

A NEW SPECIES OF SEMI-AQUATIC FRESHWATER EARTHWORM OF THE GENUS *GLYPHIDRILUS* HORST, 1889 FROM THE MEKONG RIVER (OLIGOCHAETA: ALMIDAE)

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ABSTRACT. — We report the discovery of a new semi-aquatic earthworm of the genus *Glyphidrilus* Horst, 1889 from the Mekong River, Ubon Ratchathani Province, Thailand. The description of the new species includes morphological characters of the adults, cocoons and newly-hatched juveniles. Moreover some ecological observations of the new species including the relationship with a terrestrial species *Metaphire posthuma* (Vaillant, 1868), and activities along the river banks, are reported and discussed. After comparing *Glyphidrilus mekongensis* Panha & Chanabun, new species with the closely related species described from SE Asia, we concluded that the new species differs from its congeners by having long clitellar wings (24–34). We observed no genital pores but genital markings are in 23. Seminal vesicles are presented but not atrial glands or spermathecae. Banana-shaped, deep green-black cocoons contain as many as seven young worms. Some biological activities of the new species will also be discussed.

KEY WORDS. — *Glyphidrilus*, earthworm, Almidæ, new species, Thailand

INTRODUCTION

Thailand is an integral part of the Gondwana-derived Shan-Thai terrane (Bunopas, 1981) as from the west and east Shan-Thai blocks collisions in late Triassic times together with the drifting of the nearby western Burma plate (Srinak et al., 2007). Now this region possesses a rich endemic regional (Indo-China and Indo-Burma) earthworm fauna (Gates, 1972). More recently, during the last glacial period when global sea levels were lowered, the Thai central lowland emerged (Hall & Blundell, 1996), which later furnished the habitats for Thai endemic flora and fauna (Arbhabhirama et al., 1988; Cubitt & Stewart-Cox, 1995). Our extensive surveys of the semi-aquatic freshwater earthworm genus

Glyphidrilus Horst, 1889 suggest that it is highly threatened by human transformation of riparian habitats, including forests. *Glyphidrilus* are largely restricted to the riparian areas of lowland forests and river bank mud. Some occurs only in natural habitats, but others may be found in areas modified by human activities, such as the banks of major rivers like the Mekong (Chanabun, unpublished data). There are 20 species of *Glyphidrilus* in the world, including one subspecies (Chanabun et al., 2011), all found in Asia and Africa. The records in Asia are numerous from the Indonesian islands through the Malay Peninsula and Burma, west to India and Nepal, and north to China. Up till now there has been no report of *Glyphidrilus* from Thailand. The nearest records to Thailand are two new species from Singapore and Malaysia

(Shen & Yeo, 2005), one species described from China (Chen & Xu, 1977), and one species recently described from Laos (Chanabun et al., 2011). The new species reported here was found during our recent survey in northeast Thailand. It was found along the Mekong River bank at Khong Chiam District, Ubon Ratchathani Province (Fig. 1) at low water during the beginning of the dry season in Nov.2010. Later on, collecting and biological observations were carried out every month. The worms live along the shoreline in proximity to the water but are not affected by the water current because the habitat is in a protected bay at low water, with a rock wall in front protecting it from strong currents (Fig. 2A, C). The new species is currently considered to be endemic to the lower Mekong River region.

MATERIAL AND METHODS

Earthworms were collected by carefully digging up the topsoil near casts on the shore and in the water using hand sorting and sieving the soil from a river bank of Mekong River at Khong Chiam District, Ubon Ratchathani Province, northeastern Thailand at 15°18'57.1"N, 105°30'43.9"E, 101 m elevation on 6 Nov.2010. The water level was down making the bank width 21 m. There are some small bushes of *Pericaria attenuatum* (R.Br.) Sojak ssp. *pulchra* (Blume) K.L.Wilson occurring along the bank. Adults, juveniles and cocoons were collected. The worms were killed in 30% (v/v) ethanol, photographed, transferred to 5% (w/v) formalin for fixation in approximately 12 hours, and then transferred to 70% (v/v) ethanol for longer term preservation and subsequent morphological studies (Fig. 3A–C).

Duplicate specimens and/or tissue samples (in the cases of morphotypes determined to be unique on field inspection) were preserved in 95% ethanol for molecular data and DNA barcoding analyses. About 40 adult worms were put in liquid nitrogen at –190°C for further allozyme and other molecular analyses. Cocoons were preserved in 70% ethanol, from some of which young worms have been extruded, and some cocoons were kept in liquid nitrogen for further studies.

The description of the species was made from observation under a Stemi DV 4 ZEISS stereoscopic light microscope. The following external and internal morphological structures characters were recorded: body length and segment number; the positions of clitellum and clitellar wings, genital markings, intestinal origin, gizzard, spermathecae, hearts, and seminal vesicles. The cocoons were also critically searched and studied, and young worms after hatching were also carefully investigated. Drawings were made of the body segments and the distinct external characters and internal organs, as mentioned above, and are shown in Figs. 4–7. For the new species, a newly hatched worm was also drawn. The number of segments and the body width and length were measured in both full adults and juveniles, and are presented as the range (min–max) and mean $\pm$ one standard deviation.

Comparative studies of *Glyphidrilus* type specimens were investigated at four natural history museums: Museum of

Zoology (CUMZ), Chulalongkorn University, Thailand; Zoological Reference Collection (ZRC), Raffles Museum of Biodiversity Research, National University of Singapore, Singapore; the Natural History Museum (NHM), London, UK; Biozentrum Grindel und Zoologisches Museum (ZMH), University of Hamburg, Germany. To confirm the novelty of the new species, we compared it to type specimens of *G. gatesi* Shen & Yeo, 2005 and *G. singaporensis* Shen & Yeo, 2005 preserved at the ZRC, *G. malayanus* Michaelsen, 1902 at the ZMH, and *G. vangviengensis* Panha & Chanabun, 2011 at the CUMZ. But *G. papillatus* (Rosa, 1890) and *G. birmanicus* Gates, 1958 from Burma, and *G. yunnanensis* Chen & Xu, 1977 from south China were studied from the original descriptions only.

Holotype and paratype specimens have been deposited in the CUMZ. Additional paratypes will be housed in the ZMH, NHM, and ZRC.

Anatomical abbreviations used are: gm, genital markings; he, hearts; np, nephridia; sc, spermathecae; sv, seminal vesicles; wi, wings.

SYSTEMATICS

Genus *Glyphidrilus* Horst, 1889

Type species. — *Glyphidrilus weberi* Horst, 1889, monotypy

Glyphidrilus mekongensis Panha & Chanabun, new species (Figs. 1, 2, 3A,D, 4)

Material examined. — Holotype: One adult (CUMZ 3215), Thailand, bank of Mekong River at Khong Chiam District, Ubon Ratchathani Province (15°18'57.1"N, 105°30'43.9"E), 101 m in elevation, coll. S. Panha, R. Chanabun, P. Tongkerd, C. Sutcharit & U. Bantaowong, 6 Nov.2010. 21 paratypes: 15 adults (CUMZ 3216), 3 adults (ZMH), 3 adults (NHM), and 2 adults (ZRC), same collection data as for holotype.

Diagnosis. — *Glyphidrilus mekongensis*, new species, medium to large sized semi-aquatic freshwater earthworm with distinct expanded tissues of clitellar wing organs on the lateral sides of the body in 24–34. Female pores, male pores and spermathecal pores not visible. Genital markings present in 23. Four pairs of seminal vesicles in 9–12, with the pair in segment 12 largest. Intestinal origin in 17. Ovaries in 13–14. Prostate and accessory glands and spermathecae absent.

Etymology. — This new species is named after the Mekong River, an international river. The locality is a part of this famous river and for this is the first time of this worm genus ever recorded from this river.

Description of holotype. — Dimensions: 132 mm body length by 2.9 mm at the anterior body region in segment 8 and 3.1 mm before the clitellar wings in segment 18, 3.6 mm after wings in segment 40 within the clitellum, body

cylindrical in the anterior part but quadrangular in transverse section view after clitellum, with 228 segments. The body is pale brown with variation from red to pink colouration at adjacent tissues of the so called wing portion in different individuals of newly collected specimens after placement in 30% ethanol for narcotization. Dorsal surface considerably broader than the ventral at the posterior end. The clitellar wings on ventro-lateral part of the clitellum in 24–34, 8.5 mm long, and about 0.6 mm wide on both sides. Prostomium zygotobous. Dorsal pores absent. Clitellum annular shape in 19–38. Four pairs of setae per segment from 2, setae formula aa:ab:bc:cd:dd = 2.9:1.0:1.8:1.9:2.3 in 8. Female pores, male pores, and spermathecal pores not visible. Genital markings: paired between b and c in 23.

Septa 5/6–8/9 thicker, 9/10–14/15 thick, and 15/16 to the last segment thin. Gizzard small, globular within 8. Intestine enlarged from 15. Dorsal blood vessel aborted anterior to 7. Hearts from 7–11. No nephridia distinguishable in the first 12 segments. Four pairs of seminal vesicles in 9–12, with the pair in segment 12 larger than the others. Ovaries in 13–14. Prostate and accessory glands and spermathecae absent.

Structures of cocoons and newly hatched worms

The cocoons, presumably of this worm, were collected from the river bank of Mekong River at Khong Chiam District, Ubon Ratchathani Province. The cocoons are banana-shaped and greenish brown, dark brown to black in colour (Figs.

2B, 3D). There were seven juveniles in one cocoon (Fig. 4C). The young worms are semitransparent and cylindrical in the anterior part and quadrangular in the posterior part. The lengths of the young average 6.4 mm, and about 0.7 mm in diameter, with 94–121 segments.

Variation. — The holotype measures 132 mm body length, with 228 segments; the body length of twenty one paratypes range from 125–224 mm (156.13 ± 32.57), with 223–382 segments (Table 1).

Distribution. — The new species is known only from the type locality. Our collections in nearby areas have found other *Glyphidrilus* different from the new species.

Habitat. — Found on the shore but in proximity to the river water, in the sandy mud topsoil with 94.44% sand, 3.89% silt, and 1.67% clay, and also under the water at about 10 cm depth. The river bank soil surface was covered with worm casts at Mekong River, Khong Chiam District. The terrestrial worm species *Metaphire posthuma* (Vaillant, 1868) was also found on slightly higher ground with many cocoons of the new species.

Remarks. — Seven described species from areas near Thailand, including *G. papillatus*, *G. malayanus*, *G. birmanicus*, *G. gatesi*, *G. singaporensis*, *G. yunnanensis*, and *G. vangviengensis*, are different from the new species with respect to spermathecae, genital markings, clitellum, and wing locations (Table 2). *Glyphidrilus yunnanensis* and *G. vangviengensis* are most similar to the new species. *Glyphidrilus mekongensis*, new species differs from *G. papillatus* from Burma by the shorter wings in 18–23, 24, with a longer clitellum in 14–40, the intestinal origin in 15, and the spermathecae in 14–17 in the latter species. *Glyphidrilus*

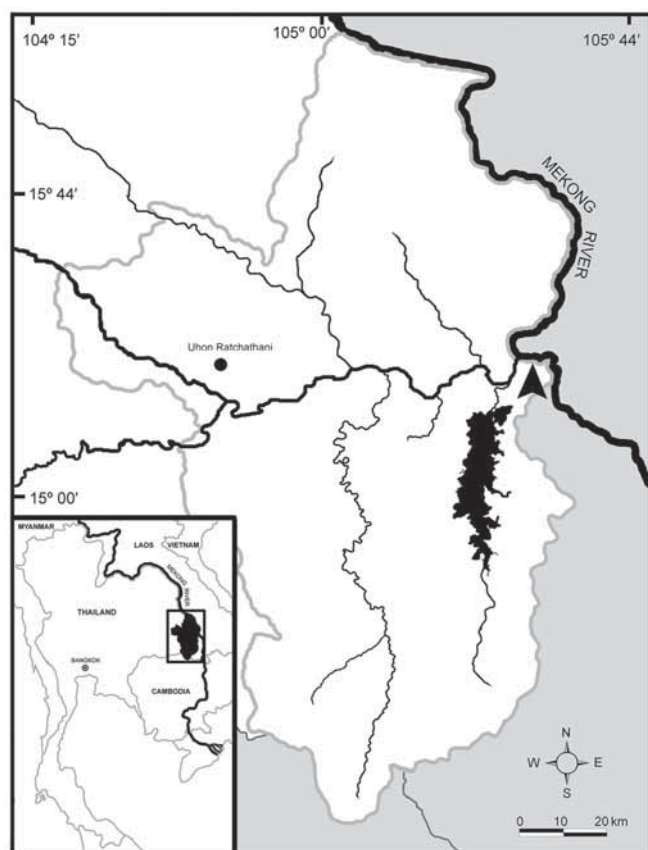


Fig. 1. Map of Ubon Ratchathani Province showing type locality of *Glyphidrilus mekongensis*, new species (arrow head) located on the shore of the Mekong River at Khong Chiam District.

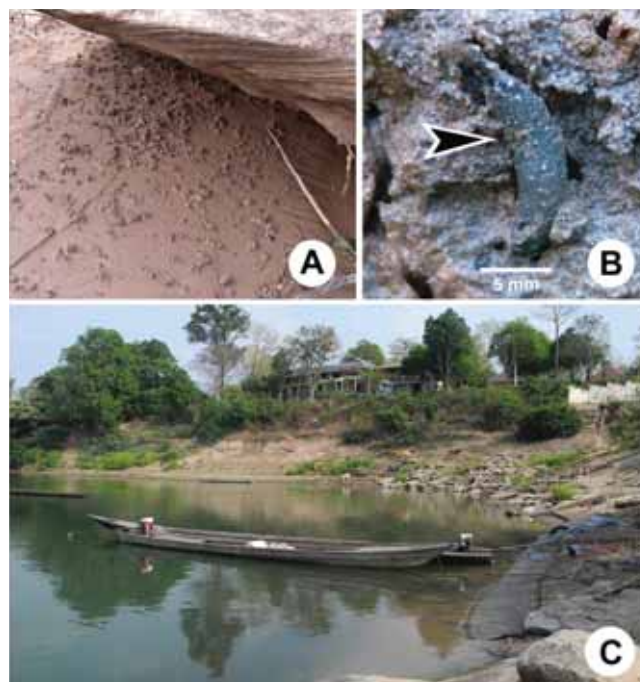


Fig. 2. Photographs showing: A, casts; B, cocoon (arrow head); C, habitat, of *Glyphidrilus mekongensis*, new species in a bay structure with rock front barrier at the Mekong River.

Table 1. Holotype and paratype dimensions and other morphological characteristics of *Glyphidrilus mekongensis* Panha & Chanabun, new species

Characters	Segments	Length	Wings location	Clitellum location	Genital markings paired in bc
Holotype CUMZ 3215	228	132	24–34	19–38	23
Paratypes CUMZ 3216					
1	229	126	24–34	19–38	23
2	252	125	24–34	19–38	—
3	306	152	24–34	19–37	23
4	223	146	24–½33, 33	19–38	23
5	277	174	24–34	19–37	—
6	298	136	24–34, ½35	19–38	—
7	382	224	24–34	19–38	23
8	271	166	24–33	19–38	23
9	129 ⁺	81 ⁺	24–34	19–38	23
10	105 ⁺	81 ⁺	24–34	19–38	23
11	178 ⁺	126 ⁺	24–34	19–38	23
12	167 ⁺	89 ⁺	24–34	19–38	23
13	142 ⁺	72 ⁺	24–34	19–38	23
14	95 ⁺	59 ⁺	24–34	19–38	—
15	148 ⁺	73 ⁺	24–34	19–38	23
16	108 ⁺	64 ⁺	24–34	19–38	—
17	60 ⁺	48 ⁺	24–½35	19–38	23
18	99 ⁺	56 ⁺	24–33	19–38	23
19	155 ⁺	82 ⁺	24–34	19–37	23
20	47 ⁺	66 ⁺	24–34	19–38	23
21	79 ⁺	49 ⁺	24–34	19–38	23

⁺ Indicates lack of tail region due to cutting during collection

mekongensis, new species differs from *G. malayanus* (Figs. 3B, 5) from Malay Peninsula by the shorter wings in ¾18, 18–21, ½22, with a clitellum in 15, 16, 17–23, 24, 25, intestinal origin in 16, three pairs of hearts in 9–11, and spermathecae in 14/15–16/17 in the latter species. *Glyphidrilus mekongensis*, new species differs from *G. birmanicus* from Burma in that *G. birmanicus* has shorter wings in 21–29 with a much longer clitellum extending from 12, 13–43, 44, the intestinal origin in 15, and the spermathecae in 13/14–17/18. *Glyphidrilus mekongensis*, new species differs from *G. yunnanensis* from Yunnan, China by the *G. yunnanensis* having wings in 22–32, with a clitellum in 18–38, and the intestinal origin in 16. *Glyphidrilus mekongensis*, new species differs from *G. gatesi* (Fig. 6) from Johor, Malaysia in the following characters: shorter and more anteriorly placed wings in 19–½24, with a shorter clitellum in 17–26, intestinal origin in 18, three pairs of hearts in 9–11, and spermathecae in 15–17 in the latter species. *Glyphidrilus mekongensis*, new species differs from *G. singaporensis* (Figs. 3C, 7) from Bukit Timah, Singapore by the shorter wings in 21–½26, 26, with a shorter clitellum in 18–27, ½28, 29, 30, 31, intestinal origin in 15 or 16, three pairs of hearts in 9–11, and spermathecae in 14–17 or 13/14–16/17 in the latter species. *Glyphidrilus mekongensis*, new species differs from *G. vangviengensis* from Vangvieng, Laos; the latter species has wing locations in 24, 25–31, 32, with clitellum location in 19, 20–35, 36, 37, the intestinal origin in 16, and genital markings median unpaired on aa

in 12, 13–14, 15 and paired between b and c in 18, 19, 20, 21–22, 23, 24 and 33, 34.

Glyphidrilus papillatus (Rosa, 1890)

Bilimba papillata Rosa, 1890: 386, Fig. 1. Type locality: Burma. Beddard, 1895: 687.

Glyphidrilus papillatus: Michaelsen, 1896: 196, 1910: 104. Stephenson, 1923: 493. Gates, 1933: 603, 1958: 60. Chen, 1938: 426. Brinkhurst & Jamieson, 1971: 763.

Remarks. — *Glyphidrilus papillatus* differs from *G. mekongensis*, new species by the shorter wings in 18–23, 24, with a clitellum in 14–40. It has genital markings median unpaired on 11–21 and 23–33, median paired on 12, 17, 18–20, 24–29, lateral paired on 12–18. Its intestinal origin is in 15, and spermathecae are in 14–17 (Table 2).

Glyphidrilus malayanus Michaelsen, 1902 (Figs. 3B, 5)

Glyphidrilus malayanus Michaelsen, 1902: 35. Type locality: Malay Peninsula: Lubock Paku, Pahang River. Michaelsen, 1910: 104. Brinkhurst & Jamieson, 1971: 762, Fig. 15.4E, F.

Material examined. — Holotype from ZMH annelid reference collections (ZMH V5875, Fig. 5A, B). Additional records: 110

Table 2. Morphological characters comparison of *Glyphidrilus mekongensis*, new species, *G. papillatus* (Rosa, 1890), *G. malayanus* Michaelsen, 1902, *G. birmanicus* Gates, 1958, *G. yunnanensis* Chen & Xu, 1977, *G. gatesi* Shen & Yeo, 2005, *G. singaporensis* Shen & Yeo, 2005, and *G. vangviengensis* Panha & Chanabun, 2011. The morphological characters are from the type specimens, except for the character with (*) which are from the original descriptions of Rosa (1890), Gates (1958), and Chen & Xu (1977).

Characters	<i>G. mekongensis</i> , new species	<i>G. papillatus</i> *	<i>G. malayanus</i>	<i>G. birmanicus</i> *	<i>G. yunnanensis</i> *	<i>G. gatesi</i>	<i>G. singaporensis</i>	<i>G. vangviengensis</i>
Length	125–224	100	52–91	95–103	123	50+	101–143	104–160
Segments	223–382	330	192–313	?	139	62+	181–341	145–229
Clitellum	19–37, 38	14–40 24, 25	15, 16, 17–23,	12, 13–43, 44	18–38	17–26 31	18–27, ½28, 29, 30,	19, 20–35, 36, 37
Wings	24–½33, 33, 34, ½35	18–23, 24	¾18, 18–21, ½22	21–29	22–32	19–½24	21–½26, 26	24, 25–31, 32
Genital markings	23	12, 13, 14, 15, 16, 17, 18–20, 24–29	13, 14, 15–17, 22, 23	12, 13–21, 22, 23, 26, 29, 30–31, 33, 34	18–21, 32–34	15, 16–18, 24, 25, 26	12–15, 20, 26, 27, 28	18, 19–23, 24, 33, 34
Unpaired	—	11–21, 23–33	12–16, 22–25	—	—	13–19, 24–27	17, 18–25, 28–29, 30	12, 13–14, 15
Hearts	7–11	7–11	9–11	7–11	7–11	9–11	9–11	7–11
Seminal vesicles	9–12	9–12	9–12	9–12	9–12	9–12	9–12	9–12
Intestinal origin	15	15	16	15	16	18	15 or 16	16
Gizzard	8	7/8	8	7/8	8	8	8	8
Spermathecae	—	14–17	14/15–16/17	13/14–17/18	—	15–17	14–17 or 13/14–16/17	—
First nephridia	13	17	14	13	14	14	12	14
Type locality	Khong Chiam, Ubon Ratchathani, Thailand	Burma	Lubock Paku, Pahang River, Malaysia	Burma	Menglun, Loso River, Yunnan, China	Sungei Kayu, River Sedili, Johor, Malaysia	Jungle Fall Valley, Bukit Timah, Singapore	Bank of Song River at Veintiane Province, Laos

— Indicates character absent

? Indicates not shown in the original description

adults and 257 juveniles (CUMZ 3219, Fig. 5C), Sungei Kelantan, Kelantan, Malaysia, 6°0'44.1"N, 102° 10'58.2"E, 24 m in elevation (27 May 2011). 17 adults and 11 juveniles (CUMZ 3220, Fig. 5D), Sungei Berkoh, Johor, Malaysia, 2°7'11.1"N, 103°2'24.9"E, 35 m in elevation (21 May 2011). 65 adults and 41 juveniles (CUMZ 3218, Figs. 3B, 5E), Tok Bok Hotspring, Kelantan, Malaysia, 5°50'19.4"N, 102°13'50.3"E, 36 m in elevation (26 May 2011).

Description. — Dimensions: 52–91 mm body length by 1.4–2.5 mm in the anterior body region, body cylindrical in the anterior part but quadrangular in transverse section after clitellum, with 192–313 segments. The body colour is pale brown. Dorsal surface considerably broader than ventral at the posterior end. The clitellar wings on ventro-lateral part of the clitellum in $\frac{3}{4}$ 18, 18–21, $\frac{1}{2}$ 22, with 2.1–2.6 mm long, and 0.5–1.2 mm wide on both sides. Prostomium zygotobous. Dorsal pores absent. Clitellum annular shape from 15, 16, 17–23, 24, 25. Four pairs of setae per segment from 2. Female pores, male pores, and spermathecal pores not visible. Genital markings: lateral series paired or asymmetrical, between line b and c in 13–15; lateral series paired or asymmetrical, lateral to line b in 16, 17, 22, 23; unpaired median series between aa in 12–16, 22–25.

Septa 4/5–8/9 thick, 9/10–14/15 moderately thick, and extremely thin from 15/16 to the end. Gizzard globular in 8. Intestine enlarged from 16. Hearts from 9–11. No nephridia distinguishable in the first 13 segments. Four pairs of seminal vesicles in 9–12, with the pair in 12 longest, parallel with intestine and extending to 15, 16 or 17. Ovaries in 13. Prostate and accessory glands absent. Spermathecae sessile, globular,

0.2–0.3 mm in diameter, on segments 14/15–16/17, two to three on each side per segment.

Habitat. — Found on the shore but in proximity to the river water, in the sandy mud topsoil and also under the water at a depth of about 10 cm. The river bank soil surface was covered with worm casts at Sungei Berkoh and Tok Bok Hotspring. Also found in the sandy clay in Sungei Kelantan.

Remarks. — *Glyphidrilus malayanus* from Malay Peninsula: Lubock Paku, Pahang River, Malaysia has clitellum in 15–25, wings in $\frac{3}{4}$ 18– $\frac{1}{2}$ 22, and the absence of median genital markings. The specimens collected from Sungei Berkoh, Tok Bok Hotspring, and Sungei Kelantan in this study were slightly different from the type specimen. The positions of the clitellum and wings of the additional specimens are slightly different from the wings location of the type specimen by one or two segments, and median genital markings were present in these specimens. Most of the morphological characteristics are quite similar to the holotype specimen. The smaller size is the only distinct character noticed. We added up our own drawings of the holotype shown in Fig. 5A, B, and of other specimens collected from different localities in Fig. 5C–E, and also the colour picture in Fig. 3B.

Glyphidrilus birmanicus Gates, 1958

Glyphidrilus birmanicus Gates, 1958: 61. Type locality: Burma. Gates, 1933: 603. Brinkhurst & Jamieson, 1971: 756.

Remarks. — *Glyphidrilus birmanicus* differs from *G. mekongensis*, new species by the shorter wings in 21–29 but the clitellum is much longer in 12, 13–43, 44. The genital markings are on setal line b in 12, 13–21, 22, 23, 26 and 29, 30–31, 33 or 34. The intestinal origin is in 15, and the spermathecae are in 13/14–17/18 (Table 2).

Glyphidrilus yunnanensis Chen & Xu, 1977

Glyphidrilus yunnanensis Chen & Xu, 1977: 181. Type locality: Menglun, Loso River, Yunnan, China.

Remarks. — *Glyphidrilus yunnanensis* differs from *G. mekongensis*, new species by the wing locations in 22–32, with clitellum location in 18–38, the intestinal origin in 16, genital markings paired in 18–21 and 32–34 (Table 2).

Glyphidrilus gatesi Shen & Yeo, 2005 (Fig. 6)

Glyphidrilus gatesi Shen & Yeo, 2005: 16, Fig. 1. Type locality: Sungei Kayu, swamp forest near River Sedili, Johor, Malaysia.

Material examined. — Holotype in the ZRC of RMBR (ZRC 1974.12.2.51).

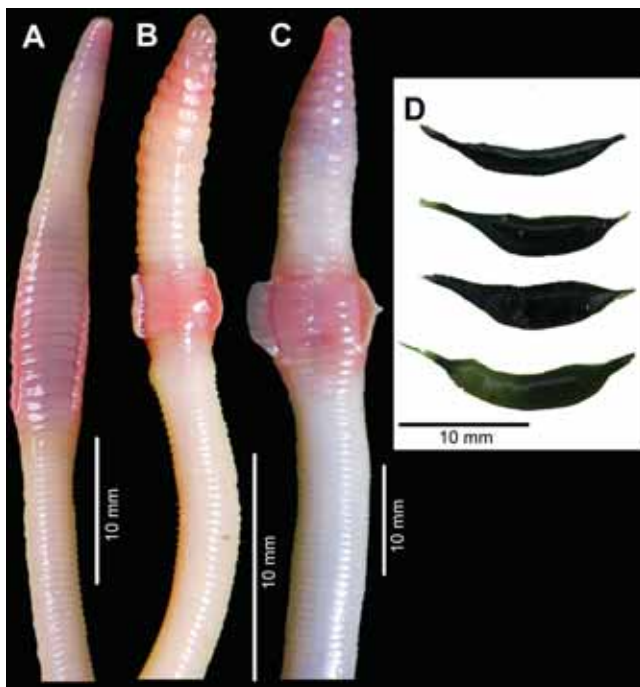


Fig. 3. The colouration of newly collected specimens just after the first step preservation in 30% ethanol: A, *Glyphidrilus mekongensis*, new species (paratype CUMZ 3216); B, *Glyphidrilus malayanus* from Tok Bok Hotspring, Kelantan, Malaysia (CUMZ 3218); C, *Glyphidrilus singaporensis* topotype specimen (CUMZ 3217); D, cocoon characters of *Glyphidrilus mekongensis*, new species (paratype CUMZ 3216).

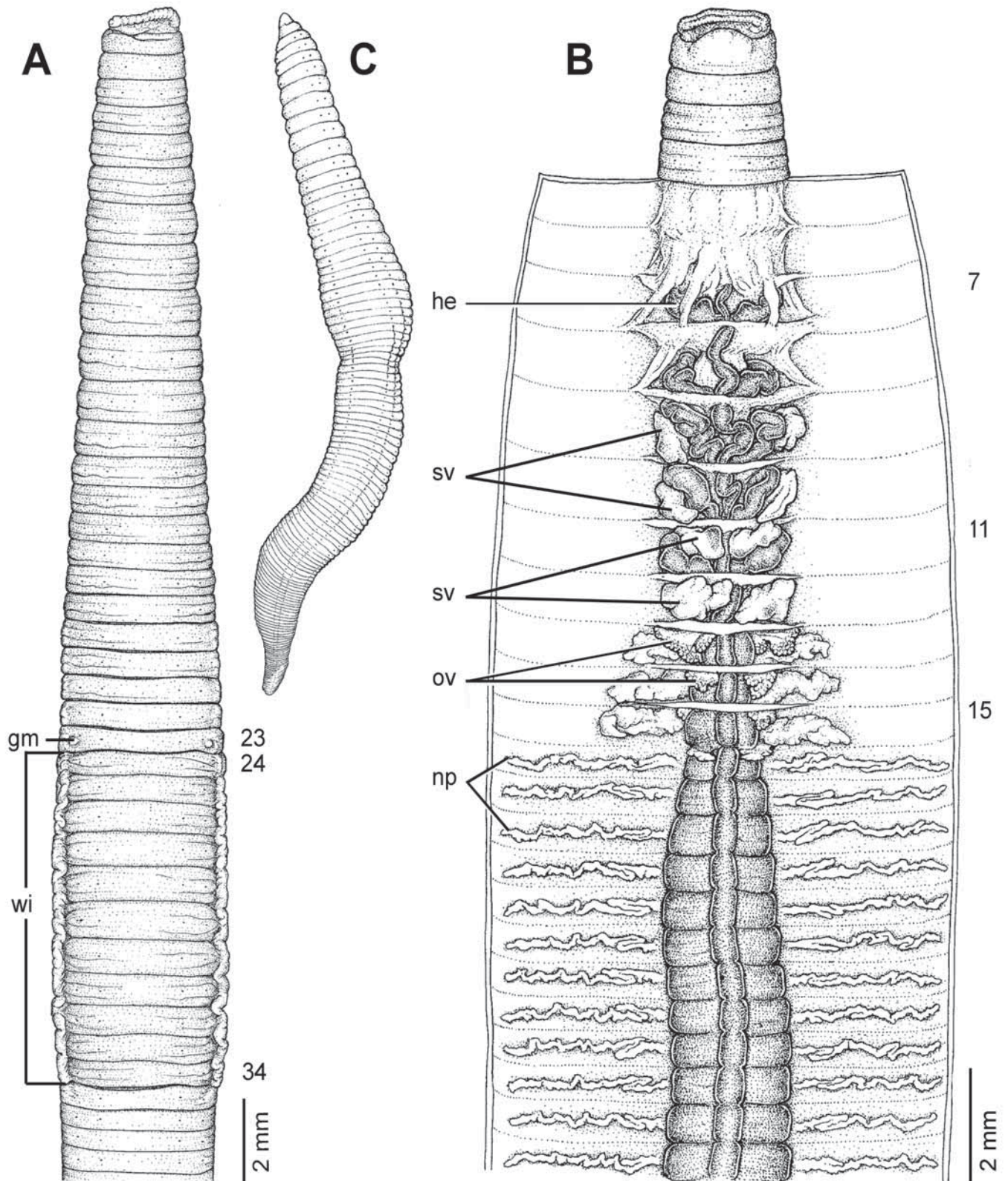


Fig. 4. External and internal morphology of holotype (CUMZ 3215) of *Glyphidrilus mekongensis*, new species: A, external ventral view; B, internal dorsal view; C, newly hatched worm characters (paratype CUMZ 3216).

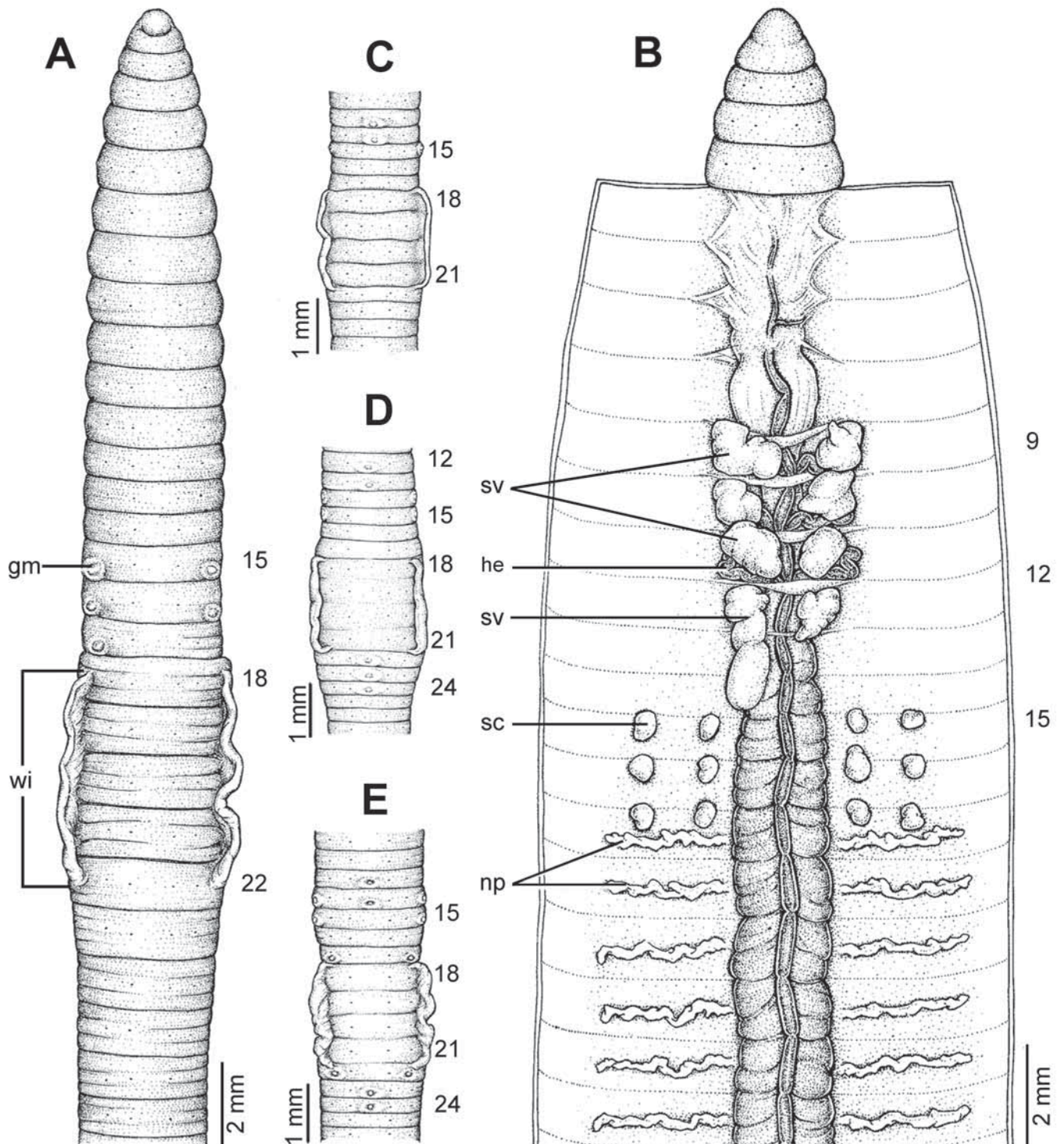


Fig. 5. External and internal morphology of holotype (ZMH V5875) of *Glyphidrilus malayanus*: A, external ventral view; B, internal dorsal view; C, specimen collected from Sungei Kelatan, Kelantan, Malaysia (CUMZ 3219); D, from Sungei Berkoh, Johor, Malaysia (CUMZ 3220); E, from Tok Bok Hotspring, Kelantan, Malaysia (CUMZ 3218), Malaysia.

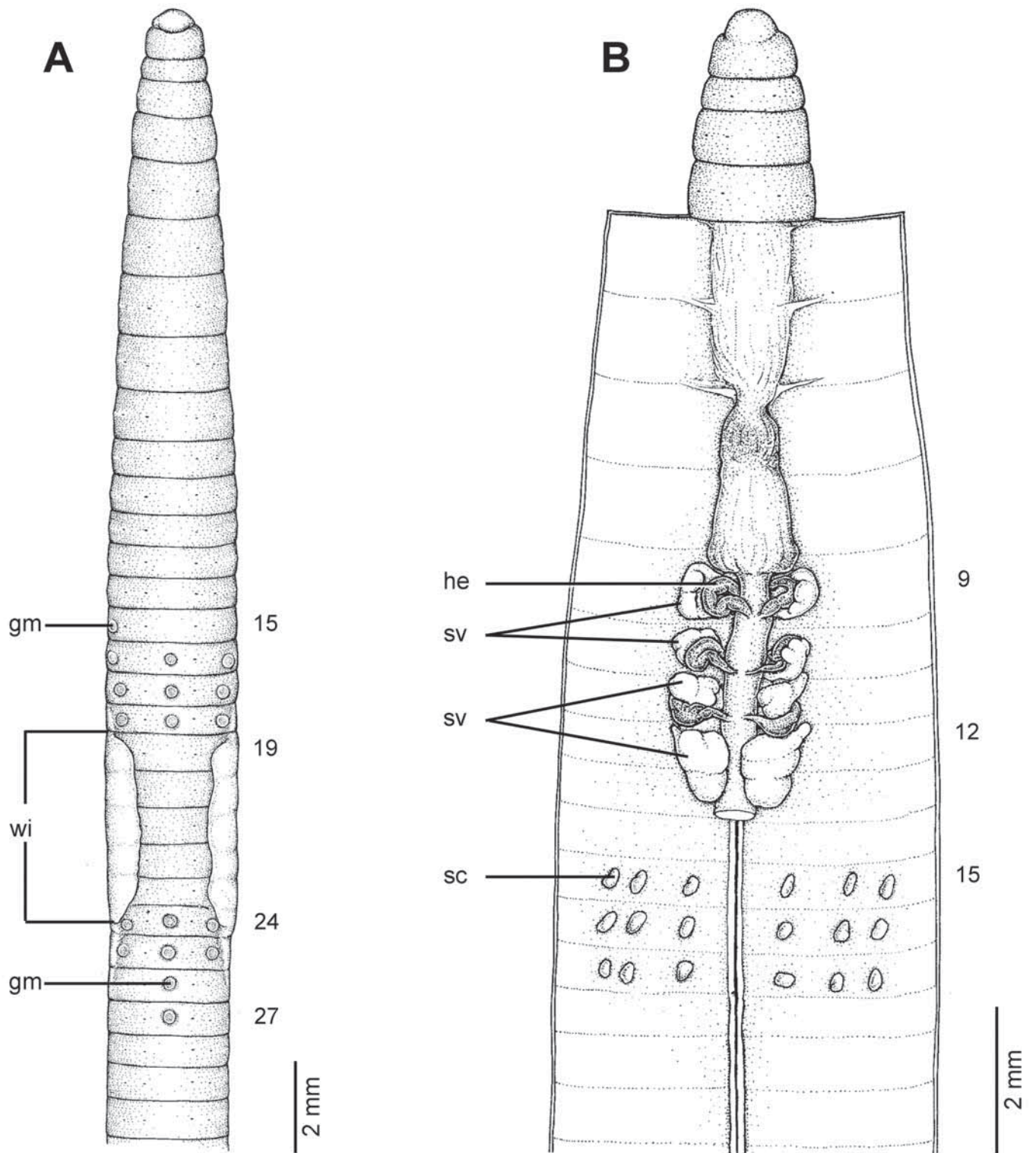


Fig. 6. External and internal morphology of holotype (ZRC 1974.12.2.51) of *Glyphidrilus gatesi*: A, external ventral view; B, internal dorsal view.

Remarks. — *Glyphidrilus gatesi* differs from *G. mekongensis*, new species in having shorter wings in 19–½24 and a shorter clitellum in 17–26, genital markings near line b in 15 on the right side only, and in 16–18 and 24–25 on both sides, and median unpaired in 13–19 and 24–27, intestinal origin in 18, three pairs of heart in 9–11, and spermathecae in 15–17.

***Glyphidrilus singaporensis* Shen & Yeo, 2005**
(Figs. 3C, 7)

Glyphidrilus singaporensis Shen & Yeo, 2005: 18, Fig. 3. Type locality: Jungle Fall Valley, Bukit Timah, Singapore.

Material examined. — Holotype in the ZRC, RMBR (Fig. 7A, B). Additional records: 10 adults (CUMZ 3217, Figs. 3C, 7C, D), Bukit Timah Nature Reserve, Singapore. 1°21'24.11"N, 103°47'6.76"E, 64 m in elevation (25 Dec.2009).

Description. — Dimensions: 101–143 mm body length by 4.2–3.8 mm at the anterior body region, body cylindrical at the anterior part but quadrangular in transverse section after clitellum, with 181–341 segments. The body colour pale brown. Dorsal surface considerably broader than ventral at the posterior end. The clitellar wings on ventro-lateral part of the clitellum in 21–½26, 26, 3.8–4.3 mm long, and 0.7–0.8 mm wide on both sides. Prostomium zygodous. Dorsal pores absent. Clitellum annular shape from 18–27, ½28, 29, 30, 31. Four pairs of setae per segment from 2. Female pores, male pores and spermathecal pores not visible. Genital markings: lateral series paired or appeared only one side, lateral to line b in 12–15, 20, 26, 27, 28; unpaired median series in 17–25, 28–30.

Septa 4/5–8/9 thick, 9/10–14/15 moderately thick and extremely thin from 15/16 to the end. Gizzard globular in 8. Intestine enlarged from 16. Hearts from 9–11. No nephridia distinguishable in the first 11 segments. Four pairs of seminal vesicles in 9–12. Ovaries in 13–14, with the portion in segment 13 larger. Prostate and accessory glands absent. Spermathecae sessile, globular, 0.2–0.4 mm in diameter, in segments 13/14–16/17, one to five on each side per segment.

Habitat. — Found in swamp forest at Bukit Timah Nature Reserve, Singapore, in the sandy loam topsoil. The soil surface was covered with organic matter and worm casts.

Remarks. — *Glyphidrilus singaporensis* from Jungle Fall Valley, Bukit Timah has clitellum in 18 or 19–27 or ½28, wings in 21–½26, 26, spermathecae in 15–17. The specimens collected from Bukit Timah Nature Reserve in this study were slightly different from the type specimen by the position of clitellum and wings, and by different numbers of spermathecae. We added up our own drawings of the holotype shown in Fig. 7A, B, and of other topotype specimens in Fig. 7C, D, and also the colour picture in Fig. 3C.

Glyphidrilus vangviengensis
Panha & Chanabun, 2011

Glyphidrilus vangviengensis Panha & Chanabun, 2011: 213. Figs. 1–3. Type locality: River bank of Song River at Vangvieng, Veintiane Province, Laos.

Material examined. — Holotype (CUMZ 3221) and paratypes (CUMZ 3222) in the CUMZ collections.

Remarks. — *Glyphidrilus vangviengensis* differs from *G. mekongensis*, new species by the wing locations in 24, 25–31, 32, with clitellum in 19, 20–35, 36, 37, the intestinal origin in 16, and genital markings median unpaired on aa in 12, 13–14, 15 and paired between b to c in 18, 19, 20, 21–22, 23, 24 and 33, 34 (Table 2).

DISCUSSION

The genus *Glyphidrilus* occurs in aquatic freshwater areas in or near rivers, streams, canals, ponds, or even in the paddy areas. They inhabit the topsoil in proximity to water, in various soil types from muddy to sandy, with pH ranging from neutral to very mild basic conditions. The worms excrete casts on the soil surface. *Glyphidrilus mekongensis*, new species lives in sandy mud habitats in banks of the Mekong River, at pH 7.1–7.3. In the dry season, during which the worms were collected, this habitat was like a small bay with no strong water current disturbance. Of interest would be a study of the worms in the rainy season at high water level. The worms were active during the survey on 9–10 Feb.2011.

The worms produce a large number of cocoons in the soil, and the young can be found in various stages of development. Cocoon characters and newly hatched young worms were found to be useful for identification. Description of this new species is the first record of the details of cocoon characters and that of the young worms. The cocoon colouration is also interesting, turning from pale yellow to black during the development of embryos to young worms within. The absence of female pores, male pores, and spermathecal pores corresponded with the lack of spermathecae. However, it may be that the female pores were so small that they escaped our notice. The lack of spermathecae and male pores could indicate a parthenogenetic condition, which should be critically investigated later. A cocoon contains 7–8 young individuals, and one could test allozyme markers to see if the many embryos per cocoon are genetically identical.

The most closely related species appears to be *G. yunnanensis* from Yunnan, south China and *G. vangviengensis* from Laos. All three species have no spermathecae. The Yunnan species has wings occurring on the same numbers of segment but differing in location. However, the Yunnan species is slightly shorter than the new species, with a longer clitellum. In addition, there is a greater number of genital markings on *G. yunnanensis*. The Laos species has shorter wings and a greater number of genital markings than the new species. Interestingly, both species occur in the Mekong River

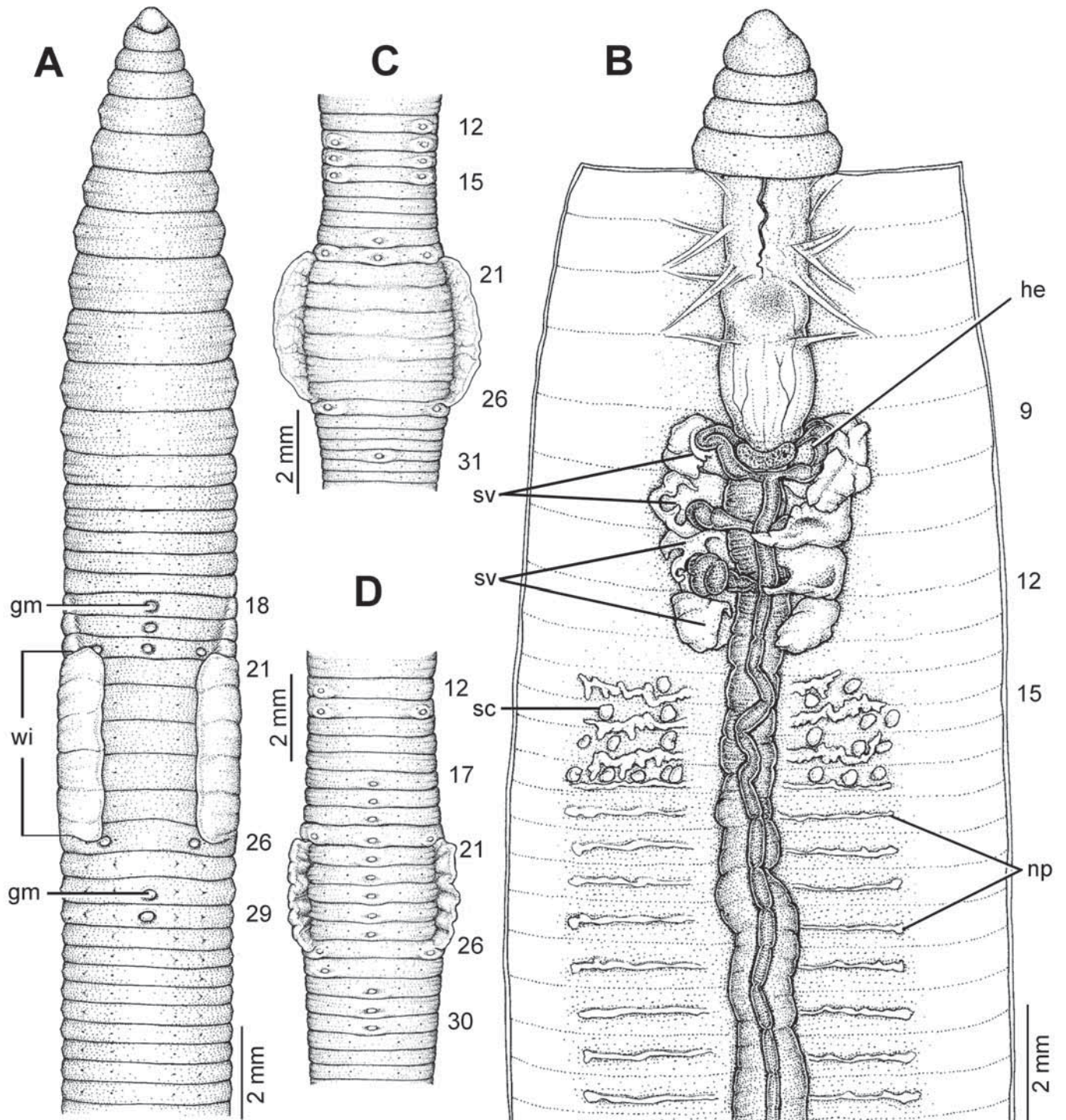


Fig. 7. External and internal morphology of holotype (ZRC) of *Glyphidrilus singaporensis*: A, external ventral view; B, internal dorsal view; C and D, topotype specimens showing variation of genital markings location (CUMZ 3217).

tributaries. This may be one reason for the similarity among the three species. The river originates in the north in Tibet and China, and flows through Burma, Laos, Thailand, Cambodia and Vietnam en route to the South China Sea. The worms or cocoons may travel down during floods, or upstream by colonising the river bank. Speciation could have occurred due to isolation by distance, or by changes in the river course. Alternative, *G. mekongensis*, new species may have diverged from some other *Glyphidrilus* lineages in this place. We plan to test these alternative hypotheses in a more extensive examination of the *Glyphidrilus* phylogeny.

Key to a new species of *Glyphidrilus* from Thailand and species described from nearby countries

1. First hearts in 9 2
- First hearts in 7 3
2. Wings from $\frac{3}{4}$ 18, 18–21, $\frac{1}{2}$ 22, spermathecae in 14/15–16/17 *G. malayanus*
- Wings from 19– $\frac{1}{2}$ 24, spermathecae in 15–17 *G. gatesi*
- Wings from 21– $\frac{1}{2}$ 26, 26, spermathecae present in 14–17 or 13/14–16/17 *G. singaporensis*
3. Unpaired genital markings on aa absent 4
- Unpaired genital markings on aa present 5
4. Spermathecae absent 6
- Spermathecae present in 13/14–17/18 *G. birmanicus*
5. Spermathecae absent *G. vangviengensis*
- Spermathecae present in 14–17 *G. papillatus*
6. Wings from 22–32, paired genital markings in 18–21, 32–34 *G. yunnanensis*
- Wings from 24– $\frac{1}{2}$ 33, 33, 34, $\frac{1}{2}$ 35, paired genital markings in 23. *G. mekongensis*, new species

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