

Two New Species of Semi-Aquatic Freshwater Earthworm Genus *Glyphidrilus* Horst, 1889 (Oligochaeta: Almidae) from Thailand and Laos

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ABSTRACT.— Material of the semi-aquatic freshwater earthworm genus *Glyphidrilus* Horst, 1889 recently collected from Thailand and Laos contains two new species: *G. panhai* Chanabun, **sp. nov.** from Song Kham River, Bueng Kan, Thailand and *G. samueljamesi* Chanabun, **sp. nov.** from Khon Phapheng Waterfall, Mekong River, Champasak, Laos. Morphological characters of both new species are distinctly different from the previous recognized species. *Glyphidrilus panhai* **sp. nov.** has wings in 23, 24–29, 30, 31, clitellum in 17, 18–32, 33, 34, 35, and spermathecae in 13/14 (14/15)–18/19. *Glyphidrilus samueljamesi* **sp. nov.** has wings in 25, 26, 27–33, 34, 35, 36, clitellum in 18, 19, 20–40, 41, 42, 43, 44, and spermathecae in 13/14 –20/21. Descriptions of all new species including illustrations of the external and internal morphological characteristics are provided.

KEYWORDS: *Glyphidrilus*, earthworm, new species, Thailand, Laos, taxonomy

INTRODUCTION

Since the Millipede-Earthworm Project in Thailand started in 2006, there have been several earthworms and millipedes that were discovered and published as new to science (e.g., Enghoff et al., 2007; Bantaowong et al., 2011a, b). The study of semi-aquatic freshwater earthworm genus *Glyphidrilus* Horst, 1889 in Thailand began since then, and then expanded to other Southeast Asian countries, e.g., Laos, Cambodia, Myanmar, Malaysia, and Singapore. Up to now, there are more than fifteen species reported from this region (Chanabun et al., 2013, 2020; Jirapatrasilp et al., 2019).

The semi-aquatic freshwater earthworm genus *Glyphidrilus* is classified in family Almidae. The earthworms live in the muddy banks of freshwater habitats (Horst, 1889; Jamieson, 1968; Michaelsen 1896, 1897; Brinkhurst and Jamieson, 1971; Shen and Yeo, 2005; Chanabun et al., 2013). The body of this earthworm is, in cross section, cylindrical in the anterior part and quadrangular in the posterior part (Beddard, 1912; Chanabun et al., 2013). A unique character of this earthworm genus is the presence of extensions of the body wall in the clitellar region called wings, which are highly vascularized and therefore alleged to assist respiration in the muddy habitats (Gates, 1972). Furthermore, worms produce a long spindle-shaped cocoon (Chanabun et al., 2013). The

distribution of *Glyphidrilus* is mostly in South and Southeast Asia (Brinkhurst and Jamieson, 1971; Chanabun et al., 2013), whereas only one species (*G. stuhlmanni* Michaelsen, 1897) and one subspecies (*G. stuhlmanni morogoronensis* Zicsi, 1996) occur in Tanzania, Africa (Michaelsen, 1897; Zicsi, 1996). Up to now, a total of 48 morphospecies and one subspecies were reported (Shen and Yeo, 2005; Chanabun et al., 2011, 2012a, b, 2013, 2017, 2020; Chanabun and Panha, 2015; Jirapatrasilp et al., 2016).

From the Mekong River Basin, a total of 11 *Glyphidrilus* species has been reported (Jirapatrasilp et al., 2019), including one species from China (Chen and Xu, 1977), four species from Laos (Chanabun et al., 2011, 2017), two species from Cambodia (Jirapatrasilp et al., 2016), and four species from Thailand (Chanabun et al., 2012a, 2013, 2017). The present study provides descriptions of two new *Glyphidrilus* species including one from Thailand and one from Laos. The species were detected in the framework of a comprehensive and integrative species-level molecular phylogenetic study of the genus *Glyphidrilus*, where 28 species were distinguished, nine of them new to science but not formally erected as new species (Jirapatrasilp et al., 2019). The purpose of this paper is to erect and to describe two of the new species detected in Jirapatrasilp et al. (2019). All DNA sequence data included here are from the cited publication.

MATERIALS AND METHODS

Semi-aquatic earthworms were collected from the river banks of freshwater habitats. The GPS coordinates of each locality was recorded. All specimens were washed, anaesthetized in 30% (v/v) ethanol, photographed, and fixed in 95% (v/v) ethanol for morphological studies. The descriptions were made from observation under an ACCU-SCOPE 3075 stereo microscope. The external and internal morphological structures were recorded: body length and segment number; the positions of clitellum and wings, genital markings, intestinal origin, gizzard and spermathecae. Illustrations of the anterior body segments and the distinct external characters and internal organs by dorsally dissected worms, and details of new species shown in Figures 2, 3. The holotype and paratypes were deposited in Chulalongkorn University, Museum of Zoology (CUMZ), The Natural History Museum, London, UK (NHMUK), and Biozentrum Grindel und Zoologisches Museum, University of Hamburg, Germany (ZMH). Animal use in this study strictly followed the protocols approved by the Institutional Animal Care and Use Committee of Khon Kaen University (IACUC-KKU) under approval number IACUC-KKU-32/65.

Taxon names

Descriptions of the new species are here solely attributed to the first author. Thus, complete citations of the authors are Chanabun in Chanabun et al.

RESULTS

Taxonomy

Family Almididae Duboscq, 1902

Genus *Glyphidrilus* Horst, 1889

Type species *Glyphidrilus weberi* Horst, 1889, by monotypy

Glyphidrilus panhai Chanabun, sp. nov.

<http://zoobank.org/urn:lsid:zoobank.org:act:D5FFAD74-8246-4AB1-8293-8121C09A0DF8>

(Figs 1, 2)

Glyphidrilus sp. 4—Jirapatrasilp et al., 2019: fig. 1, tables 2, S1.

Material examined.— Holotype: Adult specimen (CUMZ 3829), Song Khram River, Yangchum, Seka, Bueng Kan, Thailand, 17°55'50"N, 103°45'27"E, 146 m, coll. R. Chanabun, A. Aoonkum, 19 March 2022.

Paratypes: 49 specimens, 39 adults and 10 juveniles (CUMZ 3830), same collection data as for holotype; 15 specimens, 5 adults and 10 juveniles (CUMZ 3765), same locality as holotype, coll. R. Chanabun, C. Sutcharit, W. Siriwut, P. Jirapatrasilp, R. Srisonchai, & T. Seesamut, 3 May 2015; 2 adults (ZMH), 2 adults (NHMUK), same collection data as for holotype.

Other material examined.— 11 adults and 12 juveniles (CUMZ 3831), Song Khram River, Bankha, Si Songkhram, Nakhon Phanom, Thailand, 17°38'02"N, 104°14'43"E, 142 m, coll. R. Chanabun, C. Sutcharit, W. Siri-wut, & R. Srisonchai, 19 January 2022.

Diagnosis.— Body length 51–112 mm, diameter 2–4.5 mm, with 145–319 segments. The clitellar wing organs on the lateral sides of the body between segment 23, 24–29, 30, 31. Clitellum begins 17, 18–32, 33, 34, 35. Genital markings paired or asymmetrical on bc in pre-wing 17, 18, 19, 20, 21, 22, and post-wing 30, 31, 32. Genital markings paired or asymmetrical on aa in pre-wing 11, 12, 13, 14, 15, 16 and post-wing 30, 31, 32. Intestine enlarged in 16, ovaries in 13–14. Spermathecae sessile, elongated oval between 13/14 (14/15)–18/19.

Etymology.— The species name recognizes the great professor and a long-time mentor to the authors, Somsak Panha (Chulalongkorn University, Thailand).

Description of holotype.— Body length 86 mm, diameter 3.5 mm in segment 8, 4.5 mm in front of wings in segment 22, 3.5 mm behind wings in segment 31; body cylindrical in anterior part, quadrangular in cross-section behind clitellum. 266 segments. Body color pale brown with reddish from the first segment to clitellar portion. At posterior end dorsal surface broader than ventral. Clitellar wings on ventro-lateral part of clitellum in 23–30, both 4.5 mm in height and 0.3 mm wide on both sides. Prostomium zygolobous. Dorsal pores absent. Clitellum annular shape in 18–33. Four pairs of setae per segment from 2, setal formula aa: ab: bc: cd: dd = 1.0: 0.5: 1.0: 0.5: 2.0 in segment 8 and 1.0: 0.5: 1.0: 0.5: 2.5 in postclitellar segments. Female, male, and spermathecal pores not visible. Genital markings paired or asymmetrical on aa in pre-wing 13, 14, and post-wing 31, 32. Genital markings paired or asymmetrical on bc in pre-wing 18–22, and post-wing 31.

Septa 5/6–7/8 thickest, 8/9–11/12 thick and 12/13 to the last segment thin. Gizzard globular within 7/8. Intestine enlarged from 16. Dorsal blood vessel anterior to 7. Hearts in 7–11. A pair of nephridia in each segment, small in 14, and normal in 15 onwards.

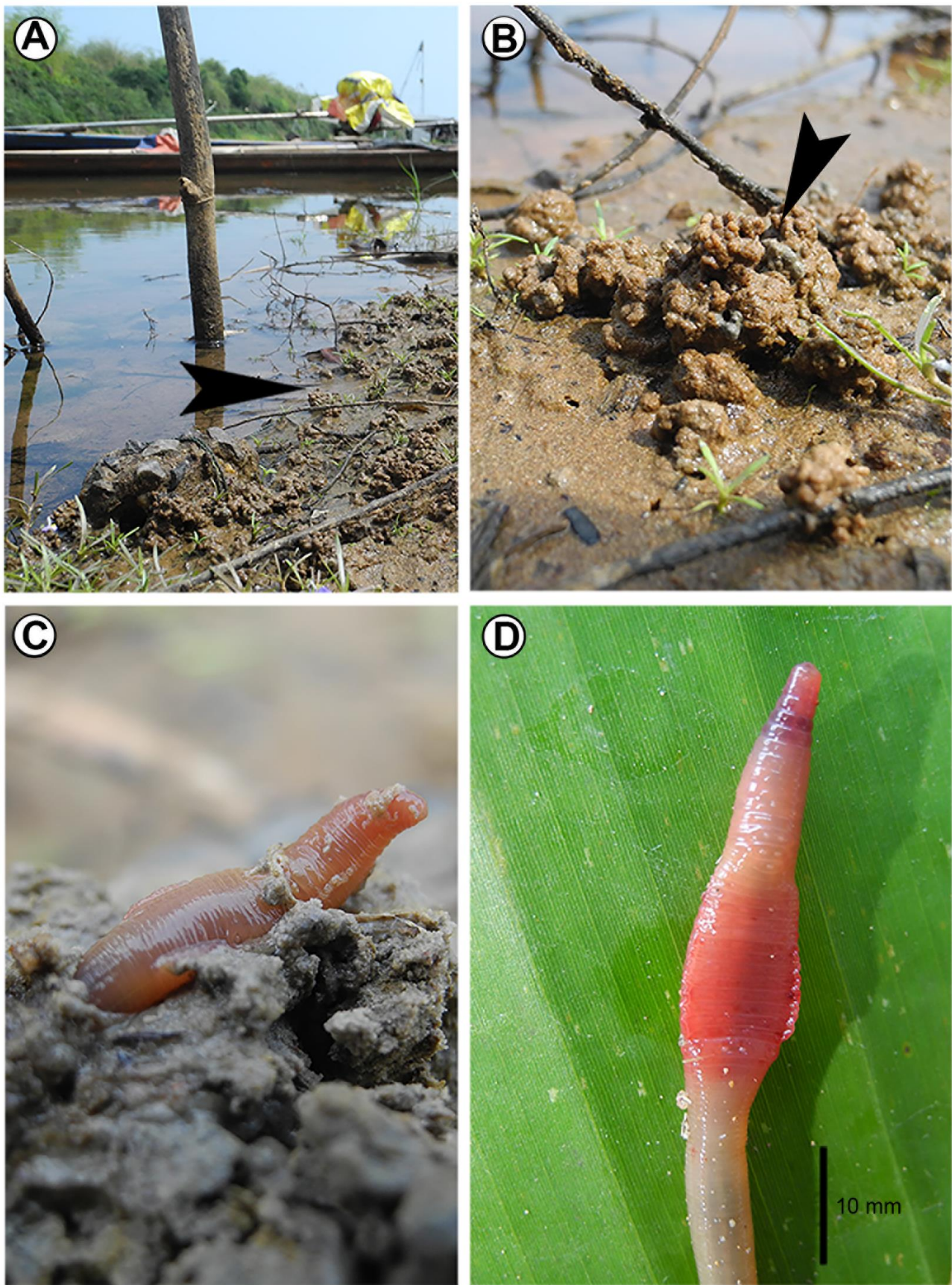


FIGURE 1. Photographs showing the (A) type locality of *Glyphidrilus panhai* Chanabun, sp. nov., with (B) casts of *Glyphidrilus panhai* sp. nov., (C) coloration of the living paratype (CUMZ 3830) and (D) after the first preservation step in 30% (v/v) ethanol.

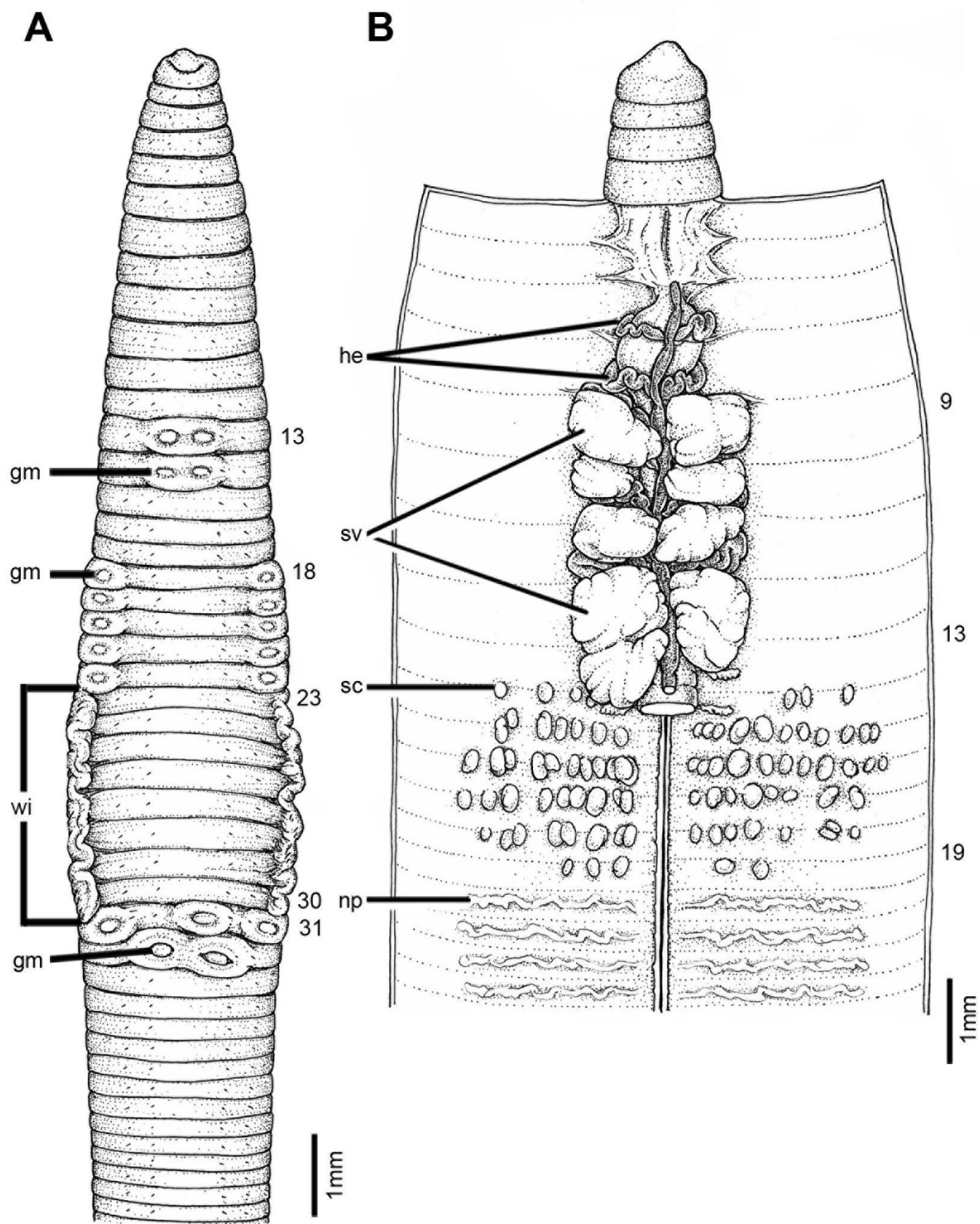


FIGURE 2. Morphology of holotype (CUMZ 3829) of *Glyphidrilus panhai* Chanabun, sp. nov. **A.** external ventral view, annular clitellum in 18–33 and **(B)** internal dorsal view.

TABLE 1. The GenBank accession numbers of DNA barcodes of *Glyphidrilus panhai* sp. nov. and *Glyphidrilus samueljamesi* sp. nov. following Jirapatrasilp et al. (2019).

Species	CUMZ no.	Type	Locality	GenBank accession no.			
				COI	16S rRNA	ITS1	ITS2
<i>G. panhai</i> sp. nov.	3765	Paratypes	Song Kham River, Yangchum, Seka, Bueng Kan, Thailand	MG923145– MG923149	MG920715– MG920719	MG921938– MG921943	MG922361– MG922365
<i>G. samueljamesi</i> sp. nov.	3797/1	Holotype	Khon Phapheng Waterfall, Mekong River, Khong, Champasak, Lao P.D.R.	MG923196	MG920766	MG922015	MG922438– MG922439
	3797/2	Paratype	Chompasak, Lao P.D.R.	MG923195	MG920765	MG922014	MG922437
	3796	Non-types	Mekong River at Ban Tha Pho, Khong, Champasak, Lao P.D.R.	MG923193– MG923194	MG920763– MG920764	MG922012– MG922013	MG922435– MG922436

Seminal vesicles in 9–12. Ovaries in 13–14. Testes in 10–11. Prostate and accessory glands absent. Spermathecae sessile, elongated oval or globular in 13/14–18/19, about 0.2–0.3 mm in diameter, two to eleven on each side per segment (Fig. 2).

Variation.— Body lengths range from 51–112 mm (77 ± 14.12), with 145–319 segments (242.89 ± 42.25). Wings begins in 23, 24–29, 30, 31, the most frequent position is 23–30. Clitellum begins in 17, 18–32, 33, 34, 35, the most frequent position is 18–33. Genital markings paired or asymmetrical on bc in pre-wing 17, 18, 19, 20, 21, 22, and post-wing 30, 31, 32. Genital markings paired or asymmetrical on aa in pre-wing 11, 12, 13, 14, 15, 16 and post-wing 30, 31, 32.

DNA barcodes.— DNA materials were extracted from 5 paratypes (CUMZ 3765) (Jirapatrasilp et al., 2019). The GenBank accession numbers are given in Table 1.

Distribution.— The new species is known from the type locality and nearby areas of the Song Kham River area.

Habitat.— The new species was found on riverbanks of Song Kham River, Northeast Thailand. The riverbank soil surface is found to be covered with earthworm casts. The earthworms occur in the loamy sand topsoil at about 5–10 cm depth.

Remarks.— *Glyphidrilus panhai* sp. nov. is similar to *G. chiensis* Chanabun and Panha, 2013 from Chi River Basins in the wing locations, but the latter has a longer clitellum in 17, 18–33, 34, 35, 36, 37, 38, widely genital markings on aa in 11, 12, 13, 14, 30, 32, 33, 34, 35, 36, on bc in 15, 16, 17, 18, 19–20, 21, 22, 23, 24, 30, 31, 33, and spermathecae in 12/13–18/19. The new species differs from *G. quadratus* Chanabun and Panha, 2013 from Mun River Basins in that *G. quadratus* has a longer clitellum in 15, 16, 17, 18–31, 32, 33, 34, 35,

36, genital markings on aa in 11, 12, 13, 14, 31, 32, 33, 34, on bc in 13, 15, 16, 17, 18, 19–21, 22, 23, 30, 31, and spermathecae in 12/13–17/18. It differs from *G. kralanhensis* Jirapatrasilp, Chanabun and Panha, 2016 from Tapan River, Cambodia in that the latter has a clitellum in 19, 20–35, 37, genital markings on aa is absent, on bc in 20, 22, 23, 24, 26–29, 30, and wings in 23, 24, ½25, 25–26, 27, ½28, 29, 30, 31. It differs from *G. jamiesoni* Jirapatrasilp, Chanabun and Panha, 2016 from stream banks near Praduk Temple, Cambodia in that the latter has longer wings in 23, 24–31, 32, 33, clitellum in 17, 18–35, 36, and genital markings on aa in 12, 13, 14, 31, 33, on bc in 15, 16, 17, 18–22, 23, 31, 32. The new species differs from *G. samueljamesi* sp. nov. (see below) from Khon Phapheng Waterfall, Laos in that *G. samueljamesi* sp. nov. has longer wings in 25, 26, 27–33, 34, 35, 36, and longer clitellum in 18, 19, 20–40, 41, 42, 43, 44, genital markings on aa is absent, on bc in 12, 13, 14–25, 26, 34, 35, 36, 37, 38–39, 40, 41, 42. For further comparison among Mekong *Glyphidrilus* species, see Table 2. This species was previously recognized as an undescribed species (sp. 4) and its status was supported by an integrative taxonomic analysis (Jirapatrasilp et al., 2019).

***Glyphidrilus samueljamesi* Chanabun, sp. nov.**

<http://zoobank.org/urn:lsid:zoobank.org:act:DF967BC3-05F6-4624-9F9C-25E98E18F37C>

(Fig. 3)

Glyphidrilus sp. 11—Jirapatrasilp et al., 2019: tables 2, S1.

Material examined.— Holotype: Adult specimen (CUMZ 3797/1), Khon Phapheng Waterfall, Mekong River, Khong, Champasak, Lao P.D.R., 13°57'46"N, 105°59'16"E, 75 m, coll. R. Chanabun, R. Srisonchai, S. Panha & K. Inkhavilay (23 July 2013). Paratypes: 4 adults (CUMZ 3797/2–5), 2 adults (ZMH), same collection data as for holotype.

TABLE 2. The comparison of taxonomic characters among *Glyphidrilus* species from Mekong River basins, based on the type specimens and original description.

Species	Length (mm)	Segment s	Cli.	Wi.	Wi. Covered	Gm.			He.	In.	Sc.	Type Locality
						Paired on bc	Paired on aa	Unpaired on aa				
<i>G. yunnanensis</i>	123	139	18–38	22–32	11	17–21, 32–34	absent	absent	7–11	16	absent	Yunnan, China
<i>G. mekongensis</i>	125–224	223–382	19–37, 38	24–½33, 33, 34, ½35	9½–11½	23	absent	absent	7–11	15	absent	Khong Chiam, Ubon Ratchathani, Thailand
<i>G. vangviengensis</i>	104–160	145–229	19, 20–35, 36, 37	24, 25–31, 32	7–9	18, 19, 20, 21–24, 33, 34	12–14, 13, 14, 15	absent	7–11	16	absent	Song River Veintiane, Laos
<i>G. chiensis</i>	61–193	122–386	17, 18–33, 34, 35, 36, 37, 38	23, 24, 25, 26–29, 30, 31, 32	4–10	15, 16, 17, 18, 19–20, 21, 22, 23, 24, 30, 31, 33	11, 12, 13, 14, 30, 32, 33, 34, 35, 36	absent	7–11	15	12/13–18/19	Mueang, Maha Sarakham, Thailand
<i>G. quadratus</i>	54–156	186–378	15, 16, 17, 18–31, 32, 33, 34, 35, 36	23, 24–28, 29, 30, 31	5–9	13, 15, 16, 17, 18, 19–21, 22, 23, 30, 31, 33, 34	11, 12, 13, 14, 31, 32, 33, 34	absent	7–11	15	12/13–17/18	Kang Sapue, Phibun Mangsahan, Ubon Ratchathani, Thailand
<i>G. jamiesoni</i>	82–114	165–307	17, 18–35, 36	23, 24–31, 32, 33	8–11	15, 16, 17, 18–22, 23, 31, 32	12, 13, 14, 31, 33	absent	7–11	16	13/14–18/19	Stream banks near Praduk Temple, Banteay Srei District, Siem Riep Province, Cambodia
<i>G. kralanhensis</i>	85–116	205–314	19, 20–35, 37	23, 24, ½25, 25–26, 27, ½28, 29, 30, 31	7–8?	20, 22, 23, 24, 26–29, 30	absent	absent	7–11	16	13/14, (14/15)–20/21 (21/22)	Tapan River, Kralanh District, Siem Riep Province, Cambodia
<i>G. chiangraiensis</i>	94–340	89–394	17, 18, 19, 20, 21–36, 37, 38, 39	23, 24–26, ½27, 27, ½28, 28	3–6	21, 22, 23, 24, 27, 28, 29–35	12–14, 15, 16	absent	7–11	16	15/16–20/21	Chiangkhong, Chiang Rai, Thailand
<i>G. sekongensis</i>	90–134	237–337	16, 17–36, 37	25–31	7	16, 17–24, 32	absent	absent	8–11	16	12/13–15/16	Ban Keangkong, Lamarm, Sekong, Laos
<i>G. namdonensis</i>	90–139	183–259	19, 20–36, 37, 38	24, 25–28, 30	5–7	22, 23, 24, 27, 28–33	13, 14	absent	7–11	16	14/15–18/19	Done River, Thakhek, Khammoua, Laos
<i>G. champasakensis</i>	167–301	248–424	19, 20–49, 50, 51, 52	23, 24–½32, 32, 33	8½–11	17, 18, 19–22, 23, 24, 32, 33, 34, 35	12, 13, 33, 34	absent	7–11	16	14/15–19/20	Mekong River at Ban Khonkhen, Champasak, Laos
<i>G. panhai</i> sp. nov.	51–112	145–319	17, 18–32, 33, 34, 35	23, 24–29, 30, 31	6–9	17, 18, 19, 20, 21, 22, 30, 31, 32	11, 12, 13, 14, 15, 16, 30, 31, 32	absent	7–11	16	13/14 (14/15)–18/19	Song Kham River, Seka, Bueng Kan, Thailand
<i>G. samueljamesi</i> sp. nov.	81–104	230–317	18, 19, 20–40, 41, 42, 43, 44	25, 26, 27–33, 34, 35, 36	8–11	12, 13, 14–25, 26, 34, 35, 36, 37, 38–39, 40, 41, 42	absent	absent	7–11	15	13/14–20/21	Khon Phapheng Waterfall, Champasak, Laos

Abbreviations: Cli., Clitellum; Wi., Wings; Gm., Genital markings; He., Hearts; In., Intestine; Sc., Spermathecae.

Other material examined.— 5 adults (CUMZ 3796), Mekong River at Ban Tha Pho, Khong, Champasak, Lao P.D.R., 14°22'29.7"N, 105°54'25.9"E, 50 m, coll. R. Chanabun, R. Srisonchai, S. Panha & K. Inkhavilay (22 July 2013).

Diagnosis.— Body length 81–104 mm, diameter 4–6 mm, 230–317 segments. The clitellar wing organs on the lateral sides of the body between segment 25, 26, 27–33, 34, 35, 36. Clitellum in 18, 19, 20–40, 41, 42, 43, 44. Genital markings paired or asymmetrical on bc in pre-wing 12, 13, 14–25, 26, and post-wing 34, 35,

36, 37, 38–39, 40, 41, 42. Intestine enlarged in 15, ovaries in 13–14. Spermathecae sessile, oval or globular between 13/14–20/21.

Etymology.— The species is named for our highly esteemed colleague Samuel Wooster James (Department of Biology, University of Iowa, USA), one of the most productive earthworm taxonomists, who encourages all new and young annelidologists in Thailand.

Description of Holotype.— Body length 104 mm, diameter 5 mm in segment 8, 6 mm in front of wings in

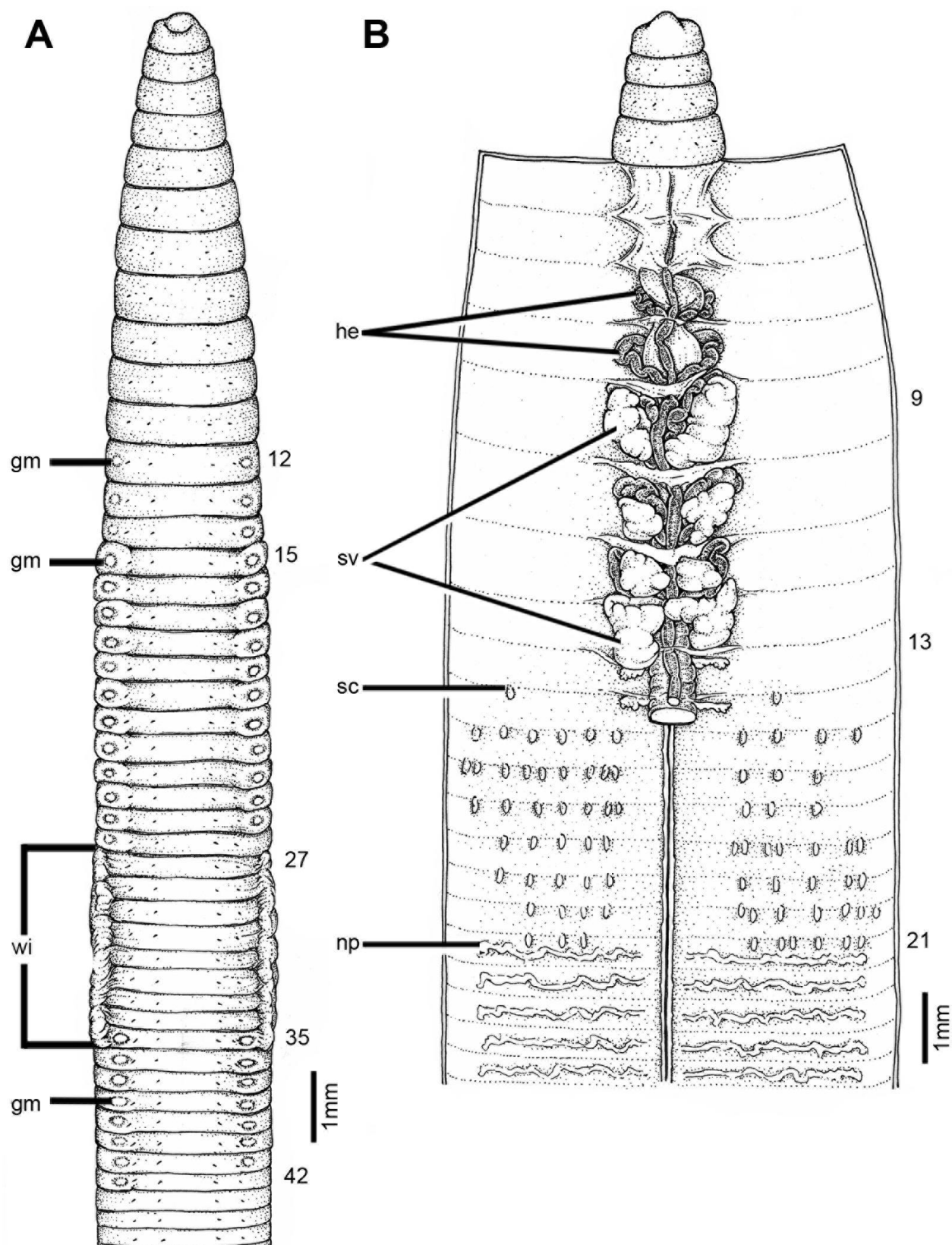


FIGURE 3. Morphology of holotype (CUMZ 3797/1) of *Glyphidrilus samueljamesi* Chanabun, sp. nov. **A.** external ventral view, annular clitellum in 20–44 and **(B)** internal dorsal view.

segment 26, 5 mm behind wings in segment 36; body cylindrical in anterior part, quadrangular in cross-section behind clitellum. 285 segments. Body color pale brown with reddish tint from the first segment to clitellar portion. At posterior end dorsal surface broader than ventral. Clitellar wings on ventro-lateral part of clitellum in 27–35, 5.0 mm height, and 0.6 mm wide on both sides. Prostomium zygotubous. Dorsal pores absent. Clitellum annular shape in 20–44. Four pairs of setae per segment from 2, setal formula aa: ab: bc: cd: dd = 1.5: 0.5: 2.0: 1.0: 2.5 in segment 8 and 2.0: 0.5: 2.5: 1.0: 3.0 in postclitellar segments. Female, male, and spermathecal pores not visible. Genital markings paired or asymmetrical on bc in pre-wing 12–26, and post-wing 35–42.

Septa 5/6–7/8 thickest, 8/9–11/12 thick and 12/13 to the last segment thin. Gizzard globular within 8. Intestine enlarged from 15. Dorsal blood vessel anterior to 7. Hearts in 7–11. A pair of nephridia in each segment, rudimentary in 13, small in 14, and normal in 15 onwards. Seminal vesicles in 9–12. Ovaries in 13–14. Testes in 10–11. Prostate and accessory glands absent. Spermathecae sessile, oval or globular in 13/14–20/21, about 0.2 mm in diameter, one to nine on each side per segment (Fig. 3).

Variation.– Body lengths range from 81–104 mm (91 ± 8.34), with 230–317 segments (289.83 ± 31.14). Wings begins in 25, 26, 27 and end in 33, 34, 35, 36, the most frequent positions are 27–35. Clitellum begins in 18, 19, 20 and ends in 40, 41, 42, 43, 44. Genital markings paired or asymmetrical on bc start in pre-wing 12, 13, 14–25, 26, and post-wing 34, 35, 36, 37, 38–39, 40, 41, 42.

DNA barcodes.– DNA materials were extracted from the holotype (CUMZ 3797/1) and one paratype (CUMZ 3797/2) from the type locality, and 2 specimens (CUMZ 3796) from Mekong River at Ban Tha Pho, Khong, Champasak, Lao P.D.R. (Jirapatrasilp et al., 2019). The GenBank accession numbers are given in Table 1.

Distribution.– The new species is known from the type locality and nearby areas of Khon Phapheng Waterfall, Mekong River.

Habitat.– The new species was found on the shore of the big waterfall and nearby banks of the Mekong River in Laos. The shore is covered with earthworm casts. The earthworms occur in the topsoil at about 10–30 cm depth.

Remarks.– *Glyphidrilus samueljamesi* sp. nov. differs from *G. mekongensis* Panha and Chanabun, 2012 in that the latter has wings in 24–½33, 33, 34, ½35, genital markings on bc in 23, and the spermathecae are absent. The new species differs from *G. jamiesoni* Jirapatrasilp, Chanabun and Panha, 2016 from stream banks near Praduk Temple, Cambodia in that the latter has shorter wings in 23, 24–31, 32, 33, a shorter clitellum in 17, 18–35, 36, and present genital markings on aa in 12, 13, 14, 31, 33, bc in 15, 16, 17, 18–22, 23, 31, 32. It differs from *G. kralanhensis* Jirapatrasilp, Chanabun and Panha, 2016 from Tapan River, Kralanh District, Cambodia in that the latter has wings in 23, 24, ½25, 25–26, 27, ½28, 29, 30, 31, genital markings on bc in 20, 22, 23, 24, 26–29, 30, and a clitellum in 19, 20–35, 37. For further comparison among Mekong *Glyphidrilus* species, see Table 2. This species was previously recognized as an undescribed species (sp. 11) and its status was supported by an integrative taxonomic analysis, (Jirapatrasilp et al., 2019).

DISCUSSION

Up to now, a total of 21 *Glyphidrilus* species have been reported from diverse habitats such as paddy field areas, waterfalls, riverbanks, and shores of the canals, streams and ponds in Thailand and Laos. In this study, we update the number of *Glyphidrilus* from the Mekong River Basin to 11 species (Chanabun et al., 2011, 2012a, 2013, 2017; Jirapatrasilp et al., 2016), including two new species. *Glyphidrilus panhai* sp. nov. occurs along the Song Kham River which is a branch of the Mekong River in Northeast Thailand. *Glyphidrilus samueljamesi* sp. nov. occurs in Khon Phapheng Waterfall, which is the largest waterfall in southeast Asia, located on the Mekong River in Champasak Province, southern Laos, and the local people call it as the jewel or the pearl of the Mekong River.

Although DNA data, especially “DNA barcodes”, becomes more important in revealing earthworm diversity (Chang and James, 2011; Decaëns et al., 2013), the traditionally-accepted original descriptions of new earthworms still require morphological data (Martinsson and Erséus, 2021). Currently, some original descriptions already include DNA barcodes as a part of the description (e.g., Chang et al., 2014; Nguyen et al., 2022), or provide a molecular phylogeny to indicate the new species’ phylogenetic relationship (e.g., Shen et al., 2019; Marchán et al., 2021; Nguyen et al., 2021; Sun et al., 2021). However, few works apply species delimitation methods for DNA data (Jeratthitikul et al., 2017; Shekhovtsov et al., 2020), and even fewer studies arrive a taxonomic decisions

based on DNA data alone (Martinsson and Erséus, 2018). Thus, this study highlights the importance of an integrative taxonomic framework incorporating both morphological and DNA data in species delimitation (Jirapatrasilp et al., 2019), which leads to the final taxonomic decision in formally describing two new *Glyphidrilus* species herein.

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