



Seven new species of the earthworm genus *Metaphire* Sims & Easton, 1972 from Thailand (Clitellata: Megascolecidae)

UEANGFA BANTAOWONG^{1,2}, RATMANEE CHANABUN³, SAMUEL W. JAMES⁴ & SOMSAK PANHA^{2,5}

¹Biological Sciences Program, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

²Animal Systematics Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
E-mail: somsak.pan@chula.ac.th and uengfa@yahoo.com

³Program in Animal Science, Faculty of Agricultural Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon 47000, Thailand
E-mail: cratmanee@yahoo.com

⁴Department of Biology, University of Iowa, Iowa City, Iowa, USA 52242. E-mail: samuel-james@uiowa.edu

⁵Corresponding author

Abstract

Earthworm specimens collected from various parts of Thailand were found to contain seven new species of the genus *Metaphire* Sims & Easton, 1972. These are *M. songkhlaensis* sp. n. in the octothecal *pulauensis* species group, *M. trangensis* sp. n. in the octothecal *ignobilis* species group, *M. khaoluangensis* sp. n. and *M. khaochamao* sp. n. in the sexthecal *houletti* species group, *M. doiphamon* sp. n. in the sexthecal *peguana* species group, *M. saxicalcis* sp. n. in the quadrithecal *planata* species group, and the bithecal *M. surinensis* sp. n. Type material of some established species from Thailand or northern Malaysia was reinvestigated and illustrated to confirm the status of the new species and to facilitate species comparisons: *M. pulauensis* (Beddard, 1900), *M. baruana* (Stephenson, 1932), both with newly designated lectotypes, and *M. planata* (Gates, 1936), illustrated and redescribed.

Key words: *Metaphire*, Clitellata, Oligochaeta, taxonomy, Thailand

Introduction

The genus *Metaphire* Sims & Easton, 1972 is one of the largest amongst the earthworm family Megascolecidae and one of the dominating genera of earthworms in East and Southeast Asia. This genus currently contains about 200 nominal species—most of them distributed among 22 informal species groups—and is characterized by copulatory pouches that contain the male pores, intestinal caeca that originate in the 27th segment, and a lack of nephridia on the spermathecal ducts (Sims & Easton 1972, Blakemore 2012).

Several surveys of terrestrial earthworms in Thailand have been published (Gates 1939, 1972; Somniam & Suwanwaree 2009; Bantaowong *et al.* 2011a, 2011b; Blakemore 2011; Bantaowong *et al.* 2014), but many localities in Thailand and nearby areas and various types of microhabitats are still unexplored. Recently, Bantaowong *et al.* (2014) summarized the knowledge about the Thailand earthworm fauna and reported 45 species. The genus *Metaphire* is very abundant, diverse and widespread, and represented by 10 nominal species: *M. anomala* (Michaelsen, 1907), *M. bahli* (Gates, 1945), *M. bipora* (Beddard, 1900), *M. grandipenes* Bantaowong & Panha, 2011, *M. houletti* (Perrier, 1872), *M. peguana* (Rosa, 1890), *M. perichaeta* (Beddard, 1900), *M. planata* (Gates, 1926), *M. posthuma* (Vaillant, 1869) and *M. virgo* (Beddard, 1900). Among these species, *M. peguana* is one of the most common indigenous species in Thailand (Bantaowong *et al.* 2011b; Prasankok *et al.* 2013).

The recently collected specimens of this study were found to be very similar to species described previously from nearby localities in North Malaysia, South Thailand and some parts of Myanmar (Burma). Thus, the type and other reference material of the species in question were reinvestigated during visits to the Natural History Museum (NHMUK), London, 2014, so that they could be redescribed to the same level of detail as in our material. As a result, our specimens were determined to belong to seven new species, morphologically similar but significantly different from those species described previously from the same or nearby areas.

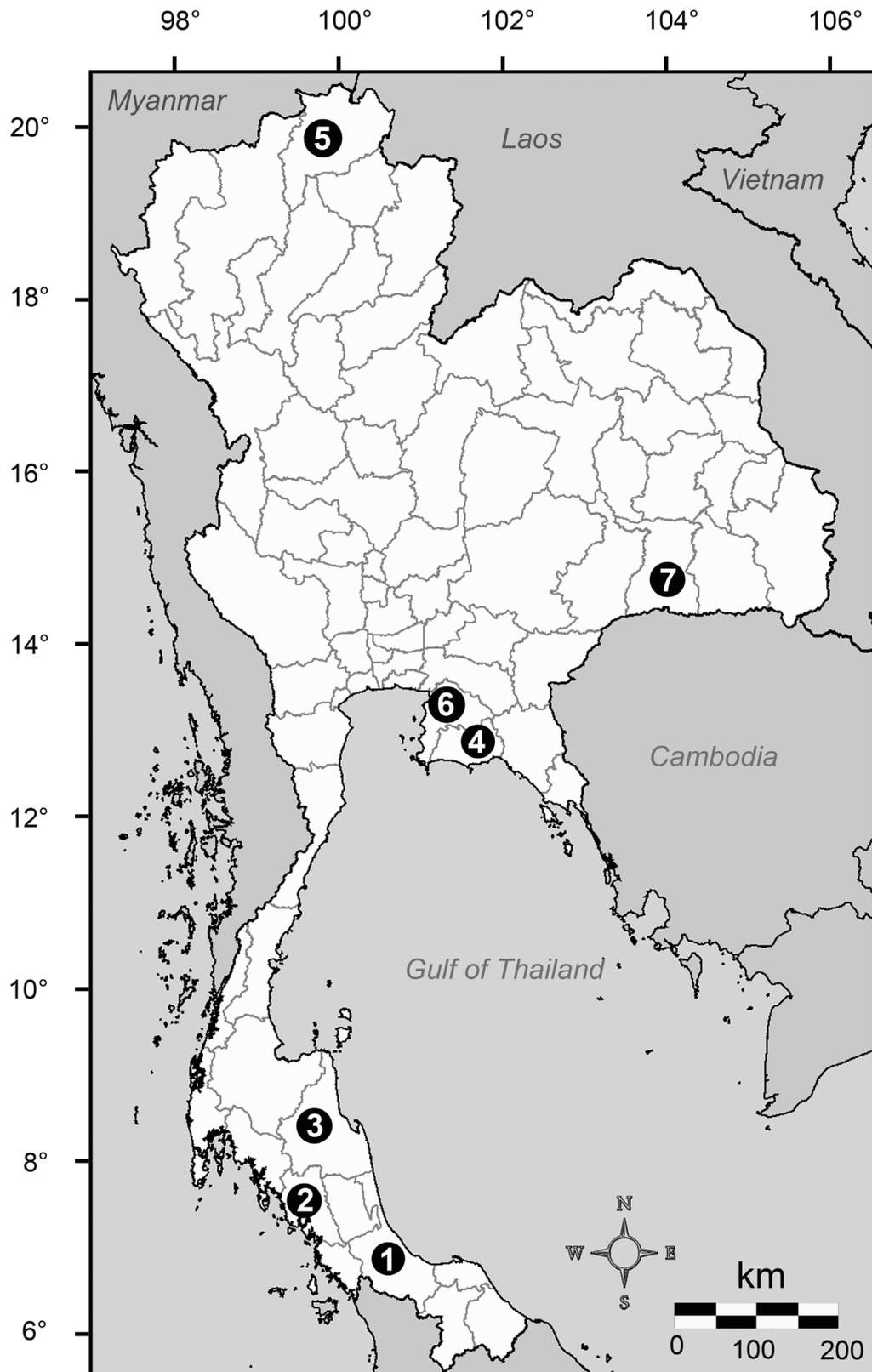


FIGURE 1. Map of type localities of the species of *Metaphire* described in this paper. (1) *Metaphire songkhlaensis* **sp. n.** from Literary Botanical Garden, Hat Yai, Songkhla. (2) *Metaphire trangensis* **sp. n.** from Peninsula Botanical Garden, Na Yong, Trang. (3) *Metaphire khaoluangensis* **sp. n.** from Ban Khlong Phod, Nopphitam, Nakorn Si Thammarat. (4) *Metaphire khaochamao* **sp. n.** from Wat Ma Dua, Khaochamao, Rayong. (5) *Metaphire doiphamon* **sp. n.** from Phu Chee Fah, Thoeng, Chiang Rai. (6) *Metaphire saxicalcis* **sp. n.** from Khao Cha Ang On, Bo Thong, Chonburi. (7) *Metaphire surinensis* **sp. n.** from Pasonnongkoo Forest Park, Sangkha, Surin.

The present study contains the descriptions of these seven new species of *Metaphire* from various parts of Thailand (Fig. 1), and lectotypes are designated for *Metaphire pulauensis* (Beddard, 1900) and *M. baruana* (Stephenson, 1932).

Materials and methods

The earthworms were collected by digging and hand-sorting from several localities in Thailand in 2012–2014 (Fig. 1). They were anesthetized in 30% (v/v) ethanol, fixed in 10% (v/v) formalin and preserved in 75% (v/v) ethanol. The specimens were examined under an Olympus SZX7 stereoscopic light microscope. The descriptions are based on observations of dorsal dissections of the specimens. In the illustrations, we have included both dorsal and ventral views containing important features. Some of the specimens in the same lot were preserved in 95% (v/v) ethanol for further molecular phylogenetic studies.

Some specimens and type specimens housed at the Natural History Museum (NHMUK), London were critically studied for comparison with the new species in this report.

The type series are deposited in the Chulalongkorn University, Museum of Zoology, Bangkok, Thailand (CUMZ). Additional paratypes will be deposited in the Natural History Museum (NHMUK), London, and at the Biozentrum Grindel und Zoologisches Museum, University of Hamburg (ZMH).

Anatomical abbreviations: fp, female pore; gm, genital marking; gmg, genital marking gland; ic, intestinal caeca; mp, male pores; pg, prostate gland; sc, spermathecae; sp, spermathecal pores; sv, seminal vesicles.

Taxonomic part

Metaphire songkhlaensis Bantaowong & Panha, sp. n.

(Figure 2, Table 1)

Material examined. Holotype: clitellate (CUMZ 3373), Thailand, Songkhla, Hat Yai, Literary Botanical Garden, 07°01'11.1"N, 100°17'34.1"E, 89 m above mean sea level (amsl), 17 January 2014, leg. U. Bantaowong, C. Sutcharit, J. Tubtimon & W. Siriwut. Paratypes: 2 adults (CUMZ 3374), 1 adult (NHMUK), same data as holotype.

Etymology. The species is named after the province where it was collected.

Diagnosis. Medium to large worms; length >165–300 mm, >89–116 segments. Setae numbering 60–71 in VII, 74–89 in XX, 20–27 between male pores. Male pores paired, prominent in XVIII, genital markings as papillae in transverse rows on VIII–IX, XIX–XXII, 3–9 papillae per row. Spermathecal pores paired at 5/6–8/9, spermathecal ampulla elongated, sac-shaped, diverticulum rod-shaped. No nephridia on the spermathecal ducts. Holandric, intestinal caeca simple, first dorsal pore in 12/13. Prostate glands large, genital marking glands sessile.

Description of holotype. Dimensions: 300 mm by 9.0 mm at VII, 9.0 mm at XX, 10.0 mm at clitellum. Body cylindrical with 116 segments. Preserved specimen reddish-brown dorsally, grayish-brown ventrally. Setae regularly distributed around segmental equators, numbering 71 at VII, 74 at XX, 20 between male pores; setal formula AA:AB:ZZ:ZY=2:1:1:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular XIV–XVI. Male apertures prominent on ventro-lateral sides in XVIII, 0.22 circumference apart ventrally, distance between apertures 6 mm, penes within small copulatory pouches or everted. Genital markings arranged in transverse rows, pre-setal in XIX–XXII, post-setal in XXI, XXII, each row consisting of 5–7 papillae. Four pairs of spermathecal pores, each marked by a whitish spot in furrows 5/6–8/9, ventral, distance between each pair about 0.37 circumference apart ventrally, distance between spermathecal pores 11 mm on body circumference. Genital papillae arranged in transverse rows centered on mid-ventral line, pre-and post-setal in VIII, IX; four per row in VIII, five per row in IX.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin in XV; the intestinal caeca originating in XXVII, simple, extending to XXIV. Typhlosole rudimentary. Oesophageal hearts four pairs in X–XIII. Holandric; testes and funnels enclosed in paired sacs in X and XI, sacs not connected. Seminal vesicles paired in XI–XII, large, well-developed. Prostate glands well-developed, large, occupying segments XVII to XIX. Prostatic duct c-shaped, copulatory pouch located within the body wall with conical penis

extending outwards to opening. Genital marking glands sessile, corresponding to external genital papillae. Ovaries and funnels in XIII. Four pairs of spermathecae in VI–IX. Ampulla as an elongate sac with short duct; diverticulum rod-shaped in terminal portion, stalk irregular.

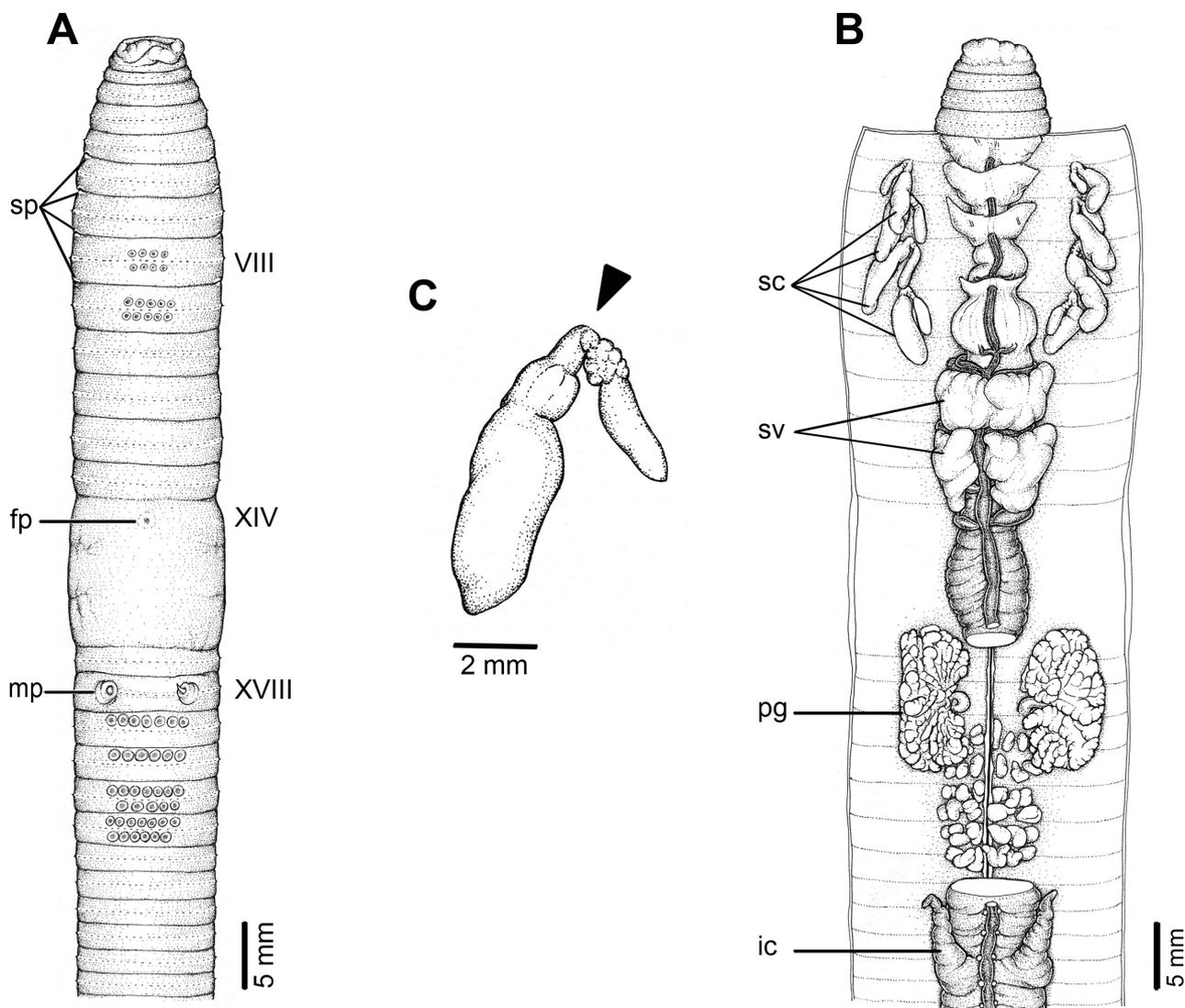


FIGURE 2. External and internal morphology of *Metaphire songkhlaensis* sp. n., holotype (CUMZ 3373). **A.** External ventral view. **B.** Internal dorsal view. **C.** Spermatheca; arrowhead: location of spermathecal pore.

Variation. The holotype is the only complete specimen, with 300 mm body length and 116 segments; all three paratypes are incomplete, longer than 165 mm and with more than 89 segments. There is variation in the number of genital papillae per row: 3–9 papillae on pre-clitellum and 6–8 papillae on post-clitellum. One paratype specimen has an additional papilla in VII and three in XXIII.

Distribution. This species lives in the top soil at about 10–15 cm depth, in the watercourse where it contributed to sedimentary organic matter accumulation, in the Literary Botanical Garden.

Remarks. *Metaphire songkhlaensis* sp. n. is octothecal with spermathecal pores in 5/6–8/9, keying out with the *pulauensis* species group in Sims and Easton (1972), which consists of two species, *M. pulauensis* (Beddard, 1900) and *M. baruana* (Stephenson, 1932) from Malaysia. *Metaphire songkhlaensis* sp. n. is easily distinguished from the two known species of this group by the higher setal number (Table 1). Further important differences are number, shape and position of the genital markings. *Metaphire songkhlaensis* sp. n. has transverse rows of papillae situated in segments VIII, IX, XIX–XXII; each papilla is associated with an internal glandular mass. *M. baruana* has only one field of papillae near the male pore area, on segment XVIII. *M. pulauensis* has numerous genital markings in sieve-like fields on VII, VIII, IX, XVII and XVIII; each of the 40–50 glands per field correspond to an

internal granular mass, but these masses are much less developed than in *M. songkhlaensis*. A further difference is the distance between the openings of the copulatory pouches and number of setae in between: *Metaphire songkhlaensis* has 20 setae between the openings; these are 0.22 body circumference apart (holotype). *M. baruana* and *M. pulauensis* have 10–17 setae between the openings; these are 0.25 body circumference apart. Table 1 summarizes information on the characters that separate the members of the *pulauensis* species group.

***Metaphire pulauensis* (Beddard, 1900)**

(Figure 3, Table 1)

Amyntas pulauensis Beddard, 1900: 904–905, Fig.5.

Pheretima pulauensis (Beddard). Stephenson 1932: 233–236, Fig. 10.

Metaphire pulauensis (Beddard). Sims & Easton 1972: 239.

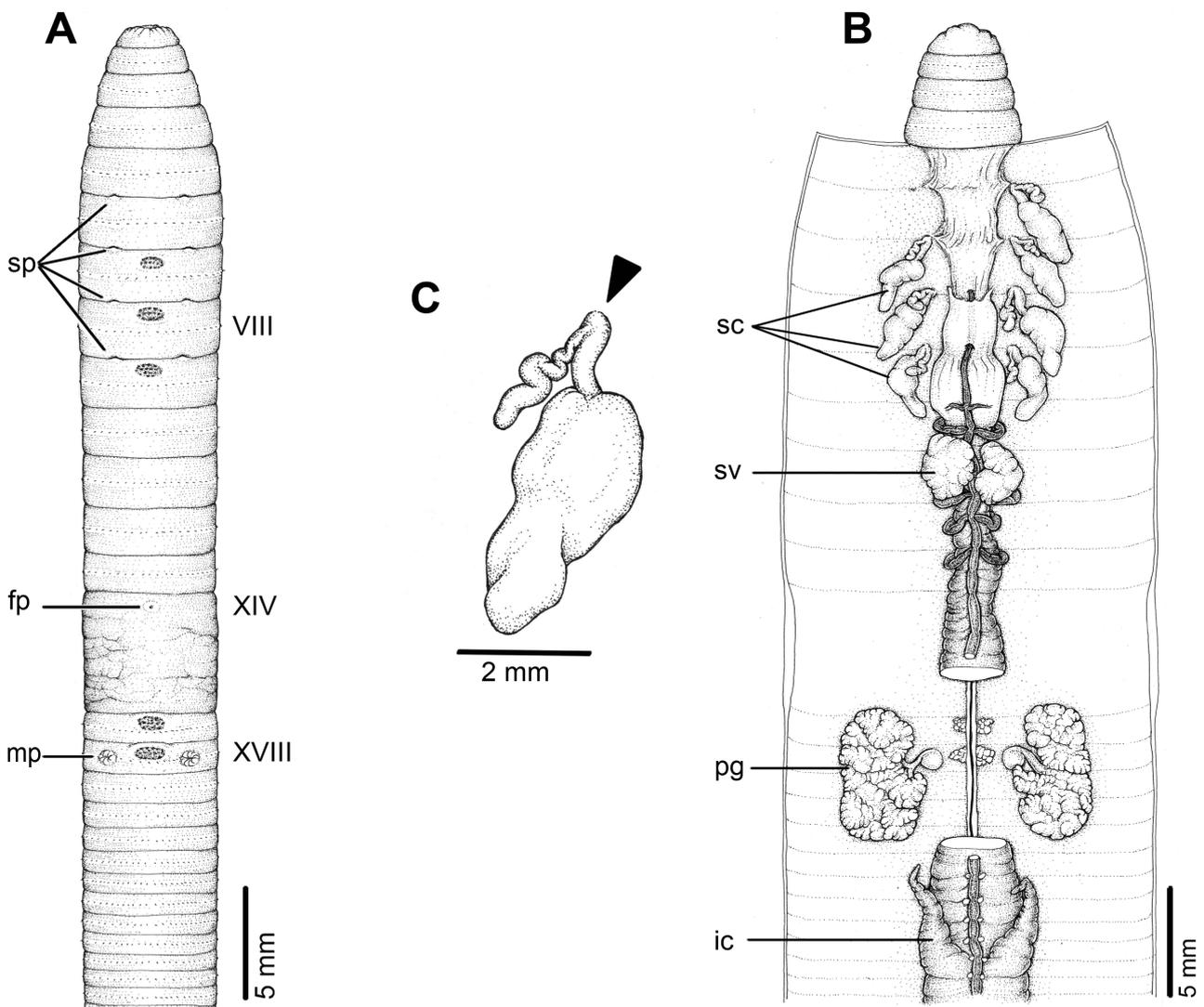


FIGURE 3. External and internal morphology of *Metaphire pulauensis* (Beddard, 1900), lectotype (NHMUK 1924.3.1.214). **A.** External ventral view. **B.** Internal dorsal view. **C.** Spermatheca; arrowhead: location of spermathecal pore.

Material examined. The specimen that contained all the important morphological features and matched the original description is designated herein as the lectotype: NHMUK 1924.3.1.214. Paralectotypes: NHMUK 1924.3.1.215, 1 adult. NHMUK 1904.10.5.1002, 1 adult; same locality as lectotype. The exact location of the type locality is unknown. The locality of all *M. pulauensis* specimens in the Natural History Museum, London, is labelled as 'Pulau Bidan, Kedah, Malay Peninsula' while Beddard (1900: 904) gives the locality as 'Pulau, Bidang,

Kelah, and Aring'. Pulau means "island" in Malay. There is a small island named "Bidan" north of the Pinang Island in Kedah State, northern Malaysia, which may be the type locality.

Diagnosis. Medium-sized, length 143–165 mm with 104–110 segments. First dorsal pore in 12/13. Male pores on XVIII, secondary openings puckered radially around margins. Genital papillae aggregated together to form slightly transversely oval patches, mid-ventral, pre-setal of VII, VIII, IX, XVII and XVIII. Spermathecal pores paired at 5/6–8/9, spermathecal ampulla elongated, sac-shaped, diverticulum twisted into short loops. No nephridia on the spermathecal ducts. Holandric, intestinal caeca simple. Prostate glands large, genital marking glands sessile.

Remarks. The original description (Beddard 1900) was based on an unspecified number of individuals that had been collected by Mr. R. Evans of Oxford, during the Skeat Expedition to the Malay Peninsula in 1899–1900; types were not designated in the original publication. Stephenson, reinvestigating the original material, states the following: "Two specimens, both already dissected, in a jar labelled "Types"; Pulau Bidan, Kedah State; Skeat Expedition; Nov. 23, 1899. A third specimen in another jar, provenance given as Malay Peninsula ... I have examined the two specimens in the first jar, both externally and internally, and the external characters of the third specimen also. The basis of the description is the larger of the two "type" specimens' (Stephenson 1932: 233). No lectotype was designated by Stephenson. We consider all three specimens to belong to the syntype series, and we designate the specimen that contains the important morphological features and matches the original description best as the lectotype (Fig. 3), and the other two as paralectotypes. Both original description (Beddard 1900) and redescription (Stephenson 1932) are detailed.

The type locality of *M. pulauensis* in northern Malaysia is quite close to the type locality of *M. songkhlaensis* sp. n. in southern Thailand. However, *M. pulauensis* can be easily distinguished from *M. songkhlaensis* by the male field puckered radially around the margins and genital markings on segments VII, VIII, IX, XVII and XVIII, while *M. songkhlaensis* sp. n. has prominent male field and no genital markings on segments XVII and XVIII.

This species is apparently known only from the type locality, but the mentioning of "Aring" in the original description may indicate that the species was also found elsewhere.

***Metaphire baruana* (Stephenson, 1932)**

(Figure 4, Table 1)

Amyntas bosschae Horst, 1893 sensu Beddard 1900: 892, nec Horst (Stephenson 1932).

Pheretima baruana Stephenson, 1932: 209–211, Fig. 3.

Metaphire baruana (Stephenson). Sims & Easton 1972: 239.

Material examined. Lectotype NHMUK 1924.10.5.14.1 (designated herein). The type locality is Khota Baru, Malay Peninsula. Paralectotype: one semi-clitellate (NHMUK 1924.10.5.14.2), same locality as lectotype.

Diagnosis. Medium, length 115–132 mm with 118–127 segments. Male pores on prominent protuberances on XVIII, genital markings on the inner slope of the left male porophore. Spermathecal pores paired at 5/6–8/9, spermathecal ampulla pear-shaped, diverticulum slender with small ovoid knob. No nephridia on the spermathecal ducts. Holandric, intestinal caeca simple, first dorsal pore in 12/13. Prostate glands large, genital marking glands sessile.

Remarks. Beddard's record of "*Amyntas bosschae*" was based on an unspecified number of individuals (Beddard 1900). Stephenson (1932: 209) found "Two specimens, both of which had been opened", and recognized them as belonging to a new species. His detailed description was mainly based on one specimen, the larger one of the two, "which may be considered as the type" (ibid.). Since this may not be understood as an explicit type designation, we designate this specimen as lectotype, and the other sub-adult specimen as paralectotype. The lectotype is illustrated in Fig. 4.

Khota Baru, northern Malaysia, the type locality of *M. baruana*, is quite close to Songkhla, south Thailand, the type locality of *M. songkhlaensis* sp. n. However, the distinct genital markings in the male pores area of *M. baruana* are absent in *M. songkhlaensis* sp. n., this being the most significant difference of the new species to *M. baruana*.

This species is known only from its type locality in Kota Bharu, north-eastern Malaysia.

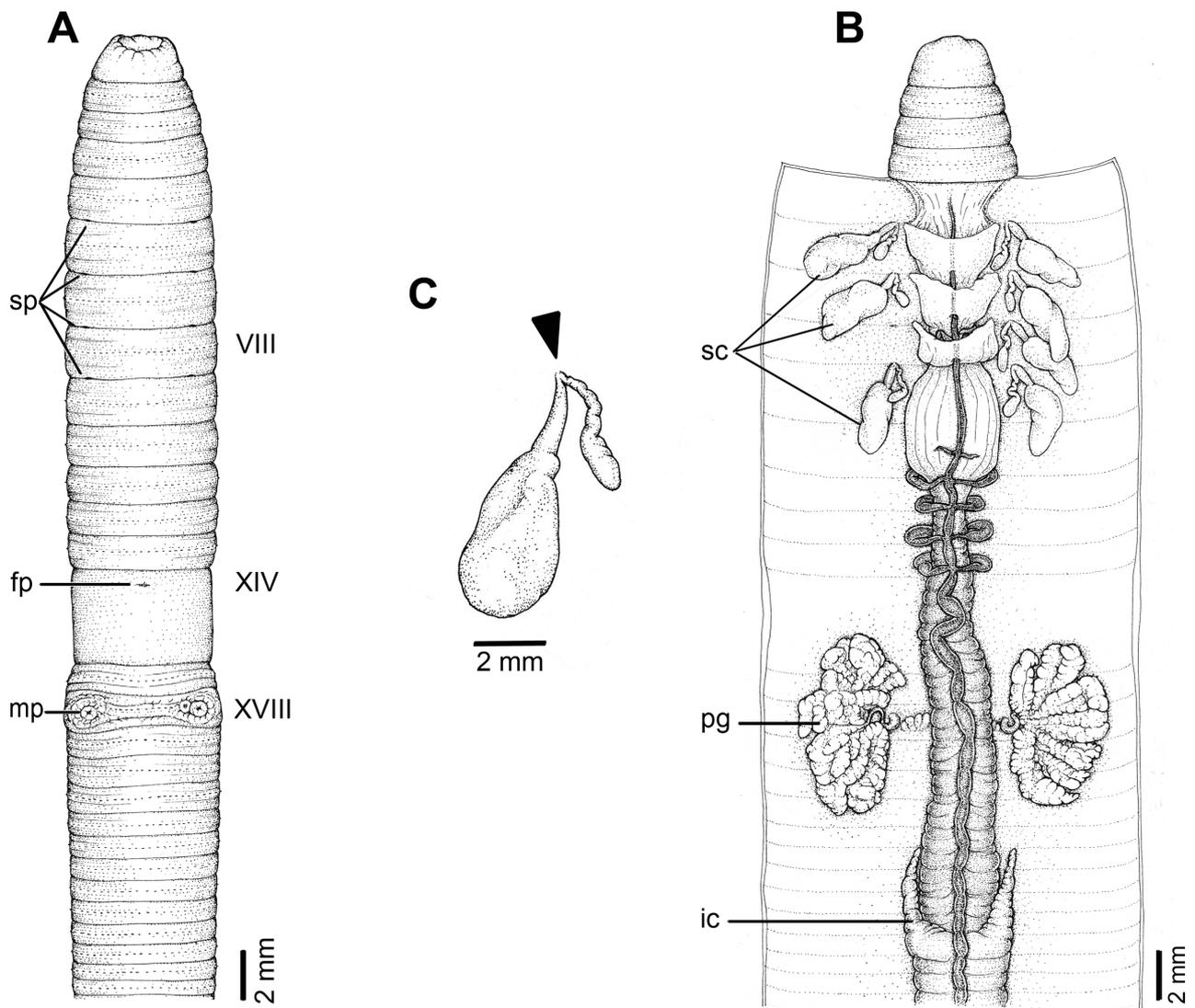


FIGURE 4. External and internal morphology of *Metaphire baruana* (Stephenson, 1932), lectotype (NHMUK 1924.10.5.14.1). **A.** External ventral view. **B.** Internal dorsal view. **C.** Spermatheca; arrowhead: location of spermathecal pore.

TABLE 1. Comparison of morphological characters in the *Metaphire pulauensis* species group. The comma is used to separate body length and width, and setal counts.

	<i>M. songkhlaensis</i> sp. n.	<i>M. pulauensis</i> (Beddard, 1900)	<i>M. baruana</i> (Stephenson, 1932)
Body length, width	300, 9.0 mm	165, 6.0 mm	132, 6.0 mm
Segment number	116	110	127
First dorsal pore	12/13	12/13	10/11, 11/12
Setae VII, XX	71, 74	44, 63	42, 58
Male pore setae	20	11	17
Male pore spacing	0.22	0.25	0.25
Genital markings	8, 9, 19, 20, 21, 22	7, 8, 9, 18, 19	18
Spermathecae	elongate	elongate	pear-shaped
Diverticulum	rod shaped	twisted curve	ovoid knob
Prostate glands in	XVII–XIX	XVII–XX	XVI–XX
Type locality	Thailand	Malaysia	Malaysia

***Metaphire trangensis* Bantaowong & Panha, sp. n.**

(Figure 5)

Material examined. Holotype: clitellate (CUMZ 3375), Thailand, Trang, Na Yong, Peninsular Botanical Garden, 7°32'57.1"N, 99°46'58.3"E, 9 m amsl, 16 January 2014, leg. U. Bantaowong, C. Sutcharit, P. Pimvichai & W. Siriwut. Paratypes: 21 adults (CUMZ 3376), 2 adults (ZMH), 2 adults (NHMUK), same data as holotype.

Etymology. This species is named after the province where it was collected.

Diagnosis. Medium-sized; length 120–169 mm with 100–152 segments. Setae numbering 70–83 in VII, 78–92 in XX, 14–20 between male pores. Male pores paired, secondary openings round, wrinkled radially around margin in segment XVIII, genital markings absent. Spermathecal pores paired in 5/6–8/9. Ampulla oval, diverticulum ellipsoidal, knob-shaped in the terminal portion. No nephridia on the spermathecal duct. Holandric, intestinal caeca simple, first dorsal pore in 11/12. Prostate glands large, its duct C-shaped, with round copulatory sac.

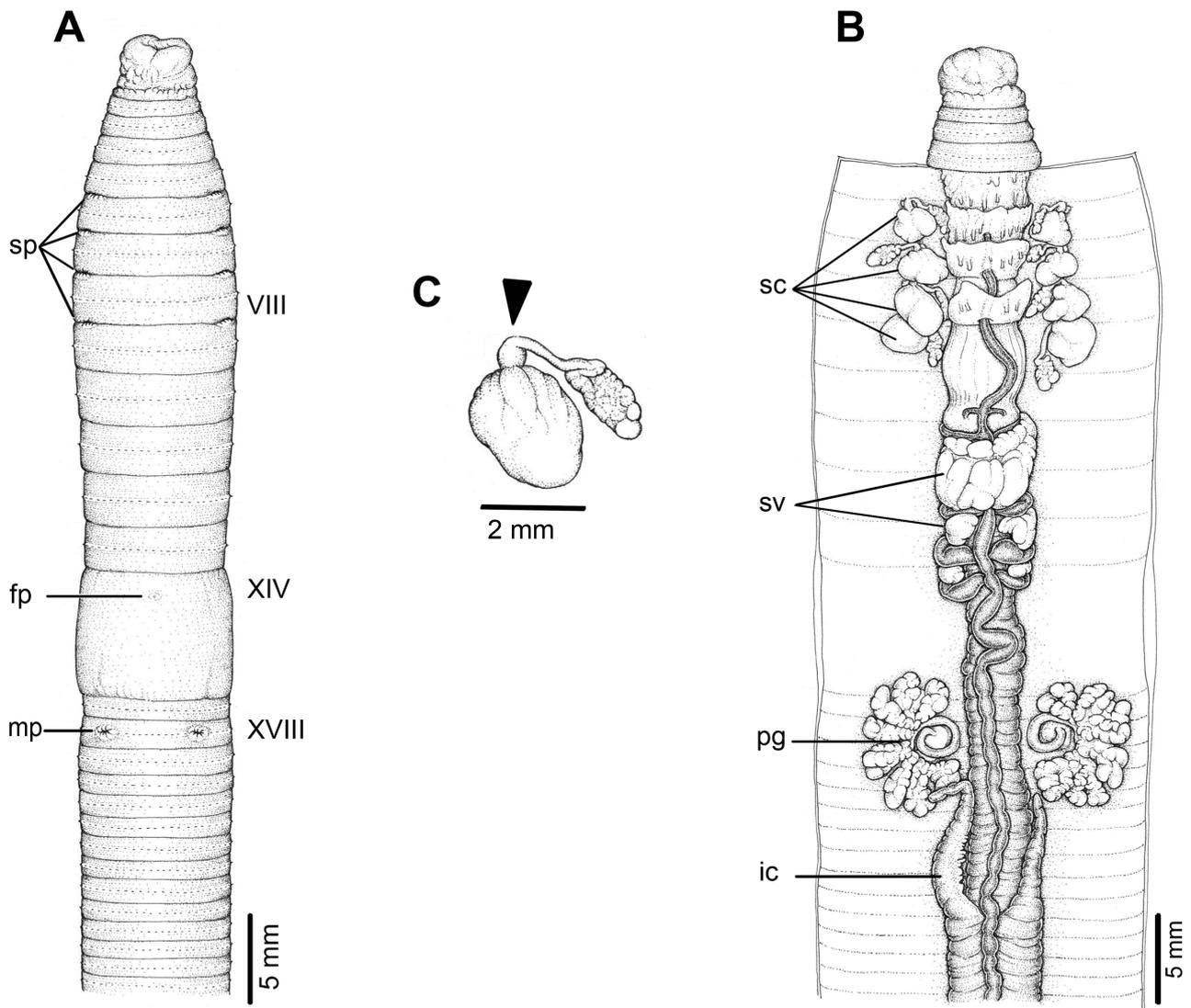


FIGURE 5. External and internal morphology of *Metaphire trangensis* sp. n., holotype (CUMZ 3375). **A.** External ventral view. **B.** Internal dorsal view. **C.** Spermatheca; arrowhead: location of spermathecal pore.

Description of holotype. Dimensions 155 mm by 7.3 mm at segment VII, 7.3 mm at segment XX, 7.6 mm at clitellum; body cylindrical with 100 segments. Preserved specimen reddish-brown dorsally, light grayish brown ventrally. Setae regularly distributed around segmental equators, numbering 70 at VII, 88 at XX, 20 between male pores, setal formula AA:AB:ZZ:ZY=1:1:1:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 11/12. Clitellum annular XIV–XVI. Male pores on XVIII, secondary openings transversely oval, wrinkled

radially around margins, 0.27 circumference apart ventrally, distance between openings of copulatory pouches 6 mm. Spermathecal pores four pairs, epidermis around each pore slightly wrinkled, in furrows 5/6–8/9, ventral, pores about 0.40 body circumference ventrally apart, distance between spermathecal pores 10 mm on body circumference. No genital markings in the spermathecal pores region.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin in XV, intestinal caeca originating in XXVII, simple, extending to XXI, typhlosole rudimentary. Oesophageal hearts four pairs in X–XIII. Holandric; testes and funnels enclosed in paired sacs in X and XI. Seminal vesicles paired in XI–XII. Prostate glands large, extending anteriorly to XVII, posteriorly to XXI. Prostatic duct C-shaped, coiled around round copulatory sac in XVIII; coiled clockwise left side, anti-clockwise right side. Ovaries in XIII. Spermathecae four pairs in VI–IX. Ampulla large, oval with much shorter duct sharply marked off from the ampulla, diverticulum ellipsoidal with tubercle of circular outline, slightly dilated to form a small ovate knob.

Variation. The 25 paratypes are 120–169 mm long (142.53 ± 15.24 mm), with 100–113 segments. The prostate glands may begin in XVI and end in XXII, and the intestinal caeca may extend to XXIV only.

Distribution. Known only from the type locality. The species was found at about 10–15 cm soil depth.

Remarks. *Metaphire trangensis* **sp. n.** is octochaetal with spermathecal pores in 5/6–8/9, and it has no genital markings. This species keys out with the *ignobilis* species group, which contains six species (Sims & Easton 1972): *M. dalatana* (Michaelsen, 1934) and *M. langbiangi* (Michaelsen, 1934) from Vietnam, *M. ignobilis* (Gates, 1935) from China, *M. feuerborni* (Michaelsen, 1932) from southern Sumatra, *M. riukuensis* (Ohfuchi, 1957) and *M. tosaensis* (Ohfuchi, 1938) from Japan. The first four species are smaller than *M. trangensis* **sp. n.**, their body length and diameter not exceeding 100 mm and 2.5–3 mm, respectively. The two species from Japan are about as large as *M. trangensis* **sp. n.** The new species differs from them in the aspect of the male field. The secondary male openings of *M. trangensis* **sp. n.** have the shape of transverse slits invaginated into segment XVIII, while *M. riukuensis* has seminal grooves from segment XVII to XIX, and *M. tosaensis* has male openings on slightly elevated oblong areas. Moreover, both *M. riukuensis* and *M. tosaensis* have serrate intestinal caeca and elongate spermathecal diverticula.

***Metaphire khaoluangensis* Bantaowong & Panha, sp. n.**

(Figure 6, Table 2)

Material examined. Holotype: clitellate (CUMZ 3377), Thailand, Nakhon Si Thammarat, Nopphitam, Ban Khlong Phod, a village in the Khaoluang mountain range, 8°48'8.4"N, 99°35'18.6"E, 140 m amsl, 17 January 2013, leg. U. Bantaowong, C. Sutcharit & W. Siriwut. Paratypes: 22 adults (CUMZ 3378), 2 adults (ZMH), 2 adults (NHMUK), same data as holotype.

Other material examined. Two adults (CUMZ 3379), Thailand, Nakhon Si Thammarat, Nopphitam, Krungching Waterfall, 8°43'34.8"N, 99°39'58.4"E, 208 m amsl, 17 January 2013. 9 adults (CUMZ 3380), Thailand, Nakhon Si Thammarat, Nopphitam, Tham Lod, 8°47'17.2"N, 99°38'40.1"E, 97 m amsl, 17 January 2013. 4 adults (CUMZ 3381), Thailand, Phatthalung, Srinagarindra, Tham Phutthakodom, 7°33'38.5"N, 99°53'5.6"E, 54 m amsl, 16 January 2013.

Diagnosis. Medium-sized; length 130–265 mm with 113–132 segments. Setae numbering 36–47 in VII, 49–59 in XX, 10–14 between male pores. Male pores paired, secondary opening with puckered margin on segment XVIII, no genital markings. Spermathecal pores paired in segments 6/7–8/9. Ampulla elongate sac-like, with short duct, diverticulum slender, constricted with round seminal chamber. No nephridia on the spermathecal duct. Holandric, intestinal caeca simple, first dorsal pore in 12/13. Prostate glands well developed, its duct embed in the copulatory sac.

Etymology. This new species was named after Mt. Khaoluang where it was collected.

Description of holotype. Dimensions 220 mm length by 10.0 mm width at segment VII, 9.0 mm at XX, 9.0 mm at clitellum; body cylindrical with 119 segments. Preserved specimen reddish-brown on head and dorsum, light-brown ventrally, and darkish purple-brown around clitellum. Setae regularly distributed around segmental equators, numbering 36 at VII, 51 at XX, 14 setae between male pores, setal formula AA:AB:ZZ:ZY=1:1:1:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular XIV–XVI. Male pores conspicuous, secondary openings with puckered margin, separated from each other by 0.28

circumference ventrally, distance between openings of copulatory pouches 7 mm. Spermathecal pores three pairs in 6/7–8/9, epidermis around each pore slightly wrinkled, about 0.44 circumference apart ventrally, distance between spermathecal pores 11 mm on body circumference.

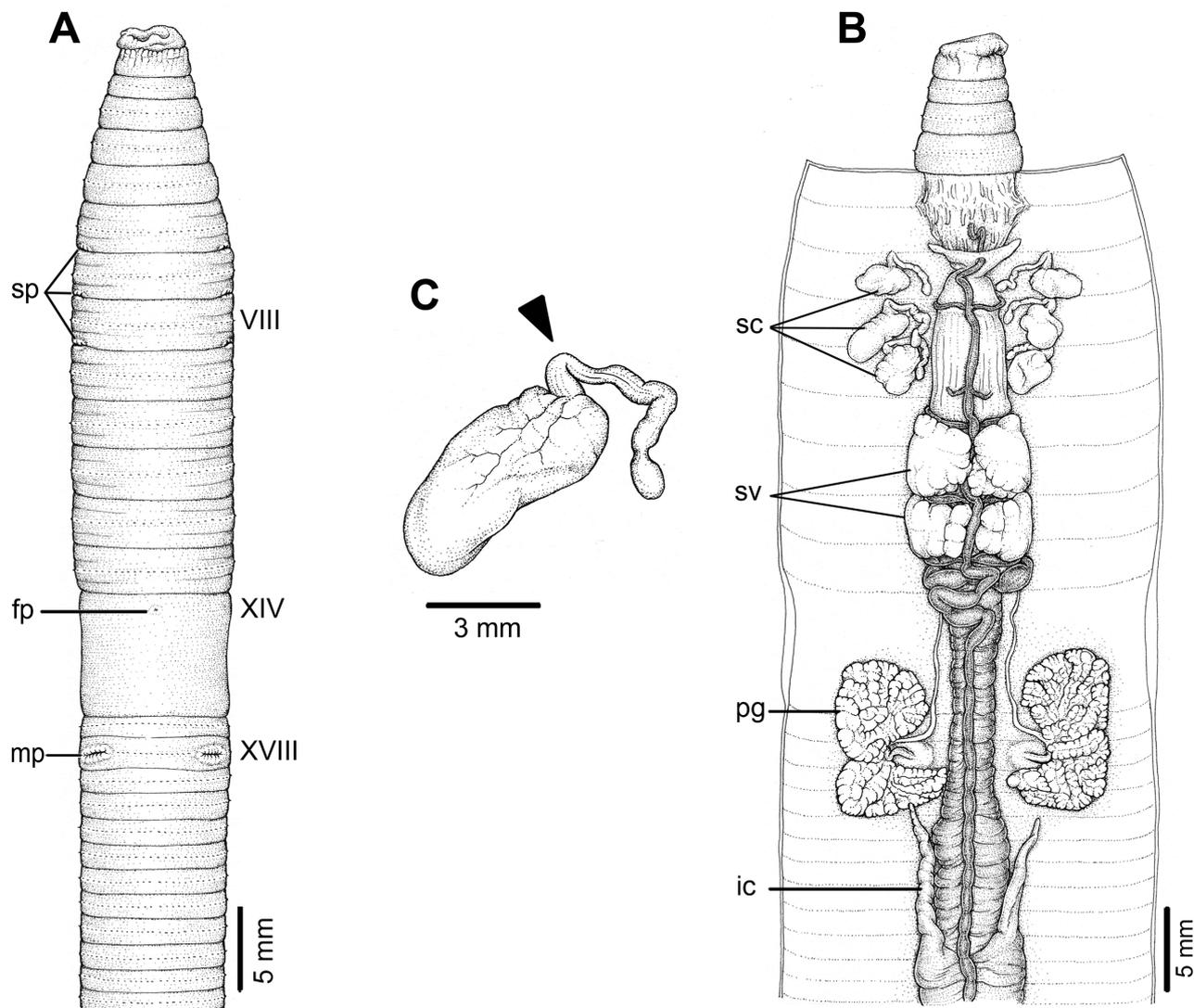


FIGURE 6. External and internal morphology of *Metaphire khaoluangensis* sp. n., holotype (CUMZ 3377). **A.** External ventral view. **B.** Internal dorsal view. **C.** Spermatheca; arrowhead: location of spermathecal pore.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin XV; intestinal caeca originating in XXVII, simple, extending to XXI. Typhlosome rudimentary. Oesophageal hearts four pairs in X–XIII. Holandric; testes two pairs in ventrally joined sacs in X and XI. Seminal vesicles paired in XI–XII. Prostate gland occupying segments XVI to XIX. Prostatic duct slightly muscular, closely attached to lateral side of copulatory sac. Ovaries in XIII. Three pairs of spermathecae in VII–IX. Ampulla elongate, sac-like, with short duct; diverticulum slender, its proximal end with a neck-like constriction; total length slightly less than ampulla.

Variation. Body length of paratypes 130–265 mm (211.6±22.6), segments 113–131. Prostate glands in XVI–XX, intestinal caeca often shorter than in holotype, ending in XXIII.

Distribution. Known from the type locality and one additional locality in Phatthalung province, the two sites being separated by approximately 240 km.

Remarks. This species is sixthcal, with spermathecal pores on 6/7–8/9, and devoid of postclitellar genital markings, so it belongs to the *houletti* species group which is one of the largest species groups in *Metaphire*, with more than 30 species (Sims & Easton 1972). In the following we will compare the new species with regional

species in the *houletti* group: *M. umbraticola* (Gates, 1932) and *M. quadrigemina* (Gates, 1932) from Myanmar, *M. amplectens* (Michaelsen, 1934) and *M. dawydovi* (Michaelsen, 1934) from Vietnam, and *M. bindjeyensis* (Michaelsen, 1899) from Sumatra.

Metaphire khaoluangensis sp. n. is similar to *M. umbraticola*, *M. dawydovi* and *M. bindjeyensis* by the aspect of the secondary male openings and the body size. They differ in the spermathecae. *M. umbraticola* has oval ampullae with long diverticulum, *M. bindjeyensis* has obclavate ampullae with zigzag diverticulum, and *M. dawydovi* has flask-shaped ampullae with small diverticulum, while *M. khaoluangensis* sp. n. has elongate sac-like ampullae with capitate diverticulum.

In Thailand, only three species of this group have been reported, *M. houletti* (Perrier, 1872), *M. virgo* (Beddard, 1900), and *M. perichaeta* (Beddard, 1900). *Metaphire khaoluangensis* sp. n. can be distinguished from the first two species by the distance between the openings of the copulatory pouches, measured as a fraction of the estimated body circumference. This measure is 0.28 in *M. khaoluangensis* sp. n., but 0.30 and 0.33 in *M. houletti* and *M. virgo*, respectively (Beddard 1900). Other differences from *M. houletti* and *M. virgo* are: first dorsal pore in 12/13 (11/12 in *M. houletti* and *M. virgo*) and spermathecal ampulla large and elongate (spherical and small sac in *M. houletti* and *M. virgo*, respectively). The new species does not have the contorted diverticulum stalk enveloped in connective tissue as found in *M. houletti*, and also lacks the typhlosole present in *M. houletti*. *M. virgo* is further distinguished from the new species by a spermathecal diverticulum stalk with multiple folds. Both *M. houletti* and *M. virgo* have genital markings bearing stalked glands in association with spermathecae and with the copulatory sacs, whereas the new species lacks these characters. This new species is fairly similar to *M. perichaeta* in male pore spacing (0.28 body circumference), but it is distinguished by elongated spermathecae with slender diverticulum and last hearts in XIII, compared to inverted pear-shape spermathecae with coiled diverticulum and last hearts in XII in *M. perichaeta* (Beddard 1900; Stephenson 1932). A comparison of characters between *M. khaoluangensis* sp. n. and other related species is presented in Table 2.

***Metaphire khaochamao* Bantaowong & Panha, sp. n.**

(Figure 7, Table 2)

Material examined. Holotype: clitellate (CUMZ 3382), Thailand, Rayong, Khao Cha Mao, Wat Ma Dua, 13°03'14.1"N, 101°36'46.6"E, 343 m amsl, 10 November 2013, leg. S. Panha, U. Bantaowong, P. Tongkerd & W. Siriwit. Paratypes: 5 adults (CUMZ 3383), 2 adults (ZMH), 2 adults (NHMUK), same data as holotype.

Other material examined. Two adults (CUMZ 3384), Thailand, Rayong, Klaeng, Wat Tham Naramitr, 12°58'20.2"N, 101°39'54.3"E, 34 m amsl, 10 November 2013. 3 adults (CUMZ 3385), Thailand, Rayong, Mueang, Wat Khao Sab, 12°36'48.1"N, 101°23'23.0"E, 9 m amsl, 10 November 2013. 77 adults (CUMZ 3386), Thailand, Rayong, Mueang, Khao Laem Ya-Mu Koh Samet National Park, 12°35'16.7"N, 101°25'10.9"E, 15 m amsl, 10 November 2013. 57 adults (CUMZ 3387), Thailand, Chonburi, Bo Thong, Khao Cha Ang On, 13°12'0.7"N, 101°34'43.6"E, 82 m amsl, 24 September 2012. 2 adults (CUMZ 3388) Thailand, Chonburi, Bo Thong, Khao Ha Yod, 13°09'46.4"N, 101°35'52.6"E, 79 m amsl, 9 November 2013. 4 adults (CUMZ 3389), Thailand, Chonburi, Si Racha, Chan Ta Then Waterfall, 13°14'12.7"N, 101°2.0'28.2"E, 168 m amsl, 13 August 2010.

Etymology. This new species is named after the type locality, Khao Cha Mao. Noun in apposition.

Diagnosis. Medium-sized; length 100–166 mm with 110–120 segments. Setae numbering 34–45 in VII, 52–65 in XX, no setae between male pores. Male pores paired, protruded with inner inclined grooves on ventro-lateral side of segment XVIII, no genital markings. Spermathecal pores paired in segments 6/7–8/9. Spermathecae with elliptic ampulla, diverticulum slender, with small round knob. No nephridia on the spermathecal ducts. Holandric, intestinal caeca simple, first dorsal pore in 12/13. Prostate glands several lobules, ducts curved.

Description of holotype. Dimensions: 166 mm by 6.0 mm at segment VII, 6.1 mm at segment XX, 5.8 mm at clitellum; body cylindrical with 118 segments. Preserved specimen brownish dorsally, light-gray ventrally. Setae regularly distributed around segmental equators, numbering 34 at VII, 60 at XX, no setae between male pores, setal formula AA:AB:ZZ:ZY=1.5:1:1:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular XIV–XVI. Male pores protruded on ventro-lateral sides of XVIII, with inner inclined grooves that run straight from tip to basal edge, about 0.30 circumference apart ventrally, distance between openings of copulatory pouch 5 mm, the median region between male pores depressed. Three pairs of spermathecal pores, each marked by a whitish spot in furrows 6/7–8/9, ventral, distance between each pair 5 mm, about 0.30 of the body circumference apart ventrally.

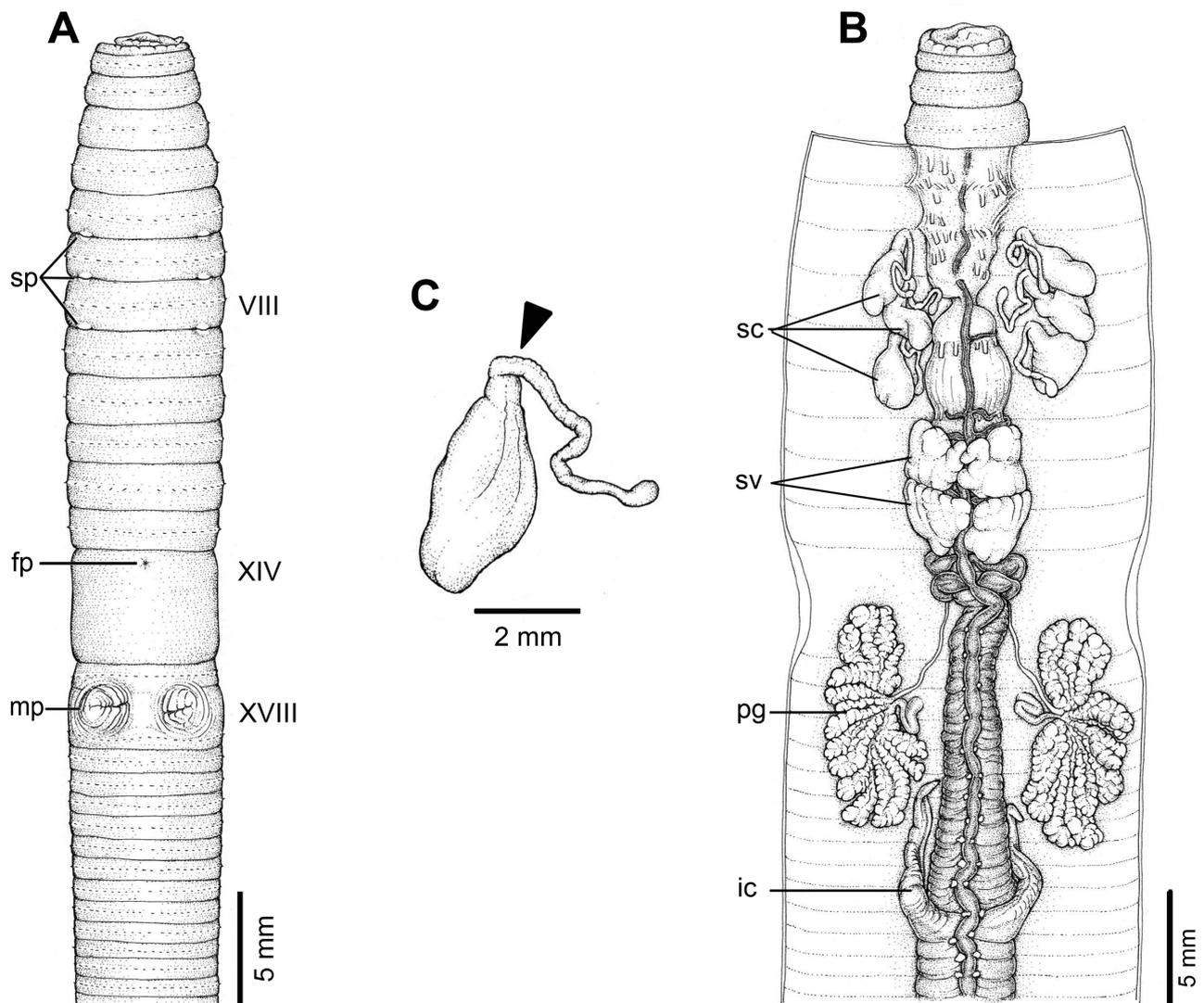


FIGURE 7. External and internal morphology of *Metaphire khaochamao* sp. n., holotype (CUMZ 3382). **A.** External ventral view. **B.** Internal dorsal view. **C.** Spermatheca; arrowhead: location of spermathecal pore.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin XV; the intestinal caeca originating in XXVII, simple, extending to XXII. Typhlosole one-fourth lumen diameter, beginning in XXVII. Oesophageal hearts four pairs in X–XIII. Holandric; testes and funnels enclosed in ventral paired sacs in X and XI, sacs not connected. Seminal vesicles paired in XI–XII. Prostate gland well developed, located in XVII to XXIII, divided into several lobules. Prostatic duct not very long, copulatory pouch compressed to the outer body wall. Ovaries in XIII. Three pairs of spermathecae in VII–IX. Ampulla elliptic with a short duct. The diverticulum stalk slender, as long as ampulla, with a small rounded seminal chamber.

Variation. The paratypes are 100–148 mm (122.6 ± 12.8) long, with 110–120 segments. The prostate glands may begin in XVI and end in XXII, and intestinal caeca may extend to only XXIII.

Distribution. Known only from Rayong and Chonburi provinces, located in eastern Thailand.

Remarks. *Metaphire khaochamao* sp. n. has protruded male pores with inner inclined grooves. This character resembles *M. formosae* (Michaelsen, 1922), but *M. formosae* has an oval pad near the male pore opening, absent in *M. khaochamao*. Moreover, the location and number of spermathecal pores of these two species are also different. *M. formosae* has four pairs of spermathecal pores located at 5/6–8/9, while *M. khaochamao* has three pairs located at 6/7–8/9. The new species also keys to the *houletti* species group in Sims and Easton (1972). Within the *houletti* species group in Thailand, which consists of *M. houletti*, *M. virgo*, *M. khaoluangensis* and *M. perichaeta*, the new species can be distinguished by the much more ventral placement of the spermathecal pores, the features of the

male field including the lateral slits associated with the male pores, absence of setae between male pores, and absence of an internally visible copulatory sac (Table 2).

***Metaphire perichaeta* (Beddard, 1900)**

(Figure 8, Table 2)

Amyntas perichaeta Beddard, 1900: 896–897.

Pheretima perichaeta (Beddard). Stephenson 1932: 227–229, Fig. 8; Gates 1939: 103.

Metaphire perichaeta (Beddard). Sims & Easton 1972: 238.

Material examined. Holotype: (NHMUK 1924.3.1.231) by monotypy. The type locality is State of Phatthalung, Malay Peninsula, without any further specification.

Diagnosis. Medium-sized, length 160 mm with 118 segments. Male pores in copulatory pouches, these with transversely slit-like, conspicuous openings, in XVIII. No genital markings. Spermathecal pores paired at 6/7–8/9, spermathecal ampullae inverted pear-shaped, diverticulum slender and greatly coiled towards distal end. No nephridia on the spermathecal ducts. Holandric, intestinal caeca simple, first dorsal pore in 12/13. Prostate glands large.

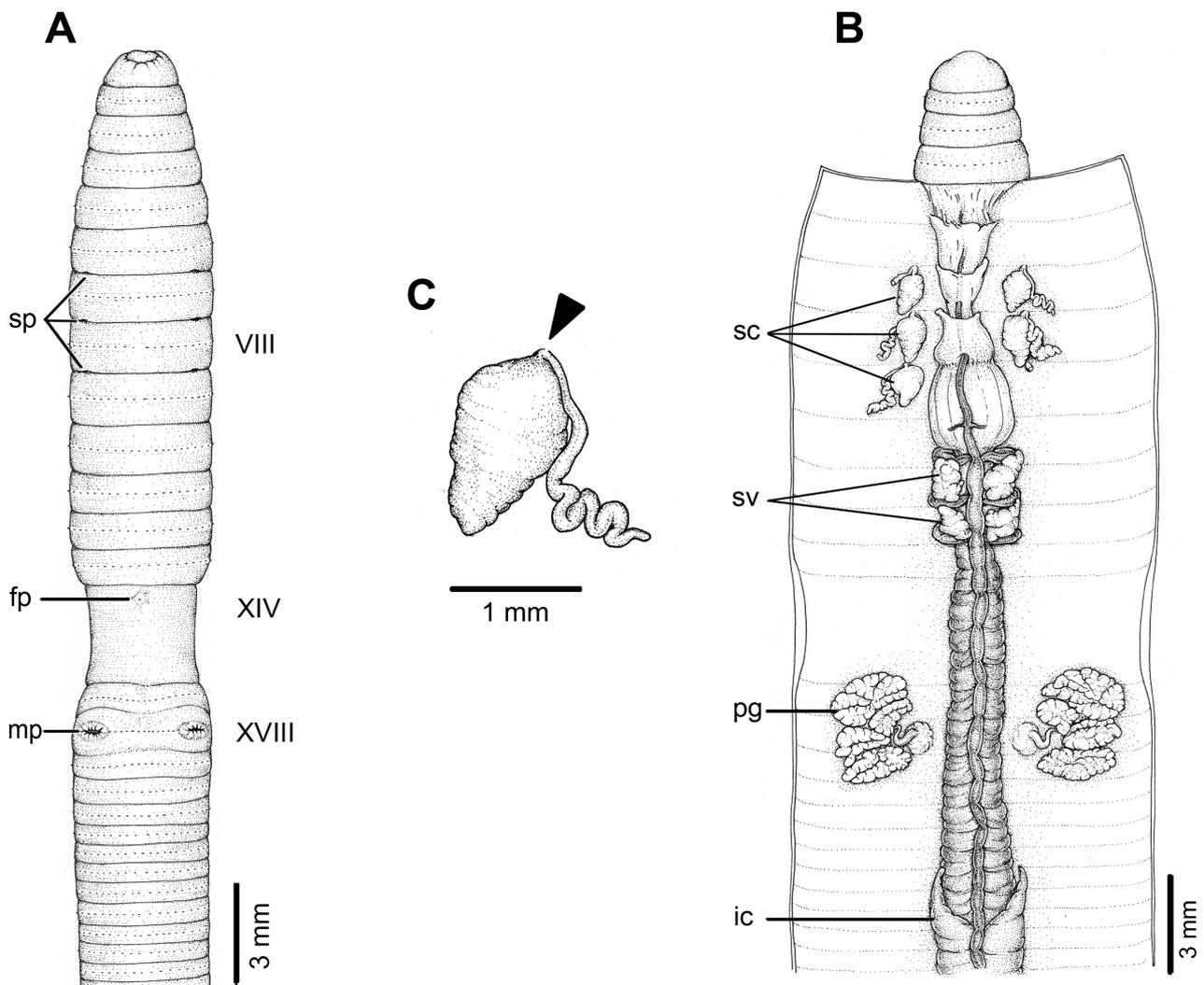


FIGURE 8. External and internal morphology of *Metaphire perichaeta* (Beddard, 1900), holotype (NHMUK 1924.3.1.231). **A.** External ventral view. **B.** Internal dorsal view. **C.** Spermatheca; arrowhead: location of spermathecal pore.

TABLE 2. Comparison of morphological characters in the *Metaphire houlleti* species group in Thailand. The comma is used to separate body length and width, and setal counts. Presence and absence of an organ is indicated by + or -. Data for *M. houlleti* are from Gates (1972), and data for *M. virgo* are from Stephenson (1932). Missing data are shown by a question mark (?).

	<i>M. khaochamao</i> sp. n.	<i>M. khaoluangensis</i> sp. n.	<i>M. perichaeta</i> (Beddard, 1900)	<i>M. houlleti</i> (Perrier, 1872)	<i>M. virgo</i> (Beddard, 1900)
Body length, width (mm)	166, 6	220, 10	160, 5	92–200, 4–7	152–157, 5
Segment number	118	119	118	92–140	129
First dorsal pore	12/13	12/13	12/13	11/12	11/12
Setae VII, XX	34, 60	36, 51	36, 46	?, 48–62	38–51(VIII), 47–56
Male pore setae	–	14	12	11–12	12
Male pore spacing	0.30	0.28	0.28	0.30	0.33
Spermathecal pore spacing	0.30	0.44	0.30	0.33	?
Genital marking	–	–	–	–	–
Spermathecae	elliptic	elongate	pear shape	large sac	small
Diverticulum	slender	slender	zigzag	looped	tubular
Prostate gland	17–23	16–19	17–19	16–21	17–18
Genital marking gland	–	–	–	stalk	–
Copulatory sac	–	+	+	+	+
Intestinal caeca	27–23	27–21	27–24	27–22	27–25
Type locality	Thailand	Thailand	Thailand	India	Thailand

Remarks. This species is known only from one specimen. The detailed descriptions of Beddard (1900) and Stephenson (1932) were without illustrations, a line drawing of the spermatheca excepted (Stephenson 1932: 229, Fig. 8). In this study, we present illustrations of both the external ventral and internal dorsal view, including important features like the male genital characters and the prostate glands.

Metaphire perichaeta was first recorded from the State of Phatthalung (previously spelled Patalung), Malay Peninsula, but the locality is actually in Thailand, and *M. khaoluangensis* sp. n. is also found in Phatthalung. Differences of *M. perichaeta* are as follows: last oesophageal hearts in XII; spermathecae inverted pear-shaped; prostate glands and intestinal caeca smaller than those of the two new species with prostate glands situated on three segments in XVII–XIX (more than three segments in the new species) and intestinal caeca in XXVII–XXIV(XXVII–XXII in the new species). Moreover, *M. perichaeta* has a narrow mid-dorsal interruption in the ring of setae, while *M. khaochamao* has a mid-ventral interruption, and in *M. khaoluangensis* the ring of setae is continuous.

Metaphire doiphamon Bantaowong & Panha, sp. n.

(Figure 9)

Material examined. Holotype: clitellate (CUMZ 3390), Thailand, Chiang Rai, Thoeng, Phu Chee Fah, Doi Phamon mountain range, 19°48'47.0"N, 100°26'20.4"E, 1205 m amsl, leg. U. Bantaowong, C. Sutcharit, & P. Tongkerd, 19 November 2012. Paratype: 1 adult (CUMZ 3391), same data as holotype.

Etymology. This species is named after the Doi Phamon mountain range, where it was collected. Noun in apposition.

Diagnosis. Large *Metaphire* worms; length 255–269 mm with 142 segments. Setae numbering 44–49 in VII, 84–89 in XX, 21–22 between male pores. Male pores paired, transverse secondary openings in XVIII, genital markings paired on 17/18 and 18/19. Spermathecal pores paired in 6/7–8/9. Spermathecae with ovoid ampulla,

diverticulum thread-like, slightly curved at tip. No nephridia on the spermathecal ducts. Holandric, intestinal caeca simple, first dorsal pore in 12/13. Prostate glands and ducts two branches, with white glandular mass on body wall where duct ends.

Description of holotype. Dimensions: 255 mm by 9.9 mm at segment VII, 11.0 mm at segment XX, 10.0 mm at clitellum; body cylindrical with 142 segments. Preserved specimen dark to grayish-brown dorsally, ventrally pale; clitellum with light brownish purple colour. Setae regularly distributed around segmental equators, numbering 49 at VII, 84 at XX, 21 between male pores, setal formula AA:AB:ZZ:ZY=1:1:1:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular XIV–XVI with three rows of setae.

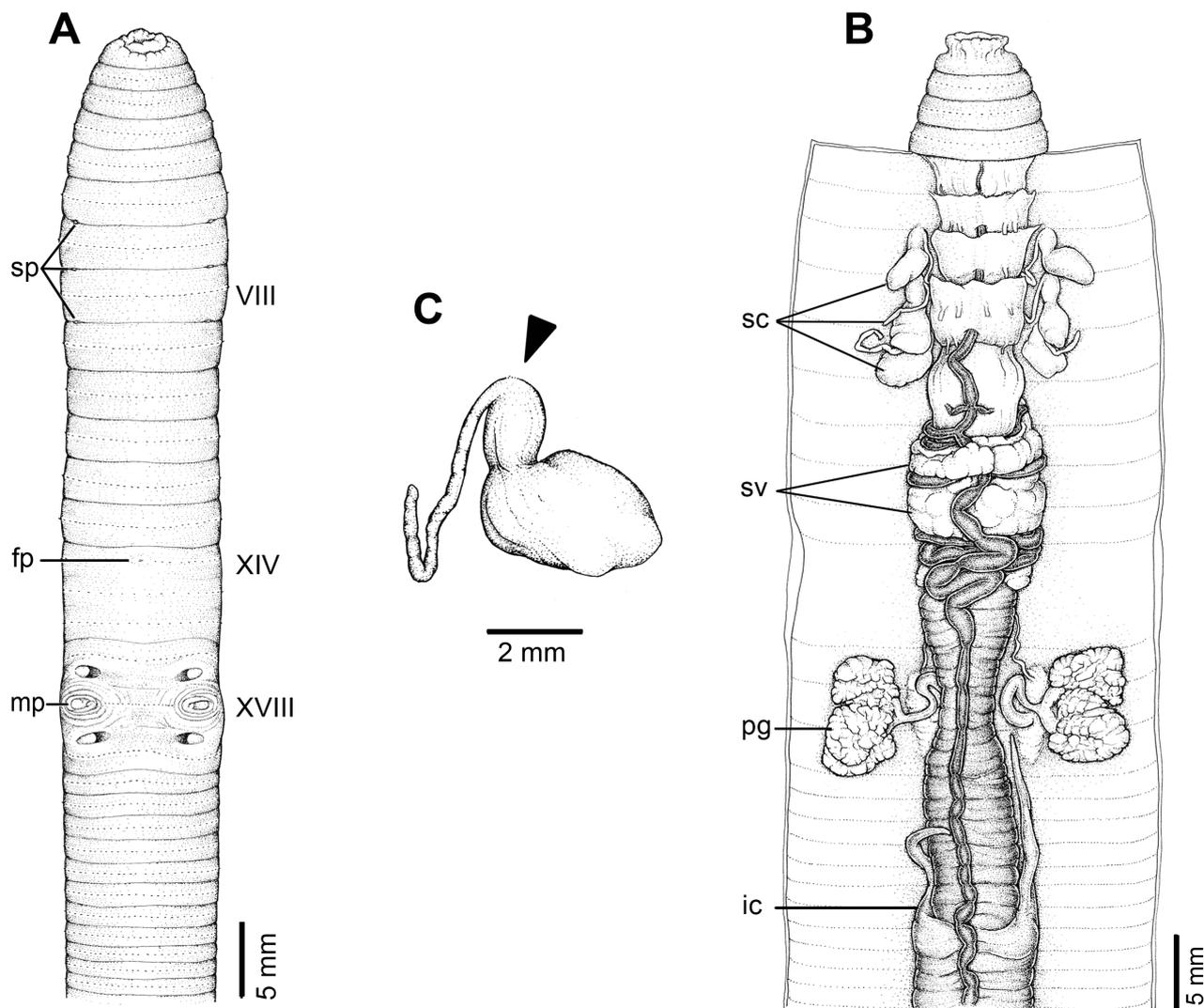


FIGURE 9. External and internal morphology of *Metaphire doiphamon* sp. n., holotype (CUMZ 3390). **A.** External ventral view. **B.** Internal dorsal view. **C.** Spermathecae; arrowhead: location of spermathecal pore.

Secondary male apertures as two transverse openings situated on ventro-lateral sides of XVIII, about 0.26 circumference apart ventrally, distance between male apertures 8 mm, each male pore in a very shallow rudimentary copulatory chamber, the whole male area surrounded by 3–4 closely adjacent concentric circular ridges. Genital markings paired, transversely elliptical, 4–5 intersetal intervals wide, located in intersegments of 17/18 and 18/19, in line with male apertures. Three pairs of spermathecal pores, transverse slits in furrows 6/7–8/9, ventral, distance between each pair 9 mm on body circumference, about 0.30 body circumference apart ventrally.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin XV; intestinal caeca originating in XXVII, simple and extending to XXII. Typhlosole simple, rudimentary. Oesophageal hearts four pairs in X–XIII. Holandric; testes two pairs in ventrally joined sacs in X and XI. Seminal vesicles paired

in XI–XII. Prostate glands usually confined to XVII–XIX, separated into two racemose lobes. Prostatic duct divided into two small branches, one per lobe. Paired, white glandular masses on copulatory sac, where prostatic ducts enter the parietes. Ovaries in XIII. Spermathecae three pairs in VII–IX. Ampulla ovoid, duct stout, one-third as long as ampulla and sharply marked off from ampulla. Diverticulum long and slender, thread-like, without a terminally enlarged seminal chamber, slightly curved at tip.

Variation. The single paratype specimen measures 269 mm in body length, with 142 segments.

Distribution. This species is known only from the type locality, where it coexists with *Amyntas phucheeifah* Bantaowong & Panha, 2014.

Remarks. *Metaphire doiphamon* sp. n. is sixthecal and presents genital markings at 17/18 and 18/19, so it keys out with the *peguana* group, which consists of three other species, *M. bahli* (Gates, 1945), *M. peguana* (Rosa, 1890) and *M. saigonensis* (Omodeo, 1957). Of these, *M. peguana* and *M. bahli* have been recorded in Thailand, whereas *M. saigonensis* is currently placed in synonymy with *M. peguana* (Blakemore 2002; Somniyam & Suwanwaree 2009; Bantaowong *et al.* 2011b; Prasankok *et al.* 2013).

This new species is distinguished from the other members of the species group by its body length of 255 mm, the form of the genital markings and the bifurcate prostatic ducts. The genital markings are transversely oval in *M. doiphamon* sp. n., they lack pores or other openings and are associated with sessile glandular masses on the copulatory pouch, while the genital markings of *M. peguana* and *M. bahli* are circular to oval, invaginated or pouch-like, and with stalked glands. Both of them have a smaller body size (range of 110–240 mm) and fewer segments. Furthermore, *M. doiphamon* sp. n. also has unique prostate glands and ducts, being divided into two main lobes each with a large and long duct, while the two former species have regular prostate glands, each consisting of a single racemose mass with many small ductlets.

***Metaphire saxicalcis* Bantaowong & Panha, sp. n.**

(Figure 10)

Material examined. Holotype: clitellate (CUMZ 3392), Thailand, Chonburi, Bo Thong, Khao Cha Ang On, 13°12'0.7"N, 101°34'43.6"E, 82 m amsl, 24 September 2012, leg. S. Panha, U. Bantaowong, R. Chanabun & W. Siriwut. Paratypes: 6 adults (CUMZ 3393), 1 adult (ZMH), 1 adult (NHMUK), same data as holotype.

Other material examined. 4 adults (CUMZ 3394), Thailand, Chonburi, Bo Thong, Khao Ha Yod, 13°9'46.5"N, 101°35'52.3"E, 93 m amsl, 24 September 2012.

Diagnosis. Large *Metaphire* worms; length 254–377 mm with 147–157 segments. Setae numbering 80–89 in VII, 89–98 in XX, 13–20 between male pores. Male pores paired, secondary apertures transversely slit-like with puckered margin on segment XVIII, no genital markings. Spermathecal pores paired in segments 6/7–7/8. Spermathecae with large paddle-shaped ampulla, duct thin, and with large saccular body at its base. Diverticulum slender, longer than ampulla, with enlarged spindle-like seminal chamber. No nephridia on the spermathecal duct. Holandric, intestinal caeca simple, first dorsal pore in 12/13. Prostate glands large, its elongate spindle-shaped duct connected to center of the large circular copulatory sac.

Etymology. The specific epithet is from the Latin "saxi calcis" = limestone. The habitat of the new species is a limestone forest.

Description of holotype. Dimensions 377 mm length by 9.6 mm width at segment VII, 9.5 mm at segment XX, 9.6 mm at clitellum; body cylindrical with 151 segments. Preserved specimen dark-brown dorsally, light-brown ventrally; clitellum brown to dark-brown. Setae regularly distributed around segmental equators, numbering 89 at VII, 96 at XX, 20 setae between male pores, setal formula AA:AB:ZZ:ZY=1:1:1:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 12/13. Clitellum annular XIV–XVI.

Male pores in copulatory pouches, these with very conspicuous, transversely slit-like apertures with puckered margins on ventro-lateral sides of segment XVIII, about 0.28 circumference apart ventrally, distance between apertures 8 mm on body circumference. Spermathecal pores two pairs, latero-ventral, each pore with transverse slits on somewhat raised glandular areas in furrows 6/7–7/8, about 0.34 body circumference apart ventrally, distance between spermathecal pores 10 mm on body circumference.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin XV; intestinal caeca originating in XXVII, simple and extending to XXII. Typhlosole rudimentary. Oesophageal hearts

four pairs in X–XIII. Holandric; testes small, two pairs in ventrally joined sacs in X and XI. Seminal vesicles paired in XI–XII. Prostate glands in XVI–XXI, expanded into a fan-shaped outline wrapped around copulatory sacs. Duct large and long, looped in a hairpin-shape with enlarged spindle-shaped ectal half, entering into the large round cushion-like copulatory sac. Ovaries in XIII. Spermathecae large, in VII and VIII; ampulla large, paddle-shaped, with saccular body at the base of spermathecal ducts, duct thin and not sharply marked off from the ampulla. Diverticulum slender, very long, length as long as main part of spermathecae, seminal chamber enlarged, spindle-like with pebbly outer texture.

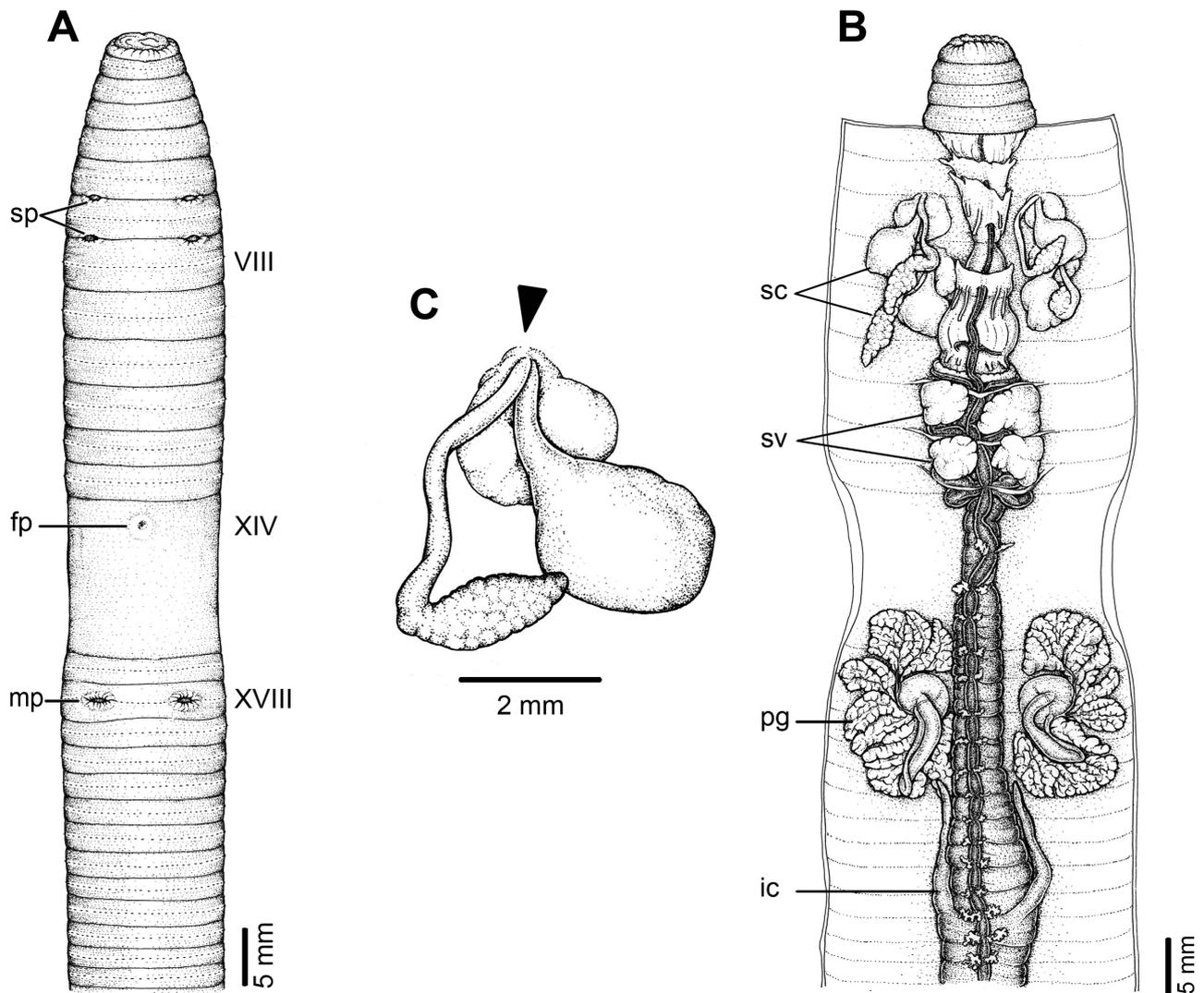


FIGURE 10. External and internal morphology of *Metaphire saxicalcis* sp. n., holotype (CUMZ3392). **A.** External ventral view. **B.** Internal dorsal view. **C.** Spermatheca; arrowhead: location of spermathecal pore.

Variation. The eight paratypes are 254–285 mm long (271.6 ± 21.9 mm) and with 147–157 segments. Setae between male pores vary from 11 to 18. Prostate glands may extend to XXII, and intestinal caeca to XXIII only.

Distribution. Known only from the type locality and one further site from Khao Ha Yod, Chonburi, which is located approximately 15 km south-west of the type locality.

Remarks. *Metaphire saxicalcis* sp. n. keys out with the *planata* species group in Sims and Easton (1972). This group consists of six species, *M. dunckeri* (Michaelsen, 1902), *M. parvula* (Ohfuchi, 1956), *M. sintangi* (Michaelsen, 1922), *M. ferdinandi* (Michaelsen, 1891), *M. planata* (Gates, 1926), and *M. decipiens* (Beddard, 1912), the latter placed in synonymy with *Pheretima darnleiensis* (Fletcher, 1886) by Blakemore *et al.* (2007). *Metaphire saxicalcis* sp. n. is easily distinguished from these species by the greater body length of 254–377 mm, while all of these five species members have body length less than 200 mm. Furthermore, none of the species have the saccular body at the spermathecal ducts that we observed in *M. saxicalcis*.

***Metaphire planata* (Gates, 1926)**

(Figure 11)

Pheretima planata Gates, 1926: 411–415, Figs 1, 2.

Pheretima planata. Gates, 1930: 320–321; 1931: 405; 1932: 411–413; 1939: 103; 1972: 211–212.

Metaphire planata (Gates). Sims & Easton 1972: 239.

Material examined. Type from NHMUK (1928.4.2.1). One semi-clitellate in ethanol, which had been dissected.

Description. Dimensions 125 mm by 4.8 mm at segment VII, body cylindrical with 138 segments. Setae regularly distributed around segmental equators, AA:AB:ZZ:ZY=1:1:1:1. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 11/12. Clitellum annular XIV–XVI with visible setae. Male pores conspicuous on XVIII, transversely slit-like secondary apertures puckered radially around its margin, 0.27 body circumference apart ventrally, distance between openings of copulatory pouch 4 mm, and 11 setae intervening between them. No genital markings. Two pairs of spermathecal pores, transverse slits, in furrows 6/7–7/8, ventral, distance between each pair about 0.25 body circumference ventrally apart, distance between spermathecal pores 4 mm on body circumference.

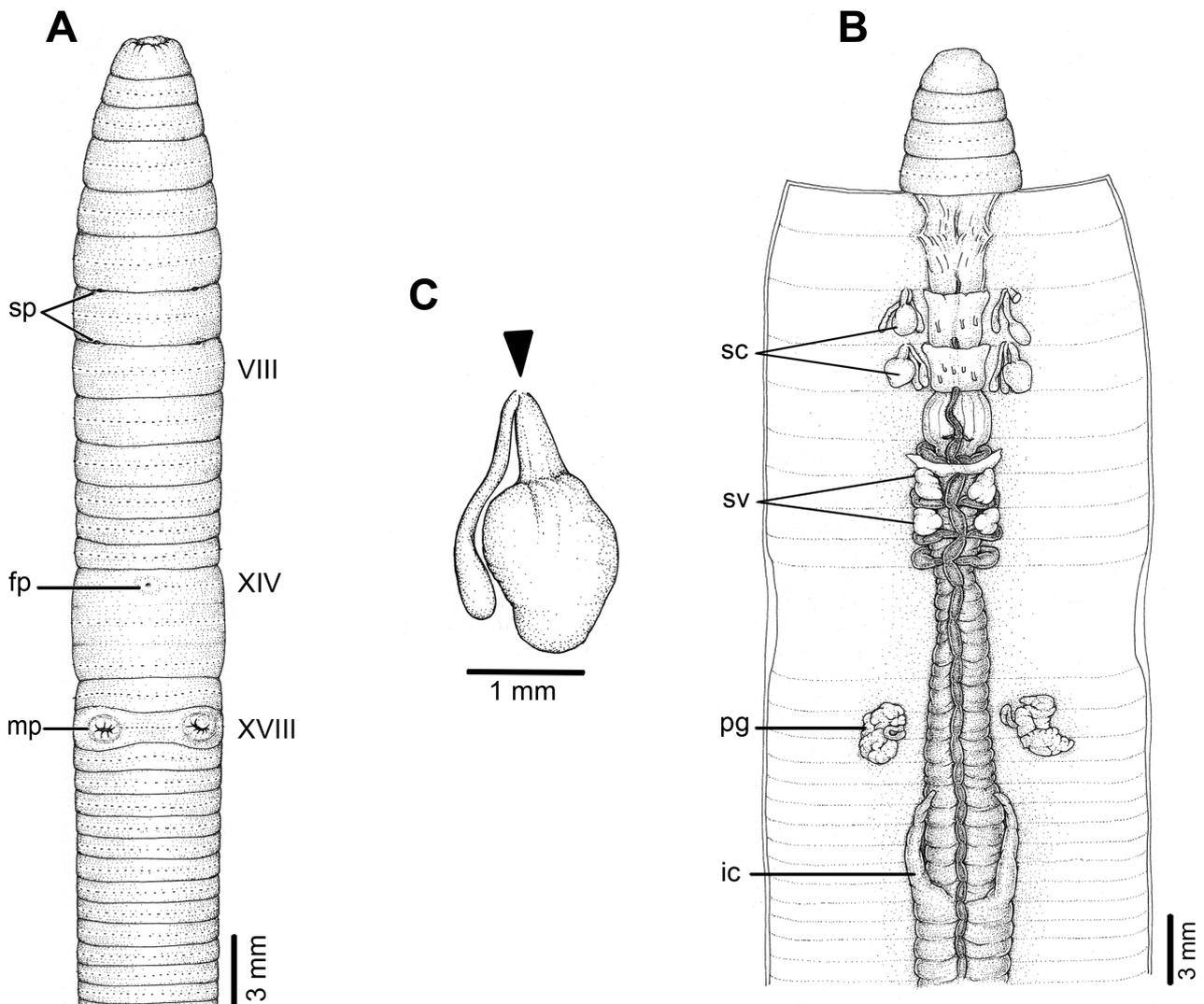


FIGURE 11. External and internal morphology of *Metaphire planata* (Gates, 1926), type specimen (NHMUK 1928.4.2.1). **A.** External ventral view. **B.** Internal dorsal view. The ampulla in VII to the right is missing. **C.** Spermatheca; arrowhead: location of spermathecal pore.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin XV; intestinal caeca originating in XXVII, simple, extending to XXII. Oesophageal hearts four pairs in X–XIII. Holandric; testes and funnels enclosed in paired sacs in X and XI. Seminal vesicles in XI and XII, small. Prostate glands small, occupying XVII–XIX. Prostatic duct C-shaped, no internally visible copulatory sacs. Ovaries in XIII. Two pairs of spermathecae in VII–VIII, long stalked genital marking gland attached near spermathecal duct. Ampulla oval sac with straight duct, diverticulum tubular, slightly dilated in tip.

Remarks. The single type specimen examined—probably the holotype—is semi-clitellate, and one of the four spermathecae is incomplete (the ampulla has been removed). The important characters are quite clear, especially the spermathecae with oval ampulla, tubular diverticulum, and genital marking gland with long stalk. *Metaphire planata* is superficially similar to *M. saxicalcis* in the position and number of spermathecae and simple intestinal caeca, but differs from *M. saxicalcis* by the smaller body and presence of a stalked genital marking gland near each spermatheca.

***Metaphire surinensis* Bantaowong & Panha, sp. n.**

(Figure 12)

Type material. Holotype: clitellate (CUMZ 3395), Thailand, Surin, Sangkha, Pasonnongkoo Forest Park, 14°41'12.0"N, 103°44'47.9" E, 165 m amsl, 16 October 2012, leg. U. Bantaowong, C. Sutcharit, R. Chanabun, J. Taptimon & W. Siritwut. Paratypes: 2 adults (CUMZ 3396), 1 adult (ZMH), 1 adult (NHMUK), same data as holotype.

Etymology. The species is named after Surin, the province where it was collected.

Diagnosis. Large *Metaphire* worms; length 275–300 mm with 153–190 segments. Setae numbering 112–130 in VII, 128–142 in XX, no setae between male pores. Copulatory pouches invaginated in a single deep transverse body wall invagination in segment XVIII, genital markings closely paired, protuberant in 18/19. Spermathecal pores closely paired in 6/7. Spermathecae with elliptical ampulla, diverticulum long, slender, with sausage-like seminal chamber. Holandric, intestinal caeca simple, first dorsal pore in 11/12. Prostate glands large, posterior to copulatory sacs, ducts slender, joining large circular copulatory sac near its center; large genital marking gland posterior to each copulatory sac.

Description of holotype. Dimensions: 275 mm by 8.8 mm at segment VII, 9.2 mm at segment XX, 9.0 mm at clitellum; body cylindrical with 190 segments. Preserved specimen brownish-red dorsally, light-gray ventrally; clitellum light-brown. Setae regularly distributed around segmental equators, numbering 123 at VII, 128 at XX, no setae between male pores, setal formula AA:AB:ZZ:ZY=1:1:1:1 at XIII. Single female pore at XIV. Prostomium epilobic. First dorsal pore at 11/12. Clitellum annular XIV–XVI.

Male pores in copulatory pouches, these, together with their apertures, enclosed in a single, deep, transverse body wall invagination; pouches invisible from the outside. Apertures of pouches inside invagination, 0.15 circumference apart ventrally, equalling 5 mm. Genital markings huge, closely paired, protuberant in 18/19. One pair of spermathecal pores, very closely paired, each on a tiny tubercle, in furrow 6/7, ventral, distance between paired pores about 0.05 body circumference, distance between spermathecal pores 1.5 mm.

Septa 5/6–7/8 thick, 8/9–9/10 absent, 10/11–11/12 thin. Gizzard large behind 7/8, intestinal origin in XV, intestinal caeca originating in XXVII, simple and extending to XXIII. Typhlosole a simple fold, one-fourth of the lumen diameter, beginning in XXVII. Oesophageal hearts four pairs in X–XIII. Holandric; testes and funnels enclosed in paired sacs in X and XI. Seminal vesicles two pairs in XI–XII, large but not conjoint mid-dorsally, nearly equal in size and in shape. Prostate glands well-developed, large, extending anteriorly to segment XX, posteriorly to XXVII. Prostate duct very long, of sigmoid shape, copulatory pouches in segment XVIII. Genital marking glands, paired cushion-like glandular masses, possibly protrusible externally. Ovaries in XIII. One pair of spermathecae in VII. Ampulla elliptical. Terminal chamber of diverticulum sausage-like, longer than ampulla, remarkable in being slightly larger than the ampulla.

Variation. The two paratypes are 208 and 330 mm long and with 153 and 169 segments, respectively. One paratype has a pair of genital markings on 17/18, with prostate glands situated on segments XVIII–XXI. The other paratype shows long prostate glands in segments XVIII–XXXIII.

Distribution. This species is known only from the type locality. They live in the soil at about 20–30 cm depth, in a forest park.

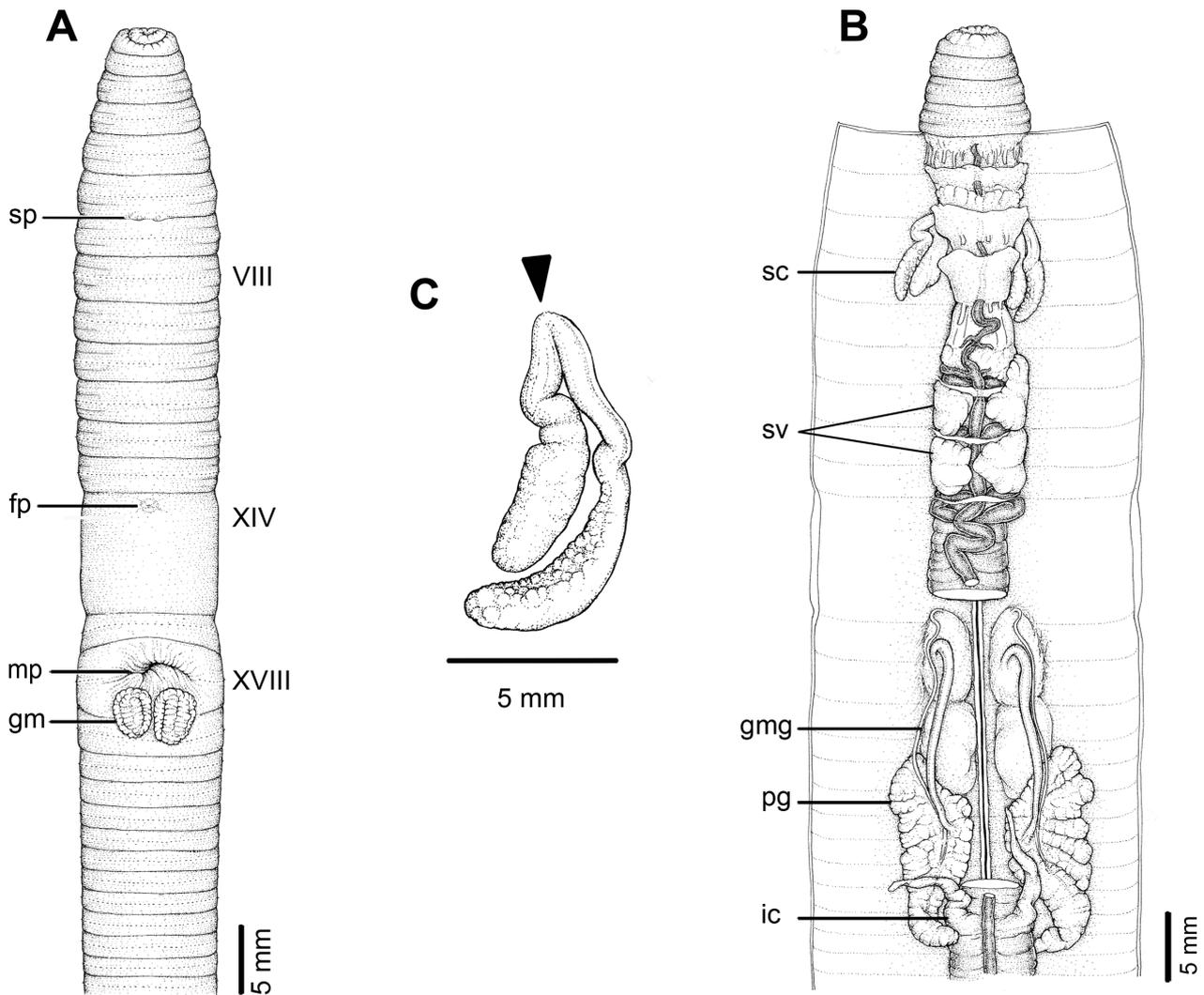


FIGURE 12. External and internal morphology of *Metaphire surinensis* sp. n., holotype (CUMZ 3395). **A.** External ventral view. **B.** Internal dorsal view. **C.** Spermatheca; arrowhead: location of spermathecal pore.

Remarks. The only bithecal species of the genus *Metaphire* known to date that has the spermathecal pores at 6/7 is *M. ladjangensis* (Ude, 1932). Differences between *M. surinensis* and *M. ladjangensis* are primarily in the body size, male field and the location of genital markings. *Metaphire ladjangensis* is a quite small earthworm, 70 mm in body length and 95 segments while *M. surinensis* has length 275–300 mm with 153–190 segments. The secondary male pores of *M. ladjangensis* are small, visible from the surface and have 14 setae between them, but in *M. surinensis* they are in a deep invagination of the body wall with no setae between them. Moreover, in *M. ladjangensis* the genital markings are situated on segment VIII and intersegments 17/18–18/19, and male pores are 0.30 body circumference ventrally apart, while in *M. surinensis* the genital markings are situated on intersegment 18/19 in the holotype and the distance between openings of copulatory pouch is about 0.15 circumference.

Discussion

The seven new species range in size, with respect to other *Metaphire* members, from moderate to large, of which *M. saxicalcis* sp. n., *M. surinensis* sp. n., *M. songkhlaensis* sp. n. and *M. doiphamon* sp. n. are the largest (255–377 mm), while the remaining three new species are medium sized (155–220 mm). *Metaphire saxicalcis* sp. n. is the largest, and *M. trangensis* sp. n. is the smallest species. All of these new species live in the top soil at a depth of 10–30 cm from the soil surface. *Metaphire doiphamon* sp. n. was observed to emerge from the soil in October 2008

and November 2012 in large numbers, where they almost covered the road, but all of them were in the juvenile stage. This is similar to the report in Gates (1961) about a mass migration of juvenile worms of the genus *Perionyx* beginning in October to November (the cold season) in Burma.

The new species were found in different habitats. Two species, *M. songkhlaensis* sp. n. and *M. trangensis* sp. n., were found in botanical gardens, while *M. khaochamao* sp. n. and *M. surinensis* sp. n. were found in dipterocarp forests. *M. saxicalcis* sp. n. was found in the top soil of a limestone forest area and *M. khaoluangensis* sp. n. was found in modified deciduous forest areas used for integrated farming. The soil pH was close to 7 in all habitat types.

The newly collected material was compared with type and other reference material at the Biozentrum Grindel und Zoologisches Museum, University of Hamburg (ZMH) and the Natural History Museum (NHMUK), London. The reinvestigations led to new findings, illustrations and redescriptions as given above. As a next step, we would welcome microhabitat analyses and molecular phylogenetic studies to clarify the ecological behaviour and the systematic position of the species and the evolution of the complicated genus *Metaphire* in general.

Acknowledgements

This research was funded by The Thailand Research Fund Senior Scholar Grant (2016–2018), RTA 5880002. Additional funding was from The National Research University Project of CHE and the Ratchadaphiseksomphot Endowment Fund (RES 560530145-FW). We are most grateful to the curators who provided specimens for our study: Emma Sherlock (NHMUK). Thanks also to Thita Krutchuen for preparing excellent illustrations, and to all members of the Animal Systematics Research Unit, Chulalongkorn University for assistance in collecting specimens. We appreciate the kind suggestions and editing of the editor and reviewers that made this manuscript publishable. Thanks to Dr. Robert Butcher for inspecting the linguistics of the manuscript.

References

- Bantaowong, U., Chanabun, R., Tongkerd, P., Sutcharit, C., James, S.W. & Panha, S. (2011a) New earthworm species of the genus *Amyntas* Kinberg, 1867 from Thailand (Clitellata, Oligochaeta, Megascolecidae). *ZooKeys*, 90, 35–62.
<http://dx.doi.org/10.3897/zookeys.90.1121>
- Bantaowong, U., Chanabun, R., Tongkerd, P., Sutcharit, C., James, S.W. & Panha, S. (2011b) A new species of the terrestrial earthworm of the genus *Metaphire* Sims and Easton, 1972 from Thailand with redescription of some Species. *Tropical Natural History*, 11 (1), 55–69.
- Bantaowong, U., Somniyam, P., Sutcharit, C., James, S.W. & Panha, S. (2014) Four new species of the earthworm genus *Amyntas* Kinberg, 1867 with redescription of the type species (Clitellata: Megascolecidae). *Raffles Bulletin of Zoology*, 62, 655–670.
- Beddard, F.E. (1900) On the earthworms collected during the “Skeat Expedition” to the Malay Peninsula, 1899–1900. *Proceedings of the Zoological Society of London*, 1900, 891–911.
- Beddard, F.E. (1912) The Oligochaeta terricolae of the Philippines. Part 1. The genus *Pheretima*. *Philippines Journal of Science*, Series D, 7, 79–203.
- Blakemore, R.J. (2002) *Cosmopolitan Earthworms – an Eco-taxonomic Guild to the Peregrine Species of the World*. VermEcology, Canberra, 500 pp. [pp. 62–237]
- Blakemore, R.J. (2011) Description of a new *Amyntas* earthworm (Megascolecidae *sensu stricto*) from Thailand. *Bulletin of the National Science Museum, Series A (Zoology)*, 37, 9–13.
- Blakemore, R.J. (2012) Restating scope of genus *Metaphire* Sims & Easton, 1972: 40 years on (Oligochaeta). *Zoology in the Middle East*, 4 (Supplementum), 5–14.
<http://dx.doi.org/10.1080/09397140.2012.10648980>
- Blakemore, R.J., Csuzdi, C., Ito, M.T., Kaneko, N., Kawaguchi, T. & Schilthuizen, M. (2007) Taxonomic status and ecology of Oriental *Pheretima darnleiensis* (Fletcher, 1886) and other earthworms (Oligochaeta : Megascolecidae) from Mt Kinabalu, Borneo. *Zootaxa*, 1613, 23–44.
- Fletcher, J.J. (1886) Notes on Australian Earthworms. Part III. *Proceedings of the Linnean Society of NSW*, Series 2, 2, 377–402.
- Gates, G.E. (1926) Note on a new species of *Pheretima* from Rangoon. *Annals and Magazine of Natural History*, Series 9, 17, 411–415.
<http://dx.doi.org/10.1080/00222932608633432>
- Gates, G.E. (1930) The earthworms of Burma I. *Records of the Indian Museum*, 32, 257–356.

- Gates, G.E. (1931) The earthworms of Burma II. *Records of the Indian Museum*, 33, 327–442.
- Gates, G.E. (1932) The earthworms of Burma III. The Megascolecinae. *Records of the Indian Museum*, 34, 357–549.
- Gates, G.E. (1935) On some earthworms from East Perak and Christmas Island. *Bulletin of the Raffles Museum*, 10, 80–95.
- Gates, G.E. (1939) Thai earthworms. *Journal of the Thailand Research Society*, 12, 65–114.
- Gates, G.E. (1945) On some earthworms from Ceylon II. *Spolia Zeylanica*, 24, 69–90.
- Gates, G.E. (1961) Ecology of some earthworms with special reference to seasonal activity. *The American Midland Naturalist*, 66, 61–86.
<http://dx.doi.org/10.2307/2422868>
- Gates, G.E. (1972) Burmese earthworms, an introduction to the systematics and biology of megadrile oligochaetes with special reference to the Southeast Asia. *Transactions of the American Philosophical Society*, 62, 1–326.
<http://dx.doi.org/10.2307/1006214>
- Horst, R. (1893) Descriptions of earthworms. No. 7. On the Malayan earthworms. *Notes from the Leyden Museum*, 15, 316–329.
- Michaelsen, W. (1891) Oligochaeten des Naturhistorischen Museum in Hamburg, IV. *Jahrbuch der Hamburgischen Wissenschaftlichen Anstalten*, 8, 1–42.
- Michaelsen, W. (1899) Terricolen von verschiedenen Gebieten der Erde. *Jahrbuch der Hamburgischen Wissenschaftlichen Anstalten*, 16, 1–122
- Michaelsen, W. (1902) Neue Oligochaeten und neue Fundorte alt-bekannter. *Jahrbuch der Hamburgischen Wissenschaftlichen Anstalten*, 19, 1–54.
- Michaelsen, W. (1907) Neue Oligochaeten von Vorder-Indien, Ceylon, Birma und den Andaman-Inseln. *Mitteilungen aus dem Naturhistorischen Museum*, 24, 143–188.
- Michaelsen, W. (1922) Oligochäten aus dem Rijks Museum van Natuurlijke Historie zu Leiden. *Capita Zoologica*, 1, 1–68.
- Michaelsen, W. (1934) Oligochaeten von Französisch-Indochina. *Archives de Zoologie Expérimentale et Générale*, 76, 493–546.
- Ohfuchi, S. (1938) New and little known forms of earthworms, *Pheretima* from Nippon. *Saito-Ho-On Kai Museum Research Bulletin*, 15, 53–66.
- Ohfuchi, S. (1956) On a collection of the terrestrial Oligochaeta obtained from the various localities in Riu-kiu Islands, together with the consideration their geographical distribution (Part I). *Journal of Agricultural Science, (Tokyo Nagyo Daigaku)*, 3, 131–176.
- Ohfuchi, S. (1957) On a collection of the terrestrial Oligochaeta obtained from the various localities in Riu-kiu Islands, together with the consideration of their geographical distribution (Part II). *Journal of Agricultural Science (Tokyo Nagyo Daigaku)*, 3, 243–261.
- Omodeo, P. (1957) Oligocheti dell'Indochina e del Mediterraneo Orientale. *Memorie del Museo Civico di Storia Naturale di Verona*, 5, 321–336.
- Perrier, E. (1872) Recherches pour servir a l'histoire des Lombriciens terrestres. *Nouvelles Archives du Museum d'histoire Naturelle*, 8, 5–198.
- 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.
<http://dx.doi.org/10.1016/j.bse.2013.07.001>
- Rosa, D. (1890) Viaggio di Leonardo Fea in Birmania e regioni vicine, XXVI. Preichetidi. *Annali del Museo Civico di Storia Naturale, Giacomo Doria*, 10, 107–122.
- Sims, R.W. & Easton, E.G. (1972) A numerical revision of the earthworm genus *Pheretima* (Megascolecidae: Oligochaeta) 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.
<http://dx.doi.org/10.1111/j.1095-8312.1972.tb00694.x>
- Somniyam, P. & Suwanwaree, P. (2009) The diversity and distribution of terrestrial earthworms in Sakaerat environmental research station and adjacent area, Nakorn Ratchasima, Thailand. *World Applied Sciences Journal*, 6, 221–226.
- Stephenson, J. (1932) A re-examination of certain Oligochaeta from the Malay Peninsula (Skeat Expedition, 1899-1900). *The Annals and Magazine of Natural History*, 9, 201–240.
<http://dx.doi.org/10.1080/00222933208673494>
- Ude, H. (1932) Beiträge zur Kenntnis der Gattung *Pheretima* und ihrer geographischen Verbreitung. *Archiv für Naturgeschichte*, 1, 114–190.
- Vaillant, L. (1868) Note sur l'anatomie de deux especes du genre *Perichaeta* et essai de classification des Annélides Lombriciens. *Annales des Sciences Naturelles*, 10, 225–256.