica* (Rosa, 1888) IN VIETNAM, WITH NOTES ON SEVERAL EARTHWORM SPECIES

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**Abstract.** *The Amynthes and Metaphire species recorded in Vietnam have been rechecked based on original descriptions and preserved specimens. As a result, Metaphire birmanica (Rosa, 1888) is recorded in Vietnam for the first time. The species is recognized by having three pairs of spermathecal pores in 5/6/7/8, male pores in xviii, presence of copulatory pouches, no genital markings, and manicate intestinal caeca. In addition, three species have been rechecked and re-assigned to different genera, namely Amynthes tripidoporophoratus (Thai & Nguyen, 1993) comb. nov., Metaphire dranfocana (Do & Huynh, 1993) comb. nov., Metaphire anhumalatana (Thai & Huynh, 1993) comb. nov.*

**Keywords:** *Amynthes, biodiversity, Megascolecidae, Metaphire, Pheretima, taxonomy, Vietnam*

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### INTRODUCTION

Almost all earthworms of Vietnam belong to the Pheretimoid group in the family Megascolecidae (207 species) (Nguyen et al., 2016a, 2016b, 2017, 2018, 2019; Nguyen & Lam, 2017; Lam et al., 2018). Prior to Nguyen et al. (2016), all Pheretimoid species were classified to only one genus *Pheretima* Kinberg, 1867 (Thai & Tran, 1986; Do, 1994; Nguyen, 1994; Le, 1995; Pham, 1995; Huynh, 1996; Thai, 2000). Although Blakemore (2007) tried to re-classify the Vietnamese earthworms in accordance with the system of Sims & Easton (1972), he seemed to have very superficial work because of lacking descriptive information and examining speci-

mens. Nguyen et al (2016) provided the comprehensive checklist of the Vietnamese earthworms, all Pheretimoid species were listed in 6 genera including two argumentative genera, *Amynthes* (112 species) and *Metaphire* (54 species). In this list, several species were still placed in the same genera following the suggestions of Blakemore (2007). After checking specimens housed in the Can Tho University, we found that the generic positions of several species were wrong, and those species need to be confirmed with new information. Therefore, this work aimed to clarify the generic position of several species and additionally contributes a new record to the earthworm fauna of Vietnam.

## MATERIALS AND METHODS

All species of two genera *Amyntas* and *Metaphire* listed in Blakemore (2007) and Nguyen et al. (2016) were rechecked using the original descriptions and preserved specimens including holotypes and paratypes. Samples observed in this study are being kept in the Laboratory of Zoology, Department of Biology, School of Education, Can Tho University. Earthworms were externally observed, dissected, and photographed under a microscope (Motic DM143-FBGG-C). For those species without preserved specimens, we used their original paper and confirmed it with species' authors. Colour images of specimens and original drawings were provided for the better illustrative comparison.

**Abbreviations:** A = Aclitellate specimen; C = Clitellate specimen; CTU = Can Tho University, Can Tho, Vietnam; SORC = Soil Organism Research Center, Hanoi, Vietnam; ag = accessory gland; amp = ampulla; dv = diverticulum; gm = genital markings; mp = male pore; pn = penis; sg = seminal groove; sp = spermathecal pore.

## RESULTS AND DISCUSSION

### Taxonomic Part

**Family:** Megascolecidae (Rosa, 1891)

**Genus:** *Metaphire* (Sims & Easton, 1972)

*Metaphire birmanica* (Rosa, 1888)

(Figure 1, Table 1)

*Perichaeta birmanica* Rosa, 1888: 164.

*Pheretima birmanica* -- Gates, 1972: 207.

*Metaphire birmanica* -- Sims & Easton, 1972: 239; Blakemore, 2007: 15.

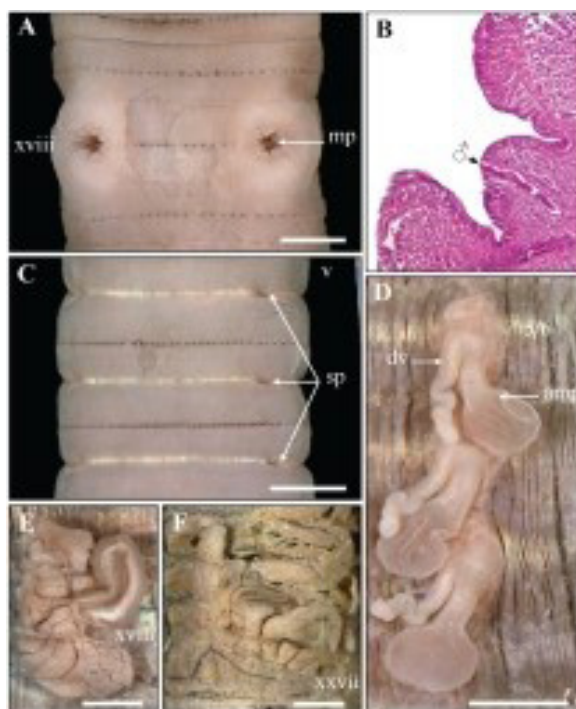


Figure 1. *Metaphire birmanica* (Rosa, 1888)(A. Male region, ventral view; B. The copulatory pouch, transverse body section; C. Spermathecal region, ventral view; E. Prostate gland, left side; F. Intestinal caecum)

**Type locality:** Myanmar (Bhamof) (Rosa, 1888).

**Type materials:** Genoa Museum, Italy.

**Examined material:** 3C (CTU-EW.165.01), Thong Thu commune, Que Phong district, Nghe An province, 21/7/1987, coll. unknown.

**History of materials:** No record

**Diagnosis:** Medium-sized worm, length 92–98 mm, diameter 4.4–4.6 mm, 141–168 segments. Prostomium epilobous. First dorsal pore in 11/12. Three pairs of spermathecal pores in ventrolateral intersegment 5/6/7/8. Male pores in xviii; copulatory pouches present. Genital markings absent in both spermathecal and male regions. Holandric. Testis sacs separated. Intestinal caeca manicate. Septa 8/9/10 aborted.

#### Re-description

External characters: Body cylindrical, medium-sized, length 92–98 mm, diameter 4.4–4.6 mm, weight 0.7–1.0 g, 141–168 segments. Dorsum greyish, ventrum paler. Prostomium epilobous. First dorsal pore in 11/12. Pre-clitellum setae thinner and denser than post-clitellar ones; setal numbers: 109–117 in viii, 89–94 in xxx, 9–11 between two male pores in ventral xviii; setal distance aa=ab, zz=zy. Clitellum annular, xiv–xvi without setae and dorsal pores. Female pore single, in midventral xiv.

Three pairs of spermathecal pores in ventrolateral intersegment 5/6/7/8. Male pores located inside copulatory pouches which opened in the setal ring xviii; ventral distance between two openings of copulatory pouches about 0.35x body circumference. Genital markings absent in both spermathecal and male regions.

Internal characters: Septa 5/6/7/8 thick, 8/9/10 absent, 10/11/12/13 thin. Oesophageal gizzard within viii–x. Intestinal origin in xv; caeca manicate within xxvii–xxiv. Last hearts in xiii. Pharyngeal micronephridia well de-

veloped in 5/6/7. Typhlosole simple, lamelli-form. Lymph glands present from 16/17.

Spermathecae paired in vi–viii. Ampulla oval-shaped, ducts about 1/2 ampulla length. Diverticula shorter than ampulla, strongly waved and directly attached to the base of ampulla duct; seminal chamber small, oval-shaped. No accessory glands.

Holandric. Testis sacs in x and xi, separated. Seminal vesicles in xi and xii. Ovaries well developed in 12/13. Ovisacs are invisible. Prostate glands deeply lobuled, paired in xvi–xxii; duct U-shaped. Accessory glands absent.

**Distribution:** Pakistan, Myanmar, China, Laos, Vietnam (Sarwar et al., 2006; Blakemore, 2006, 2007)

**Vietnamese name:** Giun miền điện

#### Remarks

Rosa (1888) described the species *Perichaeta birmanica* from poorly preserved samples from Burma (currently known as Myanmar). Those samples lack several important organs, and thus, intestinal caeca, prostomium, the ventral distance between male pores were missing in the original description. Gates (1972) re-described the species from newly collected samples in Myanmar. He provided the details of epilobous prostomium, manicate caeca, the ventral distance of male pores about 0.35 body circumference, and copulatory pouches. Recently, this species was also re-described from samples collected in Thailand (Bantaowong et al., 2011), but with the ventral distance between spermathecal pores, and between male pores of about 0.42 body circumference.

Although Blakemore (2006) listed the presence of this species in Vietnam, he has never checked specimens or explained the absence of this species in previous reports. Both the comprehensive checklists of Thai (2000) and Nguyen et al. (2016) did not list the species *Metaphire birmanica*, in Vietnam. There-

fore, we believe that the species has been never officially reported from Vietnam.

After checking the samples deposited in the Can Tho University, we found that these samples belong to the species, *Metaphire birmanica*. Our samples almost agree with the original description, with Gates (1972), and Bantaowong et al. (2011) except for several minor differences. The Vietnamese specimens slightly differ from the descriptions of Rosa

(1888) and Gates (1972) in the first dorsal pore (11/12 vs. 12/13), more segments (141-168 vs. 112), and the longest part of caeca (first vs second). Our samples are also different from Thailand ones (Bantaowong et al., 2011) in the distance of spermatheca pores (0.3X vs. 0.4X); the shape of diverticula (zigzag vs. coiled) and the presence of lymph glands (absent vs. present). The detailed comparison is presented (Table 1).

Table 1. Characteristic comparison of *Metaphire birmanica* among Vietnamese specimens and others

Characteristic	Our specimens (Vietnam)	Rosa (1888) (Myanmar)	Gates (1972) (Myanmar)	Bantaowong et al. (2011) (Thailand)
Length (mm)	92-98	130	100-160	85-210
Diameter (mm)	4.4-4.6	6	4-7	5.8-6.7
Segments	141-168	112	112	105-111
Spermathecal pores	5/6/7/8	5/6/7/8	5/6/7/8	5/6/7/8
Ventral distance between spermathecal pores	0.35X	0.42X	0.35X	0.45X
Prostomium	Epilobous	n/a	Epilobous	Epilobous
First dorsal pore	11/12	12/13	12/13	12/13
Pre-clitellum setae	109-117	70	70?	65-70
Post-clitellum setae	89-94	70	70?	63-69
Male pores	xviii, on conical penial body in small copulatory pouch	xviii, on slightly bulging pale areolas	xviii, on conical penial body in small copulatory pouch	xviii, onto the tumescent lips
Ventral distance between male pores	0.35X	0.42X	?	0.3X
Genital markings	Absent	Absent	Absent	Absent
Ampulla	Oval	Oval	?	Large sac
Shape of diverticulum	Zigzag	Convolutud tube	Looped, zigzag	Coiled
Caeca (parts)	Maniccate (4-6 parts)	?	Maniccate (3-6 parts)	Maniccate (3-6 parts)
Longest parts of caeca	First	?	Second	Second
Male organ system	Holandric	Holandric	Holandric	Holandric
Testis sacs	Separated	?	Unpaired?	Paired
Last heart	xiii	?	xiii	xiii
Origin of intestine	xv	?	xv	xv
Typhlosole	Lameliform	?	Lameliform	Present
Prostate glands	Deeply lobuled in xvi-xxii	Deeply lobuled in 3 segments	Deeply lobuled in xvi-xx	Deeply lobuled in xvii-xxi
Prostatic duct	U-shaped	Curved	U-shaped loop	U-shaped
Lymph glands	Absent	?	?	Post caecal segment



*Metaphire anhumalatana* (Thai & Huynh, 1993) comb. nov. (Figure 2-A)

*Pheretima anhumalatana* Thai & Huynh, 1993 (in: Thai et al., 1993): 14, fig. 1D; Thai, 2000: 307.

*Amyntas anhumalatanus* -- Nguyen et al., 2016: 14

*Amyntas? anhumalatanus* -- Blakemore 2007: 7.

**Type locality:** Vietnam (Dak Lak: Yok Don province) (Thai et al., 1993).

**Type materials:** lost.

**Examined material:** no

**History of materials:** SORC, Vietnam. (Nguyen et al., 2016)

**Diagnosis:** Medium-sized worm, length 110 mm, diameter 3.0–3.5 mm, 120 segments. Prostomium epilobous. First dorsal pore in 12/13. Four pairs of spermathecal pores in ventrolateral intersegment 5/6/7/8/9. Male pores in xviii; copulatory pouches present. Genital markings absent. Intestinal caeca simple. Septa 8/9/10 absent.

**Distribution:** Only known from Vietnam.

**Vietnamese name:** Giun cận nhumatat (Nguyen et al., 2016).

**Remarks:** Blakemore (2007) put the species into the genus *Amyntas*, but still with a question mark: *Amyntas? anhumalatanus*. Nguyen et al. (2016) confirmed this placement. However, it was a mistake because Thai et al. (1993) already mentioned male pores located inside copulatory pouches "*Lỗ đực trong buồng giao phối*" (Figure 2-A). They also remarked that paratypes had variations in the number of spermathecal pores. One individual has 3 pairs of spermathecal pores in 5/6/7/8 while another has 4 pairs in 5/6/7 and 8/9/10.

*Metaphire dranfocana* (Do & Huynh, 1993) comb. nov. (Figure 2-B).

*Pheretima dranfocana* Do & Huynh, 1993 (in Thai et al. 1993): 12, fig. 1A; Thai, 2000: 308.

*Amyntas dranfocanus* -- Nguyen et al., 2016: 25.

*Amyntas? dranfocanus* -- Blakemore, 2007: 31.

**Type locality:** Vietnam (Dak Lak: Yok Don province) (Thai et al., 1993).

**Type materials:** CTU, Vietnam.

**Examined material:** 1C holotype (CTU-EW.134.h01) and 1C paratype (the tail was lost) (CTU-EW.134.p02) natural forest, Drang Phok village, Krong Na commune, Buon Don district, Dak Lak province, 01/10/1989, coll. Huynh Thi Kim Hoi.

**History of materials:** 2C (SORC-HPV.040) same data as for holotype and paratype (Nguyen et al., 2016).

**Diagnosis:** Medium-sized worms. Length 110 mm, diameter ca. 3.0 mm, 75 segments. Prostomium epilobous. First dorsal pore in 12/13. Two pairs of spermathecal pores in ventrolateral intersegment 7/8/9. Male pores in xviii; copulatory pouches present. Two pre-setal pairs of genital markings in xviii and xix. Holandric. Testis sacs separated. Intestinal caeca simple. Septa 8/9/10 absent.

**Distribution:** Only known from Vietnam.

**Vietnamese name:** Giun đấng phók.

**Remarks:** Blakemore (2007) put the species into the genus *Amyntas*, but still with a question mark: *Amyntas? dranfocanus*. Nguyen et al. (2016) confirmed this placement. However, it is a mistake because Thai et al. (1993) already mentioned male pores located deeply inside copulatory pouches ("*Lỗ đực ở đáy buồng giao phối sâu*") and prostatic ducts enlarged basally and ending in a coelomic copulatory chamber ("*góc cuống nổi rõ buồng giao phối*") (Thai et al., 1993).

**Genus:** *Amyntas* Kinberg, 1867

*Amyntas tripidoporophoratus* (Thai & Nguyen, 1993) comb. nov. (Figure 2-C).

*Pheretima tripidoporophorata* Thai & Nguy-

en, 1993 (in Nguyen, 1993): 5, fig. 1A; Pham, 1995: 95; Nakamura, 1999: p. 24; Thai, 2000: 310.

*Metaphire triporophoratus* (sic!) -- Nguyen et al., 2016: 68.

*Amyntas? triporophoratus* -- Blakemore 2007: 100.

**Type locality:** Vietnam (Thua Thien Hue: Hue city) (Nguyen, 1993).

**Type material:** CTU, Vietnam.

**Examined material:** 6C (CTU-EW.141.01) An Le, Huong Dien, Hue, 15/5/1985, coll. Nguyen Van Thuan.

**History of materials:** 6C and 1A (SORC-V.095.04) same data as for (CTU-EW.141.01) (Nguyen et al., 2016).

**Diagnosis:** Medium-sized worm, length 55–71 mm, diameter 2.0–3.8 mm, 119–147 segments. Prostomium epilobous. First dorsal pore in 12/13. One pre-setal pair of spermathecal pores in ventrolateral vi. Male pore with short penis located on triangle porophore included longitudinal and transversal seminal grooves in xviii; copulatory pouches absent. Genital markings absent. Holandric. Testis sacs connected. Intestinal caeca simple. Septa 8/9/10 absent.

**Distribution:** Only known from Vietnam.

**Vietnamese name:** Giun nhú đực 3 cánh (Nguyen et al., 2016).

**Remarks:** The species was described as *Pheretima triporophorata*, then transferred to the genus *Amyntas* by Blakemore (2007) with the question mark "*Amyntas? triporophoratus*". Nguyen et al. (2016) transferred this species to the genus *Metaphire* but in the misspelled name "*Metaphire triporophoratus*". After rechecking specimens, we found that the species lacks copulatory pouches. Therefore, the species should be in the genus *Amyntas*.

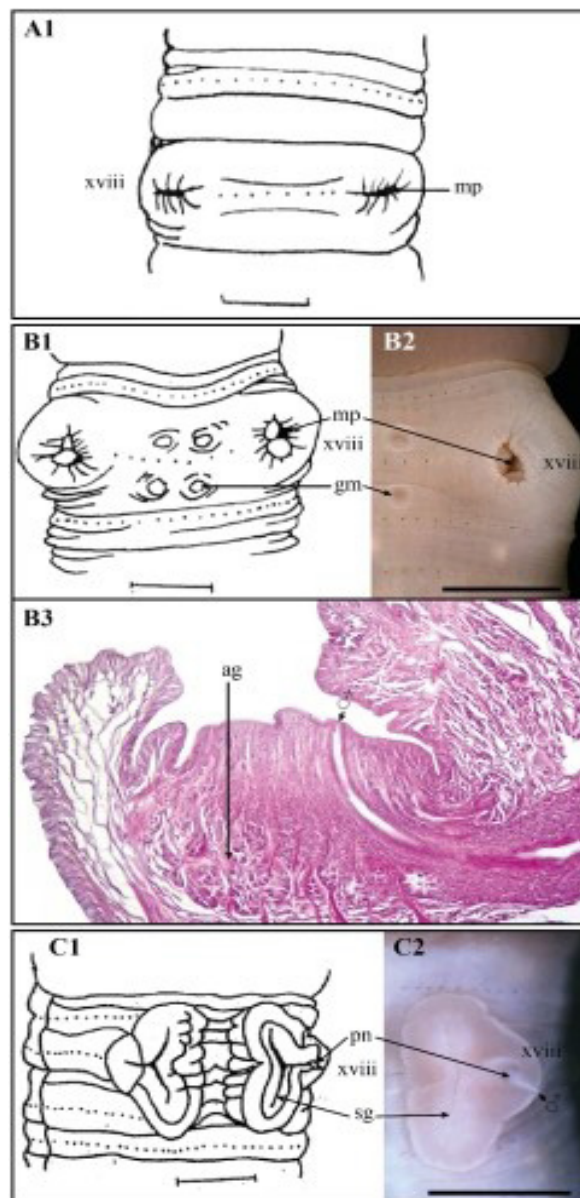


Figure 2. Male pores region of A-*Metaphire anhumalatana* (Thai & Huynh, 1993), B-*Metaphire dranfocana* (Do & Huynh, 1993) and C-*Amyntas triporophoratus* (Thai & Nguyen, 1993) (A1, B1. after Thai et al., 1993; C1. after Nguyen, 1993; B2, C2. from samples; C3. The copulatory pouch, transverse body section from sample)

With the re-confirmation of the generic position of three species and the discoveries of *Metaphire birmanica*, the number of earthworm species increases to 220 in Vietnam, of which the genus *Amyntas* has 111 species, *Metaphire* has 56 species. However, the earthworm fauna of Vietnam is far from fully understanding, more intensive studies will reveal more new discoveries.

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