



## Morphological and molecular evidence reveal a new species of the earthworm genus *Pontodrilus* Perrier, 1874 (Clitellata, Megascolecidae) from Thailand and Peninsular Malaysia

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### Abstract

A new species of the megascolecoid earthworm genus *Pontodrilus* Perrier, 1874, *Pontodrilus longissimus* sp. n., is described from seashores of Thailand and Peninsular Malaysia. The new species differs from congeners, especially the cosmopolitan *P. littoralis* (Grube, 1855) in the size of the body, number of segments and the shape of the spermathecae. *P. littoralis* is redescribed, based on specimens collected from the same region and the same type of habitat. DNA fragments of the mitochondrial cytochrome oxidase subunit I of both species were sequenced. Morphological as well as DNA sequence-based comparisons confirm that *P. longissimus* sp. n. is a lineage distinct from *P. littoralis* and in fact a new species. The illustrated descriptions are accompanied by a key to species of *Pontodrilus*.

**Key words:** littoral earthworm, systematics, taxonomy, DNA barcode

### Introduction

In Thailand, the oligochaete fauna exhibits a high species diversity, especially in terrestrial earthworms that belong to *Amyntas* Kinberg, 1867 and *Metaphire* Sims & Easton, 1972, genera within the Megascolecidae family (Gates 1972; Somniam & Suwanwaree 2009; Bantaowong *et al.* 2014, 2015, 2016). In addition, fifteen species of semi-aquatic freshwater earthworm species in the genus *Glyphidrilus* Horst, 1889 have been reported (Chanabun *et al.* 2012a, b, 2013, 2017). However, marine littoral earthworms have received no attention in Thailand until Panha *et al.* (2007) reported the first occurrence of the littoral earthworm *Pontodrilus littoralis* (Grube, 1855) in Thailand from Khanom, Nakhon Si Thammarat. This discovery supported the notion that *P. littoralis* is widely distributed in tropical coastal ecosystems (Gates 1972; Blakemore 2002). There are numerous taxonomic studies and records of *P. littoralis* from Asia, namely from the Indian subcontinent (Stephenson 1915a; Aiyer 1929; Gobi *et al.* 2004; Satheeshkumar *et al.* 2011; Narayanan *et al.* 2014), Myanmar (Gates 1972), China including Taiwan (Chen & Zhifang 1977; Shen *et al.* 2005) and Vietnam (Nguyen *et al.* 2016). *Pontodrilus littoralis* occurs in sandy habitats with a high content of organic matter and salt, and has been recorded from sandy beaches, salty mud margins of estuaries, brackish lakes, mangrove swamps and under seaweed (Gates 1972; Blakemore 2002; Gobi *et al.* 2004).

The genus *Pontodrilus* Perrier, 1874 is an earthworm taxon that primarily inhabits marine littoral ecosystems. This genus was diagnosed by the absence of holoic nephridia from the anterior segments and the distinct tubular prostatic duct structure that opens to the male pore on XVIII (Gates 1972; Easton 1984). At present, *Pontodrilus* contains five species worldwide, which are from both terrestrial and aquatic habitats. They are *P. lacustris*

(Benham, 1903) from Lake Wakatipu of New Zealand; the terrestrial species *P. agnesae* Stephenson, 1915 (Stephenson 1915b) from Horton Plains near Adam's Peak and Elk Plains, Sri Lanka; *P. sinensis* Chen & Zhifang, 1977 from Yunnan in China, which is either terrestrial or lacustrine; the littoral species *P. primoris* Blakemore, 2000 from a beach of Tasmania, Australia, and the cosmopolitan species *P. littoralis* (see Blakemore 2007). *Pontodrilus lacustris* is distinguished from *P. littoralis* by four pairs of spermathecal and penial setae, *P. agnesae* by the presence of penial setae; *P. sinensis* by the presence of four pairs of seminal vesicles in IX–XII (Easton 1984); and *P. primoris* by the short spermathecal diverticula relative to ampullae (Blakemore 2007).

DNA barcoding of the standardized region of 658 bp of the mitochondrial cytochrome *c* oxidase I (COI) gene is a powerful tool for identifying species of earthworms (Chang & James 2011; Jeratthitikul *et al.* 2017). DNA barcode analysis can sustain decisions in alpha taxonomy, including synonymies or resurrections of species names, and descriptions of new taxa (James *et al.* 2010; Decaëns *et al.* 2013); it can furthermore reveal the diversity of cryptic species (Novo *et al.* 2010; Richard *et al.* 2010). In this study, we re-describe the previously known cosmopolitan littoral earthworm species *P. littoralis* and we describe a new species of the genus *Pontodrilus*. Both species were collected from the same habitat, seashores of Thailand and Peninsular Malaysia. Specimens were compared morphologically and at the DNA level, using COI sequences. The illustrated descriptions and an accompanying key to species of *Pontodrilus* serve as an identification tool for taxonomists.

## Materials and methods

**Specimen collection and morphological examination.** Earthworms were collected throughout the coastal areas of Thailand, both the Gulf of Thailand (east coast) and the Andaman Sea (west coast), and also some parts of Peninsular Malaysia (Fig. 1) from January 2015 to February 2016. The specimens were carefully dug up along the sandy beaches at the level of tide using hand sorting. Adults, juveniles and cocoons were collected. The GPS coordinates of the sampling locality were recorded and the habitat type photographed. The worms were cleaned and then killed in 30% (v/v) ethanol, photographed, and fixed in 95% (v/v) ethanol for morphological and molecular studies. The descriptions of each species were made during observation under an OLYMPUS SZX16 stereomicroscope. Drawings were made of the external characters and internal organs. The specimens have been deposited in the Zoological Museum of Chulalongkorn University (CUMZ), The Natural History Museum, London, UK (NHMUK) and the Biozentrum Grindel und Zoologisches Museum, University of Hamburg, Germany (ZMH).

**DNA extraction, PCR and DNA sequencing.** Details of the specimens selected for molecular analysis are provided in Table 1. The genomic DNA was extracted from the integument tissue of the posterior part of the earthworms using a Geneaid™ DNA extraction kit. DNA was eluted in elution buffer and kept at -20 °C until use. For the phylogenetic analysis, a 658 bp region of the COI mitochondrial gene was used following its PCR amplification and sequencing. Each PCR amplification mixture consisted of 0.6–1 µl of DNA template, 2.5 µl (5 µM) each of the LCO1490 (forward) and HCO2198 (reverse) universal primers (Folmer *et al.* 1994), 25 µl of Ultra-Pure *Taq* PCR Master Mix with emerald dye and 19–19.4 µl of double distilled H<sub>2</sub>O. The PCR reaction mixture was heated to 94 °C for 2 min, followed by 35 cycles of 94 °C for 1 min, 48 °C for 1 min and 72 °C for 2 min, and then followed by a final extension at 72 °C for 5 min. The amplified PCR products were checked on 1% (w/v) agarose gel electrophoresis in 0.5x TBE buffer and detected with SYBR safe DNA gel staining under UV transillumination. PCR products were purified using QIAquick purification kit (QIAGEN Inc.). Purified PCR products were sent for commercial sequencing using an Applied Biosystems Automatic Sequencer (ABI 3730XL) at Macrogen, Inc. (Korea).

**Molecular analyses.** The COI sequences were aligned with MEGA version 6 (Tamura *et al.* 2013) and adjusted manually. The sequences were checked with NCBI database using the BLASTn algorithm to avoid contamination (<http://www.ncbi.nlm.nih.gov>). The phylogenetic analyses were performed using maximum likelihood (ML) and Bayesian inference (BI). The ML trees were inferred with RAxML-HPC2 on XSEDE v.8.2.4 (Stamatakis 2014) through the CIPRES Science Gateway (Miller *et al.* 2010), using 1000 bootstrap replicates to assess branch support. The BI tree was constructed by MrBayes v 3.2.2 (Ronquist *et al.* 2012) in the CIPRES Science Gateway. The analysis was run for 2 million generations (default heating parameter), and sampled every 100 generations. Then 50% of the sampled trees were discarded as a burn-in and support for nodes was defined as

posterior probabilities. Moreover, a distance-based analysis was conducted in MEGA6 using the Neighbor-Joining (NJ) algorithm with distances corrected with the Kimura-2 parameter model and a bootstrap re-analysis of 1000 pseudoreplicates. In this study, *Metaphire peguana* (CUMZ 3297) and *Metaphire bahli* (CUMZ 3298) were used as outgroups (Prasankok *et al.* 2013).

**Anatomical abbreviations.** The following abbreviations used in the figures are as appeared in Bantaowong *et al.* (2016) and Chanabun *et al.* (2017): **mp**, male pores; **fp**, female pores; **gm**, genital markings; **he**, hearts; **np**, nephridia; **sp**, spermathecal pores; **sc**, spermathecae; **sv**, seminal vesicles; **pg**, prostate glands.

**TABLE 1.** Specimens used in the molecular analysis with sampling localities, GenBank accession numbers, and abbreviations using in the molecular analysis (comp. Fig. 6), including outgroups.

Abbr.	Species	Collection locality	GenBank ID (COI)
E1	<i>P. litoralis</i>	Lung Chalerm Bungalow Koh Chang, Koh Chang, Trat, Thailand	MF488722
E2	<i>P. longissimus</i> <b>sp. n.</b>	Hat Sai Ngoen, Klong Yai, Trat, Thailand	MF488723
E3	<i>P. longissimus</i> <b>sp. n.</b>	Hat Chao Lao, Thamai, Chanthaburi, Thailand	MF488724
E4	<i>P. litoralis</i>	Hat Jomtien, Pattaya, Chonburi, Thailand	MF488725
E5	<i>P. litoralis</i>	Ao Talkoo, Laem Ngob, Trat, Thailand	MF488726
S1	<i>P. litoralis</i>	Hat Cha-am, Cha-am, Phetchaburi, Thailand	MF488727
S2	<i>P. longissimus</i> <b>sp. n.</b>	Klong Bang Siap, Patiew, Chumphon, Thailand	MF488728
S3	<i>P. litoralis</i>	Maejo University at Chumphon, Lamae, Chumphon, Thailand	MF488729
S5	<i>P. litoralis</i>	Hat Kao Seng, Mueang, Songkhla, Thailand	MF488730
S7	<i>P. litoralis</i>	Hat Bo Chet Look, La Ngu, Satun, Thailand	MF488731
S8	<i>P. longissimus</i> <b>sp. n.</b>	Hat Pak Meng, Sikao, Trang, Thailand ( <b>Holotype</b> )	MF488732
S9	<i>P. longissimus</i> <b>sp. n.</b>	Hat Mai Khao, Talang, Phuket, Thailand	MF488733
S10	<i>P. longissimus</i> <b>sp. n.</b>	Hat Koey, Kaper, Ranong, Thailand	MF488734
S11	<i>P. litoralis</i>	Hat Koey, Kaper, Ranong, Thailand	MF488735
M1	<i>P. litoralis</i>	Tanjung Bungah, Pulau Pinang, Malaysia	MF488736
M2	<i>P. litoralis</i>	Tanjung Dawai, Merbok, Kedah, Malaysia	MF488737
M3	<i>P. litoralis</i>	Jalan Klebang, Besar Dataran 1, Melaka, Malaysia	MF488738
M4	<i>P. litoralis</i>	Kampung Pasir Putih, Pasir Gudang, Johor, Malaysia	MF488739
M5	<i>P. longissimus</i> <b>sp. n.</b>	Pantai Chendor, Cukai, Terengganu, Malaysia	MF488740
M6	<i>P. litoralis</i>	Pantai Chendor, Cukai, Terengganu, Malaysia	MF488741
M7	<i>P. longissimus</i> <b>sp. n.</b>	Pantai Melawi, Sungai Ger Bachok, Kelantan, Malaysia	MF488742
M8	<i>P. litoralis</i>	Pantai Melawi, Sungai Ger Bachok, Kelantan, Malaysia	MF488743
	<i>Metaphire peguana</i>	Outgroup specimen	KC404843.1
	<i>Metaphire bahli</i>	Outgroup specimen	KC404844.1

## Results

### Systematics

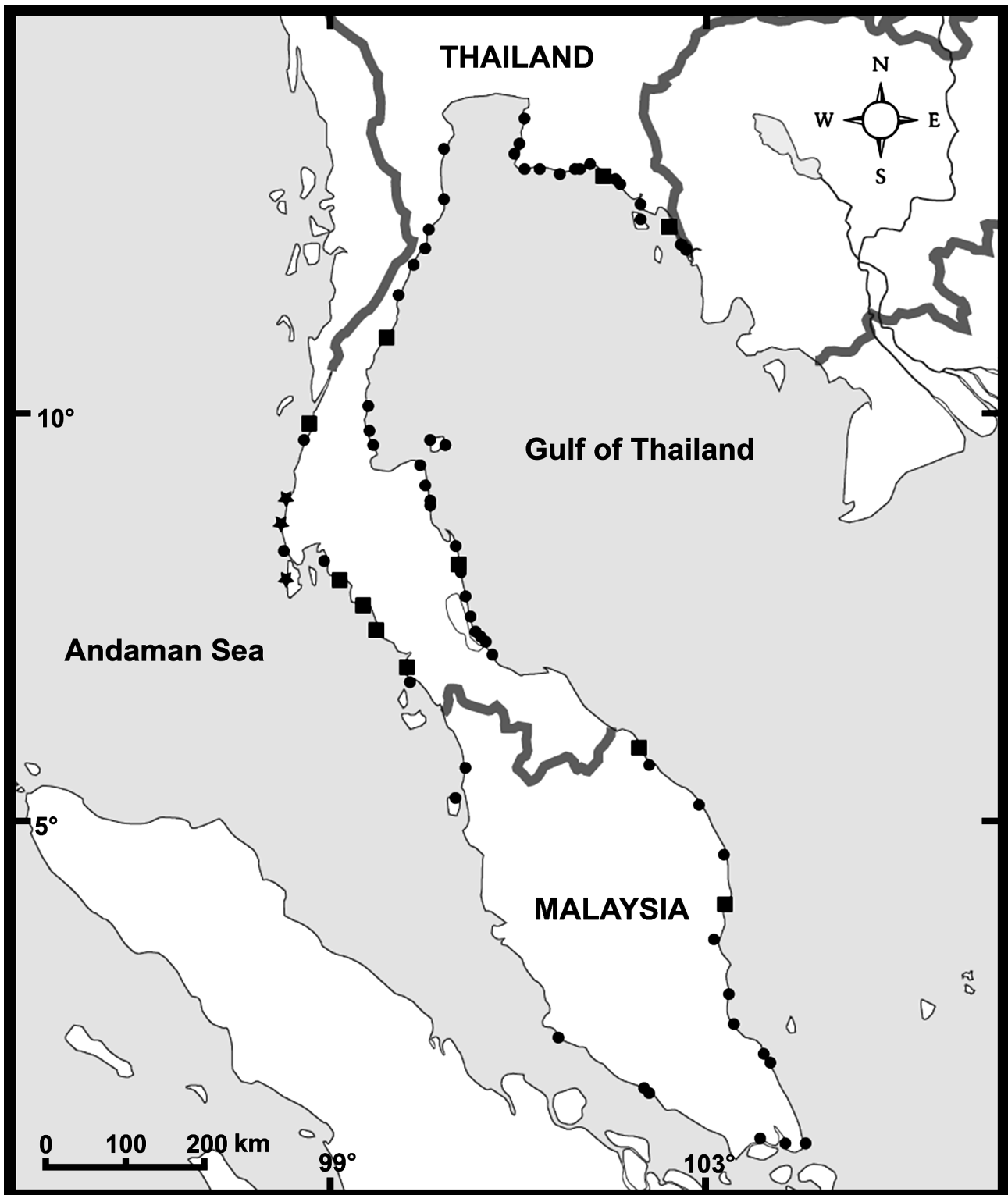
#### Family Megascolecidae Rosa, 1891

#### Genus *Pontodrilus* Perrier, 1874

**Type species.** *Lumbricus litoralis* Grube, 1855

**Diagnosis.** Dorsal pores absent. Tubular prostatic duct structure to male pore on segment XVIII. Female pores in XIV. Setae lumbricine. Nephridia absent in the anterior segments. Gizzard absent; calciferous glands absent; typhlosole absent. Penial setae present or absent. Spermathecae 2–4 pairs.

**Distribution.** One cosmopolitan species, *P. littoralis*, occurs over a very wide range of sub-temperate and tropical coastal areas all over the world. Another littoral species, *P. primoris* in Tasmania; two species, *P. agnesae*, and *P. sinensis*, found inland in Sri Lanka and China, respectively, and the lacustrine species *P. lacustris* in Lake Wakatipu, New Zealand.



**FIGURE 1.** Map showing the localities of sampling sites for *Pontodrilus*. Circle and star symbols represent the localities of *Pontodrilus littoralis* and *Pontodrilus longissimus* sp. n., respectively, while square symbols are the localities where both *Pontodrilus* species were found.

## ***Pontodrilus litoralis* (Grube, 1855)**

(Figures 2, 3, 7B; Tables 2, 3)

*Lumbricus litoralis* Grube, 1855: 127–129, pl. 5, figs 5–10.

*Pontoscolex arenicola* Schmarda, 1861: 11–12, pl. 18, fig. 157.

*Pontodrilus marionis* Perrier, 1874: 1582–1586. Perrier 1881: 176–179, pls 13–17, pl. 18; figs 40–43.

*Pontodrilus bermudensis* Beddard, 1891: 96. Michaelsen 1894: 183–184. Beddard 1895: 469–471. Michaelsen 1910: 84–89. Michaelsen 1913: 417. Michaelsen 1921: 12. Stephenson 1923: 180–182. Gates 1926: 150. Stephenson 1931: 51. Gates 1942: 90. Gates 1943: 99–100. Gates 1954: 240–241. Gates 1972: 47–48. Jamieson & Wampler 1979: 665–666. Sathianarayanan & Khan 2006: 139–144.

*Cryptodrilus insularis* Rosa, 1891: 387–388, pl. 14, fig. 11.

*Pontodrilus arenae* Michaelsen, 1892: 222–223, pl. 13, fig. 9. Michaelsen 1900: 181. Michaelsen 1903: 87.

*Pontodrilus hesperidum* Beddard, 1894: 37–40. Beddard 1895: 471. Michaelsen 1900: 182. Michaelsen 1903: 87.

*Pontodrilus litoralis* (Grube). Beddard 1895: 469. Michaelsen 1900: 180. Cognetti 1901: 16. Michaelsen 1903: 87. Michaelsen 1910: 89–91. Easton 1984: 114–116. Jamieson 2001: 1002–1007, figs 34.1–34.2. Gobi *et al.* 2004: 1712, fig. 1. James *et al.* 2005: 1022–1023. Shen *et al.* 2005: 12–13, fig. 1. Blakemore 2007: S3–S8, figs 1–3. Csuzdi & Pavlicek 2009: 12. Satheeshkumar *et al.* 2011: 406–409, fig. 1. Blakemore *et al.* 2012: 302. Narayanan *et al.* 2014: 473–476, table 1. Oba *et al.* 2015: 1–10, figs 1–2, table 1. Hara *et al.* 2016: 5–7, fig. 1. Nguyen *et al.* 2016: 10–11.

*Pontodrilus insularis* (Rosa). Beddard 1895: 471–472. Michaelsen 1897: 173–174. Michaelsen 1900: 181–182. Michaelsen 1903: 87.

*Pontodrilus michaelseni* Eisen, 1895: 73–84, pl. 33, figs 24–28; pls 34–39; pl. 40, figs 74–76. Michaelsen 1900: 182. Michaelsen 1903: 87.

*Pontodrilus ephippiger* Rosa, 1898: 281–283, pl. 9, figs 4–5. Michaelsen 1900: 180–181. Michaelsen 1903: 87. Michaelsen 1907a: 187. Michaelsen 1907b: 43. Cognetti 1908: 81–82. Stephenson 1914: 256–259.

*Pontodrilus matsushimensis* Iizuka, 1898: 21–26, pl. 2, figs 1–6. Beddard 1899: 192–193. Michaelsen 1900: 179–180. Michaelsen 1903: 87. Michaelsen 1910: 91–92. Easton 1981: 45–46.

*Pontodrilus ephippiger laysanianus* Michaelsen, 1899a: 217–220. Michaelsen 1899b: 28–29, fig. 2. Michaelsen 1900: 181.

*Pontodrilus matsushimensis chathamianus* Michaelsen, 1899a: 220–221. Michaelsen 1900: 180. Michaelsen 1910: 91–92.

*Pontodrilus michaelseni hortensis* Eisen, 1900: 241–243.

*Pontodrilus chathamensis* (*sic!* pro *chathamianus*) Michaelsen. Benham 1901: 136–140. Lee 1952: 25.

*Pontodrilus chathamianus* Michaelsen. Michaelsen 1903: 87.

*Pontodrilus laccadivensis* Beddard, 1903: 374–375.

*Pontodrilus crosslandi* Beddard, 1906: 561, fig. 78.

*Pontodrilus albanyensis* Michaelsen, 1907a: 185–187, fig. 12, pl. 2, fig. 26. Jackson 1931: 93.

*Pontodrilus bermudensis ephippiger* Rosa. Stephenson 1915a: 145. Stephenson 1915b: 61. Stephenson 1916: 311. Stephenson 1917: 375. Stephenson 1920: 202. Jackson 1931: 95–96, pl. 14, fig. 6.

*Pontodrilus albanyensis* var. *cygni* Jackson, 1931: 94–95, pl. 14, figs 2, 3, 4, 9.

*Plutellus* (*Pontodrilus*) *matsushimensis indica* Michaelsen, 1935: 106–107.

*Pontodrilus gracilis* Gates, 1943: 100–102. Gates 1954: 241.

*Pontodrilus hesperidium* (*sic!* pro *hesperidum*) Beddard. Easton 1984: 114. Jamieson 2001: 1003.

**Type locality.** Villa Franca on the French Riviera (Grube 1855).

**Materials examined. Thailand.** 112 adults and 51 juveniles, CUMZ 3462, Ao Talkoo, Laem Ngob, Trat (12° 12' 14.4" N, 102° 16' 47.1" E) on 7 August 2015. 21 adults and 56 juveniles, CUMZ 3463, Hat Laem Sing, Laem Sing, Chanthaburi (12° 28' 48.5" N, 102° 03' 46.7" E) on 7 August 2015. 8 adults and 14 juveniles, CUMZ 3464, Hat Chao Lao, Thamai, Chanthaburi (12° 33' 52.8" N, 101° 54' 25.1" E) on 8 August 2015. 38 adults and 48 juveniles, CUMZ 3465, Hat Mae Rumphueng, Ban Phe, Rayong (12° 36' 12.6" N, 101° 23' 52.2" E) on 8 August 2015. 38 adults and 8 juveniles, CUMZ 3466, Hat Nam Rin, Ban Chang, Rayong (12° 40' 31.1" N, 101° 05' 03.4" E) on 9 August 2015. 26 adults and 49 juveniles, CUMZ 3467, Ao Noi, Mueang, Prachuap Khiri Khan (11° 49' 10.7" N, 99° 47' 58.1" E) on 29 August 2015. 53 adults and 34 juveniles, CUMZ 3468, Maejo University at Chumphon, Lamae, Chumphon (9° 46' 48.8" N, 99° 08' 27.2" E) on 29 August 2015. 1 adult and 7 juveniles, CUMZ 3469, Hat Pak Meng, Sikao, Trang (7° 30' 14.3" N, 99° 19' 6.8" E) on 30 August 2015. 89 adults and 30 juveniles, CUMZ 3470, Ao Noon, La Ngu, Satun (6° 50' 16.0" N, 99° 45' 37.2" E) on 30 August 2015. 131 juveniles, CUMZ 3471, Khrua Suan Son Restaurant, Pak Phanang, Nakhon Si Thammarat (8° 10' 51.8" N, 100° 17' 30.4" E) on 1 September 2015. 1 adult and 18 juveniles, CUMZ 3472, Ban Koh Kaew Naruemit, Pak Phanang, Nakhon Si Thammarat (8° 14' 28.1" N, 100° 16' 41.2" E) on 3 September 2015. 1 adult and 29 juveniles, CUMZ 3473, Laem Talumphuk, Pak Phanang, Nakhon Si Thammarat (8° 19' 19.7" N, 100° 15' 19.9" E) on 3 September 2015. 7 adults and 60 juveniles, CUMZ 3474, Pak Mae Nam Tapa, Sichon, Nakhon Si Thammarat (8° 55' 51.2" N,

99° 54' 47.5" E) on 3 September 2015. 45 juveniles, CUMZ 3475, Klong Bang Siap, Patiew, Chumphon (10° 39' 29.9" N, 99° 18' 39.1" E) on 4 September 2015. 16 adults and 23 juveniles, CUMZ 3476, Hat Nang Kam, Don Sak, Surat Thani (9° 18' 53.7" N, 99° 45' 37.9" E) on 2 December 2015. 170 adults and 32 juveniles, CUMZ 3477, Hat Lamai, Koh Samui, Surat Thani (9° 28' 16.3" N, 100° 03' 10.3" E) on 3 December 2015. 42 adults and 16 juveniles, CUMZ 3478, Koh Samui Hospital, Koh Samui, Surat Thani (9° 31' 17.3" N, 99° 56' 08.5" E) on 3 December 2015. 24 adults and 9 juveniles, CUMZ 3479, Hat Sai Ngoen, Klong Yai, Trat (12° 02' 43.5" N, 102° 45' 01.8" E) on 15 December 2015. 16 adults and 36 juveniles, CUMZ 3480, Hat Ban Chuen, Klong Yai, Trat (11° 52' 56.9" N, 102° 47' 58.7" E) on 15 December 2015. 41 adults and 1 juvenile, CUMZ 3481, Wat Huang Som, Klong Yai, Trat (11° 50' 47.4" N, 102° 49' 31.9" E) on 15 December 2015. 86 adults and 29 juveniles, CUMZ 3482, Lung Chalerm Bungalow Koh Chang, Koh Chang, Trat (12° 05' 52.4" N, 102° 21' 27.9" E) on 16 December 2015. 81 adults and 39 juveniles, CUMZ 3483, Laem Sing, Laem Sing, Chanthaburi (12° 30' 59.0" N, 102° 01' 37.7" E) on 17 December 2015. 25 adults and 13 juveniles, CUMZ 3484, Laem Mae Nok Kaew, Na Yai Arm, Chanthaburi (12° 40' 46.7" N, 101° 48' 41.2" E) on 17 December 2015. 38 adults and 5 juveniles, CUMZ 3485, Wat Tanon Kaprao, Klaeng, Rayong (12° 41' 06.1" N, 101° 40' 27.2" E) on 17 December 2015. 6 adults and 6 juveniles, CUMZ 3486, Laem Mae Pim, Klaeng, Rayong (12° 38' 37.6" N, 101° 38' 05.1" E) on 17 December 2015. 23 adults and 4 juveniles, CUMZ 3487, Ao Dong Tai, Sattahip, Chonburi (12° 38' 46.6" N, 100° 55' 47.4" E) on 18 December 2015. 80 adults and 19 juveniles, CUMZ 3488, Hat Jomtien, Pattaya, Chonburi (12° 50' 25.1" N, 100° 54' 18.3" E) on 18 December 2015. 42 adults and 6 juveniles, CUMZ 3489, Hat Na Klua, Pattaya, Chonburi (12° 58' 08.0" N, 100° 54' 04.4" E) on 18 December 2015. 53 adults and 5 juveniles, CUMZ 3490, Hat Bang Saen, Mueang, Chonburi (13° 15' 10.9" N, 100° 55' 45.4" E) on 18 December 2015. 28 adults and 21 juveniles, CUMZ 3491, Bo Nok, Kui Buri, Prachuap Khiri Khan (11° 59' 47.8" N, 99° 51' 50.2" E) on 9 January 2016. 22 adults and 7 juveniles, CUMZ 3492, Hat Sang Arun, Tub Sakae, Prachuap Khiri Khan (11° 34' 10.9" N, 99° 39' 48.7" E) on 9 January 2016. 34 adults and 28 juveniles, CUMZ 3493, Ban Ma Phaw Resort, Bang Saphan Noi, Prachuap Khiri Khan (11° 08' 08.1" N, 99° 29' 11.9" E) on 9 January 2016. 10 adults and 5 juveniles, CUMZ 3494, Hat Koey, Kaper, Ranong (9° 37' 26.7" N, 98° 28' 08.6" E) on 10 January 2016. 16 adults, CUMZ 3495, Hat Manee Naka, Kaper, Ranong (9° 28' 40.4" N, 98° 26' 02.8" E) on 10 January 2016. 2 adults, CUMZ 3496, Wat Tet Tum Nava, Thai Muang, Phangnga (8° 21' 21.9" N, 98° 15' 29.9" E) on 10 January 2016. 36 adults and 4 juveniles, CUMZ 3623, Ao Thaleng, Mueang, Krabi (8° 09' 58.0" N, 98° 44' 53.0" E) on 11 January 2016. 8 juveniles, CUMZ 3624, Hat Yao, Nuea Klong, Krabi (7° 58' 49.4" N, 98° 56' 46.6" E) on 11 January 2016. 10 juveniles, CUMZ 3625, Hat Samran, Hat Samran, Trang (7° 14' 02.9" N, 99° 32' 19.4" E) on 12 January 2016. 52 adults and 30 juveniles, CUMZ 3626, Hat Bo Chet Look, La Ngu, Satun (6° 53' 32.6" N, 99° 41' 12.5" E) on 12 January 2016. 20 adults and 10 juveniles, CUMZ 3627, Hat Kao Seng, Mueang, Songkhla (7° 10' 58.9" N, 100° 36' 59.5" E) on 12 January 2016. 17 adults and 3 juveniles, CUMZ 3628, Hat Tumbon Kao Roob Chang, Mueang, Songkhla (7° 09' 06.5" N, 100° 38' 25.0" E) on 12 January 2016. 1 adult and 61 juveniles, CUMZ 3629, Hat Na Tub, Jana, Songkhla (7° 04' 26.3" N, 100° 41' 34.6" E) on 12 January 2016. 19 adults and 51 juveniles, CUMZ 3630, Hat Sai Kaew, Singha Nakhon, Songkhla (7° 15' 13.1" N, 100° 32' 53.1" E) on 13 January 2016. 2 adults and 1 juvenile, CUMZ 3631, Hat Ma Ha Rat, Satingpra, Songkhla (7° 28' 10.1" N, 100° 26' 50.1" E) on 13 January 2016. 40 adults and 12 juveniles, CUMZ 3632, Wat Hua Rawa, Ranod, Songkhla (7° 43' 30.3" N, 100° 22' 55.4" E) on 13 January 2016. 1 adult, CUMZ 3633, Klong Loe, Tha Sala, Nakhon Si Thammarat (8° 45' 02.0" N, 99° 56' 13.4" E) on 14 January 2016. 119 adults and 81 juveniles, CUMZ 3634, Klong Klai, Tha Sala, Nakhon Si Thammarat (8° 46' 36.1" N, 99° 56' 06.4" E) on 14 January 2016. 67 adults and 11 juveniles, CUMZ 3635, Laem Sai, Chaiya, Surat Thani (9° 25' 26.2" N, 99° 17' 05.5" E) on 14 January 2016. 49 adults and 26 juveniles, CUMZ 3636, Klong Pak Nam Thamung, Tha Chana, Surat Thani (9° 33' 15.9" N, 99° 12' 39.2" E) on 14 January 2016. 63 adults and 22 juveniles, CUMZ 3637, Hat Sam Roi Yot, Sam Roi Yot, Prachuap Khiri Khan (12° 16' 50.6" N, 99° 58' 23.8" E) on 15 January 2016. 50 adults and 93 juveniles, CUMZ 3638, Hat Cha-am, Cha-am, Phetchaburi (12° 49' 36.2" N, 99° 59' 40.3" E) on 15 January 2016.

**Malaysia.** 5 adults and 54 juveniles, CUMZ 3639, Tanjung Rhu, Sepang, Selangor (2° 38' 07.2" N, 101° 37' 26.8" E) on 21 January 2016. 154 adults and 62 juveniles, CUMZ 3640, Jalan Klebang, Besar Dataran1, Melaka (2° 13' 13.7" N, 102° 11' 09.3" E) on 22 January 2016. 71 adults and 85 juveniles, CUMZ 3641, Tanjung Kling, Pantai Kundur, Melaka (2° 14' 54.9" N, 102° 08' 15.3" E) on 22 January 2016. 64 adults and 148 juveniles, CUMZ 3642, Kampung Pasir Putih, Pasir Gudang, Johor (1° 26' 16.8" N, 103° 55' 32.9" E) on 23 January 2016. 149 adults and 65 juveniles, CUMZ 3643, Highway Senai Desaru, Sungai Johor, Johor (1° 32' 02.6" N, 104° 01' 47.8" E) on

23 January 2016. 102 adults and 57 juveniles, CUMZ 3644, Tanjung Balau, Desaru, Johor (1° 36' 55.8" N, 104° 15' 26.1" E) on 23 January 2016. 59 adults and 22 juveniles, CUMZ 3645, Sungai Jemaluang, Mersing, Johor (2° 23' 03.5" N, 103° 52' 40.8" E) on 23 January 2016. 95 adults and 96 juveniles, CUMZ 3646, Kampung Air Puteri, Mersing, Johor (2° 24' 56.0" N, 103° 51' 32.5" E) on 24 January 2016. 33 adults and 42 juveniles, CUMZ 3647, Kampung Janglau, Pantai Kuala Rompin, Pahang (2° 43' 17.2" N, 103° 34' 24.9" E) on 24 January 2016. 20 juveniles, CUMZ 3648, Kampung Pandon, Pekan, Pahang (3° 05' 40.8" N, 103° 26' 26.2" E) on 24 January 2016. 83 adults and 10 juveniles, CUMZ 3649, Kampung Tanjung Lumpur, Kuantan, Pahang (3° 48' 25.0" N, 103° 20' 29.4" E) on 25 January 2016. 38 adults and 46 juveniles, CUMZ 3650, Pantai Chendor, Cukai, Terengganu (4° 10' 36.7" N, 103° 25' 15.6" E) on 25 January 2016. 16 adults and 7 juveniles, CUMZ 3651, Teluk Bidara, Sungai Dungun, Dungun, Terengganu (4° 47' 03.9" N, 103° 25' 39.0" E) on 25 January 2016. 79 adults and 22 juveniles, CUMZ 3652, Kampung Batin Seberang Takir, Kuala Terengganu, Terengganu (5° 20' 42.7" N, 103° 07' 40.7" E) on 26 January 2016. 52 adults and 4 juveniles, CUMZ 3653, Sungai Besut Kampung Nail, Besut, Terengganu (5° 49' 25.5" N, 102° 33' 24.7" E) on 26 January 2016. 82 adults and 26 juveniles, CUMZ 3654, Pantai Melawi, Sungai Ger Bachok, Kelantan (5° 59' 35.7" N, 102° 25' 54.2" E) on 26 January 2016. 49 adults and 27 juveniles, CUMZ 3655, Tanjung Dawai, Merbok, Kedah (5° 40' 47.4" N, 100° 22' 12.8" E) on 28 January 2016. 107 adults and 46 juveniles, CUMZ 3656, Tanjung Bungah, Pulau Pinang (5° 28' 06.7" N, 100° 16' 41.0" E) on 28 January 2016.

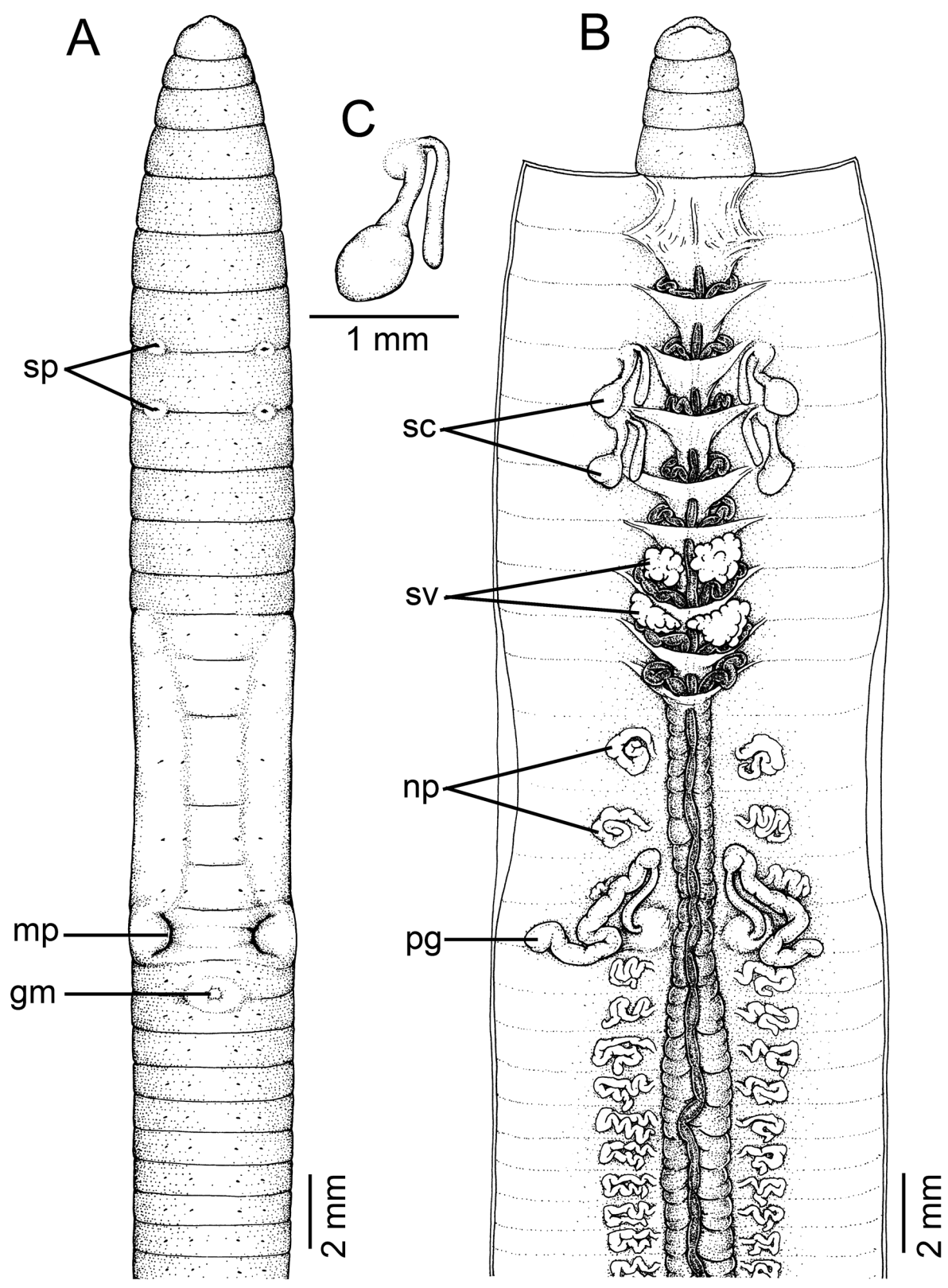
**Description of new material.** Length 28–136 mm, diameter 1–5 mm, body cylindrical, 76–128 segments. Body colour red to pink, pale brown around clitellum (newly collected specimens after placement in 30% (v/v) ethanol for narcotization). Prostomium epilobous. Clitellum saddle in XIII–XVII or XVIII, setae present. Setae lumbricine, ab absent on XVIII, aa > bc, ab < cd, aa > cd, dd > aa. Female pores paired in setal line b on XIV. Male pores minute and superficial on XVIII, close to sites of b setae, penial setae absent (or not found). Spermathecal pores minute, intersegmental, two pairs in 7/8 and 8/9, ventrolateral, in line with setae b. Dorsal pores totally absent. Genital markings large, medio-ventral, transversely oval across 19/20 (some in 20/21), center depressed.

Septa 5/6–12/13 thickened. Gizzard and calciferous glands absent. Intestine beginning in XVI or XVII. Intestinal typhlosole absent. Esophageal hearts seen in VI–XIII. Nephridia absent in the anterior segments and begin in XIII or XV with a pair of coiled tubules, smaller after clitellum. Spermathecae two pairs in VIII and IX, spermathecal diverticula slender, narrower at the junction with ampulla. Tubular prostate single pair, muscular duct curved and narrowed at each end, coil in XVIII. Ovaries present in XIII. Testes funnels in X and XI, seminal vesicles paired and large in XI–XII. Accessory glands absent.

**Distribution.** Worldwide, sub-tropical and tropical coastal areas. Easton (1984) and Blakemore (2002) provide distribution records; Figure 1 shows the locations of our finds of the species in Thailand and Peninsular Malaysia.

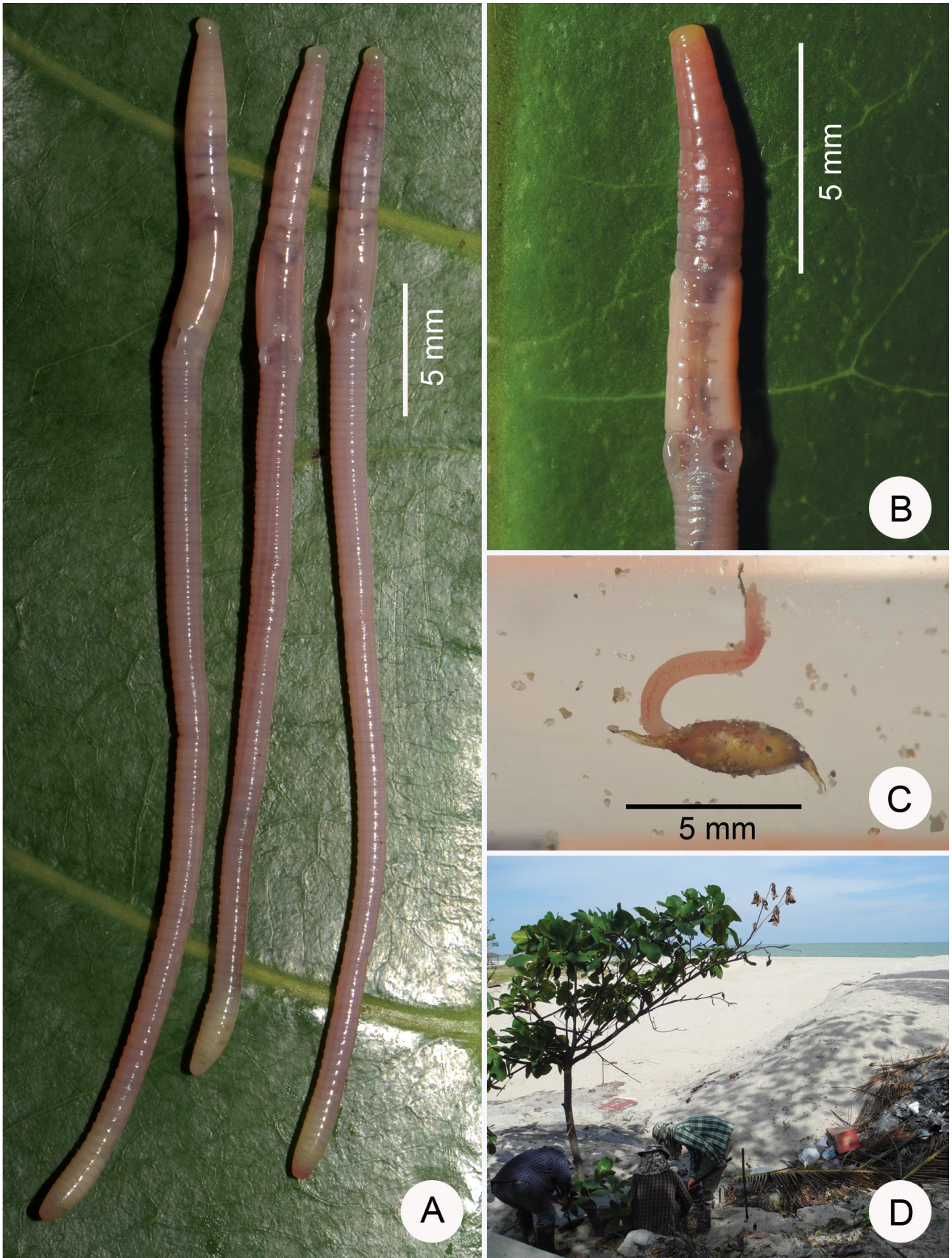
**Habitat.** Sandy beaches at the intertidal zone, mangrove swamps, coastal salt marsh of estuaries and brackish water. Found in the top soil to 20 cm depth where the salinity ranges from 1–33 ppt.

**Remarks.** *Pontodrilus litoralis* shows some variations in body dimensions, patterns of the genital markings, and shape of the spermathecal diverticula, especially if the descriptions of the many junior synonyms and redescriptions of the nominal species are taken into account. Characters from synonyms of *P. litoralis* in the Old World territory, including Australia, are presented in Table 2. Variations in body dimensions were reported by Jamieson (2001), Satheeshkumar *et al.* (2011), Shen *et al.* (2005) and Narayanan *et al.* (2014); the values are as follows: Jamieson (2001): length 32–120 mm, width 2–4 mm, segments 78–120; Satheeshkumar *et al.* (2011) and Narayanan *et al.* (2014): length 50–130 mm, clitellum width 2–4 mm, segments 81–115; Shen *et al.* (2005): length 50–130 mm, diameter 1–2 mm, segments 81–115. Considering genital markings, Jamieson (2001) notes the following variations, based on specimens from Peel Island, and including data of Jackson (1931), Gates (1972) and Easton (1984): genital markings in several furrows of 11/12–20/21. Specimens described by Jamieson (2001) from Nornalup, Western Australia, have markings at 17/18, at the anterior midventral margin of XVIII, at 18/19, or midventral at 18/19 and 19/20. Satheeshkumar *et al.* 2011; Narayanan *et al.* 2014 observed large genital markings medio-ventral at 19/20 in specimens from India; specimens of *P. litoralis* from coasts of the Penghu Island and the southwestern Taiwan have one large genital marking, transversely oval across 19/20, center depressed (Shen *et al.* 2005). Considering the diverticulum, Jamieson (2001) described it as digitiform to club-shaped, whereas in specimens from India and Taiwan it is slender. The specimens of *P. litoralis* investigated in this study are within the variation range as reported in the literature: body length 28–136 mm, diameter 1–5 mm, segments 76–128; genital markings large, medio-ventral, transversely oval across 19/20 (some in 20/21). We include drawings (Fig. 2) and colour plates (Fig. 3) of selected specimens.



**FIGURE 2.** External and internal morphology of *Pontodrilus litoralis* (CUMZ 3462) at Ao Talkoo, Laem Ngob, Trat; **A.** External ventral view; **B.** Internal dorsal view; **C.** Spermatheca.





**FIGURE 3.** Photographs showing: **A.** *Pontodrilus litoralis* (CUMZ 3462) just after the first step preservation in 30% (v/v) ethanol; **B.** Ventral view of anterior portion; **C.** Juvenile hatching from a cocoon; **D.** The microhabitat of *Pontodrilus litoralis* at Hat Cha-am, Cha-am, Phetchaburi, Thailand.

***Pontodrilus longissimus* Seesamut & Panha, sp. n.**

(Figures 4, 5, 7A; Table 3)

**Type specimens.** Holotype: CUMZ 3670 (Fig. 4), Hat Pak Meng, Sikao, Trang, Thailand (7° 30' 14.3" N, 99° 19' 06.8" E, 7 m above mean sea level), coll. T. Seesamut, C. Sutcharit, R. Srisonchai & A. Pholyotha, 30 Aug 2015. Paratypes: CUMZ 3671, 64 adults and 37 juveniles; NHMUK, 2 adults; ZMH, 2 adults; same collection data as holotype.

**Other materials examined. Thailand.** 23 adults and 57 juveniles, CUMZ 3657, Hat Chao Lao, Thamai, Chanthaburi (12° 33' 52.8" N, 101° 54' 25.1" E) on 8 August 2015. 4 juveniles, CUMZ 3658, Ban Koh Kaew Naruemit, Pak Phanang, Nakhon Si Thammarat (8° 14' 28.1" N, 100° 16' 41.2" E) on 3 September 2015. 17 juveniles, CUMZ 3659, Klong Bang Siap, Patiew, Chumphon (10° 39' 29.9" N, 99° 18' 39.1" E) on 3 September 2015. 33 adults and 67 juveniles, CUMZ 3660, Hat Mai Khao, Talang, Phuket (8° 05' 47.9" N, 98° 17' 55.5" E) on 5 December 2015. 30 adults and 40 juveniles, CUMZ 3661, Nang Thong Bay Resort, Takua Pa, Phangnga (8° 38' 43.4" N, 98° 14' 50.1" E) on 5 December 2015. 3 adults and 12 juveniles, CUMZ 3662, Hat Bang Sak, Takua Pa, Phangnga (8° 47' 03.4" N, 98° 15' 46.1" E) on 5 December 2015. 5 adults and 11 juveniles, CUMZ 3663, Hat Sai Ngoen, Klong Yai, Trat (12° 02' 43.5" N, 102° 45' 01.8" E) on 15 December 2015. 6 adults and 33 juveniles, CUMZ 3664, Hat Koey, Kaper, Ranong (9° 37' 26.7" N, 98° 28' 08.6" E) on 10 January 2016. 74 juveniles, CUMZ 3665, Hat Yao, Nuea Klong, Krabi (7° 58' 49.4" N, 98° 56' 46.6" E) on 11 January 2016. 20 juveniles, CUMZ 3666, Hat Samran, Hat Samran, Trang (7° 14' 02.9" N, 99° 32' 19.4" E) on 12 January 2016. 29 adults and 45 juveniles, CUMZ 3667, Hat Bo Chet Look, La Ngu, Satun (6° 53' 32.6" N, 99° 41' 12.5" E) on 12 January 2016.

**Malaysia.** 10 juveniles, CUMZ 3668, Pantai Chendor, Cukai, Terengganu (4° 10' 36.7" N, 103° 25' 15.6" E) on 25 January 2016. 1 juvenile, CUMZ 3669, Pantai Melawi, Sungai Ger Bachok, Kelantan (5° 59' 35.7" N, 102° 25' 54.2" E) on 26 January 2016.

**Description of holotype.** Length 161 mm, diameter 3.47 mm at segment X, 3.10 at segment XX, 3.90 mm at clitellum, body cylindrical with 183 segments. The body colour ranges from red to pink and pale brown around clitellum in newly collected specimens after placement in 30% (v/v) ethanol for narcotization. Prostomium epilobous. Clitellum saddle in XIII–XVII, setae present. Setae lumbricine, ab absent on XVIII, aa:ab:bc:cd:dd = 1.8:0.3:1.6:1.1:5.0 in XX. Female pores paired, medio-ventral in XIV. Male pores paired in XVIII, distance between male pores 2 mm, penial setae absent (or not found). Spermathecal pores intersegmental two pairs in 7/8 and 8/9, ventrolateral, in line with setae b, distance between spermathecal pores 2 mm. Dorsal pores absent. Genital markings present at intersegmental boundary in 17/18 and 18/19.

Septa 4/5–12/13 thickened. Gizzard and calciferous glands absent or not developed. Intestine enlarged from XV. Intestinal typhlosole absent. Esophageal hearts eight pairs in VI–XIII. No nephridia distinguishable in first fourteen segments, beginning in XV with a pair of coiled tubules. Two pairs of spermathecae in VIII and IX. Ampulla as large ovoid sac, without diverticulum. Two pairs of seminal vesicles in XI and XII. No mature (iridescent) sperm observed in spermathecae or seminal vesicles. Ovary in XIII. Tubular prostate single pair, muscular duct in XVIII. Accessory glands absent.

**Variation.** Holotype measures 161 mm body length with 183 segments. Body length of paratypes and non-types (adult specimens) range in size from 125–165 mm, with 160–191 segments. Clitellum in XIII, XIV–XVII. Intestine origin in XV–XVII. Nephridia from XIII or XV.

**Distribution.** The new species is known from the type locality and along the coastal areas of Thailand, both the Gulf of Thailand and the Andaman Sea, and also some parts of Peninsular Malaysia (Fig. 1).

**Habitat.** Found in the coastal salt marsh of estuaries at about 10–30 cm depth, in mud with a high content of organic matter and a salinity ranges from 1–33 ppt.

**Etymology.** The specific epithet "*longissimus*" in Latin means "the longest". This name refers to the length of this species compared to the other species in this genus.

**Diagnosis.** Length 125–165 mm, 160–191 segments. Setae lumbricine, penial setae absent. Prostomium epilobous. Dorsal pores totally absent. Clitellum saddle shape, in XIII, XIV–XVII. Male pores paired in XVIII; female pores paired in XIV. Genital markings present in 17/18 and 18/19. Spermathecae as large ovoid sacs in VIII and IX, without diverticulum. Two pairs of seminal vesicles in XI and XII. Ovary in XIII. Esophageal hearts eight pairs in VI–XIII. Tubular prostates in XVIII.



**TABLE 2.** Comparison of *Pontodrilus litoralis* from Thailand and Peninsular Malaysia with nominal species of *Pontodrilus* described from that area and now in synonymy with *P. litoralis*.

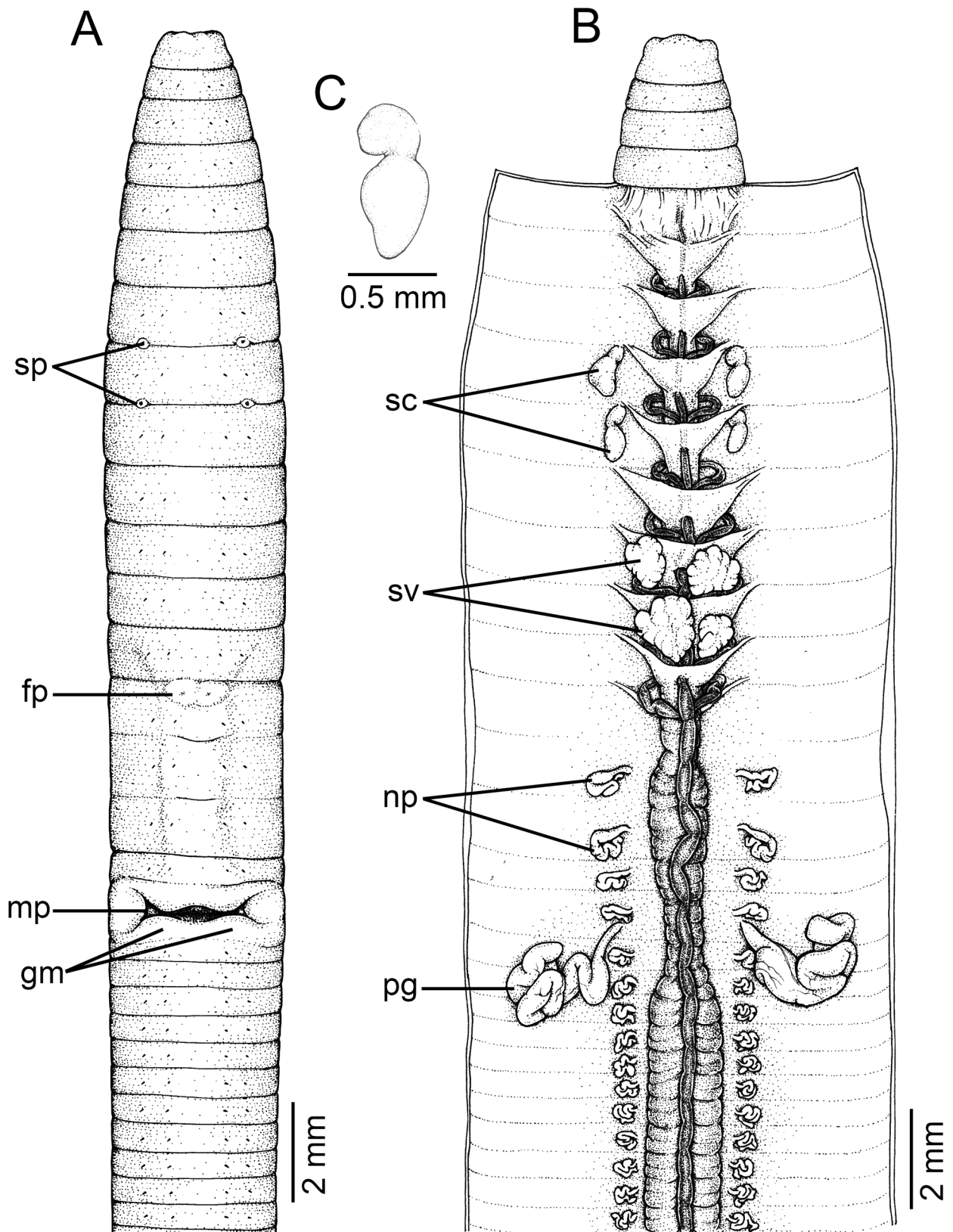
Character	<i>P. litoralis</i> (Grube, 1855)*	<i>Cryptodrilus insularis</i> Rosa, 1891	<i>P. ephippiger</i> Rosa, 1898	<i>P. matsushimensis</i> Iizuka, 1898	<i>P. laccadivensis</i> Beddard, 1903	<i>P. albanysensis</i> Michaelsen, 1907a
Length (mm)	28–136	50	43–47	90–110	90–110	50
Segment	76–128	100	85–100	100–105	?	86
Clitellum	XIII–XVII, XVIII	?	XIII–XVII	XII–XVII	XIII–XVII	?
Spermathecal pore	7/8–8/9	7/8–8/9	7/8–8/9	7/8–8/9	7/8–8/9	7/8–8/9
Genital marking	19/20 or 20/21	?	XIX–XX	XIX–XX	XII/XIII, XIV; XI/XII, XII/XIII	17/18, 18/19
First nephridia	XIII	XIII	?	XIII	XIII	?
Last heart	XIII	?	XIII	XIII	XIII	?
Intestinal origin	XVI or XVII	?	XVIII	XIV	?	?
Spermathecae	VIII–IX	VIII–IX	VIII–IX	VIII–IX	***	***
Diverticulum	slender	absent	narrow tubular	tubular	tubular	tubular
Type locality	Villa Franca on the French Riviera, France	Aru Island, Indonesia	Christmas Island, Australia	Matsushima Bay, Rikuzen, Japan	Laccadive and Maldive Islands	Princess Royal Harbor, Australia

? Characters not given in the original description; \* Specimens from Thailand and Peninsular Malaysia; \*\* Spermathecal pores at 7/8, 8/9.

**TABLE 3.** Comparison of the morphological characteristics of *P. longissimus* sp. n. with other species of the genus.

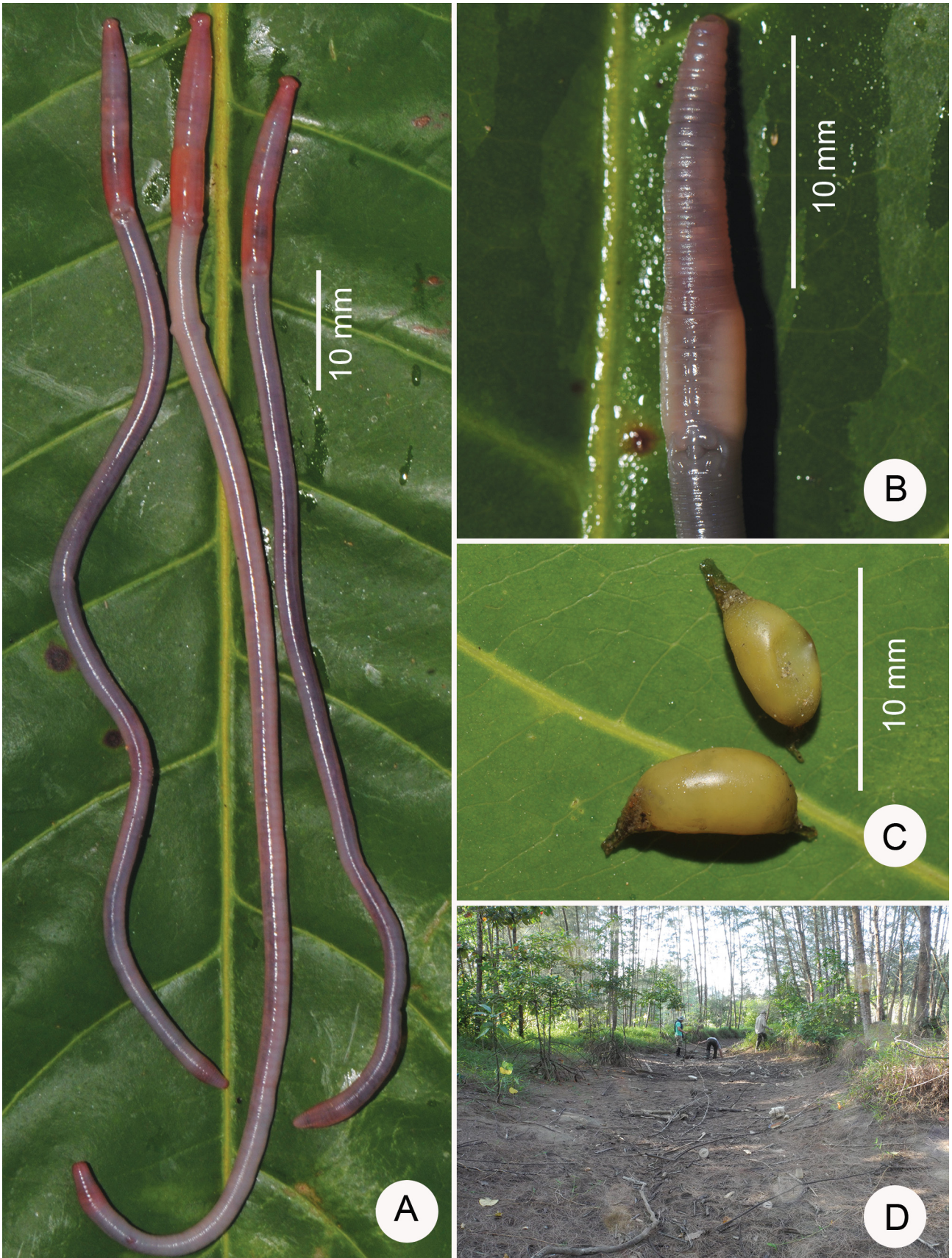
Character	<i>P. litoralis</i> * (Grube, 1855)	<i>P. lacustris</i> (Benham, 1903)	<i>P. agnesae</i> Stephenson, 1915	<i>P. sinensis</i> Chen & Zhifang, 1977	<i>P. primoris</i> Blakemore, 2000	<i>P. longissimus</i> sp. n.*
Length (mm)	28–136	35–40	65	30.5–41	60–120 (120)	125–165
Segments	76–128	85–90	116	64–94	100–150 (118)	160–191
Clitellum	XIII–XVII, XVIII	½XIII–XIV	½XIII–½XVII	XIII–XVIII	absent	XIII, XIV–XVII
Penial setae	absent	present	present	?	absent	absent
Spermathecal pore	7/8–8/9	VI–IX	7/8–8/9	7/8–8/9	7/8–8/9	7/8–8/9
Genital marking	19/20 or 20/21	?	absent	?	XIV–XVIII, 18/19– 19/20	17/18–18/19
First nephridia	XIII	XV	XII or XIII	XIII	XIV	XIII or XV
Seminal vesicle	XI and XII	IX and XI	IX and XII	IX–XII	XI and XII	XI and XII
Intestinal origin	XVI or XVII	XIII	?	XIV or XV	XV	XV or XVI or XVII
Spermathecae	VIII–IX	VI–IX	?	?	VIII–IX	VIII–IX
Diverticulum	slender	globular	club-shaped	?	short	absent
Type locality	Villa Franca, French Riviera, France	Lake Wakatipu, South Island, New Zealand	Sri Lanka	Kunming, Yunnan, China	Tasmania, Australia	Hat Pak Meng, Sikao, Trang, Thailand

? Characters not given in the original description; \* Specimen from Thailand and Peninsular Malaysia;



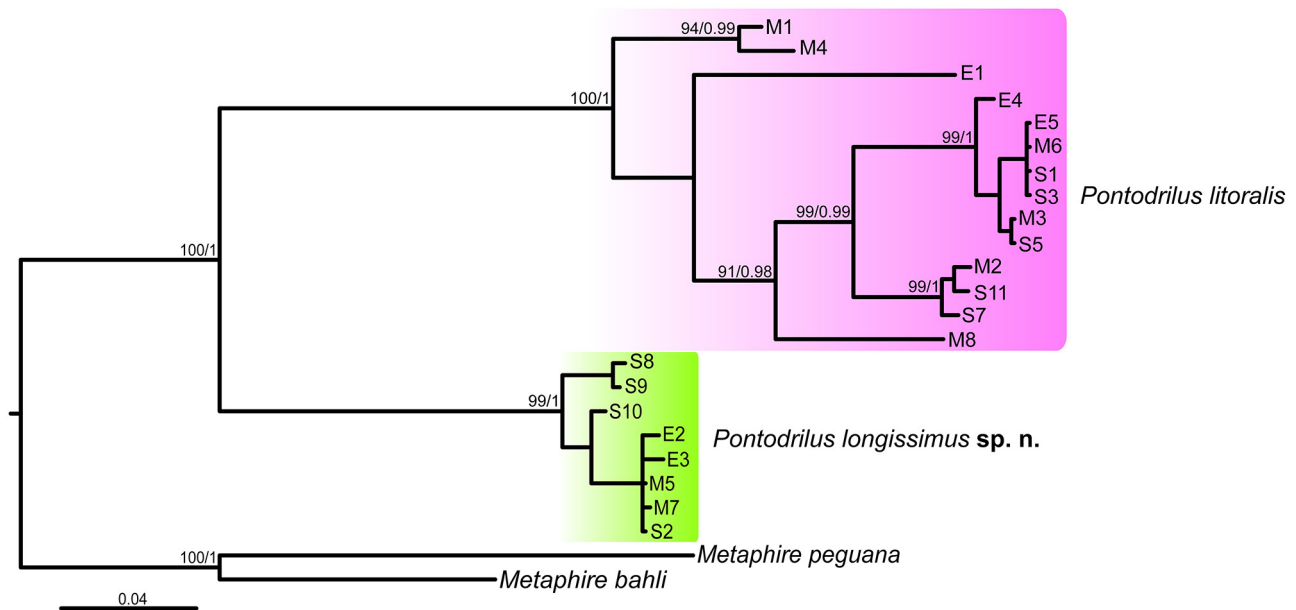
**FIGURE 4.** External and internal morphology of the holotype (CUMZ 3670) of *Pontodrilus longissimus* sp. n.; **A.** External ventral view; **B.** Internal dorsal view; **C.** Spermatheca.





**FIGURE 5.** Photographs showing: **A.** *Pontodrilus longissimus* sp. n. (CUMZ 3670, CUMZ 3671) just after the first step preservation in 30% (v/v) ethanol; **B.** Ventral view of anterior portion; **C.** Cocoons; **D.** The type locality of *Pontodrilus longissimus* sp. n. at Hat Pak Meng, Sikao, Trang, Thailand.





**FIGURE 6.** Bayesian inference tree based on the partial COI sequence (658 bp) of two *Pontodrilus* species and outgroups. Bootstrap values of >70% and posterior probability values of >0.95 are shown on the tree as ML/Bi values. Specimen names correspond to those in Table 1. Scale bar represents the number of nucleotide substitutions per site.

**Remarks.** The new species, *P. longissimus* sp. n., differs from the cosmopolitan littoral species *P. litoralis*, based on the specimens from Thailand and Peninsular Malaysia, by differences in segment number (160–191 and 76–128 for *P. longissimus* sp. n. and *P. litoralis*, respectively), and body length (125–165 mm and 28–136 mm for *P. longissimus* sp. n. and *P. litoralis*, respectively). Additionally, they are easily distinguished by the spermathecal diverticulum, absent in the new species but present in all other currently accepted species of the genus. *Pontodrilus litoralis* has a long and slender diverticulum, *P. lacustris* has a small globular diverticulum, *P. agnesae* has a spindle or club-shaped diverticulum, *P. primoris* has a short diverticulum relative to the ampulla. Among the many descriptions of *P. litoralis* and its synonyms, only *P. insularis* (Rosa, 1891) from Aru Islands, Indonesia (“Insel Aru”) is without spermathecal diverticula. Michaelsen (1897) identified tentatively two specimens from Sri Lanka (“Ceylon”) as *P. insularis* but conjectured that the absence of diverticula may be due to the fact that his and Rosa's specimens were subadult without clitellum, and he suggested synonymy with *P. bermudensis* Beddard, 1891, considering their overall similarity. Beddard (1895) also noted that the specimens were immature. However, Rosa (1898) maintained the possibility that the absence of diverticula is a taxonomic character and not caused by the immature state of the specimens, since “even in a series of sections no traces could be found of an organ which in the adult reaches so great a development” (id.: 283). Nevertheless, Michaelsen (1910) established the synonymy with *P. bermudensis*. Later, Easton (1984) synonymized *P. bermudensis* with *P. litoralis*. Even though Michaelsen's decision was not questioned in the subsequent taxonomic literature, the possibility remains that Rosa's species is distinct from *P. litoralis* (see Rosa 1898) and that it is characterized by the absence of diverticula. *Pontodrilus insularis* as originally described is much smaller than *P. longissimus* sp. n. (length 50 mm, diameter 3 mm, ca. 100 segments) and the first nephridia are found in XIII. *Pontodrilus longissimus* sp. n. is easily distinguished from other *Pontodrilus* species by the morphometric characteristics shown in Table 3. Cocoons, presumably of this earthworm, were collected from the littoral zone of the Andaman Sea at Hat Bo Chet Look, La Ngu, Satun. The cocoons are lemon-shaped, yellowish green in colour (Fig. 5C).

## Molecular analysis

The aligned COI sequence data matrix contained 22 specimens (excluding outgroups) with a length of 658 base pairs. Of these, 153 nucleotides were parsimony informative and 175 were variable sites. The majority consensus

tree of the Bayesian analysis of the combined dataset is shown in Fig. 6, but the topology was congruent with the ML analysis. Phylogenetic trees based on the NJ, ML and BI methods were divided into two well-separated clades, which agree with both species of *Pontodrilus* by 100% bootstrap values and 1.0 posterior probability supports, while the NJ tree (not shown) shows different topology within each species from ML/BI trees. The mean intraspecific variations for COI in the recognized species *P. litoralis* was 7.34% and *P. longissimus* **sp. n.** was 2.15%. Mean interspecific distance between both species was 17.77%. Comparison of *P. longissimus* sequences with published sequences of *P. litoralis* showed a similar divergence value (data not shown here). Interspecific distance between *P. longissimus* **sp. n.** and the outgroups was 18.16%.



**FIGURE 7.** Specimens of *Pontodrilus* at Hat Koe, Kaper, Ranong, Thailand, showing the comparative size and length of the two *Pontodrilus* species; **A.** *Pontodrilus longissimus* **sp. n.**; **B.** *Pontodrilus litoralis*.

## Discussion

The cosmopolitan littoral species *Pontodrilus litoralis* is widely distributed on shorelines in the tropics and warmer parts of continents and islands in all the world's oceans; further species of the genus are lacustrine in New Zealand (*P. lacustris*), terrestrial in Sri Lanka (*P. agnesae*), terrestrial or lacustrine in China (*P. sinensis*), and littoral in Tasmania, Australia (*P. primoris*) (Easton 1984; Blakemore 2002, 2007). The new littoral species described here was found in eleven locations in Thailand and two locations in Peninsular Malaysia. With respect to other *Pontodrilus* species, *P. longissimus* **sp. n.** is the longest (Fig. 7), and has the highest number of segments. Apart from the body dimensions, this new species can obviously be distinguished from the others by absence of the diverticulum.

The littoral earthworms from Thailand and Peninsular Malaysia presented in this paper, *P. longissimus* **sp. n.** and *P. litoralis*, occurred mostly on marine sandy coastal areas, which is the ecotone between terrestrial and marine habitats, in coastal salt marshes with a high organic matter and salinity ranges from 1–33 ppt. *P. longissimus* **sp. n.** was mainly found in habitats with substrates on the surface layer containing muddy sand, while *P. litoralis* occurred at habitats containing sandy mud. At Hat Pak Meng, Sikao, Trang, Thailand (type locality), *P. longissimus* **sp. n.** was found in the estuary at a salinity of 28 ppt and at 10–20 cm depth in muddy sand. Curiously, we did not

detect casts on the soil surface, yet there were some grass-like plants and some small shrubs (*Ipomoea pes-caprae* (L.) R.Br. and *Sesuvium portulacastrum* (L.) L.) growing on the soil surface. In addition, both species were found under roots of trees, leaf litter and areas that had organic matter near the shore of the sea. At some localities (Fig. 1), *P. longissimus* sp. n. was found co-existing with the cosmopolitan littoral species *P. littoralis*. Those areas might contain a high-enough load of organic matter as a food source for both species of earthworms to survive without competition. Even though we could find these earthworms in the same areas, *P. littoralis* was found at the beach surface (under seaweed and debris), while *P. longissimus* sp. n. was found deeper than *P. littoralis*; at a depth of more than 10 cm.

The molecular analysis revealed a high interspecific genetic distance between *P. littoralis* and *P. longissimus* sp. n., which is comparable to other earthworm DNA barcoding studies. Huang *et al.* (2007) indicated that the COI sequence divergence between species in earthworms in their study was greater than 15% in all cases. Furthermore, Chang & James (2011) concluded that the consensus among phylogenetic studies of earthworms showed that any two specimens with a Kimura 2-parameter distance of COI higher than 15% can be unambiguously assigned to two different species. According to Szederjesi *et al.* (2018), by comparing the molecular and morphological data, sequence divergence of 17% or higher can be treated as interspecific, but only when morphological differences have been detected. Thus, the DNA sequences of the mitochondrial COI region confirm the taxonomic status of *P. longissimus* sp. n. as a species different from *P. littoralis*. Overall, the combined evidence from both morphological and molecular results support *P. longissimus* sp. n. as a new species.

### Key to species of earthworm genus *Pontodrilus* Perrier, 1874

1	Four pairs of spermathecae	<i>P. lacustris</i> (Benham, 1903)
-	Two pairs of spermathecae	2
2	Four pairs of seminal vesicles	<i>P. sinensis</i> Chen & Zhifang, 1977
-	Two pairs of seminal vesicles	3
3	Seminal vesicles at IX and XII	<i>P. agnesae</i> Stephenson, 1915
-	Seminal vesicles at XI and XII	4
4	Spermathecal diverticula absent	<i>P. longissimus</i> sp. n.
-	Spermathecal diverticula present	5
5	Spermathecal diverticula longer than 1/2 ampulla length	<i>P. littoralis</i> (Grube, 1855)
-	Spermathecal diverticula shorter than 1/2 ampulla length	<i>P. primoris</i> Blakemore, 2000

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