

Two New Species of Semi-aquatic Earthworms Genus *Glyphidrilus* Horst, 1889 from Malaysia (Oligochaeta: Almididae)

RATMANEE CHANABUN^{1*} AND SOMSAK PANHA²

¹ Program in Animal Science, Faculty of Agriculture Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon 47000, THAILAND

² Animal Systematics Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University, 254 Phayathai Road, Pathumwan, Bangkok 10330, THAILAND

* Corresponding author. Ratmanee Chanabun (cratmanee@yahoo.com)

Received: 26 July 2015; Accepted: 6 September 2015

ABSTRACT.— Two new species of semi-aquatic freshwater earthworms of the genus *Glyphidrilus* Horst, 1889 are described from Malaysia. *Glyphidrilus perakensis* n. sp. from Sungei Kurau, Kampung, Panit Luar Geri, Perak and *G. kedahensis* n. sp. from Sungei Sedim, White fall, Kulim, Kedah. After comparing with the closely related species, the two new species have distinct different morphological characters from the previous recognized species. *Glyphidrilus perakensis* n. sp. has wings in 26, 27–30, 31, clitellum in 20–36, 37, hearts in 10–14, seminal vesicles in 12–15, and spermathecae in 16/17–18/19. *Glyphidrilus kedahensis* n. sp. has wings in 23, 24–½28, 28, clitellum in 17–33, 34, hearts in 9–12, seminal vesicles in 10–13 and spermathecae in 14/15–16/17. Descriptions of the new species including illustrations of the external and internal morphological characteristics are provided.

KEY WORDS: Taxonomy, *Glyphidrilus*, Malaysia

INTRODUCTION

The semi-aquatic earthworms genus *Glyphidrilus* Horst, 1889 have been recorded from Asia and Africa. The animals occur in appropriate aquatic margin habitats throughout most of Burma, India, Ceylon, and the Malay Peninsula (Beddard 1895; Stephenson 1923; Brinkhurst & Jamieson 1971; Gates 1972). Seven species have been described from Malaysia included *G. kukenthali* Michaelsen, 1896 from Barem River, Sarawak, *G. malayanus* Michaelsen, 1902 from Lubok Paku, Pahang River, *G. horsti* Stephenson, 1930 from Pulau Berhala, and *G. gatesi* Shen & Yeo, 2005 from swamp forest, Sedili River, Johor, *G. bisegmentus* Chanabun & Panha, 2012 from Air Banun Pandig, Perak, *G. kotatinggi* Chanabun & Panha, 2012 from Kota Tinggi waterfall, Johor and *G. peninsularis* Chanabun & Panha, 2012 from Sungei

Bantang, Johor (Michaelsen 1896, 1902; Stephenson 1930; Shen & Yeo 2005; Chanabun et al., 2012b). In June 2014 we conducted an earthworm survey in northern region of Malaysia along Kedah, Penang and Perak States. Two new species have been discovered and described.

MATERIALS AND METHODS

Earthworms were collected by carefully digging up the topsoil near casts on the shore and in the water using hand sorting. 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.

Duplicate specimens and/or tissue samples were preserved in 95% ethanol for molecular data and DNA barcoding

analyses. The descriptions were made from observation under OLYMPUS SZX16 stereoscopic light microscope. The external and internal morphological characters were recorded. 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 acitellates, and are presented as the range (min-max) and mean±one standard deviation. By convention, we use the Arabic numerals to refer to segment numbers and to indicate segmental boundaries.

To confirm the novelty of the new species, type material of morphologically similar *Glyphidrilus* species was investigated from the following collections: CUMZ, Museum of Zoology, Chulalongkorn University, Bangkok, Thailand; ZRC, Raffles Museum of Biodiversity Research, National University of Singapore, Singapore; ZMH, Biozentrum Grindel und Zoologisches Museum, University of Hamburg, Germany and NHM, The Natural History Museum, London.

Holotype and paratype specimens of the new species were deposited in CUMZ. Additional paratypes will be housed in ZMH and NHM.

The following abbreviations used in the descriptions of anatomy are as appeared in Chanabun et al. (2011, 2012a, b, 2013): wi, wings; gm, genital markings; he, hearts; sv, seminal vesicles; sc, spermathecae; ov, ovaries; np, nephridia.

SYSTEMATICS

Genus *Glyphidrilus* Horst, 1889

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

Glyphidrilus perakensis Chanabun & Panha, n. sp.

(Figs. 1, 2, 3; Table 1)

Description of Holotype. — Dimensions; 140 mm body length by 3.0 mm in the anterior body region and 3.0 mm before the clitellar wings in 26, 3.0 mm after wings in 32 within the clitellum, body cylindrical in the anterior part but after clitellum it is quadrangular in transverse section view, with 342 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 clitellar wings are attached on ventral lateral part of the clitellum in 27–31, 3.0 mm long and about 0.5 mm wide on both sides. Prostomium zygotobous. Dorsal pores absent. Clitellum annular shape in 20–37. Four pairs of setae per segment start from 2, setae formula aa:ab:bc:cd:dd = 1.0:0.5:1.5:0.5:1.0 in 8. Female pores, male pores and spermathecal pores are not visible. Genital markings lateral paired or asymmetrical on bc in 20–21; and median unpaired on aa in 20–23, 26.

Septa 10/11–14/15 thicker, 15/16 to the last segment thin. Gizzard big globular within 10. Intestine enlarged from 16. Dorsal blood vessel aborted anterior to 10. Hearts in 10–14. No nephridia are distinguishable in the first thirteen segments. Four pairs of seminal vesicles in 12–15, that of segment 14, 15 are larger than the others. The ovaries located in 16. Prostate and accessory glands absent. Spermathecae in 16/17–18/19, four to five on each side per segment.

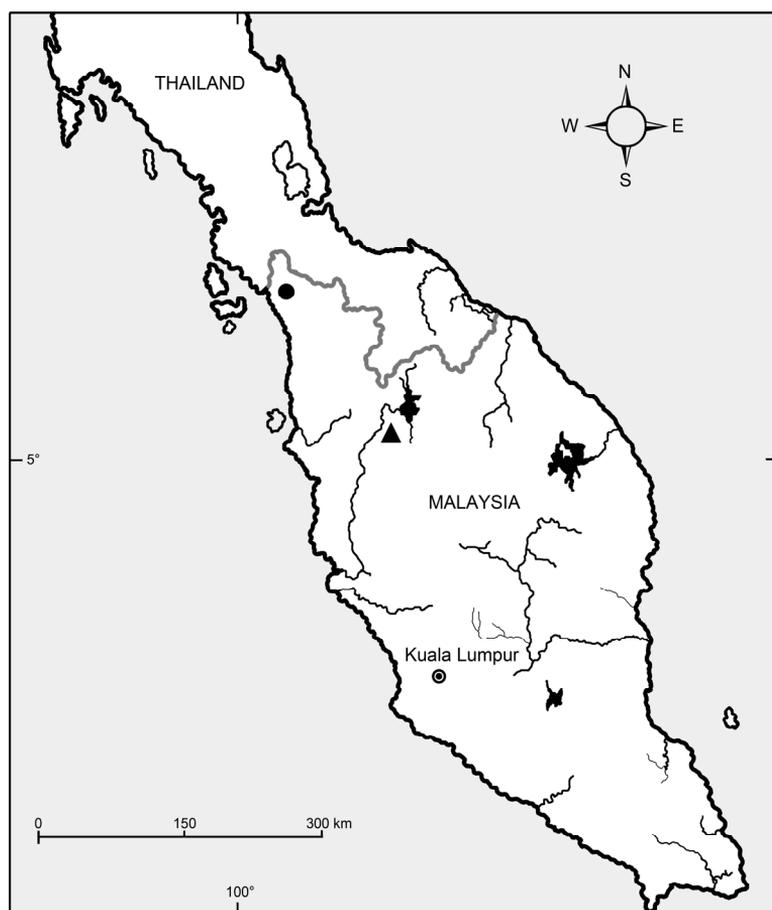


FIGURE 1. Distributions map of the new *Glyhidrilus* species from Malaysia. Filled triangular: *G. perakensis* n. sp. from Sungei Kurau near Klinik Desa, Kampung, Panit Luar Geri, Perak, Malaysia. Filled circle: *G. kedahensis* n. sp. from Sungei Sedim, White fall, Kulim, Kedah, Malaysia.

Variation. — The body length of holotype measures 140 mm body length, with 342 segments; the body length of twenty two paratypes range from 96–142 mm (122.17 ± 19.90), with 299–459 segments. Wings in 26, 27–30, 31, with annular clitellum in 20–36, 37. Genital markings: median unpaired on aa in 20–23, 24, 25, 26 and 36, 37, lateral paired or asymmetrical on bc in 20, 21.

Type locality. — Sungei Kurau near Klinik Desa, Kampung, Panit Luar Geri, Perak,

Malaysia, $05^{\circ} 36' 40.0''$ N, $101^{\circ} 03' 47.8''$ E, 182 meters elevation on 09 June 2014.

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

Etymology. — This new species was named for the type locality.

Type materials. — Holotype (CUMZ 3313) and 22 paratypes (CUMZ 3314) are deposited in Chulalongkorn University, Museum of Zoology (CUMZ).

Table 1. Comparisons of the morphological characters of *Glyptidrilus* species from Malaysia and some species described from the south of Thailand.

Species	Length (mm)	Segments	Clitellum	Wings	Genital markings		Hearts	Intestinal origin	Gizzard	Spermathecae	Type Locality
					Paired on bc	Unpaired on aa					
<i>G. kuekenhali</i>	75-90	153-300	½13-34	18, ½18, 19-24, ½25	18-25	10-15, 25-32	7-11	15 or 16	8	14-18	Borneo, Baram River, Sarawak, Malaysia Malay Peninsula
<i>G. malayanus</i>	85-90	236-356	15, 16, 17-23, 24, 25, 26	½18, 18-21, ½22	13-15, 16, 17, 22, 23	12-16, 22-25	9-11	14	8	14/15-16/17	
<i>G. horsti</i>	34.9-53.3	125-232	17, 18, ½18, 19-29, 30, ½31	23, ½23-½28, 28	18, 19, 22, 23, 27, 29	16, 17, 18-20, 22, 27, 28	8, 9-11	13 or 15 or 16	7-8	14-17, 14/15-17/18	Pulau Berhala, Straits of Malacca, Malaysia Johor, Malaysia
<i>G. gatesi</i>	34-95	42-122	½17, 17, 18-25, ½26, 26	18, ½19, 19-½24, 24	14, 15, 16-18, 24-25, 26, 27	13, 14-19, 24, 25-26, 27	9-11	18	8	15-17	
<i>G. bisegmentus</i>	40-71	152-252	16, 17-23, 24	18-19	absent	absent	9-11	15	8-9	absent	Air Bemun Pandig, Perak, Malaysia
<i>G. kotatinggi</i>	151 ⁺ -195 ⁺	221 ⁺ -415	17, 18-28, 29, 30	20, 21-½26, 26	11-15, 17, 18, 19, 20, 26-27	17, 19	9-11	14	8-9	13/14-16/17	Kota Tinggi waterfall, Johor, Malaysia
<i>G. peninsularis</i>	49-94	206-281	17, 18-31, 32	22, 23-28, ½29, 29	13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 29, 30	17, 18, 19, 20, 21, 31, 32	8-11	14	7-8	14/15-17/18	Sungei Bantang, Johor, Malaysia
<i>G. trangenensis</i>	11 ⁻ -63 ⁺	41 ⁻ -153 ⁺	17, 18-30	22, 23-27, 28	absent	18-21	8-11	16	8-9	18-21	Trang River, Nayong, Trang, Thailand
<i>G. kratuensis</i>	48-93	221-282	18-30, 31, 32	23, 24-28, 29, 30	14, 15, 16, 17, 18, 19, 22, 23, 24, 29, 30	18, 19-20, 21, 22, 23, 30-31, 32-34	8-11	14	8	14/15-17/18	Kratu waterfall, Kratu, Phuket, Thailand
<i>G. perakensis</i> n. sp.	96-142	299-459	20-36, 37	26, 27-30, 31	20, 21	20-23, 24, 25, 26, 36, 37	10-14	16	10	16/17-18/19	Sungei Kurau Kampung, Perak, Malaysia
<i>G. kedahensis</i> n. sp.	97-194	228-433	17-33, 34	23, 24-½28, 28	15, 16, 17, 18	16, 17, 18-22, 23, 30, 32	9-12	16	8-9	14/15-16/17	Sungei Sedim, Kulim, Kedah, Malaysia

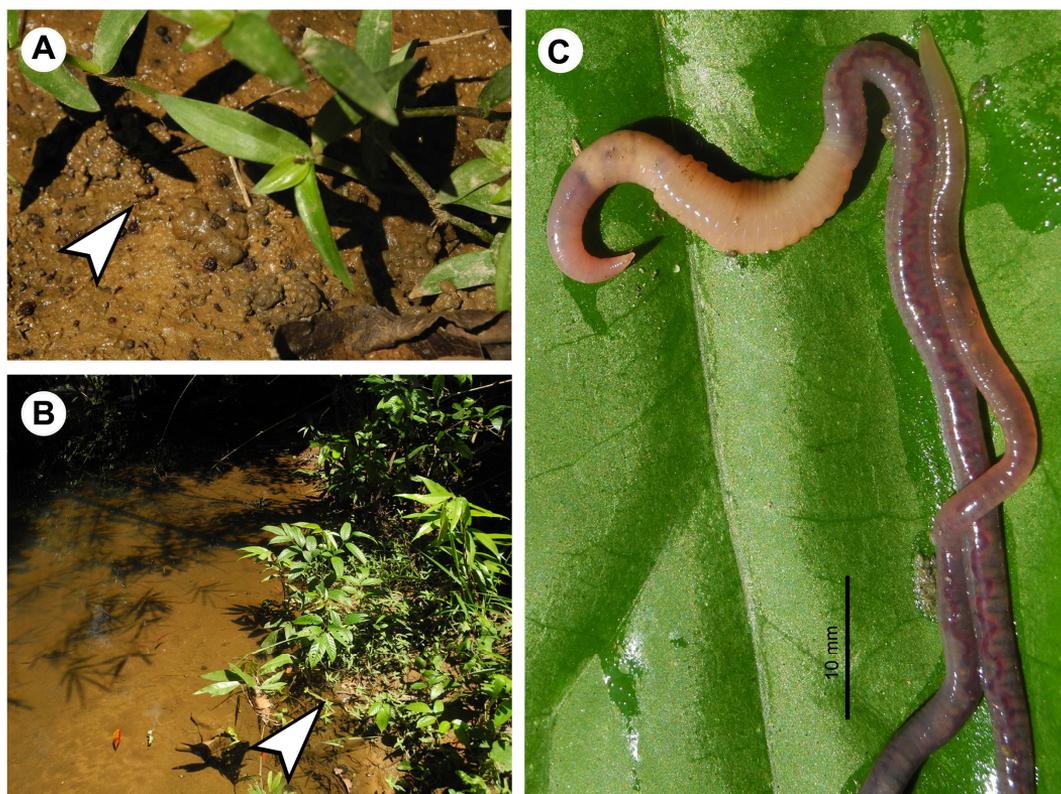


FIGURE 2. Photographs showing the (A) *Glyphidrilus perakensis*, new species casts; (B) type locality of *G. perakensis*, new species in Sungei Kurau near Klinik Desa, Kampung, Panit Luar Geri, Perak, Malaysia and (C) coloration of living paratype CUMZ 3314.

Habitat. — Found on the shore but in proximity to the river water, and under the water at about 5–10 cm depth. In the river banks soil surface was covered with worm casts at River banks of Klinik Desa, Kampong, Panit Luar Gerik, Perak, Malaysia.

Diagnosis. — *Glyphidrilus perakensis* n. sp. is a medium size semi-aquatic freshwater earthworm with distinct expanded tissues of wings in 26, 27–30, 31. No female pores, male pores and spermathecal pores were observed. But the genital markings; median unpaired on aa in 20–23, 24, 25, 26 and 36, 37, lateral paired or asymmetrical on bc in 20, 21. Hearts in 10–14. Four pairs of

seminal vesicles in 12–15, that of segment 14, 15 are largest. Intestinal origin in 16. The ovaries in 16. Prostate and accessory glands absent. Spermathecae in 16/17–18/19.

Remarks. — *Glyphidrilus perakensis* n. sp. herein have some degree of similarity to seven described species from Malaysia, however there are different with respect to spermathecae, genital markings, clitellum and wing locations. *Glyphidrilus perakensis* n. sp. differs from *G. malayanus* from Malay Peninsula, Lubock Paku, Pahang River by the latter has the wings in $\frac{3}{4}$ 18, 18–21, $\frac{1}{2}$ 22, clitellum in 15, 16, 17–23, 24, 25, 26, hearts in 9–11, seminal vesicles in

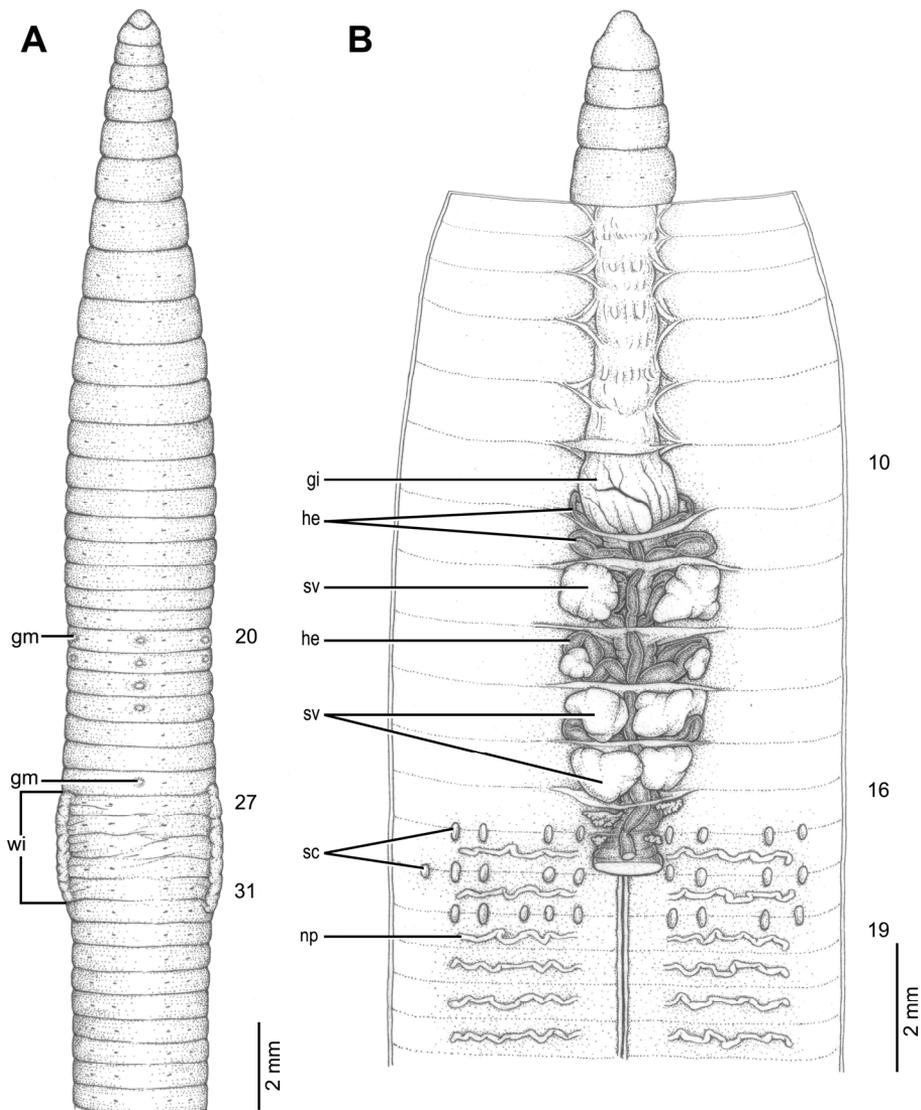


FIGURE 3. External and internal morphology of holotype (CUMZ 3313) of *Glyphidrilus perakensis*, n. sp. (A) external ventral view; (B) internal dorsal view.

9–12, intestinal origin in 14, and spermathecae in 14/15–16/17. Differs from *G. kuekenhali* from Borneo, Baram River, Sarawak by the latter has wings in 18, ½18, 19–24, ½25, clitellum in ½13–34, hearts in 7–11 and spermathecae in 14–18. Differs from *G. horsti* from Pulau Berhala, Straits of Malacca by *G. horsti* has wings in 23,

½23–½28, 28, clitellum in 17, 18, ½18, 19–29, 30, ½31, and spermathecae in 14–17, 14/15–17/18. Differs from *G. gatesi* from Sungei Kayu, swamp forest near River Sedili, Johor, Malaysia by the latter has wings in 18, ½19, 19–½24, 24, clitellum in ½17, 17, 18–25, ½26, 26, and spermathecae in 15–17. Differs from *G. bisegmentus* from

Air Banun Pandig, Perak, Malaysia by the latter has wings in 18–19, clitellum in 16, 17–23, 24, and lacks spermathecae. Differs from *G. kotatinggi* from Kota Tinggi waterfall, Johor, Malaysia by the latter has wings in 20, 21– $\frac{1}{4}$ 26, 26, clitellum in 17, 18–28, 29, 30, hearts in 9–11, seminal vesicles in 9–12, intestinal origin in 14, and the spermathecae in 13/14–16/17. Differs from *G. peninsularis* from Sungei Bantang, Johor, Malaysia by the latter has wings in 22, 23–28, $\frac{1}{2}$ 29, 29, clitellum in 17, 18–31, 32, hearts in 8–11, seminal vesicles in 9–12, intestinal origin in 14, and spermathecae in 14/15–17/18.

***Glyphidrilus kedahensis* Chanabun & Panha, n. sp.**

(Figs. 1, 4, 5; Table 1)

Description of Holotype. — Dimensions; 172 mm body length by 4.0 mm in the anterior body region and 5.0 mm before the clitellar wings in 23, 4.0 mm after wings in 29 within the clitellum, body cylindrical in the anterior part but after clitellum it is quadrangular in transverse section view, with 337 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 clitellar wings are attached on ventral lateral part of the clitellum in 24–28, 4.0 mm long and about 0.7 mm wide on both sides. Prostomium zygalobous. Dorsal pores absent. Clitellum annular shape in 17–34. Four pairs of setae per segment start from 2, setae formula aa:ab:bc:cd:dd = 1.0:0.5:1.5:0.5:1.0 in 8. Female pores, male pores and

spermathecal pores are not visible. Genital markings lateral paired or asymmetrical on bc in 17, 18; nearly setal line c in 15, 16; and median unpaired on aa in 16–23, 30, 32.

Septa 7/8–8/9 thicker, 9/10–11/12 thick, 12/13 to the last segment thin. Gizzard big globular within 8–9. Intestine enlarged from 16. Dorsal blood vessel aborted anterior to 10. Hearts in 9–12. No nephridia are distinguishable in the first thirteen segments. Four pairs of seminal vesicles in 10–13, that of segment 13 is larger than the others. The ovaries located in 13, 14. Prostate and accessory glands absent. Spermathecae in 14/15–16/17, four on each side per segment.

Variation. — The body length of holotype measures 172 mm body length, with 337 segments; the body length of fifty (paratypes) and thirty one (non-types) range from 97–194 mm (137.62 ± 35.57), with 228–433 segments. Wings in 23, 24– $\frac{1}{2}$ 28, 28, with annular clitellum in 17–33, 34. Genital markings lateral paired or asymmetrical on bc in 17, 18; nearly setal line c in 15, 16; and median unpaired on aa in 16, 17, 18–22, 23, 30, 32.

Type locality. — Sungei Sedim, White fall, Kulim, Kedah, Malaysia, 05° 24' 48.2" N, 100° 46' 51.6" E, 111 meters elevation on 10 June 2014.

Distribution. — The new species is known from the type locality and Ulu Legong Hot spring, Baling, Kedah, Malaysia, 05° 49' 0.8" N, 100° 56' 59.0" E, 141 meters elevation on 9 June 2014.

Etymology. — This new species was named for the type locality.

Type materials. — Holotype (CUMZ 3315) and 29 adults and 17 juveniles as paratypes (CUMZ 3316) are deposited in Chulalongkorn University, Museum of Zoology (CUMZ). 2 adults (ZMH 14578),

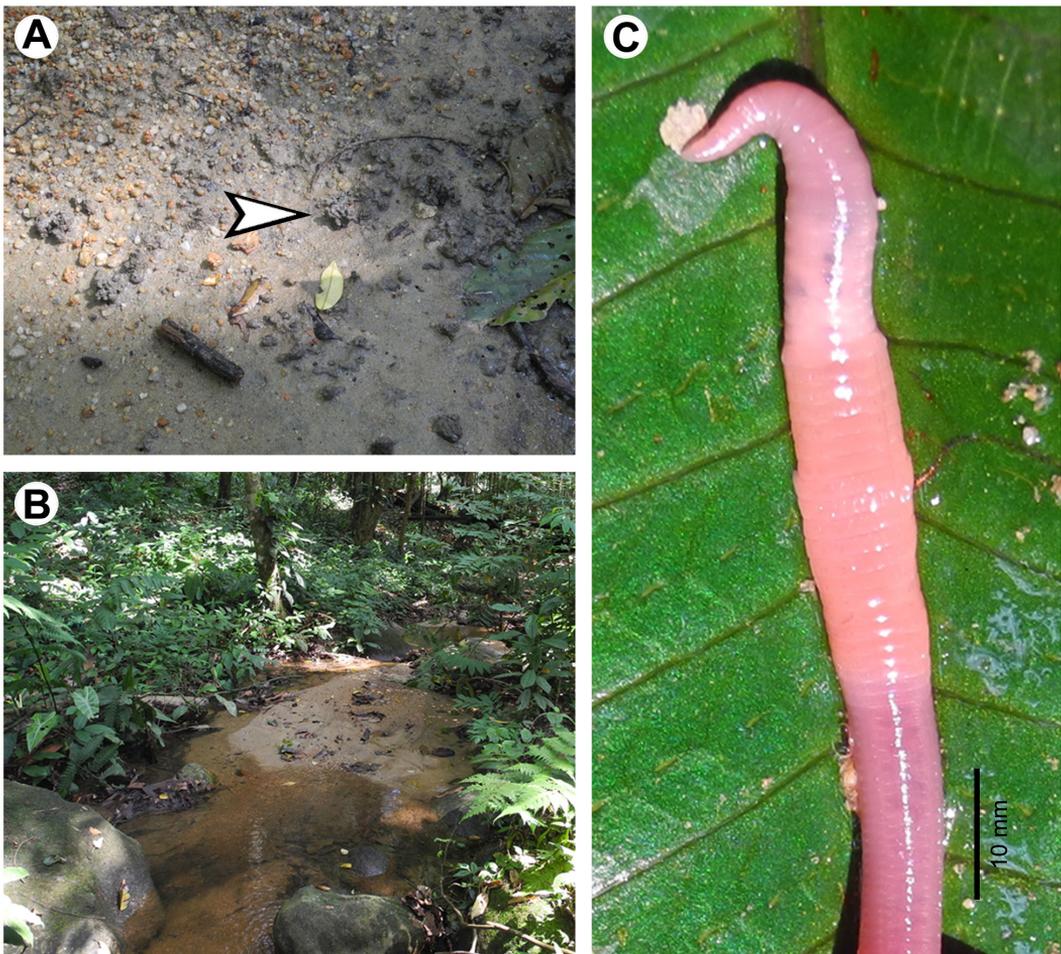


FIGURE 4. Photographs showing the (A) *Glyphidrilus kedahensis*, new species casts; (B) type locality of *G. kedahensis*, new species from Sungei Sedim, White fall, Kulim, Kedah, Malaysia and and (C) coloration of living paratype CUMZ 3316.

and 2 adults (NHM), same collection data as holotype.

Other material examined. – 20 adults and 11 juveniles (CUMZ 3317) from Ulu Legong Hot spring, Baling, Kedah, Malaysia.

Habitat. — Found on the shore but in proximity to the water, and under the water at about 5–15 cm depth. The river banks soil surface was covered with worm casts at all habitats.

Diagnosis. — *Glyphidrilus kedahensis* 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 in 23, 24– $\frac{1}{2}$ 28, 28. No female pores, male pores and spermathecal pores were observed. Genital markings lateral paired or asymmetrical on bc in 17, 18; nearly setal line c in 15, 16; and median unpaired on aa in 16, 17, 18–22, 23, 30, 32. Hearts in 9–12. Four pairs of

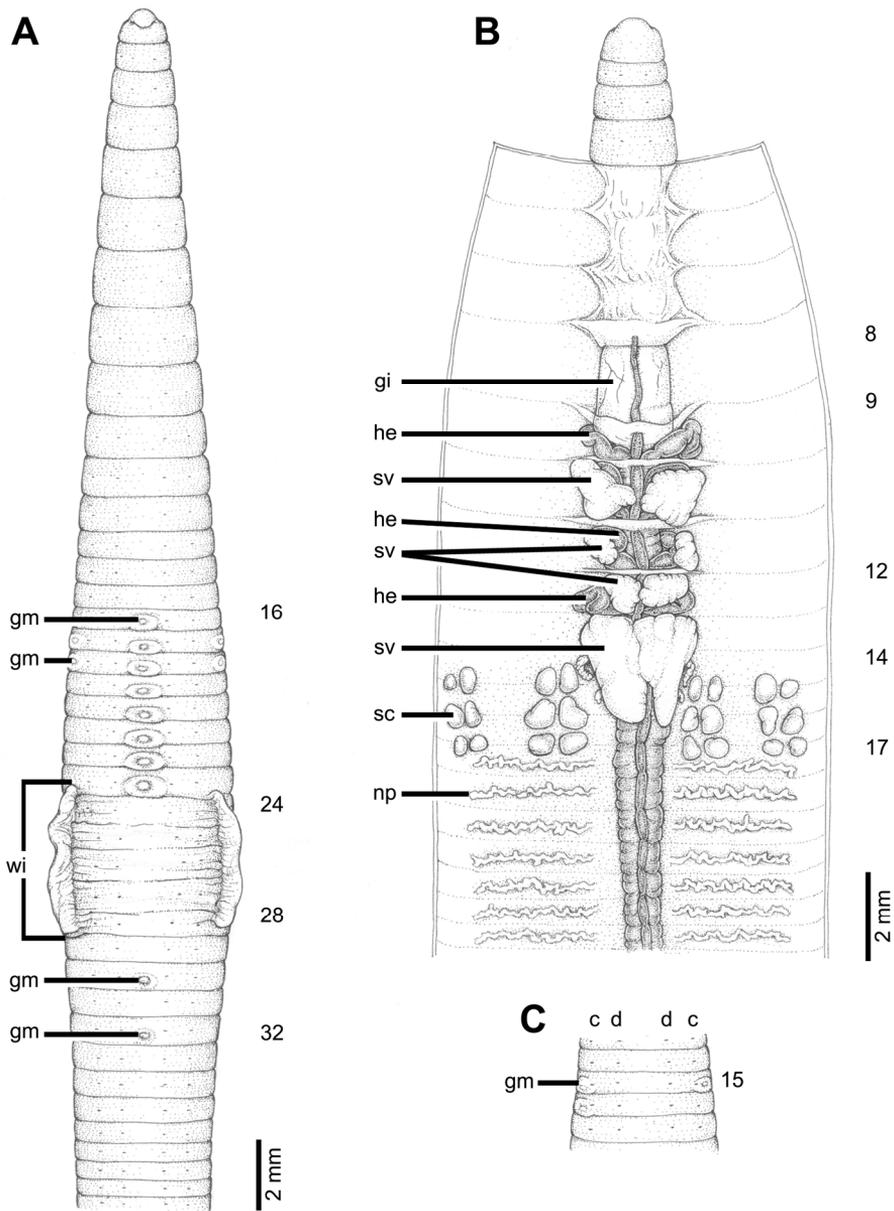


FIGURE 5. External and internal morphology of holotype (CUMZ 3315) of *Glyphidrilus kedahensis*, n. sp. (A) external ventral view; (B) internal dorsal view.

seminal vesicles in 10–13. Intestinal origin in 16. The ovaries in 13, 14. Prostate and accessory glands absent. Spermathecae in 14/15–16/17.

Remarks. — *Glyphidrilus kedahensis* n. sp. similar to *G. horsti*, *G. trangensis* and *G. kratuensis* in the location of wings. But *G. horsti* from Pulau Berhala, Straits of Malacca has smaller body, shorter clitellum

in 17, 18, $\frac{1}{2}$ 18, 19–29, 30, $\frac{1}{2}$ 31, and two pairs of spermathecae in 14–17, 14/15–17/18. *Glyphidrilus trangensis* from Trang River, Nayong, Trang, Thailand has smaller body sized with shorter clitellum in 17, 18–30, and two pairs of spermathecae in 18–21. *Glyphidrilus kratuensis* from Kratu waterfall, Kratu, Phuket, Thailand has smaller body sized with shorter clitellum in 18–30, 31, 32, and two pairs of spermathecae in 14/15–17/18. Differs from *G. kuekenthali* from Borneo, Baram River, Sarawak by *G. kuekenthali* has wings in 18, $\frac{1}{2}$ 18, 19–24, $\frac{1}{2}$ 25, with a clitellum in $\frac{1}{2}$ 13–34 and spermathecae in 14–18. Differs from *G. bisegmentus* from Air Banun Pandig, Perak, Malaysia by the latter has wings in 18–19, clitellum in 16, 17–23, 24, and lacks spermathecae. Differs from *G. gatesi* from Sungei Kayu, swamp forest near River Sedili, Johor, Malaysia by the latter has wings in 18, $\frac{1}{2}$ 19, 19– $\frac{1}{2}$ 24, 24, shorter clitellum in $\frac{1}{2}$ 17, 17, 18–25, $\frac{1}{2}$ 26, 26, and spermathecae in 15–17. Differs from *G. kotatinggi* from Kota Tinggi waterfall, Johor, Malaysia by the latter has wings in 20, 21– $\frac{1}{4}$ 26, 26, clitellum in 17, 18–28, 29, 30, and spermathecae in 13/14–16/17. Differs from *G. peninsularis* from Sungei Bantang, Johor, Malaysia by the latter has wings in 22, 23–28, $\frac{1}{2}$ 29, 29, shorter clitellum in 17, 18–31, 32, and spermathecae in 14/15–17/18. Differs from *G. perakensis* n. sp. from Sungei Kurau near Klinik Desa, Perak, Malaysia by the latter has wings in 26, 27–30, 31, clitellum in 20–36, 37, hearts in 10–14, and spermathecae in 16/17–18/19.

DISCUSSION

Glyphidrilus are recorded only in Africa and Asia. The preferred habitats are the

topsoils at the edge of aquatic freshwater habitats in various soil types, with neutral pH to slightly basic condition (Chanabun et al., 2013). The worms produce casts on the soil surfaces, which quite abundant on the shores. The earthworms treated herein were all found at the depth of 5–15 cm from the surface in wet top soil and normally locate near the casts.

The descriptions of the two new species based on specimens collected from diverse habitats. *Glyphidrilus perakensis* n. sp. was found on the shore of Sungei Kurau near Klinik Desa, Perak near agricultural area. The habitat is sandy clay covering with thick leaf litters. *Glyphidrilus kedahensis* n. sp., was found in Sungei in Sedim Nature Park, or Hutan Lipur Sungei Sedim, the recreational forest which locates at about 30 km northeast of Kulim, Kedah. The loamy sand habitat is in the 950 m canopy walk of the Sedim River Recreation Park. *G. kedahensis* n. sp. is also found along the shore of Ulu Legong Hot spring Recreational Park, the silt loam habitat which some parts have been modified for agricultural purpose.

ACKNOWLEDGEMENTS

This project was partly funded by Higher Education Research Promotion (HERP; Project Code 2558A15562001), while most of the financial support was received from The Thailand Research Fund, The TRF Senior Research Scholar RTA 5880002 (2015–2018) to SP. We are grateful to Professor Peter K.L. Ng, Dr. Daren Yeo and Dr. Kelvin Tan (ZRC, Singapore), and to Ms. Emma Sherlock (NHM, London), and Dr. Andreas Schmidt-Rhaesa (ZMH, Germany) for kindly permitting us to study the type specimens and relevant reference

material. Thanks also to Ms. Thita Krutchuen for excellent drawings, and to all members of the Animal Systematics Research Unit, Chulalongkorn University for assistance in collecting material.

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