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Description of Two New Earthworm Species (Oligochaeta: Megascolecidae) from the Mekong River, Thailand

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ABSTRACT.– Two new earthworm species are described from the Mekong River, Thailand, namely *Amynthas septuaginta* Bantaowong & Panha, sp. nov. and *Metaphire fidelis* Bantaowong & Panha, sp. nov. The former is a small Mekong earthworm in the *A. aeruginosus* species group with two pairs of spermathecal pores in 7/8-8/9. It is mainly distinguished from other *Amynthas* from the Mekong River by its small size (less than 50 mm), and with no genital markings at the male pore region. The latter is distinguished by having four pairs of spermathecal pores in 5/6-8/9, genital markings paired on xvii, ampulla paddle-shaped, and with diverticulum as a slender tube with a loose loop. Morphological description, taxonomic comments, illustrations, and distribution are provided.

KEYWORDS: *Amynthas*, *Metaphire*, taxonomy, Mekong River

INTRODUCTION

Terrestrial earthworms play a crucial role in soil ecosystem services. Their activities, such as burrowing and feeding on organic matter, improves the soil structure, promote nutrient cycling, and enhances soil fertility. Moreover, they support other living creatures in the soil, helping maintain a balanced soil food web (Lavelle et al., 2006; Decaëns et al., 2006; Blouin et al., 2013). Currently, over 6,000 species of megadrile earthworms have been identified globally, with around 5,406 recognized as valid species. (Csuzdi, 2012; Misirlioğlu et al., 2023). However, many undescribed earthworm species are still being found in numerous collections around the world, with significant gaps in biodiverse regions, particularly in tropical areas.

The Mekong River, which flows through Cambodia, Laos, Myanmar, Thailand, and Vietnam (Southeast Asia), is recognized as a biodiversity hotspot, serving as a habitat for several of the planet's most iconic and endangered species, such as the Mekong giant catfish (*Pangasianodon gigas*) (Kottelat and Whitten, 1996; Baird, 2001; Coates et al., 2003). In addition, in the last decade, new species have frequently been discovered in this region (Grootaert, 2017; Ruengsawang et al., 2012, 2024; Laudee et al., 2020; Jeratthitikul et al., 2021; Kongim et al., 2023; Sanoamuang and Watiroyram, 2023). Thus, ongoing research in the Mekong Basin is crucial for preserving the delicate balance of the environment in the midst of increasing human activity and climate change.

During our field surveys along the Mekong River in Thailand (2011–2018), we collected unknown earthworm specimens occurring on the riverbank that had a clitellum covering segments fourteen to sixteen, and clearly paired male pores on segment eighteen. These were initially presumed to be members of the genus *Amynthas* and/or *Metaphire* because these genera are the most species-rich in the Megascolecidae of Thailand, with approximately 50 species and subspecies (Bantaowong et al., 2011a, 2011b, 2014, 2015, 2016; Blakemore, 2011; Chanabun et al., 2023; Gates, 1939, 1972). In this paper, we describe two new species found in the samples from the Mekong River field surveys. *Amynthas septuaginta* sp. nov. belongs to the *A. aeruginosus* species group with intersegmental spermathecal pores in 7/8-8/9, occurring only in the bank of Mekong River in Bueng Kan Province. On the other hand, *Metaphire fidelis* sp. nov. has spermathecal pores in 5/6-8/9, and genital marking present in segment xvii. This species is quite widely distributed along the bank of Mekong River in Nong Khai, Nakhon Pranom, and Bueng Kan provinces.

MATERIALS AND METHODS

Live earthworm specimens were hand-collected along the Mekong River during 2010 to 2011. The worms were anesthetized in 30% (v/v) ethanol, transferred to 4% (v/v) formalin for approximately 12 hours, and then preserved in 75% (v/v) ethanol for long-term preservation and subsequent morphological

studies. This research was conducted under the approval of the Animal Care and Use regulation (numbers U1-09066-2563 and IACUC-KKU-32-65).

The description of each species was made using Olympus SZX16 stereoscopic microscope. Illustrations of the body segments, external characters and internal organs are shown in Figures 1 and 2 for the two new species, respectively. The number of segments and the body width and length were measured in both full adults and juveniles; measurements are presented as range (min-max) and mean ± one standard deviation. Species identification is based on original and subsequent literature (i.e., Gates, 1939, 1972; Sims and Easton, 1972; Michaelsen, 1896, 1899; Bantaowong et al., 2011a, 2011b, 2014, 2015, 2016; Chanabun et al., 2023) and compared with type specimens and/ or reference collections from the Biozentrum Grindel und Zoologisches Museum, University of Hamburg, Germany (ZMH). The convention of using Roman numerals for segments in both external and internal characters (e.g. male pore in segment xviii), and Arabic numerals for intersegmental furrows (ex. spermathecal pore in 5/6-8/9) was adhered to.

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

Holotype and paratype specimens of the new species are deposited in the Chulalongkorn University Museum of Zoology, Bangkok, Thailand (CUMZ) and the National Science Museum, Thailand (THNHM).

RESULTS

Taxonomy

Family Megascolecidae Rosa, 1891

Genus *Amynthas* Kinberg, 1867

Type species.– *Amynthas aeruginosus* Kinberg, 1867, by monotypy.

Amynthas septuaginta Bantaowong & Panha, sp. nov.

http://zoobank.org/urn:lsid:zoobank.org:act: C995E01D-251A-44AF-BA7F-7745FD78D9F0

(Fig. 1, Table 1)

Materials examined.– Holotype: adult CUMZ 3831. Paratypes: eight adults (CUMZ 3832), two adults (THNHM); same collection data as holotype. Coll. S. Panha, U. Bantaowong, R. Chanabun, W. Siriwut (1st December, 2012).

Type locality.– Mekong Riverbank, Mueang, Bueng Kan, Thailand (18°22'9.9"N, 103°39'10.4"E), elev. 164 m.

Etymology.– The species name '*septuaginta*' symbolizes the celebration of the 70th birthday of Her Royal Highness Princess Maha Chakri Sirindhorn in 2025.

Diagnosis.– Small earthworm, 36–45 mm in length, with 77–92 segments. Two tiny pairs of spermathecal pores in 7/8-8/9. Female pore mid-ventral on xiv. Male pores paired on inner side of an oval swelling porophore of segment xviii, genital marking absent. Spermathecae with round-shaped ampulla; diverticulum elongated-ovoidal seminal chamber. Testis sacs paired in x and xi. Seminal vesicles paired in xi, xii. Prostate glands paired, in xvii-xx. Intestinal caecum simple, short finger-shaped in xxvii-xxiv.

Description of Holotype.– Length 39 mm; width 2.1 mm at segment vii, 2.8 mm at segment xviii, 2.1 mm at clitellum. Body cylindrical with 82 segments. Setae regularly distributed around segmental equators, numbering 54 at vii, 56 at xx, 7 between male pores, setae formula AA:AB:ZZ:ZY=1:1:1:1 at xiii. Single female pore at xiv. Prostomium epilobic. First dorsal pore at 6/7. Clitellum annular xiv-xvi with setae on ventral side on segment xv-xvi.

Male pores paired, minute, situated ventrolaterally on setal line of xviii, about 0.28 of the circumference apart; each pore scarcely visible at the inner side of an oval swelling porophore. Spermathecal pores 2 pairs in 7/8-8/9, about 0.28 circumference ventrally apart. Each on a tiny tubercle that is sunken into the intersegmental furrow.

Internal characters: septa, in general, moderately thickened 5/6-6/7, 7/8 thin, 8/9 aborted, 9/10-13/14 very thin. Gizzard moderate in size, bell-shaped, behind 7/8. Intestinal origin in xv, no lymph glands observed. Intestinal caeca simple, short finger-shaped in xxvii-xxiv. Hearts esophageal in x-xiii as usual.

Male system holandric, paired testis sacs in x, xi. Seminal vesicles paired, large in xi-xii. Prostate glands moderate in size, paired in xvii-xx. Prostatic ducts thin but musculated, U-shaped. Ovaries paired in xiii. Spermathecae 2 pairs situated in segments vii and viii. Ampulla round, duct moderate in thickness and nearly equal to ampulla in length, sharply marked off from the latter. Diverticulum longer than the main portion, an elongated-ovoidal seminal chamber.

Variation.– Holotype measures 39 mm body length. Ten paratypes range in body length from 36–45 mm (49.50 ± 2.99 mm), with 77–92 segments.

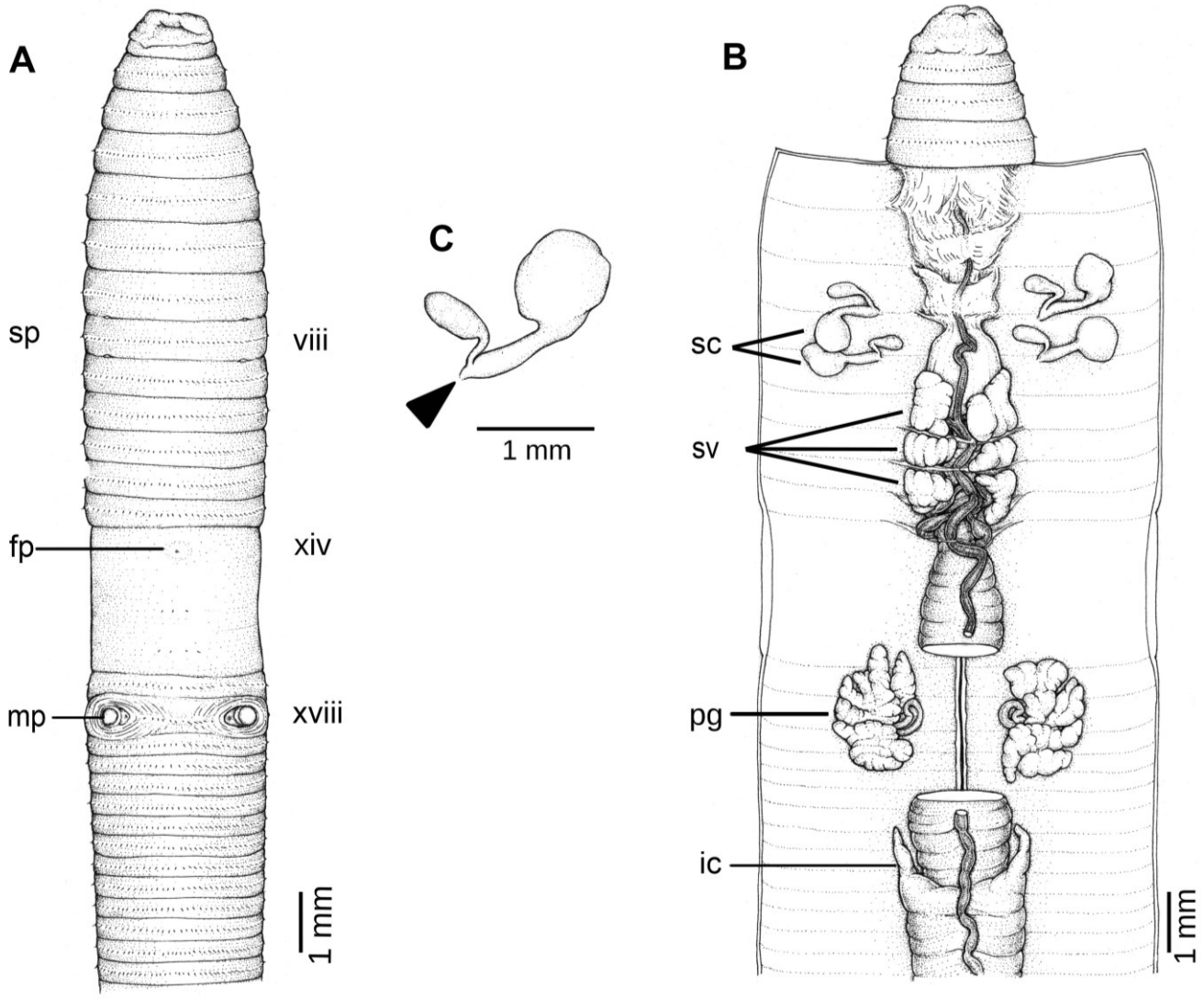

FIGURE 1. External and internal morphology of holotype (CUMZ3831) of *Amynthas septuaginta* sp. nov., **A**. external ventral view; **B**. internal dorsal view; **C**. spermathecae. Dark arrow indicates the connection of the spermathecae and spermathecal pore.

Distribution.– The new species is only known from the type locality.

Remarks.– The new species belongs to the *A. aeruginosus* species group, characterized by having spermathecal pores in 7/8-8/9, which originally included more than 60 species and subspecies (Sims and Easton, 1972). Since then, an addition of 17 more species have been reported, fourteen of which were described from Laos (Hong, 2010; Hong et al., 2018; Bantaowong et al., 2023), one from Taiwan (Shen et al., 2014), one from China (Yuan et al., 2019), and one from Vietnam (Nguyen et al., 2022). Most of them exhibit body lengths exceeding 50 mm. However, *Amynthas septuaginta* sp. nov. is similar in body size (less than 50 mm) with *Amynthas copulatrix* (Michaelsen, 1922) from Sarawak, and *A. fissigerus* (Michaelsen, 1899) and *A. pataniensis labunensis* (Michaelsen, 1896) from Sulawesi, but it is easily distinguished by the absence of genital markings, whereas the latter three species have genital markings in the male pore region. In Thailand, the only species within this species group that has been reported before is *A. hupbonensis* (Stephenson, 1931) from the eastern part of the country. *Amynthas septuaginta* sp. nov. is easily distinguished from *A. hupbonensis* by its small size of 36–45 mm body length compare to 215 mm in *A. hupbonensis*.

Among the Mekong River *Amynthas* species, which include *A. gibbosus* (Thai & Samphon, 1990), *A. mekongianus* (Cognetti, 1922), *A. polychaetiferus* (Thai & Samphon, 1990), *A. juliani* (Perrier, 1875), *A. samphoni* (Thai & Samphon, 1988), *A. unicipeniferus* (Thai & Samphon, 1988), and *A. reductus* Nguyen, Lam & Nguyen, 2022 (Blakemore et al., 2007; Nguyen et al., 2022), the new species differs from the other species by its smaller size (body length 36–45 mm vs. >100

TABLE 1. Comparison of characters among *Amynthas septuaginta* sp. nov., and other *A. aeruginosus* species group with a body size less than 50 mm: *Amynthas copulatrix* (Michaelsen, 1922), *A. fissigerus* (Michaelsen, 1899) and *A. pataniensis labunensis* (Michaelsen, 1896). Missing data are shown with a question mark (?).

Characters	*A. septuaginta* sp. nov.	*A. copulatrix*	*A. fissigerus*	*A. pataniensis labunensis*
Body length (mm)	36–45	32	33–38	40
Segment number	77–92	61	86–95	78
Spermathecal pores	7/8-8/9, genital markings absent	7/8-8/9, genital markings present: paired in vii, viii	7/8-8/9, genital markings absent	7/8-8/9, genital markings absent
First dorsal pore	6/7	12/13	12/13	?
Male pore	genital markings absent	genital markings present paired in xix,	genital markings present paired in xviii, xix, xx	genital markings present paired in xvii, xix, xx
Spermathecae	round sac	lanceolate	long oval	large sac
Prostate gland	xvii-xx	xvi-xxii	xvi-xviii	xvi-xviii
Intestinal caecum	simple, xxvii-xxiv	simple, xxvii-xxv	simple, xxvii-xxv	simple, xxvii-xxiii
Type locality	Thailand	Sarawak	Sulawesi	Sulawesi

mm) and no genital markings (vs. with genital markings) on the male region.

Genus *Metaphire* Sims & Easton, 1972

Type species. *Rhodopis javanica* Kinberg, 1867, by monotypy.

Metaphire fidelis Bantaowong & Panha sp. nov.

http://zoobank.org/urn:lsid:zoobank.org:act: E407E1CA-297A-4F68-A48C-B84ED7A38526

(Fig. 2, Table 2)

Materials examined.– Holotype: one adult (CUMZ 3833), from type locality. Paratypes: twelve adults (CUMZ 3834), two adults (THNHM); same collection data from the type locality. Coll. S. Panha, U. Bantaowong, R. Chanabun, W. Siriwut (1st December, 2012).

Other material examined.– 41 adults (CUMZ 3835), Mekong riverbank, Wat Phra Klang Tha, That Phanom, Nakhon Phanom, Thailand, (16°58'46.0"N, 104°43' 44.2"E), 162 m in elevation, 4th December, 2013; and 32 adults (CUMZ 3836), Mekong riverbank, Mueang, Nong Khai, Thailand, (17°52'33.7'N, 102°42' 31.1'E), 160 m in elevation, 7th December, 2013.

Type locality.– Mekong Riverbank, Wat Pa Thep Wimut, Mueang, Bueng Kan, Thailand (18°25'54.6"N, 103°26'21.8"E), elev. 166 m.

Etymology.– The specific name is from the Latin word '*fidelis'*, which means faithfulness, referring to loyalty to the Her Royal Highness Princess Maha Chakri Sirindhorn.

Diagnosis.– Medium-sized earthworm, 131–208 mm in length, with 110-170 segments. Four pairs of spermathecal pores at 5/6-8/9, slit-like. Female pore midventral on xiv. Male pores paired on ventral lateral of segment xviii, protrude and surrounded by 2–3 folds within a shallow circular invagination of the body wall, with a pair of transversely oval genital markings on setal line of xvii. Spermathecae: large paddle-shaped ampulla, diverticulum slender tube with loose loop. Testis sacs paired in x and xi. Seminal vesicles paired in xi, xii. Prostate glands paired, large in xvi-xxii. Intestinal caecum simple in xxvii-xxv.

Description of Holotype.– Dimensions; 162 mm by 4.3 mm at segment vii and clitellum; body cylindrical with 154 segments. Setae regularly distributed around segmental equators, numbering 89 at vii, 80 at xx, 9 between male pore, setae 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 setae on ventral on ventrally segment xiv-xvi.

Male pores paired on ventral lateral of segment xviii, protrude, and surrounded by 2–3 folds within a shallow, circular invagination of body wall, 0.25 body circumference ventrally apart, distance between male pores 3.6 mm. Genital markings paired, transversely oval on setal line of xvii, in line with male pore. Spermathecal pores: 4 pairs on ventral lateral of segment 5/6-8/9, slit-like, 0.35 body circumference ventrally apart, distance between spermathecal pores 5 mm.

Septa, 5/6-8/9 very thickened; 9/10 aborted, 10/11-13/14 very thickened. Gizzard large in ix-x. Intestinal origin in xv, no lymph glands observed. Intestinal caeca paired at xxvii extending forward to xxv, simple. Hearts esophageal in x-xiii.

Male system holandric, paired testis sacs in x, xi. Seminal vesicles paired, large in xi, xii. Prostate glands well developed, paired in xviii, large and extending from xvi-xxii. Prostatic ducts long, slender, and hair-pin-shape. Small, sessile, genital marking glands in xvii corresponding to each external genital marking.

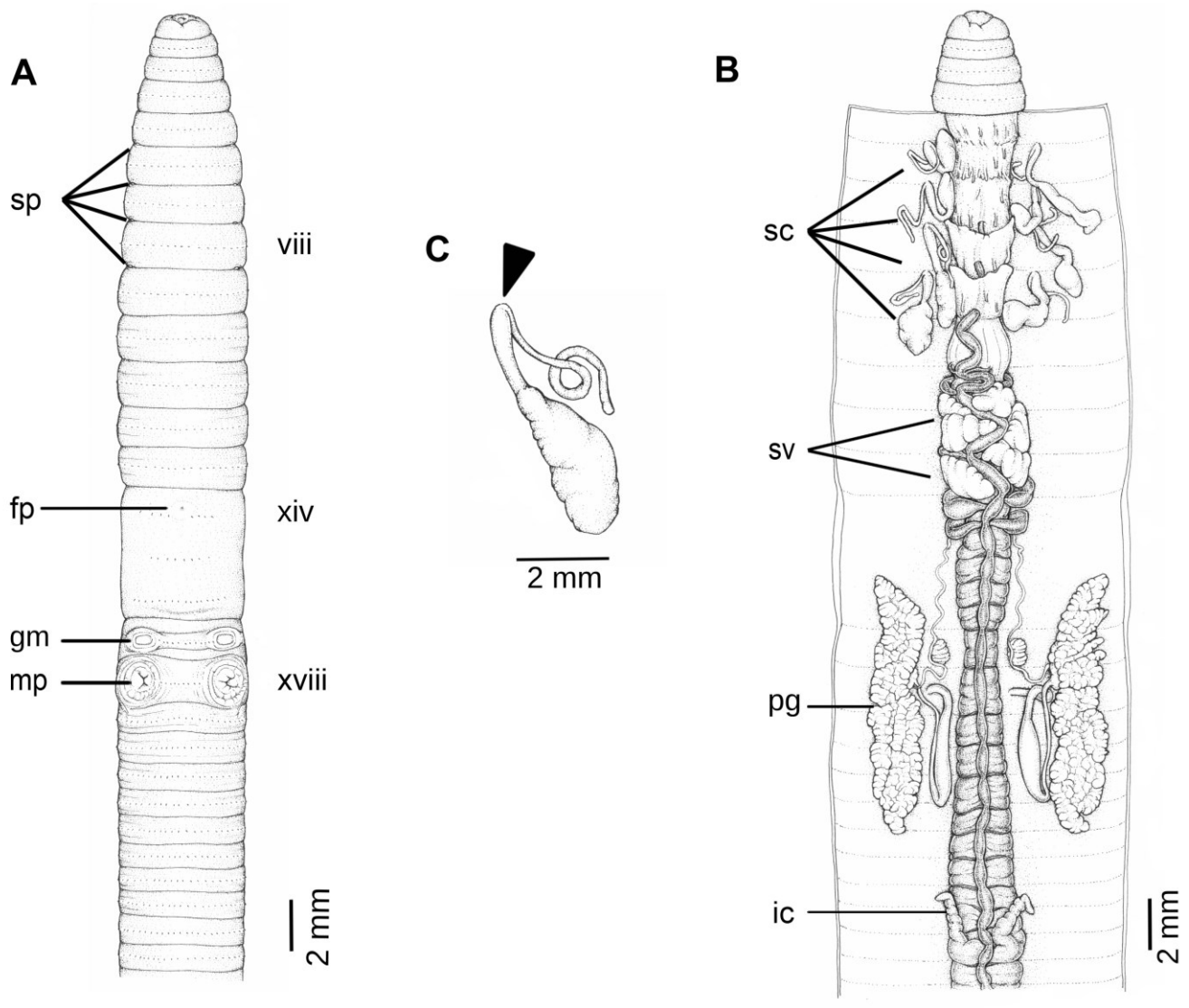

FIGURE 2. External and internal morphology of holotype (CUMZ3833) of *Metaphire fidelis* sp. nov., **A**. external ventral view; **B**. internal dorsal view; **C**. spermathecae. Dark arrow indicates the connection of the spermathecae and spermathecal pore.

Ovaries paired in xiii. Spermathecae 4 pairs situated in vi, vii, viii and ix. Ampulla paddle-shaped with a slender stalk. Diverticulum a slender tube with loose loop shorter than ampulla.

Variation.– Holotype measures 162 mm body length. Twelve paratypes range in body length from 131–208 mm (164.42 ± 21.64 mm), with 110–170 segments.

Distribution.– Currently, this new species is recorded from several localities in the Mekong riverbank in Bueng Kan, Nakhon Pranom and Nong Khai.

Remarks.– *Metaphire fidelis* sp. nov., an octothecate, holandric, and with a pair of genital markings on xvii only, is similar to *M. varellana* (Michaelsen, 1934) from Vietnam (Sims and Easton, 1972) However, the former is smaller than the new species, measuring 105 mm in length, 2–4 mm in body width, and has a pear-shaped ampulla, with a narrowly serpentine diverticulum that is not sharply defined. In the Mekong River, eight species of *Metaphire* have been reported: *Metaphire peguana* (Rosa, 1890), *M. bahli* (Gates, 1945), *M. posthuma* (Vallaint, 1867), which are widely distributed in the tidal areas of the river in Thailand, Laos, and Vietnam; *M. luongphabanganus* (Thai & Samphon, 1990), *M. packhanensis* (Thai & Samphon, 1990) from Laos; *M. thaibinhensis* (Thai, 1984), *M. fluvialoides* (Huynh, 1998) and *M. giengensis* Nguyen, Lam & Nguyen, 2022 from Vietnam (Blakemore et al., 2007; Nguyen et al., 2022). The new species is very similar in general appearance to *M. posthuma* and *M. giengensis*. However, *M. posthuma* has two pairs of genital markings in xvii and xix, an oval ampulla, and a convoluted terminal section of the diverticulum. The latter species is distinguished by having two pairs of

TABLE 2. Comparison of characters among *Metaphire fidelis* sp. nov., and other *Metaphire* species which were found in the Mekong River: *Metaphire bahli* (Gates, 1945), *M. fluvialoides* (Hoi, 1998), *M. giengensis* Nguyen, Lam & Nguyen, 2022, *M. luongphabanganus* (Thai & Samphon, 1990), *M. packhanensis* (Thai & Samphon, 1990), *Metaphire peguana* (Rosa, 1890), *Metaphire posthuma* (Vallaint, 1867), *M. thaibinhensis* (Thai, 1984). Missing data are shown with a question mark (?).

Characters	*M. fidelis* sp. nov.	*M. bahli**	*M. fluvialoides*	*M. giengensis*	*M. luongpha-banganus*	*M. packha-nensis*	*M. peguana**	*M. posthuma**	*M. thaibin-hensis*
Body length (mm)	131–208	110v150	170	39–67	125	154	140–240	111–121	125–140
Segment number	110–170	92–119	129	76–131	160	157	97–124	91–124	132–135
Spermathecal pores	5/6-8/9	6/7-8/9	5/6-8/9	5/6-6/7	6/7-8/9	6/7-8/9	6/7-8/9	5/6-8/9	5/6-6/7
First dorsal pore	13-Dec	?	13-Dec	12-Nov	13-Dec	13-Dec	13-Dec	13-Dec	?
Male pore	genital markings present one paired in xvii	genital markings present two paired in 17/18,18/19	genital markings absent	genital markings present one paired in xviii	genital markings absent	genital markings absent	genital markings present two paired in 17/18,18/19	genital markings present two paired in xvii, xix	genital markings absent
Spermathecae	paddle shape	ellipsoidal shape	ellipsoidal shape	oval shape	gourd shape	oval shape	spherical shape	ellipsoidal shape	oval shape
Prostate gland	xvi-xxii	xvii-xxi	?	xvii-xx	?	?	xvi-xxi	xv-xxi	xvii-xx
Intestinal caecum	simple	simple	simple	simple	simple	simple	simple	simple	simple
Type locality	Thailand	Sri Lunka	Vietnam	Vietnam	Laos	Laos	Myanmar	Java	Vietnam

* Data from Gates (1972)

spermathecal pores in 5/6–6/7, and two pairs of genital markings located close to male pores in segment xviii (Table 2).

DISCUSSION

At present, a total of 20 known species of earthworms has been reported in the Mekong River and its tributaries: Three species are commonly found in the tidal area of the Mekong River in Thailand, Laos and Vietnam, which include *Metaphire posthuma*, *M. peguana* and *M. bahli* (Blakemore et al., 2007); *Amynthas mekongianus* is an iconic earthworm species found in Laos and Thailand (Gates, 1939, 1972; Blakemore et al., 2007). Seven species have been reported in Vietnam, which include *A. polychaetiferus*, *A. reductus*, *Eisenia fetida*, *M. thaibinhensis*, *M. fluvialoides*, *M. giengensis* and *Polypheretima mekongmontis* (Nguyen et al., 2016, 2022); another seven species have been reported in Laos: namely *A. juliani*, *A. gibbosus*, *A. samphoni*, *A. unicipeniferus*, *M. luongphabanganus*, *M. packhanensis*, *Pheretima choana* (Blakemore et al., 2007; Thai and Samphon, 1990, 1991); in contrast, only two species have been reported in Thailand, *Amynthas septuaginta* sp. nov. and *Metaphire fidelis* sp. nov., the new species described in this study. The two new species were found in muddy soil. *Amynthas septuaginta* sp. nov. occurs only in Bueng Kan Province while *Metaphire fidelis* sp. nov. are quite wide distribution, in Nong Khai, Nakhon Phanom and Bueng Kan Provinces. Currently, almost all the banks of the Mekong River in Thailand, which provide habitat for various creatures, including earthworms, are being reinforced with concrete to prevent riverbank erosion. Such modifications may impact the habitats of these earthworms. Therefore, further studies on the molecular phylogenetics of earthworms in the Mekong River basin are necessary to clarify the systematics position of these species.

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