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A New Species of Semi-aquatic Freshwater Earthworm of the Genus *Glyphidrilus* Horst, 1889 from Laos (Oligochaeta: Almidae)

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ABSTRACT.– The discovery of a new semi-aquatic earthworm of the genus *Glyphidrilus* Horst, 1889 from the Song River, Vangvieng, Laos is reported. The description of the new species includes morphological characters of the adults. After comparing with the closely related species described from SE Asia, *Glyphidrilus vangviengensis* Panha and Chanabun, n. sp. is described. The new species differs from its congeners by having long clitellar wings at xxiv–xxxii, no genital pores but the median unpaired genital markings are observed at xii, xiii, xiv, xv; paired genital markings are at xviii, xix, xx, xxi–xxii, xxiii, xiv and xxxiii, xxxiv. Seminal vesicles are present but atrial glands and spermathecae are lacking.

KEY WORDS: *Glyphidrilus*, earthworm, Almidae, new species, Laos

INTRODUCTION

Laos is a landlocked country of South-east Asia with 235,000 sq km and bordering Thailand, Cambodia, Vietnam, China and Myanmar. Most of the country is mountains and plateaus, and two-thirds composed of forest. The Mekong River forms a large part of the western boundary with Thailand, whereas the mountains of the Annamite Chain form most of the eastern border with Vietnam. The climate is tropical and monsoon. Most of the fauna in Laos belongs to the Indochinese zoogeographic realm (Solem, 1959). Around 45% of the animal species natives to Thailand are shared by Laos (Cubitt and Stewart-Cox, 1995). Most earthworm species are in the family Megascolecidae which occur in the litter layers and soils in the forests (Hong et. al., 2008). However there have been no reports about semi-aquatic freshwater earthworms of the genus *Glyphidrilus* Horst, 1889 in this country. Our extensive surveys of earthworm genus *Glyphidrilus* 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 banks. There are 19 species and one subspecies of *Glyphidrilus* recorded in the world (Zicsi, 1996; Jamieson, 1968; Chen and Xu, 1977; Shen and Yeo, 2005), all from 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. The nearest records to Laos are two new

species from Singapore and Malaysia (Shen and Yeo, 2005), and species described from China (Chen and Xu, 1977). The new species reported here was found along the Song River bank at Vangvieng, Vientiane Province, Laos in January 2011 (Figs. 1, 2A).

MATERIALS 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 Song River at Vangvieng, Vientiane Province, Laos at 18° 54' 43.9" N, 102° 26' 34.2" E, 207 meters elevation on 12 January 2011. Adult earthworms were collected (Fig. 2B). The worms were killed in 30% (v/v) ethanol, photographed, transferred to 5% (w/v) formalin for fixation approximately 12 hours, and then transferred to 70% (v/v) ethanol for longer term preservation and subsequent morphological studies (Fig. 2C).

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.

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. Drawings were made of the body segments and the distinct external characters and internal organs. 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±one standard deviation.

Comparative studies of *Glyphidrilus* type specimens were done at the following three natural history museums: Raffles Museum of Biodiversity Research (ZRC), National University of Singapore, Singapore, The Natural History Museum, London, UK (NHM), Biozentrum Grindel und Zoologisches Museum, University of Hamburg, Germany (UHH). To confirm the novelty of the new species we compared it to type specimens of *G. gatesi* Shen and Yeo, 2005 and *G. singaporensis* Shen and Yeo, 2005 housed at ZRC, *G. malayanus* Michaelsen, 1902 at UHH. But *G. papillatus* (Rosa, 1890), *G. birmanicus* Gates, 1958 from Burma and *G. yunnanensis* Chen and Xu, 1977 from south China were studied from the original description only.

Holotype and paratype specimens have been deposited in CUMZ. Additional paratypes are housed in NHM, and UHH.

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

SYSTEMATICS

Genus *Glyphidrilus* Horst, 1889

Type species: *Glyphidrilus weberi* Horst, 1889, by monotypus.

Glyphidrilus papillatus (Rosa, 1890)

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

Glyphidrilus papillatus—Michaelsen, 1896: 196. Stephenson, 1923: 493. Gates,

1933: 603; 1958: 60. Chen, 1938: 426. Brinkhurst and Jamieson, 1971: 763.

Remarks.– *Glyphidrilus papillatus* differs from others species by the wings at xviii–xxiii, xxiv, with a clitellum at xiv–xl. The genital markings median unpaired on xi–xxi and xxiii–xxxiii, median paired on xii, xvii, xviii–xx, xxiv–xxix, lateral paired on xii–xviii. Hearts at vii–xi, seminal vesicles at ix–xii, intestinal origin in xv, and spermathecae in xiv–xvii (Table 1).

Glyphidrilus malayanus Michaelsen, 1902

Glyphidrilus malayanus Michaelsen, 1902: 35. Type locality: Malay Peninsula: Lubock Paku, Pahang River. Brinkhurst and Jamieson, 1971: 762, fig. 15.4e, f.

Remarks.– *Glyphidrilus malayanus* differs from others species by the wings at ¾xviii, xviii–xxi, ½xxii, with a clitellum at xv, xvi, xvii–xxiii, xxiv, xxv. Genital markings: lateral series paired or asymmetrical, lateral between line b to c at xiii–xv, xvi, xvii; lateral series paired or asymmetrical, lateral line b at xvii, xxii, xxiii; unpaired median series between aa at xii–xvi, xxii–xxv. Hearts at ix–xi, seminal vesicles at ix–xii, intestinal origin in xvi or xvii, and spermathecae in 14/15–16/17 (Table 1).

Glyphidrilus birmanicus Gates, 1958

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

Remarks.– *Glyphidrilus birmanicus* differs from the others by the wings at xxi–xxix, with clitellum at xii, xiii–xliii, xliv. The genital markings are postsetal above setal line b at xii, xiii–xxi, xxii, xxiii, xxvi and xxix, xxx–xxxi, xxxiii or xxxiv. Hearts at vii–xi, seminal vesicles at ix–xii, intestinal origin in xv, and the spermathecae in 13/14–17/18 (Table 1).

Glyphidrilus yunnanensis Chen and Xu, 1977

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

Remarks.– *Glyphidrilus yunnanensis* differs from the others by the wings location at xxii–xxxii, the intestinal origin in xvi, genital markings paired at xvii–xxi. Clitellum location at xviii–xxxviii. Ovaries located at xiii–xiv. Hearts at vii–xi, seminal vesicles at ix–xii, intestinal origin in xvi, and spermathecae absent (Table 1).

Glyphidrilus gatesi Shen and Yeo, 2005

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

Remarks.– *Glyphidrilus gatesi* differs from others species by the wings at xix–½xxiv, with a clitellum at xvii–xxv. The genital markings flat, rounded, locate near line b at xv on the right side only, and at xvi–xviii, xxiv–xxv on both sides, and median unpaired at xvi–xviii, xxiv–xxvii. Hearts at ix–xi, seminal vesicles at ix–xii, intestinal origin in xviii, and the spermathecae in xv–xvii (Table 1).

TABLE 1. Morphological characters comparison of *G. papillatus* (Rosa, 1890)*, *G. malayanus* Michaelsen, 1902, *G. birmanicus* Gates, 1958*, *G. yunnanensis* Chen and Xu, 1977*, *G. gatesi* Shen and Yeo, 2005, and *G. singaporensis* Shen and Yeo, 2005, and *G. vangviengensis* n. sp. 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 and Xu (1977).

Characters	*G. papillatus*	*G. malayanus*	*G. birmanicus*	*G. yunnanensis*	*G. gatesi*	*G. singaporensis*	*G. vangviengensis* n. sp.
Length	100	82	95–103	123	50+	143	160
Segments	330	241	?	139	62+	220	217
Clitellum	xiv–xl	xv–xxv	xii(xiii)–xliii(xliv)	xviii–xxxviii	xvii–xxv	xviii–xxvii	xix–xxxvii
Wings	xviii–xxiii, xxiv	¾xviii–½xxii	xxi–xxix	xxii–xxxii	xix–½xxiv	xxi–½xxvi	xxiv–xxxii
Genital markings							
Paired	xii, xiii, xiv, xv, xvi, xvii, xviii–xx, xxiv–xxix	xv–xvii	xii–xxi(xxii, xxiii), xxx–xxxi, xxxiii(xxxiv)	xviii–xxi, xxxii–xxxiv	xv–xviii, xxiv–xxv	xx, xxvi	xix–xxiii, xxxiii
Unpaired	xi–xxi, xxiii–xxxiii	xii–xvi, xxii–xxv	-	-	xvi–xviii, xxiv–xxvii	xviii–xx, xxviii–xxix	xiii–xiv
Hearts	vii–xi	ix–xi	vii–xi	vii–xi	ix–xi	ix–xi	vii–xi
Seminal vesicles	ix–xii	ix–xii	ix–xii	ix–xii	ix–xii	ix–xii	ix–xii
Intestinal origin	xv	xvi	xv	xvi	xviii	xv	xvi
Gizzard	7/8	viii	viii	viii	viii	viii	viii
Spermathecae	xiv–xvii	14/15–16/17	13/14–17/18	-	xv–xvii	xiv–xvii	-
First nephridia	xvii	xvii	xiii	xiv	xiv	xiv	xiv

Glyphidrilus singaporensis Shen and Yeo, 2005

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

Remarks.– *Glyphidrilus singaporensis* differs from others species by the wings at xxi–xxxv, ½xxvi, xxvi, with a clitellum at xviii, xix–xxvii, xxix, xxx, xxxi. Genital markings: lateral series paired or appeared only one side, lateral to line b at xii–xv, xx, xxvi, xxvi, xxvii, xxviii; unpaired median series at xvii, xviii–xx, xxv, xxviii–xxx. Hearts at ix–xi, seminal vesicles at ix–xii, intestinal origin in xv or xvi, and the spermathecae in xiv–xvii (Table 1).

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Glyphidrilus vangviengensis Panha and Chanabun, n. sp. (Figures 1, 2, 3, Table 1)

Description of Holotype.– Dimensions; 160 mm body length by 3.61 mm at the anterior body region and 5.99 mm before the clitellar wings at xxii, 5.00 mm after wings at xxxiv within the clitellum, body cylindrical in the anterior part but after clitellum it is quadrangular in transverse section view, with 217 segments. The body colour is pale brown with variation of colour from red to pink colour in expanded tissues of wing portion in different individuals of newly collected specimens after placement in 30% ethanol for narcotization. At the posterior end the dorsal surface is the most extensive and is considerably broader than the ventral. The

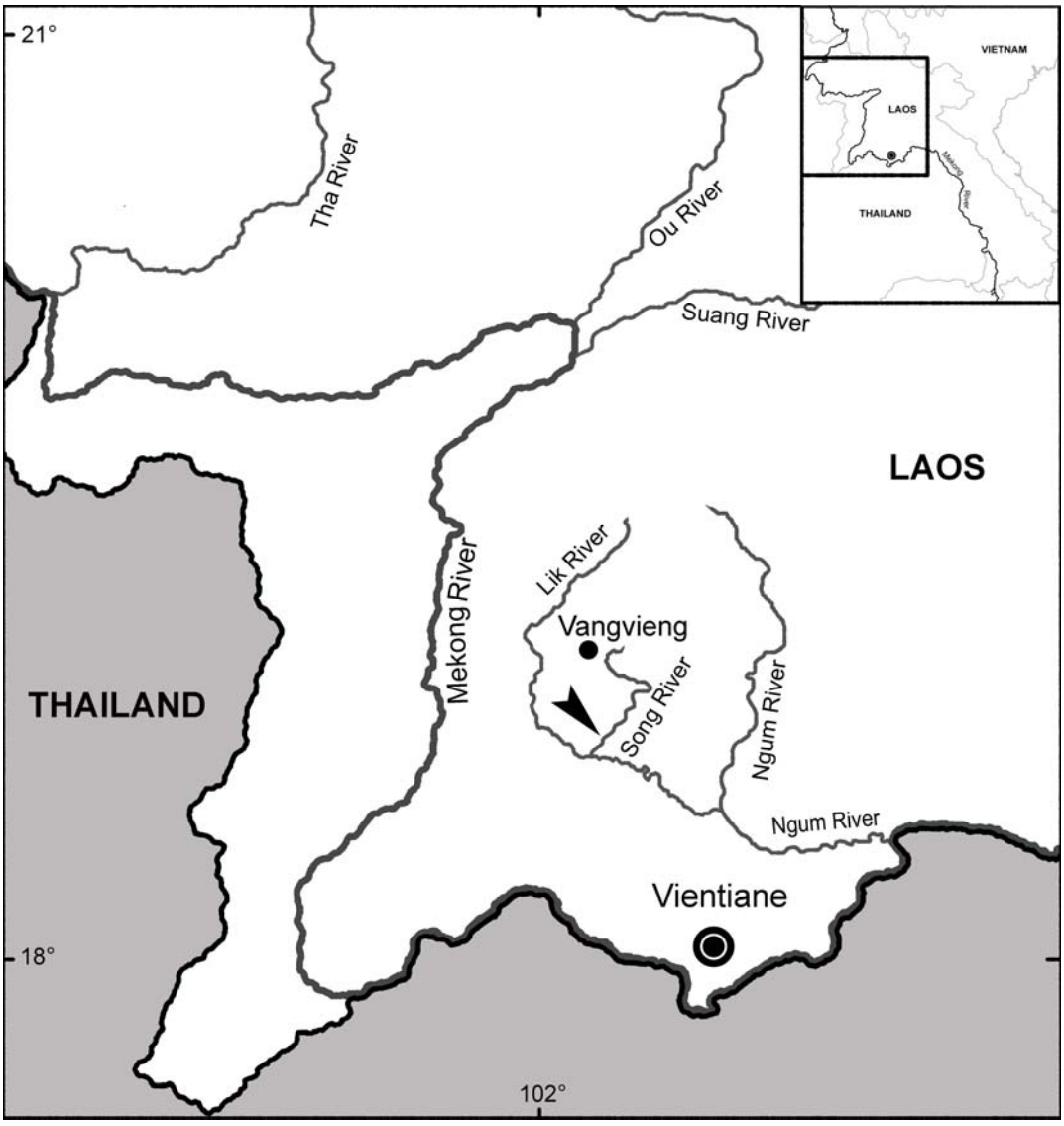

FIGURE 1. Map of Laos showing type locality of *Glyphidrilus vangviengensis* n. sp. (arrow head) locates on the shore of the Song River at Vangvieng, Vientiane Province, Laos.

clitellar wings are attached on ventral lateral part of the clitellum at xxiv–xxxii, 8.53 mm long and about 0.54 mm wide on both sides. Prostomium zygolobous. Dorsal pores absent. Clitellum annular shape at xix–xxxvii. Four pairs of setae per segment start from ii, setae formula aa:ab:bc:cd:dd = 1.39:0.83:1.46:1.05:1.80 at viii. Female pores, male pores and spermathecal pores are not visible. Genital markings: paired or unpaired genital markings near midventral in xiii, xiv and widely paired genital markings in xix–xxiii and xxxiii.

Septa 5/6–11/12 thicker, 12/13 to the last segment thin. Gizzard small globular within viii. Intestine enlarged from xvi. Dorsal

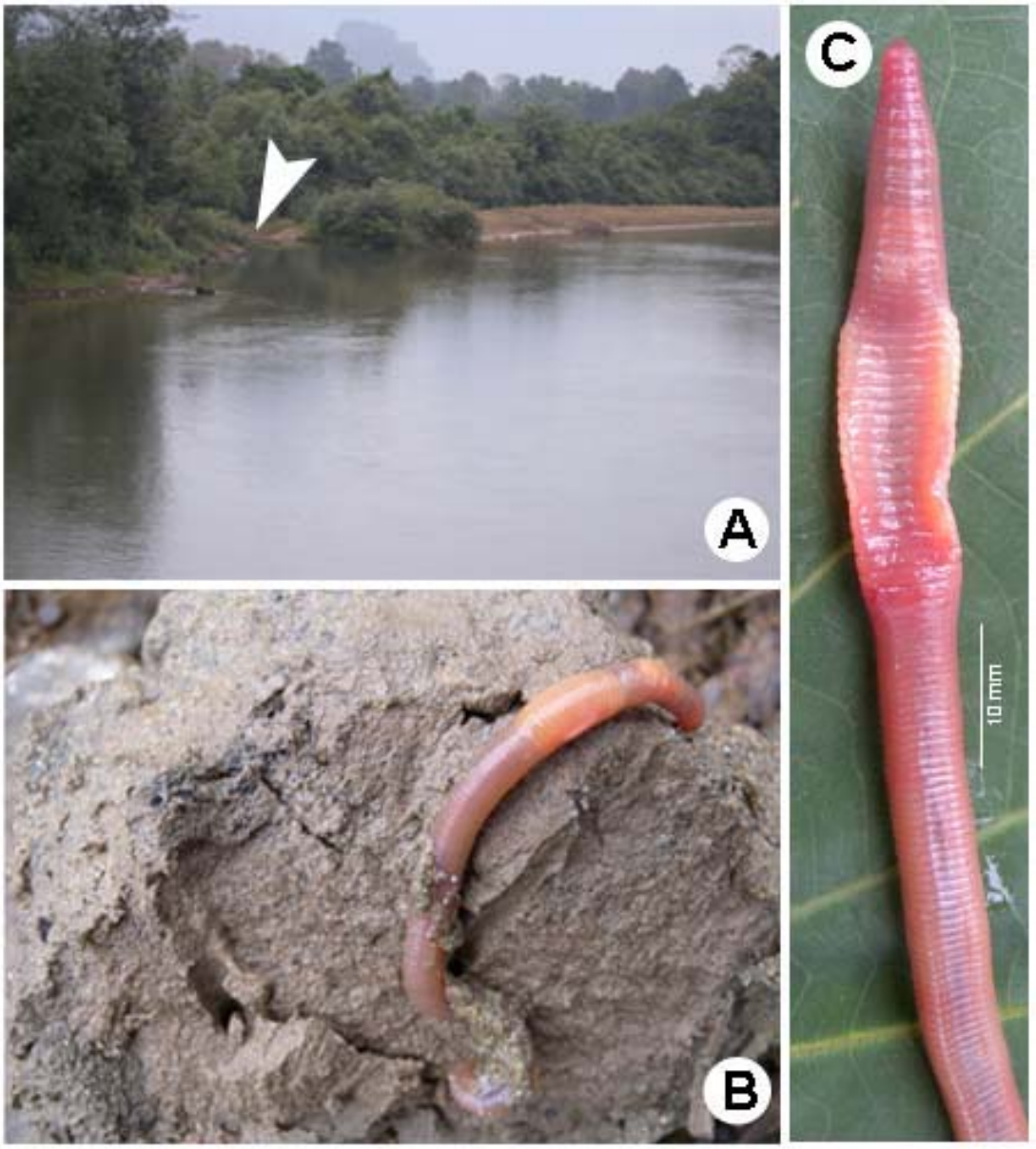

FIGURE 2. Photographs showing **(A)** habitat at the Song River, Vangvieng, Vientiane Province, Laos (arrow head) **(B)** living specimens (**C)** coloration of newly collected specimens after passing the first step preservation in 30% ethanol of *Glyphidrilus vangviengensis* n. sp. (holotype CUMZ 3221).

blood vessel aborted anterior to vii. Hearts in vii–xi. No nephridia are distinguishable in the first thirteen segments. Four pairs of seminal vesicles at ix–xii, that of segment xii is larger than the others. The ovaries located in xiii–xiv. Prostate and accessory glands and spermathecae are absent.

Variation.– The holotype measures 160 mm body length, with 217 segments; the body length of fourteen paratypes range from 104–145 mm (122.75±13.67), with 145–229 segments. The clitellar wings are attached on ventral lateral part of the clitellum at xxiv, xxv–xxxi, xxxii, with annular clitellum at xix, xx–xxxv, xxxvi, xxxvii. Genital markings: median unpaired genital markings at xii, xiii–xiv, xv; paired genital markings at xviii, xix, xx, xxi–xxii, xxiii, xxiv and xxxiii, xxxiv.

Type locality.– River bank of Song River at Vangvieng, Veintiane Province, Laos 18° 54’ 43.9” N, 102° 26’ 34.2” E, 207 meters elevation on 12 January 2011.

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.

Etymology.– This new species was named for the type locality. The locality is a part of this locally famous river and this is the first record of this worm genus ever recorded in Laos.

Type material.– Holotype (CUMZ 3221) and 10 paratypes (CUMZ 3222) are deposited in Chulalongkorn University, Museum of Zoology (CUMZ). Another two paratypes will be deposited in the Biozentrum Grindel und Zoologisches Museum, Hamburg, Germany (UHH), and two paratypes in the Natural History Museum, London (NHM).

Habitat.– Found on the shore but in proximity to the river water, in the sandy loam top soil with sand 72%, silt 24% and clay 3%, and also under the water at about 5–10 cm depth. The river bank soil surface was covered with worm casts at Song River, Vangvieng, Vientiane Province, Laos.

Diagnosis.– *Glyphidrilus vangviengensis* n. sp. is a medium to large sized semi-aquatic freshwater earthworm with distinct expanded tissues of clitellar wing organs on the lateral sides of the body at xxiv–xxxii. No female pores, male pores and spermathecal pores were observed. But the genital markings; median unpaired occur at xiii–xiv. Heart at vii–xi. Four pairs of seminal vesicles at ix–xii, that of segment xii is largest. Intestinal origin in xvi. The ovaries in xiii–xiv. Prostate and accessory glands and spermathecae absent.

Remarks.– Six described *Glyphidrilus* species from areas close to Laos have some degree of similarity but are different from the new species with respect to spermathecae, genital markings, clitellum and wing locations. *Glyphidrilus vangviengensis* n. sp. differs from *G. papillatus* from Burma by *G. papillatus* having shorter wings at xviii–xxiii, xxiv, with a clitellum at xiv–xl, the intestinal origin is in xv, and the spermathecae are in xiv–xvii. *Glyphidrilus vangviengensis* n. sp. differs from *G. malayanus* from the Malay Peninsula by the following: *G. malayanus* has shorter wings at xviii, ¾xviii–xxi, ½xxii, with a clitellum at xv, xvi, xvii–xxiii, xxiv, xxv, intestinal origin in xvi, three pairs of hearts at ix–xi, and spermathecae in 14/15–16/17. *Glyphidrilus vangviengensis*

n. sp. differs from *G. birmanicus* from Burma in that *G. birmanicus* has wings at xxi–xxix, but the clitellum is much longer, extending from xii, xiii–xliii, xliv, the intestinal origin is in xv, and the spermathecae are in xiv–xviii. *Glyphidrilus vangviengensis* n. sp. differs from *G. yunnanensis* reported from Yunnan by *G. yunnanensis* having the longer wings on xxii–xxxii and clitellum at xviii–xxxviii, the intestinal origin in xvi. *Glyphidrilus vangviengensis* n. sp. differs from *G. gatesi* from Johor, Malaysia by the following characters: *G. gatesi* has shorter and more anteriorly placed wings at xix–½xxiv, with a clitellum at xvii–xxv, intestinal origin in xviii, three pairs of hearts in ix–xi, and spermathecae in xv–xvii. *Glyphidrilus vangviengensis* n. sp. differs from *G. singaporensis* reported from Bukit Timah, Singapore by the following characters: *G. singaporensis* has shorter wings at xxi–xxv, ½xxvi, xxvi, with a clitellum in xviii, xix–xxvii, xxix, xxx, xxxi, intestinal origins in xv, three pairs of hearts in ix–xi, and spermathecae in segments xiv–xvii.

DISCUSSION

The genus *Glyphidrilus* occurs in aquatic freshwater areas in or near rivers, streams, canals, ponds or even in the paddy areas. The exact habitats are in the topsoil at the edge of the above aquatic habitats, 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 vangviengensis* n. sp. lives in the sandy loam habitats with pH 7.23 of the Song River bank. The absence of female pores, male pores and spermathecal pores corresponded with the lack of spermathecae. The lack of spermathecae and male pores could indicate a parthenogenetic condition, which should be critically investigated later. The most closely related species appears to be *G. yunnanensis* from Yunnan, south China. Both species contain no spermathecae, however the Yunnan species has a bit longer clitellum than the new species and the wing locations are longer. The number of paired genital markings between b to c of the Yunnan species are similar but there are no genital markings in aa. Interestingly the two species occur along the Mekong River and tributaries. This may be one reason for the similarity of the three species. The river originates in the north, in Tibet and China and flows through Burma, Laos, Thailand, Cambodia and Vietnam to the South China Sea. The worms or cocoons may travel down or upstream by colonizing along the river bank. Speciation may have occurred due to isolation by distance, or by changes in river course. Alternatively, *G. vangviengensis* n. sp. diverged from some other *Glyphidrilus* lineage in this place.

Molecular analyses combined with mophological data and geological history analysis could show the evolution relationships among the recorded species.

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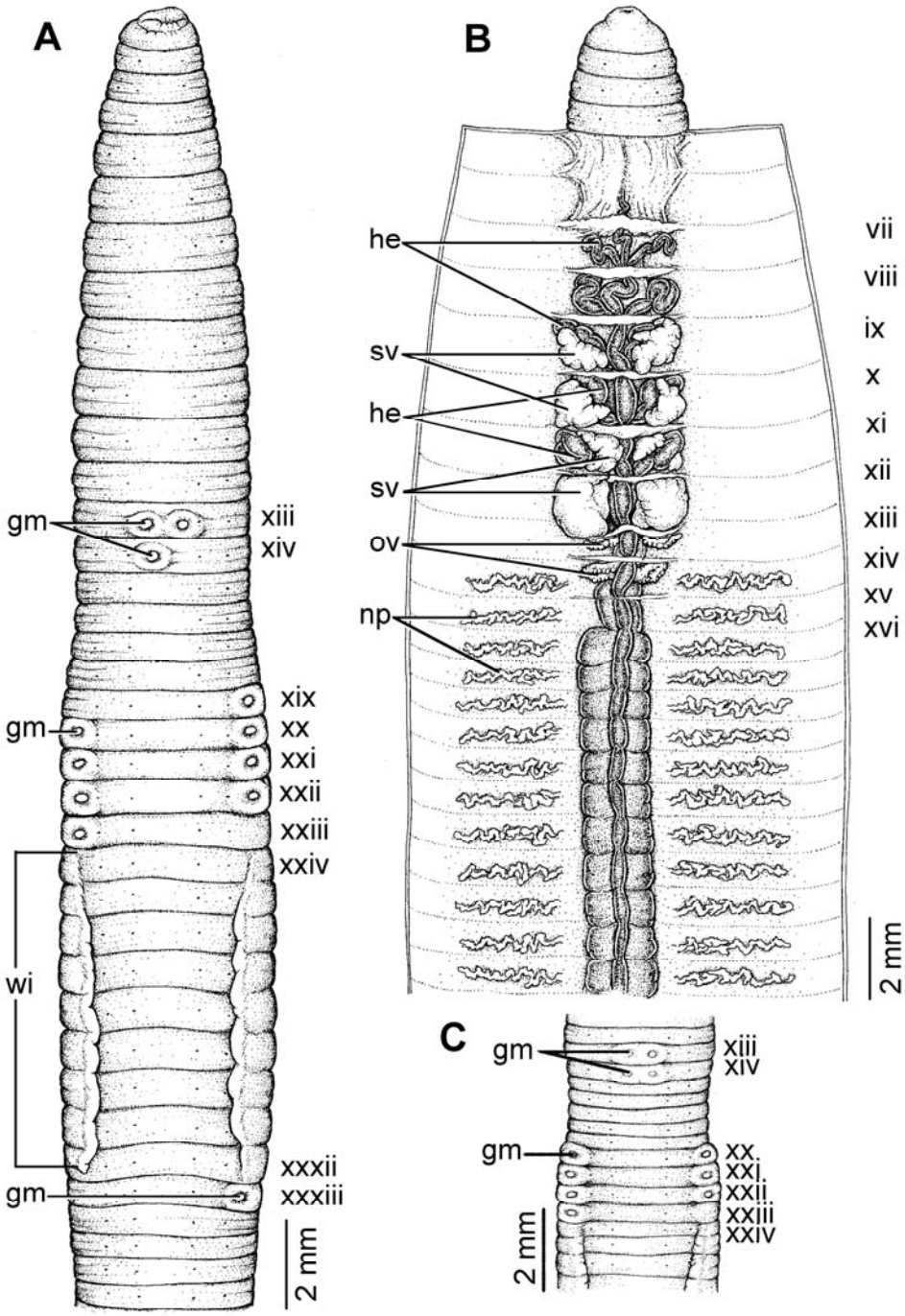

FIGURE 3. External and internal morphology of holotype (CUMZ 3221) of *Glyphidrilus vangviengensis* n. sp. **(A)** external ventral view, and **(B)** internal dorsal view, **(C)**. paratype illustration showing variation of genital markings location (CUMZ 3222).

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